

A REVIEW ON THE ENZYMATIC INDICATORS FOR MONITORING SOIL QUALITY

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Abstract

Soil is a dynamic, living, natural system that is vital to the function of terrestrial ecosystems. Soil health is maintained by physical, chemical and biological factors. Physical factors include bulk density and soil porosity, indicators of soil compaction. Chemical factors (soil pH, inorganic nitrogen, available minerals and organic carbon content) provide information for the balance of soil solution and exchange sites. Biological and biochemical factors like microbial biomass, soil respiration, potentially mineralised nitrogen and activity of soil enzymes indicate the soil's ability to function or recover from disturbance such as climate change, pest infestation, pollution and human exploitation in agriculture. The enzymes play an important role in the decomposition and recycling of nutrients from dead plants and animal tissues, the fixation of nitrogen, the maintenance of soil structure and the inhibiting effects of pollutants. Therefore, the activity of soil enzymes can be used as an indicator of soil quality. This review is focused on the activity of some defining soil enzymes like β -glucosidases, cellulases, amylases, phosphatases, ureases, dehydrogenases, arylsulfatases and peroxidases, their importance in maintaining the soil health and their sources (plants, animals, organic compounds and especially microorganisms). It also offers information on a variety of methods developed to measure enzymes activity which can give relative information about the ecological status of soils.

Key words: biological indicators, enzymes, pollutants, soil quality.

INTRODUCTION

Soil is a dynamic, living, natural system which serves as a natural medium for growth of plants.

It can be defined by a selection of parameters according to their variability in time: stable (soil depth or granularity), relatively stable (salt content, the content of organic mass in soil, heavy metal contamination), relatively dynamic (pH, the content of nutrients), and dynamic (soil humidity, temperature, microbial activity and enzymatic activity) (Fazekašová, 2012).

One of the most important components of soil is soil organic matter due to its ability to maintain soil fertility and crop production, and to prevent soil degradation, erosion and desertification (Senesi et al., 2007). A key role in the decomposition of organic matter is played by the enzymes in soil. The main source of soils enzymes are microorganisms, but they can be found as well in plants and animals.

The purpose of this study is to evaluate the enzymatic activity of some enzymes in order to

describe and understand their role as a bioindicator of the soil health.

SOIL QUALITY

Soil quality is defined by its capacity to function, within land use and ecosystem boundaries, to sustain biological productivity, maintain environmental quality and promote plant, animal and human health (Doran et al. 1997). In order to assess the quality of soil, it must be used a unique balance of physical (texture, rooting depth, infiltration rate, bulk density, water retention capacity), chemical (pH, total C, electrical conductivity, nutrient level), biological and biochemical components (C and N microbial biomass, potentially mineralizable N, soil respiration, enzymatic activity) parameters (Gil-Sotres, 2005). The biological parameters are the primary indicators of soil health because they are sensitive to the changes that may occur in the presence of any degrading agent, while physical and chemical parameters alter only when the soil is subjected

to a drastic change in the environment (Filip, 2002).

Different agrochemicals are used in conventional agriculture, in last couple of decades, in order to help the farmer to minimize the economic losses caused by weeds, insects pests and pathogens. The extensive and improper use of this practices in crop production may lead to deterioration of soil quality, pollution of soil and water, loss of biodiversity and, the most important, increase incidence of human and ecosystem health problems (Baishya, 2015). Studies have shown that only 0.1 % of a pesticide is reaching the target organism, while the remaining bulk is reaching the soil, leading to disturbance of local metabolism, enzymatic activities and soil ecosystem (Sethi, 2016).

One of the consequences of conventional agriculture is soil degradation. The soil is subjected to a series of degradation processes which are linked to agriculture: erosion due to water, wind and tillage, compaction, declining soil organic carbon and soil biodiversity, salinisation and sodification and soil contamination (heavy metals and pesticides) (Baishya, 2015).

SOIL ENZYMES AS INDICATORS OF SOIL HEALTH

Activity of soil enzymes can be used as a soil quality indicator, due to the fact that are involved in energy transfer, release of inorganic nutrients for plant growth (C, N, P, S), organic matter decomposition, transformation of native soil organic matter, nitrogen fixation, detoxification of xenobiotics and the stabilization of soil structure (Utobo, 2014).

The health or quality of contaminated and remediated soils is evaluated by several representative enzymes such as: β -glucosidase, urease, phosphatase, dehydrogenase, peroxidase, arylsulphates, amylase, cellulose and peroxidase.

Cellulases

Cellulose is the most abundant structural polysaccharide of plant cell walls with β -1,4 -glucosidic linkages and represents almost 50% of the biomass synthesized by photosynthetic fixation of CO₂ (Eriksson et al., 1990). The breakdown of cellulose requires the synergic

action of a group of hydrolytic enzymes named cellulases. The degradation of soil organic matter from cellulose to glucose, is a result of the action of three important enzymes: endoglucanase (endo-1,4-D-glucanase EC 3.2.1.4), cellobiohydrolase (exo-1,4-D-glucanase, EC 3.2.1.91) and β -glucosidase (1,4-D-glucosidase, EC 3.2.1.21). Endoglucanase acts randomly, cleaving beta 1-4 glycosidic bonds within the cellulose molecule; the cellobiohydrolase removes cellobiose units from the ends of cellulose chains (Almeida et al, 2015). Meanwhile, the cellobiose molecules are cleaved by β -glucosidase, releasing glucose in the final cellulose breakdown process (Daroit, 2007), which provide a source of energy for decomposers (Gonnet et al, 2012). Cellulose from plant debris is degraded into glucose, cellobiose and high molecular weight oligosaccharides, releasing carbon as an energy source for use by the microorganisms (White 1982).

Cellulases are synthesized by a large diversity of microorganisms including both bacteria and fungi during their growth on cellulosic materials. The genera of *Clostridium*, *Cellulomonas*, *Thermomonospora*, *Bacillus*, *Trichoderma*, and *Aspergillus* are the most extensively studied cellulases producers (Kuhad et al., 2011).

Activities of cellulases in agricultural soils are affected by several factors like temperature, soil pH, water and oxygen contents (abiotic conditions), the chemical structure of organic matter (Deng and Tabatabai, 1994; Alef and Nannipieri, 1995), the addition of fungicides (Deng and Tabatabai 1994; Arinze and Yubedee 2000) and insecticides.

β -Glucosidase

Glucosidase (EC 3.2.1.21), a predominant enzyme in soils, plays an important role in catalysing the hydrolysis of various β -glucosides present in plant debris decomposing in the soil ecosystem. It is an important C energy source of life to microorganisms in soil (Chaea, 2017) and has the following soil functions: nutrient cycling (for plant growth), biodiversity and habitat, filtering and buffering (excess nutrients and toxic chemicals from the water), and soil structure.

β -Glucosidases are distributed among animals, plants, fungi, yeasts and bacteria (Veena et al.,

2011). Their activity has been observed in various plant species such as maize and sorghum (Verdoucq et al., 2003) and in microorganisms from roots of *Panax ginseng* plant (Zhang et al., 2001), *Penicillium purpurogenum* (Dhake and Patil, 2005), *Ceriporiopsis subvermispora* (Magalhaes et al., 2006), *Flavobacterium johnsonae* (Okamoto et al., 2000), *Trichoderma harzianum* type C-4 (Yun et al., 2001), *Lactobacillus plantarum* (Spano et al., 2005) and *Dyella koreensis* spp. (An et al., 2005).

Urease

Urease (EC 3.5.1.5) is one of the most efficient of all enzymes. This enzyme catalyses the hydrolysis of urea into carbonic acid and ammonia with a reaction mechanism based on the formation of carbamate as an intermediate (Tabatabai, 1982). In 1986, Burns has shown in his study that extracellular urease associated with soil organo-mineral complexes is more stable than urease in the soil solution. In addition he has discovered that urease extracted from plants or micro-organisms is rapidly degraded in soil by proteolytic enzymes.

Urease can be influenced by some soil factors such as cropping history, organic matter content, soil depth, management practices, heavy metals, temperature and pH (Yang et al., 2006).

They are synthesized by numerous organisms, including plants (*Canavalia ensiformis*, *Glycine max*, *Gossypium hirsutum*), fungi (*Aspergillus* sp, *Schizosaccharomyces pombe*), bacteria (*Bacillus pasteurii*, *Helicobacter pylori*, *Providencia* sp, *Staphylococcus* sp, *Klebsiella aerogenes*, *Aerobacter aerogenes*, *Proteus mirabilis*), algae (*Nitellopsis obtusa*) and invertebrates (Land snail *Otialalactea*) (Krajewska, B., 2009).

Phosphatases

In soil ecosystems, these enzymes play critical roles in P cycles, possibly correlated to P stress and plant growth (Nannipieri et al, 2014).

Among the soil phosphatases, phosphomonoesterases (EC 3.1.3.1.) have been the most studied enzymes probably because they have activity both under acidic and alkaline conditions, according to its optimal pH, and because they act on low molecular P-compounds, including nucleotides, sugar

phosphates and polyphosphates (Makoi and Ndakidemi, 2008).

Microorganisms that produce phosphatases in soil includes soil fungi, particularly those belonging to the genera *Aspergillus* and *Penicillium*, along with *Pseudomonas* and *Bacillus* bacteria that produce mostly neutral phosphatase, while *Actinomycetes* produced only negligible quantities of phosphatases (Tarafdar and Chhonkar, 1979).

Dehydrogenases

Dehydrogenases (EC 1.1.1.) are a major group of the oxidoreductase enzymes class and they play a significant role in the biological oxidation of soil organic matter by transferring hydrogen from organic substrates to inorganic acceptors (Zhang et al., 2010). The soil dehydrogenases activity provides information on pesticide application, of oligoelements or of soil's processing management and on the biological activity and microbial populations in soil. These enzymes are produced by soil bacteria, genus *Pseudomonas*, with *Pseudomonas entomophila* as most abundant (Walls-Thumma, 2000).

Peroxidases

Peroxidases (EC 1.11.1) are involved in the breakdown of lignin, a component of the cell wall of plants and one of the most abundant organic polymers on Earth. The degradation of lignin contributes to soil pools of carbon and nitrogen and provides microorganisms with these essential nutrients (Sinsabaugh, 2010). Peroxidase can play a role in the detoxification of the soil as well, due to the fact they can help attenuate the toxicity of metal ions and phenolic molecules (Sinsabaugh, 2004). Peroxidase also is able to help decrease the harmful effects of reactive oxygen species that can accumulate in the soil (Sinsabaugh, 2010). Some white rot (*Basidiomycetes*) and soft rot fungi (*Ascomycetes*) produce extracellular peroxidases (Sinsabaugh, 2004).

Arylsulphatases

Arylsulphatases (3.1.6.1) are responsible for S cycling in soils because are involved in the mineralization of organic sulphur compounds to inorganic forms (SO_4^{2-}) for plant uptake (Tabatabai 1994). They are classified according to the type of the ester in: alkylsulphatases, steroid sulphatases, glucosulphatases, chondrosulphatases and myrosulphatases

(Tabatabai, 1982). Their presence in different soil systems is often correlated with the rate of microbial biomass, soil organic carbon content and rate of S immobilization (Mirleau et al, 2005). In soil they can be affected by various factors like pH changes, type and content of organic matter, heavy metal pollution and pollutants (Tyler 1981).

This group of enzymes are secreted by bacteria into the external environment (*Actinobacteria* sp., *Pseudomonas* sp., *Klebsiella* sp, *Aerobacter* sp. and *Raoultella* sp.), fungi (*Trichoderma* sp. and *Eupenicillium* sp.), plants and animals (Nicholls and Roy, 1971).

Amylases

Amylases (EC 3.2.1.) are classified in α -amylase (E.C. 3.2.1.1), β -amylase (E.C. 3.2.1.2) and glucoamylase (E.C. 3.2.1.3). Due to the fact that α -amylases are widely distributed in plants and soil, they play an important role in the breakdown of starch, converting starch like substrates to maltose, glucose and/or oligosaccharides. Instead, β -amylase converts starch limit dextrin and maltose (Thoma et al. 1971). In the end, glucoamylase hydrolyses maltose to glucose. The α -amylase can be found in microorganism such as bacteria from genus *Bacillus* (*Bacillus licheniformis*, *Bacillus stearothermophiles* and *Bacillus amyloliquefaciens*) (Padma, 2016) and fungal species such as *Aspergillusniger*, *A. oryzae*, *Thermomyces lanuginosu* and *Pencillium expansum*, plants and animals (Singh, 2016). β -amylase is synthesized mainly by plants (*Ipomea batatas*, *Arachis hypogaea* L.) (Hesam, 2015).

ENZYME ACTIVITY DETERMINATION

To give relative information about the ecological status of soil ecosystema variety of methods were developed to measure soil enzymatic activity.

The colorimetric determination of phosphatase activity involves the use of an artificial substrate, p-nitrophenyl phosphate (p-NPP). The p-nitrophenol is released by phosphatase activity after soil is incubated with buffered (pH 6.5) sodium p-nitrophenyl phosphate solution and toluene at 37° C for 1 hour (Tabatabai and Bremner, 1969).

In 1970, Tabatabai and Bremner have developed a colorimetric method for the determination of arylsulphatase. It is based on the determination of p-nitrophenol released after the incubation of soil with buffered (pH 5.8) potassium p-nitrophenyl sulfate solution and toluene at 37°C for 1 hour.

In order to determine cellulase activity in soils, it was developed a method that use 1 % carboxymethylcellulose (CMC) as a substrate, in acetate buffer (pH 5.9) and incubated for 24 hours at 37° C in order to determine the reducing sugar content in the filtrate (Deng and Tabatabai 1994).

Urease activity assay involves determination of the ammonium which is released by the enzyme after soil is incubated with tris (hydroxymethyl) aminomethane (THAM) buffer (pH 9.0), urea solution, and toluene at 37°C for 2 hour (Chae et al., 2017). The methods for the assay of amylase in soil was developed by Cole in 1977. They require the addition to the soil samples of toluene and acetate-phosphate buffer (pH - 5.5) containing 2% starch. The samples are incubated 24 hour at 30°C.

The activity of ligninolytic enzymes (peroxidase) was measured with the spectrophotometric assay, using L-dihydroxyphenylalanine (L-DOPA) as a substrat in the presence of hydrogen peroxide (Sinsabaugh et al., 1992).

The assay of β -glucosidase activity is based on the determination of the released p-nitrophenol after the incubation of soil with p-nitrophenyl- β -D-glucoside solution for 1 hour at 37° C. The colour intensity can be measured with a spectrophotometer at 400 nm (Tabatabai 1982). For the dehydrogenase activity, the most common laboratory procedure was the one developed by Casida in 1964. Dehydrogenase activity was determined with 2, 3, 5-triphenyltetrazolium chloride (TTC), after incubation at 30°C. The formed triphenylformazan has been extracted with an ethanol-acetic acid mixture and spectrophotometric evaluated at 540 nm (Wolińska and Stępniewska, 2012).

CONCLUSIONS

It is very essential to understand the activity of soil enzymes as a biochemical indicator of soil health.

Enzymes are very sensitive to major or minor changes in the environment and land management such as tillage, crop rotation, residue management, pollution. Thus they are closely related to ecological functions of soil such as biomass production, contaminated soil recovery, and ecosystem conservation.

The methods used in determination of soil enzyme activities are relatively simple, inexpensive and quick.

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