Enzymes in Agricultural Sciences

OMICS eBOOKS Group
www.esciencecentral.org/ebooks

Edited by
Liliana Gianfreda
Maria A Rao

Enzymes in Agricultural Sciences

Edited by: Liliana Gianfreda and Maria A Rao

ISBN:

DOI:

Published Date: June 2014

Printed version: June 2014

Published by **OMICS Group eBooks**

731 Gull Ave, Foster City. CA 94404, USA

A free online edition of this book is available at www.esciencecentral.org/ebooks

Additional hard copies can be obtained from orders @ www.esciencecentral.org/ebooks

Preface

In agricultural soils the presence of enzymes assures a correct, integrated and regulated course of processes at soil-plant-environment interfaces that lead to the growth and production of crops for human and animal feed. The knowledge of their main properties and functions is a need for all scientists involved in this research field.

About Editors

Liliana Gianfreda has been Full Professor of Agricultural Biochemistry at the University of Naples Federico II, Italy. She received her Ph.D. degree in Chemistry (1969) at the University of Naples Federico II, Italy. Her research focuses on Environmental and Soil Enzymology. The author, coauthor, co-editor of over 200 research articles, book chapters and books, she is member of the Italian Society of Chemistry, the Italian Society of Biochemistry, the International Union of Soil Sciences, the Italian Society of Agricultural Chemistry. Member of the Editorial Board of Enzyme Inhibition Journal, Biology and Fertility of Soils, Journal of Soil Science and Plant Nutrition.

Maria A Rao is Associate Professor of Chemistry and Biochemistry of Plant Nutrition at the University of Naples Federico II, Italy. She received her Ph.D. degree in Agricultural Chemistry (1993) from the University of Naples Federico II, Italy. Her research focuses on Soil biochemistry and Environment protection. Author or co-author of more than 75 research articles, she is member of the Italian Society of Soil Science and Italian Society of Agricultural Chemistry. She was invited speaker at numerous National and International Congresses and Conferences. She was Guest Editor for the Special Issue ISMOM 2011 for European Journal of Soil Science.

Forewords

Liliana Gianfreda was a Full Professor in Chemistry and Biochemistry of Plant Nutrition of University of Naples Federico II at the Agricultural Faculty. Her main scientific interests are in the area of environmental enzymology and biotechnology and of bioremediation of polluted environments by using natural and model systems. She was a Visiting Scientist at some universities (e.g. Pennsylvania State University). Professor Gianfreda has coordinated and has been partner of several National and International (EU) research and a member of several national and international scientific societies. She is author or coauthor of more than 200 papers and eleven book chapters and editor of some books.

Maria A Rao is Associate Professor in Chemistry and Biochemistry of Plant Nutrition of University of Naples Federico II at the Agricultural Faculty. Prof Rao carries out researches in the field of soil biochemistry and she is also interested in studies focused on the control and protection of the environment. She carries out review work for SBB, JFAC, Chemosphere, JEMA, PRBI, and she is Guest Editor for the Special Issue ISMOM 2011 for European Journal of Soil Science. She is coauthor of 60 papers published in national and international journals and books.

Anna Piotrowska-Długosz

Liliana Gianfreda and Maria Antonietta Rao have been devoting their research and academic activity to soil enzymes for more than 20 years. They represent a reference point within Italian and International scientific community involved in soil biochemistry. In particular they focused their studies on synthetic enzymatic complex dynamics and their catalytic behavior, soil enzymes as bio-indicators of soil health and quality, soil enzymes potential to reclaim polluted soils. Their research activity and results have been presented in a great number of articles (published on relevant international journals), reviews, books and international conferences.

Sara Marinari

About the book: Plant survival and crop performances are strictly related to the interplay among different factors operating in the plant-environment agro-eco-system. Knowledge on enzymes present in the soil, including those in the surrounding of the root, the intrinsic role played by enzymes in plant growth, as well as the extrinsic role of such proteins in pesticides metabolism, and ensuring beneficial interactions with soil microorganisms are crucial for a better understanding of the intricate relationship among plants, soil particles and soil living organisms. The book edited by Gianfreda and Rao aims at presenting an updated survey of data on these subjects, collected in six chapters written by researchers active in the field. This book may represent a basis for people willing to deep their knowledge on plant-soil relationship with special emphasis on the role of enzymes and may provide a stimulus for further research efforts on biochemistry of crop plants.

Prof. Liliana Gianfreda is full professor of Agricultural Biochemistry at the University of Naples Federico II and an outstanding scientist active in soil science; her competence is well recognized nationally as well as internationally. Particularly, she developed a leader position in the study of soil enzymes, environmental enzymology and bioremediation of contaminated sites; in these fields she coordinated and was partner of several committees and research projects at national and international level. Her scientific activity is supported by the wide editorial and teaching activity. Taken together these aspects make her an excellent editor for the book on Enzymes in Agricultural Sciences.

Prof. Maria A Rao is associate professor of Chemistry and Biochemistry of Plant Nutrition at the University of Naples Federico II. She has been developing for a couple of decades a deep skillfulness on soil biochemistry with special emphasis on enzymes and bioremediation of polluted environments. Numerous scientific publications, the participation in national and international research projects and the editorial activity corroborate her scientific competence and are a guarantee for an efficient co-editing activity.

Roberto Pinton

Acknowledgement

We like to thank all authors for their contributions and for their time and expertise dedicated to the book, and without them it would not be realized.

Liliana Gianfreda and Maria A Rao

Introduction

The book deals with several recent aspects on the role of enzymes in agricultural sciences such soil biochemistry as influenced by intra- and extra-cellular enzymes, soil fertility, interactions between enzymes and pesticides and/or environmental pollutants, plant growth and processes at soil-plant interface. Contributions were from 14 leading experts in the field and *Enzymes in agricultural sciences* provide a detailed discussion on the functions of soil enzymes, their capability to be good indicators of soil quality, their response to environmental contamination, their specific structural, operational and regulatory features when involved in plant growth, and their main functions in the rhizosphere.

Table of Contents

ISBN:
DOI:

Chapter: 1

Soil Enzymes

Maria A Rao*, Rosalia Scelza and Liliana Gianfreda

Department of Agriculture, University of Naples Federico II, Italy

***Corresponding author:** Maria A Rao, Department of Agriculture, University of Naples Federico II, Via University 100 – 80055 Portici Napoli, Italy; Tel: +390812539173; E-mail: mariarao@unina.it

Abstract

Soil enzymes are involved and assist all activities fundamental to agricultural sciences. Indeed, all biochemical transformations taking place in soil are dependent on the presence of enzymes. They are a composite of different intracellular and extracellular enzymatic components, produced by microbial organisms (bacteria, fungi), or derived from animal and plant sources (plant roots, lysed plant residues, digestive tracts of small animals etc.). Soil enzymes are located and/or distributed in the soil matrix and their spatial variability in a soil profile and localization in soil structural fractions of different nature and size will influence their activity and performance.

Accurate methodologies for assaying soil enzyme activities are necessary to enlarge our knowledge on the role of enzymes in soil nutrient dynamics and their response to environmental factors influencing their production and expression.

The present chapter provides a survey of recent findings dealing with methodological problems still existing in the measurement of soil enzyme activities. Information is also given on the influence on soil enzyme activities by warming, and related effects, and biochar addition, two factors recently widely considered in soil enzymology studies. Brief notices on indexes and models integrating and explaining soil enzyme activities will be also provided. Enzymatic indexes can help describing and assessing microbial functional diversity across different types of soils and monitor their changes by diverse managements or disturbances over time. Models can contribute to understanding the importance of the interactions between microbial and chemical substrates in driving soil fundamental ecological processes.

Keywords:

Enzymatic indexes; Extracellular enzymes; Models; Soil enzyme measurements

Introduction

Soil is the place where all natural and anthropogenic activities necessary to the life of humans, animal, and plants occur. Forests, plants, grasses, crops grow on soil; superficial water basins and rivers are and flow on soil; animals spend their life and move on soil, between cycles of incorporation of food and release of droppings, these latter contributing to the normal recycling of soil and its nutrients. Soil is where humans have established their residence and built all structures necessary for their life.

ISBN:
DOI:

Soil is also the place where hazardous residues deriving from anthropic activities are very often released with dangerous and frequently irreversible effects on its safety and consequently human health. Often these human activities have produced desertification, deficit or even loss of biodiversity, alteration of the soil matrix, deficiency of organic matter and nutrients. It appears therefore evident as what happens on and within soil is of paramount importance for maintaining soil health and productivity and consequently for a balanced and integrated life on earth.

An equilibrated, safe and productive soil exists when its biological, biochemical, physical and chemical properties, all correlated to each other, contribute to sustain all activities occurring in it. Among these properties the activities of soil enzymes play a fundamental role because they mediate numerous chemical reactions involved in soil nutrient cycling; transformation of plant and microbes debris; mineralization and transformation of organic matter within the carbon cycle, transformation and degradation of potentially hazardous pollutants, thus contributing to the restoration and remediation of polluted soils. Therefore, soil enzymes are involved and assist all activities fundamental to agricultural sciences and as such they may be used as useful and suitable indicators of microbial nutrient demand [1] and soil health and quality.

In literature there are several classifications of soil enzymes [2-8] from one of the earliest and very likely more exhaustive proposed by Burns in the early 1982 [3] who described up to ten categories of soil enzymes, to those recently summarized by Burns et al.[4]. Simplifying two main categories of enzymes can be recognized in a soil: Intracellular Enzymes (IEs) and Extracellular Enzymes (EEs). All enzymes occurring and functioning in living cells, i.e. microbial, plant and animal cells residing in soil belong to the first category and as such they are considered as a whole of microbial, plant and animal metabolism. Enzymes produced by living cells but secreted outside in their neighboring environment can be considered EEs. They may explicate their activities still bound or associated to their producing cell or at a distance from the parent cell, being free in the liquid phase. EEs can be in turn be divided in more categories (Figure 1) that do not have a defined borderline being interrelated with each other and possibly transforming to each other.

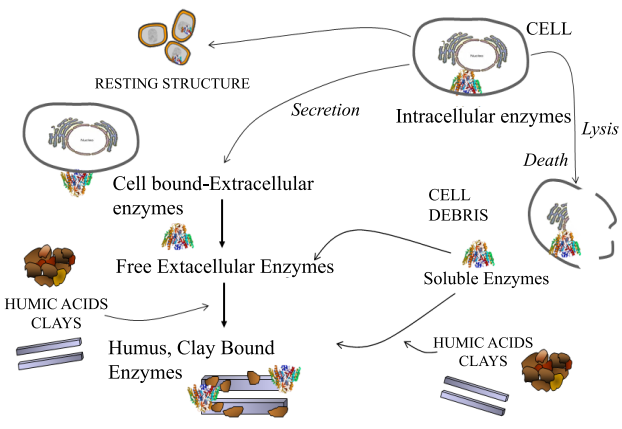

Figure 1: Complexity of enzyme activities in soil.

ISBN:
DOI:

An interesting group of soil EEs is those classified in the past by Gianfreda and Bollag [5] as "naturally immobilized enzymes" and recently renamed as stabilized enzymes (SEs) [8]. Once released into the soil from their originating cells, these enzymes are likely adsorbed, linked, anchored or embedded on/in/to solid supports such as clays, clay minerals, organic matter and organo-mineral complexes (Figure 1). Several experimental evidences derive from studies performed with synthetic enzyme systems simulating those possibly occurring in soil and their behavior, performance and features have been investigated. Several findings are available in literature on the preparation, characterization and properties of different enzymes adsorbed, entrapped and/or complexed with different natural or synthetic inorganic soil constituents by means of several mechanisms [8-10].

Although a huge amount of findings and related publications are available in literature on soil enzymes, there is still a consistent number of open, not yet resolved questions about several aspects regarding EEs such as: localization of EEs in the soil matrix, their contribution to substrates turnover and global biogeochemical processes; their regulation by biotic and abiotic soil factors and consequent effects on soil organic carbon dynamics; their relationship with producing organisms and relative counter-effects; development of suitable models to describe both the function and biomics of ecosystems; the possible development of a suitable soil enzyme index of soil status and health; and role that soil enzymes may play in the dynamic of plant nutrients, in the control and restoration of environments polluted by both pollutants and pathogenic diseases [4].

Many of these still open problems arise, as well specified by Nannipieri et al. [7] and Wallenstein and Weintraub [11], from several considerations so summarized: 1) Current and standard soil enzymatic methods usually provide limited information on the real activity of soil enzymes. Assays performed under laboratory conditions measure only potential and not real enzyme activities without giving any information on *in situ* activities or on the relationship between the two types of activities; moreover they take also into account the contribution of stabilized enzymes that could not be active under *in situ* conditions. 2) These assays do not provide information neither on the production of enzymes or their producing organisms, neither on their turnover rates or their meaning. 3) Substrates used in these assays are simple, soluble compounds whereas substrates of enzyme activities in soil are usually larger, complex insoluble polymers that are degraded in simpler monomeric products. 4) Assays under lab conditions are usually carried out at fixed pH, temperature, soil moisture etc., and thus they do not provide information on sensitivity of enzyme activities to changes of these parameters. 5) Minor modifications occurring in experimental procedures can lead to errors and affect not only the final values of measured activities but also their comparison with other data collected by different researchers. 6) Several enzymes are involved in nutrient dynamics; consequently a single enzyme activity cannot be used as valuable indicator of such dynamics. 7) Since soil enzyme activities can be influenced by several factors such as changes in soil management, plant cover, changes in environmental conditions, presence or addition of fertilizers, pesticides or contaminants, current measurement assays are often not suitable to allow a right interpretation of the response of the enzyme activities to these factors.

In this brief chapter we will try to examine some of these hot questions giving a summing up of what has been done and can be still done to clarify the even now obscure features of this fascinating field of agricultural sciences. Particular attention has been devoted to last scientific achievements.

ISBN:
DOI:

Soil Enzyme Activities: Measurement, Location and Distribution in the Soil Matrix

Measurement

As outlined by several authors and continuously stated in recent papers, the most, still present, important shortcoming, limiting a right interpretation and utilization of soil enzyme activities (and their changes by both abiotic and biotic processes) is the lacking of universal, standardized, uniform, and optimized methodologies to measure the activities of enzymes in soil. Therefore, it is difficult to use absolute soil enzyme activity values as a powerful tool for understanding soil biological processes.

It is well established that "maximum potential" and non-actual or "realized" [12] enzymatic activities are measured with assays now available. Enzymes are usually measured indirectly by determining their activity in the laboratory using biochemical assays that, therefore, do not represent true *in situ* activity levels.

Although a lot of papers, book chapters and reviews have been dedicated to this topic [2,4,5,7-10,13,14]in the last years many papers have approached again the main procedural details and problematic aspects involved in soil enzyme assays and not resolved yet (Table 1).

How to measure a soil enzyme activity?	*In situ* measurements: • histochemical techniques • electron microscopy • zymography *In vitro* measurements: • on soils in toto (soil slurry) • on soil extracts
Activity measurement problems	Sample preparation: • collection • sterilization Sample storage: • air-dried or moist • refrigerated • room temperature
Assay conditions	Determination of product formation or substrate disappearance under defined reaction conditions: • saturating substrate concentrations • optimal pH • optimal temperature • stirring

ISBN:
DOI:

Differences between *In vitro* and field conditions		*In vitro* assays	Field conditions
	Substrate	excess	limiting
		homogenous	heterogeneous
		soluble	insoluble
		artificial	natural
	Buffer	present	absent
	Temperature	optimal	variable
	Flora and fauna	absent	present
	Shaking	often	stationary
	Reproducibility	high	low
Difficulties in the determination of soil enzymatic activity	Contribution by inorganic catalysts to the reaction under investigation		
	Possible growth of microorganisms and de novo synthesis of enzymes during the assay		
	Stability of the reaction product in the reaction mixture		
	Influence of the soil treatment on the activity assay		
	Contribution of the different soil enzymatic categories		

Table 1: Methodological aspects to be considered in the evaluation of soil enzymatic activity.

In situ **measurement:** The most realistic method to detect enzyme activities in soil would be their direct visualization in undisturbed soils, i.e. *in situ* (intended as *on site*) measurements. Attempts in this way were made early by Ladd et al. [15] by using electron microscopic observations of soil sections, after their treatment with suitable compounds, which allow enzymes in soil structure to be localized, or reaction with substrates giving rise to products easily visualizable as colored compounds [15]. Histochemical staining was also considered a sensitive and suitable method to detect enzyme activity in soil as in the case of alkaline phosphatase in extra radical mycelium of arbuscular mycorrhizal (AM) fungi [16]. These methods, however, had not a large application for the measurements of enzymes associated with soil particles because of possible interferences by soil minerals and humic substances that are electron-dense components. Moreover, in the field diffusion rates may reduce the rate at which enzymes and substrate can interact to each other [17].

Visualization *in situ* of the activities of hydrolases (e.g. acid phosphatase, amino peptidase, chitinase, and b- glucosidase) in forest soils was achieved by a modified root window-based, enzyme-imprinted, membrane system [18]. Substrates giving rise to colored or fluorescent products were utilized with a good response in the field.

Recently, a different approach based on an *in situ* zymography for analysis of the two-dimensional distribution of enzyme activities in soil was utilized [19 and Chapter 4 of this book]. By means of thin gels with embedded substrates it was possible to quantify the activity of protease and amylase in the rhizosphere of lupine (*Lupinus polyphyllus*) grown in rhizoboxes [19]. The authors concluded that since zymography "does not require destruction of soil structure, it likely pictures enzyme activities more realistically than standard enzyme assays", thus offering "a promising tool for mapping distributions of enzyme activities in soils in a work- and cost-efficient way".

A very new method that allowed to rapidly measuring multiple soil biological properties simultaneously was proposed and described by Dick et al. [20]. It utilized a near infrared spectroscopy (NIRS) method requiring not expensive equipment and usable for a very large numbers of samples. The b-glucosidase and b-glucosaminidase (NAGase) activities, and soil

ISBN:
DOI:

organic C and amino sugar N concentrations in 184 diverse soils of Ohio were predicted. Values measured under laboratory conditions were calibrated against NIR spectral data with partial least squares regression analysis. Further statistical analyses gave R2 values higher than 0.8 for the majority of measured properties, thus suggesting the valuable utilization of the method.

Laboratory conditions: The majority of assays described in literature are, however, usually carried out under laboratory conditions. Soil are sampled at 0-10/20 cm layer and stored refrigerated at 4°C for 10-15 days, at the most. Debate is still present whether it is much more convenient to use soil slurries or soil extracts, to store the soil sample in the refrigerator or in freezer or also after air-drying at room temperature. Since all manipulations occurring in the sampling, preparation, handling and storage of soil samples may disturb the soil, altering several factors connected to enzyme activity (e.g. activity of microorganisms, status of the enzyme, whether immobilized or free, substrate availability, etc.) it is important to well define and describe how the soil has been sampled, handled, treated and stored prior to the determination of the enzyme activity [4,7,13]. This allows to reproduce as much as possible the procedure and to compare with other results reported in literature.

Without entering in detail, soil sampling strategy (i.e. the sampling time, the number of samples, their horizontal and vertical distribution, the sampling procedure and device) should be representative of the natural situation of studied soils and should consider temporal and spatial dynamics of biochemical and microbiological soil properties to be measured [13]. Indeed enzyme activities usually display evident temporal and spatial variations and generally decrease with increasing soil depth. An accurate sampling procedure can be easily assured if simple rules well described in literature are followed [13].

As regards storage, there are several findings demonstrating that a short storage (at the most 10-15 days or less) of field moist soils at 4°C is preferable as respect to the other storage procedures that may affect also the response of florescent substrates.

DeForest [21] evaluated whether soil storage and processing methods significantly influenced measurements of potential *in situ* enzyme activity in acidic forest soils. Six soil EEs (NAGase, phosphatase, phenol oxidase, b-glucosidase, b-xylosidase and peroxidase) were measured using 4-4-methylumbelliferone (MUF)-linked substrates and L-dihydroxyphenylalanine (L-DOPA) 2, 7, 14, and 21 days after collection on soil stored at both 4 and 20°C. Results indicated that storage temperature did not influence enzyme activity values whereas extended time in buffer did. Analyses within 2 h were considered the best measure of potential *in situ* enzyme activity and the benchmark for all statistical comparisons. Moreover, the activities of b-glucosidase, b-xylosidase and peroxidase were insensitive to storage and processing methods.

Later, Wallenius et al. [22] showed that, 16 weeks-storage of frozen or air-dried soil, humus or compost differently affected the assay of ten hydrolyzing enzyme activities determined by artificial fluorogenic substrates and the impact depended on the soil matrix and the assayed enzyme. In particular, freezing affected soil enzyme activities less than air-drying that decreased compost activity more than 50%. Similar results were obtained by Peoples and Koide [23]. Two soils with significantly different enzyme activities were tested for 1,4-β-cellobiohydrolase, acid phosphatase and β-N-acetylglucosaminidase as fresh, frozen or dried samples, and the ratios of each soil activity to that the other soil for the three storage treatments were determined. Freezing and drying significantly affected activity ratios when compared to the fresh control for all three enzymes, being the effect of freezing much less than that measured for drying.

ISBN:
DOI:

Each investigator should consider whether soil storage affects within-experiment treatment comparisons of soil enzyme activity. Therefore, the type of storage has to be considered being often dependent on the physical-chemical characteristics of the investigated materials and very often site and enzyme-specific.

Soil slurry is usually preferred to soil extract being this latter possibly disruptive of natural soil conditions and reductive as respect to the whole enzymatic activities of the investigated soil. Notwithstanding, Vancon and Keen [24] demonstrated that a new method for extracting soil β-1,4-glucanases (cellulases) and β-1,3-glucanases (laminarinases) was reproducible, could be completed in 1 day and measured twice as much enzyme activity than the standard passive soil enzyme extraction procedure. It consisted in the mechanical disruption of soil samples with acid washed ceramic and glass beads, successively processed six times with phosphate buffer (pH 7.0) by bead-beating for 30 s and 1 min incubation in an ice bath. Crude enzyme extracts were used for measuring enzyme activities. The method allowed multiplex enzymatic assays from several soil samples at one time.

As previously discussed [2,25], assay conditions such as presence or absence of a buffer, pH, substrates and their concentration, temperature, shaking of soil, inhibitors of microbial proliferation etc, markedly affect the measured activity Usually a soil enzyme assay is based on the use of a buffered solution of a synthetic, artificial substrate at a concentration high enough (saturation concentration) to be assumed constant throughout the time course of the enzymatic reaction and assuring a zero-order kinetics. Moreover, substrate concentration should be very much larger than that of the enzyme to allow a reaction rate proportional to the enzyme concentration. A buffered vs. an unbuffered condition is usually chosen when the disappearance of substrate and/or the formation of reaction products may change the pH of the soil slurry, since an optimal pH value is required during the assay.

These *In vitro* optimized conditions, very different from those existing in vivo, are usually well target for studying enzyme's characteristics. By contrast, conditions simulating as much as possible those present in nature, i.e. soil conditions, should be preferred when the goal is to study soil enzymes from an ecological point of view [4].

Substrates commonly used in the past and very often still used in recent research, either as bench or microplate assays, are those including in their molecular structure a chromophore component which, after the enzymatic reaction, achieves spectroscopic properties, i.e. becomes detectable and measurable by spectroscopic determination, or releases as product molecules itself colored or colorable under particular chemical conditions.

Classical examples of those substrates are p-nitrophenyl derivatives, used for assaying the activity of hydrolases such as phosphatases, glucosidases, and sulphatases. The main product of the enzymatic hydrolysis is p-nitro phenol that under alkaline conditions acquires a yellow color, whose intensity is detectable at 400-405 nm wavelengths.

An example of the use of these substrates was the high-throughput microplate assay implemented for simultaneous colorimetric quantification of multiple enzyme activities in soil [26]. The activities of β-N-acetyl-glucosaminidase, α-glucosidase, β-glucosidase, α-galactosidase, and β-galactosidase were measured by using p-nitrophenyl substrates. The assay being applicable to a large number of soils within hours of sampling was considered suitable for system-level evaluations.

From the introduction of substrate derivatives with enzymatic production of fluorescent end products [13 and references therein, 27, 28 several papers have been dedicated to compare results obtained with same enzymes and spectroscopic or fluorimetric based approach [29-32].

ISBN:
DOI:

Moscatelli et al. [29] by comparing fluorimetric and spectrophotometric assays of β-glucosidase in soils subjected to different management practices tried to find out reasonable answers on the role of β-glucosidase kinetic parameters as reliable indicators in detecting changes due to soil management and the capability of different analytical approaches to give the same results. Both fluorimetric and spectrophotometric approaches highlighted changes occurring under organic and conventional management. Higher V_{max} values were measured under organic cropping system although the whole catalytic behavior of β-glucosidase was not affected by organic management as indicated by the values of the specificity constant (K_a). This parameter was strongly influenced by the analytical approach. Increases of its values were detected using the fluorogenic substrate, 4-MUF-β-d-glucopyranoside, indicating a higher catalytic efficiency of the enzyme with the fluorimetric approach.

Studies on the same enzyme were also performed by Dick et al. [30] to evaluate the reliability of p-nitrophenol (pNP)-based and MUF-based microplate methods for estimating β-glucosidase activity in two soils with different characteristics. Evaluation of the two methods was also extended to the kinetics of the enzyme and the effect of different preparation procedure of soil samples (e.g., sample, sonication, soil suspension) on the two methods was studied, as well. While values of enzyme activities measured by the MUF and bench methods were comparable, more variable results in terms of reproducibility, kinetic parameters, and less sensitivity were measured with the pNP microplate method, probably because of high background absorbance that affected its reproducibility, sensitivity, and accuracy. These results suggest that the pNP microplate method is not as a valid substitute for the standard bench method.

A better efficiency of the microplate fluorimetric (F) assay was also demonstrated by Trap et al. [31] when fluorimetric assay was compared to the standard spectrophotometric (P) method. The activities of five enzymes (cellulase, N-acetyl glucosaminidase, b-glucosidase, acid phosphatase and alkaline phosphatase) in contrasting land uses, including woodland, grassland, cultivated and contaminated lands were measured. Although activities measured with the P methods were higher (around 8 times) than those measured with the F methods, significant differences in enzyme activity were revealed by the F methods in different soils. The authors concluded that “the F method improves the effectiveness and the efficiency of measuring universal soil quality indicators using enzymes”.

Further studies to define standardized and validated enzyme assays for meaningful data comparison and interpretation were carried out by Deng et al. [32]. They compared bench scale and microplated format assays of soil enzyme activities using spectroscopic (pNP) and fluorometric (MUF) based approaches. Investigations were extended to pNP-bench, pNP-microplate and MUF-microplate and three different enzymes were tested in 16 different soils. MUF-based assays were found about 14 times more sensitive and precise than pNP-based assays when soil suspensions were used, although standard errors raised by the presence of soil suspensions. Results were different with the three analytical protocols but being in the same order of magnitude and significantly correlated to each other indicated that the different protocols detected the same pool of isoenzymes.

Although the sensitivity and proved efficiency of MUF-based methods, few articles are still dedicated to these methods, indicating that their impact on soil enzymology is limited yet. Soils with a large variety of characteristics should be studied with MUF-based methods and compared with colorimetric enzyme assays. As summarized by Gianfreda and Ruggiero [13], microplate fluorimetric assay and the p-nitrophenol method have advantages and disadvantages (Table 2) and the choice between them is strongly dependent on the goal of the research is carrying on.

ISBN:
DOI:

4-methylumbelliferone micro plate fluorimetric assay	p-nitrophenol assay
Products	
Measured fluorimetrically	Measured colorimetrically
Excitation at 365 and emission at 460 nm	Absorbance at 405 nm
MUB fluorescence is pH-dependent	p-nitrophenol absorbance is pH-dependent
Highly sensitive especially at low concentrations	Low sensitivity
No known side effects	
Quenching of fluorescence by soil particles and phenolic compounds	p-nitrophenol interferes with organic material
MUB molecule is highly mobile	p-nitrophenol is adsorbed
Assay	
Soil added volumetrically	Soil added gravimetrically
Little quantity of substrate needed (μl)	Large amounts of substrate required (ml)
Short incubation time (35 min)	Medium to long incubation time (1-24 h)
Substrate difficult to dissolve	Substrates dissolve instantaneously
Continuous monitoring of product release overtime (1 read cycle min^{-1})	A single reading at the end of the incubation
Measurement directly carried out in the reaction medium	Need to stop the reaction and extract the product prior to measurement (measurement must not be delayed, otherwise there is formation of Na_2CO_3)
High numbers of samples and replicates processed at the same time (96 well $plate^{-1}$)	Maximum of 50 assays per run
Plate set-up and measurement in less than 1 h	Assay preparation, incubation, product extraction and measurement > 3.5 h
Disposable materials (micro plates, universal bottles, pipette tips)	Use of glass equipment with time-consuming preparation
Results	
Automatic calculation of activity rates (relative units of fluorescence min^{-1})	Withdrawal of background absorbance
Miscellaneous	
Expensive analytical equipment (fluorimetric plate-reader, multichannel (digital) pipettes, micro plates)	Analysis at low cost
A limited number of potential substrates available	A large number of substrates available

Table 2: Comparison between the MUB micro plate fluorimetric assay and p-nitrophenol assay for measuring soil enzyme activity [13].

The validity of different substrates for the measurement of particular soil enzymes is also under continuous investigation. Indeed, the use of different substrates may be helpful to better understand the role of an enzyme in nutrient transformations in soil.

Examples are the findings of Bach et al. [33] and Kumar et al. [34]. In these papers, different substrates were used to measure the activity of an enzyme and the effect of several parameters on the response with the different substrates was evaluated. For instance when substrate oxidation was measured for phenol oxidase and peroxidase activities with pyrogallol (PYGL), L-DOPA, and ABTS in three soils across a pH gradient from 3.0 to 10.0 to determine the pH optimum for each substrate, and further with 17 soils, results indicated that activities on the substrates followed the order PYGL > L-DOPA > ABTS and were inversely related to substrate redox potential [33]. At pH > 5 only L-DOPA was a suitable substrate. Moreover, both soil type and assay pH evaluated not only which substrate was the most suitable but also the absolute and relative oxidation rates among substrates. These results suggested that for studies devoted to particular goals it is recommended to use all the three substrates and also to perform assays

ISBN:
DOI:

at soil pH and at an optimum reference pH to evaluate the pH effect on soil oxidase activity. This could contribute to greater comparability of oxidase potential activities across studies.

The validity of the two substrates TTC and INT for soil dehydrogenase activity was reviewed by Kumar et al. [34] under different environmental conditions and with different soils to evaluate which one can be considered the most appropriate substrate. Authors examined 49 papers dealing with the measurement of soil dehydrogenase in natural and degraded soils in the presence of different factors such as moisture, temperature, soil aeration, different management regimes and others. According to other and previous findings the authors concluded that poor results are obtained with TTC and dehydrogenase measurement under controlled conditions may provide a valid, a useful index of changes in soil quality.

As reported above, enzymatic assays are usually carried out at optimal conditions that in terms of pH usually means at a time-constant and defined pH where the enzyme under investigation shows its maximum activity. Turner [35] determined the pH optima of eight hydrolytic enzymes involved in the cycles of carbon, nitrogen, phosphorus, and sulfur, in seven tropical forest soils of contrasting pH values from the Republic of Panama. Results led to individuate three classes of enzymes based on their pH optima and variations with soil pH (Table 3). As already established by other authors [36-38] the optimum pH values of acid and alkaline soil phosphatase were 4 to 5 and 9.5 to 11.5 and prevailed in acid and alkaline soils, respectively. By contrast, arylsulphatase activity showed a very acidic optimum pH in all soils (pH ≤ 3.0) irrespective of soil pH. These different behaviors can suggest that enzymes had possibly different origins or were differently stabilized on soil matrix.

Group	Enzyme	Optimum pH range
Acidic consistent among soils	cellobiohydrolase	4.0-4.5
	β-xylanase	4.5-5.5
	arylsulphatase	3.0
Acidic – sub-acidic, variable with soil pH	α-glucosidase	3.0-7.0
	β -glucosidase	3.0-4.75
	β- N-acetylglucosaminidase	3.0-5.0
Acidic or alkaline, depending on soil pH	acid phosphomonoesterase	3.0-5.0
	alkaline phosphomonoesterase	9.5-11.5
	phosphodiesterase	3.0-5.5

Table 3: Classification of enzymes by their pH optima and variation with soil pH [35].

The optimum pH value, however, can, and often is, different from the pH of tested soil. If the in-situ approach is preferred (e.g. conditions that mimic the soil environment and more approximate natural soil conditions [39], assays should be performed at pH of the bulk soil solution by keeping in mind the possible interference and artifacts arising from substrate and/or product sensitivity to pH changes as well as to strong acidic or alkaline pHs.

Attempts to use water instead of buffered solution at a given pH were made by several authors [4 and references therein, 7 and references therein, 40-43]. Recently Lessard et al. [43] compared the activities of arylsulphatase, urease, acid phosphatase and protease performing their assays at usual standard buffered conditions (acetate pH 5.8, borate pH 10, modified universal buffer pH 6.5 and THAM pH 8.1, respectively) and in water. Tests were carried out on 10 pairs of Zn-contaminated soils and buffer effects on Zn lability as well as changes of pH during water-performed assays were evaluated. No significant fluctuation of pH (only + 0.57 pH unit) and no effect by buffers on the metal concentration were observed. Moreover, similar results (except urease) were obtained under water or buffered conditions, thus suggesting that water could be used as “surrogate solvent” in some contests.

ISBN:
DOI:

Advanced methodologies: All previous results clearly indicate that variability and uncertainty are still present for the type, characteristics and features of soil enzymatic activities. They derive mainly from the absence of universal, homogeneous and standardized methodologies for enzyme assays. Some general and particular suggestions have been and can be given measuring enzyme activity in soil (Table 4). These recommendations might assist researchers in developing a unified understanding of enzyme activities in ecosystem ecology [12], although more research appears still required.

Steps to follow and to define in an accurate protocol [7]
1. the determination of concentration changes of the reaction product or the substrate
2. the use of an efficient extraction protocol for product or substrate from soil
3. the choice of the buffer
4. the monitoring of the effect of pH values on enzyme activity
5. the study of the effect of time and temperature of incubation on enzyme activity
6. the study of the effect of different amounts of soil on enzyme activity
7. the study of the effect of different substrate and end-product concentrations on the enzyme activity
8. the choice of a proper control
Recommendations [12]
1. run enzyme assays at the environmental pH and temperature
2. run proper standards, and if using fluorescent substrates with NaOH addition, use a standard time of 1 min between the addition of NaOH and reading in a fluorometer
3. run enzyme assays under saturating substrate concentrations to ensure V_{max} is being measured
4. confirm that product is produced linearly over the duration of the assay
5. examine whether mixing during the reaction is necessary to properly measure enzyme activity
6. find the balance between dilution of soil homogenate and assay variation
7. ensure that enzyme activity values are properly calculated

Table 4: Required steps and recommendations measuring soil enzyme activities [7,12].

Many of pitfalls correlated with soil enzyme measurements can be overcome by advanced and modern methodologies such as genomic and transcriptomic tools and proteomic methods. Wallenstein and Weintraub [11] proposed that genetic studies, proteomic tools and new mass-spectrometry approaches could be useful for the measurement of *in situ* activity of soil EEs. Indeed, methods to extract RNA and DNA from soil have been greatly improved and can be used to identify the genetic potential of microorganisms to produce specific enzymes in soil microbial communities [4,11].

When functional genes coding for particular enzymes are abundant and detected with a certain frequency in a soil, not necessarily it implies that those enzymes are expressed in the soil. Indeed, no correspondence was found between the presence of laccase-encoding genes of fungal origins in surface soils and their changes with season with laccase activities remaining unchanged in the same periods [44,45]. Only transcriptomic studies assessing the presence of a mRNA transcript may confirm that the enzyme has been possibly produced and regulated in that soil. Indications can also derive from phylogenetic studies. By the relationships existing between the genetic potential for enzyme production and 16S rRNA phylogeny, Zimmerman et al. [46] demonstrated that about half of sequenced prokaryotic genomes were found be capable of producing extracellularly alkaline phosphatase, chitinase and b-N-acetyl-glucosaminidase enzymes, thus suggesting that the capacity to produce EEs varies at relatively fine-scale phylogenetic resolution.

The entity of pool sizes, diversity and microbial source of soil enzymes can be, then,

ISBN:
DOI:

assessed by proteomic studies. Proteomic studies performed on a forest soil [47] and Litter [48] demonstrated a partial correspondence between proteins or enzymes in soil solution with those identified in the proteome extract. Attention has also to be devoted to factors affecting proteomic studies. For instance, Giagnone et al. [49] demonstrated in model experiments that a high content of montmorillonite greatly affected the response of microbial proteomic studies. Additionally mass-spectrometry can be an useful tool to identify and quantify the products of enzymatic degradation.

These molecular techniques, however, may fail in the estimation of unknown enzymes or the enzymatic potential of viable but non-cultivable cells. Metagenomic approaches are continuously improving [50] and they can be a powerful tool to assess the enzymatic potential of noncultivable microorganisms and help to recognize and isolate new enzymes. Additionally, metaproteomics can be used to study protein expression from a complex system and provide direct evidence of metabolic and physiological activities.

Location and distribution

Another important and complex aspect of soil enzymology is the identification of enzyme location and/or distribution in the soil matrix, i.e. the spatial variability in a soil profile and localization in soil structural fractions of different nature and size.

Extracellular enzymes extruded outside the cell may diffuse far from the cell in the soil solution or remain still attached to it by operating within the cell periplasm, associated to the cell wall being confined in biofilm or capsule of polysaccharidic nature or contained in structures such as polysomes (Figure 1) [4,7,13]. Typical examples of enzymes contained in polysomes or cellulosomes, are polysaccharidases involved in the degradation of polysaccharides and lignocellulose. It appears quite obvious that in the case of cell-bound enzymes substrates have to reach the enzymes and diffusional limitations may arise. In this case, their producing cells implement some strategies such as production of signals trough the so-called quorum-sensing system to monitor the environment and/or contact the target substrates by responding via chemotaxis to gradient concentrations. When the enzymatic action, in the majority of case macromolecule degradation, occurs at the cell-soil solution interface formed monomeric products will be easier up-taken by the cell with evident advantages for the cell metabolism.

It is widely accepted that the activities of soil enzymes usually decrease with soil depth accordingly to the decrease of microbial biomass and organic C content [7 and references therein, 13 and references therein]. Moreover, the response of enzymes to different treatments and/or disturbance may change along a soil profile and with depth.

Geng et al. [51] examined the response of six hydrolytic enzyme activities and two oxidative enzyme activities in forested ecosystems under three different treatments (intact forest controls, canopy tree thinning, and canopy tree thinning plus ericaceous stem removal and soil tilling) at different depths. All enzyme activities except for peroxidase were significantly lower at 10-20 cm than 0-10 cm soil depth. Moreover, in the 0-10 cm soil horizon the activities of protease and arylsulphatase significantly decreased in each treatment compared to controls as cellulase and phenoloxidase activities also did. By contrast, an opposite trend for peroxidase activities was observed and no significant differences between treatments for glucosaminase, glucosidase and acid phosphatase activities were detected. A different behavior occurred at 10-20 cm soil depth, where treatments affected only the activities of acid phosphatase and phenol oxidase, whereas all other soil enzyme activities remained unchanged. The effects of management or disturbance on agricultural ecosystems are usually examined in surface soils

ISBN:
DOI:

and no attention is devoted to the changes in microbial activity occurring in deep soils where large amounts of organic carbon are potentially stored with possible consequences on carbon biogeochemical cycle.

Kramer et al. [52] studied in a field experiment the response of microbial communities, in terms of microbial biomass as phospholipid fatty acids (PLFAs) and enzyme activities involved in the C-cycle (β-glucosidase, N-acetyl-β-glucosaminidase, β-xylosidase, phenoloxidase and peroxidase), to management practice such as crop type (wheat and maize) and litter amendment across a depth transect (topsoil, 0–10 cm; rooted zone beneath the plough layer, 40–50 cm; and the unrooted zone, 60–70 cm) over a period of two years. Differences were observed under the two cultivations and litter application. Higher bacterial and fungal biomass and higher enzyme activities were measured with wheat than with maize till a depth of 50 cm whereas increasing effects of the same parameters deriving from litter application were visible only in surface soils. As expected hydrolytic enzyme activities decreased with depth, by contrast, no decrease or even an increase of oxidative enzyme activities was observed with depth.

That subsoil microbial activity may contribute to nutrient cycling at rates similar to their surface counterparts was confirmed by changes in phosphatase kinetics and soil nutrients measured at 0, 20, 50, 80, 110 and 140 cm depths across two parent materials (Oxisols and Inceptisols) and two distinct forests (lower and upper montane) [53]. Both apparent kinetic parameters V_{max} and K_m as well as total carbon, nitrogen and extractable phosphorus largely decreased with soil depth.A higher variability of V_{max} compared to K_m parameter was observed but no measurable change of their ratio $V_{max}/K_m$was calculated through the first meter of soil profiles whereas 50% reduction occurred at 140 cm soil depth. These results suggested that not only microbial communities in subsoil are metabolically active; contributing to P-cycling, but also that production of the enzyme rather than substrate availability is controlled by microorganisms.

Interesting is the behavior of enzymes in the rhizosphere, an oasis of biological activity where plant properties dominate microbial and enzymatic behavior, a different gaseous regime is present and mineral solubilization and competition effects influence nutrient cycling [Chapter 4 of this book].

The distribution of enzyme activities between bulk and rhizosphere soil and the accumulation of enzymes at soil-plant root interfaces, the contribution of enzymes deriving from enzymatic proteins produced by plant roots and released in their surrounding rhizosphere soil, the effect of plant species composition and root type as well as the contribution of root mycorrhization on enzyme activities of rhizosphere soil have attracted the attention of several researchers and many findings are available in literature on these topics [13 and Chapter 4 of this book].

One of the first evidence of enzymes in the rhizosphere was their localization by *in situ* observation made by Ladd et al. [15] and Joner et al. [16], further confirmed by zymography studies [19,54].

Phosphatases are enzymes typically more abundant in the rhizosphere, particularly in rhizosphere soils of mycorrhize-infected plants, and their activity is related to P availability and/or deficiency [13] and references therein]. In their review on phosphatase activity in natural and mined soil Kumar et al. [38] demonstrated that both acid and alkaline phosphatase activities had good relationships with inorganic P fractions and their activity varied with mycorrhizal association.

ISBN:
DOI:

Further insights in the distribution of microbial- and root-derived phosphatase activities in the rhizosphere of *Lupinus albus* L and their dependence on P availability and C allocation have been recently provided by Spohn and Kuzyakov [54] in their studies on the coupling of soil zymography results with ^{14}C imaging, a technique capable of reveal the distribution of photosynthates after labeling plants with ^{14}C. Larger (5.4 times) amounts of both acid and alkaline phosphatase activity occurred in the rhizosphere than in the bulk soil, being the first mainly associated with roots although produced by roots and microorganisms. By contrast, alkaline phosphatase activity was produced only by microorganisms and was more broadly distributed in a larger area as respect to acid phosphatase. The activities of two enzymes were also differently affected by P fertilization and rhizodeposition of photosynthates, thus indicating that evidently different ecophysiological groups of organisms capable of mineralizing organic P exist and their spatial differentiation possible reduces a potential competition between them.

Not only phosphatases are clearly present in the rhizosphere. An interesting study was performed by Wu et al. [55] that correlated the activity of protease and β-glucosidase in the rhizosphere of *Citrus unshiu* with the spatial distribution of glomalin-related soil protein (GRSP), root mycorrhization, soil aggregates and carbohydrates. Positive correlations were found between two-categories (total and easily extractable) of GRSP with β-glucosidase, soil water-stable aggregates water-extractable or hydrolysable carbohydrates and mycorrhization whereas a negative correlation was found with protease.

Correlations between five enzymes involved in the transformation of C, P, and N substrates and arbuscular mycorrhizal, ectomycorrhizal, dual-colonized (arbuscular and ectomycorrhizal), and ericoid mycorrhizal plants mycorrhizospheres were found by Gartner et al. [56] in a fire chronosequence in Alaska. In particular, β-glucosidase and peroxidase were lower in arbuscular mycorrhizospheres and ericoid mycorrhizospheres, respectively, than in bulk soil. Additionally, mycorrhizosphere types influenced the entity of each enzyme activity, thus indicating that "the community composition of mycorrhizal host plants might mediate enzymatic activity in boreal soils" [56].

Additional information on rhizosphere enzymes and their relation with soil, plants and microbes was provided by studies of Bell et al. [57] in which the stoichiometry approach developed by Sinsabaugh and co-workers [1,58-60] (was applied to rhizosphere).

In their pioneering study Sinsabaugh et al. [1] assembled a comparative database of soil EEs potential for 40 ecosystems and related soil microbial function, as expressed by EE activities, to global biomass composition, nutrient dynamics and soil organic matter storage. In other words an elemental stoichiometric evaluation was derived between microbial biomass and detrital organic matter and microbial nutrient assimilation, growth and expression. The main result achieved by the authors was that a similar stoichiometry likely exists between the more common measured soil enzymatic activities and all microbial communities.

Starting from the hypothesis that "soil nutrient and microbial stoichiometry would differ among plant species and be correlated within plant rhizospheres" Bell et al. [57] studied soil C, N, P, microbial biomass C, N, and soil enzyme C, N, P nutrient acquisition activities using rhizospheres of eight different intact species-specific plants in a semiarid grassland in Wyoming, USA. Overall results indicated that strong positive correlations exist between soil and plant tissue stoichiometry, whereas these components and microbial or enzyme stoichiometry weakly correlated to each other. Indeed, contrarily to what expected no negative correlation was observed between soil microbial enzyme activities and microbial biomass stoichiometry.

ISBN:
DOI:

By contrast, plant tissue, microbial, and soil nutrient stoichiometry showed similarity among many plant species independently of plant functional type, thus indicating that no a strong conservation occurred for plant tissue and rhizosphere stoichiometry at the plant species level.

Extensive studies have been also devoted to examine the micro-scale distribution of enzyme activities in particle-size soil fractions to evaluate their catalytic potential and kinetics as affected by biotic and abiotic soil constituents as well as their variation in response to different agricultural practices [13 and references therein]. Useful information may be also achieved on the role exerted by different soil fractions on microorganisms and enzyme activities protection and in the turnover of organic C, by following the response of microbial processes to a range of long-term and short-term organic C-inputs. Usually soft fractionation methods have been utilized to guarantee the complete recovery of soil enzyme activities [13 and references therein].

For instance, Marx et al. [61] investigated the distribution of four carbohydrases (b-cellobiohydrolase, N-acetyl-b-glucosaminidase, b-glucosidase and b-xylosidase), acid phosphatase and leucine-aminopeptidase in four particle-size fractions (>200, 200–63, 63–2 and 0.1–2 mm) obtained by a combination of wet-sieving and centrifugation, after low-energy ultrasonication.The effect of the location on enzyme distribution and long-term N fertilizer management on their respective kinetics was studied as well. Carbohydrases distributed mainly in the coarser fractions while phosphatase and leucine-aminopeptidase predominated in the clay-size fractions. Moreover, the association with different particle-size fractions influenced the substrate affinity of each enzyme as revealed by Michaelis constant (K_m) measurements whereas no significant effects were observed by N fertilizer management.

Relationships between the specific location of cellulase within the matrix of a paddy soil and soil organic matter (SOM) quality and carbon turnover were found by Yan et al. [62]. In particular, aphysical fractionation procedure was used to investigate whether soil carbon was spatially isolated from degradative enzymes in the soil. In particulate organic matter fractions (POM) the activity of carboxymethyl cellulase was greatest in coarse fractions and generally decreased from the coarse to the silt-size fraction, concurrently to organic C concentrations did.

The different depth gradients shown by hydrolytic and oxidative enzymes in the study of Kramer et al. [52] could indicate that hydrolytic enzymes are very likely linked to POM whereas oxidative enzymes mainly prevail on mineral fractions. Moreover, a relationship between microbial abundance and substrate availability possibly exist only for hydrolytic enzymes.

Limitation to soil organic matter accumulation was observed in desert grassland soils where the higher and stabilized activities of phenol oxidase and peroxidase occurred as compared to temperate soils and uniformly distributed across particles ranging from >1 mm to <38 μm [63].

Environmental Factors Affecting Production and Expression of Soil Enzyme Activities

Soil enzyme activities may be affected by numerous factors of both natural (i.e. physico-geological, geographical or physico-chemical properties of soils, organic, clay or biomass contents etc.) and anthropogenic (agricultural management, environmental pollution, additives such as fertilizers, pesticides, salts, heavy metals, etc.) nature. These factors may influence the production, activity, catalytic behavior and persistence of soil enzymes through different mechanisms involving direct, either reversible or irreversible, and indirect effects. Reversible or irreversible action on the catalytic active site of soil enzymatic activities as well as alteration of

ISBN:
DOI:

the protein conformation may occur. Measurable changes in size, structure, and functionality of the microbial community as well as altered production (induction and/or repression) of enzymes may result as indirect effects on soil microbial growth and activity.

In this contest the attention has been devoted to a limited number of factors and the reader is referred to literature [5,8-10,13,64-67] and the other chapters of the present book for a comprehensive vision of the topic.

The factors selected and analyzed in this chapter are climate changes (increasing and decreasing temperature, precipitation and associated variation of soil moisture and drought, and the possible interference of nitrogen fertilization) and soil management by biochar addition. The choice has been due to the particular attention devoted in the last years to these factors and to the numerous very recent findings available in literature.

Temperature, precipitation and drought, soil moisture

Temperature strongly affects and controls soil enzyme activities, changing enzyme kinetics and stability, substrate affinity and enzyme production because it can influence the size and activity of microbial biomass. As soil hydrolytic enzymes are the main drivers of soil organic matter (SOM) degradation and litter decomposition, the dependence of these enzymes on global changes including warming, precipitation, drought and associated soil moisture will assist in understanding the relationships among SOM stock, global carbon cycle and microbial nutrient demand. Moreover, the possible interference of nitrogen demand in soil has also to be considered, being nitrogen a fundamental element not only for several metabolic routes but mainly because involved in protein and therefore enzyme synthesis.

To evaluate the role of EEs in soil and ecosystem responses to climate change, Henry [68] reviewed field studies in which the responses and inter-annual variation of soil EE activities were examined to warming and altered precipitation, seasonal variations, elevated atmospheric CO_2, increased atmospheric N deposition and changes in disturbance regimes. Henry [68] underlined that the measurement of potential soil enzyme activities, as afforded by assays performed under laboratory conditions, can be inadequate to provide a real picture of temperature and moisture dependence of enzyme activity in situ. Moreover, some criticisms about the methodologies used to simulate warming in field experiments were also evidenced. Controlled environment and field experiments usually involve open top chambers, greenhouses, retractable passive warming curtains, snow removal, heated coils/fluid filled tubes inserted into soil or overhead infrared heaters. Each of these methods, often selected by financial, logistical or spatial constraints rather than scientific reasons, may present advantages and disadvantages and introduce artifacts. In many cases they do not represent real situations occurring *in situ* following warming or precipitation or wind, mainly in cold areas where snow very often covers lands. These methodological aspects can influence the interpretation of enzyme activity responses and have to be taken in duly account [68]. The main conclusion drawn by the author was that in general soil moisture manipulations in field studies have had a much greater influence on potential EE activities than warming treatments, though no high warming effects could have been achieved in the field due to the used methodology.

Opposite conclusions were achieved by Baldrian et al. [69] in their studies in a temperate hardwood forest soil with dominant *Quercus petraea*, with large seasonal temperature differences and moderate changes in soil moisture content. The effects of soil temperature and seasonality on the sizes of EE pools and activities along with microbial biomass, soil moisture content, and pH were investigated for three years in the litter (L), organic horizon (O) and upper mineral horizon (Ah). Strong increases in enzyme activity as measured *In vitro* were observed

ISBN:
DOI:

up to temperature of 20-25 °C and higher activity levels occurred in the warm period of the year, whereas no significant changes were detected in the pools of most EEs and in the content of microbial biomass. Soil moisture did not significantly affect enzyme activities.

Contrasting results (increase or decrease) of specific enzyme activities, involved in C, Nand Pcycles, were obtained in a temperate grassland of northern China as six-year responses to warming and increased precipitation [70]. The responses were soil depth-dependent and indicated that enzymes differentially responded to warming and increased precipitation at two depths in this region. For instance, the activities of acid phosphatase and β-glucosidase dramatically decreased with warming at 10–20 cm soil depth whereas a strong increase of acid phosphatase and β-N-acetylglucosaminidase occurred in surface soils, where the patterns of enzymes were mainly driven by soil pH, ammonium and nitrate.

A different temperature-sensitivity behavior as well as low activity levels could be expected by enzymes acting in cold areas. Wallenstein et al. [71] investigated seasonal variation and temperature sensitivities of six hydrolytic enzymes at 4 and 20°C on four sampling dates in tussock, intertussock, shrub organic, and shrub mineral soils in Arctic tundra soils at Toolik Lake, Alaska. Greatest activities of β-N-acetylglucosaminidase, β-glucosidase, and peptidase were observed at the end of winter, suggesting that enzymes were produced even in frozen soils. Soil enzyme pools generally showed high sensitivity to temperature (as measured by Q_{10}) in the same period of the year and not higher activity levels were observed during Arctic summer. Temperature was found to be the strongest factor driving low *in situ* enzyme activities in the Arctic, as achieved by modeling the potential activity of *in situ* β-glucosidase for tussock and shrub organic soils based on measured enzyme activities, temperature sensitivities, and daily soil temperature data. However, limitation in nitrogen supply could have affected enzyme production in summer determining their non-expected lower values [71].

The influence of nitrogen fertilization on enzyme responses to climate changes (precipitation variability and relative varied soil drought) was successively confirmed by the findings of Alster et al. [72]. Direct and indirect negative effects of drought on litter decomposition, and faster decomposition by N-adapted microbial communities in N-fertilized plots than in non-fertilized plots were shown as measured by the activities of nine EEs in a semi-arid study site in southern California. Authors started by the hypothesis that changes in fungal biomass, considered dominant in the area, and potential EE activities would relate directly to litter decomposition responses. Contrarily to what hypothesized, bacteria and not fungi predominated in the area and they negatively responded to drought treatment. However, fungal biomass and many potential enzyme activities positively responded to drought treatment although their efficiency (measured as the mass loss of chemical substrates per unit of potential enzyme activity) declined with drought, possibly because an increase of enzyme immobilization and a reduced diffusion rate occurred due the low water availability. In N-fertilized plots, larger efficiencies of some enzymes (b-glucosidase, b-xylosidase, and polyphenol oxidase) were observed when microbes were transplanted into originating environments, thus suggesting that microbial enzymes may adapt to their local environment. Authors concluded that a link between enzyme potentials and *in situ* activities is possible if the impact of drought and N addition on the efficiencies of EEs can be predicted.

Nitrogen addition showed significant effects also on the kinetics (V_{max} and K_m parameters) of cellobiohydrolase, b-glucosidase, b-xylosidase, b-glucosidase, and b-N-acetylglucosaminidase involved in SOM degradation in two forest soils [73]. The values of K_m were considerably decreased by N addition in a soil, while variable K_m was observed in the other soil. By contrast

ISBN:
DOI:

significant increases of V_{max} were detected for all investigated enzymes in the two soils. Both kinetic parameters were temperature sensitive but a greater response from V_{max} (higher Q_{10} values) than K_m was observed. No measurable interaction between N-addition and temperature variation was detected. Overall results suggested that a larger degradation of substrates possibly occurred under N-fertilization and higher hydrolytic enzymatic activities could be expected by the simultaneous increase of temperature and N-addition.

The role of temperature sensitivities in SOM decomposition on both kinetic parameters of the same enzymes was investigated in five sites crossing from a boreal forest to a tropical rainforest [74]. Q_{10} values ranging from 1.53 to 2.27 for V_{max} and 0.90 to 1.57 for K_m were calculated and a significant ($P = 0.004$) negative relationship with mean annual temperature was observed for the K_m of b-glucosidase, suggesting that temperature can have higher effect on enzymes in cooler climates. When data were parameterized in a mathematical model it was concluded that V_{max} and K_m temperature sensitivities could counterbalance to each other for the SOM losses. Moreover, results confirmed the adaptation of microbial EEs, as measured by their kinetic behavior, to local environmental temperature [72].

Recent findings seem to partly contrast this conclusion [75] at least in an alpine grassland ecosystem. The response of five soil EE activities (phenol oxidase, b- and α-glucosidase, b-xylosidase, cellobiohydrolase) and temperature sensitivity (Q_{10}) to experimental warming was investigated by setting up a free air-temperature enhancement system. While extracellular enzymes adapted to seasonal temperature variations showing higher values during the warm period of the year, no acclimation to the field experimental warming was observed.

Similar results were obtained for four peptidases and four glycosidases when exposed to experimental climate manipulation in a long-term experiment in northern Sweden [76]. Experimental soil warming and/or winter snow addition did not show significant effect on either the potential activities or the temperature sensitivity of the two group of enzymes, whereas opposite trends of significantly season-dependent patterns occurred for the two functional enzymatic groups. This behavior may suggest that warming acts indirectly on soil processes by the seasonality of substrate supply and microbial nutrient demand rather than directly by influencing the production of enzymes. Moreover, the potential divergence between the different seasonal patterns observed for the two groups of enzymes must be considered when enzyme-based models are implemented to describe soil processes.

Detailed studies on the temperature and moisture sensitivity of *in situ* β-glucosidase enzyme activity were performed by Steinweg et al. [77] and data incorporated in a mathematical model indicated that beside temperature and moisture β-glucosidase activity was affected also by substrate concentrations and diffusion constraints, thus providing a template for analyzing the role of specific abiotic managing *in situ* enzyme activities, facilitating their incorporation in biogeochemical models. The new experimental protocol considered soil samples collected every two weeks over a 10-week period with precipitation inputs manipulated to obtain drought (50% ambient precipitation), ambient, and wet (150% ambient precipitation) treatments. Enzyme activity was assayed in soil slurries at three different temperatures (15, 25 and 35 °C) and to different moisture levels in the lab and adding substrate to homogenized dry or moist soils instead of slurries. Stable temperature sensitivity but significant variation of moisture sensitivity was detected among the five sample dates and treatments. As respect to ambient and wet plots, drought plot soils showed strong responses of the enzymatic activity to increases of moisture. When the effect of only temperature or only moisture or their simultaneous action were estimated, results indicated that the highest β-glucosidase activity occurred in ambient

ISBN:
DOI:

plots and that it responded primarily to changes in temperature except in dry soil. These results corroborated what usually retained i.e. *in situ* enzyme activity in soils can be strongly limited by low soil moisture, thus reducing any increasing effect of warming.

Further studies were extended to evaluate how climate variables (warming, precipitation and interaction between them) affected the activities of β-glucosidase, cellobiohydrolase, xylosidase, acid phosphatase, β-N-acetylglucosaminidase and leucine-aminopeptidase, and of microbial biomass carbon (MBC) in different seasons and in soils treated with the same levels of precipitation (ambient, 150% of ambient during growing season, and 50% of ambient year-round) and four levels of warming (unwarmed to ~4°C above ambient) over the course of a year [77]. Negligible or little effects by climate manipulations (warming, precipitation, moisture and season) were observed on potential enzyme activity, whereas mass-specific potential enzyme activity (calculated by potential enzyme activity/MCB ratio) raised with temperature, reached its peaking value under medium warming, then declined at the highest warming. Moreover, a stable enzyme pool under drought was observed thus indicating that mass-specific enzyme production increased with temperature and drought or that decreased enzyme turnover rates occurred in dry soils. The stoichiometry of potential enzyme acquisition activities strongly changed with season, with an increase in C-acquiring enzymes and a decline in the potential activity of N-acquiring enzymes from summer to winter when a reduction in organic N degradation likely occurred. A stable enzymatic C:P activity ratio instead was measured over the year indicating a consistent, continuous need of P sources. At low soil temperature and in frozen soils slower reaction rates as well as reduced *in situ* activities will be likely present because of the limited diffusion of substrates [77].

As compared to presence and composition of fungal communities, abiotic factors such seasonality, nutrient concentrations, moisture, pH, carbon and nitrogen content, and mean annual precipitation, showed higher and more significant effects on the activities of α- and β-glucosidase, β-xylosidase, cellobiohydrolase, β-N-acetylglucosaminidase and acid phosphatase in five sites during the growing season in March and 17 sites during the dry season in July throughout southern California. The correlations between abiotic factors, fungal community composition and soil enzyme activities were examined by analyzing the response of enzyme activities to all factors (abiotic vs. fungal composition) separately and concomitantly across a variety of ecosystems [78]. The analysis showed no correlation between fungal community composition and enzyme activities at the species, genus, family or order levels, whereas significant correlations were found between soil carbon, nitrogen and pH and hydrolytic activities, and between mean annual precipitation and oxidative activities. In dry season, fungal composition explained only 27.4% of the variation in all enzyme activities but 35.3% of it was accounted for by abiotic factors.

Biochar fertilization

Soil management is strongly related to global food security and research, then practices and policies influencing soil management are of paramount importance to assure a sustainable agriculture [79]. Soil management by addition of inorganic fertilizers and organic residues is an old and very common agricultural practice to increase soil fertility and crop production. Several are the types of inorganic fertilizers as well as organic substrates and a large literature is available on their effects on the activities of soil enzymes as one of the main and more expected results of their addition to soil [13 and Chapter 2 of this book].

Recently, a great attention has been devoted to the use of biochar to improve soil fertility by promoting sustainable resource efficiency, mitigate climate changes, increase crop yield

ISBN:
DOI:

per area, increase soil carbon (C) storage by its sequestration, reduce soil acidity, and reduce irrigation and fertilizer requirements. In addition the biochar capability to decrease soil emissions of greenhouse gases and reduce nitrous oxide (N_2O) emissions was taken into account as healthy effect [80,81].

A wide illustration of different research approaches and obtained results on biochar characteristics and its effects on several soil biological and chemical properties and environmental conditions is provided by a "Virtual special issue" of Soil Biology and Biochemistry Journal available on the web site of the Journal [82] and including 29 articles dealing with these topics and spanning from 2009 to 2012.

Biochar is a stable carbon-rich by-product synthesized through pyrolysis/carbonization of plant- and animal-based biomass that can last in soil for very long periods. Biochar chemical and physical properties depend on the raw material characteristics and pyrolysis conditions as well as its soil ameliorant capacity is dependent on the soil properties and environmental conditions. Another, interesting and valuable property of biochar is its reactivity and capability to reduce and mitigate soil pollution by contributing to the restoration of soils contaminated by both organic and inorganic contaminants when added to contaminated soils [83-37]. Biochar large surface area and cation exchange capacity, strictly dependent on the originating material and pyrolysis treatment, facilitate and improve sorption of pollutants to its surfaces, reducing not only mobility but also bioavailability and phytoavailability of organic contaminants in soil. Char materials may, however, contain toxic compounds. Therefore, to avoid undesired effects on soil biota and environmental quality, a detailed characterization of biochar nature and quality is mandatory before any application either for agricultural or environmental purposes.

It has been well established that amendment of soil with biochar affects not only microbial activity, biomass and community structure, but also other soil biota as fauna and plant roots (see the extensive review of Lehmann et al. [88] (Figure 2). Weak or strong correlations might establish among primary properties of biochar, soil processes they influence and soil biota, with resulting effects on soil behavior (Figure 2).

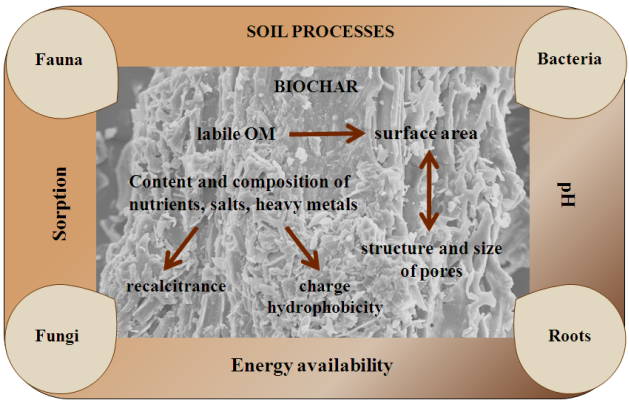

Figure 2: Interactions among soil biota, soil processes and biochar properties.

Usually, increases of enzyme activities have been detected as a result of the increase of soil microbial biomass by biochar addition, although opposite responses have been also obtained

ISBN:
DOI:

[89,90]. Stable biochar materials obtained from the weed *Parthenium hysterophorus* and used at different amounts in laboratory experiments increased the activities of soil dehydrogenase and catalase while hydrolytic enzyme activities decreased [90]. Increases of acid phosphatase, alkaline phosphatase and fluorescein hydrolase activities as well as those of dehydrogenase and catalase were instead observed with biochar obtained from water hyacinth (*Eichornia crassipes*) applied to a red soil from Dhanbad, India [89]. The increase was dependent on the amount applied and was the highest in 20 g kg^{-1} biochar treatment.

To better enhance soil productivity and biological activity biochar was co-applied with fly ash to the same soil and soil nutrients, biological properties and the yield of *Zea mays* were measured [91]. Dehydrogenase (+60.7%), alkaline phosphatase (+32.2%), and fluorescein hydrolase (+12.3%) activities increased due to co-application of the two materials. Further positive effects were great increases of soil nutrients (+110% P and +64% K), microbial biomass (+25.3%) and maize grain yield (28.1%). pH-buffering effect and sorption of SOM to mineral surfaces probably induced more reactive interactions among water, air and nutrient in soil [91]. For a clear understanding of biochar effect on soil enzyme activities, possible interaction with substrate and products should be, however, considered [92,93], because real biochar effect on soil enzymes can be hampered and masked by such interactions.

Experiments performed on sandy silt loam soils amended with different amounts of pine wood and barley straw biochar demonstrated that significant decreases (up to 80%) of substrates (pNPP and INT) and respective products (pNP) and iodonitrotetrazolium formazan (INTF) occurred in the assays of dehydrogenase and phosphatase, suggesting that solid-phase adsorption on biochar surfaces did occur [93]. Bioavailability of the substrates decreased and no saturating condition possibly arose. Statistical analysis was conducted to evaluate the effects of soil type, biochar concentration, biochar feedstock and their interactions on the concentration or extractability of enzyme assay substrates and products. Results indicated that the chemical properties and nature of biochar as well as the soil type affected the relationship between sorption and biochar concentrations, such as no an unique correlation or correction factor could be developed.

Similar sorption phenomena have been already observed by Bailey et al. [92] when effects on the activities of soil β-glucosidase, β-N-acetylglucosaminidase, lipase, and leucine aminopeptidase were investigated in biochar-amended soils or *In vitro* reaction. Indeed, to explain variable effects of biochar on soil enzymatic activities observed with field studies, *In vitro* colorimetric and fluorescent assays were performed in microcosms with tree different soils added with biochar. As compared to non-amended soils, the activity of β-glucosidase increased and that of lipase decreased as evidenced by assays performed with fluorescent substrates. When substrates were temporarily put in contact with biochar and successively tested, a reduction of the apparent activity of the enzymes was observed thus confirming that sorption of substrates on biochar surface hampered enzyme activity. By contrast, a stimulating effect (increases by 50-75%) of β-N-acetylglucosaminidase activity was measured when the purified enzyme was previously exposed to biochar. Therefore, when biochar-sorption effects are not rightly considered, soil assays can underestimate the increase of soil enzymatic activities due to biochar.

Biochar adsorption of β-glucosidase but not of its substrate cellobiose was found by Lammirato et al. [94] when both were exposed to chestnut wood char *In vitro* phosphate buffered system. A reduction by <30 % of the reaction rate occurred, indicating a slight influence of the material on the activity of the enzyme. By contrast, complete inhibition of the reaction rate occurred with activated carbon that adsorbed more than 97% of either β-glucosidase or cellobiose, probably because of its high specific surface area and high porosity.

ISBN:
DOI:

Indexes and Models Integrating and Explaining Soil Enzyme Activities

Two of the hot questions cited in the Introduction regards the possible integration of EE activities in i) an index of soil quality as a valid tool capable of properly describe and assess microbial functional diversity across different types of soils and monitor their changes by diverse managements or disturbances over time, and ii) models at global levels integrating not only carbon, nitrogen or soil nutrient dynamics but also microbial biomass and EE activities. These models can help understanding the importance of the interactions between microbial and chemical substrates in driving soil fundamental ecological processes.

Soil enzymatic indexes

As well stated in the past [95-98] and recently stressed by Cardoso et al. [99] a true and accurate description of soil health or soil quality (used synonymously) and its use in sustainability predictions require that several soil chemical, physical and biological properties should be considered and their response to soil perturbation within a reasonable timescale verified. Thus a systemic approach based on different kinds of indicators in assessing soil health would be safer than using only one kind of attribute.

Among soil biological properties, enzyme activities are considered good indicators of soil quality and its directions of change with time caused by soil management practices, soil pollution by organic and inorganic pollutants, soil perturbation by microbial inoculation and others. Indeed, enzyme activities are influenced by natural and anthropogenic factors [5], rapidly respond to ecosystem variations and changes in soil use and management and, being easily measurable, they may provide a rapid tool to follow environmental modifications [100 and references therein].

This high degree of variability of enzyme activities could be, however, a limitation to their use as indicators of soil quality because contradictory results in different studies might result [99]. Therefore, the reliability of enzyme activity measurements as indicators of changes in soil quality is severely related to the possibility of comparing information from different laboratories.

An attempt was made by Creamer et al. [101] by implementation of an inter-laboratory comparison of multiple-enzyme and multiple substrate-induced respiration assays to assess method consistency in soil monitoring. Authors compared investigations performed in three laboratories. The activities of β-cellobiohydrolase, β-N-acetylglucosaminidase, β-glucosidase, acid phosphatase, β-galactosaminidase, β-xylosidase, β-galactosidase and sulphatase were tested by the hydrolysis of 4-MUB-containing substrates from nine sampling sites providing a range of soil physical, chemical and microbiological properties. The analysis was based on testing for intrinsic variation, i.e. within-assay plate, inter-laboratory repeatability by means of geometric mean regression and correlation coefficient, and land-use discrimination (principal components analysis).Results demonstrated a large intrinsic variation, diverging patterns for the enzyme assays for inter-laboratory repeatability, and significant discrimination of soils with relatively consistent patterns, although inconsistent correspondence between the laboratories was observed. Therefore, studies confirmed that suitable biological standards should be identified if biological indicators of soil quality should be used. Indeed, less availability and widespread utility of reliable analytical standards for biological methods still limit repeatability of soil biological data.

Many efforts have been made to condense the achieved information in a numerical value, an index capable of differentiating between soils with different quality features or affected by

ISBN:
DOI:

pollution or other disturbances. As emphasized by Bastida et al. [102] the characteristic of soil quality relating to soil properties that changes as a result of soil use and management should be quantified and the index should be a "sensitive and accurate" tool "for evaluating changes in soil quality in a short time-scale".

Indexes, mainly based on enzyme activity values, have been also developed to predict and indicate the rate of a whole metabolic process. In many cases, they relied on a conceptual wrong condition, that is a single enzyme activity or just a category of activities belonging to the same enzymatic class (e.g. oxidases or hydrolases) could well describe complex soil metabolic processes [7]. In other cases, empirical relationships were derived and correlated some changes of nutrients like carbon or nitrogen with microbial biomass-C or –N, and enzyme activities involved in their transformation. Such indexes were applied and their effectiveness was tested with both agricultural and non-agricultural soils. A list of such indexes is reported in Table 5 and details on each of them, its application and effectiveness in evaluating soil quality are summarized in Bastida et al. [108] Nannipieri et al. [7] and Chapter 2 of this book.

Whichever numerical index is proposed, its applicability at a general level requires, however, validation on many soils differing in properties and history. This approach was followed by Puglisi et al. [103] who developed by canonical discriminant analysis three numerical indexes (AI 1, AI 2 and AI 3) based on all or some of seven enzyme activities (arylsuplatase, b-glucosidase, phosphatase, urease, invertase, deydrogenase and di-phenoloxidase) measured in three soils characterized by different alteration events (Table 5). The validity of each index was tested using a third experimental data set not used in index development. When tested and validated on published data sets dealing with enzymatic activities in different soils and characterized by different alteration events, the third index (AI 3) was able to discriminate altered soils from controls by higher index scores [103].

Index (*)	Purpose	Reference
Soil Alteration Index AI 1 = −21.30 arylsulphatase +35.2 β-glucosidase −10.20 phosphatase −0.52 urease −4.53 Invertase+14.3 dehydrogenase+0.003 phenoloxidase. Soil Alteration Index AI 2 = 36.18 β-glucosidase −8.72 phosphatase −0.48 urease−4.19 invertase Soil Alteration Index AI 3 = 7.87 β-glucosidase −8.22 phosphatase −0.49 urease.	Effects on the quality of agricultural soils contaminated with industrial and municipal wastes, organic fertilization or irrigation with poor quality water under different crops: *Ficus carica*, maize, tomato, etc.	[103]
Enzymatic Activity Number (EAN) = 0.2 (0.15 dehydrogenase + catalase + $1.25.10^{-5}$ phosphatase + 4.10^{-2} protease + 6.10^{-4} protease).	Effect in cultivated and forest soils and pastures Effect of soil management on its quality	[104]
Biological Index of Fertility (BIF) = (1.5 dehydrogenase + k 100 catalase)/2.	Effect in untilled management systems (natural grassland and orange grooves) comparing to tilled systems in south-eastern Sicily	[105]
Biochemical Index of Soil Fertility (B) = Corg+N_{total}+dehydrogenase+alkaline phosphatase+protease+amylase.	Effect of organic and mineral fertilization	[106]
Organic C content = −0.4008 arylsulphatase+0.4153 dehydrogenase+0.4033phosphatase+0.4916β-glucosidase.	Evaluation of soil in different states of degradation	[107]
Total N = (0.38•10−3) microbial biomass-C+($1.4.10^{-3}$) mineral N+($13.6.10^{-3}$)phosphatase+($8.9.10^{-3}$) β-glucosidase+($1.6.10^{-3}$) urease.	Evaluation of soils under climax vegetation	[108]
Total N = 0.44 available phosphate+0.017 WHC +0.410 phosphatase−0.567 urease+0.001 microbial biomass-C +0.419 β-glucosidase −0.980.	Valid for Mollisol. Evaluation of forests soils under natural vegetation without human intervention	[109]

ISBN:
DOI:

Organic C content = 4.247 available phosphate+8.185 β-glucosidase+7.949 urease+17.333.	Valid for Entisol. Evaluation of forests soils under natural vegetation without human intervention	[109]
Microbial Degradation Index (MID) = [0.89(1/1 + (dehydrogenase/4.87)$^{-2.5}$)] + [0.86(1/1+ (water-soluble carbohydrates/11.09)$^{-2.5}$)]+ [0.84(1/1+ (urease/1.79)$^{-2.5}$)] + [0.72 (1/1+ (respiration /18.01)$^{-2.5}$)].	Assessment of semiarid degraded soils	[110]
Relative Soil Stability Index (RSSI) = $\frac{\int_{day2}^{day15} \text{EA perturbed}(t)dt}{\int_{day2}^{day15} \text{EA control}(t)dt} 100$	Effects of 2,4-D herbicide on soil functional stability	[111]
Biological Quality Index (BQI) Total C = −2.924 + 0.037 hot water extractable C− 0.096 cellulase + 0.081 dehydrogenase + 0.009 respiration.	Variation in relation to the ecosystem degradation	[112]
Soil Quality Index including microbial biomass C, respiration, dehydrogenase activity, seeds germination and earthworms.	Evaluation of recuperation of hydrocarbon contaminated soils by nutrient applications, surfactants or soil agitation	[113]

Table 5: Indexes of soil quality integrating also soil enzyme activities [7,102].

C_{org} (organic carbon content, %); N_{total} (total nitrogen content, %); EA= enzyme activity (enzymes measured = arylsulphatase, β-glucosidase, urease, protease, acid and alkaline phosphatase). (*) For the units in which each enzyme activity or soil properties were expressed see references.

Recently, an interesting approach was used by Veum et al. [114] to model and assess changes in soil quality fractions and soil quality across a continuum of long-term agricultural practices in Missouri, USA. Beside several physical and chemical indicators, the activities of dehydrogenase and phenol oxidase were measured, and ^{13}C nuclear magnetic resonance (^{13}C NMR), diffuse reflectance Fourier transform (DRIFT) spectra of soil organic matter and visible, near-infrared reflectance (VNIR) spectra of whole soil were collected. All indicators strongly correlated to each other as assessed by a modified two-factor ANOVA to account for the lack of plot-level replication and to evaluate the main effects of crop and fertilization practice. The SMAF (Soil Management Assessment Framework) soil index, that incorporates several biological, physical, chemical, and nutrient data scoring curves (for details on the development and implementation of the SMAF see [115]), was used to score and rank all the 12 investigated plots for overall soil quality. Strong positive and negative correlations were observed between the SMAF index and many soil quality indicators, including dehydrogenase and phenol oxidase activities and soil organic matter composition, thus underlining the strong link existing among microbial community and its function, common indicators of soil quality, and properties of soil organic matter.

Models

The analysis of global change impacts on terrestrial systems and feedbacks to the climate system requires the development of simulation biogeochemical models, taking in account interactions between global climate changes, terrestrial carbon balance and all factors affecting them. Models are useful not only for extrapolating and understanding the behavior of functions on hearth to longer time scales not easily reachable, but also to evaluate all the interaction existing among the cycles of elements and the processes that govern them. Therefore, several efforts have been made to develop realistic, reliable models possibly founded on mechanisms really occurring on hearth and possibly taking in account several experimental data and environmental affecting factors.

ISBN:
DOI:

Since the main global pool of terrestrial carbon is soil organic matter (SOM) it is evident that many models have dealt with the decomposition of SOM and factors and actors involved in it. Although SOM decomposition is mainly operated by microbes and their EE activities, many models have ignored the integration of enzyme activities in their structure, thus disregarding their important contribution.

Microbes and fungi generate EEs to produce simple nutrients from complex organic matter but microbial enzymatic synthesis is dependent on resource availability and nutrient dynamics. Therefore, decomposition model should include enzyme dynamics and the activities of some enzymes specifically involved in C, N and P cycles thus providing insights in the biogeochemical equilibrium between microbial biomass stoichiometry and elemental composition of organic matter [116]. As previously cited [1], the activities of microbial EEs involved in the mineralization of organic carbon, nitrogen and phosphorus are stoichiometrically related to microbial biomass, detrital organic matter, microbial nutrient assimilation, growth and expression.

A simple theoretical model was elaborated by Schimel and Weintraub [117] based on first order kinetics in which a simple proportional relationship is assumed between the decomposition rate of a particular C pool and the size of the pool with a simple decomposition constant k ($dC/dt = kC$) and extended to decomposition by extracellular enzymes according to $dC/dt = kC$ x Enzymes. Nitrogen deficiency may limit microbial growth and EEs production and loss may arise by enzyme binding to soil matrices thus competing to enzymatic transformation and producing a non-linear response of decomposition rates to enzyme concentration. Therefore, both these phenomena should be considered in the development of a model. The integration of enzyme dynamics as well as of N limitation to microbial growth in a more complex version of the kinetic model led to identify that total C flow may be limited by the functioning of the EEs, and actual microbial growth may be N limited [117].

Starting from a meta-analysis of papers dealing with the measurement of acid/alkaline phosphatase (AP), b-glucosidase (BG), and b-N-acetyl-glucosaminidase (NAG) activities in tropical soils Waring et al. [116] observed that the ratios BG:AP and NAG:AP in these soils were significantly lower than those of temperate ecosystems in general. This result could indicate that in tropical soils P bioavailability is low and may constraint the growth efficiency of soil microbes; therefore microbes will invest much in the production of enzymes of organic P than of C or N degradation, with a resulting negative influence on C microbial utilization and lowering the rate of microbial growth and then of organic matter decomposition. A biogeochemical equilibrium model [59] was used to confirm that P limitation may lower microbial growth efficiency. The model included the ratios BG:AP and NAG:AP, the microbial biomass ratios C:N and C:P and two parameters L_{CN} and L_{CP} which represent "the stoichiometry of labile C versus nutrients pools in the environment". Without entering in detail [59,116] the model predicted that actually low microbial growth occurs in P-limited soils and therefore P availability should be considered when models are developed to simulate microbial enzyme allocation, biomass growth, and C mineralization.

A quite complicate decomposition model was elaborated by Moorhead and Sinsabaugh [118] founded on three functional guilds, and therefore called Guild Decomposition Model (GDM), each of them defined by different characteristics, established on common information of main physiological, growing and enzymatic properties of different microbial communities involved in various phases of litter decay. Guild 1 includes opportunist microorganisms capable to quickly colonize new available litter and consume soluble polymeric substrates; in Guild 2 decomposers that degrade polymeric substrates such as cellulose and lignocellulose by producing many hydrolytic and oxidative enzymes are enclosed, and then in Guild 3 there are

ISBN:
DOI:

microorganisms degrading recalcitrant organic matter by means of powerful oxidative enzymes capable to act on covalent bonds of aromatic rings and hydrocarbon chains, and thus to attack protected glycosides, peptides and lipids. As well summarized by Wallenstein and Weintraub [11] this model "describes how the availability of labile C and N can inhibit lignin degradation by influencing the expression of EEs responsible for microbial C acquisition, elucidating the mechanisms controlling C sequestration during decomposition".

A simpler model of carbon C dynamics was proposed by Gogo et al. [119]. It was considered to be more easily and experimentally implemented and calibrated. The model was based on a mass balance between solid C, represented by litter, water extractable organic carbon (WEOC) and gaseous C, i.e. the cumulative C-CO_2 respired. C flux from the solid to the gaseous form with time is dependent on litter decomposition in WEOC that is a consequence of extracellular enzymatic catalytic reactions at a defined rate. The mathematical expression of the model and its development is not object of this chapter and details are available in the original paper. The model was tested on three types of peatland litter and appeared robust and suitable to well describe the early stages of C dynamics in litter and efficient in measuring the whole real enzymatic catalysis rate.

A theoretical Extracellular Enzyme (EEZY) model of decomposition and producing two separate groups of C- and N-acquiring enzymes was developed by Moorhead et al. [120]. One group of enzymes was involved in the hydrolysis of only C-substrates (e.g. cellulose) and the other of substrates containing both C and N (e.g. chitin or proteins). Typical enzymes representing the two groups are b-glucosidase and b-N-acetylglucosaminidase, involved in the hydrolysis of cellulose and chitin, respectively. EEZY was able to estimate EEs behaviors obtained by field studies of terrestrial soils, aquatic sediments, fresh water biofilm and plankton communities. Moreover, its predictions of litter decay were consistent with those obtained by other models. Although the model simulated enzyme activities, it confirmed the existence of a stoichiometric 1:1 ratio of enzyme activities associated with C- and N-acquisition and indicated a great response by simulated EE activity to changes in the efficiency of microorganism in the use of carbon sources.

In many of these models it is not possible to discriminate by common enzymatic assays between free and stabilized EEs, when the presence of these latter may affect a quick response in microbial enzyme allocation determined by new or altered nutrient inputs. An integrated model was proposed by Allison [121] who considered that several situations contribute to the production and action of EEs and are dominated by economic aspects in terms of production costs. A simulation model was established in which the influence of competition by cheaters, (i.e. microorganisms that use EEs produced by other microorganisms thus competing with the producers and lowering the efficiency of the secreted enzymes for the growth and survival of their originating cells), nutrient availability, and spatial structure on microbial growth and enzyme synthesis was examined. Simulation was reiterated by changing C, N or P supply and the author concluded that cheaters were favored by conditions in which the cost of enzyme is high, whereas lower rates of enzyme diffusion favored producers. Moreover, carbon mineralization, microbial growth and enzyme production was limited by nitrogen supply because enzymes having a high content of N-units require high levels of N sources [121]. Further studies monitored the activity of phosphatase (P cycle), b-glucosidase (C cycle) and glycine amino peptidase (N cycle) in an infertile tropical soil after supplying it with C, N, and P nutrients under different combinations of complex and simple nutrient additions, and evaluated the relationships between resource availability, EEs production and nutrient dynamics. The conclusion drawn by the authors was that "microorganisms produce enzymes

ISBN:
DOI:

according to ”economic rules”, but a substantial pool of mineral-stabilized or constitutive enzymes mediates this response” [14].

A further extension of the model was made by Folse III and Allison [122] to study the correlation between depolymerization of nutrients, social interactions and diversity of microbial communities. A multi-genotype, multi-nutrient model of a community of extracellular enzyme-producing microbes was developed and included C-, N- and P- containing polymers and eight genotypes of bacteria producing different groups of polymers hydrolyzing enzymes. Possible associations between complementary types of microbes and their implications for spatial pattern formation and nutrient depolymerization were mainly considered. The model highlighted that the increase of diversity from two to eight types of microorganisms led to a decreased nutrient depolymerization rate because of the competitive interactions rising between the different groups of microorganisms which ultimately caused a minor overall enzyme production. The conclusive remark of this study was that social interactions among microbes competing for resources may strongly affect nutrient dynamics, microbial diversity, and microbial spatial distributions.

Conclusions and Future Perspectives

Although papers so far commented have examined only a reduced fraction of all variables and factors affecting the activities of soil enzymes, overall the results seem to indicate and emphasize that in soil potential enzyme activities, microbial size activity and nutrient demand are affected by abiotic and biotic factors and understanding their relationships is mandatory to better modeling and describing soil biogeochemical cycles at a global level.

Reliable, accurate and universal methodologies are, however, still needed to have a real picture of soil EE dynamics and involvement in environmental processes. Improvements of new technological approaches like *in situ* zymography or near infrared spectroscopy methods for *in situ* measurements of soil enzyme activities as well as emerging, innovative approaches based on molecular biology could overcome many of obstacles still impeding satisfactory and reasonable resolution to not yet resolved and long-standing questions about several aspects regarding EEs and their role in the mechanisms of biogeochemical processes and the controls on microbial diversity.

As concluded by Simon and Daniel [50] and Nannipieri et al. [7] the combination of DNA-based, mRNA-based, and protein-based analyses of soil microbial communities along with the assay of the respective soil enzyme activities may elucidate “the adaptive mechanisms of enzyme synthesis, the recovery and identification of the synthesized enzymes, their relative contribution to the specific soil activity and the presence of the active microbial species” [7]. Moreover, “the compositions, functions, and interactions of microbial communities and their link to environmental processes” [50] can be clarified by metagenomic studies. Improvements in metabolomic studies will allow measurement of substrate availability and product release as well as the use of isotopic labeled substrates with consequent formation of labeled products will assist in clarifying *in situ* enzyme activities and their control.

Additional assistance will derive from stoichiometry studies that may contribute to shade light on relations existing among plant community composition and below-ground soil microbial, nutrient and enzymatic characteristics.

It appears mandatory, however, to rely on data and results that could be considered valid at general level. This means that enzyme activities assays should be carried out on many soil samples and with many substrates to obtain a major repeatability between different laboratories

ISBN:
DOI:

and with soils differing for chemical and physical properties. All that is much truer, enzyme activities should behave as indicators of soil quality. As recommended by Creamer et al. [101] "Prescription of biological indicators in soil quality monitoring schemes must take due account of three potential sources of variation, viz. (1) intrinsic variation within a laboratory technique; (2) inter-laboratory repeatability and (3) discrimination between samples from different land-uses/ provenances" [101].

It is also important to take into account all factors influencing the behavior of EEs in response to the addition of fertilizers or to changes in natural situations. For instance, referring to the use of biochar as fertilizer it is of paramount importance to properly consider some aspects of this material to have a unambiguous understanding of biochar influence on soil enzyme activities and in general on soil biological activity and to contribute to implement effective soil management with biochar. Firstly, clear information on the properties and types of biochar as influenced by the originating source and temperature treatments is needed. Secondly, simple, effective methods, now not yet available, for analyzing biochar in soil should be developed as well as enzyme substrates and products sorption studies should be improved to properly describe the fate and role of biochar in soil and to estimate the true activity of enzymes in biochar-amended soils. Finally, as many of literature findings refer to short-term biochar applications, long-term soil amendment with biochar and analysis of its effect on many soil properties should be performed to exclude those small and potentially transient changes do occur [123]. Limited understanding of enzyme activities under field conditions may also reduce the capability to predict and model current and future processes of ecosystems. Pitfalls in the use of models may derive from different contradictory situations existing between modeling and experimental tests [124]. Very often, biogeochemical models are founded on a small number of inadequate data sets. Indeed, empirical data are usually assembled by experiments carried out in just one or few sites and time points whereas modeling requires to integrated data across spatial and temporal scales. Moreover, no correspondence usually exist between data determined experimentally and those needed to parameterize biogeochemical models like potential enzyme activities measured under saturating substrate conditions while limiting conditions are usually considered in models.

In conclusion, soil enzyme activities are of paramount importance to evaluate functions of microorganisms, cycling of nutrients and carbon-sources decomposition. Combination of emerging technologies with information gained by current techniques will allow the development of new, more microbially explicit biogeochemical models that will be better able to predict the impacts of enzyme-mediated soil processes. Moreover, to achieve an enhanced comprehension of carbon cycling and its availability dynamics among ecosystems, major attention has to be devoted to EE activities operating not only in terrestrial environment but also in freshwater and marine environments, where they are alike the main actors of nutrient cycling [125]. Several features are common to these different environmental enzyme groups. As underlined by Arnosti et al. [125] "While the relative importance of specific structuring factors (e.g. pH, presence of surfaces, etc.) varies dramatically among terrestrial, freshwater, and marine environments, the underlying process is the same: microorganisms produce EEs in order to gain a selective advantage; those enzymes subsequently catalyze biogeochemical cycles". Therefore, integrated enzymatic studies across these different environments may greatly assist researchers to achieve a thorough comprehension "of the ultimate controls and biogeochemical consequences of EEs across environments" [125].

References

1. Sinsabaugh R, Lauber C, Weintraub M, Ahmed B, Allison S, et al. (2008) Stoichiometry of soil enzyme activity at global scale. Ecol Lett 11: 1252-1264.

ISBN:
DOI:

2. Burns RG (1978) Soil enzymes. Academic Press, London, UK.

3. Burns RG (1982) Enzyme activity in soil: Location and a possible role in microbial ecology. Soil Biol Biochem 14: 423-427.

4. Burns RG, DeForest JL, Marxsen J, Sinsabaugh RL, Stromberger ME, et al. (2013) Soil enzymes in a changing environment: Current knowledge and future directions. Soil Biol Biochem 58: 216-234.

5. Gianfreda L, Bollag JM (1996) Influence of natural and anthropogenic factors on enzyme activity in soil. In: Stotzky G, Bollag JM (Eds.). Soil biochemistry. Marcel Dekker, New York 9: 123-194.

6. Tate RL III (2002) Microbiology and enzymology of carbon and nitrogen cycling. In: Burns RG, Dick RP (Eds.). Enzymes in the environment. Activity, ecology and applications. Marcel Dekker, New York, 227-248.

7. Nannipieri P, Giagnoni L, Renella G, Puglisi E, Ceccanti B et al. (2012) Soil enzymology: classical and molecular approaches. Biol Fertil Soils 48: 743-762.

8. Gianfreda L, Rao MA (2011) Stabilising enzymes as synthetic complexes. In: Dick RP (Ed.). Methods of soil enzymology. Soil Science Society of America, Madison 9: 319–369.

9. Gianfreda L, Rao MA, Mora M (2011) Enzymatic activity as influenced by soil mineral and humic colloids and the impact on biogeochemical processes. In: Huang PM, Li Y, Sumner ME (Eds.). Handbook of soil sciences. Resource management and environmental impacts. (2nd Edn), CRC Press, Boca Raton, 5.1-5.24.

10. Gianfreda L, Rao MA, Sannino F, Saccomandi F (2002) Enzymes in soil: properties, behaviour and potential applications. In: Violante A, Huang PM, Bollag J-M, Gianfreda L (Eds.). Soil mineral-organic matter-microorganism interactions and ecosystem health. Elsevier Science, Amsterdam 28: 301-328.

11. Wallenstein MD, Weintraub MN (2008) Emerging tools for measuring and modeling the *in situ* activity of soil extracellular enzymes. Soil Biol Biochem 40: 2098-2106.

12. German DP, Weintraub MN, Grandy AS, Lauber CL, Rinkes ZL, et al. (2011) Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biol Biochem 43: 1387-1397.

13. Gianfreda L, Ruggiero P (2006) Enzyme activities in soil. In: NannipieriP, Smalla K (Ed.). Nucleic acids and proteins in soil. Series Soil biology. Springer-Verlag, Berlin, Germany 8: 257-311.

14. Allison SD, Vitousek PM (2005) Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biol Biochem 37: 937-944.

15. Ladd JN, Forster RC, Nannipieri P, Oades JM (1996) Soil structure and biological activity. In: Stotzky G, Bollag, J-M (Eds.). Soil biochemistry. Marcel Dekker, New York, 9: 23-78.

16. Joner EJ, Van Aarle I, Vosatka M (2000) Phosphatase activity of extra-radical arbuscular mycorrhizal hyphae: A review. Plant Soil 226: 199-210.

17. Steinweg JM, Dukes JS, Wallenstein MD (2012) Modeling the effects of temperature and moisture on soil enzyme activity: Linking laboratory assays to continuous field data. Soil Biol Biochem 55: 85-92.

18. Dong S, Brooks D, Jones MD, Grayston SJ (2007) A method for linking *in situ* activities of hydrolytic enzymes toassociated organisms in forest soils. Soil Biol Biochem 39: 2414-2419.

19. Spohn M, Carminati A, Kuzyakov Y (2013) Soil zymography - A novel *in situ* method for mapping distribution of enzyme activity in soil. Soil Biol Biochem 58: 275-280.

20. Dick WA, Thavamani B, Conley S, Blaisdell R, Sengupta A (2013) Prediction of b-glucosidase and b-glucosaminidase activities, soil organic C, and amino sugar N in a diverse population of soils using near infrared reflectance spectroscopy. Soil Biol Biochem 56: 99-104.

21. DeForest JL (2009) The influence of time, storage temperature, and substrate age on potential soil enzyme activity in acidic forest soils using MUB-linked substrates and L-DOPA. Soil Biol Biochem 41: 1180-1186.

22. Wallenius K, Rita H, Simpanen S, Mikkonen A, Niemi RM (2010) Sample storage for soil enzyme activity and bacterial community profiles. J Microbiol Meth 81: 48-55.

23. Peoples MS, Koide RT (2012) Considerations in the storage of soil samples for enzyme activity analysis. Appl Soil Ecol 62: 98-102.

24. Vancov T, Keen B (2009) Rapid isolation and high-throughput determination of cellulase and laminarinase activity in soils. J Microbiol Meth 79: 174-177.

ISBN:
DOI:

25. Alef K, Nannipieri PV (1995) Methods in applied soil microbiology and biochemistry. Academic Press, London.

26. Popova IE, Deng S (2010) A high-throughput microplate assay for simultaneous colorimetric quantification of multiple enzyme activities in soil. Appl Soil Ecol 45: 315-318.

27. Marx MC, Wood M, Jarvis SC (2001) A microplate fluorimetric assay for the study of enzyme diversity in soils. Soil Biol Biochem 33: 1633-1640.

28. Stemmer M (2004) Multiple-substrate enzyme assays: a useful approach for profiling enzyme activity in soils? Soil Biol Biochem 36: 519-527.

29. Moscatelli MC, Lagomarsino A, Garzillo AMV, Pignataro A, Grego S (2012) β-Glucosidase kinetic parameters as indicators of soil quality under conventional and organic cropping systems applying two analytical approaches. Ecol Indic 13: 322-327.

30. Dick LK, Jia G, Deng S, Dick RP (2013) Evaluation of microplate and bench-scale β-glucosidase assays for reproducibility, comparability, kinetics, and homogenization methods in two soils. Biol Fert Soils 49: 1227-1236.

31. Trap J, Riah W, Akpa-Vinceslas M, Bailleul C, Laval K, et al. (2012) Improved effectiveness and efficiency in measuring soil enzymes as universal soil quality indicators using microplate fluorimetry. Soil Biol Biochem 45: 98-101.

32. Deng S, Popova IE, Dick L, Dick R (2013) Bench scale and microplate format assay of soil enzyme activities using spectroscopic and fluorometric approaches. Appl Soil Ecol 64: 84-90.

33. Bach CE, Warnock DD, Van Horn DJ, Weintraub MN, Sinsabaugh RL, et al. (2013) Measuring phenol oxidase and peroxidase activities with pyrogallol, L-DOPA, and ABTS: Effect of assay conditions and soil type. Soil Biol Biochem 67: 183-191.

34. Kumar S, Chaudhuri S, Maiti SK (2013) Soil dehydrogenase enzyme activity in natural and mine soil - A review. Middle East J Sci Res 13: 898-906.

35. Turner BL (2010) Variation in ph optima of hydrolytic enzyme activities in tropical rain forest soils. Appl Environ Microbiol 76: 6485-6493.

36. Eivazi F, Tabatabai MA (1977) Phosphatases in soils. Soil Biol Biochem 9: 167-172.

37. Dick WA, Cheng L, Wang P (2000) Soil acid and alkaline phosphatase activity as pH adjustment indicators. Soil Biol Biochem 32: 1915-1919.

38. Kumar S, Chaudhuri S, Maiti SK (2011) Phosphatase activity in natural and mined soil - A review. Indian J Environ Prot 31: 955-962.

39. Saiya-Cork KR, Sinsabaugh RL, Zak DR (2002) The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. Soil Biol Biochem 34: 1309-1315.

40. Taylor JP, Wilson B, Mills MS, Burns RG (2002) Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques. Soil Biol Biochem 34: 387-401.

41. Dussault M, Bécaert V, François M, Sauvé S, Deschênes L (2008) Effect of copper on soil functional stability measured by relative soil stability index (RSSI) based on two enzyme activities. Chemosphere 72: 755-762.

42. Chaer GM, Myrold DD, Bottomley PJ (2009) A soil quality index based on the equilibrium between soil organic matter and biochemical properties of undisturbed coniferous forest soils of the Pacific Northwest. Soil Biol Biochem 41: 822-830.

43. Lessard I, Renella G, Sauvé S, Deschênes L (2013) Metal toxicity assessment in soils using enzymatic activity: Can water be used as a surrogate buffer? Soil Biol Biochem 57: 256-263.

44. Luis P, Kellner H, Zimdars B, Langer U, Martin F, et al. (2005) Patchiness and spatial distribution of laccase genes of ectomycorrhizal, saprotrophic and unknown basidiomycetes in the upper horizons of a mixed forest Cambisol. Microb Ecol 50: 570-579.

45. Kellner H, Luis P, Zimdars B, Kiesel B, Buscot F (2009) Diversity of bacterial laccase-like multicopper oxidase genes in forest and grassland Cambisol soil samples. Soil Biol Biochem 40: 638-648.

46. Zimmerman AE, Martiny AC, Allison SD (2013) Microdiversity of extracellular enzyme genes among sequenced prokaryotic genomes. ISME J 7: 1187-1199.

47. Schulze WX, Gleixner G, Kaiser K, Guggenberger G, Mann M, et al. (2005) A proteomic fingerprint of dissolved organic carbon and of soil particles. Oecologia 142: 335-343.

48. Schneider T, Keiblinger K, Schmid E, Sterflinger-Gleixner K, Ellersdorfer G, et al. (2012) Who is who in litter decomposition? Metaproteomics reveals players and their biogeochemical functions. ISME J 6: 1749-1762.

ISBN:
DOI:

49. Giagnoni L, Migliaccio A, Nannipieri P, Renella G (2013) High montmorillonite content may affect soil microbial proteomic analysis. Appl Soil Ecol 72: 203-206.

50. Simon C, Daniel R (2011) Metagenomic analyses: Past and future trends. Appl EnvironMicrob 77: 1153-1161.

51. Geng Y, Dighton J, Gray D (2012) The effects of thinning and soil disturbance on enzyme activities under pitch pine soil in New Jersey Pinelands. Appl Soil Ecol 62: 1-7.

52. Kramer S, Marhan S, Haslwimmer H, Ruess L, Kandeler E (2013) Temporal variation in surface and subsoil abundance and function of the soil microbial community in an arable soil. Soil Biol Biochem 61: 76-85.

53. Stone MM, Plante AF (2014) Changes in phosphatase kinetics with soil depth across a variable tropical landscape. Soil Biol Biochem 71: 61-67.

54. Spohn M, Kuzyakov Y (2013) Distribution of microbial- and root-derived phosphatase activities in the rhizosphere depending on P availability and C allocation – Coupling soil zymography with ^{14}C imaging. Soil Biol Biochem 67: 106-113.

55. Wu QS, He XH, Zou YN, KP He, YH Sun, et al. (2012) Spatial distribution of glomalin-related soil protein and its relationships with root mycorrhization, soil aggregates, carbohydrates, activity of protease and β-glucosidase in the rhizosphere of *Citrus unshiu*. Soil Biol Biochem 45: 181-183.

56. Gartner TB, Treseder KK, Malcolm GM, Sinsabaugh RL (2012) Extracellular enzyme activity in the mycorrhizospheres of a boreal fire chronosequence. Pedobiologia 55: 121-127.

57. Bell C, Carrillo Y, Boot CM, Rocca JD, Pendall E, et al. (2014) Rhizosphere stoichiometry: Are C: N: P ratios of plants, soils, and enzymes conserved at the plant species-level? New Phytol 201: 505–517.

58. Sinsabaugh R, Follstad Shah J (2011) Ecoenzymatic stoichiometry of recalcitrant organic matter decomposition: the growth rate hypothesis in reverse. Biogeochemistry 102: 31-43.

59. Sinsabaugh R, Follstad Shah J (2012) Ecoenzymatic stoichiometry and ecological theory. Annu Rev Ecol Evol Syst 43: 313-343.

60. Sinsabaugh R, Hill B, Shah J (2009) Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462: 795-799.

61. Marx MC, Kandeler E, Wood M, Wermbter N, Jarvis SC (2005) Exploring the enzymatic landscape: distribution and kinetics of hydrolytic enzymes in soil particle-size fractions. Soil Biol Biochem 37: 35-48.

62. Yan J, Pan G, Li A, Quan G (2009) Cellulase activity in physically isolated fractions of a paddy soil. Proceedings of the International Conference on Environmental Science and Information Application Technology 3: 613-616.

63. Stursova M, Sinsabaugh RL (2008) Stabilization of oxidative enzymes in desert soil may limit organic matter accumulation. Soil Biol Biochem 40: 550-553.

64. Burns RG, Dick RP (2002) Enzymes in the environment: activity, ecology and applications. Marcel Dekker, New York.

65. Gianfreda L, Rao MA (2004) Potential of extra cellular enzymes in remediation of polluted soils: a review. Enz Microb Technol 35: 339-354.

66. Gianfreda L, Rao MA (2008) Interaction between xenobiotics and microbial and enzymatic soil activity. Crit Rev Environ SciTechnol 38: 269-310.

67. Schaffer A (1993) Pesticide effects on enzyme activities in the soil ecosystems. In Bollag J-M, Stotzky G (Eds.). Soil biochemistry. Marcel Dekker, New York, 8: 273–340.

68. Henry HAL (2012) Soil extracellular dynamics in a changing climate. Soil Biol Biochem 47: 53-59.

69. Baldrian P, Šnajdr J, Merhautová V, Dobiášová P, Cajthaml T, et al. (2013) Responses of the extracellular enzyme activities in hardwood forest to soil temperature and seasonality and the potential effects of climate change. Soil Biol Biochem 56: 60-68.

70. Zhou X, Chen C, Wang Y, Xu Z, Han H, et al. (2013) Warming and increased precipitation have differential effects on soil extracellular enzyme activities in temperate grassland. Sci Total Environ 444: 552-558.

71. Wallenstein MD, Mcmahon SK, Schimel JP (2009) Seasonal variation in enzyme activities and temperature sensitivities in Arctic tundra soils. Glob Change Biol 15: 1631-1639.

72. Alster CJ, German DP, Lu Y, Allison SD (2013) Microbial enzymatic responses to drought and to nitrogen addition in a southern California grassland. Soil Biol Biochem 64: 68-79.

ISBN:
DOI:

73. Stone MM, Weiss MS, Goodale CL, Adams MB, Fernandez IJ, et al. (2012) Temperature sensitivity of soil enzyme kinetics under N-fertilization in two temperate forests. Glob Change Biol 18: 1173-1184.

74. German DP, Marcelo KRB, Stone MM, Allison SD (2012) The Michaelis-Menten kinetics of soil extracellular enzymes in response to temperature: A cross-latitudinal study. Glob Change Biol 18: 1468-1479.

75. Jing X, Wang Y, Chung H, Mi Z, Wang S, et al. (2014) No temperature acclimation of soil extracellular enzymes to experimental warming in an alpine grassland ecosystem on the Tibetan Plateau. Biogeochemistry 117: 39-54.

76. Weedon JT, Aerts R, Kowalchuk GA, van Bodegom PM (2014) No effects of experimental warming but contrasting seasonal patterns for soil peptidase and glycosidase enzymes in a subarctic peat bog. Biogeochemistry 117: 55-66.

77. Steinweg JM, Dukes JS, Paul EA, Wallenstein MD (2013) Microbial responses to multi-factor climate change: Effects on soil enzymes. Front Microbiol.

78. Kivlin SN, Treseder KK (2014) Soil extracellular enzyme activities correspond with abiotic factors more than fungal community composition. Biogeochemistry 117: 23-37.

79. Powlson DS, Gregory PJ, Whalley WR, Quinton JN, Hopkins DW, et al. (2011) Soil management in relation to sustainable agriculture and ecosystem services. Food Policy 36: 72-87.

80. Cayuela ML, van Zwieten L, Singh BP, Jeffery S, Roig A, et al. (2013) Biochar's role in mitigating soil nitrous oxide emissions: A review and meta-analysis. AgricEcosystems Environ.

81. Nelissen V, Saha BK, Ruysschaert G, Boeckx P (2014) Effect of different biochar and fertilizer types on N_2O and NO emissions. Soil Biol Biochem 70: 244-255.

82. http://www.journals.elsevier.com/soil-biology-and-biochemistry/virtual-special-issues/virtual-special-issue-on-biochar/

83. Beesley L, Moreno-Jiménez E, Gomez-Eyles JL, Harris E, Robinson B, et al. (2011) A review of biochars' potential role in remediation, revegetation and restoration of contaminated soils. Environ Pollut 159: 3269-3282.

84. Mackenzie J, Denyes V, Langlois S, Rutter A, Zeeb BA (2012) The use of biochar to reduce soil PCB bioavailability to Cucurbita pepo and Eisenia fetida. Sci Total Environ 437: 76-82.

85. Tong H, Hu M, Li FB, Liu CS, Chen MJ (2014) Biochar enhances the microbial and chemical transformation of pentachlorophenol in paddy soil. Soil Biol Biochem 70: 142-150.

86. Jones DL, Edwards-Jones G, Murphy DV (2011) Biochar mediated alterations in herbicide breakdown and leaching in soil. Soil Biol Biochem 43: 804-813.

87. Yu XY, Ying GG, Kookana RS (2009) Reduced plant uptake of pesticides with biochar additions to soil. Chemosphere 76: 665-671.

88. Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, et al. (2011) Biochar effects on soil biota – A review. Soil Biol Biochem 43: 1812-1836.

89. Masto RE, Kumar S, Rout TK, Sarkar P, Joshy G, et al. (2013) Biochar from water hyacinth (*Eichornia crassipes*) and its impact on soil biological activity. CATENA 111: 64-71.

90. Kumar S, Masto RE, Ram LC, Sarkar P, George J, et al. (2013) Biochar preparation from Parthenium hysterophorus and its potential use in soil application. Ecol Eng 55: 67-72.

91. Masto RE, Ansari MdA, George J, Selvi VA, Ram LC (2013) Co-application of biochar and lignite fly ash on soil nutrients and biological parameters at different crop growth stages of *Zea mays*. Ecol Eng 58: 314-322.

92. Bailey VL, Fansler SJ, Smith JL, Bolton H (2011) Reconciling apparent variability in effects of biochar amendment on soil enzyme activities by assay optimization. Soil Biol Biochem 43: 296-301.

93. Swaine M, Obrike R, Clark JM, Shae LJ (2013) Biochar alteration of the sorption of substrates and products in soil enzyme assays. Appl Environ Soil Sci.

94. Lammirato C, Miltner A, Kaestner M (2011) Effect of wood char and activated carbon on the hydrolysis of cellobiose by b-glucosidase from Aspergillus niger. Soil Biol Biochem 43: 1936-1942.

95. Doran JW, Parkin TB (1994) Defining and assessing soil quality. In: Doran JW, Coleman DC, Bezdicek DF, Stewart BA (Eds.). Defining Soil quality for a sustainable environment. SSSA Special Publication, Madison, 35: 1-21.

96. Dick RP (1997) Soil enzyme activities as integrative indicators of soil health. In: Pankhurst CE, Doube BM, Gupta VVSR (Eds.). Biological indicators of soil health. CAB International, Wallingford, 121-156.

ISBN:
DOI:

97. Doran JW, Saffley M (1997) Defining and assessing soil health and sustainable productivity. In Pankhurst CE, Doube BM, Gupta VVSR (Eds.) Biological indicators of soil health. CAB International, Wallingford, 1-28.

98. Doran JW, Zeiss MR (2000) Soil health and sustainability: managing the biotic component of soil quality. Appl Soil Ecol 15: 3-11.

99. Cardoso EJBN, Vasconcellos RLF, Bini D, Miyauchi MYH, dos Santos CA, et al. (2013) Soil health: Looking for suitable indicators. What should be considered to assess the effects of use and management on soil health? Sci Agric 70: 274-289.

100. Rao MA, Scelza R, Acevedo F, Diez MC, Gianfreda L (2014) Enzymes as useful tools for environmental purposes. Chemosphere.

101. Creamer RE, Bellamy P, Black HIJ, Cameron CM, Campbell CD, et al. (2009) An inter-laboratory comparison of multi-enzyme and multiple substrate-induced respiration assays to assess method consistency in soil monitoring. Biol Fert Soils 45: 623-633.

102. Bastida F, Zsolnay A, Hernández T, García C (2008) Past, present and future of soil quality indices: A biological perspective. Geoderma 147: 159-171.

103. Puglisi E, Del Re AAM, Rao MA, Gianfreda L (2006) Development and validation of numerical indexes integrating enzyme activities of soils. Soil Biol Biochem 38: 1673-1681.

104. Beck TH (1984) Methods and application of soil microbiological analysis at the Landensanstalt fur Bodenkultur und Pflanzenbau (LBB) for determination of some aspects of soil fertility. In: Nemes MP, Kiss S, Papacostea P, Stefanic C, Rusan M (Eds.). Proceedings of the Fifth Symposium on Soil biology. Rumanian National Society of Soil Science, Bucharest, Rumania, 13–20.

105. Stefanic F, Ellade G, Chirnageanu J (1984) Researches concerning a biological index of soil fertility. In: Nemes MP, Kiss S, Papacostea P, Stefanic C, Rusan M (Eds.). Proceedings of the Fifth Symposium on Soil biology. Rumanian National Society of Soil Science, Bucharest, Rumania, 35–45.

106. Koper J, Piotrowska A (2003) Application of biochemical index to define soil fertility depending on varied organic and mineral fertilization. Electron J Pol Agric Univ 6.

107. De la Paz-Jiménez M, De la Horra AM, Pruzzo L, Palma RM (2002) Soil quality: a new index base microbiological and biochemical parameters. Biol Fertil Soils 35: 302–306.

108. Trasar-Cepeda C, Leirós C, Gil-Sotres F, Seoane S (1998) Towards a biochemical quality index for soils: an expression relating several biological and biochemical properties. Biol Fertil Soils 26: 100–106.

109. Zornoza R, Mataiz-Solera J, Guerrero C, Arcenegui V, García-Orenes F, et al. (2007) Evaluation of soil quality using multiple lineal regression based on physical, chemical and biochemical properties. Sci Total Environ 378: 233–237.

110. Bastida F, Moreno JL, Hernández T, García C (2006) Microbiological degradation index of soils in a semiarid climate. Soil Biol Biochem 38: 3463–3473.

111. Bécaert V, Samson R, Deschênes L(2006) Effect of 2,4-D contamination on soil functional stability evaluated using the relative soil stability index (RSSI). Chemosphere 64: 1713–1721.

112. Armas CM, Santana B, Mora JL, Notario JS, Arbelo CD, et al. (2007) A biological quality index for volcanic Andisols and Aridisols (Canary Islands, Spain): variations related to the ecosystem development. Sci Total Environ 378: 238–244.

113. Dawson JJC, Godsiffe EJ, Thompson IP, Ralebitso-Senior TK, Killham KS, et al. (2007) Application of biological indicators to assess recovery of hydrocarbon impacted soils. Soil Biol Biochem 39: 164–177.

114. Veum KS, Goyne KW, Kremer RJ, Miles RJ, Sudduth KA (2014) Biological indicators of soil quality and soil organic matter characteristics in an agricultural management continuum. Biogeochemistry 117: 81-99.

115. Andrews SS, Karlen DL, Cambardella CA (2004) The soil management assessment framework: a quantitative soil quality evaluation method. Soil Sci Soc Am J 68:1945–1962.

116. Waring BG, Weintraub SR, Sinsabaugh RL (2014) Ecoenzymatic stoichiometry of microbial nutrient acquisition in tropical soils. Biogeochemistry 117: 101-113.

117. Schimel JP, Weintraub MN (2003) The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biol Biochem 35: 549-563.

118. Moorhead DL, Sinsabaugh RL (2006) A theoretical model of litter decay and microbial interaction. Ecol Monogr 76: 151-174.

119. Gogo S, Francez AJ, Laggoun-Défarge F, Gouélibo N, Delarue F, et al. (2014) Simultaneous estimation of actual litter enzymatic catalysis and respiration rates with a simple model of C dynamics in Sphagnum-dominated peatlands. Ecosystems 17: 302-316.

120. Moorhead DL, Lashermes G, Sinsabaugh RL (2012) A theoretical model of C- and N-acquiring exoenzyme activities, which balances microbial demands during decomposition. Soil Biol Biochem 53: 133-141.

ISBN:
DOI:

121. Allison SD (2005) Cheaters, diffusion, and nutrients constrain decomposition by microbial enzymes in spatially structured environments. Ecol Lett 8: 626-635.

122. Folse HJ 3rd, Allison SD (2012) Cooperation, competition, and coalitions in enzyme-producing microbes: social evolution and nutrient depolymerization rates. Front Microbiol 3: article 338.

123. Jones DL, Rousk J, Edwards-Jones G, DeLuca TH, Murphy DV (2012) Biochar-mediated changes in soil quality and plant growth in a three year field trial. Soil Biol Biochem 45: 113-124.

124. Wallenstein M, Stromberger M, Bell C (2012) Bridging the gap between modelers and experimentalists. Eos Trans AGU 93: 312.

125. Arnosti C, Bell C, Moorhead DL, Sinsabaugh RL, Steen AD, et al. (2014) Extracellular enzymes in terrestrial, freshwater, and marine environments: perspectives on system variability and common research needs. Biogeochemistry 117: 5-21.

ISBN:
DOI:

Chapter: 2

Enzymes and Soil Fertility

Anna Piotrowska-Długosz*

Department of Soil Science and Soil Protection, Division of Biochemistry Faculty of Agriculture and Biotechnology, University of Technology and Life Sciences, Bernardyńska, Bydgoszcz, Poland

***Corresponding author:** Anna Piotrowska-Długosz, Department of Soil Science and Soil Protection, Division of Biochemistry Faculty of Agriculture and Biotechnology, University of Technology and Life Sciences, Bernardyńska St., 85-089 Bydgoszcz, Poland, Tel: +48 52 374 9555; E-mail: apiotr@utp.edu.pl

Abstract

Soil is a fundamental resource in the agricultural production system and monitoring its fertility is an important objective in the sustainable development of agro-ecosystems. In order to evaluate soil fertility, changes in its physical, chemical and biological properties must be taken into account. Among the biological features, soil enzymes are often used as index of soil fertility since they are very sensitive and respond to changes in soil management more quickly than other soil variables. Thus, the objective of this work was to review some of the aspects that are connected with using soil enzymes as indicators of agricultural practices impact (e.g., soil fertilization, crop rotation, tillage) and soil fertility. The results that are discussed in the works listed in the bibliography showed no consistent trends in enzymatic activity as being dependent on farming management practices that have stimulated, decreased or not affected this activity. The influence of inorganic fertilization and organic amendments on the soil enzyme activities depended on the dose of this amendment, the time of its application, the content of harmful substances (e.g., heavy metals), the soil type and climatic conditions. Soil fertility indicators including soil enzymes as well as some advantages and shortcomings concerning the use of these indices are also discussed. Single enzyme activities are often used as indicators of soil fertility, which is considered to be a conceptual mistake, since they usually take part in only one specific process and therefore they cannot reflect the rate of all of the metabolic processes in soil. Complex expressions, in which different properties are combined, are thought to be more suitable for estimating soil fertility, although their use is limited to the area and the conditions in which they have been described.

Introduction

Soil is an important component of all terrestrial ecosystems as well as a main source of production in agriculture. Proper soil functioning is essential for the maintenance of the global biochemical cycles for all required nutrients and thus, the processes in soils affect many other biotic and abiotic components of ecosystems [1]. To understand the functioning of soils and to prevent soil damage due to both natural and anthropogenic factors, it is important to have suitable tools for predicting and assessing soil changes that are caused by environmental factors and management practices. Strategies based on biological indicators would be a suitable tool to evaluate the sustainability of the soil ecosystem. Studies of soil enzymes are important since

ISBN:
DOI:

they indicate the potential of the soil to support the biochemical processes that are essential for the maintenance of soil fertility [2]. Soil enzymes regulate the functioning of the ecosystem and play key biochemical functions in the overall process of organic matter transformation and nutrient cycling in the soil system [3-6]. The overall enzyme activity in soil consists of various intracellular and extracellular enzymes that originate from microorganisms (e.g., bacteria, fungi) or from plants and animals (e.g., plant rots or residues, digestive tracts of small animals) [6]. The same enzyme can originates from different sources and the exact origin as well as the temporal and spatial variability of the activity is difficult to identify [7]. Intracellular enzymes exist in different parts of living and proliferating cells, while extracellular enzymes are produced and secreted by living cells and act outside the parent cells as free enzymes in a soil solution or as enzymes that are still associated with the external surface of the root epidermal or microbial cell wall (so-called ectoenzymes). When secreted outside the cell, enzymes can be free in a soil solution or they can be adsorbed by soil mineral constituents or complexed with humic substances or both. The amount of free extracellular enzymes in soil is very low compared with that in the adsorbed state due to their short life span in an inhospitable environment such as [8]. Adsorbed enzymes are resistant to proteolysis, thermal and chemical denaturation [9], but immobilization usually protects enzymes against degradation at the cost of some loss of activity. Although bounded enzymes reveal less activity than free enzymes, the most important part of their activity is being responsible for the transformation of organic matter and the availability of nutrients.

Numerous factors can influence enzyme activity in soil. Natural parameters (e.g., seasonal changes, geographic location, in situ distribution, physical-chemical properties, content of organic matter and clay) usually affect the enzyme activity level by influencing both the production of enzymes by plants and microorganisms and their persistence under natural conditions. The physical and chemical properties of a soil are involved in the immobilization and stabilization processes of most extracellular enzymes. A high content of clay or humus colloids is usually associated with stable but less active enzymes. Agricultural activities and environmental pollution (e.g., fertilizers, pesticides, tillage, heavy metals, PAHs) may affect the chemical composition and structural characteristics of soil, which in turn will influence the species composition and abundance of soil microorganisms and/or their metabolic activity, the enhancement or suppression of enzyme production and the overall activity of an enzyme in soil [6].

Soil enzymes are important in soil functioning because of the following features: 1) they play a critical role in the decomposition of organic materials and the transformation of organic matter, 2) they release available nutrients to plants, 3) they participate in N2 fixation, nitrification and denitrification processes, and 4) they take part in the detoxification of xenobiotics, such as pesticides, industrial wastes, etc. [10].

Soil enzyme activities have been suggested as sensitive indicators of soil fertility since they catalyze the principal biochemical reaction that involves nutrient cycles in soil, are very sensitive and respond to changes caused by natural and anthropogenic factors easily and can be easily measured since large number of samples can be analyzed within a few days using a small amount of soil [3,4,11,12].

Soil Fertility – Definitions and Evaluation of Concepts

The quality and fertility of soils play an important role in the sustainable development of the terrestrial ecosystem. Soil quality has been defined as 'the continued capacity of soil to function as a vital living system, within ecosystem and land use boundaries, to sustain

ISBN:
DOI:

biological productivity, promote the quality of air and water environments and maintain plant, animal and human health' [13]). Soil fertility is an integral part of soil quality that focuses more on the productivity of the soil, which is a measure of the soil's ability to produce a particular crop under a specific management system. All productive soils are fertile for the plant being grown, but many fertile soils are unproductive because they are subjected to same unbeneficial natural factors (e.g., drought) or management practices [14].

A variety of definitions and approaches have been proposed for the term 'soil fertility' [15,16]. In 1931 Waksman [17] wrote that 'the measure of soil fertility is the crop itself' but he did not succeed in distinguishing between the concept of soil fertility and that of soil productivity. The biological concept of the term 'soil fertility' was, however, presented in his consideration about the nature of this phenomenon 'soil fertility and the rate of oxidation were found to be influenced by the same factors and to the same extent so that it was suggested that the later could be used as a measure of the former'. The importance of humus in the assessment of soil fertility was emphasized in the definition given by Howard in 1940 [18] 'soil fertility is the condition of the soil rich in humus in which the growth processes are getting on fast and efficiently' Nearly 50 years later, a definition of soil fertility connected with plant nutrition was proposed by Foth and Ellis [14], who stated that soil fertility is 'the status of a soil with respect to its ability to supply elements essential for plants growth without a toxic concentration of any element'. Thus, soil fertility focuses on an adequate and balanced supply of elements or nutrients to satisfy the needs of plants under different climatic and soil conditions. A few years later, Ştefanic's [19] definition again dealt with the most fundamental biological feature of soil fertility: 'fertility is the fundamental feature of the soil, that results from the vital activity of micro population, of plant roots, of accumulated enzymes and chemical processes, generators of biomass, humus, mineral salts and active biological substances. The fertility level is related with the potential level of bioaccumulation and mineralization processes, these depending on the programme and conditions of the ecological subsystem evolution and on anthropic influences'. This complex definition was often replaced by the one proposed by Persson and Otabbong [20], who simply wrote that soil fertility is 'the long-term capacity of a soil to produce good yields of high quality on the basis of chemical, physical and biological quality factors'. Furthermore, they discussed the concept of soil fertility thoroughly and specified three main components of soil fertility – physical, chemical and biological. These components continuously interact with each other under the influence of climatic factors, soil type and management practices [21]. The fertility of soil can be improved, maintained or decreased, depending on the cultivation practices that are used. For example, soil potassium or magnesium content can be increased or decreased. However, some fertility factors cannot be modified because of cultivation managements, e.g., soil type and topography. For instance, soil pH and the susceptibility of the soil to compaction are dependent on the constituents of the original parent rock. Subsequent events, including the growth of plants and the addition of fertilizers, modify the soil's characteristics and alter its fertility. For instance, the original soil pH can be modified by legumes, which increase soil acidity (i.e. decrease soil pH). In relation to the statements above, actual and potential soil fertility can be distinguished. Potential fertility is when all variable fertility factors are optimized. In this situation, the unchangeable factors alone manage soil fertility. Persson and Otabbong [20] themselves concluded that yield level is an imprecise definition of soil fertility because of the complexity of the soil and soil processes.

Soil fertility cannot be assessed directly; it must be determined on the basis of changes in soil properties. Soil fertility is significantly affected by physical, chemical, microbiological and biochemical properties, which are sensitive to changes in the environment and land

ISBN:
DOI:

management. When soil fertility is considered in terms of the highest level of productivity, the emphasis is mainly on the physical and chemical properties of the soil. Among the chemical properties, total carbon and nitrogen content, soil reaction and the content of available nutrients are the most important in evaluating soil fertility, while as regards to the physical properties, the most important are bulk density, porosity, water retention, soil temperature,, etc. Recently, concern regarding the long-term productivity and sustainability of agro-ecosystems has been concentrated on various bio-indicators and the application of biological methods, particularly in the development and protection of soil resources [22]. Biological indicators are used to assess soil quality or fertility because of their central role in nutrient transformations and their rapid response to changes in management practices. The biological properties of soil such as microbial biomass (C) [13], ecophysiological quotients [23], specific biochemical properties such as the activity of hydrolytic soil enzymes related to C, N and P cycles [10] and the composition of the microbial community [24] have been proposed as indicators of soil fertility.

Potential Role of Soil Enzymes in Maintaining Soil Fertility

Among the many biological properties that have potential as sensitive indicators of soil quality and fertility, enzyme activities often provide a unique integrative biological assessment of soil function, especially those that catalyze a wide range of soil biological processes, such as dehydrogenase, urease and phosphatase [11]. Soil enzymes play key biochemical functions in the overall process of the transformation of organic matter and soil nutrient cycling in the soil system [4,5]. The agricultural significance of soil enzymes has been progressively expanded since the first report on soil enzymes was written about a century ago [25]. Soil enzymes, which were once used as descriptive parameters, are now appreciated for their multiple functions in microbial activities, soil processes and ecosystem responses to management and global environmental change [26]. Selected enzymes that are of great agricultural significance are presented below and in Table 1.

Class/EC number*	Recommended name	Soil function and agriculture significance	Reaction	References
Oxidoreductases				
1.11.1.6	Catalase	Release oxygen from hydrogen peroxide; has a detoxification function in cells	$2H_2O_2 \rightarrow 2H_2O+O_2$	[27,28]
1.11.1.7	Peroxidase		S+H_2O_2 → oxidized S+H_2O	[29,30]
1.18.6.1	Nitrogenase	N fixation, catalyses the conversion of atmospheric, gaseous dinitrogen (N_2) and dihydrogen (H_2) into NH_3	$N_2+3H_2 \rightarrow 2NH_3$	[31]
1.10 1.13 1.14	Phenol oxidases	Oxidize phenolic compounds and are involved in humification of organic matter	e.g. laccase: 2 *p*-diphenol+O_2 → *p*-quinone+$2H_2O$	[29,30]
Transferases				
2.8.1.1.	Rhodanese	Performs intermediate step in oxidation of elemental S which is found in small amounts in soil or is added as a S fertilizer	S_2O3_2–+CN → SCN–+SO_3^{2-}	[32,33]
Hydrolases				
3.1.4.1	Phosphodiesterase	Indicator for P cycle, revealed to be a good index of the soil P availability to the plant	$R_2NaPO_4+H_2O$ → ROH+R-$NaHPO_4$	[34,35]
3.6.1.1	Pyrophosphatase	Indicator for P cycle, interest in this enzyme activity derives from the fact that ammonium polyphosphate, an inorganic salt of polyphosphoric acid and ammonia, is one of the frequently used phosphoric fertilizers	Pyrophosphate+H_2O → $2PO_4^{3-}$	[36]

ISBN:
DOI:

3.5.1.1 3.5.1.2.	L-Asparaginase L-Glutaminase	Act on C-N bonds (other than peptide bonds) on respective amino acids releasing NH_3, important in N mineralization to provide plant available N	Asparagine → aspartic acid+NH_3 Glutamine → glutamic acid+NH_3	[37,38]
3.5.1.13	Aryl acylamidase	Hydrolyses propanil, which is use as a component of herbicide	Anilide+H_2O → carboxylate+aniline	[39]
3.5.1.4	Amidase	Hydrolysis of C-N bonds other than peptide bond in linear amides releasing NH_3, important for N mineralization to provide plant available N form	Monocarboxylacid amide+H_2O → monocarboksyl acid+NH_3	[37]
3.4.11.2	Arylamidase	Indicator of N cycle, hydrolysis of a N-terminal amino acid from peptides, amides and arylamides, an index of N mineralization in soils		[40,41]
3.2.1.26	Invertase	Indicator for C cycle, catalyzes the hydrolysis of sucrose to glucose and fructose; its substrate, sucrose, is one of the most abundant soluble sugar in plants, partially responsible for the breakdown of plant litter in soil	$C_{12}H_{22}O_{11}+H_2O$ → $C_6H_{12}O_6+C_6H_{12}O_6$	[42,43]
3.2.1.8	Xylanase	Responsible for decomposition of xylan, a polysaccharide found with cellulose in soil	Hydrolysis of β-1,4-xylan bonds	[43,44]
Broad spectrum enzymes assay	Fluorescein diacetate hydrolysis	Provides general indicators of soil hydrolytic activity, which is carried out by proteases, lipases, and esterases; energy and nutrients for microorganisms, the measure of microbial biomass, organic matter decomposition and nutrient cycling		[45,46]

Table 1: Agronomically important soil enzymes and their functions. Parts adopted from Gianfreda and Bollag [6], Gianfreda and Ruggiero [7] and Dick [10].

*The enzymes are classified according to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB); ROH: Hydroxylated compound; R: either alcohol or phenol group; S: reduced organic substrate.

Dehydrogenases activity (DHA) (EC 1.1): Soil dehydrogenases are the major representatives of the oxidoreductase enzymes class. Lenhard [47] was first to introduce the concept of determining the metabolic activity of soil microorganisms by measuring the activity of dehydrogenases because of its simplicity as compared to other quantitative methods. The activity of the DHA reflects the total range of the oxidative activity of soil microorganisms and may be considered a good indicator of the oxidative metabolism in soils, and thus, of microbiological activity [48]. Dehydrogenases oxidize soil organic matter by transferring protons and electrons from organic substrates to inorganic acceptors [49]. Many specific dehydrogenases transfer hydrogen on either the nicotinamide adenine dinucleotide or the nicotinamide adenine dinucleotide phosphate. Throughout mentioned co-enzymes hydrogen atoms are involved in the reductive processes of biosynthesis. These processes are part of the respiration pathways of soil microorganisms and are closely related to the type of soil and air-water conditions [50].

Nitrate reductase activity (NR) (EC 1.7.99.4): Nitrate reductase is an important enzyme in the process of denitrification, which catalyzes the reduction of NO_2– to N_2O under anaerobic conditions. The nitrogen that is present in the structure of this enzyme acts as a terminal electron acceptor by bacteria rather than molecular O_2 and this is irreversible once NO is formed [51]. The systematic name for nitrate reductase is reduced NADP – nitrate oxidoreductase. Flavoprotein (FAD), which contains molybdenum, creates a prosthetic group for this enzyme [52]. Nitrate reductase is an adaptive enzyme and is synthesized only in the presence of NO_3–

ISBN:
DOI:

ions, while in a soil solution it is repressed not by NH_4+ per se, which was suggested earlier [53] but by L-glutamine, which is formed by the microbial assimilation of NH_4+ [54].

The Urease (UR) enzyme is responsible for the hydrolysis of urea fertilizer into NH_3 and CO_2 with the concomitant rise in soil pH and N loss to the atmosphere through NH3 volatilization [55]. Urease can be produced by bacteria, yeasts, fungi and algae, as well as plants [56]. Urease may be synthesized constitutively in some organisms, but most often urease expression is under N regulation [57]. The enzyme synthesis is inhibited when cells grow in the presence of a preferred N source such as NH_4+ [58]. In contrast, urease production is activated in the presence of urea or alternative N sources [57]. Due to its role in the regulation of N supply to plants after urea fertilization, soil urease activity has received a great deal of attention since it was first reported [59]. Urease has been widely used to evaluate changes in soil fertility since its activity increases with organic fertilization and decreases with soil tillage [60].

Cellulases (EC 3.2.1.4): Cellulose is the most abundant organic compound in the biosphere comprising almost 50% of the biomass that is synthesized by the photosynthetic fixation of CO_2 [61]. The growth and activity of soil microorganisms depends on the carbon source that is contained mainly as plant residues that occur in the soil [62]. However, for carbon to be released as an energy source for use by the microorganisms, cellulose in plant debris has to be degraded into high molecular weight oligosaccharides, cellobiose and glucose by cellulase enzymes [63]. Cellulases is a group of hydrolytic enzymes that catalyze the breakdown of b-1,4 linked bonds in cellulose. Complete degradation of cellulose requires at least three enzymes: endo-b-1,4-glucanase, which attacks the cellulose chains at random, exo-b-1,4-glucanase, which removes glucose or cellobiose from the non-reducing end of the cellulose chains and b-D-glucosidase, which hydrolyzes cellobiose and other water soluble cellodextrins to glucose [62]. Since cellulase enzymes play an important role in the global recycling of the most abundant polymer in nature, more research should be done to understand the nature of this enzyme better, so that it may be used more regularly as a predictive tool in the assessment of soil fertility.

Glucosidases (EC 3.2.1.20/21) are the group of C-cycling enzymes that should be investigated as a function of the application of an organic amendment as they play a key role in the breakdown of low molecular weight carbohydrates. The most common and predominant among soil glucosidases is b-glucosidase [64]. This enzyme plays an important role in soils because it is involved in catalyzing the hydrolysis and biodegradation of various b-glucosides that are present in plant debris and which are decomposed in the ecosystem [65]. Glucose, the final product of b-glucosidase activity, is an important carbon source for the growth and activity of soil microorganisms [64,66]. The activity of b-glucosidase is particularly stable and has a low seasonal variability [67] and is very sensitive to soil pH and soil management practices [68,69]; therefore, it can be used as a good biochemical indicator for measuring ecological changes that result from soil acidification and various agricultural practices. Additionally, changes in its activity could be indications of the fungi/bacteria ratio in soil [70].

Phosphatases are a group of enzymes that are of great agronomic value because they catalyze the hydrolysis of organic phosphorus compounds and transform them into an inorganic form of P, which is then assimilated by plants and microorganisms [71]. Agricultural soils contain phosphatases in varying amount depending on the microbial count, the amount of organic materials, mineral and organic fertilizers, tillage and other agricultural practices [72]. The relationship between the available P content and phosphatase activity in soil is complex. A positive, negative or no relationship can be observed between these properties. Generally, a significant and positive relationship between phosphatase activity and P availability [6,73] is

ISBN:
DOI:

obtained in soils that are not fertilized and/or those that have small amounts of nutrients in which a P deficiency occurs. An inverse relationship between these two parameters is usually observed in soils that are fertilized with P and/or those with a sufficient content of available P. There are studies that show that phosphatase activity is inversely proportional to the plant available P content [71,73,74], which confirms the thesis that the production and activity of soil phosphatases is connected with the demand of microorganisms and plants for P. Phosphatases are typical adaptive enzymes and their activity increases when the plant available P content decreases [75]. Kinetics studies indicate that orthophosphate ions, which are the product of the reaction that is conducted by the phosphatases, are competitive inhibitors of their activity in soil [76]. When no relationship is seen, P may not limit the study system and some other factors may influence the enzyme production and activity [77].

Arylsulphatase (EC 3.1.6.1) plays an important role in the mineralization of organic S in soils. This enzyme is involved in the hydrolysis of aromatic sulphate esters ($R\text{-}O\text{-}SO_3^-$) into phenols (R-OH) and sulfate sulfur (SO_4^{-2}) by splitting the oxygen-sulphur (O-S) linkage and that is why it is involved in the mineralization of ester sulfate in soil [64]. In most aerobic soils ester sulfates account for up to 70% of the organic S, and therefore are the most important organic S reserve in soil [78]. Additionally, that enzyme can be an indicator of the presence of fungi since only fungi contain ester sulfates, a substrate of arylsulfatase activity [69,79]. Arylsulfatase has been detected in plants, animals, microorganisms and soils [64] and is only one of the many types of sulfatases involved in the mineralization of ester S compounds. Arylsulfatase is a mostly adaptive enzyme and its synthesis by microorganisms may be controlled by the C and S content in the soil environment [10,64].

Proteases: The application of organic nitrogen (N) to soil may be an important economic alternative to the application of fertilizer N due to the reduced cost of the production of inorganic N fertilizers. It has been estimated that about 40% of the total soil N, including proteins, glycoproteins, peptides and amino acids, is proteinaceous material [80]. Organic amendments that are applied in agriculture, such as farmyard manure, municipal solid waste or sewage sludge are characterized by the presence of a high load of organic N forms, such as proteins, nucleic acids and amino-polysaccharides [e.g. 81]. Thus, protein transformation in soil has a considerable influence on soil ecology and agriculture [82].

Protein that is added to soil is readily decomposed by proteases and peptidases into smaller, membrane-permeable peptides and amino acids. The latter are further metabolized with the release of NH_4+ [83]. Proteases are ubiquitous and originate from a number of different sources in soil, including microorganisms (bacteria, actinomycetes, and fungi), plants and animal excrements [84]. Many of these enzymes are extracellular, as a large number of native proteins are too large to be absorbed by living cells [83]. Proteolysis is an important process in many ecosystems with regard to N-cycling because it is considered to be a rate-limiting step during N mineralization in soils due to the much slower primary phase of protease activities during N mineralization compared with amino acids mineralization [85].

Amylases (EC 3.2.11/2): Amylases together with cellulases and invertase form the group of enzymes that are responsible for the rate and course of the decomposition of plant material in soil [86]. Amylase is the name of a group of starch hydrolyzing enzymes in which the most important is α-amylase, which converts starch into glucose and/or oligosaccharides and b-amylase, which converts starch into maltose [87]. The α-amylases are synthesized by plants, animals and microorganisms, while b-amylase is produced mainly by plants [87]. Studies have indicated that the activities of soil amylases may be influenced by different agricultural practices, the type of soil and vegetation [42,86]. Plants may influence soil amylase activity

ISBN:
DOI:

by supplying enzymes directly from their residues or excreted compounds, or by indirectly providing substrates for the synthesis enzymes by microorganisms [86].

Soil Enzymes as Indicators of Agricultural Practice

Enzymes play an important role in the cycling of nutrient in nature and because their activity is sensitive to agricultural practices they can be used as an index of soil microbial activity and fertility [88]. Earlier, the emphasis had been placed on the conventional physical and chemical properties as indicators of soil fertility rate, but often these properties responded slowly to management practices and were found to be not sensitive enough to detect changes in soil properties that are caused by agricultural management practices, especially in the short-term [89]. Therefore, there is a need to find suitable tools that reflect the influence of management practices in order to observe possible changes [90]. Biological indicators, such as soil microbiological biomass and enzymatic activity, seem to be better indicators since they respond much more quickly to both natural and anthropogenic factors in comparison with other variables [91]. Thus, they may be useful as early indicators of biological changes in soil [69,92]. Soil enzyme activities are strongly affected by the agricultural management practices and have been used as indicators of irrigation [93], the application of inorganic fertilizers and organic amendments [e.g., 94-98], different management and farming systems [e.g., 37,99,100] and soil tillage [e.g., 40,101-103]. Enzymatic activity was found to be the most strongly influenced soil property under intensive agricultural practices as compared with other biochemical parameters [60].

Inorganic fertilization, positive and negative feedback mechanisms

Among the different farming practices, the management of mineral fertilizers and organic amendments could have a major impact on soil fertility, thus influencing the quantity and quality of organic residues and nutrient inputs that enter the soil and the rate at which the residues and organic matter are decomposed [104]. The influence of inorganic fertilization on the soil enzyme activity depends on the dose of the fertilizer and the time of its application, the soil type, climatic conditions and the enzyme itself [6]. Studies on the effect of inorganic N fertilization on enzyme activities have led to contradictory results [7]. Some previous studies have shown that N fertilization can accelerate the activity of some C, N and P cycling enzymes, like cellulases [94], urease [105] and phosphatases [98,105] or decrease the activity of urease [106], cellulases [94], peroxidase [107], proteases [108], while some other enzymes are not affected with increasing N fertilizer application [99,109]. More often, however, enzyme activities increased when organic and inorganic N fertilizers were added together [e.g., 110]. Mineral N can directly affect the microbial production of soil enzymes but the effect varies with the type of soil and the enzyme as well as with the kind of enzymatic reaction [97], which is possibly due to changes in the composition of the soil microbial community and, therefore, the enzyme production [4,97]. On the other hand, N fertilization, especially in mineral forms, may have an indirect effect on the activities of soil enzymes via changes in soil properties, such as soil reaction [7].

Most often soil enzyme activities significantly depend on the dose and the frequency Repeated application of inorganic N fertilizers over a period of 305 days showed no significant effect on the activities of b-glucosidase and proteases [111]. However, the enzyme activities (b-glucosidase, urease, arginine deaminase, acid and alkaline phosphatases) were 10-26% lower at a rate of 160 kg N ha^{-1} $year^{-1}$ compared to the highest activity, which was noted in the case of a rate of 40 and/or 80 kg N ha^{-1} $year^{-1}$ [90]. Siwik-Ziomek et al. [112] stated that the optimal rate of ammonium nitrate that coincided with the highest activity of arylsulfatase

ISBN:
DOI:

was 100 kg N ha^{-1}, while higher doses (150 and 200 kg N ha^{-1}) decreased this activity. In the study of Šlimek et al. [113], dehydrogenase activity decreased with an increase in the dose of NPK fertilizer and was further decreased in the absence of lime. In the study of Giacometti et al. [108], a significant reduction of dehydrogenase activity was observed at 200 kg ha^{-1} of mineral N compared to control (N0) plots. This suggested that dehydrogenase activity was highly sensitive to the inhibitory effects that are associated with large additions of mineral fertilizers. According to Iyyemperumal and Shi [97], the activities of cellulase, cellobiohydrolase and acid phosphatase increased with an increasing rate of ammonium nitrate (0-600 kg PAN ha^{-1} $year^{-1}$) and were inversely related to the soil microbial biomass. The activities of peroxidase, phenolooxidase, cellobiohydrolase and protease activities were similar at the highest fertilization rate and in the unfertilized control. The above data was similar to that obtained by Sinsabaugh et al. [94], where increasing the available soil N generally stimulated soil cellulase activity but inhibited the activity of oxidative enzymes, such as phenol oxidase and peroxidase. Dick at al. [114] showed that amidase and urease activities decreased with the increased application of ammonia-based N fertilizer. They stated that the addition of the end product of the enzymatic reaction (NH_4+) suppressed enzyme synthesis.

In fact, a feedback mechanism such as suppressing the production of enzymes whose reaction product was continually supplemented with inorganic fertilizers is generally known for some enzymes, such as urease or phosphatases [76]. Perhaps extracellular enzymes are produced by microorganisms only when the enzymes can help to better use the resource and therefore lead to optimal microbial growth and metabolism. This cost-efficient strategy predicts that the production of microbial enzymes can be low when the end products of enzymatic reactions, e.g., nutrients, are abundant or when the end products of enzymatic reactions, e.g., complex organic substances, are limited [115]. End product suppression on enzymes has been mentioned by many authors and the activity of a nutrient-mineralization enzyme is inversely related to the availability of nutrients [25]. It was shown that negative feedback was indeed the cause of a reduction in phosphatase and chitinase activities after long-term phosphorus and nitrogen fertilization [77]. In the case of high nutrient availability, enzyme activities can be low and thus fertilization will not cause additional suppressive effects [77]. When nutrient availability is low, the nutrient may limit microbial growth and metabolism and then fertilization may stimulate microbial biomass and in turn microbial enzyme production.

Phosphorus fertilization under field conditions has been shown to depress phosphatase activity in agricultural systems [116], although the influence of fertilization on the enzyme was dependent on the content of soil organic matter. A low organic matter content increased phosphatase activity with P fertilization, but soil with a high organic matter content that was amended with P fertilizer showed no changes in the enzyme activity. In the study of Mijangos et al. [89], acid phosphatase did not decrease when P was added as a mineral fertilizer, probably due to the relatively low P dose that was applied (i.e. the highest values of Olsen P were around 30 mg kg^{-1}). According to Chunderova and Zuberts [117], phosphatase activity appears to be inhibited at 100 mg Olsen P kg^{-1} soil. Similarly, in the study of Criquet and Braud [118], the application of both Na and K phosphate salts at a rate to provide 0.22 g kg^{-1} of P total quantity (equivalent to 0.5 g P_2O_5) (supplied as NaH_2PO_4 and KH_2PO_4, respectively) did not show any significant effect on acid and alkaline phosphatases and phosphodiesterase activities, although it resulted in a significant increase in the available P content up to 112.2 µg P g^{-1} soil.

The application of lime to soil most often causes a significant increase in pH and thus significantly affects other physical, chemical and biological soil properties such as microbial biomass and diversity, and therefore, enzyme activities [e.g. 40,66]. According to Haynes and

ISBN:
DOI:

Swift [119] additions of lime generally increased protease and sulfatase activity, but decreased phosphatase activity. In the study of Ekenler and Tabatabai [120], acid phosphatase decreased with increasing pH because of liming and also found that liming increased the activities of alkaline phosphatase and phosphodiesterase. The different responses of acid and alkaline phosphatases to liming supported the previous finding that phosphatases are inducible enzymes and that the intensity of their release by microorganisms and plants is determined by their requirement for orthophosphate, which is strongly affected by soil pH [3]. The optimum soil pH for crop production and the amount of lime required to achieve this optimum can be investigated by using alkaline (Pal) and acid phosphatase (Pac) activities [121]. The ratio of both of these enzyme activities (Pal/Pac) responded immediately to changes in pH that was caused by the addition of $CaCO_3$ and the ratio of approximately 0:5 divided soils into those with a proper pH and those that still needed an additional lime treatment (Figure 1). In the study of Lemanowicz [122] with long-term nitrogen fertilization (ammonium nitrate at the following rates: 0, 50, 100, 150, 200 and 250 kg N ha^{-1}), the Pal/Pac ratio decreased from 0.44 to 0.21 with increasing doses of the nitrogen ap¬plication. Dick et al. [121] concluded that measuring the Pal/Pac ratio may be preferable to chemical approaches for evaluating the effective soil pH and liming needs.

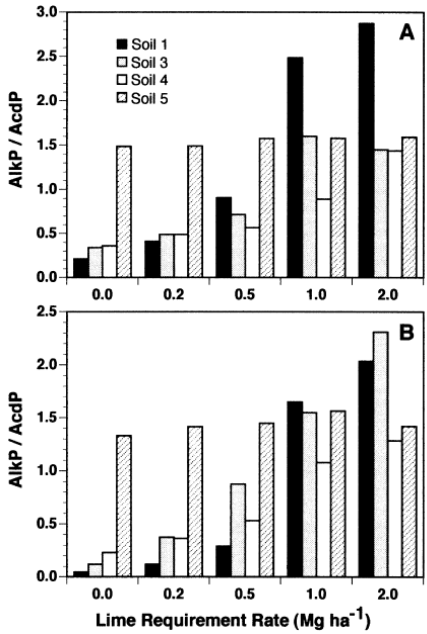

Figure 1: Changes in alkaline phosphatase/acid phosphatase (AlkP/AcdP) activity ratios in soils amended with (A) chicken manure and (B) alfalfa residue after adjusting pH with C_aCO_3 according to the lime requirement of each soil. $LSD_{0.05}$ values used for mean comparisons for Soils 1, 3, 4 and 5 are 0.277, 0.236, 0.215 and 0.341, respectively. Adopted from Dick et al. [121].

Organic amendments

The application of organic amendments (straw, farmyard manure, green manure, sewage

ISBN:
DOI:

sludge, olive mill waste and other waste and by-products) to soil as a nutrient source is a management practice that can increase the soil organic matter content, improve soil biological activity and consequently increase the soil nutrient status with a simultaneous reduction in the dependence on mineral fertilization [e.g. 123-126]. Among the organic amendments that can be used as potential fertilizers, special attention has been paid to urban residues such as municipal solid waste and sewage sludge, the generation of which has been growing rapidly in recent decades and its management has become one of the key tasks in environmental policy of many countries [127,128].

Contradictory results have often been observed when the short- and long-term effects of organic amendments on soil enzyme activities were compared [129,130]. The short-term increase in soil enzyme activities after the application of organic amendments can be attributed to a greater microbial biomass due either to the addition of microorganisms and enzymes in the amendment or indirectly to the addition of available organic substrates that promote the growth of indigenous microorganisms [e.g., 88,95]. The long-term effect of organic amendments on enzyme activities is probably the combined effect of a higher degree of the stabilization of enzyme to humic substances and an increase in the microbial biomass with an increased soil C concentration [131] as well as the inhibitory effect of heavy metals and other toxic substances. The increase of enzymatic activity shortly after the addition of organic amendments, probably caused by exhausting the easily degradable substrates, has been noted in many studies [e.g. 70,96,132,133], Perucci [70] found significantly higher values of the activities of eight different groups of soil enzymes within 30 days after the application of compost from municipal residues as well as during the following three years. In the study of Kizilkaya and Bayrakh [96], a sudden and significant increase in enzymatic activity (urease, alkaline phosphatases, arylsulfatase and b-glucosidase) was observed after the addition of sewage sludge in sludge-amended soils followed by a progressive decrease in this activity. Positive influence of manure and vermicompost application on enzyme activity was found by Marinari et al. [126] (Table 2).

Treatments	Dehydrogenase (μg INTF g-1 h-1)	Protease BAA (μg NH3 g-1 h-1)	Acid phosphatase (μg pNP g-1 h-1)
Control	4.1^{a}	75.1^{a}	266^{a}
NH_4NO_3	6.1^{b}	87.8^{ab}	457^{b}
Manure	20.7^{c}	73.5^{a}	727^{d}
Vermicompost	1.7^{b}	91.8^{b}	613^{c}

Table 2: Enzyme activities in soil fertilized and unfertilized, three months after fertilization Adopted from: Marinari et al. [126]. The values with the same letter are not significantly different ($P \leq 0.05$)

Because olive mill waste water contains a high level of organic matter and nutrient content, its recycling as fertilizer may be an alternative to its disposal that also improves soil fertility and productivity [134]. All nine enzymes that were tested in a ten-year field study in semi-arid Mediterranean areas increased even with the application of the higher de-oiled two-phase olive mill waste (DW) (54 Mg ha^{-1}). Compared to the control, the addition of crude OMW (olive mill wastewater) and dephenolized OMW to the soil increased dehydrogenase activity suddenly and sharply, which was 8 and 4 times higher as compared to the control soil [133]. In the same study [133] diphenol oxidase activity, another enzyme that is involved in redox soil reactions was inhibited by dephenolized OMW as compared with crude OMW. This was probably due to the presence of available diphenol substrates in crude OMW, which could enhance the synthesis of the enzymes by the microorganisms [95].

The rapid development of biogas production is resulting in the increased use of biogas residues as an organic fertilizer. In the study of Chen et al. [135], the extracellular enzyme

ISBN:
DOI:

activities in agricultural soil amended with biogas residues (BGR) versus maize straw (MST) were assessed. The influence of given treatments on enzymatic activity is presented on Figure 2. Conversely, MST significantly increased the activity of these three enzymes. The contrasting effect of treatments of BGR and MST on these enzymes may be attributed to the lower availability of C in biogas residue compared with maize straw. The high lignin content in BGR indicated a low C availability due to the formation of lingo-cellulose or lignin-polysaccharide complexes, which may resist the attack of enzymes [125]. Biogas residue significantly promoted the activities of chitinase and leucine amino peptidase, two enzymes that are related to the N-cycle. These enzymes were promoted by an N-enriching organic component, e.g., peptidoglucan, which was accumulated as microbial residue during the fermentation of the biogas [136].

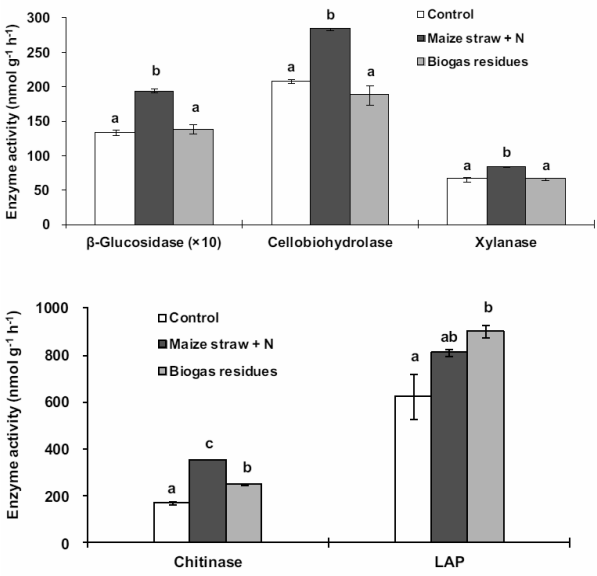

Figure 2: Extracellular enzyme activity as influenced by the addition of biogas residue, maize straw+mineral N. C-cycle related enzymes β-glucosidase, cellobiohydrolase and xylanase are at the top and N-cycle related enzymes chitinase and leucine amino peptidase (LAP) are at the bottom. The values of β-glucosidase activity were divided by ten in order to fit the scale. Data are means of 3 replicates ± standard errors. Different letters show significant differences between the treatments ($P<0.05$). Adopted from Chen et al. [135].

A decrease of some enzyme activities after the application of organic amendments was also shown [e.g., 133,137] because they may also contain pollutants (e.g., heavy metals and toxic organic compounds) which, if present in an inhibitory concentration, may decrease soil enzymatic activity [6,96]. Sewage sludge may especially inhibit soil enzyme activities drastically due to a higher content of heavy metals [e.g. 138,139]. The inhibitory effect of heavy metals can overcome the stimulatory effects of the addition of municipal solid waste compost to soil [75] and the effect is dependent on the individual sensitivity of enzymes to heavy metals [70,129]. In the study of Moreno et al. [140] no microbial activity, which was indicated by the total lack of dehydrogenase activity, was found with sewage sludge that contained a high Cd content (815 mg kg^{-1}) or when the toxic olive-mill solid waste was applied to the soil. Olive waste

ISBN:
DOI:

shows a high toxicity to microorganisms, directly related to the presence of different types of polyphenols [95]. In fact, in the study of Piotrowska et al. [133], some enzymes (fluorescein diacetate hydrolase: FDAH, nitrate reductase, urease), decreased significantly after the addition of crude olive mill waste water (OMW) compared with dephenolized OMW.

In agricultural practice, the importance of farmyard manure as an organic fertilizer that has great value has gradually decreased in recent years due to new techniques in animal production. On the other hand, green manure or straw residues are still an important source of organic matter and nutrient input into arable soils [124,125,141,142]. According to Perucci and Scarponi [137], the majority of crop residues (wheat straw, maize, sunflower, tobacco, capsicum, sorghum, and tomato) generally caused the activation of phosphodiesterase in untreated soil, while it inhibited acid phosphatase activity. Only the incorporation of tobacco residues caused a significant increase in all of the phosphatases that were tested (neutral, acid and alkaline). In Turkey, it is possible to use tobacco waste as a soil amendment due to its high organic matter and low content of toxic elements [143]. In the study of Okur et al. [143], the highest enzyme activities were observed in soil with 25% of farmyard manure and 75% of tobacco waste compost. Detailed results of enzyme activities are presented in Table 3.

Treatments	DH (μg TPF g^{-1} 16 h^{-1})	Pal (μg *p*NP g^{-1} h^{-1})	UR (μg S g^{-1} 3 h^{-1})
Control	143.4 (9.1)^{c}	625.3 (78.2)^{b}	50.9 (2.9)^{b}
25% FYM+75% TWC	230.3 (13.8)^{a}	824.2 (82.4)^{a}	71.3 (6.2)^{a}
50% FYM+50% TWC	176.1 (13.8)^{bc}	716.4 (64.4)^{ab}	51.1 (11.6)^{b}
75% FYM+25% TWC	180.1 (14.5)^{bc}	767.0 (62.6)^{ab}	58.9 (4.0)^{ab}
100 % FYM	166.2 (19.5)^{c}	701.2 (79.8)^{ab}	49.5 (7.9)^{b}
100% TWC	208 (8.3)^{ab}	762.2 (73.9)^{ab}	68.2 (10.3)^{a}

Table 3: Dehydrogenase (DH), alkaline phosphatase (Pal) and urease (UR) activity in the soil samples (0-20 cm). Adopted from Okur et al. [143].

Number in parentheses are standard deviation (n=3). Mean value followed by the same letter are not significantly different between different treatments, according to Tukey's test ($P<0.05$); FYM: farmyard manure; TWC: tobacco waste compost; TPF: triphenyl formazan; pNP: p-nitrophenol; S: saligenian.

The increase in enzymatic activities that is observed because of the application of green manure has been mentioned in many studies [69,141,142,144]. Debosz et al. [145] reported that the activities of b-glucosidase, cellobiohydrolase and endo-cellulase in a fertilization system with green manure were significantly higher than in a system with exclusive mineral nitrogen fertilization. Tejada et al. [142] studied the effect of incorporating three green manures originating from the residues of *Trifolium pratense* L (TP), *Brasicca napus* L (BN) and a mixture of them (TP+BN). All of the green manures had a positive effect on the biological properties including enzymatic activities. The activities of dehydrogenase, urease, b-glucosidase, phosphatase and arylsulfatase increased more significantly in the TP amended soils (from 79 to 96%), followed by the TP+BN and BN amended sites, thus suggesting the more easily decomposable components of TP, and hence an improvement in their microbial activity. In the study of Kautz et al. [141], the highest dehydrogenase and cellulases activities were observed in treatments with straw and green manure as compared to the control, farmyard manure and mineral N fertilization. It was stated that the higher the dose of the organic amendment the greater the enzymatic activity due to the higher microbial biomass produced in response [123]. As was stated Geisseler et al. [146], plant residue is composed of many polymeric molecules that must be broken down into available units by extracellular enzymes. Therefore, it was no

ISBN:
DOI:

surprise that protease, b-glucosidase and exocellulase activities were significantly increased with the addition of the oats-legume plant residue. According to Pancholy and Rice [86], dehydrogenase activity was influenced by the quality rather than by the quantity of organic matter that was incorporated into the soil. Thus, the stronger effects of green manures on dehydrogenase activity might be due to the more easily decomposable components of crop residues on the soil microorganisms. Under field conditions, however, the decomposition of green manures is complex and is controlled by many factors, such as carbon and nitrogen content and the C/N ratio, the biochemical nature of the plant residues, soil climatic factors, etc. [142]. The C/N ratio of the organic amendments will largely determine the balance between the mineralization and humification processes [123]. The C/N ratio is considered to be the best parameter for predicting the potential amount of N that can be mineralized from a plant material [147].

Straw incorporation into the soil is considered to be an important strategy to improve soil fertility and to reduce the dependence on mineral fertilization [148]. Although the use of plant residues has long-term positive effects on soil properties, in the short term (several weeks following straw incorporation), this residue may cramp root penetration and cause an N deficiency. The reason for an N deficiency is that more soil N is being immobilized by microorganisms due to the incorporation of the straw [149]. Therefore, it is important to find a way to enhance the decomposition of straw shortly after its incorporation. One promising method is accelerating straw decomposition through the application of exogenous cellulose to soil [148]. In the study of Han and He [148], 70 U g^{-1} was considered to be the optimal cellulase concentration for plant growth, while 50 U g^{-1} was acknowledged as being economically beneficial. Earlier, Fontaine et al. [150] found that exogenous cellulase accelerated the decomposition of cellulose in soil significantly. Similarly, the short-term effects of the application of exogenous protease on soil fertility with the incorporation of rice straw were studied by the same authors [151]. After 120 days of incubation with rice straw, soil protease activity, available N, available P and electrical conductivity of treatments with at least 0.5% added protease were significantly ($P<0.05$) greater than the no-protease control. Protease activities potentially increase the soil available N because they promote the release of amino acids from straw protein through hydrolysis. Protease application increased the available P content because microbial activity can increase as a result of the increase in the available N content. The soil organic matter and pH of treatments with added protease were lower than the non-protease control. Without the incorporation of straw, protease amendments only affected the soil protease activity itself, but not other soil properties. The authors concluded that the addition of exogenous protease can reduce an N deficiency and may be used in fields when straw is applied [151]. Therefore, the application of exogenous cellulase and protease during the incorporation of straw may be a new strategy that will help farmers manage plant residues in the field and increase soil fertility [148,151].

Bio-fertilizers

Although bio-fertilizers have been known for many years, relatively little research has been done to document the effects (or non-effects) of many bio-fertilizers on crop production, or to provide evidence of their potential effects on soil processes [e.g. 152-214]. Moreover, only a few studies exist on the effects of bio-fertilizers on enzymatic activities [152,155,156]. Valarini et al. [155] concluded that the incorporation of “Effective Microorganisms” (EM) together with animal manure and fresh plant debris significantly improved the soil biological activity measured as alkaline phosphatase, esterases and polysaccharidases activities, due to the quick humification of fresh organic matter. The four-year application of two preparations

ISBN:
DOI:

based on EM in an arable organic farming crop rotation in the temperate climate of Central Europe caused no effects on soil dehydrogenase activity [152]. No clear tendency in changes in dehydrogenase activity was noted in a three-year study with UGmax biofertilizer [156]. The results of the study of Piotrowska et al. [156] showed that the microbiological fertilizer UGmax accelerated the initial phase of the decomposition of post-harvest residues, which was confirmed by a significant decrease in cellulase activity in the soil that had been taken from a field where UGmax was applied as compared with the control field. One of the possible explanations is that the cellulase activity increased directly after UGmax treatment and therefore the post-harvest residues decomposed faster than in the control field. As the result of this fact, five months after the second UGmax application (soil samples were always taken shortly before the autumn UGmax treatment), the post-harvest residue content was lower than in the control soil and simultaneously the cellulase activity had decreased (Figure 3). This suggests that UGmax is probably a medium that determines the decomposition rate of post-harvest residues and that the activity of cellulase was a distinct indicator of soil changes after the application of UGmax. Results presented by Chen et al. [154] suggested that the two agricultural biostimulants used (Z93 which is marketed in USA under the trade name GroZyme® and W91, which is not yet marketed) significantly augmented cellulase activity, which was measured as the rate of filter paper weight loss that systematically increased up to the end of the incubation (56 days).

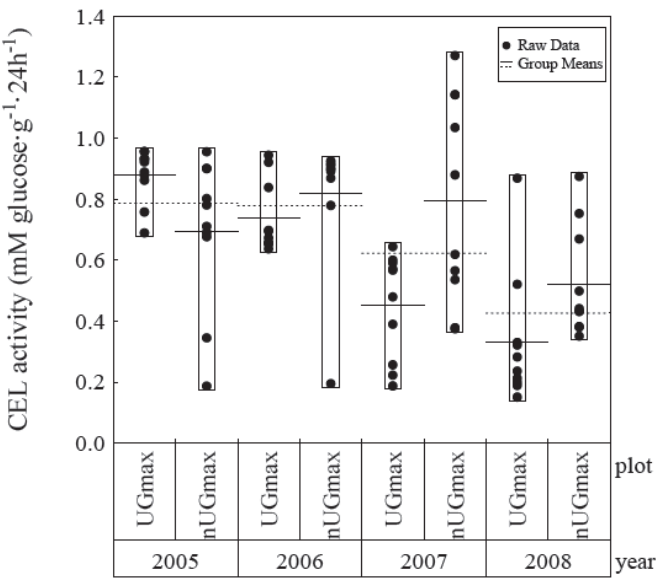

Figure 3: Cellulase activity (CEL) as influenced by UGmax treatment; – mean value for treatment (UGmax vs. control); mean values for both UGmax and control in succeeding years. Adopted from Piotrowska et al. [156].

Tillage

Tillage may change soil fertility due to altering the physico-chemical [157] as well as microbiological and biochemical properties, and thus the soil enzymes [40]. Tillage influences

ISBN:
DOI:

the soil nutrient level and its availability the distribution of organic matter in the soil profile [158] and the soil water and oxygen content [159]. Tillage causes the acceleration of soil organic matter mineralization and consequently leads to a rapid loss of its content. This in turn, causes a decrease in the soil biological and enzymatic activity [101]. In fact, some authors have reported a decrease in enzyme activities due to the decrease in organic matter content as a result of the mixing of horizons by plowing [e.g. 160].

In some studies, no-tillage system increased soil enzymatic activity as compared to conventional tillage practice [75,161]. Deng and Tabatabai [162] showed that the activity of L-asparaginase, L-glutaminase and urease were generally greater under a zero-till system than under a conventional tillage practice. Mina et al. [102] observed the same trend for soil dehydrogenase, alkaline phosphates and protease activities. The activity of dehydrogenase, urease, protease, phosphatase and b-glucosidase was significantly higher in a no-tillage system with varying percentages of surface residue coverage (0, 3, 66, 100%) and a no-tillage system with 33% residue coverage together with cover crops of *Vicia* sp. or *Phaseolus vulgaris* L. as compared with conventional tillage [161]. Usually, the enzymatic activity of the surface layer of a zero-tilled soil are greater than those of the same layer of tilled soils, while the opposite occurs for the deepest soil horizons [e.g. 69,163], which is due to the fact that microbiological activity of surface no-tilled soils is higher than in conventional tillage [6], which is caused by the fact that organic matter is more thoroughly distributed in soil under conventional cultivation compared with that in soils under reduced tillage in which crop residues, which are substrates for soil microorganisms, are concentrated on the soil surface [120].

Some other authors have indicated that plowing causes an increase in enzymatic activity in agricultural soils due to the exposure of new surfaces as soil aggregates are broken [e.g. 164]. In fact in the study of Ulrich et al. [103], the arginine ammonification showed high activity down to a depth of 30 cm of soil profile due to the high aeration in the plow treatment. Similarly, Seifert et al. [165] stated that conventional tillage accelerates the microbial oxidation of organic matter thus stimulating a greater microbial activity. For example, microbial activity, estimated by dehydrogenase activity and by FDA hydrolysis, was higher under conventional tillage than by incorporating plant residues onto surface soil, due to more labile C substrates, which support microbial activity.

In turn, tillage practice (none vs. conventional) had no effect on the enzyme activities in a multi-location field study carried out in Colorado, Kansas and Kentucky (USA) in a loam soil [66]. Similarly, no significant effects of tillage practice on the activities of dehydrogenase, phosphatase and urease activities were noted in soil over four years of spring barley cultivation [166].

The effect of tillage on enzymatic activity in soil depends on the enzyme itself and that is why phosphatase activity was more sensitive to negative changes that were caused by the tillage system [167] than was the urease activity. According to Ekenler and Tabatabai [168], L-glutaminase was the most sensitive N-cycle enzyme followed by amidase, L-asparaginase, L-aspartase, urease, arylamidase for discriminating the effect of tillage (no-till, ridge-till, chise-till). Earlier, the same authors [120] indicated the activity of b-glucosidase and β-glucosaminidase as the most sensitive enzymes that are affected by liming and tillage systems. Moreover, the response of enzyme activity to different tillage can be annually and seasonally dependent [159,163].

Vegetation cover and cropping system

Soil enzyme activity can be affected by the presence and nature of plant cover [6]. Although

ISBN:
DOI:

most of the soil enzyme activities originate from microorganisms, plant roots are an important source of extracellular enzymes in soil. Juma and Tabatabai [169] showed that sterile corn and soybean roots contain acid phosphatase, but not alkaline phosphatase activity. Although there was no clear relationship between arylsulfatase activity and root distance; enzyme activity tended to be higher close to the root surface (distance 0.25 mm) as compared with the distance of 0.75 mm in all of the crop species (*Barssica napus, Sinapus album, Triticum aestivum, Lolium perenne*) [170]. Plants actively respond to an insufficient S supply by producing and excreting sulfatases, which may help them to exploit the organic soil S, compounds [170]. The arylsulfatase activity that is found in the root protein extracts cannot originate from soil bacteria, since the seeds and seedlings had no contact with the soil. This could be explained by the fact that higher plants possess their own arylsulfatases that are inducible under an S deficiency or that seed-borne bacteria that colonize the intercellular sites within the root such as endophytes are responsible for the enzyme activity [170].

Plant roots stimulate enzyme activity by creating advantageous conditions for microbial activity [e.g. [171]. Highest higher enzymatic activity in the plant rhizosphere than in bulk soils was found in many researches, which may be explained by the development of a large population of soil microorganisms in the vicinity of roots that metabolize amino acids, sugars, organic acids and other compounds that are exuded by roots [e.g 170,172]. Leguminous plants have the potential for biological N_2 fixation and this could stimulate the activity of the enzymes that are involved in the N cycle (urease and protease-BBA) [161]. In crop systems that involve both leguminous and non-leguminous plants, the leguminous rhizosphere showed a higher activity of acid, neutral and alkaline phosphatase as compared with the non-leguminous plant rhizosphere [172]. The highest catalase activity was recorded in soil under wheat, soybean and winter legume crops, while the lowest activities were found in soil bearing corn and cotton and during the winter fallow period in the rotation system at the Agronomy farm of the Alabama Agricultural Experimental Station (USA) [27]. The highest activity of arylsulfatase among different crop species was with Cruciferae due to their high S demand [170]. Soil that was under permanent grassland had 1.5 times higher dehydrogenase activity and almost a two times higher acid phosphatase activity than no-till and conventionally tilled soils [173]. Higher arylsulfatase activity was noted in soils from a permanent pasture and an alfalfa field than from cultivated wheat fields [174]. Both long-term leguminous cover cropping and the direct incorporation of green manure increase the soil protease activity due to the enhancement of soil organic matter and the stimulation of soil microbial activity [175].

Much research is devoted to the influence of cropping system on enzymatic activities [33,37,100,109]. The type of cropping system may also influence soil enzyme activities. Crop rotation systems, which over time provide greater plant diversity than monoculture systems, generally have a positive effect on soil enzyme activities [176]. This effect may be due to the stimulation of microorganisms in the rhizosphere and improved physical conditions of soils in crop rotation, due to the high input and diversity of organic materials entering the soil, particularly when the rotations contain legume species, whereas, a monoculture causes the physical degradation of soil, which in turn has a negative effect on soil microbial and enzymatic activities [7,37,109]. Gajda and Martyniuk [100] found higher dehydrogenase and phosphatases activities in organic and conventional systems than in monocultures. In the study of Klose et al. [99], the total intracellular and extracellular arylsulfatase activities in the soils of Iowa (USA) were significantly affected by crop rotation and plant cover. Generally, the highest arylsulfatase activities were obtained in soil under cereal-meadow rotations (corn-soybean, corn-corn-oat-meadow) and the lowest under continuous cropping systems (corn,

ISBN:
DOI:

soybean). As stated by the authors, the results could be due to the positive effects of diversified crop rotations, improved soil structure, a nearly year-round rhizosphere and plant cover, a stabilized microclimate and higher root density. Other studies from long-term field experiments have shown that crop rotations with higher C inputs contribute to higher microbial activities in soils [e.g. 177]. Each rotation produced a different amount of residues, which have different decomposition rates and various contributions to the easily decomposable soil organic matter fraction. Decomposition rates vary among plant materials depending on their content of N, S, soluble C, lignin and carbohydrates [178].

With the development of transgenic crops, there is an increasing concern about the possible adverse effects of their vegetation and residues on the quality of the soil environment. The dehydrogenase and phosphatase activities of soil transgenic alfalfa were significantly lower than those of soil sampled from the parental alfalfa [179]. In the study of Fang et al. [180], the possible effects of the vegetation of transgenic Bt rice lines Huachi B6 (HC) and TT51 (TT) were studied followed by the effect of the return of their straw to the soil on soil enzymes, such as catalase, urease, neutral phosphatase and invertase under field conditions. The results obtained in this study indicated that the vegetation of the two transgenic rice lines and their subsequent straw amendment had few adverse effects on soil enzymes when compared with non-transgenic plants. No different pattern of impact due to plant species was found between the HC and TT rice [180]. The results were in agreement with previous studies in which some significant differences were found in the activity of some enzymes (arylsulfatases, phosphatases, dehydrogenases, urease, invertase and protease) between the soils with Bt and their near-isogenic non-Bt plants [181]. Some studies have reported that there were no significant differences in the activities of phosphatases and catalase between soil cultivated with Bt and non-Bt maize [182]. Similarly, salinity-tolerant MCM6 transgenic tobacco revealed no significant (or only minor) alterations on soil dehydrogenase and acid phosphates as compared with non-transgenic tobacco [183].

Soil Enzyme Activities as Possible Indicators of Nutrient Dynamics and Soil Fertility – Advantages and Shortcomings

Single enzymes

The increasing amount of research on soil enzymes in the 1950s delivered numerous empirical observations of soil enzyme activities with respect to amendments, cultivation practices and responses to environmental and climatic factors. It was hoped that information on extracellular enzymes would provide a suitable tool for determining the total biological activity in soil and consequently a 'fertility index' of soils that would be useful for practical purposes in agriculture [3]. Since it was stated that the determination of soil enzyme activity was more important to soil fertility assessment than its microbiological properties, in the 1950s and 1960s the use of single enzyme activities such as invertase, protease, asparaginase, urease, phosphatase and catalase was a common approach that was used in determining soil fertility [3].

As more information on enzyme activities in soils becomes available, it became more difficult to support the correlation with soil fertility and the generalization was more difficult to make [3]. Soil enzymes were investigated because of the more widely differentiated natural and anthropogenic factors and these observations produced many conflicting and confusing data indicating that the use of soil enzymatic activity as a soil fertility index was limited. More and Russel [184] criticized dehydrogenase activity as a general index of soil fertility because

ISBN:
DOI:

they did not find any useful correlation between that activity and either the soil properties that were known to influence plant growth or plant yield and also because of the poor response of dehydrogenase to the addition of nutrients to soil that was known to be of a low nutrient status.

The data on the relationship between enzymatic activity and plant productivity, which is closely related to soil fertility, has led to contradictory results as well. Some studies have shown no close relationship between soil enzyme activity and crop yields [e.g. 185], but in many other studies soil enzyme activities were often considered to be the index of soil fertility since they correlated significantly with plant yield [e.g. 186-188]. However, in managed systems this kind of correlation is questionable because other factors may disturb the relationship between enzyme activity and plant productivity. This is likely to be true for agro-ecosystems in which the external input of nutrients and water can greatly increase plant growth and development without a corresponding response in soil microbial and enzymatic activity [10]. The study of Yaroschevich [189] showed that manure-amended soil increased soil enzymatic activity while inorganic fertilization decreased this activity. Crop yields, however, were the same when adequate nutrients were supplied from either inorganic or organic sources. This indicated that the activity of some enzymes is more closely related to plant production under native conditions and highly disturbed landscapes [86,190] than in managed agricultural systems [189]. In the study of de Castro Lopes et al. [188], the enzymatic activities were, among other microbial properties, interpreted as a function of the relative cumulative yields (RCYs) of corn and soybean using linear regression models. Adequacy classes for each enzyme as a function of the RCY were established based on the following criteria – a value of an enzyme that was higher than the relative cumulative yield (RCY) of 80% corresponds to the production of maximum economic efficiency. The values of the enzymes that corresponded to an RCY between 41% and 80% were classified as moderate and values that corresponded to an RCY of ≤ 40% were classified as low. The interpretative classes for enzymatic activities in a clayey Red Latosol of the Cerrado region as a function of the RCY are presented in Table 4.

Enzyme indicator	Classes as a function of RCY		
	Low	Moderate	Adequate
Cellulase activity (mg glucose kg^{-1} soil d^{-1})	≤ 70	71-105	>105
β-glucosidase (mg glucose kg^{-1} soil d^{-1})	≤ 65	66-115	>115
Acid phosphatas (mg glucose kg^{-1} soil d^{-1})	≤ 680	681-1160	>1160
Arylsulfatase (mg glucose kg^{-1} soil d^{-1})	≤ 40	41-90	>90

Table 4: Interpretative classes for enzymatic indicators in a clayey red Latosol of the Cerrado region (Brazil) as function of the relative cumulative yield (RCY). Adopted from de Castro-Lopes et al. [188].

Despite its many drawbacks, the use of soil enzymes as a soil fertility index still remains an elusive goal and it has not been abandoned [e.g. 191-194]. There has been growing interest in the application of soil enzymes as early indicators of changes in soil fertility under contrasting agricultural management practices [195, Chapter 1 of this book]. According to Gil-Sotres et al. [193], dehydrogenase, FDA hydrolyzing capacity, urease and phosphatase were the enzymes that are most often used as soil fertility indicators. Masciandaro et al. [196] proposed an index expressed as the ratio between dehydrogenase activity and the water-soluble C content. This ratio was initially used to get quantitative information about soil degradation that is caused by intensive soil use [196]. Later, this index was used by several authors to assess the effects of different crops or management practices on soil fertility [60,197]. Perucci [70] proposed the Hydrolyzing Coefficient as the quantity of fluorescein diacetate that is hydrolyzed after incubation divided by the total quantity before the hydrolysis. This index was mainly used to

ISBN:
DOI:

test the activity of a soil that was amended with compost, although the author did not establish the minimal level for this ratio [194]. Two hydrolases, namely, urease and phosphates, have been widely used in the evaluation of changes in soil fertility due to soil management. Their activity increased due to organic fertilization [198,199] and after the addition of cattle slurry to the soil and decreased as a consequence of plowing [60]. Because both practices are normally carried out at the same time in agricultural soils, it is clear that the use of urease as the fertility index is limited [193]. Soil phosphatase activity is widely used as an indicator of inorganic P availability for plants and microorganisms [71] and is also considered to be a good index of the quality and quantity of organic matter in soils and can be very high in arable soil as long as the content of organic matter is maintained [200] Among the enzymes that are involved in the carbon cycle, b-glucosidase has been the most widely used in the evaluation of soil quality and fertility indicators in soil that has been subjected to different management practices. b-glucosidase activity was significantly lower in arable soils than in woodland and meadow soils [60,69]. Some agricultural practices, such as organic fertilization can increase the activity of this enzyme [69,198].

As was mentioned earlier, the use of individual properties such as soil fertility indicators has led to conflicting and confusing data and conclusions, not only because the adopted methodologies were sometimes questionable, but also because it is conceptually incorrect to use a single enzyme activity to determine plant productivity or soil fertility, what has been widely criticized [e.g. 3,132], and summarized by Nannipieri [75] and more recently by Nannipieri et al. [12]:

1. Soil enzyme activities catalyze a specific reaction and therefore, they cannot be related to the overall soil microbiological activity, which includes a broad range of different enzymatic reactions. The synthesis of a particular enzyme can be repressed by a specific compound, while the overall microbiological activity of soil or crop productivity is not affected.

2. Since a given enzyme is substrate specific, it cannot reflect the total nutrient status of the soil. However, an individual soil enzyme may answer questions regarding a specific decomposition process in the soil or questions about specific nutrient cycles. For example, cellulase complexes and other carbohydrates might indicate the decomposition rate of plant materials, whereas urease is important agriculturally as an enzyme that might limit the content of nitrogen that is available to plants from fertilizers or natural sources [3]. The urease activity is often considered to be an indicator of organic N mineralization although the enzyme is involved in urea hydrolysis and urea is not an important component of soil organic nitrogen, particularly when urea is not used as a fertilizer. An enzyme activity that is frequently used as indicator of C mineralization is b-glucosidase. Also in this case, as was stated by Nannipieri et al. [12], the assumption is conceptually incorrect because the mineralization of plant residues involves the mineralization of cellulose and lignin, which are the main components of plant residues. b-glucosidase is only one of the enzyme complexes that are responsible for cellulose degradation.

3. Soil enzyme activities show a high degree of spatio-temporal variability that is due to climate, season, geographical location and pedogenetic factors. Usually, enzyme activities fluctuate with the seasons, decreasing in summer and winter with moisture and temperature, respectively, being the limiting factors for their activities.

4. It is difficult to compare the data obtained in one experiment with those from another because they are usually obtained using different protocols, either because there are no standardized methods or because the soil samples have been subjected to different pretreatments (sample collection and storage) prior to the analysis [193].

ISBN:
DOI:

5. Some chemical compounds in soil can inhibit or activate the synthesis and the activity of an individual enzyme without having any influence on total microbial activity.

6. The overall activity of any single enzyme in soil depends on enzymes in different locations including extracellular enzymes that are immobilized by soil colloids. The activity of immobilized enzymes may not be as sensitive to environmental factors as are those directly associated with microbial activity. On the other hand, this could be an advantage since bound enzymes are less variable, for example in the vegetation period.

These methodological problems along with the inherent complexity of soil systems indicate that no estimation of soil fertility using simple indicators can be considered to be reliable. Efforts to use soil biochemical properties as indicators of soil fertility should be focused on the search for complex expressions that are capable of describing the complexity of soil much more accurately [193].

Simple and complex indicators

Due to the complexity of soil structure and function, a good soil quality and fertility indicator must be the integrative combination of a number of measurements into an easily understood and quantitative measure [195]. Moreover, since soil enzymes differ in origin, function and location in soil and respond differently to environmental factors, it would be useful to condense the information they give into one single numerical value. That is why, complex indicators, which are calculated with algebraic operations of different soil biochemical properties [11,193] or multivariate analysis such as Principal Component Analysis (PCA) and factorial analysis [201,202] are frequently used. This approach might better reflect both the release of nutrients during organic matter decomposition and the relative availability of inorganic nutrients compared to the activity of a single enzyme.

A few attempts have been made to integrate different enzyme activities in single and complex indexes that can be used in the assessment of agricultural soils. The biological index of soil fertility (BIF) [203] and Enzyme Activity Number [204] was proposed and widely discussed earlier. Both indexes have been tested by other authors to show the effect of soil management on quality [60,70,75,197]. A serious limitation to the use of EAN as an index of microbiological activity is that the alkaline phosphate assay can be used only in neutral or alkaline soils [75]. In the study of Perucci [70] using municipal refuse in loamy soil, the EAN index was correlated with amylase, arylsulphatase, deaminase, dehydrogenase, alkaline phosphatase and protease activity but not with catalase and phosphodiesterase activity. The BIF was only correlated with catalase activity. Thus, it appears that EAN can give a more realistic indication of the microbial activities of soil than BIF. Saviozzi et al. [60] observed lower values of these indexes for cultivated soils than for other soils (Table 5). Similarly, Riffaldi et al. [197] observed an increase in EAN and BIF in untilled management systems compared to tilled systems in southeastern Sicily (Italy). According to Nannipieri [75] the indexes used by Stefanic et al. [203] and Beck [204] was not as sensitive as those that are currently used to determine enzyme activities in soil.

Type of management	BIF	EAN
C	1.2 (0.08)	1.1 (0.10)
G	10.2 (1.00)	4.1 (0.33)
F	8.8 (0.97)	3.3 (0.27)

Table 5: Empirical indexes for comparing quality of cultivated (C) and adjacent native grassland (G) and forest (F) soils. Confidence limits (P=0.05) are reported in brackets. Adopted from Saviozzi et al. [60].

BIF: biological index of soil fertility, EAN: enzyme activity number

ISBN:
DOI:

A more accurate and focused selection of enzyme activities was carried out by Sinsabaugh et al. [205] who measured six enzyme activities that work in a cascade in the degradation of lignocelluloses material, which is quantitatively the most important component of plant debris. Using principal component analysis (PCA), Sinsabaugh et al. [205] obtained the lignocelluloses factor (LF), which was calculated using the following enzyme activities: β-1,4-glucosidase, β-1,4-endoglucanase or endocellulase, β-1,4-exoglucanase, β-xylosidase, phenolxidase and peroxidase activities. The LF factor was significantly correlated with the percentage of weight-loss of the plant remains over time. The cascade of enzyme activities approach (i.e. the LF factor) has been considered by some authors to be one of the best among those using biochemical properties as indicators of soil quality due to its accurate and focused selection of enzyme activities [11]. The only criticism that could be made about this approach is that it only considers the enzymes that are involved in the C cycle, and not those of the N, P or S transformation. That is why the factor can be a good indicator of a soil's capacity to degrade lignocellulosic material, but not as an indicator of the global capacity of the soil to degrade organic compounds.

A similar approach was that of Monreal and Bergstrom [206], who obtained a decomposition factor that was able to explain that 96% of the variation in soil enzymatic activity was due to the cropping system and tillage using PCA and that it was mainly caused by changes in b-glucosidase, dehydrogenase and L-glutaminase activities. Fioretto et al. [207], who studied decomposition factors, preferred to consider only the determination of b-amylase and b-amylase among all of the enzymes that are implicated in the latter decomposition process.

Other indicators that consist exclusively of enzyme activities were those proposed by Puglisi et al. [191]. The authors proposed three indexes of soil alteration using different enzymatic activities to establish an index of soil degradation that was the result agricultural practices, including crop density and the application of organic fertilizers in different parts of Italy. These indexes were developed using a data reduction technique (canonical discriminant analysis, CDA). The first index (AI 1) was developed by considering seven enzyme activities (arylsulfatase, β-glucosidase, phosphatase, urease, invertase, dehydrogenase and phenoloxidase). The second index (AI 2) was constructed with β-glucosidase, phosphatase, urease and invertase activities that had been automatically selected by CDA as the most capable of discriminating between altered and non-altered soils. Finally, the third index (AI 3) was developed by considering the enzyme activities that were most studied (β-glucosidase, phosphatase and urease) according to the bibliography and it was tested on several published data sets. The Al 3 index was able to discriminate soils that had been subjected to irrigation with brackish waste, intensive agriculture, contamination by a tannery, landfill effluents and heavy metals [191].

The above-presented indexes, which are based exclusively on enzymatic activities, do not take into account the content of the main nutrients in soil, and thus its nutrient-supplying capacity, which is important for plant growth. That is why Kang et al. [192] proposed a trigonometric approach that is based on three sub-indexes (a nutrient index, a microbial index of the soil and a crop-related index) to establish a Sustainability Index in soil under wheat that had been amended with manures in Punjab (India), and noted that the quality increased with the amendment. The microbial index (MI_i) was based on the microbial biomass C and N, potentially mineralizable N, soil respiration, bacterial population, mycorrhizal infection of corn roots and finally dehydrogenase and phosphatase activities. In one of the experiments that were carried out by authors, the treatment with farmyard manure (100% NPK+FYM), which had been added to the soil for 29 years, was the most sustainable for the corn-wheat system. Conversely, the 100% NPK treatment was unsustainable for the same cropping system. The

ISBN:
DOI:

lack of sustainability of the inorganic fertilizer-treated plots was due to the low microbial (0.91) and crop (0.71) indexes. In contrast, the application of FYM gave higher nutrient (1.25), microbial (1.22) and crop (1.66) indexes than the application of inorganic fertilizers, thus making the system more sustainable (sustainability index of 2.43).

The following equation, which was developed by Paz-Ferreiro et al. [208] for native grassland soils in Galicia, was used to assess the biochemical equilibrium of different grassland soils under contrasting management systems [organic slurry (25 kg N ha^{-1} and 8 kg P ha^{-1}) and inorganic fertilizer (80 kg N ha^{-1} and 20 kg P ha^{-1})] [209]:

Total carbon (%)=0.764+(2.304 x 10^{-3} microbial biomass C – expressed as mg kg^{-1})+(0.936 catalase activity – expressed as mM H_2O_2 g^{-1} h^{-1})+(0.017 urease activity – expressed as mM $N\text{-}NH_4^+$ g^{-1} h^{-1})+(0.206 phosphomonoesterase activity – expressed as mM pNP g^{-1} h^{-1}).

The biochemical equilibrium of the soil was estimated by comparing the total C content, which was measured using the dichromate oxidation method (Cr), with the total C content as determined from the equation (Ct). Theoretically, the value of the Ct/Cr ratio in soils in the biochemical equilibrium should be 100. In the study of Paz-Fereiro et al. [209], the Ct/C index varied widely between 40 and 180 and the values mainly depended on the soil management system and revealed that the unmanaged grassland was in biochemical equilibrium throughout the study period, while no such balance was observed in the managed grassland.

The geometric mean of the assayed enzyme activities (GMea) was used as an index of soil quality in order to compare 18 pairs of organic and neighboring conventional olive orchards in southern Spain [195]. The GMea was calculated as the geometric mean of the enzymes tested as:

$$GMea=\sqrt[6]{AcP\times AIP\times Glu\times Ary\times DEhy\times PN}$$

Where, AcP, AlP, Glu, Ary, Dehy and PN are acid phosphatase, alkaline phosphatase, b-glucosidase, arylsulphatase and dehydrogenase activities and potential nitrification rate, respectively. The results of García-Ruiz et al. [195] showed that the soil GMea index was able to discriminate between organic and conventional management practices at eight of the 18 sites (Figure 4). Relationship between the increase of the GMea in relation to years since accreditation of organic farms and tillage intensity was shown on Figure 5. The calculation of this index was based on soil enzymes except for the potential nitrification rate and specific physical and chemical properties of the soil were not included.

ISBN:
DOI:

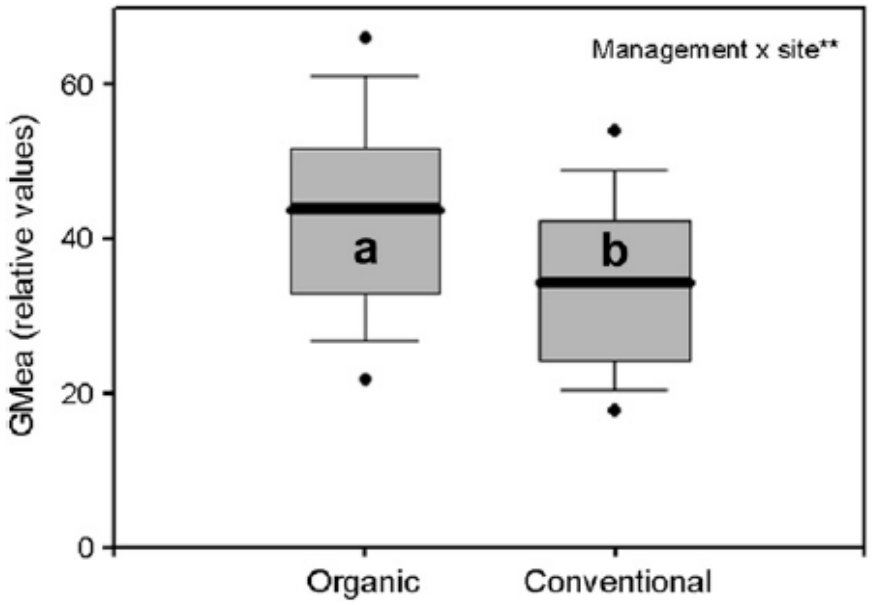

Figure 4: Box-plot representation of the geometric mean of the assayed enzyme (GMea) activities in the soils of the organic and conventional olive oil farms. Boundaries of the boxes closest to, and furthest from zero indicate the 25th and 75th percentiles, respectively. The thin and thick lines within the box mark the median and average, respectively. Bars above and below the box indicate the 90th and 10th percentiles, respectively. Outliers are represented as black dots. Average values with the same letter in each figure indicate no significantly differences between management types ($P<0.05$). Probability of the effect of interaction ''management practices x site'' on GMea is indicated and was lower than 0.01 (**). Adopted from García-Ruiz et al. [195].

ISBN:
DOI:

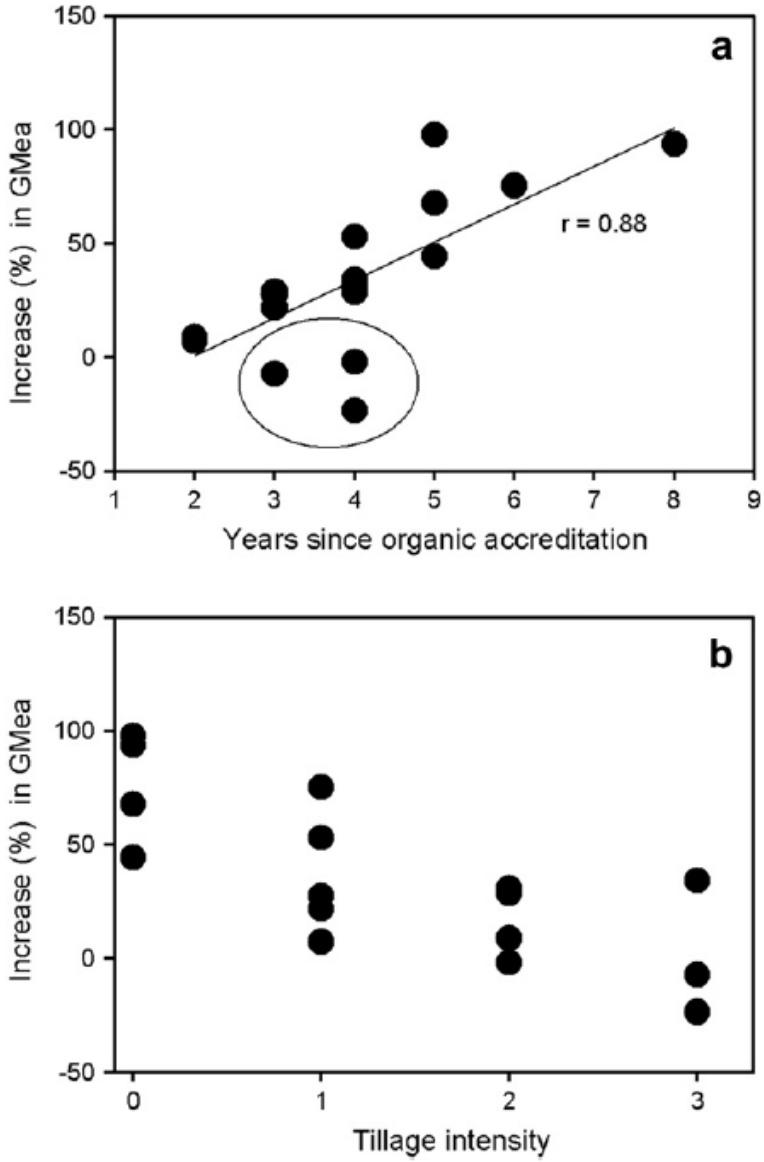

Figure 5: Relationship between the increase in the GMea in the organic relative to the comparable conventional farms and (a) years since organic accreditation of the organic farms, and (b) tillage intensity. Farms within the circle in (a) were excluded or the calculation of the coefficient of correlation because of the high tillage intensity. The coefficient of correlation was significant at $P<0.01$. Adopted from García-Ruiz et al. [195].

ISBN:
DOI:

In conclusion, the use of complex expressions in which biochemical (enzymatic) along with various chemical properties seems to be a promising direction in developing a universal fertility index. The inclusion of different properties makes it possible to better reflect the complexity of a soil system, at least for the condition in which they have been designed [193]. The main problem with the currently available indicators is that they usually have not been tested in locations or under conditions other than those for which they were developed. As a result, they can be applied only on a regional but not on a global scale [194]. According to Burns et al. [210], the development of a soil enzyme index that can be used as a reliable measure of soil fertility is one of the key research priorities in soil enzymology today. One absolute indicator for the evaluation of soil fertility under different soil management systems and under various climatic conditions and geographic regions is difficult to develop due to the intrinsic variability of biological properties and several site-specific factors that affect soil enzyme activity. The attempt to develop a global indicator of soil fertility should be undertaken at the international level, taking into account some important site-specific factors for the study area, such as climatic parameters, different soil types and vegetation cover.

Conclusions and Future Challenges

The sustainability of agricultural systems has recently become an important issue all over the world. Many issues of sustainability are related to soil fertility and its changes over time. The activities of soil biological properties have been proposed as one of the important indicators of soil fertility. Among these biological properties, soil enzymes have been suggested as potential indicators of soil fertility due to the fact that they are involved in the cycling of the most important nutrients and that they correlate well with nutrient availability. Extracellular enzymes, especially, often catalyze the rate-limiting step of decomposition and nutrient cycling, thus making their expression and kinetics potential useful parameters for nutrient turnover models. Moreover, soil enzymes quickly respond to changes in soil management when compared with other biological and physicochemical properties and are simple to measure. Therefore, in most of the papers cited here, it was stated that soil enzymes have a great value as early and sensitive indicators of soil fertility that are induced by different agricultural management systems. Due to their sensitivity and capacity to provide information that integrates many environmental factors, enzymes are useful tools to assess the effect of farming practices on the capacity of the land to remain productive and on soil fertility. In fact, enzymes provide early warnings of a system's collapse and allow us to react before irreversible damage is done to the integrity and functioning of the soil ecosystem.

The bibliography cited in this review, especially those that date from last ten years, has indicated that the interest in enzyme measurements due to their possible use as soil fertility indicators is still high. Many studies have analyzed soil fertility in agricultural soils using individual enzyme activities, which was highly criticized because single enzyme activities cannot represent the rate of all of the metabolic processes unless they usually catalyze one specific reaction. That is why it has been proposed that several enzyme activities be measured, sometimes along with other biological or chemical properties and that they are integrated in an index [191,192,195,205,206,208] that better reflects all of the soil metabolic processes. In addition, the currently available indexes have not been widely used on a large scale, even in similar types of soils and under similar climatic condition and management strategies. The shortcomings of the use of soil fertility indicators in order to compare the results obtained in different researches are due to the lack of a standard methodology that is used in all laboratories, which is as a fundamental problem when interpreting the results; differences in

ISBN:
DOI:

sample collection, storage and the pre-treatment of soil samples and finally, the high degree of variability in the data because it is affected by seasonal and edaphic factors. Additionally, there is the lack of reference values or a broad database for high-quality soils that could be used to make comparisons [193]. The advantages and disadvantages of the existing indicators suggest that a good soil fertility index should be sensitive enough to the presence of the greatest possible number of management practices, but that it should not be too sensitive to seasonal and among-sites variation, since this could mask any changes that are caused by different management strategies. Secondly, a good indicator should display consistency in the direction of the change that has been undergone in response to a given factor and should clearly reflect the gradual increase or decrease in the level of a given factor. Apart from this, it should be easily interpretable and easy and inexpensive to obtain [193].

Soil enzymes are not only used as indicators of soil status that is due to diverse agricultural practices. One of the key research priorities in soil enzymology is to develop methods of manipulating soil extracellular enzymes for ecosystem services, e.g., in agriculture to enhance the plant nutrient content or to control pathogens and pests [210]. Another option is the application of exogenous enzymes directly into soil in order to regulate the rate and direction of certain soil processes. For example, the application of exogenous cellulase and protease was used effectively to accelerate straw decomposition in soil (reference). Immobilized carbonic anhydrases are being tested for their feasibility for sequestering CO_2 into bicarbonate [211], whereas purified phytase improved maize seedling growth when P was supplied in the form of phytase [212]. The problem is the loss of enzyme activity over time, which may occur when enzymes are immobilized by or entrapped within clays, organic matter or organo-mineral complexes [213] or degraded by soil proteases. This is an economic issue due to the high costs of producing the exogenous enzymes for the applications may be unprofitable unless the enzyme has a prolonged activity.

Although molecular properties have not been included in soil quality and fertility indicators, the development of genomic, transcriptomic or proteomic methodologies could be important in the evaluation of such indicators. These methods could provide information about what the role of specific microorganisms and their enzymes are in the key processes that are related to soil functionality [194]. Since the soil is complex and dynamic biological system, it is difficult to determine which microbial genotypes are responsible for the production of certain enzymes. It is necessary to understand the relationship between genetic diversity and community structure and function [214] and because all extracellular enzymes have corresponding genes, they represent an ideal model system for linking microbial identity to specific and critical ecosystem processes. That is why, advances in proteomics, metabolomics and transcriptomics are of great potential [210]. The metagenomic approach can reveal potential gene coding for an enzyme catalyzing targeted reaction, while only transcriptomics and proteomics can assess the actual levels of enzyme expression and indicate which enzyme can be used as an ecological soil indicator [12].

References

1. Baldrian P (2009) Microbial enzyme-catalyzed processes in soils and their analysis. Plant Soil Environ 55: 370-378.
2. Baležentienė L (2012) Hydrolases related to C and N cycles and soil fertility amendment: responses to different management styles and agro-ecosystems. Pol J Environ Stud 21(5): 1153-1159.
3. Skujins J (1978) History of abiontic soil enzymes research: Soil enzymes. (1stedn), Academic Press, New York, USA.
4. Burns RG (1978) Enzyme activity in soil. In: Some theoretical and practical considerations. In: Soil Enzymes. (1stedn), Academic Press, New York, USA.

ISBN:
DOI:

5. Burns RG (1983) Extracellular enzyme-substrate interactions in soil. In: Microbes in Their Natural Environment. (1stedn), Cambridge University Press, London, UK.

6. Gianfreda L, Bollag JM (1996) Influence of natural and anthropogenic factors on enzyme activity in soil. In: Soil Biochemistry. (1stedn), Marcel Dekker Inc., New York, Basel, Hong Kong.

7. Gianfreda L, Ruggiero P (2006) Enzyme Activities in Soil. In: Nucleic Acids and Proteins in Soil. (1stedn), Springer-Verlag, Berlin, Heidelberg, Germany.

8. Nannipieri P, Gianfreda L (1998) Kinetics of enzyme reactions in soil environments. In: Environmental particles – structure and surface reactions of soil particles. John Wiley, Chichester.

9. Nannipieri P, Sequi P, Fusi P (1996) Humus and enzyme activity. In: Humic substances in terrestrial ecosystems Elsevier Science, London.

10. Dick RP (1997) Soil enzyme activities as integrative indicators of soil health. In: Biological indicators of soil health (1stedn), CAB International, New York, USA.

11. Nannipieri P, Kandeler E, Ruggiero P (2002) Enzyme activities and microbiological and biochemical processes in soil. In: Enzymes in the environment. Activity, ecology and application. (1stedn), Marcel Dekker, New York. USA.

12. Nannipieri P, Giagnoni L, Renella G, Puglisi E, Ceccanti B, et al. (2012) Soil enzymology: classical and molecular approaches. Biol Fertil Soils 48: 743-762.

13. Doran JW, Parkin TB (1994) Defining and assessing soil quality. In: Defining soil quality for a sustainable environment. Soil Sci Soc Am, Special Publication 35, SSSA-ASA, Madison, WI.

14. Foth HD, Ellis BG (1988) Soil fertility and plant nutrition: Soil fertility. Wiley & Sons, New York.

15. Ştefanic G, Gheorghiţă N (2006) Soil fertility or soil quality? Rom Agric Res 23: 57-63.

16. Feller C, Blanchart E, Bernoux M, Lal R, Manley R (2012) Soil fertility concepts over the two past centuries: the importance attributed to soil organic matter in developed and developing countries. Arch Agron Soil Sci 58(1): 3-21.

17. Waksman SA (1931) Principles of soil microbiology. (2ndedn), Bailliére, Tindall and Cox, London.

18. Howard A (1940) Testament agricole - pour une agriculture naturelle. (1stedn), Oxford University Press, London, UK.

19. Ştefanic G (1994) Biological definition, quantifying method and agricultural interpretation of soil fertility. Rom Agric Res 2: 107-116.

20. Persson J, Otabbong E (1994) Fertility of cultivated soils. In: Soil fertility and regulating factors. Report 4337, Swedish EPA, Stokholm.

21. Abbott LK, Murphy DV (2003) What is soil biological fertility? In: Soil biological fertility – a key to sustainable land use in agriculture. Kluwer Academic Publisher, The Netherlands.

22. Svirskiené A (2003) Microbiological and biochemical indicators of anthropogenic impact of soils. Eurasian Soil Sci 36: 192-200.

23. Anderson TH, Domsch KH (1990) Application of eco-physiological quotients (qCO_2 and qD) on microbial biomasses from soil of different cropping histories. Soil Biol Biochem 22: 251-255.

24. Verstraete W, Mertens B (2004) Integrative approaches in soil biology. The key role of soil microbes. In: Vital Soil-Function, Value and Properties. Developments in Soil Science 29. Elsevier, Amsterdam.

25. Shi W (2011) Agricultural and ecological significance of soil enzymes: soil carbon sequestration and nutrient cycling. In: Soil enzymology. (1stedn), Springer-Verlag Berlin Heidelberg, Germany.

26. Finzi AC, Sinsabaugh RL, Long TM, Osgood MP (2006) Microbial community responses to atmospheric carbon dioxide enrichment in a warm-temperate forest. Ecosystems 9: 215-226.

27. Rodríguez-Kábana R, Truelove B (1982) Effects of crop rotation and fertilization on catalase activity in a soil of the southeastern United States. Plant Soil 69: 97-104.

28. Uzun N, Uyanöz R (2011) Determination of urease and catalase activities and CO_2 respiration in different soils obtained from Konya, Turkey. Trends Soil Sci Plant Nutr 2(1): 1-6.

29. Sinsabaugh RL (2010) Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol Biochem 42: 391-404.

30. Bach CE, Warnock DD, Van Horn DJ, Weintraub MN, Sinsabaugh RL et al. (2013) Measuring phenol oxidase and peroxidase activities with pyrogallol, L-DOPA, and ABTS: effect of assay conditions and soil type. Soil Biol Biochem 67: 183-191.

31. Egamberdieva D, Kucharova Z (2008) Cropping effects on microbial population and nitrogenase activity in saline arid soil. Turk J Biol 32: 85-90.

ISBN:
DOI:

32. Tabatabai MA, Singh BB (1976) Rhodanese activity of soils. Soil Sci Soc Am J 40: 381-385.

33. Szajdak L (1996) Impact of crop rotation and phonological periods on rhodanese activity and free sulfuric amino acids concentrations in soils under continuous rye cropping and crop rotation. Environ Internat 22(5): 563-569.

34. Browman MG, Tabatabai MA (1978) Phosphodiesterase activity of soils. Soil Sci Soc Am J 42(2): 284-290.

35. Nannipieri P, Giagnoni L, Landi L, Renella G (2011) Role of phosphatase enzymes in soil. In: Phosphorus in action, Springer-Verlag, Berlin Heidelberg.

36. Dick RP, Tabatabai MA (1978) Inorganic pyrophosphatase activity of soils. Soil Biol Biochem 10: 58-65.

37. Dodor DE, Tabatabai MA (2003) Aminohydrolases in soil as affected by cropping systems. Appl Soil Ecol 24: 73-90.

38. Bhattacharyya P, Chakrabarti K, Tripathy S, Chakrabarti A, Kim K, et al. (2007) L-asparaginase and L-glutaminase activities in submerged rice soil amended with municipal solid waste compost and decomposed cow manure. J Environ Sci Health B 42(5): 593-598.

39. Zablatowicz RM, Hoagland RE, Wagner SC (1998) 2-Nitroacetanilide as substrate for determination of aryl acylamidase activity in soils. Soil Biol Biochem 30: 679-686.

40. Acosta-Martínez V, Tabatabai MA (2001) Tillage and residue management effects on arylamidase activity in soils. Biol Fertil Soils 43: 21-24.

41. Dodor DE, Tabatabai MA (2007) Arylamidase activity as an index of nitrogen mineralization in soils. Commun Soil Sci Plant Nutr 38(15-16): 2197-2207.

42. Ross DJ (1975) Studies on climosequence of soils in tussock grassland. Invertase and amylase activities of topsoil and their relationship with other properties. New Zeal J Sci 18: 511-518.

43. Stemmer M, Gerzabek MH, Kandeler E (1998) Invertase and xylanase activity of bulk soil and particle-size fraction during maize straw decomposition. Soil Biol Biochem 31: 9-19.

44. Rodríguez-Kábana R (1982) The effects of crop rotation and fertilization on soil xylanase activity in a soil of the southeastern United States. Plant Soil 64: 237-247.

45. Adam G, Duncan H (2001) Development of sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. Soil Biol Biochem 33: 943-951.

46. Sánchez-Monedero MA, Mondini C, Cayuela ML, Roig A, Contin M, et al. (2008) Fluorescein diacetate hydrolysis, respiration and microbial biomass in freshly amnded soils. Biol Fertil Soils 44: 885-890.

47. Lenhard G (1956) The dehydrogenase activity in soil as a measure of the activity of soil microorganisms. Z. Pflanzenernaehr Dueng Bodenkd 73: 1-11.

48. Gu Y, Wang P, Kong C (2009) Urease, invertase, dehydrogenase and polyphenol activities in paddy soils influenced by allelophatic rice variety. Eur J Soil Biol 45: 436-441.

49. Kumar S, Chaudhuri S, Maiti SK (2013) Soil dehydrogenase activity in natural and mine soil – a review. Middle-East J Sci Res 13(7): 898-906.

50. Wolińska A, Stępniewka Z (2012) Dehydrogenase activity in the soil environment: Dehydrogenases, In Tech.

51. Knowles R (1982) Denitrification. Microbiol Rev 46: 43-70.

52. Paul EA, Clark FE (1996) Soil microbiology and biochemistry. (2ndedn), Academic Press, San Diego.

53. Rice CW, Tiedje J M (1989) Regulation of nitrate assimilation by ammonium in soils and in isolated soil microorganisms. Soil Biol Biochem 4: 597-602.

54. McCarty GW, Bremner JM (1992) Regulation of assimilatory nitrate reductase activity in soil by microbial assimilation of ammonium. Proc Natl Acad Sci USA 89: 453-456.

55. Fazekašová D (2012) Evaluation of soil quality parameters development in terms of sustainable land use: Sustainable development - authoritative and leading edge content for environmental management In Tech, Rijeka.

56. Follmer C (2008) Insights into the role and structure of plant ureases. Phytochemistry 69: 18-28.

57. Mobley HLT, Island MD, Hausinger RP (1995) Molecular biology of microbial ureases. Microbiol Rev 59: 451-480.

58. Geisseler D, Horwath WR, Joergensen RG, Ludwig B (2010) Pathways of nitrogen utilization by soil microorganisms – a review. Soil Biol Biochem 42: 2058-2067.

ISBN:
DOI:

59. Rotini OT (1935) La trasformazione enzimatica dell'urea nel terreno. Ann Labor Ric Ferm Spallanrani 3: 143-154.

60. Saviozzi A, Levi-Minzi R, Cardelli R, Riffaldi R (2001) A comparison of soil quality in adjacent cultivated, forest and native grassland soils. Plant Soil 233: 251-259.

61. Eriksson KEL, Blanchette RA, Ander P (1990) Biodegradation of cellulose. In: Microbial and enzymatic degradation of wood and wood components. Springer-Verlag, New York.

62. Deng SP, Tabatabai MA (1994) Cellulase activity of soils. Soil Biol Biochem 26: 1347-1354.

63. White AR (1982) Visualisation of cellulases and cellulose degradation. In: Cellulose and other natural polymer systems: biogenesis, structure, and degradation. (1stedn), Plenum, New York, USA.

64. Tabatabai MA (1994) Soil enzymes. In: Methods of soil analysis (part 2) Microbiological and biochemical properties. SSSA Book Series No. 5. Soil Sci Soc Am, Madison, Wisconsin.

65. Ajwa HA, Tabatabai MA (1994) Decomposition of different organic materials in soils. Biol Fertil Soils 18: 175-182.

66. Acosta-Martinez V, Mikha MM, Sistani KR, Stahlman PW, Benjamin JG, et al. (2011) Multi-location study of soil enzyme activities as affected by types and rates of manure application and tillage practices. Agriculture 1: 4-21.

67. Turner BL, Hopkins DW, Haygartha PM, Ostlec N (2002) β-glucosidase activity in pasture soils. Appl Soil Ecol 20: 157-162.

68. Acosta-Martínez V, Tabatabai MA (2000) Enzyme activities in a limed agricultural soil. Biol Fertil Soils 31: 85-91.

69. Bandick AK, Dick RP (1999) Field management effects on soil enzyme activities. Soil Biol Biochem 31:1471-1479.

70. Perucci P (1992) Enzyme activity and microbial biomass in a field soil amended with municipal refuse. Biol Feril Soils 14: 54-60.

71. Amador JA, Glucksman AM, Lyons JB, Görres JH (1997) Spatial distribution of soil phosphatase activity within a riparian forest. Soil Sci 162: 808-825.

72. Banerjee A, Sanyal S, Sen S (2012) Soil phosphatase activity of agricultural land: A possible index of soil fertility. Agric Sci Res J 2(7): 412-419.

73. Šarapatka B (2003) Phosphatase activities (ACP, ALP) in agroecosystem soils. Doctoral thesis. Swedish University of Agricultural Sciences, Uppsala.

74. Sinsabaugh RL, Antibus RK, Linkins AE, McClaugherty CA, Rayburn L, et al. (1993) Wood decomposition: nitrogen and phosphorus dynamics in relation to extracellular enzyme activity. Ecology 74(5): 1586-1593.

75. Nannipieri P (1994) The potential use of soil enzymes as indicators of productivity, sustainability and pollution. In: Soil biota: Management in Sustainable Farming Systems. CSIRO, Adelaide.

76. Juma NG, Tabatabai MA (1978) Distribution of phosphomonoesterases in soils. Soil Science 126: 101-108.

77. Olander LP, Vitousek PM (2000) Regulation of soil phosphatase and chitinase activity by N and P availability. Biogeochemistry 49: 175-190.

78. Freney JR (1986) Forms and reactions of organic sulfur compounds in soils. In: Sulfur in agriculture, Agronomy 27. American Society of Agronomy, Madison WI, USA.

79. Kertesz MA, Mirleau P (2004) The role of soil microbes in plant sulphur nutrition. J Exp Bot 55: 1939-1945.

80. Schulten RH, Schnitzer M (1998) The chemistry of soil organic nitrogen: a review. Biol Fertil Soils 26: 1-15.

81. He XT, Traina SJ, Logan TJ (1992) Chemical properties of municipal solid waste composts. J Environ Qual 21: 318-329.

82. Geisseler D, Horwath WR (2008) Regulation of extracellular protease activity in soil in response to different sources and concentration of nitrogen and carbon. Soil Biol Biochem 40: 3040-3048.

83. Loll MJ, Bollag JM (1983) Protein transformation in soil. Adv Agron 36: 351-382.

84. Vranova V, Rejsek K, Formanek P (2013) Proteolytic activity in soil : a review. Appl Soil Ecol 70: 23-32.

85. Jan TM, Roberts P, Tonheim SK, Jones DL (2009) Protein breakdown represents a major bottleneck in nitrogen cycling in grassland soils. Soil Biol Biochem 41: 2272-2282.

86. Pancholy SK, Rice EL (1972) Soil enzymes in relation to old field succession. In: Amylase, cellulase, invertase, dehydrogenase, and urease. Soil Sci Soc Am Proc 37: 47-50.

87. Thoma JA, Spradlin JE, Dygerd S (1971) Plant and animal amylases. In: The enzymes. (5th Edn), Academic, New York.

ISBN:
DOI:

88. Benitez E, Melgar R, Sainz H, Gómez M, Nogales R (2000) Enzymes activities in rhizosphere of pepper (*Capsicum annum* L) grown with olive cake mulches. Soil Biol Biochem 32: 1829-1835.

89. Mijangos I, Pérez R, Albizu I, Garbisu C (2006) Effects of fertilization and tillage on soil biological parameters. Enzyme Microbiol Technol 40: 100-106.

90. Piotrowska A, Wilczewski E (2012) Effects of catch crops cultivated for green manure and mineral nitrogen fertilization on soil enzyme activities and chemical properties. Geoderma 189-190: 72-80.

91. Garcia C, Hernandez T, Pascual JA, Moreno JL, Ros M (2000) Microbial activity in soils of SE Spain exposed to degradation and desertification processes: Research and perspectives of soil enzymology in Spain. CEBAS-CSIC, Spain.

92. Masciandaro G, Ceccanti B, Benedito S, Lee HC, Cook F (2004) Enzyme activity and C and N pools in soil following application of mulches. Can J Soil Sci 84: 19-30.

93. Zhang YL, Wang YS (2006) Soil enzyme activities with greenhouse subsurface irrigation. Pedosphere 16: 512-518.

94. Sinsabaugh RL, Gallo ME, Lauber C, Waldrop MP, Zak DR (2005) Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. Biogeochemistry 75: 201-215.

95. Benitez E, Melgar R, Nogales R (2004) Estimating soil resilience to a toxic organic waste by measuring enzyme activities. Soil Biol Biochem 36(10): 1615-1623.

96. Kizilkaya R, Bayrakli B (2005) Effects of N-enriched sewage sludge on soil enzyme activities. Appl Soil Ecol 30: 192-202.

97. Iyyemperumal K, Shi W (2008) Soil enzyme activities in two forage systems following application of different rates of swine lagoon effluent or ammonium nitrate. Appl Soil Ecol 38: 128-136.

98. Guo P, Wang C, Jia Y, Wang Q, Han G, et al. (2011) Responses of soil microbial biomass and enzymatic activities to fertilizations of mixed inorganic and organic nitrogen at a subtropical forest in East China. Plant Soil 338: 355-366.

99. Klose S, Moore JM, Tabatabai MA (1999) Arylsulfatase activity of microbial biomass in soils as affected by cropping systems. Biol Fertil Soils 29: 46-54.

100. Gajda A, Martyniuk S (2005) Microbial biomass C and N and activity of enzymes in soil under winter wheat grown in different crop management systems. Pol J Environ Stud 14: 159-163.

101. Madejon E, Moreno F, Murillo JM, Pelagrin F (2007) Soil biochemical response to long-term conservation tillage under semiarid Mediterranean conditions. Soil Till Res 94: 346-352.

102. Mina BL, Saha S, Kumar N, Srivastova AK, Gupta HS (2008) Changes in soil nutrient content and enzymatic activity under conventional and zero-tillage practices in an Indian sandy clay loam soil. Nutr Cycl Agroecosyst 82: 273-281.

103. Ulrich S, Tischer S, Hofmann B, Christen O (2010) Biological soil properties in a long-term tillage trial in Germany. J Plant Nutr Soil Sci 173: 483-489.

104. Gerzabek MH, Antil RS, Kögel-Knabner I, Knicker H, Kirchmann H (2006) How are soil use and management reflected by soil organic matter characteristics: a spectroscopic approach. Eur J Soil Sci 57: 485-494.

105. Saiya-Cork KR, Sinsabaugh RL, Zak DR (2002) The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. Soil Biol Biochem 34: 1309-1315.

106. Burket JZ, Dick RP (1998) Microbial and soil parameters in relation to N mineralization in soils of diverse genesis under differing management systems. Biol Fertil Soils 27: 430-438.

107. DeForest JL, Zak DR, Pregizer KS, Burton AJ (2004) Atmospheric nitrate deposition, microbial community composition, and enzyme activity in northern hardwood forests. Soil Sci Soc Am J 68: 132-138.

108. Giacometti C, Deyman MS, Cavani L, Marzadori C, Ciavatta C, et al. (2013) Chemical and biochemical soil quality indicators and their potential to differentiate fertilization regimes in temperate agroecosystems. Appl Soil Ecol 64: 32-48.

109. Klose S, Tabatabai MA (2000) Urease activity of microbial biomass in soils as affected by cropping systems. Biol Fertil Soils 31: 191-199.

110. Eivazi F, Bayan MR, Schmidt K (2003) Selected soil enzyme activities in the historic sanborn field as affected by long-term cropping systems. Comm Soil Sci Plant Anal 34: 2259-2275.

111. Fauci MF, Dick RP (1994) Soil microbial dynamics: short- and long-term effects of inorganic and organic nitrogen. Soil Sci Soc Am J 58: 801-806.

112. Siwik-Ziomek A, Lemanowicz J, Koper J (2013) Arylosulphatse activity and the content of total sulphur and its forms under the influence of fertilization with nitrogen and other macroelements. J Elem 3: 437-447.

ISBN:
DOI:

113. Šlimek M, Hopkins DW, Kalčik J, Picek T, Šantrůčková, et al. (1999) Biological and chemical properties of arable soils affected by long-term organic and inorganic fertilizer application. Biol Fertil Soils 29: 300-308.

114. Dick RP, Rasmunsen PE, Kerle EA (1988) Influence of long-term residue management on soil enzyme activities in relation to soil chemical properties of a fallow system. Biol Fertil Soils 6: 159-164.

115. Allison SD, Vitousek PM (2005) Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biol Biochem 37: 937-944.

116. Speir GA, Mc Gill WB (1979) Effects of phosphorus addition and energy supply on acid phosphatase production and activity in soils. Soil Biol Biochem 11: 3-8.

117. Chunderova AI, Zuberts T (1969) Phosphatase activity in dernopodzolic soils. Pochvovedenie 11: 47-53.

118. Criquet S, Braud A (2009) A comparison between short-term effects of sewage sludge compost and phosphate salt fertilizations on microbial activities involved in P turn-over of two calcareous and siliceous Mediterranean vineyard soils: Soil fertility. (1stedn), Nova Science Publisher, Inc., New York, USA.

119. Haynes RJ, Swift RS (1988) Effect of lime and phosphate additions on changes in enzyme activities, microbial biomass and levels of extractable nitrogen, sulphur and phosphorus in acid soil. Biol Fertil Soils 6(2): 153 – 158.

120. Ekenler M, Tabatabai MA (2003) Response of phosphaatses and arylsulfatase in soils to liming and tillage systems. J Plant Nutr Soil Sci 166: 281-290.

121. Dick RP, Cheng L, Wang P (2000) Soil acid and alkaline phosphatase activity as pH adjustment indicators. Soil Biol Biochem 32: 1915-1919.

122. Lemanowicz J (2013) Mineral fertilisation as a factor determining selected sorption properties of the soil against the activity of phosphatases. Plant Soil Environ 59(10): 439-445.

123. Tejada M, Gonzalez JL (2006) Crushed cotton gin compost on soil biological properties and rice yield. Eur J Agron 25: 22-29.

124. Elfstrand S, Hedlund K, Můrtensson A (2007) Soil enzyme activities, microbial community composition and function after 47 years of continuous green manuring. Appl Soil Ecol 35: 610-621.

125. Elfstrand S, Bååth B, Mårtensson A. (2007) Influence of various forms of green manure amendment on soil microbial community composition, enzyme activity and nutrient levels in leek. Appl Soil Ecol 36(1):70-82.

126. Marinari S, Masciandaro G, Ceccanti B, Grego S (2000) Influence of organic and mineral fertilizers on soil biological and physical properties. Bioresource Technol 72: 9-17.

127. Damghani AM, Savarypour G, Zand E, Deihimfard R (2008) Municipal solid waste management in Teheran: Current practices, opportunities and challenges. Waste Manage 28: 929-934.

128. Singh RP, Agrawal M (2008) Potential benefits and risk of land application of sewage sludge. Waste Manage 28: 347-358.

129. García-Gil JC, Plaza C, Soler-Rovira P, Polo A (2000) Long-term effect of municipal solid waste compost application on soil enzyme activities and microbial biomass. Soil Biol Biochem 32: 1907-1913.

130. Crecchio C, Curci M, Mininni R, Ricciuti P, Ruggiero P (2001) Short-term effects of municipal solid waste compost amendments on soil carbon and nitrogen content, some enzyme activities and genetic diversity. Biol Fertil Soils 34: 311-318.

131. Goyal S, Mishra MM, Dankar SS, Kapoor KK, Batra R (1993) Microbial biomass turnover and enzyme activities following the application of farmyard manure to field soils with and without previous long-term applications. Biol Fertil Soils 15: 60-64.

132. Nannipieri P, Ceccanti B, Grego S (1990) Ecological significance of biological activity in soil: Soil Biochemistry 6. (1stedn), Marcel Dekker, New York, USA.

133. Piotrowska A, Rao MA, Scotti R, Gianfreda L (2011) Changes in soil chemical and biochemical properties following amendment with crude and dephenolized olive mill waste water (OMW). Geoderma 161: 8-17.

134. López-Piñeiro A, Albarrán A, Rato Nutes JM, Peña D, Cabrera D (2011) Long-term impact of de-oiled two-phases olive mill waste on soil chemical properties, enzyme activities and productivity in an olive grove. Soil Till Res 114: 175-182.

135. Chen R, Blagodatskaya E, Senbayram M, Blagodatsky S, Myachina O, et al. (2012) Decomposition of biogas residues in soil and their effects on microbial growth kinetics and enzyme activities. Biomass Bioenerg 45: 221-229.

136. Hernández DL, Hobbie SE (2010) The effects of substrate composition, quantity, and diversity on microbial activity. Plant Soil 335: 397-411.

137. Perucci P, Scarponi L (1985) Effect of different treatment with crop residue on soil phosphatase activity. Biol Fertil Soils 1: 111-115.

ISBN:
DOI:

138. Frankenberger WT, Johanson JB, Nelson CO (1983) Urease activity in sewage sludge-amended soils. Soil Biol Biochem 15: 543-549.

139. Karaca A, Naseby DC, Lynch JM (2002) Effect of cadmium contamination with sewage sludge and phosphate fertilizer amendments on soil enzyme activities, microbial structure and available cadmium. Biol Fertil Soils 35: 428-434.

140. Moreno JL, Hernandez T, Garcia C (1999) Effects of cadmium contaminated sewage sludge compost on dynamics of organic matter and microbial activity in an arid soil. Biol Fertil Soils 28: 230-237.

141. Kautz T, Wirth S, Ellmer F (2004) Microbial activity in a sandy arable soil is governed by the fertilization regime. Eur J Soil Biol 40: 87-94.

142. Tejada M, Gonzalez JL, García-Martíez AM, Parrado J (2008) Effects of different green manures on soil biological properties and mainze yield. Bioresource Technol 99: 1758-1767.

143. Okur N, Kayikçioglu HH, Okur B, Delibcak S (2008) Organic amendments based on tabacco waste compost and farmyard manure: influence on soil biological properties and butter-head lettuce yield. Turk J Agric For 32: 91-99.

144. Zhao Y, Wang P, Li J, Chen Y, Ying X, et al. (2009) The effects of two organic manures on soil properties and crop yields on a temperate calcareous soil under a wheat–maize cropping system. Eur J Agron 31: 36-42.

145. Debosz K, Rasmunsen PH, Pedersen AR (1999) Temporal variations in microbial biomass C and cellulolytic enzyme activity in arable soils: effects of organic matter input. Appl Soil Ecol 13: 209-218.

146. Geisseler D, Horwath WR, Scow KM (2011) Soil moisture and plant residue addition interact in their effect on extracellular enzyme activity. Pedobiologia 54: 71-78.

147. Chaves B, DeNeve S, Hofman G, Doeckx P, Van Cleemput O (2004) Nitrogen mineralization of vegetable root residues and green manures as related to their (bio)chemical composition. Eur J Agron 21:161-170.

148. Han W, He M (2010) The application of exogenous cellulase to improve soil fertility and plant growth due to acceleration of straw decomposition. Bioresource Technol 101: 3724-3731.

149. Shindo H, Nishio T (2005) Immobilization and remineralization of N following addition of wheat straw into soil: determination of gross N transformation rates by ^{15}N-ammonium isotope dilution technique. Soil Biol Biochem 37: 425-432.

150. Fontaine S, Bardoux G, Benest D, Verdier B, Mariotti A, et al. (2004) Mechanisms of the priming effect in a savannah soil amended with cellulose. Soil Sci Am J 68: 125-131.

151. Han W, He M (2010) Short-term effects of exogenous protease application on soil fertility with rice straw decomposition. Eur J Soil Biol 46: 144-150.

152. Mayer J, Scheid S, Widmer F, Fließbach A, Oberholzer HR (2010) How effective are 'Effective microorganisms® (EM)'? Results from a field study in temperate climate. Appl Soil Ecol 46: 230-239.

153. Fatunbi AO, Ncube L (2009) Activities of Effective Microorganisms (EM) on the nutrient dynamics of different organic materials applied to soil. Am-Eur J Agron 2: 26-35.

154. Chen SK, Subler S, Edwards CA (2002) Effects of agricultural biostimulants on soil microbial activity and nitrogen dynamics. Appl Soil Ecol 19: 249-259.

155. Valarini PJ, Cruz Diaz Alvarez M, Gascó JM, Guerrero F, Tokeshi H (2002) Integrated evaluation of soil quality after the incorporation of organic matter and microorganisms. Braz J Microbiol 33: 35-40.

156. Piotrowska A, Długosz J, Zamorski R, Bogdanowicz P (2012) Changes in some biological and chemical properties of an arable soil treated with the micobial biofetilizer UGmax. Pol J Environ Stud 21(2): 455-463.

157. Rahman MH, Okuba A, Sugiyama S, Mayland HF (2008) Physical, chemical and microbiological properties of an Andisol as related to land use and tillage practice. Soil Till Res 101: 10-19.

158. Kandeler E, Tscherko D, Spiegel H (1999) Long-term monitoring of microbial biomass, N mineralization and enzyme activities of a Chernozem under different tillage management. Biol Fertil Soils 28: 343-351.

159. Curci M, Pizzigallo MDR, Crecchio C, Mininni R (1997) Effects of conventional tillage on biochemical properties of soils. Biol Fertil Soils 25: 1-6.

160. Dick RP (1994) Soil enzyme activities as indicators of soil quality. In: Defining soil quality for a sustainable environment. Soil Science Society of America, Special Publication, 35, Madison, Wisconsin.

161. Roldán A, Caravaca F, Hernández MT, García C, Sánchez-Brito et al. (2003) No-tillage, crop residue addition, and legume cover cropping effects on soil quality characteristics under maize in Patzcuaro watershed (Mexico). Soil Till Res 72: 65-73.

ISBN:
DOI:

162. Deng SP, Tabatabai MA (1996) Effect of tillage and residue management on enzyme activities in soils: I. Aminohydrolases. Biol Fertil Soils 22: 202-207.

163. Kandeler E, Böhm KE (1996) Temporal dynamics of microbial biomass, xylanase activity, N-mineralisation and potential nitrification in different tillage systems. Appl Soil Ecol 4: 181-191.

164. Khan AR (1996) Influence of tillage on soil aeration. J Agron Crop Sci 177: 253-259.

165. Seifert S, Shaw DR, Zablatowicz RM, Wesley RA, Kingery WL (2001) Effect of tillage on microbial characteristics and herbicide degradation in a Sharkey clay soil. Weed Sci 49: 685-693.

166. Corchran VL, Elliott LF, Lewis CE (1989) Soil microbial biomass and enzyme activity in subarctic agricultural and forest soils. Biol Fertil Soils 7: 283-288.

167. Palma RM, Arrigo NM, Saubidet MI, Conti ME (2000) Chemical and biochemical properties as potential indicators of disturbances. Biol Fertil Soils 32: 381-384.

168. Ekenler M, Tabatabai MA (2004) Arylamidase and amidohydrolases in soils as affected by liming and tillage systems. Soil Till Res 77: 157-168.

169. Juma NG, Tabatabai MA (1988) Phosphatase activity in corn and soybean roots: conditions for assay and effects of metals. Plant Soils 107: 39-47.

170. Knauff U, Schulz M, Scherer HW (2003) Arylsulfatase activity in the rhizosphere and roots of different crop species. Eur J Agron 19: 215-223.

171. Castellano SD, Dick RP (1991) Cropping and sulfur fertilization influence on sulfur transformation in soil. Soil Sci Soc Am J 54: 114-121.

172. Tarafdar JC, Chhonkar PK (1978) Status of phosphatases in the root-soil interface of leguminous and non-leguminous crops. Z Pflanzenernähr Bodenkd 141: 347-351.

173. Carpenter-Boggs L, Stahl PD, Lindstrom MJ, Schumacher TE (2003) Soil microbial properties under permanent grass, conventional tillage, and no-till management. Soil Till Res 71: 15-23.

174. Germida JJ, Wainwright M, Gupta VVSR (1992) Biochemistry of sulfur cycling in soil: Soil Biochemistry. Marcel Dekker, New York.

175. Dinesh R, Suryanarayana MA, Chaudhuri SG, Sheeja TE (2004) Long-term influence of leguminous cover crops on the biochemical properties of a sandy clay loam Fluventic Sulfaquent in a humid tropical regions of India. Soil Till Res 77: 69-77.

176. Dick RP (1984) Influence of long-term tillage and crop rotation combinations on soil enzyme activities. Soil Sci Soc Am J 48: 569-574.

177. Friedel JK, Munch JC, Fischer WR (1996) Soil microbial properties and the assessment of available soil organic matter in a halpic luvisol after several years of different cultivation and crop rotation. Soil Biol Biochem 28: 479-488.

178. Janzen HH, Kucey RMN (1988) C, N, and S mineralization of crop residues as influenced by crop species and nutrient regime. Plant Soil 106: 35-41.

179. Donegan KK, Seidler RJ, Doyle JD, Porteous LA (1999) A field study with genetically engineered alfalfa inoculated with recombinant *Sinorhizobium meliloti*: effects on the soil ecosystem. J Appl Ecol 36: 920-936.

180. Fang H, Dong B, Yan H, Tang F, Wang B, et al. (2012) Effects of vegetation of transgenic Bt rice lines and their straw amendment on soil enzymes, respiration, functional diversity and community structure of soil microorganisms under field conditions. J Environ Sci 24(7): 1259-1270.

181. Liu W, Lu HH, Wu WX, Wei QK, Chen YX, et al. (2008) Transgenic Bt rice not affect enzyme activities and microbial composition in the rhizosphere during crop development. Soil Biol Biochem 40(2): 475-486.

182. Icoz I, Stotzky G (2008) Fate and effects of insect-resistant Bt crops in soil ecosystems. Soil Biol Biochem 40(3): 559-586.

183. Chaudhry V, Dang HQ, Tran NQ, Mishra A, Chauhan PS, et al. (2012) Impact of salinity-tolerant MCM6 transgenic tabacco on soil enzymatic activities and the functional diversity of rhizosphere microbial communities. Res Microbiol 163: 511-517.

184. Moore AW, Russel JS (1972) Factors affecting dehydrogenase activity as an index of soil fertility. Plant Soil 37: 675-682.

185. Herrero O, Canet R, Albiach R, Pomares F (1998) Enzymatical activities and content of mineral nitrogen in soil after the application of two rates of different organic products. Agrochimica 42(6): 296-303.

186. Zhang YL, Chen LJ, Sun CX, Wu ZJ, Chen ZH, et al. (2010) Soil hydrolase activities and kinetic properties as affected by wheat cropping systems of Northeastern China. Plant Soil Environ 56: 526-532.

ISBN:
DOI:

187. Wang JB, Chen ZH, Chen LJ, Zhu AN, Wu ZJ (2011) Surface soil phosphorus and phosphatase activities affected by tillage and crop residue input amounts. Plant Soil Environ 57: 251-257

188. De Castro Lopes AA, Gomes de Suosa DM, Montandon Chaer G, dos Reis FB, Goedert WJ, et al. (2012) Interpretation of microbial soil indicators as function of crop yield and organic carbon. Soil Sci Soc Am J 77: 461-472.

189. Yaroschevich IV (1966) Effect of fifty years' application of fertilizers in a rotation on the biological activity of a chernozem. Agrokhimiya 6: 14-19.

190. Pancholy SK, Rice EL, Turner JA (1975) Soil factors preventing revegetation of a denuaded area near an abandoned zinc smelter in Oklahoma. J Appl Ecol 12: 337-342.

191. Puglisi E, Del Re AAM, Rao MA, Gianfreda L (2006) Development and validation of numerical indexes integrating enzyme activities of soils. Soil Biol Biochem 38: 1673-1681.

192. Kang GS, Beri V, Sidhu BS, Rupela OP (2005) A new index to assess soil quality and sustainability of wheat-based cropping systems. Biol Fertil Soils 41: 389-398.

193. Gil-Sotres F, Trasar-Cepeda C, Leirós MC, Seoane S (2005) Different approaches to evaluating soil quality using biochemical properties. Soil Biol Biochem 37: 877-887.

194. Bastida F, Zsolnay A, Hernández T, García C (2008) Past, present and future of soil quality indices: a biological perspective. Geoderma 147: 159-171.

195. García-Ruiz R, Ochoa V, Hijanosa MB, Carreira JA (2008) Suitability of enzyme activities for the monitoring of soil quality improvement in organic agricultural systems. Soil Biol Biochem 40: 2137-2145.

196. Masciandaro G, Ceccani B, Gallardo-Lancho JF (1998) Organic matter properties in cultivated versus set-aside arable soils. Agric Ecosyst Environ 67: 267-274.

197. Riffaldi R, Saviozzi A, Levi-Minzi R, Cardelli R (2002) Biochemical properties of a Mediterranean soil as affected by long-term crop management systems. Soil Till Res 67: 109-114.

198. Pascual JA, García C, Hernandez T (1999) Lasting microbiological and biochemical effects of the addition of municipal solid waste to an arid soil. Biol Fertil Soils 30: 1-6.

199. Chakrabarti K, Sarkar B, Charkraborty A, Banik P, Bagchi DK (2000) Organic recycling for soil quality conservation in a sub-tropical plateau region. J Agron Crop Sci 184: 137-142.

200. Dick RP, Sandor JA, Eash NS (1994) Soil enzyme activities after 1500 years of terrace agriculture in the Colca Valley, Perú. Agric Ecosyst Environ 50: 123-131.

201. Melero S, Porras JC, Herencia JF, Madejon E (2006) Chemical and biochemical properties in a silty loam soil under conventional and organic management. Soil Till Res 90: 162-170.

202. Shukla MK, Lal R, Ebinger M (2006) Determining soil quality indicators by factor analysis. Soil Till Res 87: 194-204.

203. Beck T (1984) Method and application domain of soil microbiological analysis at the Landesanstalt fur Bodenkultur und Pflanzenbau (LBP) in Munich for the determination of some aspects of soil fertility. Proceedings of the 5th Symposium on Soil Biology. Romanian National Society of Soil Science, Bucharest.

204. Sinsabaugh RL, Antibus RK, Linkins AE, McClaugherty CA, Rayburn L, et al. (1992) Wood decomposition over a first-order watershed: mass loss as function of lignocellulase activity. Soil Biol Biochem 24: 743-749.

205. Monreal C M, Bergstrom D W (2000) Soil enzymatic factors expressing the influence of land use, tillage system and texture on soil biochemical quality. Can J Soil Sci 80: 419-428.

206. Fioretto A, Papa S, Sorrentino G, Fuggi A (2001) Decomposition of *Cistus incanus* leaf litter in a Mediterranean maquis ecosystem: mass loss, microbial enzyme activities and nutrient changes. Soil Biol Biochem 33: 311-321.

207. Paz-Ferreiro J, Trasar-Cepeda C, Leirós MC, Seoane S, Gill-Sotres F (2007) Biochemical properties of acid soils under native grassland in a temperate humid zone. New Zeal J Agr Res 50: 537-548.

208. Paz-Ferreiro J, Trasar-Cepeda C, Leirós MC, Seoane S, Gill-Sotres F (2011) Intra-annual variation in biochemical properties and the biochemical equilibrum of different grassland soils under contrasting management and climate Biol Fertil Soils 47: 633-645.

209. Burns RG, DeForest JL, Marxsen J, Sinsabaugh RL, Stromberger ME, et al. (2013) Soil enzymes in a changing environment: Current knowledge and future directions. Soil Biol Biochem 58: 216-234.

210. Ramanan R. Kannan K, Sivanesan SD, Mudliar S, Kaur S, et al. (2009) Bio-sequestration of carbon dioxide using carbonic anhydrase enzyme purified from Citrobacter freundii. World J Microbiol Biotechnol 25: 981-987.

ISBN:
DOI:

211. Idriss EE, Makarewicz O, Farouk A, Rosner K, Greiner R, et al. (2002) Extracellular phytase activity of Bacillus amyloliquefaciens FZB45 contributes to its plant-growth-promoting effect. Microbiology 148: 2097-2109.

212. Gianfreda L, Rao MA (2004) Potential of extra cellular enzymes in remediation of polluted soils: a review. Enzyme Microbial Technol 35: 339-354.

213. Suenaga H (2011) Targeted metagenomics: a high-resolution metagenomics approach for specific gene clusters in complex microbial communities. Environ Microbiol 14: 13-22.

214. van Elsas JD, Boersma FGH (2011) A review of molecular methods to study the microbiota of soil and the mycosphere. Eur J Soil Biol 47: 77-87.

ISBN:
DOI:

Chapter: 3

Enzymes in Plant Growth

Roberto Pinton* and Nicola Tomasi

Department of Agricultural and Environmental Sciences, University of Udine, via delle Scienze 208, Udine, Italy

***Corresponding author:** Roberto Pinton, Department of Agricultural and Environmental Sciences, University of Udine, via delle Scienze 208, I-33100 Udine, Italy, Tel: +39 0432 558641; Fax: +39 0432 558603; E-mail: Roberto.pinton@uniud.it

Abstract

Living organisms, including plants, rely on metabolic processes for growth and development; enzymes play a pivotal role in growth, as they are biological catalysts of metabolic reactions. As autotroph organisms, plants absorb mainly inorganic elements from the external environment. Furthermore, converting light energy into chemical bonds, they can energize fixation of carbon dioxide into carbohydrates; these will be, in turn, used for the synthesis of all the organic molecules needed, including proteins.

In this context, mechanisms of ions uptake, carbon fixation and nitrogen assimilation, are crucial for ensuring plants with essential nutrients and building blocks for biomolecules. To accomplish these tasks plants have evolved specific enzymes with distinctive structural and operational (including regulatory) features. Among these some master enzymes can be indicated, whose function is crucial for the overall process.

In this chapter the role of plasma membrane proton pumps (PM H^+-ATPase), ATP synthase, photosynthetic carbon fixing enzymes (Rubisco) and nitrogen assimilation enzymes (Glutamine synthase) will be described, considering aspects of their function, structure and regulation.

Introduction

Plants are sessile organisms that became adapted to different environments by developing appropriate mechanisms for the use of natural resources in order to sustain growth and reproduction. Cultivated crops have been developed considering an unlimited supply of nutrients and water; nowadays limitation in nutrient availability, environmental concerns and climate change ask for the adoption of sustainable agricultural managements of cultivated land.

As autotroph organisms, plants acquire water and mineral nutrients (generally in ionic forms) from the soil, while oxygen and carbon dioxide come from the atmosphere. Generally essential elements need to be accumulated in plant tissues at concentrations higher than those found in the external media (e.g. due to soil constraints) or the concentration in the environment may be not suitable for optimal use efficiency by internal mechanisms (CO_2 vs.

ISBN:
DOI:

O_2 concentration in the air). These factors can limit plant growth and crop yield; furthermore, this situation might become even worse due to intensive agriculture and global warming. This implies a profound knowledge of mechanisms that plants evolved to acquire water and nutrients and to build up their structural and functional biomolecules; understanding how these mechanisms are regulated by internal and exogenous (environmental) factors is also crucial for the development of new efficient crops genotypes.

Nutrients' uptake from the soil solution, translocation and transport of solutes to different organs and cells within the plant are processes that need specific protein structures located at the cell plasma membranes allowing the passage of uncharged solutes and ions and, especially, capable to generate an electric potential favorable for trans membrane transport. The plasma membrane (PM) H^+-pumping ATPase has a unique role in solute transport in plant cells [1]. On the other hand, ATP used in metabolic processes needs to be previously synthesized by photophosphorylation in the chloroplast or oxidative phosphorylation in the mitochondrion via the ATP synthase proteins.

Fixation of CO_2 is the key step in the photosynthetic biomass accumulation in higher plants. This task is accomplished by Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase), a chloroplast-located enzyme that maintained highly conserved function during evolution; although partly inefficient due to the double catalytic function (carboxylase/oxygenase); this enzyme play a central role in carbon fixation even in plants that developed variants to this mechanism [2]. Ammonia assimilation into glutamine allows the formation of glutamate and further synthesis of plant amino acids. This reaction is catalyzed by glutamine synthase, an enzyme with different isoforms localized in plastids/chloroplasts and in the cytosol [3]. Structural and functional features of these enzymes will be described and discussed in relation to their role in plant growth and response to environmental conditions.

Plasma Membrane H^+-ATPase: A Master Enzyme for Plant Nutrition

Movement of uncharged solutes and ions across biological membrane is essential to ensure metabolic reactions. This movement can be a passive as well an active process and involves the operation of channels and carriers. Both passive and active transport of ions across biological membranes need that a transmembrane electric potential is generated and maintained. In plants, this task is accomplished by the plasma membrane (PM) H^+-ATPase that is an electrogenic enzyme coupling the hydrolysis of ATP to the transport of H^+ from the cytosol into the apoplast. This activity creates a transmembrane gradient of electric potential (negative inside) and pH (more acidic outside), which can be exploited in a variety of physiological processes such as the transport of nutrients and metabolites, preservation of intra- and extra-cellular p^H, cell turgor and related processes [1]. In roots, that are exposed to changing external conditions, such as p^H, concentration of nutrients and toxic elements and rhizosphere signals, the PM H^+-ATPase plays a pivotal role for mineral nutrition and the response to abiotic stress.

The plasma membrane H^+-ATPase belongs to the family of P-type ATPases, which use ATP and form a phosphorylated aspartyl intermediate during the reaction cycle. It is a single subunit protein with molecular mass of about 100 KDa containing ten trans membrane helices and four cytoplasmic domains (Figure 1): the nucleotide binding domain (N-domain), the phosphorylation domain (P-domain), the phosphatase domain (A-domain) and the regulatory domain (R-domain, consisting of the C-terminal of the protein). The N-terminal end is directly involved in controlling the pump activity state interacting with the C-terminal end [4]. The

ISBN:
DOI:

C-terminal region exerts a self-inhibiting function on the enzyme activity by binding to the large cytoplasmic domain. This inhibition is removed by binding of 14-3-3 proteins, that is dependent on the phosphorylation of the penultimate Thr residue [5-7]. The fungal phytotoxin, fusicoccin stimulates H^+ pumping by blocking the complex of 14-3-3 proteins and the PM H^+-ATPase.

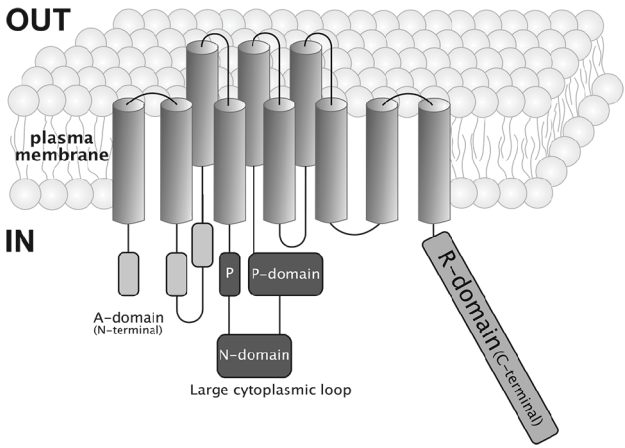

Figure 1: Structure of PM H^+-ATPase.

In several plant species, it has been found that the PM H^+-ATPase is encoded by a multigene family (about 10 genes) belonging to 5 sub-families [8]. It has been suggested that the heterogeneity of isoforms can be linked to the multicellular nature of plants and the need for a fine regulation of the enzymatic activity [9]. Analysis of expression patterns based on available micro array data shows that most of the H^+-ATPase isoforms are expressed at a relative constant level and expression level does not change when a related isoform is deleted or reduced [10]. Regulatory events involving modifications of activity, amount and gene expression have been reported in response to change in the concentration of ionic nutrients in the root external solution.

A close relationship between the activity of the PM proton pump of the root cells and transmembrane transport of anionic nutrients has been demonstrated in the case of NO_3-. Nitrate uptake is an energy-dependent proton-coupled process. Exposure of maize roots to nitrate can cause the induction of the high-affinity transport system; this behavior is paralleled by a concomitant increase in activity and amount of the PM H^+-ATPase and by the preferential expression of the genes MHA3 and MH4, belonging to sub-family II of the PM H^+-ATPase in maize [11]. Spatial and temporal expression patterns of the two genes were also observed along primary maize roots that paralleled changes in enzyme activity and anion transport rates [12]. Other isoforms of the proton pump (MHA1, belonging to sub-family I) have been suggested to be involved in nitrate transport in maize [13].

Another example of regulation of the PM H^+-ATPase has been described in response to limited amounts of phosphate. When grown at low available phosphate white lupin (*Lupinus albus*, L.) release substantial amounts of carboxylates from specialized root structure, called cluster roots, and concomitantly acidify the rhizosphere. The burst of citrate exudation is

ISBN:
DOI:

accompanied by a strong acidification of the external medium and alkalization of the cytosol. The increase in proton secretion is due to both an increased transcription level of one of the two PM H^+-ATPase genes found in white lupin (LHA1 and LHA2) and a post-translational modification of H^+-ATPase protein involving binding of activating 14-3-3 protein [14]. More recently, an RNA-Seq study showed that six H^+-ATPase transcripts exhibited Pi -dependent expression changes in white lupin roots [15].

Rhizosphere acidification mediated by the activity of PM H^+-ATPase has been reported to be also part of the response to limited iron availability in roots of dicots and non-graminaceous plants. It has been shown that under conditions of Fe deficiency, the activity and the quantity of the PM H^+-ATPase increase [16], with the enzyme, which appears to be, concentrated particularly in the rhizodermal and root hair cells of the sub-apical root area [17]. It has also been found that in cucumber plants the expression of the PM H^+-ATPase CsHA2 gene, found both in roots and leaves, was not influenced by the Fe nutritional status of the plant, while the CsHA1 gene, expressed exclusively in the roots, was up-regulated by Fe deprivation [18].

Increases in PM H^+-ATPase enzyme activity have also been observed in the roots of plants adapted to acidic soils [19] and, particularly in response to Al toxicity, a condition which often occurs in that kind of soils. It has been shown that resistant plants release a high amount of organic acid anions from the sub-apical regions of the roots and that the PM H^+-ATPase could be involved in this process [20]. The observed activation of the enzyme due to the presence of Al, has been ascribed to an increased phosphorylation of a threonine residue localized in the auto inhibitory (C-terminal) domain of the PM H^+-ATPase, suggesting an involvement of 14-3-3 proteins. However, it is worth to note that citrate exudation in P-deficient white lupin plants exposed to Al was suggested to be uncoupled from PM H^+-ATPase activity and linked to K^+ rather than to H^+ extrusion; furthermore, a poor relationship between Al-induced oxalate exudation and PM H^+-ATPase activity was reported in tomato roots [21].

There is a number of reports indicating that PM H^+-ATPase could be involved in cell elongation; this function would imply a direct action of auxin on activity and, possibly, synthesis of the proton pump or an indirect one through induced changes in cytoplasmic pH or altered ion fluxes across plasma membrane [22]. Whatever the mechanism, other signal compounds, like those present in the soil solution (e.g. humic molecules or hormone-like compounds released by rhizosphere micro-organisms) have been shown to affect enzyme activity with different mechanisms and, in some cases, acting on the amount of the protein and of gene transcripts [13,23,24]; these effects have been related to root growth [24], apoplast acidification [25] and nutrient uptake [23,26,27].

ATP Synthase: An Extraordinary Nano-Engine

To directly sustain the energy necessary for growth and metabolism, living organisms use mainly that trapped in the phosphoranhydride bound contained in ATP molecules. The majority of ATP is regenerated from ADP and inorganic phosphate (Pi) by the ATP synthase complex. In plants, ATP is mainly produced via two mechanisms: the photophosphorylation in chloroplasts and oxidative phosphorylation in mitochondria. In chloroplasts, plants use the photosynthetic reaction centers to transport protons from the stroma into the lumen and create an electrochemical potential through the membrane of the thylakoids. In mitochondria, a series of complexes present in the respiration chain pumps protons and charge the inner mitochondrial membrane. Nevertheless in both organelles, the final step is catalyzed by the same type of enzyme, the ATP synthase, which transforms the energy of the electrochemical gradient across the membrane of the thylakoid or across the internal membrane of the mitochondrion into the chemical bound contained in the ATP molecule.

ISBN:
DOI:

The structure of the ATP synthase is a complex of two main subunits, F_0 and F_1. The complex (F_0, F_1) forms a rotary engine that is able to convert the transport of protons, or sodium ions in bacteria, into chemical energy and this complex can also work in reverse mode as an H^+(or Na^+)-ATPase. F0 is embedded in the membrane and consists of at least 1 a, 1 b_2 and 10-15 c subunits; the c subunits form a ring in the membrane which spins with the passage of protons. The F_1 part is a water-soluble complex of proteins, which binds ADP and Pi, catalyze the synthesis of ATP and by conformational changes induced by the rotation of the F_0 counterpart release ATP [28,29]. Many animations illustrating the structure and the rotational mechanism of the ATP synthase can be seen on this website (Mitochondrial Biology Unit).

Due to the dual activity of the ATP synthase a complex regulation mechanism controls its activity. In mitochondria, when the availability of oxygen drops, the electrochemical gradient of protons across the inner mitochondrial membrane decreases and the ATP synthase starts to consume ATP to reestablish the gradient. Under these conditions, the ATP production is sustain by the glycolysis and the factor regulating the ATP synthase, natural inhibitor protein IF1, binds to the ATP synthase due to the drop of pH in the mitochondria matrix [30].

In chloroplast, where no IF1 protein has been identified, the ATPase activity of the ATP synthase is repressed in the dark to prevent wasteful consumption of ATP. Under these conditions, accumulation of Mg^{2+}-ADP and different modifications on the F_1 subunits, including a disulfide bound, inhibit the ATPasic activity [31-33]. Reversely when plants are exposed back to light, photosynthesis centers acidify the stroma, which induce the removal of the inhibiting ADP and the disulfide bound is reduced. Moreover the C-terminal domain of the Epsilon subunit, within the F_1 part, seems to act similarly to the auto inhibitory (C-terminal) domain of the PM H^+-ATPase (see above), inhibiting the ATP hydrolyzing activity the chloroplastic ATP synthase [33]. To sum up, the ATP synthase regulation is just starting to be revealed, but still needs further research.

Another interesting aspect about the functionality of the ATP synthase is the relative importance of the pH gradient and of the electrical potential across the membrane. It is a common believe that it is the pH gradient, which is the main driven force for the synthesis of ATP; however, it depends on the organelles considered. For example, in chloroplast, the electrical potential across the membrane is small but the pH gradient is high. In these conditions, chloroplastic ATP synthase has a large c ring (14 units) [34], as each c unit transport one proton, this enzyme needs many protons for each ATP synthetize but less electrical potential per protons. On the other hand, mitochondrial one has a smaller c ring (10) [35], thus a better proton per ATP ratio, due to the high electrical potential maintained by the respiratory chain for a similar, in comparison to chloroplast, pH gradient across the membrane. Therefore in mitochondria, the electrical potential is the main driving force and in chloroplast is the pH gradient [36].

Rubisco: An Old Enzyme for Future Challenges

Biomass accumulation and crop yield is strictly related to photosynthetic rate and efficiency. Plants have been grouped depending on the first compound that is generated upon incorporation of CO_2 in a pre-existing carbon skeleton.

C3 plants fix carbon of CO_2 into ribulose 1,5-bisphosphate (RuBP) generating primarily two molecules of 3-phosphoglycerate (PGA, a 3-carbon compound). Other variants of photosynthetic carbon assimilation are represented by C4 (the first compounds has 4 C atoms) and

ISBN:
DOI:

Crassulacean acid metabolism (CAM) plants, which rely on CO_2-concentrating mechanisms. Notwithstanding these differences Rubisco (ribulose-1,5 bisphosphate carboxylase/oxygenase) plays a central role in CO_2 fixation.

Rubisco of higher plants belongs to the form I of the enzyme, found also in algae and in most photosynthetic bacteria. It is a complex protein, with eight large subunits (four large subunit mass of about 50-52 kDa) and eight small subunits (mass of about 14-15 kDa) arranged in a L_8S_8 structure (four large subunit dimers along with eight non-catalytic small subunits capping the large ones).

The large subunits have the catalytic sites. Each subunit comprise an N-terminal domain and a larger C-terminal domain that forms a α /β -barrel; L2 dimers, formed by head-tail arrangement, have two active sites located at the L-L interface. The small subunits consist of four stranded antiparallel β-sheets with two α-helices; they are not essential for catalysis but provide structural stability to the Rubisco complex [37]. L-subunits are synthesized from the single rbcL gene of the plastid genome; nucleus-encoded factors [38], chaperones [39] and post-translational modification of N-terminal domain [40] would help avoiding misfolding and protect the newly forming protein from proteolytic degradation.

Multiple copies of the rbcS gene, coding for the S-subunit, are located in the nucleus. An N-terminal transit peptide allows transfer of the S-subunits synthesized in the cytosol through the chloroplast envelope translocon complexes into the plastid [41]. Within the stroma, the S-subunits undergo further posttranslational modification (transit peptide cleavage, Met-1 aN- methylation) prior to assembly into L_8S_8 complexes [42].

The activity of Rubisco is highly regulated. The enzyme is inactive in the dark and is converted to an active form upon illumination. Activation is mediated by several environmental and systemic factors, including temperature, pH, light, heavy metal concentrations, natural inhibitors, and by the activity of an ancillary protein: Rubisco activase [43].

Prior to catalysis, Rubisco needs to be preactivated; activation is the result of the binding of CO_2 to the 201-lysine residue near the catalytic site (position may slightly change depending on the species). The carbamate that is formed is then stabilized by Mg^{2+} binding [37]. Carbamylation changes the conformation of the large subunit activating the enzyme that can bind RuBP and catalyzes a complex five-step reaction involving a CO_2 and a water molecule before the release of two 3-phosphoglycerate (3PGA) molecules. Carbamylation is essential for Rubisco activation, as the non-carbamylated Rubisco binds RuBP too tightly to allow catalysis. The first, rate-limiting, step in carboxylation is the enolization of RuBP via the carbamate side chain; pH values lower than 8.0 may lead to the generation of Xylulose-1,5-bisphosphate that inhibit the enzyme activity (see below).

Another protein, Rubisco activase, is also involved in mediating the light activation of Rubisco. This nucleus-encoded protein uses the energy of ATP to remove active-site bound sugar-phosphate inhibitors, such as 2 carboxyarabinitol 1-phosphate (CA1P) or xyulose-1,5-bisphosphate (XuBP), d-glycero-2,3-pentodiulose-1,5-bisphosphate (PDBP) and, under some conditions, RuBP itself [44]. While XuBP and PDBP can be by-products of reaction intermediates, CA1P occurs naturally in the leaves of several plants and is a strong inhibitor of Rubisco. The affinity of Rubisco for CA1P is much stronger than that for RuBP, the substrate. As a result, CA1P, which accumulates in leaves during the night, inactivates Rubisco by blocking the binding sites. During the day (or on illumination), the bound CA1P is released from Rubisco by the concerted action of Rubisco activase and CA1P phosphatase.

The action of Rubisco activase may be crucial for maintaining Rubisco activity under low

ISBN:
DOI:

CO_2 supply and the sensitivity of Rubisco activase to high temperature might explain the decrease in Rubisco efficiency under these environmental conditions [45,46] (Figure 2).

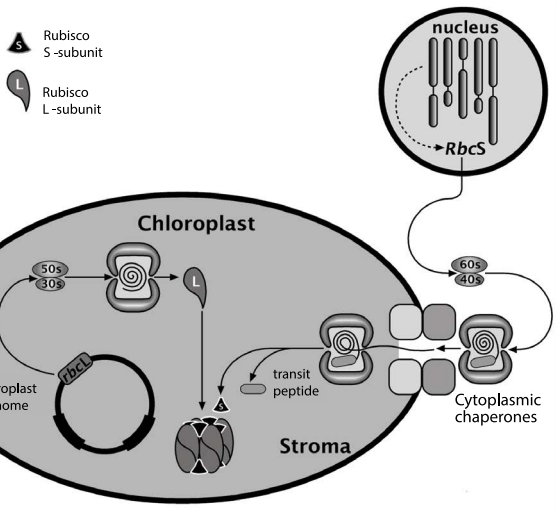

Figure 2: Assembly of large (L) and small (S) subunits of Rubisco.

Besides the carboxylation reaction, Rubisco reacts with oxygen to form one molecule of 2-phosphoglycolate and one of PGA; this reaction is the first step of the photorespiration pathway that leads to the release of previously fixed CO_2, NH_3 and energy. Due to photorespiration, C-fixing reaction has a reduced efficiency and a large amount of protein is needed to support adequate photosynthetic rates (Rubisco accounts for an average of 50% of leaf protein). Photorespiration if favoured by prolonged drought stress conditions and high temperature.

C4 plants have evolved biochemical mechanisms to elevate levels of CO_2 that rely on spatial separation of the initial fixation of atmospheric CO_2 from the Calvin cycle. Phosphoenolpyruvate carboxylase (PEPC) catalyzes CO_2 fixation to PEP in mesophyll cells producing oxaloacetate. Four-C acids (malate and aspartate) are then transported to bundle sheath cells, where they provide CO_2 to Rubisco, after undergoing decarboxylation. A pyruvate, phosphate dikinase (PPDK) catalyses the regeneration of PEP from pyruvate in mesophyll cells [47,48].

As compared to C3 plants, in C4 plants Rubisco shows lower affinity for CO_2 but higher carboxylation rates with minimal photorespiration; this would in turn lead to a higher biomass accumulation for a given amount of energy derived from sunlight. C4 plants can sustain high photosynthetic rates with a lower level of Rubisco; this implies that a lower amount of nitrogen is needed (higher nitrogen use efficiency). C4 plants can operate efficiently under low CO_2 levels, alleviating the need for wide stomata apertures, thereby reducing water loss [49].

Increasing CO_2 concentration associated with global warming might render C3 plants more efficient with respect to their Rubisco activity, although this might require increasing nitrogen supplies. Due to the low efficiency of Rubisco in C3 plants and the huge demand of Nitrogen to sustain the enzyme, strategies to improve Rubisco activity have received much attention in the last years.

ISBN:
DOI:

Indeed it has been demonstrated that a high variability exists among vascular plant with respect to the catalytic properties of Rubisco; for example plants adapted to dryer environments showed a higher selectivity between CO_2 and O2 [50]. On the other hand, it has also been noted that an increased specificity for the substrate may be accompanied by a decrease in the catalytic rate. Indeed, Rubisco of C4 plants show lower affinity than in C3 plants, as they are adapted to a relative high CO_2 concentration.

The possibility to introduce new Rubiscos in crops by conventional breeding has been exploited [2]; furthermore biotechnological approaches have been tempted to modify Rubisco content and performance [51]. Plastome transformation has evidenced that changes in L-subunit determine changes in photosynthetic rate; although not directly involved in catalysis, transformation of the nucleus-encoded S-subunits are also attractive. Another possible way to increase Rubisco efficiency is through introduction of CO_2-concentrating mechanisms (or C4-like features) in C3 plants. Bioengineering plants for improved thermal tolerance of Rubisco activase [52], and a limited abundance of naturally occurring Rubisco inhibitors [53] may represent indirect targets for improving Rubisco performance.

Glutamine Synthetase: The Eye of the Needle in Nitrogen Assimilation

Plant nitrogenous compounds, including proteins, are all virtually built up starting from glutamine that is the product of the ATP-dependent ammonia addition to a glutamate molecule. This reaction is catalyzed by glutamine synthetase (GS), the first enzyme of nitrogen assimilation (and re-assimilation), which plays a key role in plant growth and productivity as well as in nitrogen use efficiency (NUE) [54,55].

In higher plants, with the exception of conifers, the enzyme is present as plastidial (GS1) and cytolsolic (GS2) isoforms encoded by a multigene family. The presence of isoenzymes in different plant organs has been referred to specific functions [56]. Due to its abundance, this protein can be also used as nitrogen storage in plants [57].

Ammonia for GS activity derives from a wide variety of primary and secondary metabolic processes, including nitrate and ammonium uptake, N2 fixation, photorespiration, protein and amino acid catabolism and phenylpropanoid biosynthetic pathway [58]. This implies that different isoenzymes are involved in production of organic nitrogen in source tissues and the subsequent N assimilation in sink tissues.

Plant GS has been categorized as the type II commonly occurring in prokaryotes. Using X-ray crystallography it has demonstrated that the enzyme has a decameric structure composed of two face-to-face pentameric rings and possesses 10 active sites, each localized at the interface between the N-terminal and C-terminal domains of two neighbouring subunits [59]. The subunit of cytosolic GS1 has a molecular mass of 38–40 kDa, while the plastid GS2 form range from 42 to 45 kDa. Glutamine synthesis is a two-step reaction involving the production of the activated intermediate γ-glutamyl phosphate from ATP and glutamate and the deprotonation of a bound ammonium ion to form ammonia, which attacks the carbonyl C to form glutamine (Figure 3).

ISBN:
DOI:

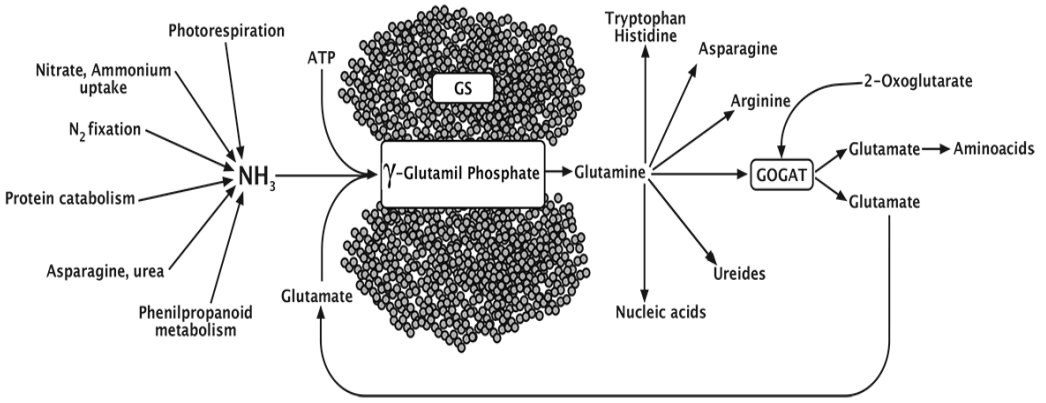

Figure 3: Reaction of Glutamine Synthetase (GS) and fate of glutamine.

Due to its role as a cornerstone in nitrogen metabolism, it is not surprising that GS is a highly regulated enzyme, with regulation occurring at transcriptional and post-translational level.

Using different plant species, a number of 3 to 5 genes encoding for the cytosolic GS1 has been generally identified; on the other hand, a single gene encodes the chloroplastic GS2. Each of the GS genes appears to participate in different metabolic processes, based on where and how they are expressed [60]. The expression of GS1 genes and the presence of the enzyme differ considerably at the tissue and cellular levels [54]. The GS2 gene is of nuclear origin and is targeted to the plastid; it is highly expressed in the mesophyll of leaves and other photosynthetic tissues [61]. Expression is influenced by developmental and environmental cues, such as soil N availability, plant N status, external and internal C status, as well as changes in plant hormones [62,63].

A cytosolic gene has been shown to be highly expressed in infected cells of leguminous root nodules where ammonium produced by N_2 fixation has to be assimilated [64]. The localization of a specific cytosolic GS isoenzyme in the vascular tissues has been reported for several species and related to N transport function [65]. The plastidic GS2, besides its major role in ammonia assimilation for amino acid synthesis, is involved in re-assimilation of ammonium released by photorespiration in photosynthetic tissues [66].

GS1 has been implicated in assimilation of nitrogen in sink tissues. Increasing importance of GS1 relative to GS2 in senescing leaves suggests a role for the former in the mobilization of N to be delivered to different sinks (e.g. developing seeds) [67]. QTL analyses have shown that cytosolic GS is necessary for grain filling [68]. It has also been shown that cytosolic GS protein can accumulate in mesophyll cells of plants in response to stress, such as pathogen attack [69], suggesting a role for this isoform in the re-assimilation of the nitrogen released during the disassembly of the photosynthetic apparatus.

Conifers do not possess GS2 gene, but rather they show two GS1 genes (GS1a and GS1b); based on light stimulation and parallelism between GS1a expression and chloroplast development it has been proposed that this gene might act similarly to GS2 in angiosperms [70]. Interestingly the overexpression of cytosolic GS1a resulted in improved chemical characteristics of field grown hybrid poplars [71].

ISBN:
DOI:

Understanding the role for the cytosolic GS1 and plastidic GS2 isoforms are being elucidated, mainly using mutant plants. This task is particularly difficult for GS1, due to the variable number of genes found in different plant species. Experiments using model and cultivated plants point to a non-redundancy of the different proteins, rather to a specific role for each of them [58]. These aspects still deserve further research efforts.

Different kinds of post-translational modifications have been reported, involving Ca^{2+}-dependent kinases and phosphorylation [72] or binding of 14-3-3 proteins [73]; furthermore selective phosphorylation of Ser^{97} residue and subsequent binding to 14-3-3 proteins, which causes proteolytic breakdown to an inactive product has been reported for plastidic GS2 [74]. Recently, ubiquitination of GS polypeptides has also been proposed as a reversible post-translational regulatory mechanism [75].

The possibility to alter the expression of either GS1 or GS2 and the enzyme activity has attracted attention of researcher due to the possible effects on N metabolism and NUE [76]. Several transgenic approaches have been used, mainly based on overexpression or knockdown mutants [55].

Several studies have reported increased biomass and yield when GS genes are over-expressed in greenhouse and hydroponics experiments (e.g. [77]). Although the outcomes of these studies have been variable, they have clearly indicated that cytosolic GS1 can be important for efficient nitrogen assimilation, plant growth and biomass accumulation [78]. As compared to GS2, GS1 might be a key component of plant NUE [78].

However it has also been evidenced that post-translational modification of GS might significantly affects the over-expression of this enzyme, thereby influencing its ability to increase NUE in the field. Further research is needed on these aspects; moreover, it has been proposed that gene stacking experiments utilizing GS along with other genes of interest for N metabolism might be a suitable strategy to get a clear effect on NUE. The variable effects of environmental conditions, especially N supply, observed in gene expression experiments [60] should be taken also into account when designing for transgenic approaches to evaluating NUE.

Concluding Remarks

Adaptation of plants to different environments and an efficient use of natural resources to sustain adequate crop productivity are strictly related to the coordinated action of metabolic processes within the plant. Several enzymes play crucial roles in this framework guaranteeing the basis for autotrophic behavior of higher plants. The enzymes analyzed in this chapter amply demonstrate this feature, as they catalyze key reactions in nutrient acquisition, energy production, carbon and nitrogen assimilation. Advances in protein structure definition, genomic approaches and identification of regulatory routes have made it possible to better understand the function of these enzymes, paving the way for designing plants adapted to changing environment and able to respond to new challenges of modern agriculture. Further improvement of our knowledge could conceivably derive from exploiting natural variation and by using transgenic approaches.

References

1. Palmgren MG (1998) Proton gradients and plant growth: Role of the plasma membrane H^+-ATPase. Advances in Botanical Research 28: 1-70.

2. SM, Houtz RL, Alonso H (2011) Advancing Our Understanding and Capacity to Engineer Nature's CO_2-Sequestering Enzyme, Rubisco. Plant Physiol 155: 27-35.

ISBN:
DOI:

3. Edwards JW, Walker EL, Coruzzi GM (1990) Cell-specific expression in transgenic plants reveals nonoverlapping roles for chloroplast and cytosolic glutamine synthetase. PNAS 87: 3459-3463.

4. Ekberg K, Palmgren MG, Veierskov B, Buch-Pedersen MJ (2010) A Novel Mechanism of P-type ATPase Autoinhibition Involving Both Termini of the Protein. J Biol Chem 285: 7344-7350.

5. Fuglsang AT, Visconti S, Drumm K, Jahn T, Stensballe A et al. (1999) Binding of 14-3-3 protein to the plasma membrane H^+-ATPase AHA2 involves the three C-terminal residues Tyr^{946}-Thr-Val and requires phosphorylation of Thr^{947}. J Biol Chem 274: 36774-36780.

6. Olsson A, Svennelid F, Ek B, Sommarin M, Larsson C (1998) A Phosphothreonine Residue at the C-Terminal End of the Plasma Membrane H^+-ATPase Is Protected by Fusicoccin-Induced 14-3-3 Binding. Plant Physiol 118: 551-555.

7. Svennelid F, Olsson A, Piotrowski M, Rosenquist M, Ottman C et al. (1999) Phosphorylation of Thr-948 at the C Terminus of the Plasma Membrane H^+-ATPase Creates a Binding Site for the Regulatory 14-3-3 Protein. Plant Cell 11: 2379-2392.

8. Palmgren MG (2001) Plant Plasma Membrane H^+-ATPases: Powerhouses for Nutrient Uptake. Annu Rev Plant Physiol Plant Mol Biol 52: 817-845.

9. Duby G, Boutry M (2009) The plant plasma membrane proton pump ATPase: a highly regulated P-type ATPase with multiple physiological roles. Pflugers Arch - Eur J Physiol 457: 645-655.

10. Haruta M, Sussman MR (2012) The Effect of a Genetically Reduced Plasma Membrane Protonmotive Force on Vegetative Growth of *Arabidopsis*. Plant Physiol 158: 1158-1171.

11. Santi S, Locci G, Monte R, Pinton R, Varanini Z (2003) Induction of nitrate uptake in maize roots: expression of a putative high-affinity nitrate transporter and plasma membrane H^+-ATPase isoforms. J Exp Bot 54: 1851-1864.

12. Sorgonà A, Lupini A, Mercati F, Di Dio L, Sunseri F et al. (2011) Nitrate uptake along the maize primary root: an integrated physiological and molecular approach. Plant Cell Environ 34: 1127-1140.

13. Quaggiotti S, Ruperti B, Pizzeghello D, Francioso O, Tugnoli V et al. (2004) Effect of low molecular size humic substances on nitrate uptake and expression of genes involved in nitrate transport in maize (*Zea mays* L.). J Exp Bot 55: 803-813.

14. Tomasi N, Kretzschmar T, Espen L, Weisskopf L, Fuglsang AT et al. (2009) Plasma-membrane H^+-ATPase-dependent citrate exudation from cluster roots of phosphate-deficient white lupin. Plant Cell Environ 32: 465-475.

15. O'Rourke JA, Yang SS, Miller SS, Bucciarelli B, Liu J et al. (2013) An RNA-Seq Transcriptome Analysis of Orthophosphate-Deficient White Lupin Reveals Novel Insights into Phosphorus Acclimation in Plants. Plant Physiol 161: 705-724.

16. Dell'Orto M, Santi S, De Nisi P, Cesco S, Varanini Z et al. (2000) Development of Fe-deficiency responses in cucumber (*Cucumis sativus* L.) roots: involvement of plasma membrane H^+-ATPase activity. J Exp Bot 51: 695-701.

17. Dell'Orto M, Pirovano L, Villalba JM, Gonzalez-Fontes A, Zocchi G (2002) Localization of the plasma membrane H^+-ATPase in Fe-deficient cucumber roots by immunodetection. Plant Soil 241: 11-17.

18. Santi S, Cesco S, Varanini Z, Pinton R (2005) Two plasma membrane H+-ATPase genes are differentially expressed in iron-deficient cucumber plants. Plant Physiol Biochem 43: 287-292.

19. Yan F, Feuerle R, Schäffer S, Fortmeier H, Schubert S (1998) Adaptation of Active Proton Pumping and Plasmalemma ATPase Activity of Corn Roots to Low Root Medium pH. Plant Physiol 117: 311-319.

20. Shen H, He LF, Sasaki T, Yamamoto Y, Zheng SJ et al. (2005) Citrate secretion coupled with the modulation of soybean root tip under aluminum stress. Up-regulation of transcription, translation, and threonine-oriented phosphorylation of plasma membrane H^+-ATPase. Plant Physiol 138: 287-296.

21. Yang J, Zhu X, Peng Y, Zheng C, Ming F et al. (2011) Aluminum regulates oxalate secretion and plasma membrane H+-ATPase activity independently in tomato roots. Planta 234: 281-291.

22. Hager A (2003) Role of the plasma membrane H^+-ATPase in auxin-induced elongation growth: historical and new aspects. J Plant Res 116: 483-505.

23. Tomasi N, De Nobili M, Gottardi S, Zanin L, Mimmo T et al. (2013) Physiological and molecular characterization of Fe acquisition by tomato plants from natural Fe complexes. Biol Fertil Soil 49: 187-200.

24. Canellas LP, Olivares FL, Okorokova-Facanha AL, Facanha AR (2002) Humic Acids Isolated from Earthworm Compost Enhance Root Elongation, Lateral Root Emergence, and Plasma Membrane H^+-ATPase Activity in Maize Roots. Plant Physiol 130: 1951-1957.

25. Zandonadi D, Canellas L, Facanha AR (2007) Indolacetic and humic acids induce lateral root development through a concerted plasmalemma and tonoplast H^+ pumps activation. Planta 225: 1583-1595.

ISBN:
DOI:

26. Pinton R, Cesco S, Iacolettig G, Astolfi S, Varanini Z (1999) Modulation of NO_3- uptake by water-extractable humic substances: involvement of root plasma membrane H^+ATPase. Plant Soil 215: 155-161.

27. Garcia-Mina JM, Antolin MC, Sanchez-Diaz M (2004) Metal-humic complexes and plant micronutrient uptake: a study based on different plant species cultivated in diverse soil types. Plant Soil 258: 57-68.

28. Capaldi, RA, Aggeler, R (2002) Mechanism of the F1F0-type ATP synthase, a biological rotary motor. Trends Biochem Sci 27: 154-160.

29. Kayalar C, Rosing J, Boyer PD (1977) An alternating site sequence for oxidative phosphorylation suggested by measurement of substrate binding patterns and exchange reaction inhibitions. J Biol Chem 252: 2486-2491.

30. Cabezon E, Butler PJ, Runswick MJ, Walker JE (2000) Modulation of the Oligomerization State of the Bovine F1-ATPase Inhibitor Protein, IF1, by pH. J Biol Chem 275: 25460-25464.

31. Nalin CM, McCarty RE (1984) Role of a disulfide bond in the gamma subunit in activation of the ATPase of chloroplast coupling factor 1. J Biol Chem 259: 7275-7280.

32. Dimroth P, von Ballmoos C, Meier T (2006) Catalytic and mechanical cycles in F-ATP synthases. EMBO reports 7: 276-282.

33. Nowak KF, McCarty RE (2004) Regulatory Role of the C-Terminus of the Epsilon Subunit from the Chloroplast ATP Synthase. Biochemistry 43: 3273-3279.

34. Seelert H, Poetsch A, Dencher NA, Engel A, Stahlberg H et al. (2000) Structural biology: Proton-powered turbine of a plant motor. Nature 405: 418-419.

35. Stock D, Leslie AGW, Walker JE (1999) Molecular Architecture of the Rotary Motor in ATP Synthase. Science 286: 1700-1705.

36. von Ballmoos C, Cook GM, Dimroth P (2008) Unique Rotary ATP Synthase and Its Biological Diversity. Annu Rev Biophys 37: 43-64.

37. Andersson I, Backlund A (2008) Structure and function of Rubisco. Plant Physiol Biochem 46: 275-291.

38. Shiina T, Tsunoyama Y, Nakahira Y, Khan MS (2005) Plastid RNA Polymerases, Promoters, and Transcription Regulators in Higher Plants. Kwang WJ. International Review of Cytology, A Survey of Cell Biology. Academic Press.

39. Nishimura K, Ogawa T, Ashida H, Yokota A (2008) Molecular mechanisms of RuBisCO biosynthesis in higher plants. Plant Biotechnology 25: 285-290.

40. Houtz RL, Magnani R, Nayak NR, Dirk LMA (2008) Co- and post-translational modifications in Rubisco: unanswered questions. J Exp Bot 59: 1635-1645.

41. Jarvis P (2008) Targeting of nucleus-encoded proteins to chloroplasts in plants. New Phytol 179: 257-285.

42. Grimm R, Grimm M, Eckerskorn C, Pohlmeyer K, Röhl T et al. (1997) Postimport methylation of the small subunit of ribulose-1,5-bisphosphate carboxylase in chloroplasts. FEBS Lett 408: 350-354.

43. Andersson I (2008) Catalysis and regulation in Rubisco. J Exp Bot 59: 1555-1568.

44. Parry MAJ, Keys AJ, Madgwick PJ, Carmo-Silva AE, Andralojc PJ (2008) Rubisco regulation: a role for inhibitors. J Exp Bot 59: 1569-1580.

45. Galmés J, Aranjuelo I, Medrano H, Flexas J (2013) Variation in Rubisco content and activity under variable climatic factors. Photosynth Res 117: 73-90.

46. Feller U, Crafts-Brandner SJ, Salvucci ME (1998) Moderately High Temperatures Inhibit Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase (Rubisco) Activase-Mediated Activation of Rubisco. Plant Physiol 116: 539-546.

47. Sage RF (2004) The evolution of C4 photosynthesis. New Phytol 161: 341-370.

48. Edwards GE, Franceschi VR, Voznesenskaya EV (2004) Single-cell C4 photosynthesis versus the dual-cell (Kranz) paradigm. Annu Rev Plant Biol 55: 173-196.

49. Ghannoum O, Evans JR, Chow WS, Andrews TJ, Conroy JP et al. (2005) Faster Rubisco Is the Key to Superior Nitrogen-Use Efficiency in NADP-Malic Enzyme Relative to NAD-Malic Enzyme C4 Grasses. Plant Physiol 137: 638-650.

50. Galmés J, Flexas J, Keys AJ, Cifre J, Mitchell RAC et al. (2005) Rubisco specificity factor tends to be larger in plant species from drier habitats and in species with persistent leaves. Plant, Cell & Environment 28: 571-579.

51. Parry MAJ, Andralojc PJ, Scales JC, Salvucci ME, Carmo-Silva AE et al. (2012) Rubisco activity and regulation as targets for crop improvement. J Exp Bot.

ISBN:
DOI:

52. Carmo-Silva AE, Gore MA, Andrade-Sanchez P, French AN, Hunsaker DJ et al. (2012) Decreased CO_2 availability and inactivation of Rubisco limit photosynthesis in cotton plants under heat and drought stress in the field. Environmental and Experimental Botany 83: 1-11.

53. Pearce FG (2006) Catalytic by-product formation and ligand binding by ribulose bisphosphate carboxylases from different phylogenies. Biochem J 399: 525-534.

54. Miflin BJ, Habash DZ (2002) The role of glutamine synthetase and glutamate dehydrogenase in nitrogen assimilation and possibilities for improvement in the nitrogen utilization of crops. J Exp Bot 53: 979-987.

55. Xu G, Fan X, Miller AJ (2012) Plant Nitrogen Assimilation and Use Efficiency. Annu Rev Plant Biol 63: 153-182.

56. Lea PJ, Miflin BJ (2010) Nitrogen Assimilation and its Relevance to Crop Improvement. Annual Plant Reviews Volume 42. Wiley-Blackwell.

57. Good A, Beatty P (2011) Biotechnological approaches to improving nitrogen use efficiency in plants: alanine aminotransferase as a case study. Hawkesford MJ and Barraclough PB. The Molecular and Physiological Basis of Nutrient Use Efficiency in Crops. John Wiley and Sons, Inc., Sussex.

58. Forde BG, Lea PJ (2007) Glutamate in plants: metabolism, regulation, and signalling. J Exp Bot 58: 2339-2358.

59. Unno H, Uchida T, Sugawara H, Kurisu G, Sugiyama T et al. (2006) Atomic Structure of Plant Glutamine Synthetase: A key enzyme for plant productivity. J Biol Chem 281: 29287-29296.

60. Hirel B, Martin A, Tercé-Laforgue T, Gonzalez-Moro MB, Estavillo JM (2005) Physiology of maize I: A comprehensive and integrated view of nitrogen metabolism in a C4 plant. Physiol Plantarum 124: 167-177.

61. Lightfoot DA, Green NK, Cullimore JV (1988) The chloroplast-located glutamine synthetase of Phaseolus vulgaris L.: nucleotide sequence, expression in different organs and uptake into isolated chloroplasts. Plant Mol Biol 11: 191-202.

62. Vidal EA, Tamayo KP, Gutierrez RA (2010) Gene networks for nitrogen sensing, signaling, and response in *Arabidopsis thaliana*. WIREs Syst Biol Med 2: 683-693.

63. Castaings L, Marchive C, Meyer C, Krapp A (2011) Nitrogen signalling in Arabidopsis: how to obtain insights into a complex signalling network. J Exp Bot 62: 1391-1397.

64. Forde BG, Day HM, Turton JF, Shen WJ, Cullimore JV et al. (1989) Two glutamine synthetase genes from *Phaseolus vulgaris* L. display contrasting developmental and spatial patterns of expression in transgenic *Lotus corniculatus* plants. The Plant Cell Online 1: 391-401.

65. Carvalho H, Lima L, Lescure N, Camut S, Salema R et al. (2000) Differential expression of the two cytosolic glutamine synthetase genes in various organs of *Medicago truncatula*. Plant science 159: 301-312.

66. Wallsgrove RM, Turner JC, Hall NP, Kendall AC, Bright SWJ (1987) Barley Mutants Lacking Chloroplast Glutamine Synthetase-Biochemical and Genetic Analysis. Plant Physiol 83: 155-158.

67. Tabuchi M, Abiko T, Yamaya T (2007) Assimilation of ammonium ions and reutilization of nitrogen in rice (*Oryza sativa* L.). J Exp Bot 58: 2319-2327.

68. Hirel B, Bertin P, Quilleré I, Bourdoncle W, Attagnant C et al. (2001) Towards a Better Understanding of the Genetic and Physiological Basis for Nitrogen Use Efficiency in Maize. Plant Physiol 125: 1258-1270.

69. Pérez-García A, Pereira S, Pissarra J, García Gutiérrez A, Cazorla FM et al. (1998) Cytosolic localization in tomato mesophyll cells of a novel glutamine synthetase induced in response to bacterial infection or phosphinothricin treatment. Planta 206: 426-434.

70. Cantón FR, Suárez M-F, Josè-Estanyol M, Cánovas FM (1999) Expression analysis of a cytosolic glutamine synthetase gene in cotyledons of Scots pine seedlings: developmental, light regulation and spatial distribution of specific transcripts. Plant Mol Biol 40: 623-634.

71. Coleman HD, Cánovas FM, Man H, Kirby EG, Mansfield SD (2012) Enhanced expression of glutamine synthetase (GS1a) confers altered fibre and wood chemistry in field grown hybrid poplar (*Populus tremula* X *alba*) (717-1B4). Plant Biotechnol J 10: 883-889.

72. Li RJ, Hua W, Lu YT (2006) *Arabidopsis* cytosolic glutamine synthetase AtGLN1;1 is a potential substrate of AtCRK3 involved in leaf senescence. Biochemical and Biophysical Research Communications 342: 119-126.

73. Riedel J, Tischner R, Mäck G (2001) The chloroplastic glutamine synthetase (GS-2) of tobacco is phosphorylated and associated with 14-3-3 proteins inside the chloroplast. Planta 213: 396-401.

74. Lima L, Seabra A, Melo P, Cullimore J, Carvalho H (2006) Post-translational regulation of cytosolic glutamine synthetase of *Medicago truncatula*. J Exp Bot 57: 2751-2761.

ISBN:
DOI:

75. Seabra A, Silva L, Carvalho H (2013) Novel aspects of glutamine synthetase (GS) regulation revealed by a detailed expression analysis of the entire GS gene family of *Medicago truncatula* under different physiological conditions. BMC Plant Biology 13: 137.

76. McAllister CH, Beatty PH, Good AG (2012) Engineering nitrogen use efficient crop plants: the current status. Plant Biotechnol J 10: 1011-1025.

77. Brauer EK, Rochon A, Bi YM, Bozzo GG, Rothstein SJ et al. (2011) Reappraisal of nitrogen use efficiency in rice overexpressing *glutamine synthetase1*. Physiol Plantarum 141: 361-372.

78. Bernard SM, Habash DZ (2009) The importance of cytosolic glutamine synthetase in nitrogen assimilation and recycling. New Phytol 182: 608-620.

ISBN:
DOI:

Chapter: 4

Enzymes at Plant-Soil Interface

Sara Marinari[1]*, Cristina Moscatelli[1] and Stefano Grego[2]

[1]Department for Innovation Systems Biological, Agri and Forestry, Italy

[2]Department of Science and Technology for Agriculture, Forestry, Nature and Energy, Italy

***Corresponding author:** Sara Marinari, Department for Innovation Systems Biological, Agri and Forestry, Via San Camillo de Lellis s.n.c.- 01100 Viterbo, Italy, Tel: +39 0761 357288; E-mail: marinari@unitu.it

Abstract

The plant-soil interface is called rhizosphere; it is defined as the volume of soil around living roots and influenced by root activities. The spatial patterns of microbial activity in the rhizosphere have attracted significant scientific interest. Due to inputs of easily degradable organic compounds from the roots, microbial biomass and activity are high in the rhizosphere, making this plant-root interface an important hotspot of nutrient cycling. Plant rhizodeposition may promote microbial biomass and activity, which could elevate overall enzyme production. However, the enzyme activity in the rhizosphere depends not only on the stimulation of microbial activity by rhizodeposition but also on the release of enzymes by plant roots. Therefore, the enzyme activity profile is a footprint of plant-microorganisms interactions. Information deriving from different enzyme activities may be combined in diversity indexes to calculate microbial functional diversity and obtain an overall picture of microbial activity and metabolic capabilities. Functional diversity may be a more relevant component of total biodiversity providing information on actual microbial processes performed in the rhizosphere. The enzyme activities at plant-soil interface may reflect improvement of the highly integrated microorganisms-plant associations (symbiotic and plant growth promoting rhizobacteria) and control of plant pathogens and pests. Moreover, soil enzymes have an important role on regeneration of degraded and polluted soils using the genetically plants modified in a way that influences the composition and activity of the rhizosphere community. For this reason, the biotechnology aimed to improve enzyme activities profile in the rhizosphere represents a new challenge for future research on soil remediation and environmental protection.

Introduction

The rhizosphere is known to be a hot spot of plant-microbial interactions and a driving force of soil processes. Plant species could affect carbon resources in the rhizosphere, which would influence the amount and activity of microbial biomass in these environments. Different plant species can promote proliferation of different microbial communities by releasing different amount and types of root exudates. The exudation of carbon by tree roots stimulates microbial activity and the production of extracellular enzymes in the rhizosphere. Labile C inputs to soils by roots are thought to increase microbial exo-enzyme production, which increases organic matter decomposition [1]. The feedback to microbial enzyme production and nutrient supply is often inferred; it remains unclear exactly how the belowground flux of C affects the activity

ISBN:
DOI:

of microorganisms, exo-enzyme production and the depolymerization of N [2]. More than one-third of the photosynthates reaching healthy roots are lost into the soil [3] as sloughed cap cells, mucilages, soluble exudates and lysates, and decaying root hairs and outer cortical cells. When plant rhizodeposition increases, microbial biomass often increases, which could elevate overall enzyme production. However, the higher enzyme activity of the rhizosphere than bulk soil depends not only on the stimulation of microbial activity by rhizodeposition but also on the release of enzymes by roots or by lysis of root cells. Microbial activities are affected by the many plant responses to environmental changes. For example, elevated CO_2, which induces qualitative variations of plant C efflux into the rhizosphere, could induce an acceleration of metabolic (respiration) and co-metabolic (enzymes) activity defined as priming effect [4]. Moscatelli et al. [5] reported an increased nutrient acquisition activity (e.g. enzymes) induced by elevated atmospheric CO_2 in the rhizosphere of a poplar plantation growing under limited N availability (Figure 1).

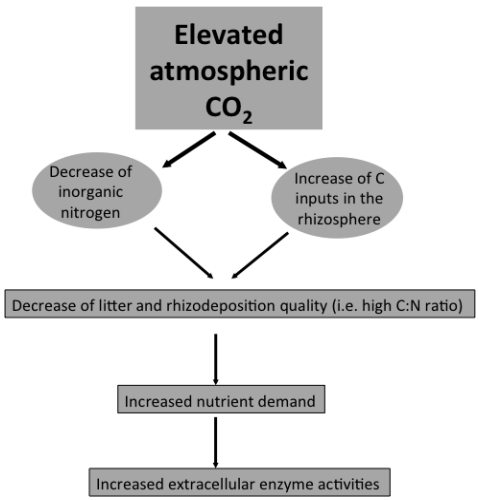

Figure 1: The effects of elevated atmospheric CO2 on enzyme activities at plant-soil interface.

Moreover, expression and repression of extracellular enzyme activity in the rhizosphere may be important to understand the relationship between enzyme activity and intensity/direction of priming effect due to exudates. Marinari et al. [6] supposed that the input of a substance inducing priming effect can activate the microbial synthesis of intracellular and extracellular enzymes; while Schimel and Weintraub [7] suggest that the fresh available substrate inducing priming effect can serve as an energy source for the production of extracellular enzymes with the subsequent increase in the decomposition of native soil organic matter (priming effect). It is known that agricultural practices (fertilization, crop rotation, irrigation) may promote enzyme activities as a result of improved crops growth (Chapter 2 of this book). Usually, crops with higher root developments than other can stimulate enzyme activities by the rhizosphere effect [8]. For instance the continuous presence of vegetation and the extensive root system of grasses may increase the rhizosphere effect [9]; therefore this effect and lack of tillage probably contribute to consistently higher enzyme activities measured in grasslands over comparable cultivated soils [10]. Moreover, soil-plant-microorganisms interactions have been widely studied as strategies to improve the efficiency of agriculture [11]. One of the main evidence

ISBN:
DOI:

on those nutritional strategies is given by the mineralization of organic P through the activity of phosphatase enzymes. Substantial increases in phosphatase activity have been shown to occur in the rhizosphere of plants often associated with a depletion of soil organic P [12-14] (Figure 2).

Some authors reported that, although microbial biomass supported by rhizodeposition increased the production of peptidases to hydrolyze enzymes and other proteins, C-degrading enzymes did not increase [15-17]. Overall, these findings suggest that cellulases are not driving increased mineralization of soil organic carbon in response to increased rhizodeposition [18]. An increased degradation of xenobiotic compounds in plant rhizospheres is ascribed to the root-associated microflora and/or root-exuded enzymes. In fact, it is known that roots of certain members of *Fabaceae, Gramineae, and Solanaceae* release enough oxido-reductases to take part in the oxidative degradation of certain soil constituents [19]. Rhizomediation is a technology that uses growing plant roots to distribute exudates throughout the soil that stimulate growth and activity of contaminant degraders [20]. In this context, the plants genetically modified in a way that influences the composition and activity of the rhizosphere community, may represent engineering for rhizomediation as ecosystem services [18,21].

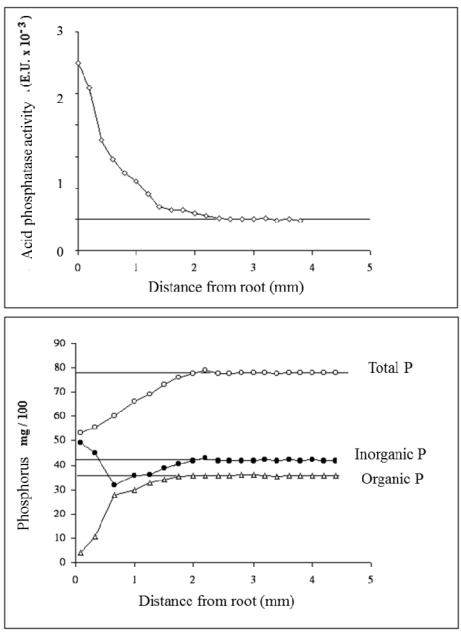

Figure 2: Gradient of acid phosphatase activity in the plant soil-interface associated. with a depletion of soil organic phosphorus [Modified from 15].

Soil Enzyme and Functional Diversity in the Rhizosphere

Functional diversity is a component of overall soil biodiversity that possibly provides a more practical and ecologically relevant measure of microbial diversity [22]. According to the conceptual model of Zak et al. [22], total biodiversity relates to genetic, taxonomic and functional

ISBN:
DOI:

diversity. The functional diversity results from genetic variability within a taxon, environmental effects on gene expression and ecological interactions among taxa. Zak et al. [22] point out that there is a general lack of information relating taxonomic diversity to ecosystem functions. Microbial functional diversity represents "the sum of the ecological processes developed by the organisms of a community and it can be expressed through species or important groups to maintain several functions in the soil, while the genetic one represents gene and genotype variations" [23]. Distinct from the genetic diversity of the soil microbial biomass [24,25] which assess potential diversity, functional diversity is thus related to the actual activities resulting from that potential so that "functional rather than taxonomic diversity may provide greater insight to microbial roles in ecosystems" [22].

In terrestrial environments, *in situ* enzyme activities are dynamic [26] and responsive to changes in microbial biomass and community structure (i.e. production) [27]. Soil enzyme assays are useful for assessing microbial community function to answer questions related to soil decomposition [i.e. C-cycling) and nutrient cycling [i.e. N and/or P-cycling). Microbes produce specific enzymes [i.e. C-, N-, or P-degrading enzymes) to meet nutrient demands within their soil environments [28]. In addition, the study of different hydrolases is important since they indicate the soil potential to carry out specific biochemical reactions and also maintain soil fertility; for that reason they have been widely used in the evaluation of soil quality changes due to soil management and in response to different agricultural practices [29].

The relationship between soil enzymes and microbial functional diversity was first proposed by Kandeler et al. [30] who introduced a novel approach aimed to characterize functional diversity of microbial communities in soil. According to these authors the actual rate of enzyme production and the fate of produced enzymes are modified by environmental effects as well as by ecological interactions. Thus, they related the functional diversity approach to the enzymes synthesized by the soil microflora, including endocellular as well as exocellular enzymes.

Soil enzymes are produced by plants and microbes; enzymes location and activity rate are thus strictly related to the presence of these organisms, mutual interactions and their specific needs in terms of basal metabolism, nutrient acquisition activity, protection mechanisms etc.

Plant communities directly affect soil microbial community composition and activity through alteration of the physical environment during root growth and substrate availability through root exudation [31]. These effects are evidently strongest in the rhizosphere [32], the soil zone directly impacted by roots, and used by plants to exploit soil organic nutrient pools.

The rhizosphere is that particular environment that provides a source of labile carbon input and is thought to prime microbial decomposition of more recalcitrant organic matter [33]. As a result, root carbon inputs to the soil are the driver of microbial nutrient acquisition and enzyme production [34].In the rhizosphere, the main driving factors for changes in bacterial community composition and activity are likely to be root exudate amount, type and composition. Consequently, each plant species may modify the conditions in the rhizosphere in order to maximize nutrient acquisition from organic matter by promoting particular functional groups of microorganisms. For example, the composition and functions of the microbial community plays a significant role in controlling C cycling in the rhizosphere and in organic detritus (litter or dead roots). In the rhizosphere, microbial community composition is regulated by the specific substrates and chemical signals released by the plant root, and by the specific physical and biotic environment created by the plant root in terms of O_2, p^H, and other chemical variables [35]. These select for a distinct group of microbes, some of which act as plant growth-promoting rhizobacteria or as pathogens and so have powerful feedbacks to plant

ISBN:
DOI:

growth and C cycling [36].In the rhizosphere environment, while the microbial community composition will determine the potential for enzyme synthesis, the release of root exudates will modify the actual rate of enzyme production and the fate of produced enzymes [37]. Higher activity of several enzymes can be interpreted as a greater functional diversity of the microbial community [30] in the rhizosphere.

To assess the functional diversity of soil microbial communities enzyme activities have been recently widely used in diverse soil conditions in relation to land use [38], agricultural management [39], and forest ecosystems [40].

Kandeler et al. [30] suggested the estimations of soil enzyme activities involved in the C-, N-, P-, and S-cycle for the calculation of functional diversity of soil microbial communities. Multiple assays like this allow for the use of composite indices or multivariate statistics for the comparison of samples. Thus measuring soil enzymes combined in diversity indexes or microbial indexes may constitute a valid approach to study microbial functional diversity.

Microbial functional diversity measured by means of enzyme activities can be expressed using popular diversity indexes such as the Shannon index [H'= -∑pi log2 pi; 41] or the Simpson–Yule, [CE = 1/∑ pi2; 42] where pi is the ratio of the single enzyme activity to the sum of all enzyme activities. The combined use of microplates and of fluorimetric techniques, proposed by Marx et al. [43], found a wide consensus throughout the scientific literature due to the rapid assays, high sensitivity and possibility to assess eight enzyme activities at the same time. Multiple enzyme fluorimetric approach can inform on several ecological processes within a single assay. An increasingly wide range of fluorescently labeled substrates are available which enable sensitive measurements to be made on small samples, permitting high-throughput assay systems [43]. The most common fluorogenic methylumbelliferyl (MUF)-substrates are4-MUF-β-D-cellobioside, 4-MUF- N-acetyl-β-glucosaminide, 4-MUF-β-Dglucoside, 4-MUF-α-D-glucoside, 4-MUF-phosphate, 4-MUF-sulphate, 4-MUF-7-β-D-xyloside and 4-MUF-butyrate/acetate to test, respectively, β-cellobiohydrolase, N-acetyl-β-glucosaminidase, β-glucosidase, α-glucosidase, acid phosphatase, arylsulfatase, xylosidase and butyrate/acetate esterase. Acetate and/or butyrate esterase are included in the set as proxy of intracellular enzymatic activities.

Many studies assessed functional diversity in rhizosphere soils to study the effect of different plant species, land use, agricultural practices, heavy metals [44-48]. Gardner et al. [46] demonstrated that organic farming system under different rhizospheres can have implications in soil health and metabolic functioning, and the yield and nutritional value of each crop. The different rhizospheres showed in fact differences in the group of enzyme activities evaluated, which revealed variations in C, N, P, and S biogeochemical cycling.

Kandeler et al. [37] showed significant different enzymatic patterns under maize rhizosphere. Principal component analysis showed increased acid phosphatase and invertase activities being responsible for a clear rhizosphere effect whereas differences in protease and alkaline phosphatase activities mainly caused the separation of planted and unplanted microcosms.

Marschner et al. [47] found changes in community composition associated with an increase in activities of enzymes involved in N and C cycling (β-glucosidase and protease) in the rhizosphere of Banksia trees. Upland and irrigated rice production systems showed important differences in rhizospheric enzymatic patterns due to the different redox conditions in rice paddies [44].

Montes Borrego et al. [49] study, on olive orchards under organic management, showed higher microbial diversity (measured by catabolic capability and functional indexes of the

ISBN:
DOI:

BiologEcoPlate and enzymatic assays) compared to conventionally managed orchards.

Moreno et al. [48] found that microbial functional diversity (measured by 6 enzyme activities) was more sensitive than other microbiological properties in evidencing differences between organic and conventional olive orchards in Spain.

In conclusion, the complexity of rhizosphere chemistry and biology continues to present a multitude of "black boxes" to be opened. Many aspects concerning microbial biomass content and diversity in the soil still need to be clarified; thus the link between the structure of soil microbes and their function still makes difficult to predict and manipulate rhizosphere ecology [20].

Burns et al. [18] conclude that as knowledge on extracellular enzymes (EE) function, regulation and expression increases, and manipulation of EE for ecosystem services becomes possible. The likely strategies range from manipulating 'natural' microorganisms and their enzymes to genetically modified organisms. As far as the rhizospheric processes, they range from improving the already highly specialized symbiotic associations to controlling plant pathogens and pests and reclamation of degraded and contaminated soils.

Enzyme Activity in the Rhizosphere and Plant Growth Promotion

Microorganisms that colonize the rhizosphere can be classified according to their effects on plants and the way they interact with roots, some being pathogens whereas other triggering beneficial effects. Rhizobacteria inhabit plant roots and exert a positive effect ranging from direct influence mechanisms to an indirect effect. So, the bacteria inhabiting the rhizosphere and beneficial to plants are termed PGPR. Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly and/or indirectly.

PGPR can affect plant growth by different direct and indirect mechanisms [50]. Some examples of these mechanisms, which can probably be active simultaneously or sequentially at different stages of plant growth, are i) increased mineral nutrient solubilization and nitrogen fixation, making nutrients available for the plant; ii) repression of soil borne pathogens (by the production of hydrogen cyanide, siderophores, antibiotics, and/or competition for nutrients); iii) improving plant stress tolerance to drought, salinity, and metal toxicity; iv) production of phytohormones such as indole-3-acetic acid (IAA); and v) modulating the content of ethylene [50].

The increased level of resistance using external agents, without modifying the genome of the plant, is known as induced or acquired resistance. Induction of resistance promoted by PGPR is active and signaling in the route of salicylic acid with induction of PR-proteins (proteins related to the pathogenesis) or route of the jasmonic acid and ethylene [51]. In addition to the PR-proteins, the plants produce other enzymes of the defense, including peroxidases, phenylalanine ammonia-lyase (PAL), and polyphenoloxidase (PPO). Peroxidase and PPO are catalysts in the formation of lignin [52]. PAL and other enzymes are involved in the formation of phytoalexins. In several studies, the quantification of activity of enzymes involved in the induction of resistance has been used as a parameter to assess the induction mechanism (biotic or abiotic) involved [51]. Thus, the application of PGPR in agriculture via soil or seed inoculation can be characterized as clear rhizosphere effect creating a beneficial component in the integrated management of diseases.

A number of bacterial species belonging to genera *Azospirillum, Alcaligenes, Arthrobacter,*

ISBN:
DOI:

Acinetobacter, Bacillus, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Pseudomonas, Rhizobium and *Serratiaare* associated with the plant rhizosphere and are able to exert a beneficial effect on plant growth interacting at rhizosphere level. Similarly, mycorrhiza formation may promote plant growth. However, the interaction of mycorrhiza with other specific microorganisms may cause the attenuation of the positive effect on plant growth [53]. Free-living microbial inoculants could stimulate mycorrhizal colonization [54], but also mycorrhiza formation can affect the microbial population in the rhizosphere through changes in root exudation patterns, or through fungal exudates. In this context, changes of the microbial patterns in the rhizosphere, due to the formation of arbuscular mycorrhizal, have been assesses through measurements of different soil enzyme activities [55].

The important role is played by plants in selecting and enriching the types of microorganisms by the constituents of their root exudates. Thus, the microbial community in the rhizosphere develops depending on the nature and concentrations of organic constituents of exudates, and the corresponding ability of the microbes to utilize these as sources of energy [56].

Several microorganisms help to derive maximum benefit from root exudates by their ability to attach to the root surfaces (rhizoplane). Since associative interactions of plants and microorganisms must have come into existence as a result of co-evolution, the use of latter group as bio inoculants must be pre-adapted, so that it fits into a long-term sustainable agricultural system.

PGPR are commonly used as inoculants for improving the growth and yield of agricultural crops and offer an attractive way to replace chemical fertilizers, pesticides, and supplements depending on the nature and concentrations of organic constituents of exudates, and the corresponding ability of the bacteria to utilize these as sources of energy synthesizing the appropriate enzyme pattern. There is a continuum of bacterial presence in soil rhizosphere, rhizoplane and internal of the plant tissues [56].

The biological nitrogen fixation by PGPR is very important in enhancing the soil fertility. In addition to biological nitrogen fixation, mineral and organic phosphate solubilization is equally important. Phosphorus (P) is a major essential macronutrient for biological growth and development. Microorganisms offer a biological rescue system capable of solubilizing the inorganic or organic P of soil and make it available to the plants. The ability of some microorganisms to convert insoluble phosphorus (P) to an accessible form, like orthophosphate, is an important trait in a PGPB for increasing plant yields [57,58].

Induced Systemic Resistance (ISR) of plants against pathogens is a widespread phenomenon that has been intensively investigated with respect to the underlying signaling pathways as well as to its potential use in plant protection. Elicited by a local infection, plants respond with a salicylic-dependent signaling cascade that leads to the systemic expression of a broad spectrum and long-lasting disease resistance that is efficient against fungi, bacteria and viruses.

Enzymatic pathways involving hydrolytic, oxidative, reductive, and substitution/transfer reactions are implicated in detoxification of cyanide by bacteria and fungi. The enzyme rhodanese from cyanogenic bacterium *Pseudomonas aeruginosa* involved in transfer reactions causes cyanide detoxification. The enzymes like chitinase, β-1,3-glucanase and cellulose are involved in antagonistic action of *Pseudomonas* against fungal pathogens. The enzyme formamide hydro-lyase is involved in HCN detoxification in sorghum infected by *Gloeocercospora sorghi*. [56].

Other mechanisms of growth promotion involve modulation of plant regulatory mechanisms

ISBN:
DOI:

through the production of hormones or other compounds that influence plant development. Many bacterial species are capable of producing auxin and/or ethylene, and synthesis of gibberellins and cytokines has also been documented [59].

Ethylene is usually considered an inhibitor of plant growth, but at low levels can actually promote growth in several plant species. Bacteria possessing ACC deaminase activity reduce the level of stress ethylene conferring resistance and stimulating growth of plants under various biotic and abiotic stresses. Some plant-growth-promoting rhizobacteria (PGPR) promote plant growth by lowering the endogenous ethylene synthesis in the roots through their 1-aminocyclopropane-1-carboxylate (ACC)-deaminase activity. Ethylene is well thought-out stress hormone because its synthesis is induced by a variety of stresses. Nodulation and subsequent nitrogen fixation by lentil plants are inhibited by accelerated ethylene concentration in the root zone. The isolates also showed compatibility with *Rhizobium leguminosarum* under axenic conditions and also promoted root/shoot growth in lentil seedlings. Results reveal that inoculation with PGPR containing ACC-deaminase and Rhizobium could be utilized as expeditious bio fertilizers to increase the growth as well as nodulation in legume plants [60].

In the rhizosphere of rice plants grown in coastal saline soils the efficiency of ACC deaminase producing PGPR strains has been studied to quantify the effect of bacteria on rice seed germination and seedling growth under salinity stress. Inoculation with selected PGPR isolates had considerable positive impacts on different growth parameters of rice including germination percentage, shoot and root growth and chlorophyll content as compared to uninoculated control. Inoculation with the ACC deaminase producing strains reduced ethylene production under salinity stress. It is evident the effectiveness of rhizobacteria containing ACC deaminase for enhancing salt tolerance and consequently improving the growth of rice plants under salt-stress conditions [61].

Effect of Transgenic Crops on Enzyme Activity in Rhizosphere and Bulk Soils

Whether genetically modified (GM) plants have any substantial influence on the soil microbial communities has never been an easy question to answer. Statistically significant changes might be transient and biologically insignificant. Lottmann et al. [62] suggest that the impacts of GM plants should focus on functional markers rather than monitoring the structure and genetic diversity of microbial communities. In their studies on field-grown trees monitored for two consecutive years they monitored both bacterial and fungal communities including ectomycorrhizal fungi. Their conclusion was that long-term studies on field-grown GM trees at different locations are needed to provide the baseline data necessary to relate potential population shifts to natural variability caused by growth stage, soil type, climate, season, or tree species. In addition, the long life span of trees makes the evaluation of environmental risks difficult.

The soil ecosystem is much more complex and the multitude of micro-driven soil properties forces one to make choices as to what organisms and properties might best be examined. When attempting to determine the effects of GM crops on soil ecosystems, it makes sense to start first with an assessment of what is known about the GM crop and the system into which it is to be introduced [63]. However, it has been demonstrated that field site influences microbial community composition and interact with plant varieties in their influence on the microbial community [57]. The effect of plant variety on microbial community at one field site was sometimes entirely different at another field site, suggesting that the environment will

ISBN:
DOI:

play a major role in determining the potential ecological significance of growing genetically modified plants [64]. On the other hand, rhizosphere-mediated effects may also arise from root-specific modifications due to altered root exudation patterns. In rice exudates are known to influence plant-promoting rhizobacteria and the modification of rice plants leads to multiple effects on plant biochemistry. It is likely that there is a change in the rate or composition of the exudates, affecting soil organisms and plant-rhizosphere interactions [65]. For the detection of less predictable effects on microbial groups and processes, more general analyses would need to be applied, such as activity measurements enzyme activities, soil fungi balance or decomposition of recalcitrant organic compounds [63].

Early studies have been concentrated to genetically modified microorganisms (GMMs) and it has been reported by many authors that the introduction of large numbers of GMMs into the soil could alter microbial populations and disturb microbiological driven soil processes. Effects of introduced GMMs on soil ecosystems have been studied mainly in microcosm experiments. Transient perturbations have been observed in indigenous bacterial [66], fungal [67] and protozoal [68] populations, and in carbon turnover [69]. Naseby and Lynch [70] studied the effect of genetically modified microorganisms, realized as bio control agents, on soil enzyme activities and indigenous microbial populations. They suggest that measurements of soil enzyme activities may be useful to reach a better understanding of the nature of perturbation caused to ecosystem function. Using a range of methods for assessing the impact of introducing a genetically modified *Pseudomonas* into soil Naseby and Lynch [70] showed that the nature of the genetic modification is important and that some modification of functional genes in the genome of *Pseudomonas* strains can have an impact on rhizosphere population and function [70]. However, these microcosms lack the full biotic and abiotic components of a field environment. It is known that the functional capacity of the soil microbial community, as reflected in the activities of enzymes involved in nutrient mineralization processes, varies among soils dominated by different plant species. Many studies with annual and perennial plants have shown that plant species differ in exudate amount and composition and also in rhizosphere community composition [71]. Lynch et al. [2004] focused on the impact of GM plants and GM microorganisms on soil microflora. Transgenic plants do not always affect bacterial composition and when these effects have been observed, they have not been related to natural fluctuations. Indeed, relevant effects of transgenic plants on microbial communities should cause deeper changes than the commonly accepted changes due to environmental factors related to season, field site and year. They suggest that the rhizospheres could be altered in response to plant genetic transformation through HGT from GM plants to the indigenous soil microbes [72].

Soil microorganisms are the primary producers of soil enzymes and the analysis of the soil enzymatic activity is one of microbiological indicators of soil quality. Enzymes participate in numerous biochemical processes occurring in the soil and they are sensitive to all environmental changes caused by natural and anthropogenic factors. Enzymes are secreted by floral and faunal organisms, but most often they are produced by microorganisms in soil. The assessment of microbial populations in combination with their activity provides more sensitive information than either activity or population analysis alone. The impacts on soil microbial communities are therefore an important aspect of environmental risk assessment especially to monitor transgenic plants [73]. Blackwood and Buyer [74] used phospholipid fatty acid (PLFA) profiles and community-level physiological profiles of microbes to determine whether growing Bt corn had any effect on soil microbial communities as compared with the growth of non-Bt corn. They found that the profiles of the microbial communities were heavily affected by soil

ISBN:
DOI:

type, but the effect of expression of the Bt gene in corn was small.

In pot experiment in greenhouse the changes in the rhizospheric soil of two different locations of India and soil microbial communities colonizing the rhizosphere of PDH45 transgenic rice plant in comparison to its non-transgenic counterpart (WT) in the presence and absence of salt have been studied [75]. The effects of PDH45-transgene on rhizospheric soil dehydrogenase activity (DHA), phosphatase, urease and nitrate reductase activities were also evaluated. Results show that the PDH45 transgenic rice plants had no detectable adverse effects on the soil microbial community composition, physico-chemical properties and enzymatic activities of the soil rhizosphere as compared with their WT counterpart. The rhizospheric soil bacterial populations and enzyme activities revealed minor alterations among transgenic and non-transgenic rice plants. These studies have shown the possibility that there was no evidence to indicate any adverse effects of transgenic PDH45 on the native soil microflora. Similar results were obtained by on transgenic poplars have been developed to enhance the bioremediation of heavy metals [69]. Due to the important role of fungi for plant growth in natural environments and they role in ecological equilibrium, they studied the structural changes of indigenous fungal communities in rhizosphere soils of GM and wild type (WT) poplars at a range of growth stages, together with unplanted, contaminated soil. The results show that the overall structure of the rhizosphere fungal community was not significantly influenced by GM poplars. However, the presence of GM specific taxa, and faster rate of community change during poplar growth, appeared to be characteristic of the GM plant-induced effects on soil-born fungal communities. The results of this study provide additional information about the potential effects of GM poplar trees aged 1.5-3 years, on the soil fungal community [76].

Density and physiological profiles of aerobic bacteria in the rhizosphere were studied in transgenic alfalfa (*Medicago sativa* L.) plants, in comparison to their non-transgenic counterpart [77]. Plants of transgenic alfalfa expressing the AMVcp-s gene coding for Alfalfa Mosaic Virus coat protein were cultivated in a climatic chamber and different methods were used to determine the microbial diversity in rhizospheres of transgenic plants, the cultivation-dependent plating method, based on the determination of the density of colony-forming bacteria, and second, a biochemical method using the Biolog™ system, based on the utilization of different carbon sources by soil microorganisms. Results suggest that both, the community metabolic diversity and the utilization of C-sources increased in all alfalfa lines with culture time and regardless of transgenic or non-transgenic nature of lines.

Bacterial communities associated with the rhizosphere and rhizoplane regions of tobacco of wild-type (WT) plants were compared to communities found in transgenic tobacco lines (CAB1, CAB2 and TRP). The analyses revealed the presence of fairly common rhizosphere organisms with the main groups: Alpha proteobacteria, Beta proteobacteria, Actinobacteria and Bacilli. Analysis of the total bacterial communities using PCR-DGGE (denaturing gradient gel electrophoresis) revealed that shifts in bacterial communities occurred during early plant development, but the reestablishment of original community structure was observed over time. The effects were smaller in rhizosphere than in rhizoplane samples, where selection of specific bacterial groups by the different plant lines was demonstrated. Results revealed that, although rhizosphere/rhizoplane microbial communities can be affected by the cultivation of transgenic plants, soil resilience may be able to restore the original bacterial diversity after one cycle of plant cultivation [78].

The rapid development of agricultural biotechnology and release of new transgenic plants for agriculture has provided many economic benefits, but has also raised concern over the potential impact of transgenic plants on the environment. Considerable research has now been

ISBN:
DOI:

conducted on the effects of transgenic plants on soil microorganisms. These effects include unintentional changes in the chemical compositions of root exudates, and the direct effects of transgenic proteins on non-target species of soil microorganisms. Most studies to date suggest that transgenic plants that have been released causes minor changes in microbial community structures that are often transient in duration. However, due to our limited knowledge of the linkage between microbial community structure and function, more work needs to be done on a case-by-case basis to further evaluate the effects of transgenic plants on soil microorganisms and soil ecosystem functions.

Method for Mapping Distribution of Enzyme Activity in Soil: From Rhizosphere to Bulk Soil

The rhizosphere studies have been challenged by the lack of a satisfying method for obtaining sufficient soil samples for subsequent laboratorial analysis. Several procedures based on shaking or washing-off soil particles adhering to roots have been proposed for the separation of rhizosphere soil from bulk soil [79,80]. However, it is known that soil texture and moisture strongly influence the amount of soil adhering to the root system, and root induced changes on some soil variables have been observed [81]. Therefore, rhizosphere studies often involve sampling procedures that permit assessment of this gradient variation. The spatial distribution of enzyme activity has been explored in soils at different distances from root zones [37,82,83]. The common procedure consists of slicing soil in multiple segments, and determining enzyme activities in each of them. In addition, various types of rhizoboxes were proposed by several authors as experimental tools to growth plants for studying rhizosperic and bulk soils [84-86].The basic element of most rhizoboxes designs is a porous membrane (30-50 μm mesh) that separates the roots from a rootless rhizosphere compartment. The rhizosphere is then divided into several thin sections such that the chemical and biochemical properties can be analyzed at increasing distances from the root surface.

Besides being very labor intensive, the approach only provides a one-dimensional vision into enzyme activity with a very irregular spatial resolution [83,87,88].

Recently, there has been growing interest in the spatial distribution of microbial activity and enzyme activities in soil hotspots such as rhizosphere. An *in situ* zymography technique for localization and quantification of enzyme activities in soil has been recently studied [89]. Zymography has mostly been used to localize enzymatic activity in electrophoresis gels and in tissue sections, but also a zymography technique has been used for analysis of the two-dimensional distribution of enzyme activities in soil. The technique was applied to map and quantify protease and amylase activity in the rhizosphere of lupine (*Lupinus polyphyllus* Lindl.) grown in rhizoboxes. A specimen, in which a certain enzyme occurs, is incubated attached to a gel that contains the enzyme's substrate. Subsequently, the gel is stained in order to visualize the substrate and to identify the areas in which the substrate has been enzymatically degraded during the incubation. Since zymography is an *in situ* method, it likely pictures enzyme activities more realistically than standard enzyme assays.

Conclusion

The rhizosphere is a soil hot spot where the physiological footprint of plant-microorganisms interaction might be expressed by the enzyme activities profile. The enzyme activities are the driving force of degradation processes of natural compounds and xenobiotics. Scientific articles on soil enzymes in the rhizosphere constitute a very small amount within the total

ISBN:
DOI:

body of literature on soil enzymes. A web-search performed on Scopus on March, 6th, 2013, crossing the keywords “soil enzymes” and “rhizosphere” (title, abstract and keywords search fields), sorted a No. of articles ranging from 1.7% in 1993 to 8.0% in 2013 of total literature having “soil enzymes” as keyword.

When analyzed separately, all articles having “soil enzymes” or “rhizosphere” as keywords show an increasing trend in the last 20 years being much steeper for soil enzymes issues (by 32%) (Figure 3). This suggests a lower interest in rhizosphere issues in the same period. For this reason, the enzyme activity as an expression of plant-microorganisms interaction is a new challenge for future research in biotechnology applied to soil remediation and environmental protection. However, in order to achieve progress in research, a standard tool for mapping distributions of enzyme activities in soils is desirable to work in widely accessible and cost-efficient way.

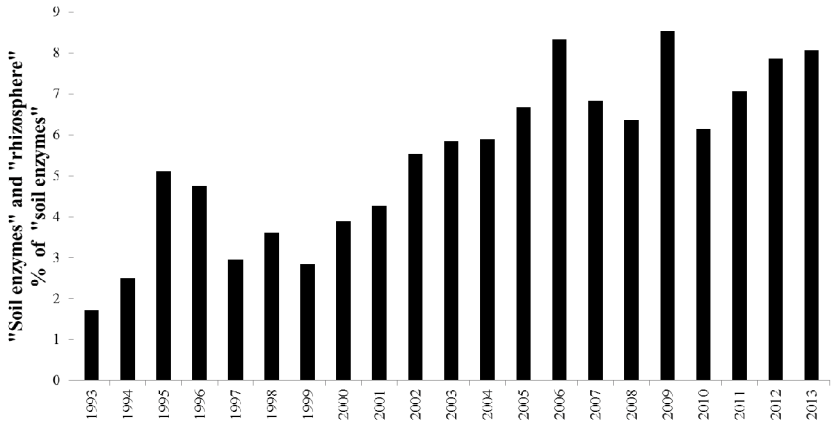

Figure 3: % of articles with both keywords “soil enzymes” and “rhizosphere” on total articles with only “soil enzymes” as keyword. The bibliographic search was performed on Scopus on March, 6th 2014.

References

1. Asmar F, Eiland F, Nielsen NE (1994) Effect of extracellular enzyme activities on solubilization rate of soil organic nitrogen. Biol Fertil Soils 17: 32-38.
2. Frank DA1, Groffman PM (2009) Plant rhizospheric N processes: what we don’t know and why we should care. Ecology 90: 1512-1519.
3. Barber DA, Martin JK (1976) The release of organic substances by cereal roots into soil. New Phytol. 76: 69-80.
4. Kuzyakov Y, Friedel JK, Stahr K (2000) Review of mechanisms and quantification of priming effects. Soil Biol Biochem 32: 1485–1498.
5. Moscatelli MC, Lagomarsino A, De Angelis P, Grego S (2005) Seasonality of soil biological properties in a poplar plantation growing under elevated atmospheric CO_2. Appl Soil Ecol 30: 162–173.
6. Marinari S, Masciandaro G, Ceccanti B, Grego S (2000) Influence of organic and mineral fertilisers on soil biological and physical properties. Bioresource Technol 72: 9–17.
7. Schimel JP, Weintraub MN (2003) The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biol Biochem 35:549–563.

ISBN:
DOI:

8. Nannipieri P, Giagnoni L, Renella G, Puglisi E, Ceccanti B, et al. (2012) Soil enzymology: Classical and molecular approaches. Biol Fertil Soils 48: 743-762.

9. Dell EA, Seth Carley D, Rufty T, Shi W (2012) Heat stress and N fertilization affect soil microbial and enzyme activities in the creeping bentgrass (Agrostis stolonifera L.) rhizosphere. Appl Soil Ecol 56: 19-26.

10. Bandick AK, Dick RP (1999) Field management effects on soil enzyme activities. Soil Biol Biochem 31: 1471-1479.

11. Richardson AE, Lynch JP, Ryan PR, Delhaize E, Smith FA, et al. (2011) Plant and microbial strategies to improve the phosphorus. Plant Soil 349: 121–156.

12. Asmar F, Gahoonia TS, Nielsen NE (1995) Barley genotypes differ in activity of soluble extracellular phosphatase and depletion of organic phosphorus in the rhizosphere soil. Plant Soil 172: 117–122.

13. Gahoonia TS, Nielsen NE (1992) The effect of root induced pH changes on the depletion of inorganic and organic phosphorus in the rhizosphere. Plant Soil 143: 185–191.

14. Chen CR, Condron LM, Davis MR, Sherlock RR (2002) Phosphorus dynamics in the rhizosphere of perennial ryegrass (Loliumperenne L.) and radiata pine (Pinus radiate D. Don). Soil Biol Biochem 34: 487–499.

15. Cocucci M, Varanini Z, Pinton R (2005) Il suolo, la pianta e la rizosfera. In: Patron (Ed.). Fondamenti di Chimica del Suolo, Bologna, Italy.

16. Ebersberger D, Niklaus PA, Kandeler E, (2003) Long term CO_2 enrichment stimulates N-mineralisation and enzyme activities in calcareous grassland. Soil Biol Biochem 35: 965-972.

17. Weintraub MN, Scott-Denton LE, Schmidt SK, Monson RK (2007) The effects of tree rhizodeposition on soil exoenzyme activity, dissolved organic carbon, and nutrient availability in a subalpine forest ecosystem. Oecologia 154: 327-338.

18. Burns RG, De Forest JL, Marxsen J, Sinsabaugh RL, Stromberger ME, et al. (2013) Soil enzymes in a changing environment: Current knowledge and future Directions. Soil Biol Biochem 58: 216-234.

19. Gramss G, Voigt KD, Kirsche B (1999) Oxidoreductase enzymes liberated by plant roots and their effects on soil humic material. Chemosphere 38: 1481-1494.

20. Kuiper I, Lagendijk EL, Bloemberg GV, Lugtenberg BJ (2004) Rhizoremediation: a beneficial plant-microbe interaction. Mol Plant Microbe Interact 17: 6-15.

21. Ryan PR, Dessaux Y, Thomashow LS, Weller DM (2009) Rhizosphere engineering and management for sustainable agriculture. Plant Soil 321: 363–383.

22. Zak JC, Willig MR, Moorhead DL, Wildman HG, (1994) Functional diversity of microbial communities: A quantitative approach. Soil Biol Biochem 26: 1101-1108.

23. Insam H, Parkinson D, Domsch KH (1989) Influence of macroclimate on soil microbial biomass. Soil Biol Biochem 21: 211-221.

24. Emmerling C, Schloter M, Hartmann A, Kandeler E (2002) Functional diversity of soil organisms - A review of recent research activities in Germany. J Plant Nutr Soil Sci 165: 408-420.

25. Wellington EM, Berry A, Krsek M (2003) Resolving functional diversity in relation to microbial community structure in soil: exploiting genomics and stable isotope probing. Curr Opin Microbiol 6: 295-301.

26. Bell TH, Henry HAL (2011) Fine scale variability in soil extra- cellular enzyme activity is insensitive to rain events and temperature in a mesic system. Pedobiology 54: 141–146.

27. Waldrop MP, Balser TC, Firestone MK (2000) Linking microbial community composition to function in a tropical soil. Soil Biol Biochem 32: 1837–1846.

28. Arnosti C, Bell C, Moorhead DL, Sinsabaugh RL, Steen AD, et al. (2014) Extracellular enzymes in terrestrial, freshwater, and marine environments: perspectives on system variability and common research needs. Biogeochemistry 117: 5–21.

29. Pajares S, Gallardo JF, Masciandaro G, Ceccanti B, Etchevers JD (2010) Enzyme activity as an indicator of soil quality changes in degraded cultivated Acrisols in the Mexican Trans-volcanic Belt. Land Degrad Dev 22: 373–381.

30. Kandeler E, Kampichler C, Horak O (1996) Influence of heavy metals on the functional diversity of soil microbial communities. Biol Fertil Soils 23:299-306.

31. Bird JA, Herman DJ, Firestone MK (2011) Rhizosphere priming of soil organic matter by bacterial groups in a grassland soil. Soil Biol Biochem 43: 718–725.

32. de Graaff MA, Classen AT, Castro HF, Schadt CW (2010) Labile soil carbon inputs mediate the soil microbial community

ISBN:
DOI:

composition and plant residue decomposition rates. New Phytol 188: 1055-1064.

33. Kuzyakov Y (2002) Factors affecting rhizosphere priming effects. Journal of Plant Nutrition and Soil Sci 165: 382-396.

34. Drake JE, Darby BA, Giasson MA, Kramer MA, Phillips RP, et al. (2013) Stoichiometry constrains microbial response to root exudation-insights from a model and a ?eld experiment in a temperate forest. Biogeosciences 10: 821–838.

35. Hinsinger P, Bengough AG, Vetterlein D, Young IM (2009) Rhizosphere: biophysics, biogeochemistry and ecological relevance. Plant Soil 321: 117-152.

36. Schimel JP, Schaeffer SM (2012) Microbial control over carbon cycling in soil. Front Microbiol 3: 348.

37. Kandeler E, Marschner P, Tscherko D, Singh Gahoonia T, Nielsen NE (2002) Microbial community composition and functional diversity in the rhizosphere of maize. Plant Soil 238: 301-312.

38. Gavrichkova O, Moscatelli MC, Kuzyakov Y, Grego S, Valentini R (2010) Influence of defoliation on CO_2 efflux from soil and microbial activity in a Mediterranean grassland. Agr Ecosyst Environ 136: 87–96.

39. Bending GD, Turner MK, Rayns F, Marx MC, Wood M (2004) Microbial and biochemical soil quality indicators and their potential for differentiating areas under contrasting agricultural management regimes. Soil Biol Biochem 36: 1785–1792.

40. Pignataro A, Moscatelli MC, Mocali S, Grego S, Benedetti A (2012) Assessment of soil microbial functional diversity in a coppiced forest system. Appl Soil Ecol 62: 115-123

41. Kennedy AC, Smith KL (1995) Soil microbial diversity and the sustainability of agricultural soils. Plant Soil 170: 75-86.

42. Magurran AE (1988) Ecological Diversity and its Measurement. Croom-Helm, London.

43. Marx MC, Wood M, Jarvis SC (2001) A microplatefluorimetric assay for the study of enzyme diversity in soils. Soil Biol Biochem 33: 1633-1640.

44. Vanegas J, Landazabal G, Melgarejo LM, Beltran M, Uribe-Vélez D (2013) Structural and functional characterization of the microbial communities associated with the upland and irrigated rice rhizospheres in a neotropical Colombian savannah. Eur J Soil Biol 55: 1-8.

45. Li J, Jin Z, Gu Q (2011) Effect of plant species on the function and structure of the bacterial community in the rhizosphere of lead-zinc mine tailings in Zhejiang, China. Can J Microbiol 57: 569-577.

46. Gardner T, Acosta-Martinez V, Senwo Z, Dowd SE (2011) Soil Rhizosphere Microbial Communities and Enzyme Activities under Organic Farming in Alabama. Diversity 3: 308-328.

47. Marschner P, Grierson PF, Rengel Z (2005) Microbial community composition and functioning in the rhizosphere of three Banksia species in native woodland in Western Australia. Appl Soil Ecol 28: 191–201.

48. Moreno B, García-Rodríguez S, Canizares R, Castro J, Benitez E (2009) Rainfed olive farming in south-eastern Spain: long-term effect of soil management on biological indicators of soil quality. Agric Ecosyst Environ 131: 333–339.

49. Montes-Borrego M, Navas-Cortés JA, Landa BB (2013) Linking microbial functional diversity of olive rhizosphere soil to management systems in commercial orchards in southern Spain. Agr Ecosyst Environ 181: 169-178.

50. Gupta A, Gopal M, Tilak KV (2000) Mechanism of plant growth promotion by rhizobacteria. Indian J Exp Biol 38: 856-862.

51. Saikia R, Kumar R, Arora DK, Gogoi DK, Azad P (2006) *Pseudomonas* aeruginosa inducing rice resistance against Rhizoctonia solani: production of salicylic acid and peroxidases. Folia Microbiol (Praha) 51: 375-380.

52. do Vale Barreto Figueiredo M, Seldin L, de Araujo FF, de Lima Ramos Mariano R (2010) Plant Growth Promoting Rhizobacteria: Fundamentals and Applications. In: Maheshwari DK (Ed.). Plant Growth and Health Promoting Bacteria, Microbiology Monographs. Springer-Verlag Berlin Heidelberg.

53. Vázquez MM, César S, Azcón R, Barea JM (2000) Interactions between arbuscula rmycorrhizal fungi and other microbial inoculants (*Azospirillum, Pseudomonas, Trichoderma*) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. Appl Soil Ecol 15: 261–272.

54. Vosátka M, Gryndler M (1999) Treatment with culture fractions from *Pseudomonas* putida modifies the development of Glomus fistolosum mycorrhiza and the response of potato and maizeplants to inoculation. Appl Soil Ecol 11: 245–251.

55. Camprubì A, Calvet C, Estaún V (1995) Growth enhancement of *Citrus reshni* after inoculation with Glomus intraradices and Trichoderma aureoviride and associated effects on microbial populations and enzyme activity in potting mixes. Plant Soil 173: 233–238.

56. Saharan BS, Nehra V (2011) Plant growth promoting rhizobacteria: a critical review. Life Sciences and Medicine Research, Volume

ISBN:
DOI:

2011: LSMR-21.

57. Rodríguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17: 319-339.

58. Rodriguez H, Fraga R, Gonzalez T, Bashan Y (2006) Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil 287: 15-21.

59. van Loon LC (2007) Plant responses to plant growth-promoting rhizobacteria. Eur J Plant Pathol 119: 243–254.

60. Saini P, Khanna V (2013) Preliminary screening for ACC-deaminase production by plant growth promoting rhizobacteria. J Pure Appl Microbiol 7: 573-576.

61. Bal HB, Nayak L, Das S, Adhya TK (2013) Isolation of ACC deaminase producing PGPR from rice rhizosphere and evaluating their plant growth promoting activity under salt stress. Plant Soil 355: 93-105.

62. Lottmann J, O'Callaghan M, Baird D, Walter C (2010) Bacterial and fungal communities in the rhizosphere of field-grown genetically modified pine trees (Pinus radiata D.). Environ Biosafety Res 9: 25-40.

63. Bruinsma M, Kowalchuk GA, vanVeen JA (2003) Effects of genetically modified plants on microbial communities and processes in soil. Biol Fertil Soils 37: 329–337.

64. Dumfield KE, Geremida JJ (2001) Diversity of bacterial communities in the rhizosphere and root-interior of field-grown genetically modified Brassica napus. FEMS Microbiol. Ecol. 38: 1-9.

65. Aulakh MS, Wassmann R, Bueno C, Kreuzwieser J, Rennenberg H (2001) Characterization of root exudates at different growth stages of ten rice (*Oryza sativa* L.) cultivars. Plant Biol 3: 139–148.

66. Natch A, Keel C, Hebecker N, Laasik E, Défago G (1997) Influence of the biocontrol strain *Pseudomonas fluorescens* CHAO and its antibiotic overproducing derivative on the diversity of resident root colonising pseudomonads. FEMS Microbiol Ecol 23: 341–352.

67. Short KA, Seidler RJ, Olsen RH (1990) Survival and degradative capacity of *Pseudomonas* putida induced or constitutively expressing plasmid-mediated degradation of 2,4-dichlorophenoxyacetate (TFD) in soil. Can J Microbiol 36: 821–826.

68. Austin HK, Hartel PG, Coleman DC (1990) Effects of genetically altered *Pseudomonas solanacearum* on predatory protozoa. Soil Biol Biochem 22: 115–117.

69. Wang ZM, Crawford DL, Magnuson TS, Bleakley BH, Hertel G (1991) Effects of bacterial lignin peroxidase on organic-carbon mineralization in soil, using recombinant Streptomyces strains. Can J Microbiol 37: 287–294.

70. Naseby DC, Lynch JM (1998) Impact of wild type and genetically-modified *Pseudomonas* fluorescens on soil enzyme activities and microbial population structure in the rhizosphere of pea. Mol Ecol 7: 617–625.

71. Marschnera P, Pauline F, Grierson B, Rengel Z (2005) Microbial community composition and functioning in the rhizosphere of three Banksia species in native woodland in Western Australia. Appl Soil Ecol 28: 191–201.

72. Lynch JM, Benedetti A, Insam H, Nuti MP, Smalla K, et al. (2004) Microbial diversity in soil: ecological theories, the contribution of molecular techniques and the impact of transgenic plants and transgenic microorganisms. Biol Fertil Soils 40: 363–385.

73. Sun T, Wang YP, Wang ZY, Liu P, Xu GD (2013) The effects of molybdenum and boron on the rhizosphere microorganisms and soil enzyme activities of soybean. Acta Physiol Plant 35: 763–770.

74. Blackwood CB, Buyer JS (2004) Soil microbial communities associated with Bt and non-Bt corn in three soils. J Environ Qual 33: 832-836.

75. Sahoo RK, Tuteja N (2013) Effect of salinity tolerant PDH45 transgenic rice on physicochemical properties, enzymatic activities and microbial communities of rhizosphere soils. Plant Signal Behav 8.

76. Hur M, Lim YW, Yu JJ, Cheon SU, Choi YI, et al. (2012) Fungal community associated with genetically modified poplar during metal phytoremediation. J Microbiol 50: 910-915.

77. Faragova N, Gottwaldovà K, Farago J (2011) Effect of transgenic alfalfa plants with introduced gene for Alfalfa Mosaic Virus coat protein on rhizosphere microbial community composition and physiological profile. Biologia 66: 768-777.

78. Andreote FD, Mendes R, Dini-Andreote F, Rossetto PB, Labate CA, et al. (2008) Transgenic tobacco revealing altered bacterial diversity in the rhizosphere during early plant development. Antonie van Leeuwenhoek, International J Gen Molec Microbiol 93: 415-424.

79. Luster J, Go¨ ttlein A, Nowack B, Sarret G (2009) Sampling, defining, characterising and modeling the rhizosphere–the soil science tool box. Plant Soil 321: 457–482.

80. Yanai RD, Majdi H, Park BB (2003) Measured and modeled differences in nutrient concentrations between rhizosphere and bulk

soil in a Norway spruce stand. Plant Soil 257: 133–142.

81. Jungk A, Claassen N (1997) Ion diffusion in the soil–root system. Adv Agron 61: 53–110.

82. Haase S, Philippot L, Neumann Marhan S, Kandeler E (2008) Local response of bacterial densities and enzyme activities to elevated atmospheric CO_2 and different N supply in the rhizosphere of *Phaseolus vulgaris* L. Soil Biol Biochem 40: 1225-1234.

83. Tarafdar JC, Jungk A (1987) Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. Biol Fertil Soils 3: 199-204.

84. Aviani I, Laor Y, Raviv M (2006) Limitations and potential of *in situ* rhizobox sampling for assessing microbial activity in fruit tree rhizosphere. Plant Soil 279: 327–332.

85. Wenhao Y, Hong H, Mei R, Wuzhong N (2013) Changes of microbial properties in (near-) rhizosphere soils after phytoextraction by Sedum alfredii H: A rhizobox approach with an artificial Cd-contaminated soil. Appl Soil Ecol 72: 14–21.

86. Azimzadeh Y, Shirvani M, Shariatmadari H (2014) Green manure and overlapped rhizosphere effects on Pb chemical forms in soil and plant uptake in maize/canola intercrop systems: a rhizobox study. Soil Sedim Contamin Int J 23: 677-690.

87. Gahoonia TS, Nielsen NE (1991) A method to study rhizosphere processes in thin soil layers of different proximity to roots. Plant Soil 135: 143-146.

88. Kuzyakov Y, Raskatov AV, Kaupenjohann M (2003) Turnover and distribution of root exudates of *Zea mays*. Plant Soil 254: 317-327.

89. Spohn M, Carminati A, Kuzyakov Y (2013) Soil zymography a novel *in situ* method for mapping distribution of enzyme activity in soil. Soil Biol Biochem 58: 275-280.

ISBN:
DOI:

Chapter: 5

Enzymes and Pesticides

M Srinivasulu* and V Rangaswamy

Department of Microbiology, Sri Krishnadevaraya University, Anantapuram, Andhra Pradesh, India

***Corresponding author:** M Srinivasulu, Department of Microbiology, Sri Krishnadevaraya University, Anantapuram- 515003, Andhra Pradesh, India, Tel: 91-8554-255759; E-mail: mandalasrinivasulu@yahoo.in

Abstract

Pesticides are usually applied simultaneously or one after another for crop protection, and this type of pesticide application often leads to a combined contamination of pesticide residues in soil environment. Entry of pesticides in soils due to agricultural practices may disturbs the activities of soil enzymes, such as, arylamidase, dehydrogenase, myrosinase, phosphatase, protease, urease, amylase, cellulase, invertase, arylsulfatase and fluorescein diacetate hydrolase; thus affecting recycling of nutrients and soil fertility. Soil enzymes, that represent the major living organism activities, are involved in catalyzing various biochemical reactions necessary for organic matter decomposition, nutrient cycling, energy transfer and productivity. Literature survey reveals that pesticides decrease the activities of enzymes that are key indicators of soil health, whereas some studies show that pesticides enhance the enzymatic activities only at particular concentrations (2.5 to 5.0 kg ha^{-1}) in soils. In this chapter, we attempt to analyze the effect of pesticides on soil enzyme activities.

Introduction

The overall biochemical reactions of soil results from a series of reactions catalyzed by enzymes, either as intracellular components of the microbial community or as extracellular (cell-free) enzymes. Cell-free enzymes exist in soil as a result of their excretion into the soil by living cells or after lyses of dead plant or microbial cells. Soil enzymes catalyze physico-chemical and biochemical reactions involved in nutrient cycling [1]. Measurements of several enzymatic activities have been used to establish indices of soil fertility [2]. Arylamidase activity of soils has an intensive role in N-mineralization [3]. Activity of myrosinase enzyme in soils is responsible for hydrolysis of glucosinolates to glucose in microorganisms [4]. Dehydrogenases oxidize and release inorganic nutrients from organic carbon [5]. Phosphatases are hydrolytic enzymes that cleave organic P to inorganic forms [6]. The enzyme protease is essential for mineralization of N [7], whereas, urease enzyme influences the availability of plant utilizable N in soils fertilized with urea [8]. Amylase enzyme mainly involved in the hydrolysis of polysaccharides in soil. Invertase catalyzes the hydrolysis of sucrose to glucose and fructose, and is abundantly available in soil microorganisms and plants. Cellulase is a key enzyme in the carbon cycle; it acts on the cellulosic material present in soil ecosystem. Arylsulfatase is responsible for the hydrolysis of sulfate esters in the soil [9]. Enzymes may react to changes in soil management more quickly than other variables and therefore may be useful as early indicators of biological changes [10].

ISBN:
DOI:

The activities of microorganisms in soil are crucial to the global cycling of carbon, nitrogen, sulphur, phosphorus and other elements, because of many substances cannot be degraded by organisms other than microbes [11]. Microorganisms are the foremost producers of enzyme activities in soil [12]. Accordingly, the biochemical activity of accumulated enzymes for certain reactions has been estimated to be more important than that of the microbial cells [13]. Soil enzymes play an important role in catalyzing reactions for the decomposition of organic matter and nutrient recycling in ecosystems [14] involving a range of plants, microorganisms, animals and their debris [15].

Rapid raise in population density and advancement in agricultural technology has led to a greater release of xenobiotic compounds into the environment. The major sources of xenobiotics applied are pesticides, which are in high demand these days to control pests and diseases as well as to protect the agricultural products from microbial spoilage during transition. Pesticides, which include insecticides, fungicides and herbicides, have become an integral part of modern agricultural systems because their use has benefited modern society by improving the quantity and quality of the world's production while keeping the cost of that food supply reasonable. Because of continuous pest problems, their usage possibly cannot be discontinued in the near future since the greatest contribution toward the control of these pests has come from the use of pesticides. Different pesticides used in agriculture exhibit different time in the environment, with some persisting even indefinitely. Repeated and extensive application of the pesticides ultimately reaches the soil, which in turn may interact with soil organisms and their metabolic activities [16]. Microbial processes play an important role in bringing about the biological transformation of pesticides. Therefore, the behavior of the total microflora and their biological activity (enzyme activities) under continue pesticide input is an important aspect in the agricultural ecology [17]. Microorganisms are the dominant producers of enzyme activities in soil. Due to widespread use of agricultural chemicals in crop production, considerable attention has been given to soil biochemical and microbial testing programs to assess possible side effects [11]. Interaction between pesticides on non-target microflora present in soil, may eventually lead to not only alteration in life function of individual microflora but also disturbance in equilibrium among the soil enzymes thereby affecting the primary function (fertility) of the soil.

Impact of Pesticides on Soil Enzyme Activities

Soil enzyme activities have been suggested as suitable indicators of soil quality because; a) they are a measure of soil microbial activity and therefore they are strictly related to the nutrient cycles and transformations b) they are easy to measure [18,19]. According to several authors [20,21] soil enzyme activities may be considered early and sensitive indicators to measure the degree of soil degradation in both natural and agro-ecosystems. Many studies have shown that biological parameters have been used to assess soil quality and health as affected by agricultural practices [22-24]. In this respect, soil enzymes can be used as potential indicators of soil quality for sustainable management because they are sensitive to ecological stress and land management practices [25].

Negative impact of pesticides on soil enzymes like hydrolases, oxidoreductases and dehydrogenase activities has been widely reported in the literature [26-29]. Pesticides reaching the soil may disturb local metabolism or enzyme activities [30-32]. There is also evidence that soil enzymatic activities and ATP contents are increased by some pesticides [33,34]. ATP contents are correlated with specific soil enzyme activities and may provide valuable information on trends in transformation of pesticides in soils [35]. Enzyme activity in soils

ISBN:
DOI:

reflects not only enzymes in soil solution and living tissue, but also enzymes bound to soil colloids and humic substances [36,37]. Enzyme activity is influenced by soil conditions such as organic matter content [38-40], moisture [41,42]), and temperature [43]. A number of factors, for example, chemical nature of pesticides, concentration used, microbial community structure, type of soil, and soil conditions can contribute to divergent research findings. Malkomes [44] accredited such differences to the dual behaviour of pesticides (both harmful and beneficial for soil enzymes), diversity and various stages of the processes taking place in soil that are recurrently overlapped. The impact of pesticides on soil enzymes particularly extracellular enzymes are not clear due to their multidimensional behavior in complex soil medium and the greater complexity of soil microbial and biochemical interactions. For these reasons, researchers are faced with difficulties in discriminating the effects of pesticides on extracellular enzyme activities in soil [45].

Arylamidase enzyme

The enzyme arylamidase [α-aminoacylpeptide hydrolase (microsomal) catalyzes the release of an N-terminal amino acid from peptides, amides, or arylamides. Arylamidase is widely distributed in the tissues and body fluids of all animals [46]), plants, and microorganisms [47]. The chemical nature of N in soils is such that a large proportion (15–25%) of organic N is often released as NH_4^+ by 6 M HCl hydrolysis. Several papers have been published on linear amidase in soils and on the enzyme involved in hydrolysis of amides and aminoacids such as aspargine, aspartic acid, and glutamine [48], but the possibility of the presence of arylamidase in soils has not been explored. The activity of this enzyme in soils deserves investigation because present knowledge indicates that a variety of arylamides are present in soils [49]. Arylamidase may play an important role as an initial limiting step in mineralization of organic N in soils. Thus understanding the environmental controls on the activity of this enzyme in soil is important for better understanding the N-cycling process. This enzyme is capable of hydrolyzing the neutral amino acid β-naphtylamides or p-nitroanilides according to the following reaction (Using the amino acid L-leucine as an example).

Arylamidase activity increased in all individual and binary mixtures of pesticide treated and 10-day incubated soils up to 2.5 or 5.0 kg ha^{-1} than the controls. The enzyme activity continued up to 20 days and then decreased gradually after 30 and 40 days of incubation [50]. Floch et al. [51] reported that arylamidase activity, varied with incubation time, but tended to return to its initial soil background level after prolonged period (12 months) of incubation with pesticides at 100 μg g^{-1} soil (10 kg ha^{-1}). Monocrotophos either singly or in combination improved arylamidase activity significantly in 10-day incubated soils. Monocrotophos at concentrations ranging from 1.0 to 5.0 kg ha^{-1} increased the arylamidase activity gradually and reached maximum at combination improved the arylamidase activity significantly in 10-day incubated soils. Monocrotophos at concentrations ranging from 1.0 to 5.0 kg ha^{-1} increased the arylamidase activity gradually and reached maximum at the concentration of 5.0 kg ha^{-1} in both soils. Application of monocrotophos above 5.0 kg ha^{-1} showed negative effect on arylamidase activity and exhibited minimum activity at 10.0 kg ha^{-1}. Chlorpyrifos at the concentrations of 1.0 and 2.5 kg ha^{-1} showed marked increase in arylamidase activity and above this concentration the activity decreased gradually and reached minimum at 10.0 kg ha-1 in vertisol and laterite soils. The combination of monocrotophos with mancozeb and chlorpyrifos with carbendazim increased arylamidase activity at 1.0 and 2.5 kg ha^{-1} of each pesticide in both soils. However, amendment of the soils with higher concentrations of pesticides (7.5–10 kg ha^{-1}) resulted in minimum enzyme activity [50].

ISBN:
DOI:

Dehydrogenase enzyme

Soil dehydrogenase activity is considered as a valuable parameter for assessing the side effects of pesticide treatments on the soil microbial biomass and can also be used as an indicator of the micrbiological redox system [52]. Dehydrogenases conduct a broad range of oxidative activities that are responsible for the degradation, i.e. the dehydrogenation, of organic matter. In a cascade of events involving specific carriers, electrons are transferred from substrate to oxygen as the final acceptor [11]. Many specific dehydrogenases transfer the liberated hydrogen atoms to either NAD or NADP, thus taking part in the oxidoreductive processes of biosynthesis. Dehydrogenase can be regarded as an indicator of the overall microbial activities of soil [36]. Dehydrogenase activity has been recommended as a useful indicator for testing the side effects of agrochemicals [53].

Mayanglambam et al. [54] studied the effect of organophosphate insecticide (quinalphos) on dehydrogenase activity (DHA) in soil and observed 30% ($p<0.05$) inhibition in DHA after 15 days. DHA was recovered after 90 days of treatment which may be due to adaptation of soil microbes to counter the effect of chemical stress in hostile conditions. Similar observations were made with the application of insecticides [26,55] fungicides [27,56], herbicides [30,57], and fumigants [58,59]. Klose et al. [60] reported that soil fumigation reduced DHA activity to 35% over a period of 90 days. However, there are reports which reveal the stimulatory effects of pesticides on soil DHA [61,62]. Singh and Kumar [63] revealed that acetamiprid increased dehydrogenase activity up to 22% after first insecticide application. Other reports show variable results. Chen et al. [64] studied three fungicides and all three fungicides had different effects on DHA in the amended soils. Metalaxyl (fungicide) application initially increased and then decreased the DHA in fungicide-treated soil [65]. He et al. [66] did not observe any inhibiti on of dehydrogenase enzyme with the application of herbicide. The strong inhibition of DHA was also found as a microbial response to soil amendment with mefenoxam and metalaxyl [27] as well as azoxystrobin, tebuconazole and chlorothalonil [67].

Tu [68] reported no inhibition of dehydrogenase activity with treatments of chlorfenvinphos, chlorpyrifos, diazinon, ethion, ethoprophos, fensulfothion, fonofos, leptophos, malathion, parathion, phorate, thionazin, triazophos, trichloronate, terbufos and permethrin all at 5 and 10 mg kg^{-1} within 7 days in a clay loam soil. When the same pesticides were applied to an organic soil with 26.8% organic carbon and a pH of 7.2, dehydrogenase activity was reduced by 60% with terbufos, triazophos and trichloronate after one week, but after 2 weeks several of the pesticides stimulated dehydrogenase activity [69]. Application of fenamiphos at rates from 0 to 5.0 kg ha^{-1} soil throughout the 20-day incubation had no inhibitory effect on dehydrogenase activity whereas soil with higher rate of application at 10 kg ha^{-1} showed slight inhibition only at 10 days [70]. Furthermore, stimulation in the dehydrogenase activity in soil with lower concentrations at 20 days may probably be due to active metabolism of the compound by microbes.

However, significant increase in dehydrogenase activity was noticed with the lower concentrations of 0.5 and 5 µg g^{-1} of five pyrethroid insecticides, permethrin (FMC 33297), FMC 45498, Shell WL41706, Shell WL43467 and Shell WL43775 after 3 weeks of incubation [71]. The dehydrogenase activity was increased significantly with increasing concentrations of two organophosphorus insecticides, monocrotophos and quinalphos and two pyrethroids cypermethrin and fenvalerate up to the concentration of 2.5 kg ha^{-1}, but higher rates were either innocuous or toxic to the dehydrogenase activity during 7 days of incubation [72]. Likewise, methyl parathion at 15 kg ha^{-1} stimulated soil dehydrogenase activity [73], but complete

ISBN:
DOI:

inhibition of the activity by the same insecticide at 150-300 kg ha^{-1} at temperatures of 12-15°C in soils was noticed [74]. Similarly, insecticide such as tefluthrin and two experimental phosphorothioates DOWCO 429X and DPX 43898 at the concentration of 10 mg kg^{-1} stimulated dehydrogenase activity in a sandy loam soil during 2 weeks while dehydrogenase activity was initially reduced by tefluthrin and unaffected by the other pesticides in an organic soil after 2 weeks [75]. In an alluvial soil, methidathion, methoate and parathion reduced the dehydrogenase activity after 6 weeks at recommended field rates [76]. Dehydrogenase activity was decreased by chlorpyrifos at 2-10 kg ha^{-1} but recovered after 14 days to levels of control [77]. Insecticides alone and in combination with fungicides, in cultivation of groundnut, at field application rates (2.5–5.0 kg ha^{-1}) enhanced the activity of dehydrogenase significantly in vertisol and laterite soils. However, higher concentrations (7.5 and 10 kg ha^{-1}) of the pesticides were either innocuous or toxic to the enzyme activities in soils [50].

Myrosinase enzyme

Myrosinase is found in all glucosinolate-containing plants such as *Brassica* species and possibly in some bacteria and fungi [78]. Glucosinolates are sugar anionic thioesters containing β-thioglucoside-type bonds [79]. Myrosinase is normally segregated from glucosinolates in plant tissues, but when plant cells are damaged or decomposed; myrosinase is released and catalyzes the hydrolysis of glucosinolates. The products of glucosinolate hydrolysis include glucose, sulfate, and a number of active allelochemicals such as isothiocyanates, nitriles, thiocyanates, cyanides, and others depending on substrates and reaction conditions used [80]. These allelochemicals have been found to inhibit weed seed germination and some pathogens in soil [81,82]. Recently, several studies have proposed the use of glucosinolate-containing plants a cover crop to reduce the use of synthetic pesticides [83].

Glucosinolates by themselves are not biologically active but must be enzymatically hydrolyzed by myrosinase to the allelochemicals capable of suppressing weeds and pathogens [84]. Myrosinase is, thus, the key factor for allelochemical expression derived from glucosinolates and hence its study in soil is of interest. Myrosinase is thought to be released to soils cropped with *Brassica* or the related *Sinapis* via root exudation, disruption and decomposition. Soils cropped with Brassica, therefore, are expected to have enhanced myrosinase activity. Currently, no assay has been developed to measure myrosinase activity in soil although Borek et al. [85] have studied this enzyme in soil extracts. Extraction of enzymes from soil, however, is a demanding procedure and is often incomplete [86]. Moreover, the conditions used by Borek et al. [85] to extract myrosinase possibly altered its activity [87]. The procedure to measure myrosinase activity in soil extracts also involved the use of a gas chromatograph coupled to a mass spectrometry, which is not commonly available in many laboratories.

Myrosinase activity increased in all individual and binary mixtures of pesticides treated soils up to 7.5 kg ha^{-1} than the controls in 10-day incubated soil samples. The enzyme activity continued up to 20 days and then decreased gradually after 30 and 40 days of incubation. Monocrotophos either singly or in combination improved the myrosinase activity significantly in 10-day incubated soil samples. Monocrotophos, chlorpyrifos alone and combination with mancozeb and carbendazim respectively, at concentrations ranging from 1.0 to 5.0 kg ha^{-1} increased the myrosinase activity gradually and reached maximum at the concentration of 5.0 kg ha^{-1} in both the soil samples. Above 5.0 kg ha^{-1} monocrotophos, chlorpyrifos alone and in combination with mancozeb and carbendazim, respectively, showed minimum enzyme activity and exhibited negative effect at 10.0 kg ha^{-1}. All four pesticides exhibited maximum stimulation in enzyme activities at 2.5 or 5.0 kg ha^{-1} throughout the incubation period

ISBN:
DOI:

suggesting synergistic interaction. Chlorpyrifos alone and in combination with carbendazim at 10 kg ha^{-1} showed 27 and 33% inhibition in arylamidase activity respectively in vertisol, whereas in laterite soil same pesticides exhibited 29 and 13% inhibition in enzyme activity in comparison to control indicating antagonistic interaction [50].

Phosphatase enzyme

Phosphatase is an exocellular enzyme produced by many soil microorganisms and is responsible for the hydrolysis of organic P compounds to inorganic P [27]. Several researchers have shown either unchanged, increase or decrease in urease activity following various pesticide applications [57,64,88,89]. Demanou et al. [56] did not observe significant effect of ridomil fungicide on phosphatases activities in soil. These enzymes may be protected from degradation by adsorption to clays or to humic substances in soil [90]. This protection of these exoenzymes may result their insensitivity toward the fungicide application [56]. Phosphatases represent a broad range of intracellular as well as soil-accumulated activities that catalyse the hydrolysis of both the esters and anhydrides of phosphoric acid [91]. Klose et al. [60] reported that soil fumigation reduced the activity of acid phosphatase to 22% over a period of 90 days. However, decrease in this enzyme may be ascribed to the suppression of a sensitive fraction of soil biota. The activities of both phosphatases were also inhibited in a sandy loam soil amended with captan during 94 days of incubation [92]. Other studies showed different responses of phosphatases to the addition of metalaxyl and mefenoxam into soil, indicating the significance of chemical nature of fungicides for the results ascertained [27].

Phosphatases find widely in bacteria to mammals, and indicate their importance in fundamental biochemical processes [93]. The term phosphatase in soil is used to describe a group of enzymes that are responsible for the hydrolytic cleavage of a variety of ester-phosphate bonds of organic phosphates and anhydrides of orthophosphoric acid (H_3PO_4) into inorganic phosphate. Acid and alkaline phosphatases particularly hydrolyse the ester bonds binding P to C (C-O-P ester bonds) in organic matter. During the process, inorganic P is released from organically bound P such as leaf litter, dead root systems, and other organic debris without concomitant release of carbon [94]. Phosphatase is concentrated in the surface layer and rhizosphere where most of the fresh and less humified organic matter is prevailing [95,96]. Phosphatases play a crucial role in the phosphorous acquisition of plants and microorganisms, and thus in the cycling of it within the soil [97].

Chlorpyrifos, terbufos and fonofos increased activities of acid phosphatase in loam soil sites in the field [98]. Two organophosphorus insecticides, monocrotophos and quinalphos and two pyrethroids, cypermethrin and fenvalerate all at the lower concentrations of 1-5 kg ha^{-1} was significantly stimulatory to the phosphatase activity but these insecticides were inhibitory to the activity at higher concentrations i.e. 7.5 kg ha^{-1} in four experimental soils [99]. Four insecticides, tefluthrin, DOWCO 429X and DPX 43898, when applied at 10 mg kg^{-1}, induced a reduction of activity in an organic soil but stimulation in a sandy soil [75]. Chlorpyrifos inhibited the phosphate-mobilizing bacteria temporarily over a period of 3 months in a clay soil [100]. Pesticide, fenitrothion [101] had no effect on the activity of phosphatase in soil. Acid and alkaline phosphatase activities significantly decreased initially at levels of 2-10 kg ha^{-1} by chlorpyrifos but recovered after 14 days to levels similar to those in control soil without chlorpyrifos [77].

Phosphatase activity was increased in all individual and binary mixtures of pesticide-treated soils up to 7.5 kg ha^{-1} than the controls in 10-day-incubated soil samples. The enzyme activity continued up to 20 days and then gradually decreased after 30 and 40 days

ISBN:
DOI:

of incubation [102]. On the contrary, application of mancozeb in potato field soils showed inhibition on phosphatase activity throughout the incubation periods (15, 30, 45 and 65 days) [34]. Monocrotophos either singly or in combination significantly improved the phosphatase activity in 10–day-incubated soil samples. Monocrotophos at concentrations ranging from 1.0 to 5.0 kg ha^{-1} gradually increased the phosphatase activity and reached maximum at the concentration of 5.0 kg ha^{-1} in both the soil samples. Beyond 5.0 kg ha^{-1} monocrotophos showed negative effect on phosphatase activity and showed minimum activity at 10.0 kg ha^{-1}. At the end of the 10-day incubation, about 28–90% increase in phosphatase activity was observed in black soil and 12–95% increase was observed in red soil treated black soil and 54–126% increase was observed in red soil treated with chlorpyrifos in comparison with control soil samples. Rangaswamy and Venkateswarlu [99] reported comparable stimulatory effects of two organophosphate pesticides, monocrotophos and quinolphos, and two pyrethroids, cypermethrin and fenvalerate, at 1–5 kg ha^{-1} on the phosphatase activity. Similar stimulatory effect by cholorpyrifos on phosphatase activity in the field conditions was noticed [98]. On the contrary, tefluthrin, DOWCO 429X and DPX 43,898, when applied even at 10 mg kg^{-1} in induced a reduction in phosphatase activity in an organic soil, but stimulation in a sandy soil was reported [75]. The combination of monocrotophos along with mancozeb and chlorpyrifos along with carbendazim showed increase in phosphatase activity at 1.0 and 2.5 kg ha^{-1} of each pesticide in both the soils. But higher concentrations of pesticides at the level of 7.5-10 kg ha^{-1} have inhibitory effect on the phosphatase activity and represents antagonistic interaction. In black soil, monocrotophos along with mancozeb showed 38–83% increase, and same combination in red soil showed 58–117% inphosphatase activity at the end of 10-day incubation. Similar stimulation on the activity of phosphatase with mancozeb alone was demonstrated [103]. The combination of chlorpyrifos and carbendazim showed 24–64% increase in phosphatase activity in black soil, and in red soil, the activity increased from 46 to 104% over controls.

Protease enzyme

Protease enzymes contribute to the breakdown of proteinaceous substances in soil to simpler nitrogen compounds that are available for plant nutrition. It has been shown that proteases in soil can hydrolyse not only added [104] but also native soil proteins and peptides [105]. Proteases, which are widely distributed among soils, show a wide range of activities [106] and properties [107]). Discharge of effluents from sugar industry, enhanced the soil protease activity but it declined with the time. In a study of Rangaswamy et al. [72], insecticides, monocrotophos and quinalphos of organophosphates and cypermethrin and fenvalerate of pyrethroid within a range of 2.5 kg ha^{-1} significantly stimulated the protease activity in a soil but these insecticides at higher concentration were toxic to the protease activity.

Protease activity drastically decreased at higher concentrations (5.0, 7.5, 10.0 kg ha^{-1}) of endosulfan and profenophos treated soils than the untreated controls throughout the experiment, suggesting that the enzyme is rather sensitive to endosulfan and profenophos. Interestingly, a stimulatory effect was observed at 10-25 ppm concentrations with individual increments of two insecticidal treatments, than the control, they are as follows: 13-47% and 2-15% in black clay soil after10 days of incubation. This trend follows up to 20 days of incubation, when further prolonged in the period of incubation up to 40 days; a decline in enzyme activity was observed [108]. The impact of different concentrations (1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1}) of two selective fungicides, propiconazole and chlorothalonil on protease activity has been studied in two groundnut soils (laterite and vertisol) supplemented with 1% casein. Interestingly, stimulatory effect was observed with all concentrations tested at 10-

ISBN:
DOI:

day incubation period in both soils. The percentages of increasing in protease activity of the two fungicidal treatments, over control are as follows: 14-47% and 2-15% in laterite soil and 7-65%, 17-48% in vertisol soil respectively at 10-day interval over control (fungicides treated at 10, 25, 50 ppm level). However, stimulatory effect was more pronounced at 5.0 kg ha^{-1} of propiconazole and chlorothalonil in both soils incubated for 10-days. This trend follows up to 20 days of incubation further prolong in period of incubation up to 40 days decline in enzyme activity was observed [109].

Urease enzyme

Urease catalyzes the hydrolysis of urea to ammonium and carbon dioxide. In as much as the ammonium formed represents a bioavailable form of nitrogen for plant uptake, this ubiquitous activity has a primary role in the cycling of nitrogen. Urease activity in soil originates predominantly from microorganisms and is correlated with the soil organic matter content [110]. Urease, in particular, has attracted a good deal of attention due, in part, to the increasing agricultural importance of its substrate, urea [111]. Urease activity is a useful indicator to evaluate the soil pollution situation. Decreased urease activity in soil with the application of pesticides reduces urea hydrolysis which is generally beneficial, because it helps to maintain N in a form (NH4+) less leachable [88]. Yang et al. [112] showed that chlorimuron ethyl and furadan activated urease in the four soils. The chlorimuron ethyl and furadan enhanced urease activity up to 14–18% and 13–21%, respectively. Contrarily, acetamiprid reduced up to 35% urease activity in soil at 43 days after crop sowing [63]. Similar observations have been reported in case of phosphatase activity in soil [27,56,99,113]. Acid and alkaline phosphatases are mostly found in microorganisms and animals [114,115].

Urease activity was unchanged by glyphosate, paraquat, trifluralin and atrazine at 5.4, 2.2, 3.4 and 2.2 kg ha^{-1}, respectively in a sandy loam soil during the 8-week experiment [116]. Similarly, 0.5 and 5 μg g^{-1} levels of pyrethroids, permethrin (FMC 33297), FMC 45498, Shell WL41706, Shell WL43467 and Shell WL43775 were found to have no effect on urease activity in a sandy loam soil [71]. No effect was observed on urease activity with four herbicides including glyphosate and one insecticide malathion at 11.5 and 14.8 mg kg^{-1}, respectively [117]. The addition of the phosphorothioates (organophosphorus), fenitrothion, malathion and phorate at elevated doses between 50 and 1000 mg kg^{-1} strongly inhibited the urease activity by 40-50% especially at the highest doses throughout 60 days in a sandy clay loam sand and a silt loam soil [118]. Complete metabolism of the parent compound, malathion within 60 days in this study suggested that the inhibition of activity was mediated by one or several metabolites of malathion rather than by the parent compound. Profenofos at 6.4 and 38.4 μg g^{-1} accelerated urease activity for 6 weeks after soil treated but inhibited the urease activity after longer periods [119]. Several pesticides at 5 and 10 mg kg^{-1} stimulated the urease activity but trichloronate at 5 mg kg^{-1} reduced the urease activity significantly in a clay loam soil [68]. In a similar study, in an organic soil, urease activity was inhibited by all of these pesticides at 10 mg kg^{-1}. Urease activity was significantly enhanced by monocrotophos, quinalphos, cypermethrin and fenvalerate all at the concentrations ranging from 1 to 5 kg ha^{-1}, especially the activity was more pronounced or striking at 2.5 kg ha^{-1} but above this level i.e. 7.5 and 10 kg ha^{-1} affected a significant inhibition in four soils under laboratory conditions [120]. Similarly, slightly increased activity occurred in a sandy loam and an organic soil treated with tefluthrin, DOWCO 429X and DPX 43898 at 10 mg kg^{-1} [75]. Glyphosate at 0.3 and 1.5 mM enhanced the urease activity of soils by 1.1-1.4-fold and soil extracts by 2.59-6.73-fold whereas no significant effects were detected on the activity of jackbean urease, either free in solution or absorbed on montmorillonite [121]. Parathion, methidathion and methoate at recommended

ISBN:
DOI:

field rates had no effect on urease activity during about 6 weeks [76]. Fenamiphos at 18.6 kg ha^{-1} reduced the activity of urease under field conditions, but after 5 months activity was same as in control while no effect was observed under laboratory conditions [42,122]. Reduced urease activity was observed with fenamiphos but recovered after one month [123].

Amylase enzyme

Amylase catalyzes the hydrolytic depolymerisation of polysaccharides in soil [124]). Starch-hydrolysing enzymes are usually extracellular and inducible, but the activity of microorganisms to form amylolytic enzymes depends on the type of starch [125]. Two amylases α-and β-amylases may be concerned in the breakdown of starch and related oligo- and poly-saccharides that contain D-glucose units [11]. Both enzymes accumulate in soil, with β-amylase activity prevailing. Amylase enzyme assay is based on the hydrolysis of soluble starch and subsequent analysis of the reducing sugar [126]. Amylase was not affected by tefluthrin, DOWCO 429X and DPX 43898 at 10 mg kg^{-1} in a sandy loam soil, but the activity was enhanced by the pesticides when applied to an organic soil [75]. Similarly, fenamiphos at 18.6 kg ha^{-1} [122] had no adverse effects on amylase activity.

Amylase activity was more pronounced by two organophosphorus insecticides, monocrotophos and quinalphos and two pyrethroids, cypermethrin and fenvalerate all at the concentrations ranging from 1 to 2.5 kg ha^{-1}, but these insecticides at higher concentrations of 5 and 10 kg ha^{-1} were toxic to amylase activity in groundnut soils [127]. Tu [128] reported that malathion and permethrin at a high level after 3 days were stimulatory in the formation of glucose from added starch. Stimulation in the activity of amylase was observed with chlorfenvinphos, chlorpyrifos, diazinon, ethion, ethoprophos, fensulfothion, fonofos, leptophos, malathion, parathion, phorate, thionazin, triazophos, trichloronate, terbufos, permethrin and glyphosate all at 5 and 10 mg kg^{-1} with maximum stimulation by chlorpyrifos and phorate [126].

Amylase activity showed a variable pattern in response to different insecticide concentration after 10 days of incubation. The activity of amylase increased at lower dosage and decreased at higher concentration of pesticides in comparison to controls in black and red clay soils. The maximum activity was observed at 2.5 kg ha^{-1} (stimulatory) for flubendiamide, spinosad. Amylase activity showed an individual increment of 35–61, 29–58, 0–45, 8–50 %, in black clay soil and 61–133, 31–100, 39–50, 15–85 %, in comparison to control at 24 and for 72 hours received 2.5 and 5.0 kg ha^{-1} respectively in red clay soil. With the increase in incubation periods, the stimulated enzyme activities were also increased up to 20-days further increase in the incubation decrease in the enzyme activity was noticed [129]. These results were in contrast with the several researcher works [68,69,126,128,130,131], triazophos, a phosphorothioate triazole is stimulated for amylase at 5 and 10 mg/kg incubated for 3 days in an organic soil. As per the observation made by the Prasad and Mathur [132] the amylase activity increased during germination in both control, and Cuman treated seeds at 0.25, 0.5, 0.75 and 1% respectively. Interaction effects on soil enzyme activities, including amylase activity received least attention. There were only isolated reports on interaction effects between two chemical compounds in axenic culture studies with algae, cyanobacteria and fungi [133-135]. Kennedy and Arathan ([136] reported that application of carbofuran at 1 and 1.5 kg ha^{-1} significantly reduced the activity of soil enzymes, viz., alpha -amylase, beta -glucosidase, cellulase, urease and phosphatase up to 30 days after carbofuran application. However, application of carbofuran at the recommended level (0.5 kg ha^{-1}) had no significant effect upon the activity of soil enzymes, which are biologically significant as they play an important role not only in the

ISBN:
DOI:

soil chemical and biological properties but also affect the nutrient availability to plants. The activity of amylase enhanced significantly at first and then decreased with increase in period of incubation [120,137].

Invertase enzyme

Invertase catalyse the hydrolysis of sucrose to D-glucose and D-fructose, and is widely distributed in microorganisms, animals and plants [138]. Awfully little information is available regarding interaction of pesticides on soil invertase activity. Tu [126] postulated that application of ethion, phorate and trichloronate at 5 and 10 mg/kg stimulated invertase activity for 3 days in an organic soil. Similar evidence was noted in sandy clay soils [128]. According to Srimathi and Karanth [139], hexachlorocyclohexane and its isomers had a steady increase on soil enzyme activity after 2 weeks at 10 and 100 ppm. Rate of invertase activity followed same trend of increase to that of amylase activity in two agriculture soils as reported by Rangaswamy and Venkateswarlu [120] which was further observed by Gianfreda, et al. [121] with carbaryl at 1 mM in soils. Conversely Endo et al. [140] reported that enzyme activity was not affected on treatment with cartap HCl at higher concentrations of 10, 100 and 1000 ppm in soils. Similar reports were obtained by Ross et al. [42] and Ross and Speir [122], at 18.6 kg/ha of fenamiphos. The non-toxic nature was further revealed by Tu [75] in soil samples receiving experimental insecticides like 2,4-D, dieldrin, permethrin, carbofuran and chlorfenvinfos. Singh et al. [141] observed similar result with fenamiphos and chlorpyrifos on invertase activity.

On the contrary, Palaniappan and Balasubramanian [142] observed inhibition at 2, 5 and 10 ppm of carbofuran on invertase activity in lab conditions. Suppressed activity of invertase for 1 day was noticed by Tu [143] which disappeared after 2 days in sandy loam soil with captan and chlorothalonil. Similarly, El-Hamady and Sheloa [144] found that the insecticidal activity of imidacloprid significantly reduced invertase activity in Egyptian soil. On the other hand, Tu [123] stated initial inhibiton followed by recovery with imidacloprid in sandy soil.

Invertase activity was depressed in flubendiamide and spinosad treated soils throughout the experiment when compared to the controls in soils incubated for 10 days. The maximum enzyme activity was observed at 2.5 kg ha^{-1} (stimulatory) for flubendiamide and spinosad. The individual increments of invertase activity ranged from a low increase 2–69, 1–74% and 0–80, 87–100% for black clay soil and for red clay soil, 43–81, 27–40% and 38–43, 65–125% received 2.5 kg ha^{-1} respectively in comparison to control at 24 and 48 hours. The results reveal that invertase enzyme is quite sensitive to flubendiamide and spinosad. Although enzyme activities of samples were lower than the control, significant differences were found among the enzyme activities between treated and untreated soil samples. With the increase in incubation periods, the enzyme activities were also increased up to 20-day, further increase in the incubation decrease in the enzyme activity was noticed [129]. These results appeared to be consistent with previous reports, in which it is demonstrated that pesticides stimulated invertase activity of soils [130,145].

Cellulase enzyme

Cellulase is an important enzyme in the carbon cycle, it acts on the cellulosic material present in soil ecosystem [146]. They acts on the β-1,4-glucan bonds in cellulose, the most abundant carbonaceous polymer in the Nature and, thus, is involved in one of the major processes of the natural carbon cycle [147]. Assays of activity have been based on the decomposition of cellophane disks, cellulose powder, and the hydrolysis of carboxymethyl cellulose [148]. The products of cellulose degradation are glucose, cellobiose, and higher molecular weight

ISBN:
DOI:

oligosaccharides [147]. However, the rate of cellulose degradation in natural organic litter is not exclusively related to extra-and intracellular cellulase activity, but is also related to activity of other hydrolytic as well as oxidative enzymes [149,150].

When formulations of the nematicide fenamiphos at the concentration of 18.6 kg ha^{-1} was applied to a fine silty montmorillonite soil (6.5% organic matter, pH 6.1) under graneclover pasture in the field, the cellulase activity increased upto 50%, particularly after treatment with fenamiphos [42]. In a similar experiment under laboratory conditions [122], pesticide, fenamiphos at the recommended field rates was innocuous but at higher application rates 37 and 930 mg kg^{-1} had deleterious effect on cellulase activity with 24 and 48% inhibition, respectively, even 62 days after treatment. Furthermore, the observed effects were less masked in the field than under laboratory conditions [122]. The production of cellulase from *Trichoderma harzianum* was drastically reduced by the incorporation of 3 insecticides including phorate at 5, 10, 50, 100 and 500 ppm. In this study, phorate was more inhibitory [151]. No effect of methamidophos was observed on cellulase activity in clay soil whereas 25% and 38% reduction in cellulase activity was observed in clay loam and loam sand, respectively, after 6 weeks. It is usually stated that high enzymatic activities are associated with high organic matter contents but different results have been reported by Gianfreda et al. [152].

Cellulase activity was significantly increased at the level of 0.1 to 2.5 kg ha^{-1} whereas the activity was decreased at higher concentrations (5.0–10.0 kg ha^{-1}) of pesticides in black and red soils. The enzyme activity was significantly enhanced at 2.5 kg ha^{-1} in both soils for flubendiamide and spinosad and showed individual increments of cellulase activity ranging from a low increase 15–29, 11–19 and 2–18, 12–36% in comparison to control. The stimulatory concentration (2.5 kg ha^{-1}) induces the highest cellulase activity after 20, 30 and 40-day of incubation in black clay soils with flubendiamide and spinosad when compared to control, whereas in red clay soil a similar trend was followed by flubendiamide, which induces the highest cellulase activity after 20, 30 and 40 days of incubation but spinosad showed a variable pattern at 30 and 40-day, cellulase enzyme activity remained same with control [129]. The relatively low activity of cellulase might result from the toxic effect of flubendiamide and spinosad on soil microorganisms, which in turn produces cellulase. The inhibition of cellulase activity by flubendiamide and spinosad could be attributed to the properties of flubendiamide and spinosad. Similal reports were shown by Mohiddin et al. [131] with imidacloprid and acephate. Identical observations were made by Katayama and Kuwatsuka [153], Madhuri and Rangaswamy [113] on cellulase activity. Analogous report was obtained by Ismail et al. [154,155] on application of metolachor to Malaysian soil. Gigliotti et al. [156] also reported that bensulfurn methyl at 16 and 160 µg/g inhibited cellulase activity in soil samples. In a diverse study made by Gherbawy and Abdelzaher [157], indicate that alteration in the activity of cellulase by metalaxyl was marked in pure fungal cultures. Similarly, Arinz and Yubedee [158], show that kelthane and fenvalerate caused inhibition cellulase activity.

Arylsulfatase enzyme

Arylsulfatases are usually widespread in soils [159,160]. They are responsible for the hydrolysis of sulfate esters in the soil [9] and are secreted by bacteria into the exterior environment as a response to sulfur limitation [161]. The same ephemeral effect on arylsulfatase was observed with the fungicide metalaxyl [65]. The effects of pesticides on arylsulfatase activity in soil are meagrely documented from the year 2000. Generally, the pesticides do not seem to affect the activity of this enzyme [25,162,163]. The endosulfan (insecticide) applied at elevated level (100 ppm) significantly increased arylsulfatase activity. This increase in arylsulfatase

ISBN:
DOI:

activity was temporary and declined with the depletion of applied endosulfan. The short-term effect of endosulfan may be due to its degradation or its gradual adsorption by the soil colloid, making it unavailable for microbes [164].

Fluorescein diacetate hydrolase enzyme

The fluorescein di-acetate hydrolase activity usually represents soil enzymatic activities and combined biological effects. Fluorescein di-acetate is a substrate hydrolysed by various types of enzymes, such as protease, lipase and esterase and its hydrolysis was observed among a wide range of primary decomposers, bacteria and fungi [165]. There is no clear indication of fluorescein diacetate hydrolase to pesticides input, but it seems to be more influenced by insecticides [166,167] than herbicides [10]. Fluorescein diacetate hydrolase activity enhanced by the supply of pesticides of imidazolines (Imazethapyr) and organochlorines (endosulfan) families [164,168] which have been less studied than other families of pesticides. The application of organophosphate pesticides (chlorpyrifos and ethion) at different concentrations had the similar effect on this enzymatic activity [167,169]. Slight and transitory increases in fluorescein diacetate hydrolase activity were observed at the highest applied pesticide rates (tenfold field rate). Fluorescein diacetate hydrolase activity in soil is poorly influenced by herbicides or insecticides applications, except endosulfan applications, which seems to stimulate this activity [170].

Conclusions

The investigation by several researchers on the effect of various pesticides on soil enzyme activities clearly indicates that, pesticides show positive or negative or no effect on the activities of enzymes in soils. Either positive or negative effect of pesticides on soil enzyme activities is dose dependent; the behaviour of pesticides in soils is influenced by the physicochemical properties, presence organic matter and total nitrogen content. The increase or decrease in soil enzyme activities is also dependent on the period of incubation with pesticides in soils. Few studies proved that pesticides used in agriculture at filed application rates (at 2.5 or 5.0 kg ha^{-1}), typically improves the soil enzymatic activities, which are important in nutrient cycling and soil fertility. Application of mixtures of pesticides became a common trend in modern agriculture in order to eradicate multiple pests. Very few reports are available on the effect of agrochemical combinations on biological activities in soils. Hence, understanding on the effects of combinations of pesticides on soil enzyme activities could be useful for elucidating the risk assessment of pesticides in soils.

References

1. Nannipieri P, Landi L (2000) Soil enzymes. Microbially mediated processes. In: M E Smner (Ed.). Hand book of soil science. CRC Press, Boca Raton, London, New York & Washington DC.
2. Moreno JL, Garcia C, Hernandez T (2003) Toxic effect of cadmium and nickel on soil enzymes and the influence of adding sewage sludge. Euro J Soil Sci 54: 377-386.
3. Acosta-Martinez V, Tabatabai MA (2000) Arylamidase activity in soils. Soil Sci Soc Am J 64: 215-221.
4. Turki AI, Dick AW (2003) Myrosinase activity in soil. Soil Sci Soc Am J 67: 139-145.
5. Ramesh PR, Kotur SC, Sudhir K (2003) Relationship of activities of urease and dehydrogenase with physico-chemical properties of some coffee-growing soils of Karnataka. J Ind Soc Soil Sci 51: 78-80.
6. Shukla AK (2000) Impact of fungicides on soil microbial population and enzyme activities. Acta Botanica Indica 28: 55-87.
7. Handa SK, Agnihothri MP, Kulshresta G (2000) Effect of pesticides on soil fertility. In: Pesticide residue analysis and significance. Research Periodicals and Publishing House, New Delhi, India.

ISBN:
DOI:

8. Suryanarayana Reddy M (2002) Relationship between soil enzymes and physico-chemical properties of soils. 39: 143-146.

9. Kertesz MA, Mirleau P (2004) The role of soil microbes in plant sulphur nutrition. J Exp Bot 55:1939–1945.

10. Zabaloy MC, Garland JL, Gomez MA (2008) An integrated approach to evaluate the impacts of the herbicides glyphosate, 2,4-D and metsulfuron-methyl on soil microbial communities in the Pampas region, Argentina. Appl Soil Ecol 40: 1–12.

11. Schaffer A (1993) Pesticide effects on enzyme activities in the soil ecosystem. In: Soil Biochemistry, Marcel Dekker, Inc., New York.

12. Burns RG (1982) Enzyme activity in soil: location and a possible role in microbial ecology. Soil Biol Biochem 14: 423-427.

13. Burns RG (1986) Interaction of enzymes with soil minerals and organic colloids. In: Interactions of soil minerals with natural organics and microbes. Soil Science Society of America, Madison, WI.

14. Johansson E, Krantz-Rulcker C, Zhang BX, Oberg G (2000) Chlorination and biodegradation of ligning. Soil Biol Biochem 32: 1029-1032.

15. Gramss G, Voigt KD, Kirschc B (1999) Oxidoreductase enzyme liberated by plant roots and their effects on soil humic material. Chemosphere 38: 1481-1494.

16. Sharma H.C, Roomiro Ortiz (2002) Host plant resistance to insects: An eco-friendly aqpproach for pest management and environment conservation. J Environ Biol 23: 111-135.

17. Andrea MM, Peres TB, Luchini LC, Pettinelli JA (2000) Impact of long term pesticide applications on some soil biological parameters. J Environ Sci Health 35: 297-307.

18. Colombo C, Palumbo G, Sannino F, Gianfreda L (2002) Chemical and biochemical indicators of managed agricultural soils. 17th World Congress of Soil Science Bangkok Thailand 1740: 1-9.

19. Nannipieri P, Kandeler E, Ruggiero P (2002) Enzyme activities and microbiological and biochemical processes in soil. In: Enzymes in the environment Marcel Dekker, New York.

20. Van Beelen PV, Doelman P (1997) Significance and application of microbial toxicity tests in assessing ecotoxicological risks of contaminants in soil and sediments. Chemosphere 43: 455-499.

21. Trasar-Cepeda C, Leiros MC, Scoane S, Gil-Stores F (2000) Limitations of soil enzymes as indicators of soil pollution. Soil Biol Biochem 32: 1867-1875.

22. Gianfreda L, Rao MA, Piotrowska A, Palumbo G, Colombo C (2005) Soil enzyme activities as affected by anthropogenic alterations: intensive agricultural practices and organic pollution. Sci Total Environ 341: 265-279.

23. Truu M, Truu J, Ivask M (2008) Soil microbiological and biochemical properties for assessing the effect of agricultural management practices in Estonian cultivated soils. Eur J Soil Biol 44: 231–237.

24. Garcia-Ruiz R, Ochoa V, Vinegla B, Hinojosa MB, Pena-Santiago R, et al. (2009) Soil enzymes, nematode community and selected physico-chemical properties as soil quality indicators in organic and conventional olive oil farming: Influence of seasonality and site features. Appl Soil Ecol 41: 305–314.

25. Tejada M (2009) Evolution of soil biological properties after addition of glyphosate, diflufenican and glyphosate + diflufenican herbicides. Chemosphere 76: 365–373.

26. Menon P, Gopal M, Parsad R (2005) Effects of chlorpyrifos and quinalphos on dehydrogenase activities and reduction of Fe^{3+} in the soils of two semi-arid fields of tropical India. Agri Ecosyst Environ 108: 73–83.

27. Monkiedje A, Ilori MO, Spiteller M (2002) Soil quality changes resulting from the application of the fungicides mefenoxam and metalaxyl to a sandy loam soil. Soil Biol Biochem 34: 1939–1948.

28. Schuster E, D Schroder (1990) Side-effects of sequentially- and simultaneously-applied pesticides on non-target soil microorganisms: laboratory experiments. Soil Biol Biochem 22: 375-383.

29. Tu CM (1992) Effect of three newer insecticides on microbial and enzymatic activities in soil. Bull Environ Toxicol 49:120-128.

30. Engelen B, Meinken K, Von Wintzingerode F, Heuer H, Malkomes HP, et al. (1998) Monitoring impacts of a pesticide treatment on bacterial soil communities by metabolic and genetic fingerprinting in addition to conventional testing procedures. Appl Environ Microbiol 64: 2814–2821.

31. Liu J, Xie J, Chu Y, Sun C, Chen C, et al. (2008) Combined effect of cypermethrin and copper on catalase activity in soil. J Soils Sed 8: 327–332.

32. Topp E, Vallaeys T, Soulas G (1997) Pesticides: Microbial degradation and effects on microorganisms. In Modern Soil Microbiology, Marcel Dekker, New York.

ISBN:
DOI:

33. Megharaj M, Singleton I, Kookana R, Naidu R (1999) Persistence and effects of fenamiphos on native algal populations and enzymatic activities in soil. Soil Biol Biochem 31: 1549-1553.

34. Shukla PK, Mishra RR (1997) Influence of herbicides on microbial population and enzyme activities in potato (*Solanum tuberosum*) field soil. J Agric Sci 67: 610-611.

35. Kanazawa S, Filip Z (1986) Distribution of microorganisms, total biomass, and enzyme activities in different particles of brown soil. Microbiol Ecol 12: 205–215.

36. Nannipieri P, Grego S, Ceccanti B (1990) Ecological significance of the biological activity in soil. In Soil Biochemistry, Marcel Dekker, New York.

37. Skujins J (1976) Extracellular enzymes in soil. Crit Rev Microbiol 4: 383–421.

38. Kandeler E, Luxh J, Tscherko D, Magid J (1999) Xylanase, invertase and protease at the soil–litter interface of a loamy sand. Soil Biol Biochem 31: 1171–1179.

39. Lalande B, Gagon B, Simard RR (1998) Microbial biomass C and alkaline phosphatase activity in two compost amended soils. Can J Soil Sci 78: 581–587.

40. Pancholy SK, Rice E L (1973) Soil enzymes in relation to old field succession: Amylase, cellulase, invertase dehydrogenase and urease. Soil Sci Soc Am J 37: 47–50.

41. Bergstrom DW, Montreal CM, Millette JA, King DJ (1998) Spatial dependence of soil enzyme activities along a slope. Soil Sci Soc Am J 62: 1302–1308.

42. Ross DJ, Speir TW, Cowling JC, Whale KN (1984) Influence of field applications of oxamyl and fenamiphos on biochemical activities of soil under pasture. New Zealand. J Sci 27: 247-254.

43. Tscherko D, Kandeler E, Jones TH (2001) Effect of temperature on below-ground N-dynamics in a weedy model ecosystem at ambient and elevated atmospheric CO2 levels. Soil Biol Biochem 33: 491–501.

44. Malkomes HP (1997) Applications of ecotoxicity tests to assess side effects of pesticides in soils. In: Soil Ecotoxicology.

45. Nannipieri P (1994) The potential use of soil enzymes as indicators of productivity, sustainability and pollution. In ‘‘Soil Biota: Management in Sustainable Farming Systems’’ CSIRO, East Melbourne.

46. Hiwada K, Ito T, Yokoyama M, Kokubu T (1980) Isolation and characterization of membrane-bound arylamidases from human placenta and kidney. J Biochem104:155-165.

47. Appel W (1974) Peptidases. In: Bergmeyer HU (Ed.). Methods of enzymatic analysis. Volume 2, Academic Press, New York.

48. Senwo ZN, Tabatabai MA (1996) Aspartase activity of soils. Soil Sci Soc Am J 60: 1416–1422.

49. Stevenson FJ (1994) Humus chemistry: Genesis, composition, reactions. (IInd edn). John Wiley & Sons, New York.

50. Srinivasulu M, Rangaswamy V (2013) Influence of insecticides alone and in combination with fungicides on enzyme activities in soils. Int J Environ Sci Tech 10: 341–350.

51. Floch C, Anne-Celine C, Karine J, Yvan C, Steven C (2011) Indicators of pesticide contamination: Soil enzyme compared to functional diversity of bacterial communities via Biolog Ecoplates. Euro J Soil Biol 47: 256-263.

52. Gundi VAKB, Narasimha G, Reddy BR (2005) Interaction Effects of soil insecticides on microbial populations and dehydrogenase activity in a black clay soil. J Env Sci Heal 40: 269-281.

53. Gerber HR, Anderson JPE, Bugel-Mogenson B, Castle D, Domsch KH, et al. (1991) Revision of recommended laboratory tests for assessing side-effects of pesticides on the soil microflora. Toxicol Environ Chem 30: 249-261.

54. Mayanglambam T, Vig K, Singh DK (2005) Quinalphos persistence and leaching under field conditions and effects of residues on dehydrogenase and alkaline phospho monoesterases activities in soil. Bull Environ Contam Toxicol 75: 1067–1076.

55. Xie XM, Liao M, Huang CY, Liu WP, Abid S (2004) Effects of pesticides on soil biochemical characteristics of paddy soil. J Environ Sci 16: 252-255.

56. Demanou J, Monkiedje A, Njine T, Foto SM, Nola M, et al. (2004) Changes in soil chemical properties and microbial activities in response to the fungicide Ridomil gold plus copper. Int J Environ Res Public Health 1: 26–34.

57. Nowak A, Nowak J, Klodka D, Pryzbulewska K, Telesinski A, et al. (2004) Changes in the microflora and biological activity of the soil during the degradation of isoproturon. J Plant Dis Protec 19: 1003–1016.

58. Klose S, Ajwa HA (2004) Enzymes activities in agricultural soils fumigated with methyl bromide alternatives. Soil Biol Biochem 36: 1625–1635.

ISBN:
DOI:

59. Schutter M, Ajwa H, Orosco L, Wright A (2001) Changes in soil microbial activities under drip fumigation. In ‘‘Annual Meetings Abstracts.’’ ASA–CSSA–SSSA, Madison, WI.

60. Klose S, Acosta-Martınez V, Ajwa HA (2006) Microbial community composition and enzyme activities in a sandy loam soil after fumigation with methyl bromide or alternative biocides. Soil Biol Biochem 38: 1243–1254.

61. Min H, Ye Chen ZY, Wu WX, Yufeng D (2001) Effects of butachlor on microbial populations and enzymes activities in paddy soil. J Environ Sci Health B 36: 581–595.

62. Singh J, Singh DK (2005) Bacterial, azotobacter, actinomycetes, and fungal population in soil after diazinon, imidacloprid, and lindane treatments in groundnut (*Arachis hypogaea* L.) fields. J Environ Sci Heal B 40: 785-800.

63. Singh DK, Kumar S (2008) Nitrate reductase, arginine deaminase, urease and dehydrogenase activities in natural soil (ridges with forest) and in cotton soil after acetamiprid treatments. Chemosphere 71: 412–418.

64. Chen SK, Edwards CA (2001) A microcosm approach to assess the effects of fungicides on soil ecological processes and plant growth: Comparison of two soil types. Soil Biol Biochem 33: 1981–1991.

65. Sukul P (2006) Enzymatic activities and microbial biomass in soil as influenced by metalaxyl residues. Soil Biol Biochem 38: 320–326.

66. He Y H, Shen DS, Fang CR, He R, Zhu YM (2006) Effect of metsulfuronmethyl on the microbial population and enzyme activities in wheat rhizosphere soil. J Environ Sci Health B 41: 269–284.

67. Bending GD, Rodrıguez-Cruz MS, Lincoln SD (2007). Fungicide impacts on microbial communities in soils with contrasting management histories. Chemosphere 69: 82-88.

68. Tu CM (1981) Effect of pesticides on activity of enzymes and microorganisms in a clay loam soil. J Env Sci Health 16: 179-181.

69. Tu CM (1981) Effect of some pesticides on enzyme activities in an organic soil. Bull Environ Contam Toxicol 27: 109-114.

70. Megharaj M, Boul HL, Thiele JH (1999) Effects of DDT and its metabolites on soil algae and enzymatic activity. Biol. Fertil Soils 29: 130–134.

71. Tu CM (1980) Influence of pesticides and some of the oxidised analogues on microbial population, nitrification and respiration activities in soil. Bull. Environ. Contam Toxicol 24: 13-19.

72. Rangaswamy V, Reddy BR, Venkateswarlu K (1994) Activities of dehydrogenase and protease in soil as influenced by monocrotophos, quinalphos, cypermethrin fenvalerate. Agric Ecosys Environ 47: 319-326.

73. Naumann K (1970) Zur Dynamik der Bodenmikroflora nach Anwendung von Pflanzenschutzmitteln. IV. Untersuchungen uber die Wirkung von Parathion-Methyl auf die Atmung und die Dehydrogenase-Aktivitat des Bodens. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg Abt II 125: 119-133.

74. Naumann K (1972) Die Wirking einiger Umweltfaktoren auf die Reaktion der Bodenmikroflora gegenuber Pflanzenschutz Zentrabl. Bakteriol. Parasitenkd. Infektionskr. Hyg Abt 11: 379-396.

75. Tu CM (1990) Effect of four experimental insecticides on enzyme activities and levels of adenosine in mineral and organic soils. J Environ Sci Health 25: 787-800.

76. Bayer H, Mitterer M, Schinner F (1982) The influence of insecticides on microbial processes in Ah-materials of an agricultural soil. Pedobiologia 23: 311-319.

77. Pozo C, Martinez-Toledo MV, Salmeron V, Rodelas B, Gonzalez-Lopez J (1995) Effect of chlorpyrifos on soil microbial activity. Environ Toxicol Chem 14: 187-192.

78. Rask L, Andreasson E, Ekbom B, Eriksson S, Pontoppidan B, et al. (2000) Myrosinase: gene family evolution and herbivore defense in Brassicaceae. Plant Mol Biol 42: 93-113.

79. Brown PD, Morra MJ (1996) Hydrolysis products of glucosino- lates in *Brassica napus* tissues as inhibitors of seed germination. Plant Soil 181:307-316.

80. Gil V, Macleod AJ (1980) Studies on glucosinolate degradation in *Lepidium sativum* seed extract. Phytochemistry 19:1369–1374.

81. Angus, JF, Grander, PA, Kirkegaard JA, Desmarchelier JM (1994) Biofumigation: Isothiocyanates released from *Brassica* roots inhibit growth of the take-all fungus. Plant Soil 162: 107-112.

82. Brown PD, Morra MJ (1997) Control of soil-borne plant pests using glucosinolate-containing plants. Adv Agron 61: 167-231.

83. Yenish JP, Worsham AD, York AC (1996) Cover crops for herbicide replacement in no-tillage corn (*Zea Mays*). Weed Technol 10: 815-821.

ISBN:
DOI:

84. Brabban AD, Edwards C (1995) The effects of glucosinolates and their hydrolysis products on microbial growth. J Appl Bacteriol 79: 171-177.

85. Borek V, Morra MJ, Caffrey JPM (1996) Myrosinase activity in soil extracts. Soil Sci Soc Am J 60: 1792-1797.

86. Tabatabai MA, Fu M (1992) Extraction of enzymes from soils. pp. 197-227. In: G Stotzky and JM Bollag (Ed.). Soil Biochemistry, Marcel Dekker, New York.

87. Ludikhuyze L, Ooms V, Weemaes C, Hendrickx M (1999) Kinetic study of myrosinase from Broccoli (*Brassica oleracea* L. Cv. Italica). J.y of the irreversible thermal and pressure inactivation. Agric Food Chem 47: 1794-1800.

88. Antonious GF (2003) Impact of soil management and two botanical insecticides on urease and invertase activity. J Environ Sci Health B 38: 479-488.

89. Ingram CW, Coyne MS, Ann Williams DW (2005) Effects of commercial diazinon and imidacloprid on microbial urease activity in soil. J Environ Qual 34:1573–1580.

90. Boyd SA, Mortland MM (1990) Enzymes interactions with clays and clay–organic matter complexes. In: Soil Biochemistry. Marcel Dekker, New York.

91. Speir TW, Ross DJ (1978) Soil phosphatase and sulfatase. In: Soil Enzymes. Academic Press, New York.

92. Piotrowska-Seget Z, Engel R, Nowak E, Kozdroj J (2008) Sucessive soil treatment with captan or oxytetracycline affects non-target microorganisms. World J Microbio Biotech 24: 2843-2848.

93. Posen S (1967) Alkaline phosphatase. Annals of Internal Medicine 67: 183–203.

94. Harrison AF (1983) Relationship between intensity of phosphatase activity and physico-chemical properties in woodland soils. Soil Biol Biochem 15: 93-99.

95. Rojo MJ, Carcedo SG, Mateos MP (1990) Distribution and characterization of phosphatase and organic phosphorus in soil fraction. Soil Biol Biochem 22: 169-174.

96. Tarafdar JC, Yadav RS, Meena SC (2001) Comparative efficiency of acid phosphatase originated from plant and fungal source. J Plant Nutrit Soil Sci 164: 279-282.

97. Schneider K, Turrion MB, Grierson BF, Gallardo JF (2001) Phosphatase activity, microbial phosphorus, and fine root growth in forest soil in the Sierra de Gata, western central Spain. Biol Fertil Soils 34: 151-155.

98. Sikora LJ, Kaufman DD, Horng LC (1990) Enzyme activity in soils showing enhanced degradation of organophosphate insecticides. Biol Fertil Soils 9: 14-18.

99. Rangaswamy V, Venkateswarlu K (1996) Phosphatase activity in groundnut soils as influenced by selected insecticides. J Environ Biol 17: 115-119.

100. Sivasithamparam K (1970) Some effects of insecticide (Dursban) and a weedkiller (Linuron) on the microflora of submerged soil. Riso 19: 339-346.

101. Nakamura T, Mochida K, Ozoe Y, Ukawa S, Sakai M, et al. (1990) Enzymological properties of three soil hydrolases and effects of several pesticides on their activities. J Pestic Sci 15: 593-598.

102. Srinivasulu M, Mohiddin GJ, Subramanyam K, Rangaswamy V (2012) Effect of insecticides alone and in combination with fungicides on nitrification and phosphatase activity in two groundnut (Arachis hypogeae L.) soils. Environ Geochem Health 34: 365–374.

103. Nazima R, Zafar Reshi A (2010) Effect of the fungicide mancozeb at different application rates on enzyme activities in a silt loam soil of the Kashmir Himalaya, India. Tropic Ecol 51: 199-205.

104. Kiss S, Dragon-Bularda M, Radulescu D (1975) Biological significance of enzymes in soil. Adv Agron 27: 25-87.

105. Dedeken M, Voets JP (1965) Recherches sur le metabolisme des acides amines dans le sol. 1. Le metabolisme de glycine, alanine, acids aspartque et acide glutaminique, Annales de 1' Institut Pasteur, Paris 10g 3: 103-111.

106. Ladd JN, Butler HA (1972) Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. Soil Biol Biochem 4: 19-30.

107. Watanabe K, Hayano K (1993) Distribution and identification of proteolytic Bacillus sp. in paddy field under rice cultivation. Can J Microbiol 39: 674-680.

108. Nasreen C, Mohiddin GJ, Srinivasulu M, Rekha Padmini A, Ramanamma P, et al. (2012) Interaction effects of insecticides on enzyme activities in black clay soil from groundnut (*Arachis hypogaea* L.) fields. Environ Res Eng Management 2:21-28.

ISBN:
DOI:

109. Ramudu AC, Srinivasulu M, Mohiddin GJ, Rangaswamy V (2012) Effect of fungicides on urease and protease activities in two groundnut (*Arachis hypogaea* L.) soils. Int J Environ Protection 2: 23-28.

110. Beri V, Goswami KP, Brar SS (1978) Urease activity and its Michaelis constant for soil systems. Plant Soil 49: 105-115.

111. Cooke GW (1969) Fertilizers in 2000 AD. In: Phosphorus in Agriculture Bull. Doc, No. 53. Internation Superphosphate and Compound Manufacturers' Association, Paris.

112. Yang C, Sun T, He W, Chen S (2006) Effects of pesticides on soil urease activity. Ying Yong Sheng Tai Xue Bao 17: 1354–1356.

113. Madhuri RJ, Rangaswamy V (2002) Influence of selected insecticides on activity in groundnut (*Arachis hypogaea* L.) soils. J Env Biol 23: 393–397.

114. Tabatabai MA (1982) Soil enzyme. In methods of Soil Analysis, Part 2, American society of Agronomy, Soil Science Society of America, Madison, WI.

115. Tabatabai MA (1994) Soil enzymes. In: Methods of soil analysis. Part 2. Microbial and Biochemical properties, Soil Sci Soc Am J, Madison, Wisconsin.

116. Davies HA, Greaves MP (1981) Effects of some herbicides on soil enzyme activities. Weed Res 21: 205-209.

117. Lethbridge G, Bull AT, Burns RG (1981) Effects of pesticides on 1,3-α-glucanase and urease activities in soil in the presence and absence of fertilizers, lime and organic materials. Pestic Sci 12: 147-155.

118. Lethbridge G, Burns RG (1976) Inhibition of soil urease by organophosphorus insecticides. Soil Biol Biochem 8: 99-102.

119. Abdel-Mallek AY, Moharram MA, Abdel-Kader MIA, Omar SA (1994) Effect of soil treatment with the organophosphorus insecticide Profenofos on the fungal flora and some microbial activities. Microbiol Res 149: 167-171.

120. Rangaswamy V, Venkateswarlu K (1992) Effect of monocrotophos, quinalphos, cypermethrin and fenvalerate on urease activity in groundnut soils. Env Ecol 10:429-433.

121. Gianfreda I, Sannino F, Ortega N, Nannipieri P (1994) Activity of free and immobilized urease in soil: Effects of pesticides. Soil Biol Biochem 26: 777-784.

122. Ross DJ, Speir TW (1985) Changes in biochemical activities of soil incubated with the nematicides oxamyl and fenamiphos. Soil Biol Biochem 17: 123-125.

123. Tu CM (1995) Effect of five insecticides on microbial and enzyme activities in sandy soil. J Environ Sci Health 30: 289-306.

124. Tu CM, Miles JRW (1976) Interactions between insecticides and soil microbes. Res Rev 64: 17-65.

125. Alexander M (1977). Introduction to Soil Microbiology. (2nd Edn.), Wiley, New York.

126. Tu CM (1982) Influence of pesticides on activities of amylase, invertase and level of adenosine triphosphate in organic soil. Chemosphere 2:909-914.

127. Rangaswamy V, Venkateswarlu K (1992) Degradation of selected insecticides by bacteria isolated from soil. Bull Environ Contam Toxicol 49: 797-804.

128. Tu CM (1988) Effect of selected pesticides on activities of amylase, invertase and microbial respiration in sandy soil. Chemosphere 17: 159-163.

129. Mohiddin GJ, Subramanyam K, Rangaswamy V, Srinivasulu M, Madakka M, et al. (2013) Influence of insecticides flubendiamide and spinosad on biological activities in tropical black and red clay soils. 3 Biotech DOI 10.1007/s13205-013-0188-3.

130. Srinivasulu M, Rangaswamy V (2006) Activities of invertase and cellulase as influenced by the application of tridemorph and captan to groundnut (*Arachis hypogaea* L.) soil. Afr J Biotechnol 2: 175–180.

131. Mohiddin GJ, Srinivasulu M, Madakka M, Rangaswamy V (2010) Influence of insecticides on the activity of amylase and cellulase in groundnut (*Arachis hypogaea* L.) soil. Ecol Environ Conserv 3: 383–388.

132. Prasad BN, Mathur SN (1983) Effect of metasystox and cumin-l on seed germination, reducing sugar content and amylase activity in *Vigna mungo* (L.) Hepper. Ind J Plant Physiol 24: 209-213.

133. Megharaj M, Venkateswarlu K, Rao AS (1989) Interaction effects of insecticide combinations towards the growth of *Scenedesmus bijugatus* and *Synechococcus elongatus*. Plant Soil 114: 159-163.

134. Stratton GW, Corke CT (1982) Toxicity of the insecticide permethrin and some degradation products towards algae and cyanobacteria. Environ Pollut A 29: 71-80.

135. Stratton GW, Corke CT (1982) Comparative fungitoxicity of the insecticide permethrin and ten degradation products. Pestic Sci 13: 679-685.

ISBN:
DOI:

136. Kennedy Z J, Arathan SS (2002) Influence of carbofuran on the activity of soil enzymes in submerged rice soil ecosystem. In: Rajac RC (ed) Biotechnology of microbes and sustainable utilization. India.

137. Gundi VAKB, Viswanath B, Subhosh Chandra M, Narahari Kumar V, Rajasekhar Reddy B (2007) Activities of cellulose and amylase in soils as influenced by insecticide interactions. Ecotoxicol Environ Safety 68: 278–285.

138. Alef LK, Nannipieri P (1995) Methods in applied soil microbiology and biochemistry. Academic Press, Harcourt Bjace & Company Publishers, London.

139. Srimathi MS, Karanth MGK (1989) Influence of hexachlorocyclohexane isomers on soil enzymes. J Soil Biol Ecol 9: 65-71.

140. Endo T, Taiki K, Nobutsura T, Michihiko S (1982) Effect of insecticide cartap hydrochloride on soil enzyme activities, respiration and on nitrification. J Pestic Sc 7: 101-110.

141. Singh BK, Allan W, Denis JW (2002) Degradation of chlorpyrifos, fenamiphos, and chlorothalonil alone and in combination and their effects on soil microbial activity. *Environ*. Toxicol Chem 21: 2600-2605.

142. Palaniappan SP, Balasubramanian A (1985) Influence of two pesticides on certain soil enzymes. Agric Res J Kerala 23: 189-192.

143. Tu CM (1993) Effect of fungicides, captafol and chlorothalonil, on microbial and enzymatic activities in mineral soil. J Environ Sci Health 28: 67-80.

144. El Hamady SEE, Sheloa MKAA (1999) Field evaluation of imidacloprid applied as seed treatment to control thrips tabaci lind on cotton with regard to soil pollution. Arab Univ. J Agric Sci 7: 561-574.

145. Sannino F, Gianfreda L (2001) Pesticide influence on soil enzymatic activities. Chemosphere 45: 417–425.

146. Mallik MK, Sharma SG (2002) An in situ study on the activity of cellulase enzyme fractions during anaerobic digestion of agricultural wastes and weeds. Raj J Res 12: 79-81.

147. White AR (1982) Visualization of cellulases and cellulose degradation. In Cellulose and Other Natural Polymer Systems: Biogenesis, and Degradation, Plenum Press, New York.

148. Ladd JN (1978) Origin and range of enzymes in soil. In: Soil Enzymes. Academic Press, London.

149. Eriksson KE, Wood TM (1985) Biodegradation of cellulose. In Biosynthesis and biodegradation of wood components. Academic Press, New York.

150. Ljungdahl LG, Eriksson KE (1985) Ecology of microbial cellulose degradation. Adv Microb Ecol 8: 237-299.

151. Jairaj J, Ramabadram R (1996) Effect of certain soil insecticides on in vitro growth, phosphatase sporulation and cellulase production by Trichoderma harzianum Rifai. J Biol Cont 10:111-115.

152. Gianfreda L, Sannino F, Violante A (1995) Pesticide effects on the activity of free immobilized and soil invertase. Soil Biol Biochem 27: 1201-1208.

153. Katayama A, Kuwatsuka S (1991) Effects of pesticides on cellulose degradation in soil under upland and flooded conditions. Soil Sci Plant Nutr 37: 1-6.

154. Ismail BS, Fugon D, Omar O (1996a) Effect of metolachlor on soil enzymes in Malaysian soil. J Environ Sci Health 31:1267–1278.

155. Ismail BS, Omar O, Ingon O (1996b) Effects of metolachlor on the activities of four soil enzymes. Microbios 87: 239–248.

156. Gigliotti C, Allievi L, Salandi C, Ferrari F, Farini A (1998) Microbial ecotoxicity and persistence in soil of the herbicide bensulfuron methyl. J Environ Sci Health 33: 381–398.

157. Gherbawy YA, Abdel Zaher HMA (1999) Isolation of fungi from tomato rhizosphere and evaluation of the effect of some fungicides and biological agents on the production of cellulose enzymes. Czech Mycol 51: 157–170.

158. Arinz AE, Yubedee AG (2000) Effect of fungicides on Fusarium grain rot and enzyme production in maize (*Zea mays* L.). Glob J Appl Sci 6: 629–634.

159. Tabatabai MA, Bremner JM (1970) Arylsulfatase activity of soils. Soil Sci Soc Am Pro.

160. Ganeshamurthy AN, Takkar PN (1997) Residual management of sulfur applied to soybean or wheat in a soybean-wheat system on Vertisols. Aust J Soil Res 35: 199–208.

161. McGill WB, Cole CV (1981) Comparative aspects of cycling of organic C, N, S and P through soil organic-matter. Geoderma 26: 267–286.

162. Niemi RM, Heiskanen I, Ahtiainen JH, Rahkonen A, Mantykoski K, et al. (2009) Microbial toxicity and impacts on soil enzyme activities of pesticides used in potato cultivation. Appl Soil Ecol 41: 293–304.

163. Vavoulidou E, Avramides E, Wood M, Lolos P (2009) Response of soil quality indicators to the pesticide cadusaphos. Commun Soil Sci Plant Anal Doi:10.1080/00103620802697996.

164. Kalyani SS, Sharma J, Dureja P, Singh S, Lata (2010) Influence of endosulfan on microbial biomass and soil enzymatic activities of a tropical Alfisol. Bull Environ Contam Toxicol 84: 351–356.

165. Janvier C, Villeneuve F, Alabouvette C, Edel-Hermann V, Mateille T, et al. (2007) Soil health through soil disease suppression: which strategy from descriptors to indicators. Soil Biol Bioch 39: 1–23.

166. Das P, Pal R, Chowdhury A (2007) Effect of novaluron on microbial biomass, respiration, and fluorescein diacetate-hydrolyzing activity in tropical soils. Biol Fert Soils 44: 387–391.

167. Bishnu A, Chakraborty A, Chakrabarti K, Saha T (2012) Ethion degradation and its correlation with microbial and biochemical parameters of tea soils. Biol Fert Soils 48: 19-29.

168. Perucci P, Dumontet S, Bufo SA, Mazzatura A, Casucci C (2000) Effects of organic amendment and herbicide treatment on soil microbial biomass. Biol Fertil Soils 32:17–23.

169. Dutta M, Sardar D, Pal R, Kole RK (2010) Effect of chlorpyrifos on microbial biomass and activities in tropical clay loam soil. Environ Monit Assess 160: 385–391.

170. Riah W, Laval K, Mougin C, Laroche-Ajzenberg E, Latour X, et al. (2014) Effects of pesticides on soil enzymes: A review. Environ Chem Lett.

ISBN:
DOI:

Chapter: 6

Enzymes and Environmental Contaminants Significant to Agricultural Sciences

Diana Bello[1]*, Carmen Trasar-Cepeda[1] and Fernando Gil-Sotres[2]

[1]Department of Biochemistry, Soil IIAG-CSIC, Santiago de Compostela, Spain

[2]Soil Science retired profesor, formerly working at Department of Soil Science and Agricultural Chemistry, Group Quality Assessment Soil CSIC Associated Unit, Faculty of Pharmacy, University of Santiago de Compostela, Santiago de Compostela, Spain

***Corresponding author:** Diana Bello, Department of Biochemistry, Soil IIAG-CSIC, Apartado 122, E-15780 Santiago de Compostela, Spain, Tel: +34 981590958, Ext 275; E-mail: dianabello@iiag.csic.es

Abstract

Contamination of soils is a particularly serious problem because of the impact that it has on soil functioning, and on the whole ecosystem. Although some contamination is due to natural processes (e.g. volcanic eruptions and weathering of the soil parent material), most is generated by daily human activity, such as industrial processes, transportation, construction, uncontrolled discharges, waste generation and agriculture. Agricultural soils, which are continually exploited to produce food and fodder, are particularly sensitive to contamination as agricultural practices affect many basic soil properties. The filtering and degradative functions of agricultural soils are intensely, often irreversibly, modified. Agricultural soils generally display poor resilience, i.e. they are incapable of recovering from any type of aggression, and any type of contamination that they suffer is likely to lead to their complete degradation.

As the effects of pesticides and fertilizers on soil enzyme activity are considered in previous chapters, the aim of this chapter is to reflect the modifications in commonly used contamination indicators (soil enzymes) in response to the other contaminants most frequently encountered in the agricultural sector (heavy metals, petroleum derived products and persistent organic pollutants).

Keywords

Agricultural soils; Chlorophenols; Heavy metals; Persistent organic pollutants (POPs); Petroleum; Polycyclic aromatic hydrocarbons (PAHs); Soil contamination; Soil enzymes

General Introduction

Contamination of soils is a particularly serious problem because of the impact that it has on the whole ecosystem, as well as on soil functioning [1]. Although some contamination is due to natural processes (e.g. volcanic eruptions and weathering of the soil parent material), most of it is generated by daily human activity, such as industrial processes, transportation,

ISBN:
DOI:

construction, uncontrolled discharges, waste generation and agriculture [2].

Of the anthropogenic activities that cause contamination, industrial and agricultural processes are particularly important sources of potentially toxic compounds. Contamination via agricultural activity occurs as a result of the ever more frequent use of fertilizers, pesticides and other compounds. These types of substances may persist in the soil for years as they are often highly resistant to natural degradation and their toxic effects may therefore be prolonged [3]. Industrial processes release immeasurable amounts of substances, most of which are deposited on soils. Moreover, the land on which large factories are installed is directly affected by any waste or by uncontrolled discharges generated and is therefore likely to be contaminated. The transportation of industrial products is also a potential source of contamination due to accidental discharges. Daily urban life is yet another source of soil contamination via the generation of municipal solid waste, emission of gases to the atmosphere and production of sewage sludge [2].

Agricultural soils, which are continually exploited to produce food and fodder, are particularly sensitive to contamination as agricultural practices affect many basic soil properties [4]. Removal of natural vegetation interrupts the natural cycling of elements. Moreover, because plant remains are not returned to the soil, the organic matter becomes depleted and the soil characteristics are altered by the consequent acceleration of mineralization processes. During ploughing, the topsoil is turned and homogenized, thus destroying the soil structure and altering the flow of air and water within the soil. The filtering and degradative functions of agricultural soils are therefore intensely, often irreversibly, modified [5]. Agricultural soils generally display poor resilience, i.e. they are incapable of recovering from any type of aggression, and any type of contamination that they suffer is likely to lead to their complete degradation [6].

Contaminants can be classified in many different ways, on the basis of the source or nature of the compounds or by the routes whereby they reach the soil [7-9]. There are also many ways of further classifying the contaminants within these groups. The four types of contaminants that usually affect agricultural soils are heavy metals (often associated with the addition of fertilizer to the soil), pesticides, oil-based compounds and persistent organic pesticides (POPs). The effects of fertilizers and pesticides on soil enzyme activity are considered in previous chapters of this book (chapters 2 and 5 respectively). In this chapter, we will consider the effects of heavy metals, oil derivatives and POPs on soil enzymatic activity. The aim of this chapter is to consider the modifications in commonly used contamination indicators in response to the contaminants most frequently encountered in the agricultural sector.

Contamination by Heavy Metals

General concepts and sources of heavy metals in agricultural soils

Heavy metals are sometimes defined, on the basis of the density of the elemental form of the metal, as those chemical elements of density greater than 6 g cm^{-3} [10]. The most important of these elements from an environmental viewpoint are Cd (density 8.65 g cm^{-3}), Co (8.90 g cm^{-3}), Hg (13.6 g cm^{-3}), Ni (8.90 g cm^{-3}), Zn (7.10 g cm^{-3}), Pb and Cu (both 11.30 g cm^{-3}). Despite their lower densities, As (5.72 g cm^{-3}) and Se (4.79 g cm^{-3}) are also considered as heavy metals.

Some of these elements, such as Co, Cu, Mn and Zn, are essential for living organisms, while others (Cd, Pb, Hg and As) are not essential and can be highly toxic. Essential elements are required at very low concentrations and are therefore also known as trace elements or microelements. Non-essential elements, which can be toxic to both plants (phytotoxic) and animals, are generally denominated toxic elements [10]. Some of these elements, such as Co,

ISBN:
DOI:

Cu, Mn and Zn, are essential for living organisms, while others (Cd, Pb, Hg and As) are not essential and can be highly toxic.

The two main sources of heavy metals in agricultural and other types of soils are natural processes and anthropogenic sources. The latter source is the main cause of pollution as inputs via this route are approximately 100 times higher than inputs generated by natural contamination [2].

In natural contamination processes, heavy metals accumulate in agricultural soils as a result of weathering of rocks and their constituent minerals. In general, this source of input is more important in soils derived from igneous or metamorphic rocks than in those derived from sedimentary rocks. Thus, soils derived from basic and ultrabasic rocks (e.g. serpentinites) accumulate metals such as Mn, Co, Ni Cu and Zn, and soils derived from parent materials containing sulphides can accumulate Pb (galena, PbS) and Hg (cinnabar, HgS).

Heavy metals usually reach agricultural soils as a result of different human activities, either via the use of common agricultural practices (fertilization) or via atmospheric deposition.

Agricultural practices such as the application of organic fertilizers, inorganic fertilizers, lime, pesticides and even irrigation waters, lead to the addition of trace metals to soil, often at concentrations that cause toxicity [2]. Thus, heavy metals such as As, Cu, Mn and Zn may be added to soil via organic fertilizers, whereas As, Cd, Mn, V and Zn may be added via inorganic fertilizers, and As and Pb via lime. The main elements added via pesticides would be Cu, Mn, Zn, As and Pb, and those added via irrigation water, Cd, Pb and Se. These types of amendments are usually carried out periodically, and the input of heavy metals can therefore be considerable, even when the agents applied do not contain large amounts of metals. Compost derived from urban waste is a major source of heavy metals, particularly Zn, Cd and Pb, and also Cr, Cu and Hg [2]. Pig slurry can be an important source of Cu, as this element is included in the pig diet to accelerate the growth of pig livestock. The presence of metals in these fertilizers has led to the appearance of regulations concerning the maximum amounts of metals that can be added to soil to minimize toxic effects and the risk of the transfer of such metals from the soil to other parts of the ecosystem via water. Unfortunately, the laws establishing the maximum amounts of metals that a soil can receive before being considered as contaminated differ between countries and are often not based on the same parameters.

Inorganic phosphate fertilizers are an important source of Cd and other heavy metals such as Cr and Pb. These metals occur naturally in phosphate fertilizers, as they are present in phosphate rock. However, application of this type of fertilizer to agricultural soils that are used intensively during several consecutive years has generated high levels of Cd (potentially toxic) in the soil [11]. However, the addition of Cd to soils via fertilizer is not generally considered problematical as this element is usually present in phosphate rock as the trivalent cation (Cr^{3+}), which is considered non-toxic [12,13].

Atmospheric deposition of metals to agricultural soils generally occurs in particulate form, via dry, wet or occult deposition (fog and mist). Transportation of metals via the atmosphere may involve long distances, and the source may be far from the soil where the metals are finally deposited. However, atmospheric deposition is usually more important in soils close to industrial sites, high temperature ovens, open cast mines and roads with heavy traffic. Although all of the previously mentioned sources are of human origin, metals can also enter the atmosphere via natural sources, including volcanic activity, which is an important source of Hg, Pb and Ni. Agricultural soils close to areas where volcanic activity prevails will obviously be particularly affected by this type of emission.

ISBN:
DOI:

Heavy metals and soil enzymes

The effects of heavy metals on soil enzymes have been investigated in numerous studies, particularly in the past 25 years. However, as the effects are conditioned by methodological factors, which vary widely between studies, it is difficult to reach any general conclusions. The methods used in such studies have not been standardized and they generally vary in terms of the location (laboratory or field), the nature of contamination (artificial or real), the amount of metal that reaches the soil (sometimes indeterminate, particularly when involving waste discharge), the interval between the contamination event and analysis of the soil, the metal causing the contamination (often complicated by the simultaneous involvement of several metals), and the enzyme(s) considered.

More than 30% of recently published studies refer to real situations of contamination in the field, produced by accidental discharges (Figure 1). This indicates the great variety of information available as it implies an almost total lack of information about the amounts and types of metals that reach the soil, and sometimes the timing of the contamination event is not even known. Although field data are generally considered to reflect real situations better than laboratory-derived data [14], only some 10% of published studies have been carried out in experimental plots in which all parameters (both environmental and contamination-related, i.e. metal, dose, timing) are known (Figure 1). By contrast, more than 50% of the publications report studies carried out in controlled laboratory conditions (Figure 1). Although all of the factors that affect the contamination are controlled, these studies do not, however, reflect the real changes that will take place under natural conditions [14].

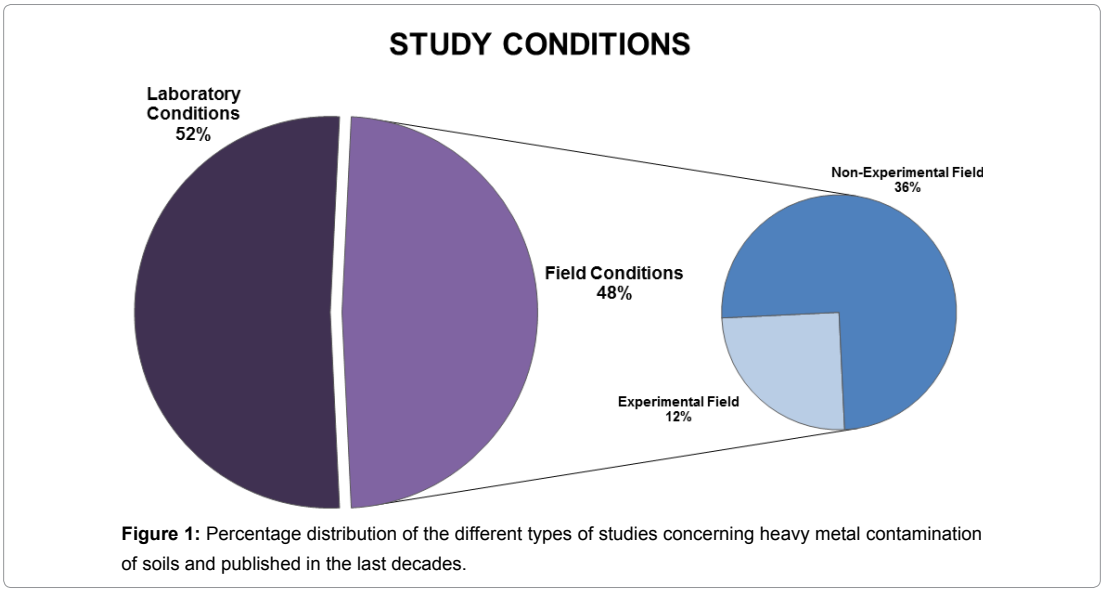

Figure 1: Percentage distribution of the different types of studies concerning heavy metal contamination of soils and published in the last decades.

One of the main differences between studies carried out under controlled conditions (either in the laboratory or in an experimental plot) and under field conditions is the length of time between the addition of metal to the soil and the analysis of the biochemical properties used as contamination indicators. Under controlled conditions, the effects of metal are usually studied

ISBN:
DOI:

at the initial moment of contamination and also after some days [15-17] or even weeks [18]. Different periods of exposure (contact time between metal and soil) are often used to determine the temporal changes in enzymatic activity, as done by [15] and [17], who carried out 3 measurements in an incubation period of 28 days. In exceptional cases, the study period may span several years, although this is not common in laboratory experiments as the properties of the control soil would be greatly affected by the incubation, thus complicating interpretation of any effects observed in the contaminated soils. By contrast, in many studies carried out under field conditions, the kinetics of the reactions are not considered (although with some exceptions, [19]), and the information is limited to a single observation time that varies greatly between studies, between 2 days [20] and 33 years [21,22].

Most studies published in the last decades have focused on the effects of only 4 metals, two of which are toxic (Cd and Pb) and two of which are essential elements or micronutrients (Cu and Zn), as these metals are often found as soil contaminants. Other metals such as Cr and Ni have been studied on some occasions, whereas studies involving V, As, Co, Hg, Ag, Mn and Se are relatively scarce (Figure 2). The studies also vary widely in regard to the enzyme(s) used as indicators of the effects of the metals (Figure 3). Phosphatase (both acid and alkaline) is the enzyme most commonly considered in these studies (25 % of studies), followed by dehydrogenase (19%). Urease and arylsulphatase activities have been measured in 15% of the studies, while the activities of ß-glucosidase, proteases, cellulases, invertases, lipases and xilanases are rarely measured. In other words, most authors seem to consider that the metal toxicity basically affects the P cycle (of which phosphatase is the key enzyme) and microbial activity, which would be represented by dehydrogenase activity as this oxidoreductase enzyme is only present in active microorganisms.

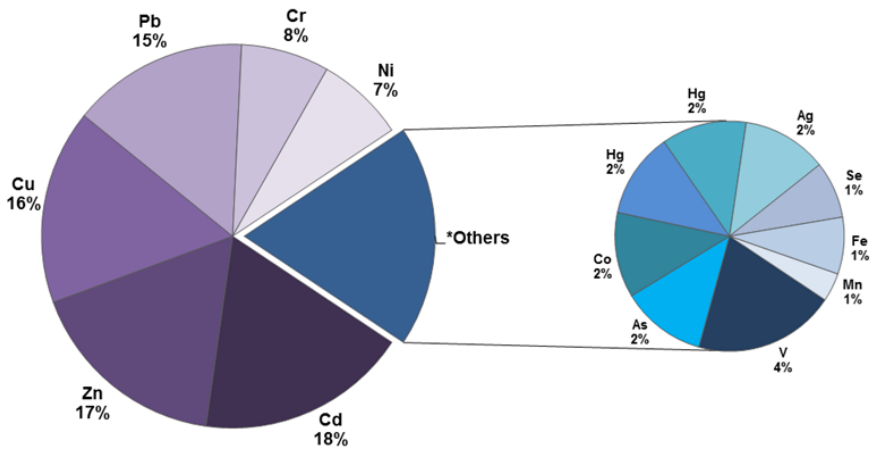

Figure 2: Relative importance of the metals investigated in studies concerning heavy metal contamination of soils and published in the last decades.

ISBN:
DOI:

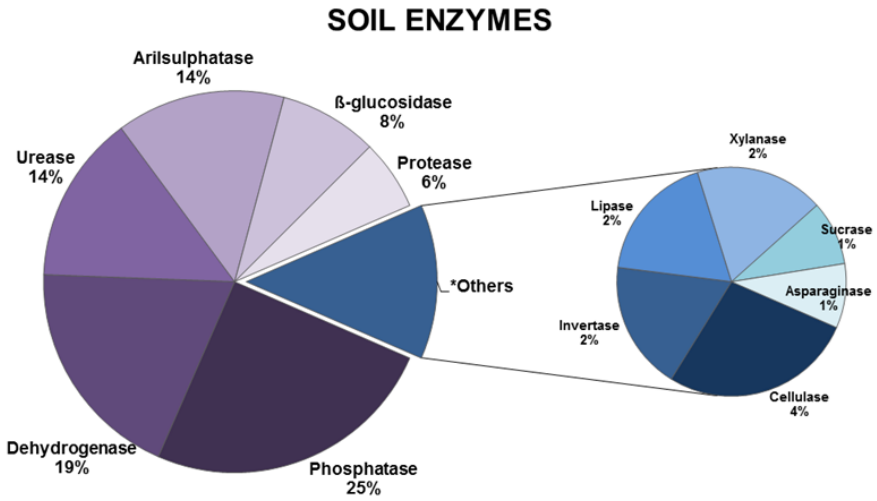

Figure 3: Percentage distribution of different enzymes analyzed in studies evaluating the effect of heavy metal contamination of soils and published in the last decades.

This type of study is also complicated by the dose of metal used. Different doses of metals are used in all studies (in some cases the differences are of one or two orders of magnitude), and very few researchers express their results on the basis of toxicological parameters, such as e.g. ED_{50}, which would enable immediate comparison of the data. The ED_{50} has been defined as the concentration of the compound that causes the value of a particular soil property to decrease by half of the value observed in control (uncontaminated) soil [23,24]. The fact that different units are used to express the dose of metal also hinders comparison of the results of different studies. Moreover, in some studies the metal is only added on a single occasion, whereas in others the metal is added at several different times. Likewise, although many authors only use a single metal, others add various metals (as mixtures or individually), sometimes each at different concentrations.

Despite the above-mentioned difficulties, some general findings can be highlighted. For example, oxidoreductase-type enzymes, such as dehydrogenase, are more sensitive to metals than hydrolytic enzymes [25,26]; this may be because dehydrogenase is an intracellular enzyme and is thus only found in active cells, and death of the microorganisms will cause an immediate decrease in the concentrations of the enzyme. The lower sensitivity of hydrolytic enzymes may be explained by the fact that a fraction of the total enzyme content is usually stabilized on soil colloids and is thus protected from the action of the metal [25]. However, dehydrogenase is not always more sensitive than hydrolytic enzymes, particularly in Cu-contaminated soils, as the presence of this metal affects measurement of the dehydrogenase activity, thus producing erroneous data [27]. With respect to hydrolytic enzymes, although urease is usually strongly affected by the presence of metals [15,19,28,29], the effects on phosphatase and ß-glucosidase are more variable [17,29], and they are often either only slightly affected [22,30-32] or not affected at all [10,33-35]. Nonetheless, the information published so far suggests that general conclusions cannot be reached about the behaviour of the enzymes, as the results reported by different authors are somewhat inconsistent. Enzymes do not always behave in the same way

ISBN:
DOI:

in response to different metals. Phosphatase has been found to be sensitive to Pb [36], but not to a mixture of metals [35,37]. Moreover, different enzymes are sometimes affected in different ways by the same metal, even in the same experimental study. Thus, Moreno et al. [38,39] report that in soils contaminated with increasing doses of Cd, dehydrogenase is negatively and directly affected by Cd, whereas protease-BAA and ß-glucosidase are not affected and the activity of urease increases only very slightly, at least 7 days after the contamination event. Likewise, Bellas et al. [29] found that urease and phosphomonoesterase were highly sensitive to contamination with increasing doses of Zn, while ß-glucosidase was less sensitive, as its activity was only modified by very high doses of Zn.

The concentrations at which a particular metal is toxic and may affect edaphic enzymes also vary depending on the metal, although some authors consider that any metal can be toxic if present at high enough concentrations [31,40]. The metals usually considered to be most toxic to the edaphic microbiota are Cu (+3), Cr (+5) and Ni (+5), and those considered the least toxic are Zn (+2), Cd (+1) and Pb (+4), although the concentrations at which these metals are reported to be toxic vary greatly, possibly because of the different methods used in different studies. Metal concentrations higher than those established as the legal limits often do not affect the enzymatic activity, suggesting the existence of toxicity buffering mechanisms in soil [41]. The toxicity of Pb and particularly Cd (which is considered the most hazardous in terms of environmental effects) differs in soil and in ecosystems. These differences may be associated with the high mobility of this element, which can easily be transferred to plants and animals [39,42].

The effects of the metal on an enzyme, and therefore the concentrations at which the metal is toxic, vary depending on the time that has elapsed since the contamination event. This will be reflected by decreased toxicity of the metal, as a result of the interaction between the metal and the soil components, and by the presence in the soil of microorganisms that are resistant to the metal [43,44], as is clearly reflected in some kinetic-type studies reported in the literature. Thus, Moreno et al. [15] indicated that in soils contaminated with Cd, the ED_{50} value for dehydrogenase increased by between 2.1 and 4.2 times (depending on the soil characteristics) from 3 hours after the initial contamination up to 25 days later. Other authors, when investigating different metals and at different concentrations and contact time, also observed increases of ED_{50} [18,45]. However, the time of contact between the soil and contaminant does not always affect the enzymatic activity in the same way. Thus, several authors have observed that in some cases as the time of contact increases, the negative effect of the metal on the enzyme becomes more acute, and the ED_{50} value decreases [17,18,36,45].

Enzyme activities are often studied after the addition of several metals to soil, as occurs in accidental discharges or when organic fertilizers (e.g. sewage sludge) are applied to land. The most common combinations of metals considered are Cd and Zn [32,46] and these metals plus Cu [17] or plus Cu and Pb [21,30]. A synergistic effect between the metals is usually observed [46]. However, studies carried out under controlled conditions are usually very different from each other and numerous factors can be considered. It is therefore not possible to reach general conclusions about whether the decrease in enzyme activity in the presence of different metals is due to additive effects or whether the toxic effects of some metals are enhanced by the presence of other metals. This obviously also applies to studies in which the conditions are not controlled, in which it is almost impossible to separate the individual effects of each metal. However, in soils that are heavily contaminated with mixtures of heavy metals, the negative effects on enzyme activity are usually evident and related to the concentrations of metals present [21,22,32]; in some cases, the activity of enzymes such as urease, phosphatase and

ISBN:
DOI:

dehydrogenase is almost totally inhibited [21].

The great diversity of results regarding the effect of metals of soil enzyme activity can be at least partly explained by considering the mechanisms involving the metals in the soil and those involving the enzymes in the presence of metals. As already mentioned the effects of metals on enzyme activity are known to be the result of the effects on the edaphic microbiota and on stabilized enzymes. The toxic effects of metals on soil microorganisms are mainly attributed to the denaturation of proteins or destruction of the integrity of microbial cells [47], which kills the microbiota. Some microbial process may also be negatively affected by exposure to heavy metals, although such effects will not necessarily kill the microorganisms [48]. The metal must enter the microbial cells to exert any effect, and it must therefore be present in the ionic form. Metals affect extracellular enzymes directly via modification of the interaction between the enzyme and its substrate, denaturation of the enzyme, and interaction with the active groups on the enzyme [48,49]. Indirect effects on the synthesis of enzymes by microbial cells may also occur as a result of the changes in microbial communities brought about by the presence of metals. All of these mechanisms also require the presence of ionic forms of the metals. In soil, the metals react strongly with colloidal soil components (clay, organic matter, extractable oxides) to form rather insoluble compounds, thus reducing the presence of the ionic forms of the metal in the soil solution [2,29]. Many authors believe that only the bioavailable fraction of metals, rather than the total metal content should be considered when evaluating the effects on enzymes, [50,51, among others]. The size of this fraction relative to the total content is determined by the physical and chemical characteristics of the soil. Thus, for those metals with different oxidation numbers, the redox state will determine the predominant ionic form at a particular soil pH. The soil pH will, in turn, influence the solubilization processes and determine the concentration of the soluble ionic form of the metal. On the other hand, the organic matter content, the degree of transformation and the particle size distribution of the inorganic soil fraction will affect the retention of different exchangeable forms of the metal, as well as the fractions that are stabilized on organic matter, bound to oxides or precipitated. The solubility of each of these fractions will also differ. The soil texture will also affect the mobility of metals in the soil, thus determining whether the metal will remain in the surface horizon or move to deeper horizons. The importance of the soil properties in determining the effects of metals on enzymes has been confirmed in numerous studies. The effects are greater in acidic soils than in alkaline soils, as the metals are more soluble at low pH [14,40].

The influence of the type and amount of soil organic matter has been considered in different studies as one of the main factors regulating the toxicity of heavy metals, because it is one of the soil properties that determine the concentration of heavy metals in the soil solution and therefore its bioavailability [29,38,52]. The soil texture also determines the effect of the metal on the soil enzymatic activity, and metals have been found to be most toxic in sandy and sandy-loam soils [18]. These later authors also reported that the ED_{50} values increase (i.e. the toxicity of the metal decreases) as the organic matter content and CEC increase. Despite this basic effect of the soil properties on the metal toxicity, analytical data on the soils used in the experiments are often not provided, which hinders interpretation of the data reported.

The different soil pools in which the metals are retained will be continuously modified by the soil dynamics during the time of contact, and the solubility of the metals tends to decrease over time. These changes may explain the increases in ED_{50} that occur as the time of contact between the soil and metal increases. However, the changes depend on the soil properties and do not always occur in the same direction, particularly if there are any alterations in the soil physicochemical conditions. This type of amendment often occurs in agricultural soils as a

ISBN:
DOI:

result of management practices such as liming, fertilization and the mixing of soil horizons by ploughing. The application of organic fertilizers that are rich in metals, such as pig manure and sewage sludge, is particularly problematical as mineralization of the organic matter increases the bioavailability of metals in soil [38].

Contamination by Petroleum Products

General concepts and sources of petroleum products in agricultural soils

Ever since coal, crude oil (petroleum) and natural gas were first extracted, the production, storage, refinement and transportation of these fossil fuels have led soils being contaminated with hydrocarbons [53]. Petroleum products include combustible derivatives (petrol, diesel, fuel oil, asphalt, etc.) as well as petrochemical derivatives (waxes, oils, benzene, etc.) and natural gas. These derivatives occur in solid or semisolid states and also in liquid and gaseous forms. Many of them contain a wide variety of compounds, such as polycyclic aromatic hydrocarbons (PAHs), benzene and derivatives, cycloalkanes, and aliphatic hydrocarbons [54].

Crude oil is usually released to the soil as a result of breakages in pipelines, whereas the derivatives usually reach ecosystems via discharges produced during transportation and storage, or as a result of accidents involving agricultural machinery [55]. The physical, chemical and biological effects of these discharges on soils are diverse as oil-based compounds differ greatly with respect to their physical state, composition and contents of toxic products. These discharges are known to affect the health of plants, animals and humans, as well as soil quality and productivity.

Petroleum products and soil enzymes

Interest in the effects of hydrocarbons on soil enzyme activity first arose in the 1960s [56], after which there was surge in the number of studies focused on the alterations to soil functioning brought about by crude oil. Of the studies reviewed, some 73% of those published in the last decades (Figure 4a) analyzed the changes in soil enzyme activity caused by the presence of crude oil, whereas only 19% involved more refined products such as diesel (Figure 4a). Studies concerning the impact of lighter products such as petrol, kerosene and motor oil are much less common (< 5%).

Almost half of the studies reviewed (43%) were carried out under controlled laboratory conditions e.g. [57,58] (Figure 4b). However, many researchers [59-64] have evaluated the effects of accidental discharges (mainly caused by leaks from damaged or broken pipelines) of hydrocarbons, and especially of crude oil, in the field (39%, Figure 4b) [62-64]. However, studies carried out in experimental plots and in which all parameters related to the contamination event (dose, time of contact, etc.) are considered are much less common, and only 18% of the studies reviewed (Figure 4b) were carried out under field conditions in the strictest sense, and in all of these the contaminant was crude oil e.g. [65-67].

A wide variety of enzymes are used to evaluate the extent of soil degradation caused by the presence of hydrocarbons. As shown in Figure 4c, most studies carried out in the past few decades have focused on the effect of hydrocarbons on dehydrogenase activity (22%), followed by urease (16%) and phosphatase (13%) activity [68-71]. The activity of enzymes such as catalase, invertase and protease has been measured in approximately 10% of studies, to evaluate the degree of soil contamination caused by different types of hydrocarbons, and the activity of ß-glucosidase, cellulase and peroxidase has been considered in less than 5% of studies [62,72,73].

ISBN:
DOI:

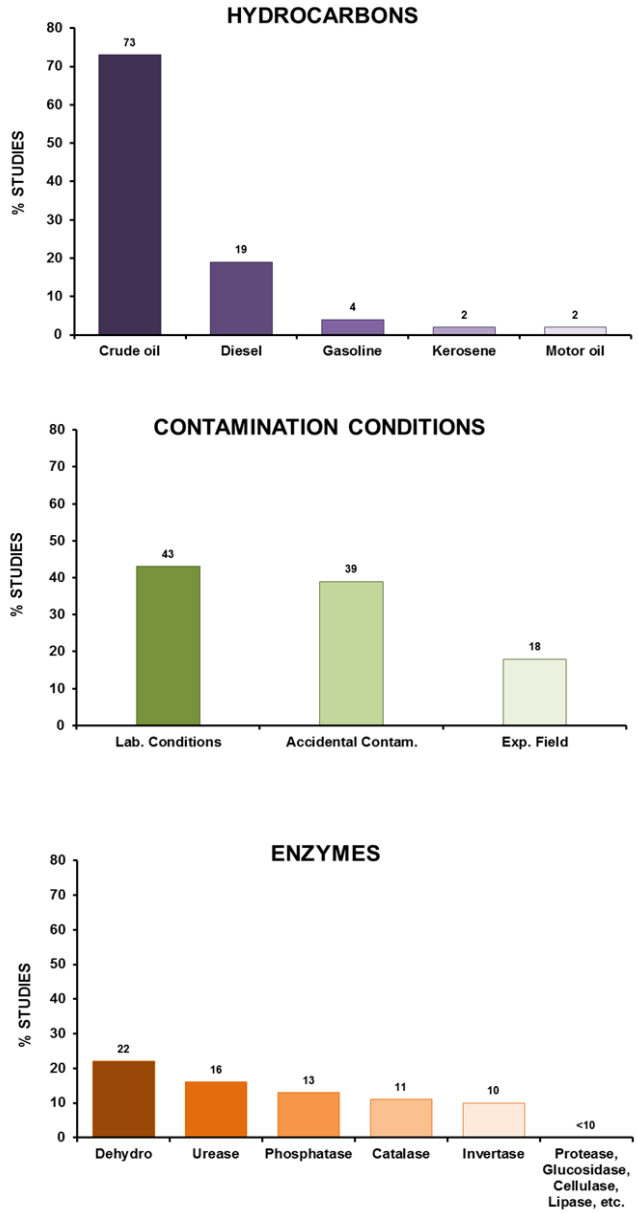

Figure 4: Percentage distribution of (a) type of compound investigated, (b) experimental conditions and (c) enzymes investigated in studies concerning contamination of soils with oil-derived products and published last decades.

ISBN:
DOI:

There is a lack of consistency in the behaviour of the different enzymes, and even in that of the same enzyme, in response to contamination with hydrocarbons. Thus, dehydrogenase activity can decrease, increase or be unaffected by the presence of hydrocarbons, depending on the soil characteristics [60,61] or the type of hydrocarbon involved [63,74]. Likewise, the dose of contaminant [59,75,76] and the time of contact between the contaminant and the soil [64,70,77] also affect the activity of enzymes such as dehydrogenase, urease, phosphatase and catalase. In most of the studies reviewed, the dehydrogenase activity in soils contaminated with the lowest doses of diesel tended to decrease, whereas the activity tended to increase at the highest doses (Table 1). However, for contamination by crude oil, the percentage of soils in which the dehydrogenase activity decreased and increased was almost the same, for all doses used (Table 1). The inhibitory effect of the presence of hydrocarbons on the intracellular enzyme activity is usually related to the death of microbial populations that are sensitive to these contaminants [70,78]. However, stimulation of enzyme activity indicates that, after a certain amount of time, some adaptation takes place and microbial populations that are resistant to the presence of hydrocarbons are able to grow and multiply and, therefore, the number of soil microorganisms and intracellular activity both increase [79,80]. The decrease in soil enzymatic activity in response to contamination may be caused by non-polar organic compounds covering both organic-mineral and cell surfaces, thus hindering the interaction between enzyme active sites and soluble substrates, with adverse effects on the expression of enzyme activity [55,81].

	CRUDE OIL		DIESEL	
	Low doses	High doses	Low doses	High doses
Dehydrogenase				
No effect	13%	4%	0%	33%
Reduction	50%	52%	75%	0%
Estimulation	37%	44%	25%	67%
Urease				
No effect	13%	0%	0%	0%
Reduction	47%	50%	50%	50%
Estimulation	40%	50%	50%	50%
Phosphatase				
No effect	0%	0%	0%	0%
Reduction	57%	58%	50%	60%
Estimulation	43%	42%	50%	40%
Catalase				
No effect	33%	7%	---	50%
Reduction	34%	60%	---	0%
Estimulation	33%	33%	---	50%
Invertase				
No effect	10%	12%	---	---
Reduction	60%	47%	---	---
Estimulation	30%	11%	---	---

Table 1: Percentage of studies involving the enzyme activity measured in response to the presence of low and high doses of crude oil and diesel.

The proportion of studies in which the presence of hydrocarbons inhibited the activity of hydrolytic-type enzymes is almost the same as that in which the activity (especially of urease and phosphatase) was stimulated (Table 2). Hydrocarbon-derived inhibition of the activity of

ISBN:
DOI:

enzymes associated with N, P, S and C cycling [82-86] has been related to the interaction between the active centre of the enzyme and the petroleum product involved [70,79]. On the other hand, in soils contaminated with hydrocarbons the resistance or stimulation is attributed to the stabilized fraction of the enzyme, which is protected from the action of the contaminant [79]. Stimulation of the activity of hydrolytic-type enzymes has also been related to the ability of certain microbial populations to degrade peptide and phosphate compounds in contaminated soils [58], which indicates that the fraction that depends on the edaphic microbiota will be responsible for the recovery. Another argument used to explain the increase in the hydrolytic activity of certain enzymes in soils contaminated with petroleum products is the presence of certain compounds in the hydrocarbons that could be used as enzyme substrates [79].

	PAH's	Chlorophenols
Dehydrogenase		
No effect	8%	17%
Reduction	62%	83%
Estimulation	30%	0%
Urease		
No effect	10%	0%
Reduction	70%	100%
Estimulation	20%	0%
Phosphatase		
No effect	20%	29%
Reduction	40%	43%
Estimulation	40%	28%

Table 2: Proportion of studies involving the enzyme activity measured in response to the presence of PAHs or chlorophenols.

When crude oil or oil-based products are released to the environment they are subjected to a series of processes such as evaporation, dissolution, dispersion, emulsification, adsorption, microbial degradation and photooxidation [87]. Thus, in kinetic studies concerning the changes in functioning of hydrocarbon-contaminated soils, it was found that although the enzyme activity (dehydrogenase phosphatase and urease) decreased immediately after the contamination event, the toxic effect diminished and in some cases the activity reached similar values to those measured in uncontaminated soils [64,70]. This suggests that the contaminants undergo some type of transformation that reduces the degree of toxicity to soil.

As already mentioned and indicated in Table 1, the presence of petroleum derivatives has highly variable effects on the soil. The different effects of hydrocarbons on soil enzyme activity will depend on the site characteristics, as well as on the composition of the organic compound and the dose of the product reaching the soil [55]. The site characteristics include climatic aspects, such as vegetation and, in particular, the soil characteristics. Climate affects the biological activity of the soil [88,89], and plants excrete compounds that may interact with the contaminants (e.g. the root exudates of different legumes) [77].

The influence of the soil is expressed by means of the physical properties, which are determined by the size of the organic fraction and the inorganic particle size distribution, amongst others [70,81]. Petroleum derivatives may decrease the exchange of oxygen between the soil and the atmosphere, thus decreasing the availability of oxygen for microbiota [90]. A lower content of oxygen in the soil atmosphere would lead to alteration of the redox state [85], yielding more reduced conditions. The immediate effect of these changes would be a decrease in aerobic populations, particularly nitrifying microorganisms [85,90-92]. Peña et al. [85] sug-

ISBN:
DOI:

gested that at high doses of contaminant, the organic compounds may block soil pores and also form films around nitrifying bacteria, thus hampering the passage of oxygen to the cells and the involvement of these bacteria in ammonia nitrification. The possibility that microbiota become covered by organic compounds has also been suggested by Serrano et al. [70]. Some authors have reported a large decrease in the presence of cellulose-degrading microorganisms as a result of the lower availability of oxygen in the soil, with a consequent decrease in cellulase activity [79,92], mainly exocellulase activity [78,93]. Another effect of a lower oxygen content would be an alteration in microbial communities, leading to changes in the relationships between diverse groups of microbiota [71,76,78,90,91].

The organic matter content and the particle size distribution of the mineral component of the soil are also important factors and will determine whether the components of the contaminant will be degraded by the soil microbiota or whether they will exert toxic effects on the microbiota. It is known that small molecular weight (i.e. more soluble) compounds in oil derivatives can be strongly adsorbed on organic and mineral soil colloids, thus making them less accessible to microorganisms [54,70,79,86]. Thus, the soils that are richest in organic matter and clay will be most capable of decreasing the toxic effects of the contaminant by eliminating the most toxic compounds from the soil solution [58,79]. Thus, in clayey soils neither dehydrogenase activity nor respiration is affected by the contamination [94]. However, some authors consider that the adsorption is not related to the physical composition of the soil [70]. These later authors also suggest that it is difficult to evaluate the real impact of the adsorbed fraction, as although adsorption of the contaminant decreases its toxicity in the short term, it also leads to greater persistence of harmful substances in the soil, which will affect the edaphic microbiota in the long term as these substances are gradually desorbed [79]. Adsorption of the contaminant on soil colloids will also inhibit the activity of enzymes stabilized on these colloids [70].

Although the site conditions are important, the effects of petroleum derivatives on the soil enzyme activity will mainly depend on the composition of the derivatives. This effect is manifested in two ways. On the one hand, the composition will affect the presence of degradable products, which may stimulate the soil activity. On the other hand, it will affect the amount of toxic products present, which will obviously decrease or even totally inhibit the activity. Stimulation of the biological and biochemical activity of the soil has been indicated by authors such as Aliev and Gadzhiev [72], who described how low doses of contaminants stimulate microbial growth, thus generating greater enzyme activity and by [95], who suggested that crude oil is used by the edaphic microbiota at a greater rate than that at which they would degrade plant remains. Berry and Burton [94] report that concentrations of crude oil lower than 5000 mg kg^{-1} will not have any impact on soil, and Bauer et al. [58] report that the continuous presence of crude oil will cause an increase in dehydrogenase activity in the soil, related to stimulation of the microbiota, and in urease, protease-BAA and phosphatase activity. Some authors have also suggested that degradation of the crude oil components is closely linked to soil characteristics such as neutral pH and nutrient availability [54,59,79]. Interestingly, not all authors agree about the timing of the response of soil microbiota to the presence of crude oil. Mikkonen et al. [77] reported that the stimulation occurs immediately after the contamination event and then decreases gradually over time. By contrast, Labud et al. [79] described lag-type effects indicative of adaptation by the microbiota, possibly due to the substitution of microbial populations that are not adapted to crude oil by other resistant types that are also capable of degrading the oil. Margesin et al. [78] reported that the addition of diesel causes an increase in all enzyme activities as a result of the development of heterotrophic microorgan-

ISBN:
DOI:

isms in contaminated soil.

The greater or lower stimulation of soil biochemical activity as a result of contamination by hydrocarbons is not only related to the dose of compound that reaches the soil, but also to the composition of the oil-derived product. Thus, aliphatic compounds tend to be more readily degraded, and therefore more rapidly degraded, than aromatic compounds [63,96]. Shorter and straighter aliphatic chains will be more readily degraded those longer, more complex chains [96]. Compounds between C_{10} and C_{19} are considered as potentially dangerous when they reach the soil [97]. Microorganisms are also less able to degrade compounds with a high boiling point and that are poorly soluble in water, and these compounds will therefore remain in the soil for longer [96]. The solubility of organic compounds is usually expressed by the K_{ow} parameter (coefficient of the distribution of the compound between an organic phase, octanol, and an aqueous phase): compounds with high K_{ow} values are considered to be more persistent, and indirectly more toxic, than compounds with lower K_{ow}values [97].

The toxic effect of discharges of oil derivatives is associated both with the characteristics of the oil compounds and the presence of minor components that are extraordinarily toxic to the edaphic microbiota [59,98,99]. These compounds are generally metals and organic compounds such as PAHs, which are either initially present in the crude oil or are incorporated as additives during transformation processes [98,100]. Earlier publications indicate the lead added to increase the octane rating of petrol as the main component responsible for the negative effects of petrol discharges on soil [82]. However, the addition of lead to petrol is now prohibited, at least in the EU, and therefore this risk has almost disappeared within the European community. Special care must be taken with PAHs, which may be incorporated into petroleum products during transformation in petrochemical plants. The presence of PAHs will inhibit the activity of dehydrogenase and most hydrolases. More specifically, the microbial populations involved in C, N, P and S cycling will be almost totally decimated, i.e. the metabolic capacity of the soil will be almost totally paralyzed [70]. The presence of organic acids can have a similar effect as these are also highly toxic to microorganisms [63]. However, some authors suggest that the presence of certain additives in petrol and diesel may increase the soil biochemical activity, thus increasing the dehydrogenase activity [98].

Finally, there is no agreement about the effects of the presence of mineral oils in the refined products. Some researchers suggest that the presence of these oils will lead to development of lipases in the contaminated soil, which suggests that the mechanism of degradation of these oils is similar to that of fatty acids [84]. However, others state that the presence of oils in products that reach the soil would have more negative effects on the biochemical activity than the discharge of diesel products.

Persistent Organic Pollutants (POPS)

General concepts and sources of persistent organic pollutants in agricultural soils

In addition to the organic compounds used as pesticides (considered in a previous chapter of this book and therefore not considered here), other persistent organic pollutants (POPs) can occur in soils (both agricultural and natural soils) in significant amounts; these include phenolic compounds, mainly chlorophenols, and polyaromatic hydrocarbons (PAHs) [101]. Both of these groups include stable compounds that persist in the environment because of their structural properties. Many of these compounds are volatile and can circulate widely by a process known as the “grasshopper effect”. Once released by repeated (seasonal) processes

ISBN:
DOI:

of evaporation and deposition, these compounds are transported over long distances via the atmosphere [101]. Moreover, these compounds are biochemically, chemically and physically recalcitrant, which enables them to accumulate in live organisms and thus reach humans through the food chain.

Phenolic compounds are highly toxic and widespread environmental contaminants and are therefore dangerous for living organisms. Although these compounds occur naturally in soils and plants, as contaminants they are usually of anthropogenic origin and derived from agricultural or industrial waste [101].

Among the phenolic compounds, chlorophenols (Figure 5) are common contaminants of soils and water [102]. They are synthetic compounds that are obtained on commercial scale via the chlorination of phenols or by hydrolysis of chlorobenzenes [103]. They can also be formed as intermediate products during the bleaching of paper pulp [103]. Chlorophenols can also reach soils as degradation products of some herbicides and pesticides [103,104].

Chlorophenols are used as fungicides, bacteriocides and insecticides in the preservation of wood and leather, although they are also used as herbicides and as protective agents or antiseptics in plant production [103]. Most studies on the effects of these compounds on soil biochemical properties involve pentachlorophenol (PCP) [105-108]. Some studies have also investigated the effects of some of the tetrachlorophenols [103] and less substituted phenols such as 2,4-dichlorophenol and/or 2,4,5-trichlorophenol [109-111]. The general use of PCP, the most highly chlorinated phenolic compound, is possibly due to the capacity of this compound to decouple oxidative phosphorylation [104], which also makes it highly toxic, persistent and apparently ubiquitous in the environment [112,113].

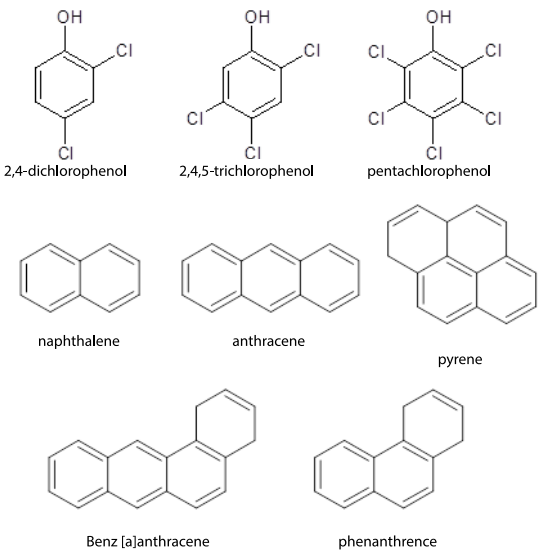

Figure 5: Persistent Organic Pollutants (POPs) most commonly found in the environment and considered in this review.

ISBN:
DOI:

Chlorophenols can reach the environment, particularly the soil, as a result of the use of chemical products containing these compounds, the uncontrolled discharge of industrial waste, including the waste generated during the manufacturing of leather, paper and paper pulp, combustion of municipal, industrial and chemical waste, and also the degradation of some pesticides used in agriculture [104].

PAHs are a type of POPs that are characterized by containing 2 or more aromatic rings with shared C atoms [114]. The PAHs most commonly found in the environment are anthracene, fluoranthene, pyrene, benzoanthracene crysene, benzofluoranthene and naphthalene (Figure 5). These toxic, carcinogenic and hydrophobic compounds tend to bind to soil organic matter, and thus more than 90% of these compounds that are present in the environment accumulate in the surface horizon of soils [115]. The high toxicity of these compounds has important consequences for human and environmental health [116]. The physical and chemical characteristics lead to the already-mentioned high biological and chemical persistence of the compounds and their accumulation in large amounts in the environment where they enter food chains and potentially affect human health. PAHs are usually therefore considered to be the most dangerous soil contaminants [117].

The PAHs found in the environment are derived from both natural and anthropogenic sources. Natural sources include volcanic eruptions, forest fires and combustion of fossil fuels [117]. The most common anthropogenic sources are wood combustion, waste incineration, combustion of petrol and crude oil, pyrolysis of fats and oils, vehicle emissions, industrial carbon distillation processes and other processes associated with the petrochemical industry [101,116,118-120], and also biochar production, which has increased greatly in recent years [121,122]. PAHs can also reach agricultural soils via the application of sewage sludges [117].

Interactions between POPs and soil enzymes

Chlorophenols and soil enzymes

The most notable finding regarding contamination of soil with chlorophenols is the reduction, or even disappearance, of dehydrogenase activity (Figure 6). This effect has been observed by several researchers, even in soils with very different physical and chemical characteristics [110,111,124]. Dehydrogenase is an oxidoreductase enzyme that only acts intracellularly and thus reflects the microbial activity [49,135]. The strong reduction in activity therefore reflects the death of some or all of the soil microorganisms. The mortality is associated with the toxicity of chlorophenols to the edaphic microbiota [107], which is caused by the presence of the chlorophenolate anion. This anion is generated by the dissociation of chlorophenol and because of its negative charge and small size, it passes through cell membranes and once inside the cytoplasm, causes cell death [110,111,136]. The proportion of dissociated forms depends on the pKa value, and therefore the effect of the compound on the soil will increase as the soil pH approaches the pKa of chlorophenol dissociation [137]. This explains the greater effect on the dehydrogenase activity that chlorophenols such as 2,4-DCP and 2,4,5-TCP have on alkaline soils than on acidic soils [111]. Therefore, soil pH is one of the most important properties that should be taken into account when determining the effects of chlorophenols.

The intermediate products of the microbial degradation of chlorophenol may also have toxic effects on soil. Chlorophenol can be degraded by both autochthonous soil microorganisms [107] and by microorganisms added to the soil specifically for this purpose [108]. However, in a kinetic study of the effect of the action of PCP on several enzymes, Diez et al. [124] observed a strong initial decrease in dehydrogenase activity after the addition of PCP, followed by a stationary phase and then by recovery of the activity. These authors attributed the initial de-

ISBN:
DOI:

crease in activity to the toxicity of PCP (or its reaction products), leading to the death of the soil microorganisms and the consequent decrease in dehydrogenase activity. As the PCP (and/or its products) is adsorbed by the soil, the toxicity will decrease, enabling growth of the microorganisms and recovery of the enzyme activity. In a bioremediation trial with bacteria capable of degrading PCP, McGrath and Singleton [105] observed a sharp decrease in dehydrogenase activity after the addition of PCP to the soil and no recovery of the enzyme activity after 6 weeks, even though the PCP had disappeared. These authors suggested that this was due to the formation of toxic products derived from the degradation of PCP or to the inability of the microorganisms to recover after the initial addition of PCP.

The effects of chlorophenols on soil may be further complicated by the fact that these compounds can be adsorbed onto soil colloids. This would considerably decrease the bioavailability of the compound, including the availability of the compound to interact with the soil microbiota, thus reducing its toxicity [138]. However, the adsorption would also lead to greater persistence of the compound, with the possible slow desorption into the soil solution and interaction with the adsorbed forms of soil enzymes. All of this would contribute to the high toxicity associated with chlorophenols [110,111].

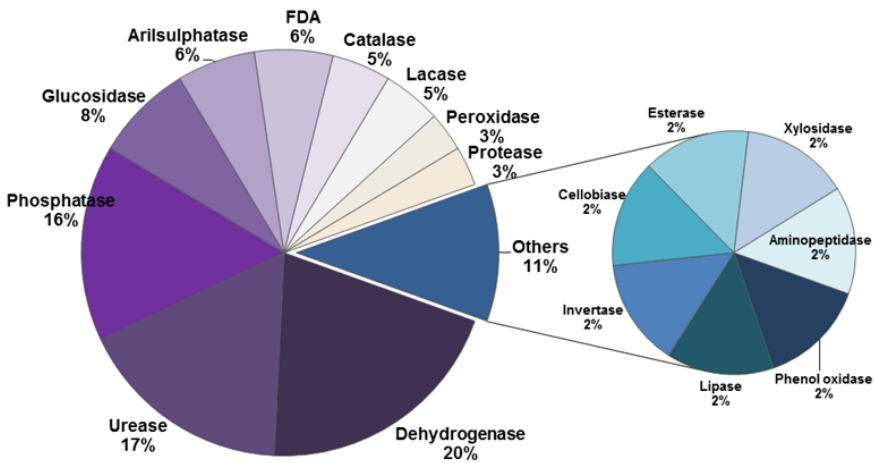

Figure 6: Percentage distribution of the different enzyme activities measured in studies concerning soil contamination with Persistent Organic Pollutants (POPs) and published in the last decades.

The adsorption of chlorophenols on soil components partly depends on the composition and physicochemical characteristics of the compound and partly on the physical and chemical characteristics of the soil, such as pH, organic matter content, specific surface area, cation exchange capacity, clay content and amorphous components [49,139-142]. The colloidal soil components play a fundamental role in the retention of contaminants via sorption processes, either by physical processes or chemical interactions. Physical adsorption mainly occurs on the soil organic matter as a result of weak hydrophobic bonding [143]. Adsorption via chemical interactions between soil colloids and chlorophenols is due to the positive charge on both organic and mineral soil colloids, as well as to the proportion of phenolate forms in the soil

ISBN:
DOI:

solution [144,145]. In other words, the soil pH (which affects the sign of the charge on the colloids) and the pKa of chlorophenol (which together with the soil pH determine the proportion of ionized forms of the compound) will determine the degree of adsorption. Other possible mechanisms of chemical interaction include the formation of cationic bridges between the negatively charged soil components and the phenolate group, and also binding by ligand exchange processes. Both of these are fundamental processes in soils in which negatively charged surfaces predominate [142,143].

Other characteristics of the compound that should be taken into account in the adsorption processes are the number and location of chlorine substituents. These characteristics also determine the adsorption of the compound, because the value of the coefficient of octanol/water distribution (Kow) increases with the number of chlorine atoms. The resulting increased hydrophobicity favours the adsorption of chlorophenols on polar components, as the presence of phenol in the aqueous phase is hampered [146-149]. The adsorption also depends on the position in which the chlorine atoms are located in the ring relative to the hydroxyl group, a characteristic of the compound that also determines its toxicity [150,151].

These adsorption processes indirectly affect the soil enzymes that are stabilized on soil colloids [111]. These authors showed that, in acidic and neutral soils, the main process that regulates the toxicity of the chlorophenols is the adsorption by organic matter, which limits the concentration of the contaminant in the soil solution and determines that the adsorbed fraction will basically be a non-dissociated fraction of the contaminant. They also showed that the pH is of relatively minor importance as regards the decrease in the activity of enzymes that are sensitive to the contaminants and, thus, that dissociation and action of compounds such as phenolate are less important for the toxicity of the compound.

Polycyclic aromatic hydrocarbons and soil enzymes

Studies of the influence of PAHs on the soil enzymatic activity are scarce. Nonetheless, the few studies carried out to date clearly show that the effect of PAHs on soil enzyme activity depends on the type of contaminant and on soil characteristics such as the pH, content of inorganic colloids, soil nutritional status and particularly the organic matter content [129,117].

The effects of PAHs on soil are similar to those described for other organic compounds. Thus, depending on the type of compound and its complexity, different processes may occur in the soil and the presence of the compound will therefore affect different enzymes in different ways. In most cases, the presence of PAHs in soil leads to a strong reduction in the activity of enzymes such as urease and dehydrogenase. This can be attributed to the high toxicity of these compounds to soil microorganisms: these compounds are generally recognised as a potential health risk due to their intrinsic chemical stability, high recalcitrance to different types of degradation and high toxicity to living organisms [152]. Nonetheless, in some cases the presence of PAHs can stimulate soil enzyme activity. This is because (as with hydrocarbons) some PAHs can be degraded by soil microorganisms (mainly bacteria, usually Actinobacteria, and some fungi). The degree of inhibition or stimulation of soil enzyme activity is generally thought to depend on the molecular weight of the PAHs and the number of benzene rings in the molecule [78,117]. Thus, PAHs with more than four benzene rings will be more difficult for microorganisms to degrade and may be toxic to these, thus decreasing the soil enzyme activity [117]. By contrast, PAHs with two, three or four benzene rings (naphthalene, phenanthrene, anthracene and pyrene) are more readily degraded by soil microorganisms. Nonetheless, even when the PAHs can be used by microorganisms as a source of energy, they may initially have a toxic effect, leading to a reduction in the number and activity of microorganisms over a certain

ISBN:
DOI:

period of time. After this period of stagnation, and in the absence of competitors, the resistant microorganisms can undergo a period of enormous growth, giving rise to a period of strong metabolic activity during which the PAHs will be degraded, leading to a stimulatory effect on some intracellular enzymes such as dehydrogenase [128,129]. However, some authors have also suggested that PAH-contaminated soils may be colonized by microorganisms capable of degrading these organic compounds [117].

In addition to microbial degradation, PAHs may also be subjected to other processes in soil. One of the main processes is adsorption on organic matter, which leads to the formation of stable combinations known as "bond residue" [117]. The adsorption is favoured by the low solubility and the polar nature of the PAHs, characteristics that do not favour the occurrence of the compounds in the soil solution. On the other hand, PAHs may also undergo adsorption on the inorganic soil colloids, or they may be volatized, a process that is limited by high soil moisture contents. All of these processes reduce the availability of the PAHs in the soil, which will reduce their toxicity [129], but will also prevent their use by the microorganisms as substrate [117]. This will favour persistence of the compounds in the soil and, therefore, the potential risk to microorganisms associated with the potential release of the compounds as a result of a change in the soil conditions. Only those PAHs that are present in the soil solution will be available for interacting with enzymes, and the distribution of the contaminants in the soil phases is crucial for interactions with enzymes [128]. Thus, if the soil is contaminated with several contaminants, competition for adsorption sites may occur and those compounds with a lower adsorption capacity will be displaced to the soil solution and will exert greater toxicity than those compounds with a higher adsorption capacity.

In soils contaminated with different types of PAHs, the degree of persistence of these compounds in the soil, and their bioavailability to soil microorganisms will generally influence the enzyme activity and the overall microbial activity. However, once again the results of different studies are very variable. The discrepancies are probably due to the very different types of compounds (or mixtures of these) investigated, the different doses added to the soil, and the different enzymes studied. Thus, in a study of the contamination of soil with phenathrene and naphthalene, Margesin et al. [78] observed an initial increase in protease activity and a decrease in urease activity, whereas catalase, dehydrogenase and lipase activities, which were low in the uncontaminated soil, were scarcely affected. These authors also observed a decrease in the concentration of PAHs over time, which may have been due to volatilization (as occurs with naphthalene) or irreversible sorption, in addition to biodegradation. However, as the enzyme activities either did not recover or only increased slightly (urease), the authors concluded that these enzymes are not good indicators of PAHs contamination [78].

In a later study, Shen et al. [128] observed that after a strong initial reduction, the activity of enzymes such as dehydrogenase tended to recover, while other enzymes such as urease increased during the later stages of incubation. These authors suggested possible explanations for their findings, including a slow ageing process in which the bioavailability of the contaminant is presumed to decrease due to the association of the contaminant with the native organic matter [153] and penetration within the soil micropores [123,154]. Shen et al. [128] also suggested that recovery of the enzyme activity may be due to tolerance and adaptation of the microorganism to the PAHs, to their biodegradation or to their sorption on soil colloids, all of which would reduce the bioavailability of the compounds.

Contamination with PAHs usually involves other contaminants such as heavy metals. Thus, the joint effects of pollutants may be similar to (additive) or stronger (synergistic, more than additive) or weaker (antagonistic, less than additive) than the effects expected from exposure to

ISBN:
DOI:

each substance [123]. The effects depend on the constituents of the contaminant mixture and can vary significantly [155]. Studies, in which the combined effects of one or more PAHs with different metals are investigated, generally show that the mixtures have stronger effects on soil enzyme activity and exert stronger toxicity than the PAHs by itself. Dehydrogenase is generally considered to be the enzyme that is most sensitive to combined contamination with metals and PAHs [127,128], although urease is also sensitive to this type of contamination [123]. Maliszewska-Kordybach and Smreczak [127] also suggest that the mixtures are more toxic, as indicated by decreased dehydrogenase activity, in soils with a lower affinity for metals, i.e. soils with low organic matter and silt contents. Other authors [131] also report that the organic matter content is negatively correlated with the effect of mixtures of pyrene and different metals (Pb, Cr, Cd and Cu) on dehydrogenase activity, whereas the content of amorphous compounds is positively related to the reduction in dehydrogenase activity caused by these metals.

The higher toxicity of mixtures of metals and PAHs than the metals by themselves is usually attributed to the fact that some lipophilic compounds, such as PAHs, exert a narcotic effect whereby they interact with the cytoplasmic membranes of microorganisms, thus affecting their permeability and structure. This would facilitate the entry of the metals accompanying the PAHs into the microbial cells, negatively affecting the cell functions [127,128]. Furthermore, according to some authors, if the metals exert toxic effects, the microorganisms will not be able to degrade the PAHs as they would in the absence of the metals and the PAHs will thus remain in the soil at higher concentrations than otherwise [156]. Moreover, at high concentrations of heavy metals, non-specific binding of these elements to cell surface may preclude interaction of PAH with bacterial membranes leading to decrease of their toxic effects [157].

Finally, some field studies have been carried out in areas close to biochar production plants, where continual, long-term contamination with PAHs (in this case bound to biochar) occurs. In these studies, it was found that the presence of PAHs, even at high concentrations, did not have any effect on the activity of various soil enzymes. The authors of these studies suggested that this could be due to adaptation to the high amounts of PAHs (which act as an additional source of carbon), or to minimization of the negative effect of the PAHs by the biochar or to a reduction in the bioavailability of the PAHs that was bound to the biochar before reaching the soil [158]. These authors also suggested that PAHs of pyrogenic origin will bind more closely to biochar particles than those of petrogenic origin, as in the former case the binding occurs at the moment of formation, while in the latter case it is a secondary process.

Final Remarks

From the information given in this chapter, it may appear that soil enzymes are of limited value as contamination indicators. Many of the contradictory results reported in the literature are difficult to explain, as many researchers do not provide detailed information about the conditions under which the contamination occurred or about the soil characteristics. Many field studies involving accidental discharges do not include control values for uncontaminated soils. This lack of data is a serious omission as the dynamics of the contaminants will depend on the soil characteristics, as recognised by most authors.

The initial decrease in dehydrogenase activity appears to be one of the most common effects observed. This reflects the lethal effects of the compounds on the soil microbiota. The variations in this activity in response to the contaminant depend greatly on the characteristics of the compound, the type of soil and the experimental conditions. Urease is usually the most sensitive of the hydrolytic enzymes. The urease activity usually decreases to a lesser extent than that of dehydrogenase, indicating that a large part of the urease in soil is stabilized on

ISBN:
DOI:

soil colloids and that in these locations; the enzyme suffers less degradation than the microbiota. However, some compounds can inactivate stabilized enzymes, either by inactivation of the active centre or by other mechanisms.

The toxic effects of contaminants in the soil are greatly affected by the colloidal fractions (both organic and inorganic), which favour sorption processes and removal of the contaminant from the soil solution. These properties are diminished in agricultural soils, relative to natural soils, as a result of agricultural practices. Agricultural soils are therefore extraordinarily sensitive to contamination, indicating the urgent need for reliable indicators of degradation. Although soil enzymes may be suitable as contamination indicators, methods of studying and determining enzyme activities must be developed as rapidly as possible to enable different research groups to select the best indicator(s) for their purposes. Thus, despite the possible value of using expressions that reflect the toxicity of a compound in relation to the enzymatic activity (such as $ED_{50,}$ $DL_{50,}$ REC_{50} and other ecotoxicological measures), there is not yet any standardized method of calculating these expressions. If more data on these parameters were available, we would perhaps have a more positive perception of the capacity of soil enzyme activities to act as contamination indicators.

Acknowledgements

The authors are grateful to the Spanish Ministerio de Ciencia e Innovación for financial support (Project No. CTM2011-25664, co-financed with FEDER founds from EU).

References

1. Adriano DC, Bollag JM, Frankenberger WT, Sims RC (1999) Bioremediation of Contaminated Soils. ASA, Madison.
2. Ross SM (1994) Sources and forms of potentially toxic metals in soil-plant systems. In: Ross SM (Ed.). Metals in soil-plant systems, 3-25. Wiley & Sons Ltd, New York.
3. Morrill LG, Mahilum BC, Mohiuddin SH (1982) Organic Compounds in Soils: Sorption, Degradation and Persistence. Ann Arbor Science Publishers, Ann Arbor.
4. Leita L, de Nobili M, Mondicini M, Muhlbachova G, Marchiol L, et al. (1999) Influence of inorganic and organic fertilization on soil microbial biomass, metabolic quotient and heavy metal availability. Biol Fertil Soils 28: 371-376.
5. Doran JW, Parkin TB (1994) Defining and Assessing Soil Quality. In: Doran JW, Coleman DC, Bezdicek DF, Stewart BA (Eds.). Defining Soil Quality for a Sustainable Environment, 3-21, SSSA-ASA, Madison.
6. Blum WEH (1990) The challenge of soil protection in Europe. Env Conserv 17: 72-74.
7. Pepper IL, Gerba CP, Brusseau ML (1996) Pollution Science. Academic Press Inc, New York.
8. Knox AS, Gamerdinger AP, Adriano DC, Kolka RK, Kaplan DI (1999) Sources and Practices Contributing to Soil Contamination. In: Adriano DC, Bollag JM, Frankenberger WT, Sims RC (Eds.). Bioremediation of Contaminated Soils, 53-88, ASA, Madison.
9. Pierzynski GM, Sims JT, Vance GF (2004) Soils and Environmental Quality. CRC Press, Boca Raton.
10. Naidu R, Krishnamurti GSR, Wenzel W, Megharaj M, Bolan NS (2001) Heavy Metal Interactions in Soils and Implications for Soil Microbial Diversity. In: Prasad MNV (Ed.). Metals in the Environment: Analysis by Biodiversity, 401-431, Marcel Dekker Inc, New York.
11. Alloway BJ (1990) Cadmium. In: Alloway BJ (Ed.). Heavy Metals in Soils, 100-124, Chapman & Hall, Glasgow.
12. McGrath SP, Smith S (1990) Chromium and Nickel. In: Alloway BJ (Ed.). Heavy Metals in Soils, 125-150, Chapman & Hall, Glasgow.
13. Samborska A, Stepniewska Z, Stepniewsk W (2004) Influence of different oxidation states of chromium (VI, III) on soil urease activity. Geoderma 122: 317-322.
14. Giller KE, Witter E, McGrath SP (1998) Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: A review. Soil Biol Biochem 30: 1389-1414.

ISBN:
DOI:

15. Moreno JL, García C, Landi L, Falchini L, Pietramellara G, et al. (2001) The ecological dose value (ED_{50}) for assessing Cd toxicity on ATP content and dehydrogenase and urease activities of soil. Soil Biol Biochem 33: 483-489.

16. Chander K, Klein T, Eberhardt U, Joergensen RG (2002) Decomposition of carbon-14-labelled wheat straw in repeatedly fumigated and non-fumigated soils with different levels of heavy metal contamination. Biol Fertil Soils 35: 86-91.

17. Renella G, Ortigoza ALR, Landi L, Nannipieri P (2003) Additive effects of copper and zinc on cadmium toxicity to phosphomonoesterase activities and ATP content of soil as estimated by the ecological dose (ED_{50}). Soil Biol Biochem 35: 1203–1210.

18. Haanstra L, Doelman P (1991) An ecological dose-response model approch to short- and long-term effects of heavy metals on arylsulphatase activity. Biol Fertil Soils 11: 18-23.

19. Wang Q-Y, Zhou D-M, Cang L (2009) Microbial and enzyme properties of apple orchard soil as affected by long-term application of copper fungicide. Soil Biol Biochem 41: 1504–1509.

20. Hinojosa MB, Carreira JA, García-Ruíz R, Dick RP (2004) Soil moisture pre-treatment effects on enzyme activities as indicators of heavy metal-contaminated and reclaimed soils. Soil Biol Biochem 36: 1559-1568.

21. Lee S-H, Kim E-Y, Hyun S, Kim J-G (2009) Metal availability in heavy metal-contaminated open burning and open detonation soil: assessment using soil enzymes, earthworms, and chemical extractions. J Hazard Mater 170: 382–388.

22. Niemeyer JC, Lolata GB, de Carvalho GM, da Silva EM, Sousa JP, et al. (2012) Microbial indicators of soil health as tools for ecological risk assessment of a metal contaminated site in Brazil. Appl Soil Ecol 59: 96–105.

23. Babich H, Bewley RJF, Stotzky G (1983) Application of the ecological dose concept to the impact of heavy metals on some microbe-mediated ecological processes in soil. Arch Environ Con Tox 12: 421–426.

24. Haanstra L, Doelman P, Oude Voshaar JH (1985) The use of sigmoidal dose-response curves in soil ecotoxicological research. Plant Soil 84: 293–297.

25. Brookes PC (1995) The use of microbial parameters in monitoring soil pollution by heavy metals. Biol Fertil Soils 19: 269–279.

26. Brookes PC (2000) Changes is soil microbial properties as indicators of adverse effects of heavy metals. Rendiconti Accademia Nazionale delle Scienze detta dei XL: Memorie di Scienze Fisiche e Naturali 118º XXIV: 205-227.

27. Chander K, Brookes PC (1991) Is the dehydrogenase assay invalid as a method to estimate microbial activity in Cu-contaminated soils? Soil Biol Biochem 23: 909-915.

28. Kandeler E, Tscherco D, Bruce KD, Stemmer M, Hobbs PJ, et al. (2000) Structure and function of the soil microbial community in microhabitats of a heavy metal polluted soil. Biol Fertil Soils 32: 390-400.

29. Bellas R, Trasar-Cepeda C, Gil-Sotres F, Leirós MC (2012) Changes in some hydrolase activities in agricultural soils in response to zinc contamination. In: Trasar-Cepeda C, Hernández MT, García C, Rad C, González-Carcedo S (Eds.). Soil Enzymology in the Recycling of Organic Wastes and Environmental Restoration, 181-193, Springer-Verlag, Heidelberg.

30. Tyler G (1976) Heavy metal pollution, phosphatase activity, and mineralization of organic phosphorus in forest soils. Soil Biol Biochem 8: 327-332.

31. Wuertz S, Mergeay M (1997) The Impact of Heavy Metals on Soil Microbial Communities and their Activities. In: Van Elsas JD, Trevors JT, Wellington EMH (Eds.). Modern Soil Microbiology, 607-642, Marcel Dekker Inc, New York.

32. Jiang J, Wu L, Li N, Luo Y, Liu L, et al. (2010) Effects of multiple heavy metal contamination and repeated phytoextraction by *Sedum plumbizincicola* on soil microbial properties. Eur J Soil Biol 46: 18–26.

33. Brookes PC, McGrath SP, Klein DA, Elliott ET (1984) Effects of heavy metals on microbial activity and biomass in field soils treated with sewage sludge. In: Environmental Contamination International Conference, 574-583, CEP Consultants Ltd, Edinburgh.

34. Reddy GB, Faza A, Bennett R (1987) Activity of enzymes in rhizosphere and nonrhizosphere soils amended with sludge. Soil Biol Biochem 19: 203-205.

35. Gzik A, Kuehling M, Schneider I, Tschochner B (2003) Heavy metal contamination of soils in a mining area in South Africa and its impact on some biotic systems. J Soil Sediment 3: 29-34.

36. Marzadori C, Ciavatta C, Montecchio D, Gessa C (1996). Effects of lead pollution on different soil enzyme activities. Biol Fertil Soils 22: 53–58.

37. Tyler G (1974) Heavy metal pollution and soil enzymatic activity. Plant Soil 41: 303-311.

38. Moreno JL, Hernández MT, García C (1999) Effects of cadmium-contaminated sewage sludge compost on dynamics of organic

ISBN:
DOI:

matter and microbial activity in an arid soil. Biol Fertil Soils 28: 230-237.

39. Moreno JL, Hernández T, Pérez A, García C (2002) Toxicity of cadmium to soil microbial activity: effect of sewage sludge addition to soil on the ecological dose. Appl Soil Ecol 21: 149–158.

40. Bååth E (1986) Effects of heavy metals in soil on microbial processes and populations (A review). Water Air Soil Poll 47: 335-379.

41. Dumestre A, Sauvé A, McBride M, Baveye P, Berthelin J (1999) Copper speciation and microbial activity in long-term contaminated soils. Arch Environ Con Tox 36: 124–131.

42. Landi L, Renella G, Moreno JL, Falchini L, Nannipieri P (2000) Influence of cadmium on metabolic quotient, L-D-glutamic acid respiration ratio and enzyme activity: microbial biomass ratio under laboratory conditions. Biol Fertil Soils 32: 8-16.

43. Yamamoto H, Tatsuyama K, Uchiwa T (1985) Fungal flora of soil polluted with copper. Soil Biol Biochem 17: 785–790.

44. Díaz-Raviña M, Bååth E, Frostegard A (1994) Multiple heavy metal tolerance of soil bacterial communities and its measurement by a thymidine incorporation technique. Appl Environ Microb 60: 2238–2247.

45. Speir TW, Kettles HA, Parshotam A, Searle PL, Vlaar LNC (1995) A simple kinetic approach to derive the ecological dose value, ED50, for the assessment of Cr(VI) toxicity to soil biological properties. Soil Biol Biochem 27: 801–810.

46. Moreno JL, Bastida F, Ros M, Hernández MT, García C (2009) Soil organic carbon buffers heavy metal contamination on semiarid soils: Effects of different metal threshold levels on soil microbial activity. Eur J Soil Biol 45: 220–228.

47. Dai J, Becquer T, Rouiller JH, Reversat G, Bernhard-Reversat F, et al. (2004) Influence of heavy metals on C and N mineralisation and microbial biomass in Zn-, Pb-, Cu-, and Cd-contaminated soils. Appl Soil Ecol 25: 99-109.

48. Landi L, Renella G, Moreno JL, Falchini L, Nannipieri P (2001) Influence of cadmium on the metabolic quotient, L- :D-glutamic acid respiration ratio and enzyme activity : microbial biomass ratio under laboratory conditions. Biol Fertil Soils 32: 8-16.

49. Nannipieri P (1994) The potential use of soil enzymes as indicators of productivity, sustainability and pollution. In: Pankhurst CE, Double BM, Gupta VVSR, Grace PR (Eds.). Soil biota: management in sustainable farming systems, 238-244, CSIRO, Sidney.

50. McGrath SP (1994) Effects of heavy metals from sewage sludge on soil microbes in agricultural ecosystems. In: Ross SM (Ed.). Toxic metals in soil-plant systems, 247-274, John Wiley & Sons, New York.

51. Chaperon S, Sauvé S (2007) Toxicity interaction of metals (Ag, Cu, Hg, Zn) to urease and dehydrogenase activities in soils. Soil Biol Biochem 39: 2329-2338.

52. Renella G, Zornoza R, Landi L, Mench M, Nannipieri P (2011) Arylesterase activity in trace element contaminated soils. Eur J Soil Sci 62: 590–597.

53. Saterback A, Toy RJ, McMain BJ, Willians MP, Dorn PB (2000) Ecotoxicological and analytical assessment of effects of bioremediation on hydrocarbon-containing soils. Environ Toxicol Chem 19: 2643-2652.

54. Franco I, Contin M, Bragato G, de Nobili M (2004) Microbiological resilience of soils contaminated with crude oil. Geoderma 121: 17–30.

55. Kiss S, Pasca D, Dragan-Bularda M (1998) Development in Soil Science 26: Enzymology of Disturbed Soils, Elsevier, Amsterdam.

56. Steubing L (1967) Studies on the change in microflora of a forest soil by penetrated heating oil. Angew Bot 40: 275-286.

57. Bauer E, Pennerstorfer C, Kandeler E, Braun R (1992) Evaluation of biological soil decontamination procedures - experiments on laboratory scale. In: ALVA-Tagung 1992, Fachgruppe Boden Vortrdge, 47-56, Bundesanstalt für Bodenwirtschaft, Vienna.

58. Bauer E, Pennerstorfer C, Kandeler E. Braun R (1994) Biological soil decontamination. Mitteilungen der Osterreichischen Bodenkundlicher Gesellschaft, No. 48/49, Vienna.

59. Kaigorodova SYu (1996) Biological activity of the petroleum-polluted bog soils in the Tyumen region. Programma i Tezisy 4. Nauchnoi Konferentsii Instituta Biologii 'Aktual'nye Problemy Biologii" (Syktyvkar in Russian).

60. Caravaca F, Roldán A (2003) Assessing changes in physical and biological properties in a soil contaminated by oil sludges under semiarid Mediterranean conditions. Geoderma 117: 53–61.

61. Wang J, Zhan X, Zhou L, Lin Y (2010) Biological indicators capable of assessing thermal treatment efficiency of hydrocarbon mixture-contaminated soil. Chemosphere 80: 837–844.

62. Mukatanov AKh, Rivkin PR (1980) Effect of petroleum on properties of soils. Neftyanoe Khozyaistvo 4: 53-54 (in Russian).

ISBN:
DOI:

63. Andreson RK, Khaziev FKh (1981) Fighting against petroleum pollution of soils. Korroziya i Zashchita v Neftegazovoi Promyshlennosti (Moscow) 7: 1-46 (in Russian).

64. Antonenko AM, Zanina OV (1992) Effect of oil on the enzymatic activity of alluvial soils in West Siberia. Pochvovedenie 1: 38-43.

65. Ismailov NM (1982) Petroleum pollution and biological activity of soils. In: Dobycha Poleznykh Iskopaemykh i Geokhimiya Prirodnykh Ekosistem, 227-235, IzdateFstvo Nauka, Moscow (in Russian).

66. Khaziev FKh, Tishkina EI, Kireeva NA (1988) Effect of petroleum products on the biological activity of soil. Biologicheskie Nauki (Moscow) 10: 93-99 (in Russian).

67. Kireeva NA, Novoselova EI, Khaziev FKH (1997) Phosphohydrolase activity of petroleum-polluted soils. Pochvovedenie 6: 723-725 (In Russian).

68. Tishkina EI, Kireeva NA (1986) Cultivation of petroleum-polluted grey forest soils. In: Khaziev FKh (ed) Povyshenie Plodorodiya Pochv V Usloviyakh Intensivnoi Sistemy Zemledeliya, 145-151, Bashkirskii Filial Akademii Nauk SSSR, Institut Biologii, Ufa (in Russian).

69. Kandeler E, Pennerstorfer C, Bauer E, Braun R (1994) Microbiological evaluation of the biological soil decontamination procedures in model experiment. Z Pflanz Bodenkunde 157: 345-350.

70. Serrano A, Tejada M, Gallego M, González JL (2009) Evaluation of soil biological activity after a diesel fuel spill. Sci Total Environ 407: 4056–4061.

71. dos Santos EDC, Silva IS, Simões THN, Simioni KCM, Oliveira VM, et al. (2012) Correlation of soil microbial community responses to contamination with crude oil with and without chromium and copper. Int Biodet Biodegr 70: 104–110.

72. Aliev SA Gadzhiev DA (1977) Effect of pollution by petroleum organic matter on the activity of biological processes of soils. Izvestiya Akademii Nauk Az.SSR, Seriya Biologicheskikh Nauk 2: 46-49 (in Russian).

73. Zhou L-K, Zheng Q-Y, Song M (1990) Biodegradation of petroleum hydrocarbons and phenol substances in soil and soil enzyme activities. Journal of Applied Ecology (Shenyang X) 1: 149-155.

74. Kireeva NA (1995) Microbiological evaluation of the soil polluted by petroleum hydrocarbons. Bashkirskii Khimicheskii Zhurnal 2: 65-68 (in Russian).

75. Zimenka TG, Kartyzhova LY (1986) Effect of petroleum pollution on the biological activity of a soddy-podzolic light loam soil. Vestsi Akademii Navuk BSSR, Seryya Biyalagichnykh Navuk 6: 52-55 (in Russian).

76. Megharaj M, Singleton I, McClure NC, Naidu R (2000) Influence of petroleum hydrocarbon contamination on microalgae and microbial activities in a long-term contaminated soil. Arch Environ Con Tox 38: 439–445.

77. Mikkonen A, Kondo E, Lappi K, Wallenius K, Lindström K, et al. (2011) Contaminant and plant-derived changes in soil chemical and microbiological indicators during fuel oil rhizoremediation with Galega orientalis. Geoderma 160: 336–346.

78. Margesin R, Zimmerbauer A, Schinner F (2000) Monitoring of bioremediation by soil biological activities. Chemosphere 40: 339–346.

79. Labud V, García C, Hernández T (2007) Effect of hydrocarbon pollution on the microbial properties of a sandy and a clay soil. Chemosphere 66: 1863–1871.

80. Serrano A, Gallego M, González JL, Tejada M (2008) Natural attenuation of diesel aliphatic hydrocarbons in contaminated agricultural soil. Environ Pollut 151: 494–502.

81. Andreoni V, Cavalca L, Rao M, Nocerino G, Bernasconi S, et al. (2004) Bacterial communities and enzymatic activities in PAHs polluted soils. Chemosphere 57:401-412.

82. Frankenberger WT (1988) Use of urea as a nitrogen fertilizer in bioreclamation of petroleum hydrocarbons in soil. B Environ Contam Tox 40: 66–68.

83. Kireeva NA (1996) Restoration of the fertility of petroleum-polluted soils. Neftyanaya i Gazovaya Promyshlennost 7: 21-22 (in Russian).

84. Margesin R, Zimmerbauer A, Schinner F (1999) Soil lipase activity – a useful indicator of oil biodegradation. Biotechnol Tech 13: 859–863.

85. Peña W, Trasar-Cepeda C, Gil-Sotres F, Leirós MC (2007) Modification of the degradative capacity of a soil artificially contaminated with diesel. Chemosphere 67: 1057–1063.

86. Guo H, Yao J, Cai M, Qian Y, Guo Y, et al. (2012) Effects of petroleum contamination on soil microbial numbers, metabolic activity and urease activity. Chemosphere 87:1273–1280.

87. Wang Z, Fingas M, Lambert P, Zeng G, Yang C, et al. (2004) Characterization and identification of the Detroit river mystery oil spill

ISBN:
DOI:

(2002). J Chromatogr A 1038: 201-214.

88. Duchaufour P (1977) Pédogenèse et classification. Masson, Paris.

89. Voroney RP (2007) The soil habitat. In: Paul EA (Ed.). Soil Microbiology, Ecology and Biochemistry. (3rd Edn), 25-49, Elsevier Inc, Oxford.

90. Ismailov NM (1983) Effect of petroleum pollution on the nitrogen cycle in soil. Mikrobiologiya 52: 1003-1007 (in Russian).

91. Janke S, Schamber H, Kunze C (1992) Effects of heating oil on the soil biological activity. Angew Bot 66: 42-45.

92. Kireeva NA, Vodop'yanov VV (1996) Mathematical modelling of the microbiological processes in petroleum-polluted soils. Pochvovedenie 10: 1222-1226 (in Russian).

93. Linkins AE, Fetcher N (1983) Effect of surface-applied Prudhoe Bay crude oil on vegetation and soil processes in tussock tundra. Proceedings 4th International Conference on Permafrost, 723-728, Fairbanks.

94. Berry KAT, Burton DL (1997) Natural attenuation of diesel fuel in heavy clay soil. Can J Soil Sci 77: 469-477.

95. Linkins AE, Atlas RM, Gustin P (1978) Effect of surface applied crude oil on soil and vascular plant root respiration, soil cellulase, and hydrocarbon hydroxylase at Barrow, Alaska. Arctic 31: 355-365.

96. Fan C-Y, Krishnamurthy S (1995) Enzymes for enhancing bioremediation of petroleum-contaminated soils: a brief review. J Air Waste Manage 45: 453-460.

97. Tang J, Wang M, Wang F, Sun Q, Zhou Q (2011) Eco-toxicity of petroleum hydrocarbon contaminated soil. J Env Sci 23: 845–851.

98. Frankenberger WT, Johanson JB Jr (1982) Influence of crude oil and refined petroleum products on soil dehydrogenase activity. J Environ Qual 11: 602-607.

99. Maila MP, Cloete TE (2005) The use of biological activities to monitor the removal of fuel contaminants-perspective for monitoring hydrocarbon contamination: a review. Int Biodeter Biodegr 55: 1–8.

100. Dawson JJC, Godsiffe EJ, Thompson IP, Ralebitso-Senior TK, Killham KS, et al. (2007) Application of biological indicators to assess recovery of hydrocarbon impacted soils. Soil Biol Biochem 39: 164–177.

101. Rao M, Scelza R, Acevedo F, Diez MC, Gianfreda L (2014) Enzymes as useful tools for environmental purposes. Chemosphere.

102. Rubilar O, Diez MC, Gianfreda L (2008) Transformation of chlorinated phenolic compounds by white rot fungi. Crit Rev Env Sci Tec 38: 227–268.

103. Czaplicka M (2004) Sources and transformations of chlorophenols in the natural environment. Sci Total Environ 322: 21–39.

104. Boyd SA, Mikesell MD, Lee J (1989) Chlorophenols in soils. In: Sawhney SA, Brown K (eds.) Reactions and Movement of Organic Chemicals in Soils, 209-228, SSA-ASA, Madison.

105. McGrath R, Singleton I (2000) Pentachlorophenol transformation in soil: toxicological assessment. Soil Biol Biochem 32: 1311-1314.

106. Saavedra G (2002) Determinación de la lacasa, Mn-peroxidasa, deshidrogenasa y fosfatasa del suelo para evaluar la detoxificación de un suelo Andisol de la IX Región, Tesis de Ingeniería Ambiental, Universidad de La Frontera, Temuco.

107. Scelza R, Rao MA, Gianfreda L (2008) Response of an agricultural soil to pentachlorophenol (PCP) contamination and the addition of compost or dissolved organic matter. Soil Biol Biochem 40: 2162–2169.

108. Cea M, Jorquera M, Rubilar O, Langer H, Tortella G, et al. (2010) Bioremediation of soil contaminated with pentachlorophenol by Anthracophyllum discolor and its effect on soil microbial community. J Hazard Mater 181: 315–323.

109. Moscoso F, Bouzas S, Gil-Sotres F, Leirós MC, Trasar-Cepeda C (2007) Suitability of the OCDE tests to estimate contamination with 2,4-dichlorophenol of soils from Galicia (NW Spain). Sci Total Environ 378: 58–62.

110. Bello D, Trasar-Cepeda C, Leirós MC, Gil-Sotres F (2008) Evaluation of various tests for the diagnosis of soil contamination by 2,4,5-trichlorophenol (2,4,5-TCP). Environ Pollut 156: 611-617.

111. Bello D, Trasar-Cepeda C, Leirós MC, Gil-Sotres F (2013) Modification of enzymatic activity in soils of contrasting pH contaminated with 2,4-dichlorophenol and 2,4,5-trichlorophenol. Soil Biol Biochem 56: 80-86.

112. Dercová K, Kysel'ová Z, Baranckova G, Sejaková Z, Mal'ová A (2003) Biodegradation and bioremediation of pentachlorophenol. Chemické Listy 97: 991–1002.

ISBN:
DOI:

113. Dercová K, Certík M, Mal'ová A, Sejaková Z (2004) Effect of chlorophenols on the membrane lipids of bacterial cells. Int Biodeter Biodegr 54: 251-254.

114. Zhan X, Wu W, Zhou L, Liang J, Jiang T (2010) Interactive effect of dissolved organic matter and phenanthrene on soil enzymatic activities. J Environ Sci 22: 607–614.

115. Wild SR, Jones KC (1995) Polynuclear aromatic hydrocarbons in the United Kingdom environment: a preliminary source in inventory and budget. Environ Pollut 88: 91–108.

116. Johnsen AR, Wick LY, Harms H (2005) Principles of microbial PAH-degradation in soil. Environ Pollut 133: 71–84.

117. Lipińska A, Kucharski J, Wyszkowska J (2013) Urease activity in soil contaminated with polycyclic aromatic hydrocarbons. Pol J Environ Stud 22: 1393–1400.

118. Gibson DT, Subramanian V (1984). Microbial degradation of aromatic hydrocarbons. In: Gibson DT (ed) Microbial Degradation of Organic Compounds, pp 181-252, Marcel Dekker Inc, New York.

119. Saim N, Dean JR, Abdullah MP, Zakaria Z (1997) Extraction of polycyclic aromatic hydrocarbons from contaminated soil using Soxhlet extraction, pressurised and atmospheric microwave-assisted extraction, supercritical fluid extraction and accelerated solvent extraction. J Chromatogr A 791: 361–366.

120. Jaouen-Madoulet A, Abarnou A, Le Guellec A-M, Loizeau V, Leboulenger F (2000) Validation of an analytical procedure for polychlorinated biphenyls, coplanar polychlorinated biphenyls and polycyclic aromatic hydrocarbons in environmental samples. J Chromatogr A 886: 153–173.

121. Meyer S, Glaser B, Quicker P. (2011) Technical, economical, and climate-related aspects of biochar production technologies: a literature review. Environ Sci Technol 45: 9473-9483.

122. Stavi I, Lal R (2012) Agroforestry and biochar to offset climate change: a review. Agron Sustain Dev 33: 81-96.

123. Shen G, Lu Y, Hong J (2006) Combined effect of heavy metals and polycyclic aromatic hydrocarbons on urease activity in soil. Ecotoxicol Environ Safe 63: 474–480.

124. Diez MC, Gallardo F, Saavedra G, Cea M, Gianfreda L, et al. (2006) Effect of pentachlorophenol on selected soil enzyme activities in a Chilean andisol. J Soil Sci Plant Nutr 6: 40-51.

125. Gianfreda L, Rao M, Piotrowska A, Palumbo G, Colombo C (2005) Soil enzyme activities as affected by anthropogenic alterations: intensive agricultural practices and organic pollution. Sci Total Environ 341: 265–279.

126. Kähkönen M, Tuomela M, Hatakka A (2007) Microbial activities in soils of a former sawmill area. Chemosphere 67: 521–526.

127. Maliszewska-Kordybach B, Smreczak B (2003) Habitat function of agricultural soils as affected by heavy metals and polycyclic aromatic hydrocarbons contamination. Environ Int 28: 719–728.

128. Shen G, Lu Y, Zhou Q, Hong J (2005) Interaction of polycyclic aromatic hydrocarbons and heavy metals on soil enzyme. Chemosphere 61: 1175–1182.

129. Baran S, Bielińska JE, Oleszczuk P (2004) Enzymatic activity in an airfield soil polluted with polycyclic aromatic hydrocarbons. Geoderma 118: 221–232.

130. Thavamani P, Malik S, Beer M, Megharaj M, Naidu R (2012) Microbial activity and diversity in long-term mixed contaminated soils with respect to polyaromatic hydrocarbons and heavy metals. J Environ Manage 99: 10–17.

131. Irha N, Slet J, Petersell V (2003) Effect of heavy metals and PAH on soil assessed via dehydrogenase assay. Environ Int 28: 779-782.

132. Ahtiainen J, Valo R, Jarvine M, Joutti A (2002) Microbial toxicity tests and chemical analysis as monitoring parameter at composting of creosote-contaminated soil. Ecotoxicol Environ Saf 53: 323–329.

133. Koch C, Wilke B-M (1996) Effect of PAH, PCB and heavy metals on the microbial activity in irrigated field soils. Mitt D Bodenkundlichen Gesell 81: 295-298.

134. Diez MC, Quiroz A, Ureta-Zañartu S, Vidal G, Mora ML, et al. (2005) Soil retention capacity of phenols from biologically pre-treated kraft mill wastewater. Water Air Soil Poll 163: 325–339.

135. Skujins J, Burns RG (1976) Extracellular enzymes in soil. Crit Review Microbiol 4: 383–421.

136. Packham ED, Duxbury CL, Mayfield CI, Inniss WE, Kruuv J, et al. (1982) Quantitative Analysis of Pollutant-induced Lethal and Sublethal Damage in Cultured Mammalian Cells. Bull Environ Contam Toxicol 29: 739-746.

ISBN:
DOI:

137. Diez MC, Mora ML, Videla S (1999) Adsorption of phenolic compounds and colour from bleached kraft mill effluent using allophanic compounds. Water Res 33: 125–130.

138. Ogram AV, Jessup RE, Ou LT, Rao PSC (1985) Effects of sorption on biological degradation rates of (2,4-dichlorophenoxy) acetic acid in soils. Appl Environ Microb 49: 582–587.

139. Bollag JM, Myers CJ, Minard RD (1992) Biological and chemical interactions of pesticides with soil organic matter. Sci Total Environ 123/124: 205–217.

140. Senesi N (1992) Binding mechanisms of pesticides to soil humic substances. Sci Total Environ 123/124: 63–76.

141. Benoit P, Barriuso E, Huout S, Calvet R (1996) Influence of the nature of soil organic matter on the sorption-desorption of 4-chlorophenol, 2,4-dichlorophenol and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D). Eur J Soil Sci 47: 567-578.

142. Cea M, Seaman JC, Jara AA, Fuentes B, Mora ML, et al. (2007) Adsorption behaviour of 2,4-dichlorophenol and pentachlorophenol in an allophanic soil. Chemosphere 67: 1354–1360.

143. McBride MB (1994) Environmental Chemistry of Soils. Oxford University Press, New York.

144. Peuravuori J, Paaso N, Pihlaja K (2002) Sorption behaviour of some chlorophenols in lake aquatic humic matter. Talanta 56: 523–538.

145. He Y, Xu J, Wang H, Ma Z, Chen J (2006) Detailed sorption isotherms of pentachlorophenol on soils and its correlation with soil properties. Environ Res 101: 362–372.

146. Liu D (1981) A rapid biochemical test for measuring chemical toxicity. Bull Environ Contam Toxicol 26: 145–149.

147. You CN, Liu JC (1996) Desorptive behaviour of chlorophenols in contaminated soils. Water Sci Technol 33: 263–270.

148. Dentel SK, Bottero JY, Khatib K, Demougeot H, Duguet JP, et al. (1995) Sorption of tannic acid, phenol, and 2,4,5-trichlorophenol on organoclays. Water Res 29: 1273–1280.

149. Pandiyan T, Martínez Rivas O, Orozco Mez J, Burillo Amescua G, Martínez Carrillo MA (2002) Comparison of methods for the photochemical degradation of chlorophenols. J Photoch Photobiol A 146: 149–155.

150. Liu D, Thompson K, Kaiser KLE (1982) Quantitative structure-toxicity relationship of halogenated phenols on bacteria. B Environ Contam 29: 130–136.

151. Saito H, Sudo M, Shigeoka T, Yamauchi F (1991) In vitro cytotoxicity of chlorophenols to goldfish GF-scale (GSF) cells and quantitative structure-activity relationships. Environ Toxicol Chem 10: 235–241.

152. Alexander M (1999) Biodegradation and Bioremediation. Academic Press Inc, San Diego.

153. Carrol KM, Harkness MR, Bracco AA, Balcarcel RR (1994) Application of a permeant/polymer diffusional model to the desorption of polychlorinated biphenyls from Hudson River sediments. Environ Sci Technol 28: 253–258.

154. Wu S Ch, Gschwend PM (1986) Sorption kinetics of hydrophobic organic compounds to natural sediments and soils. Environ Sci Technol 20: 717–725.

155. Jensen J, Sverdrup LE (2002) Joint toxicity of linear alkylbenzene sulfonates and Pyrene on Folsomia fimetaria. Ecotoxicol Environ Saf 52: 75–81.

156. Wild SR, Obbard JP, Munn CI, Berrow ML, Jones KC (1991) The long-term persistence of polynuclear aromatic hydrocarbons (PAHs) in an agricultural soil amended with metal-contaminated sewage sludges. Sci Total Environ 101: 235-253.

157. Gogolev A, Wilke B-M (1997) Combination effects of heavy metals and fluoranthene on soil bacteria. Biol Fertil Soils 25: 274-278.

158. Oleszczuk P, Jośko I, Kuśmierz M, Futa B, Wielgosz E, et al. (2014) Microbiological, biochemical and ecotoxicological evaluation of soils in the area of biochar production in relation to polycyclic aromatic hydrocarbon content. Geoderma 213: 502-511.