Review Article

Activities of β-glucosidase, Phosphatase and Dehydrogenase as Soil Quality Indicators: A Review

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A B S T R A C T

Keywords Enzyme activity, Phosphatase, Soil quality, Dehydrogenase, β-glucosidase **Article Info** *Accepted:* 10 May 2019 *Available Online:* 10 June 2019	Soil biochemical properties are indicators of soil quality, but there is still no consensus as to how they should be used. Generally, biochemical properties related to the biocycles of the elements (C, N, P and S) are used to diagnose soil quality. These properties include both general biochemical parameters (i.e. microbial biomass C, dehydrogenase activity and N mineralization potential) and specific biochemical parameters (i.e. the activity of hydrolytic enzymes, such as phosphatase, Dehydrogenase, urease and b-glucosidase). Soil is a fundamental resource and it is crucial to manage its quality in order to enhance agricultural productivity and environmental quality. Soil enzymes are act as catalyzer in several biochemical reactions which result in the transformation of organic matter release of inorganic nutrients for plant growth and nutrient cycling. Soil enzyme activities are useful biological soil quality indicators since they are operationally practical, very sensitive, integrative, easy to measure more responsive to soil tillage and structure than other soil variables. There are several enzymes in soil, but those involved in hydrolases and the degradation of main litter components are used most often for evaluating soil quality. This paper reviews the roles of soil enzymes such as β-glucosidase, phosphatase and dehydrogenase as well as the implications of their activities for soil quality.

Introduction

The soil is a living, dynamic and non-renewable resource. Soil conditions influence food production environmental efficiency and the global ecological balance (Binkley and Fisher, 2012). Soil quality is an important indicator of good crop yield in various land use contexts (Almeida *et al.,* 2015). Doran and Parkin (1994) define soil quality as 'the continued capacity of soil to function as a vital living system, within ecosystem and land use boundaries to sustain biological productivity, promote the quality of air and water environments and maintain plant, animal and human health'. To manage soil quality, it is essential to have appropriate tools for predicting and evaluating soil changes. A number of methods have been used to predict changes in soil quality. The chemical, physical and biological properties of soil can serve as indicators of quality (Abbott and Murphy, 2003). Indicators that are sensitive and make rapid, incisive and

efficient responses concerning soil quality are preferred (Matsuoka *et al.,* 2003). Soil quality indicators that are linked to microbial activity may respond to disturbances over a shorter period of time than those linked to physical or chemical properties (Garciagil *et al.,* 2000). Thus, soil ecosystem sustainability can be suitably evaluated through the use of biologically based indicators (Piotrowska - Dlugosz and Charzynski, 2015). In recent years, soil biology has turned to assessing the degrading capacity of microorganisms by evaluating their enzyme activity (Fioretto *et al.,* 2000). Microbial species release enzymes into the environment in order to degrade complex organic molecules into absorbable simple molecules (Almeida *et al.,* 2015). Thus, soil enzymes catalyze and increase several biochemical reactions that bring about the decay of organic residues, transformation of native soil organic matter, mineralization of nutrients for plant growth, and soil aggregation (Balezentiene, 2012). Decomposition rates are therefore linked to the enzymes that act directly on the main structural parts of plant material and can supply useful information on definite features of the microbial community and succession (Fioretto *et al.,* 2000). This paper reviews the charastistics and of three enzymes – β-glucosidase, phosphatase and dehydrogenase as indicators of soil quality. It also reviews the factors affecting their activity in the soil as well as their application in agriculture.

General characteristics and use of soil enzymes

All soils contain a group of intracellular and extracellular enzymes with different origins that may be synthesized by plants, animals and microorganisms (Gianfreda *et al.,* 1996). Intracellular enzymes can be found in various parts of proliferating living cells (Nannipieri *et al.,* 1998). Living cells, however, produce and secrete extracellular enzymes which function 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 (Gianfreda, 2015). These enzymes are not only available in dead cells, but may also be taken up on clays or integrated into humic substances. Enzymes play a vital role in agriculture and in nutrient cycling, in particular, since they are constantly being synthesized, accumulated, in activated and decomposed in the soil. The choice to use enzymes to assess soil quality is based on their sensitivity to soil management, organic matter decomposition, and relative ease of analysis (Balota and Chaves, 2010).

The determination of soil fertility and plant yield using a single enzyme activity has been proven to be inaccurate. This is because soil enzyme activities catalyze a particular reaction and cannot therefore be linked to the general soil microbiological activity, which comprises a wide range of different enzymatic. Furthermore, a given enzyme cannot reflect the whole nutrient status of the soil because it is substrate specific (Nannipieri *et al.,* 2012).

Thus the enzymes most widely used for evaluating the factors controlling plant litter de-composition and soil quality are those involved in the degradation of main litter decomponents and hydrolases, which are associated with the carbon (β-glucosidase and β-galactosidase), nitrogen (Dehydrogenase), phosphorus (phosphatase) and sulphur (arylsulphatase) cycle (Karaca *et al.,* 2010). Other soil enzymes may include amylase amidase, phenol oxidase, cellulose, chitinase, dehydrogenase and protease (Karaca *et al.,* 2010). Soil enzyme activity can be estimated and serve as a valuable pointer to nutrient cycling potential, nitrification, oxidation, and other processes crucial to soil quality (Almeida *et al.,* 2015). This review therefore

focuses on the enzymes β-glucosidase, phosphatase and dehydrogenase known to play crucial roles in C, P and N cycling respectively, which are important nutrients for plant growth and microbial metabolism. The three enzymes are widely distributed in nature and are very sensitive to the environment and management induced changes in the soil ecosystem.

β-Glucosidase

Characteristics and role of β-glucosidases

Glycosidases are a group of enzymes that catalyze the hydrolysis of glycosides (Martinez and Tabatabai, 1997). They are highly diverse enzymes owing to the wide diversity of glycosidic bonds and variations in their substrates (Almeida *et al.,* 2015).

Among the glycosidases, α- and β-glucosidase, as well as α- and β-galactosidase are the main members, widely dis-tributed in the soil. The α-glucosidase (maltase) catalyzes the hydrolysis of α-D-glucopyranosides while β-glucosidase (cellobiase) hydrolyzes maltose and cellobiose (Utobo and Tewari, 2015). β-glucosidase is, however, the most common, important and widely used soil quality indicator (Bandick and Dick, 1999). β-glucosidase is predominantly found among plants, animals, fungi, bacteria, and yeasts. Its role in soils is crucial since it is involved in catalyzing the hydrolysis and biodegradation of various β-glucosides that are present in plant debris (Martinez and Tabatabai, 1997). β-glucosidase acts in the last phase of the cellulose degradation process by hydrolyzing the cellobiose residue (GilSotres *et al.,* 2005).

These reactions produce glucose as the final product, an important C energy source for the growth and activity of soil microorganisms (Merino *et al.,* 2016). β-glucosidase

involvement in C cycling has remarkably facilitated its adoption for soil quality testing.

Factors affecting β-glucosidase activity

The activity of β-glucosidase decreased as soil pH increased from 4.5 to 8.5 and 4.3 to 7.4 in a paddy soil (Xiao-Chang and Qin, 2006). The sensitivity of β-glucosidase to pH changes can serve as a reliable biochemical indicator for assessing environmental changes caused by soil acidification (Acosta-Martinez and Tabatabai, 2000). Soil moisture can influence the biochemical processes of soil carbon transformation catalyzed by β-glucosidase (Zhang *et al.,* 2011). β-glucosidase activity decreased by 10-80% and 35-83% when soil moisture was reduced by 10% and 21% respectively, depending on the soil depth (Sardans and Penuelas, 2005). Thus drought influences β-glucosidase activity and its catalytic features, causing a slower nutrient turnover and a reduced nutrient supply to plants. Increased soil salinity and solidity led to an exponential and linear decline in β-glucosidase activity, respectively (Rietz and Haynes, 2003). It has been reported that plant residues in a soil polluted with heavy metals neither decompose nor indicate β-glucosidase activities (Geiger *et al.,* 1993), resulting in less glucose for soil microbes. The response of β-glucosidase activity to soil salinity and heavy metal contamination can serve as a good pointer to soil quality status. Several studies have revealed that β-glucosidase activity decreases with soil depth (Xiao -Chang and Qin, 2006). This is because β-glucosidase activity greatly depends on substrate supply and the microorganisms that mainly produce this enzyme are active in the top soil (Xiao - Chang and Qin, 2006). Therefore, β-glucosidase activity can be used to indicate the presence of higher simple sugars for microbial population in the soil surface layer.

Application of β-glucosidase activity in agriculture

The activity of β-glucosidase is influenced by crop residue quality and a number of soil management practices. A no till system and reduction in tillage frequency results in an increase in the activities of β-glucosidase as well as microbial biomass C and N in contrast to a conventional tillage system (Pandey *et al.,* 2014). The reduction in tillage intensity favours and increases β-glucosidase activity due to improvement in microbial biomass, more substrate availability and reduced soil disturbance (Sinsabaugh *et al.,* 2008).Therefore a conventional tillage system may cause soil organic matter depletion. which can result in a reduction in simple sugars for microbial functioning owing to a decrease in β-glucosidase activity.

The activity of β-glucosidase was reported to be lower in arable soils than in woodland and meadow soils (Bandick and Dick, 1999). This may be as a result of varying substrate and organic matter composition associated with each soil. Incorporation of residues of vetch, oat+legume, *Trifolium pratense* L, *Brassica napus* L and *Trifolium pratense* L+*Brassica napus* L, led to an increase in the activity of β-glucosidase compared to oats (Piotrowska-Dlugosz and Wilczewski, 2014a). β-glucosidase activity increased because the soils amended with lower C:N crop residue favours its function, resulting in quick organic matter decomposition and nutrient release.

The high capacity to respond to substrate and soil type makes β-glucosidase activity an efficient soil quality indicator. Several studies have shown that β-glucosidase activity was higher in fertilization treatments with compost, vermicompost, municipal solid waste compost and straw mulch, than in those without compost as well as those with synthetic fertilizer and herbicide (Saha *et al.,*

2008). Furthermore, β-glucosidase activity increased remarkably in various soils amended with sewage sludge and irrigated with winery wastewater rather than municipal water (Mulidzi and Wooldridge, 2016). B-glucosidase activity increased due to the inducement caused by the addition of simple organic substrates contained in those residues, which makes this enzyme a reliable indicator of soil quality. I

n general, β-glucosidase activity is closely related to soil organic matter, biological activity and C cycling, and it can provide an advanced sign of alterations in organic carbon long before this can be correctly determined by other routine techniques. These qualities have significantly enabled its adoption for soil quality testing in agriculture. More knowledge is however needed to show the dynamics of β-glucosidase and other factors influencing their activity, to enhance the understanding of biological soil fertility management in agriculture.

The β-glucosidase activity as influenced by fertilization options is presented in (Fig. 1). The highest value of β-glucosidase activity (45.1 μg p - nitrophenol g^{-1} h^{-1}) was found associated with 100% NPK + FYM and lowest value (25.9 μg p-nitrophenol g^{-1} h^{-1}) was recorded in control.

The activity of β-glucosidase increases with organic matter content and this is why it is considered as a very sensitive biological indicator of the effect of soil management practices. The β-glucosidase enzyme activity was greater in the FYM-treated plots compared with the plots receiving sole fertilizers (Patel *et al.,* 2018).The application of FYM significantly increased β-glucosidase activity, as the enzyme activity is strongly correlated with soil organic carbon content (Patel *et al.,* 2018).

Phosphatases

Characteristics and role of phosphatises

Phosphatises are a group of enzymes that catalyze the hydrolysis of esters and anhydrides of phosphoric acid (Condron *et al.,* 2005). Plants and micro organisms are the main sources of phosphatase enzymes in the soil. The amount of phosphatase present in the soil varies with the microbial count and the extent of organic materials, mineral and organic fertilizer tillage and other agricultural practices (Banerjee *et al.,* 2012). Since plants make use of only inorganic P and a large amount of soil P is organically bound, the mineralization of this organic portion can be a vital influence in plant nutrition (Nannipieri *et al.,* 2011). When phosphorus is lacking in the soil, plant roots and microorganisms increase the secretion of phosphatase to intensify the solubilisation and remobilization of phosphate, therefore influencing the ability of the plant to cope with phosphorus-stressed conditions (Kai *et al.,* 2002). This shows that the demand for phosphorus by plants and micro organisms can be linked to the production and activity of soil phosphatase (Condron *et al.,* 2005). Phosphatase activity can therefore be used as an indicator of inorganic phosphorus availability for plants and microorganisms (Piotrowska-Dlugosz and Charzynski, 2015). Phosphomon esterase is the most studied among the phosphatases present in the soil. It hydrolyses phosphate monoester to produce free phosphate for biological uptake (Makoi and Ndakidemi, 2008). Phosphomon esterase is active under acidic and alkaline conditions, depending on its optimal pH, and acts upon low molecular P compounds with monoester bonds, including nucleotides, sugar phosphates and polyphosphates (Dodor and Tabatabai, 2003).Acid phosphatase activity is therefore found mainly in acid soils, while alkaline phosphatase dominates in alkaline soils

(Dodor and Tabatabai, 2003), with ranges of 4-6 and 9-11, respectively.

Factors affecting phosphatase activity

Soil pH influences the rate of synthesis, release, and stability of phosphatase (Acosta-Martinez and Tabatabai, 2000). As the soil pH increases, the activity of alkaline phosphatase increases, while acid phosphatase activity decreases (Dick *et al.,* 2000). Alkaline (Pal) and acid (Pac) phosphatase activities can be used to examine the optimum soil pH for crop production and the amount of lime required to achieve it (Dick *et al.,* 2000). Thus, determination of the Pal/Pac ratio may be a better way of evaluating the effective soil pH and liming needs than the chemical method (Acosta-Martinez *et al.,* 2003b). The activity of phosphatase was influenced in soils affected by forest fire, increasing over the years as the soil recovered (Staddon *et al.,* 1998). The effect of drought has also been reported. When soil moisture was reduced by 21%, there was a 31-40% reduction in acid phosphatase activity (Sardans and Penuelas, 2005). The presence of lead and other heavy metals in the soil decreased phosphatase activity (Kandeler *et al.,* 1996). Phosphatases are a good soil quality indicator since their activity reflects the situation of the soil.

Application of phosphatase activity in agriculture

In agricultural soils, phosphatases play a crucial role in phosphorus cycles and because their activity is sensitive to management practices they can be used as an index of soil quality (Makoi and Ndakidemi, 2008). To improve soil quality management and agricultural productivity, it is important to evaluate the effect of different management practices on phosphatase activity in the soil. Agricultural management methods responsible for phosphorus stress in the soil

may influence the production of these enzymes in the ecosystem (Ndakidemi, 2006). Previous studies have reported that legumes such as chickpea, cowpea, *Cyclopia* and *Aspalathus* release more phosphatase enzymes than non legumes (Maseko and Dakora, 2013). This is because legumes require more phosphorus in the symbiotic nitrogen fixation process than cereals do (Makoi and Ndakidemi, 2008). The increase in phosphatase activity in the legume roots and soils leads to a significant increase in plant avail-able P (Makoi *et al.,* 2010). Thus phosphorus supply and assimilation can be estimated by acid and alkaline phosphatase activity in the low -P soils of legume crops (Maseko and Dakora, 2013). However the activity of phosphatases was higher in a crop rotation system comprising oats or meadow than in a monoculture system of corn or soybean (Mukumbareza *et al.,* 2015) revealed that the rotation of maize with vetch and fertilized oat cover crops increased microbial biomass carbon and the activities of phosphatase in a South African deep alluvial soil. The increased microbial biomass carbon and phosphatase activity in the bicultural as compared to the monocultures showed that the cover crops have synergistic effects in bicultures, and could be valuable for improving P cycling and soil physiochemical properties (Mukumbareza *et al.,* 2016). Acid and alkaline phosphatase activity increased under various soil management practices where organic fertilizers like plant residues, sewage sludge, manure, compost and vermicompost were applied (Nannipieri *et al.,* 2011; Piotrowska Dlugosz and Wilczewski, 2014b). The increase in phosphatase activity associated with soils amended with organic materials can be attributed to stimulation of microbial growth and soil organic matter enrichment. Phosphatase activity can be considered to be a good index of the quality and quantity of organic matter in soils several studies have reported that the activity of

phosphatases increases in soils supplemented with organic matter and inoculated with mycorrhizal species (Joner and Jakobsen, 1995; Van Aarle and Plassard, 2010). The association of mycorrhizal with phosphatase activity supports the role this enzyme plays in the degradation of soil bound phosphorus (Van Aarle and Plassard, 2010). Phosphatase probably requires a considerable amount of N, since nitrogen fertilization has been shown to increase acid phosphatase activity and reduced alkaline phosphatase activity in soils cultivated with corn and wheat (Kalembasa and Symanowicz, 2012). Furthermore, the joint application of vermicompost or municipal solid waste compost and mineral N fertilizer revealed higher phosphate activity than the separate application of the fertilizers to soil (Srivastava *et al.,* 2012). Phosphatase activity increased when P fertilizer was added to soils with low organic matter, yet there were no changes in the activity of this enzyme when P fertilizer was amended with soils containing high organic matter (Piotrowska-Dlugosz and Wilczewski, 2014a). Thus, the synthesis and activity of phosphatase can be influenced by P from mineral fertilization in the soil (Saha *et al.,* 2008). This confirms that phosphatase increases the available reserves of P when this nutrient is limited, and that the addition of P to the soil is an alternative for increasing the availability of this element (Bautista-Cruz and Ortiz-Hernandez, 2015). Phosphatase activity can therefore serve as a good soil quality indicator because of its strong correlation with soil organic matter, organic P, inorganic P, and N availability in the soil. No tillage and conventional tillage systems affect soil biological activity and aggregate stability. Greater acid phosphatase activity was observed under a no till and reduced tillage system compared to a continuous tillage system in Brazilian Cerrado Oxisol and rice grown soil (Pandey *et al.,* 2014). Thus, phosphatase activity is a reliable soil quality indicator since it promptly detects

changes in soil organic matter caused by tillage. Tillage should be reduced to increase the biological activity of surface soils in order to improve P nutrient cycling processes and soil structure. A larger amount of the inorganic P assimilated by plants is produced from the mineralization of organic P through phosphatase activity. The functions of phosphatase activity and its ability to rapidly detect management changes, as described above indicate the importance of this enzyme as a biological soil quality indicator. More understanding of the roles of phosphatase and better ways of optimizing its activities in soils managed organically will result in improved soil conservation, P release and increased agricultural sustainability in the ecosystems.

The data showed significant effect of graded level of fertilizers (50 to 100% NPK) on acid phosphatase activity (Fig. 2). The highest value for acid phosphatase activity was found in 100% NPK + FYM (8.5 µg p-nitrophenol g^{-1} $h^{-1)}$ and lowest in control (5.6 µg p-nitrophenol g^{-1} h^{-1}). With regard to alkaline phosphatase activity in soil (Fig. 2), 100% NPK+FYM treatment showed significantly highest value (27.9 µg p-nitrophenol g^{-1} h^{-1}) compared with 100% NPK (21.6 µg p-nitrophenol g^{-1} h^{-1}), whereas control plot showed the lowest value (14.4 µg pnitrophenol g^{-1} h^{-1}). Activity of phosphatases is important in studying the P cycle because this can provide a route for P mineralization and plant uptake (Patel *et al.,* 2018). However, activities of these enzymes were not persistent, and sometimes appeared contrasting. The acid phosphatase activity was much lower than alkaline phosphatase activity, irrespective of the treatments, which may be due to the alkaline reaction of the soil. Earlier studies also proved that phosphatase activity was strongly influenced by soil pH (Dick 1994). The significantly greater activity of alkaline phosphatase in the FYM treated plots may be due to enhanced microbial

activity and perhaps diversity of phosphate solubilising bacteria consequent to manure input over the years (Mandal *et al.,* 2007).

Dehydrogenase activity

The dehydrogenase activity of the soil is considered to be an indicator of the microbial redox system and of the oxidative activities of the soil (Trevors, 1984). Like microbial biomass C, this property has been used mainly to assess the influence of management on soil quality and contradictory results have also been obtained. For example, ploughing can both increase and decrease dehydrogenase activity (Bergstrom *et al.,* 1998), whilst the addition of organic fertilizers, landfill effluents and industrial waste generally increases dehydrogenase activity (Langer and Gunther, 2001). In general, this activity is not affected by the presence of heavy metals, unless in very high doses (Kandeler *et al.,* 1996; Filip, 2002). Dehydrogenase activity has also been used to evaluate the degree of recovery of degraded soils, being considered as a good indicator, even in soils that have been contaminated by petroleum spillage (Margesin *et al.,* 2000).

Dehydrogenase activity

The DHA increased with graded levels of fertilizers from 50 to 100% NPK (Fig. 3). Application of FYM with 100% NPK recorded significantly higher DHA (10.0 µg TPF g^{-1} $24h^{-1}$) compared with other treatments. The increase in DHA was to the extent of 12.3 per cent under INM over 100% NPK (Patel *et al.,* 2018). The results are in agreement with the findings of Bhattacharyya *et al.,* (2008) that reported 4-5 folds increase in DHA due to FYM application along with N or NPK. The addition of FYM coupled with fertilization exerted a stimulating influence on the preponderance of bacteria (Selvi *et al.,* 2004). It was significantly higher under 100%

NPK (8.9 µg TPF g^{-1} 24 h^{-1}) compared with control (6.6 µg TPF g^{-1} 24 h^{-1}), suggesting importance of balanced fertilization. Mandal *et al.,* (2007) showed that easily decomposable components of crop residues may have a strong effect on DHA and metabolism of soil microorganisms. Similarly, Katkar *et al.,* (2011) also reported that the DHA increased by 18.6 per cent due to integrated nutrient management over 100% NPK under 20-year old long-term fertilizer experiment in a Vertisol**.**

Fig.1 Effect of continuous application of fertilizers and FYM on β-glucosidase activities under soybean-wheat cropping system
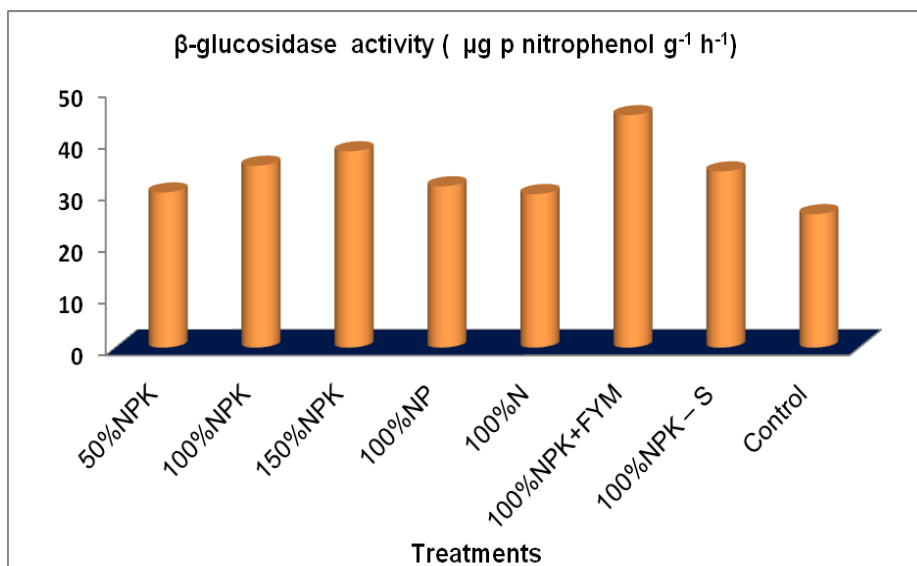

Fig.2 Effect of continuous application of fertilizers and FYM on phosphatise activities under soybean-wheat cropping system. Acid and alkaline phosphatase activity
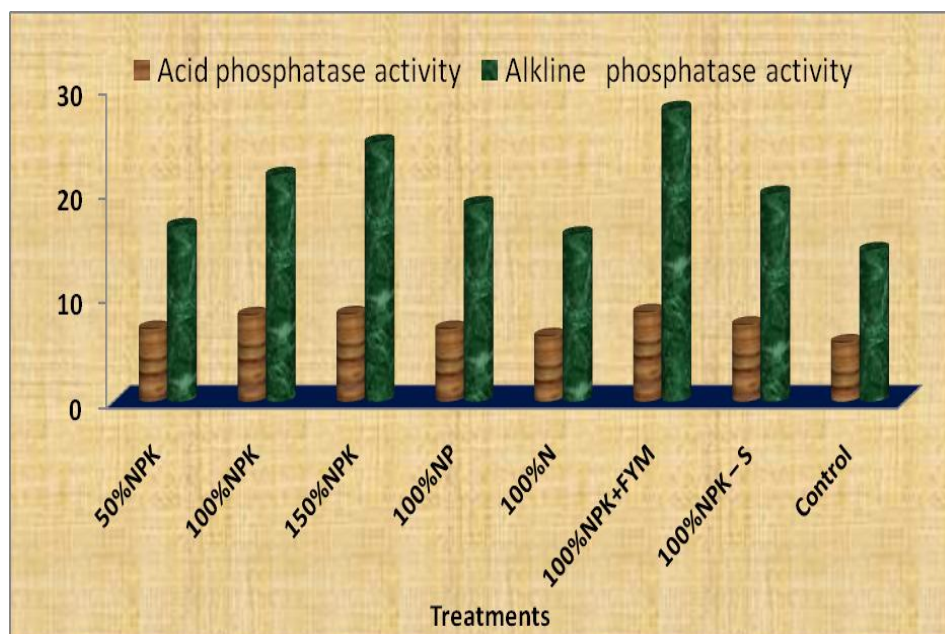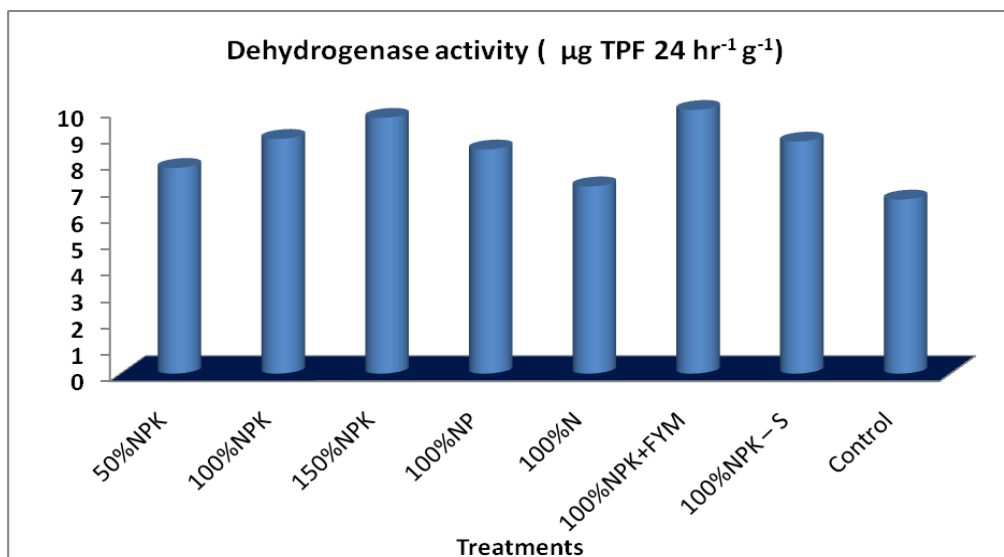
Fig.3 Effect of continuous application of fertilizers and FYM on dehydrogenase activity activities under soybean-wheat cropping system

Relationship β-glucosidase, dehydrogenase, phosphatase activity with crop productivity

The biological parameters i.e. dehydrogenase assay (r=0.910**), alkaline phosphatase activity (r=0.909**), acid phosphatase activity (r=0.946**) and β-glucosidase activity (r=0.868**) were significant and positively correlated with total productivity (Patel *et al.,* 2018). This indicates that total productivity of soybean and wheat grown in Typic Haplusterts was enhanced and controlled by these soil parameters (Mandal *et al.,* 2007; Patel *et al.,* 2018).

In conclusion, soil enzymes have been successfully used as indicators of soil quality in different agricultural systems. A better understanding of the function of soil enzymes and the factors influencing their activity is crucial for improving soil management, soil quality and food production. Soil enzymes catalyze and facilitate decomposition and nutrient cycling, thereby rendering their activities a biological index of soil quality. The soil enzymes are operationally practical, integrative, easy to measure, and they respond to soil management changes long before other soil quality indicator changes are detectable. Their activities may be influenced by soil depth and type, temperature, moisture, pH, the quality and quantity of available substrate, as well as management regimes. Individual enzyme activity does not reflect soil quality status since single enzyme activities cannot represent the rate of all metabolic processes (unless they catalyze one specific reaction). Thus, several enzyme activities should be assessed in order to measure soil quality effectively.

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