

# Assessment of Soil Enzyme Activity in Soybean Fields under Long-Term Fertilization and Manuring Practices"

## Abstract

The experiment carried out in this field study was between at the (AICRP) research farm in the outward-bound farm of the College of Agriculture, Indore. Indore is a small city in the western part of Madhya Pradesh, Central India, situated on the Malwa Plateau. An experiment was conducted with three replications by using different combination of N, P levels and crop residues in RBD design. Experiment field was medium to black soil with pH of 7.63 and medium in organic carbon 0.58%. The soil was low in available nitrogen (208.0 kg/ha) medium in available phosphorus (21.0 kg/ha) and high in potassium (585 kg/ha) and divided into gross plot size of 10 x 7.2 m<sup>2</sup> and a net plot size of 9 x 6.4 m<sup>2</sup>, respectively. During crop harvest four different depths were used to gather the soil samples: 0–10, 10–20, 20–30, and 30–40 cm. All the collected samples are examined separated for Dehydrogenase, Urease enzyme, Acid & alkaline phosphate, Arysulphatase activity. The finding showed that maximum soil DHA, Urease, Acid & alkaline phosphate, Arysulphatase activity was observed with the application of treatment T6 treatment (FYM 6 t ha<sup>-1</sup> + N20 P13).

**Keyword:** *Dehydrogenase (DHA), Urease enzyme, Acid phosphate, & alkaline phosphate, Arysulphatase activity*

## Introduction

Soybean (*Glycine max* L.) is a vital oilseed and pulse crop globally, belonging to the Leguminosae family and the Papilionaceae subfamily. Often referred to as the "Golden Bean" of the 21st century, soybean is a major legume crop used extensively as a vegetable protein source. Over recent decades, the shift towards intensive cropping systems and the cultivation of high-yielding varieties have led to a decline in natural soil fertility, posing a significant threat to sustainable agriculture. Concurrently, widespread nutrient deficiencies have emerged as a major concern (Singh et al., 2013; [1] Bhagwat et al., 2018) [2]. Soybean is rich in protein (40-42%) and oil (18-20%), adding considerable nutritional value. In Madhya Pradesh, soybean dominates in both area and production among oilseeds, contributing nearly 60% of India's soybean cultivation, and has earned the state the title of "Soya State."

The microbiological activity of soil plays a crucial role in soil quality and fertility. Soil enzyme activity is particularly important as it influences nutrient transformation through the mineralization of soil organic matter. Dehydrogenase activity, among other soil enzymes, provides valuable insights into biological activity and microbial populations (Waksman et al., 1992) [3]. Soil enzymes are essential for the breakdown of organic matter, catalyzing various biochemical processes necessary for the survival of soil microorganisms, the stabilization of soil structure, and the degradation of substances. Agronomic practices significantly impact soil enzyme activity, as enzymes and substrates serving as energy sources for microorganisms are vital for these

**Comment [mm1]:** On abstract: please add introduction, prolems, aims, methods, results, and novelty

**Comment [mm2]:** Alphabetic

**Formatted:** Font: (Default) Times New Roman

**Formatted:** Font: (Default) Times New Roman, Italic

biochemical reactions (Kiss et al., 1978)[4]. Soil enzymes, which are derived from plants, animals, organic substances, and microorganisms, include amylase, arylsulphatases, dehydrogenase, phosphatases, and urease.

#### Materials and Methods:

A field experiment carried out at Research Farm, College of Agriculture (Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya), Indore, during kharif season on a medium to black soil with pH of 7.63 and organic carbon 0.58%. The soil was low in available nitrogen (208.0 kg/ha) medium in available phosphorus (21.0 kg/ha) and high in potassium (585 kg/ha). The treatment was laid out in randomized block design (RBD) in three replications with twelve treatments, namely T1 : Control, T2: N20 P13 (Fertilizer N and P @ 20 and 13 kg ha<sup>-1</sup>), T3: N30 P20 (Fertilizer N and P @ 30 and 20 kg ha<sup>-1</sup>), T4 : N40 P26 (Fertilizer N and P @ 40 and 26 kg ha<sup>-1</sup>), T5: N60 P35 (Fertilizer N and P @ 60 and 35 kg ha<sup>-1</sup>), T6: FYM 6 t ha<sup>-1</sup>+ N20 P13 (FYM @ 6 t ha<sup>-1</sup> in rainy season on Only plus fertilizer N and P @ 20 and 13 kg ha<sup>-1</sup>), T7:Residues 5 t ha<sup>-1</sup>+ N20 P13 (Crop residues of soybean @ 5 t ha<sup>-1</sup> plus fertilizer N and P @ 20 and 13kg ha<sup>-1</sup>), T8:FYM 6 t ha<sup>-1</sup>(Farmyard manure @ 6 t/ha) and T9: Residues 5 t ha<sup>-1</sup>(Crop residues of soybean @5t/ha). The crop residues and FYM are applied 7 days before sowing and mixed well in soil. Inorganic fertilizers are applied as basal dose at sowing. The produce is sold @ 4 rs per kg of grain yield. The soil samples were collected and an assay for soil enzymatic activities viz. urease, dehydrogenase, at initial sample (kharif sesason), maximum tillering stage, flowering stage and at harvest. 2.1 Collection of Soil Samples Soil samples (wet samples) were collected from the surface soil of experimental area from the different treatments at different growth stages of the rice crop viz: initial sample , maximum tillering stage, flowering stage and at harvest. The samples were stored in the refrigerator at 4°C. Enzymatic activity viz: urease and dehydrogenase activity were determined by procedures as mentioned below Soil enzymes viz. urease activity was assayed by Measuring the absorbance of the blue coloured complex at 638 nm on the spectrophotometer and quantifying the amount of ammonium released and expressed as  $\mu\text{g NH}_4^+$  released g<sup>-1</sup> soil h<sup>-1</sup> described by Tabatabai and Bermner[5]. And also assay techniques used by "Dehydrogenase assays based on the 2,3,5- triphenyl tetrazolium chloride (TTC) to the creaming red coloured triphenylformazone (TPF) and it was quantifying the amount TPF released and expressed as of  $\mu\text{g TPF}$  produced g<sup>-1</sup> soil day<sup>-1</sup> described by Cassida et al., (1964)[6].

Comment [mm3]: On methods: add references on all parameters

#### Results and Discussion

Formatted: Font: (Default) Times New Roman

##### Dehydrogenase activity (DHA) ;

The results presents Table 1 the Dehydrogenase activity (DHA) influenced by manure and fertilizer applications at various soil depths (0-10 cm, 10-20 cm, 20-30 cm, and 30-40 cm), including an unfertilized control. The results indicate that DHA is higher in surface soils compared to subsurface soils. The DHA values ranged from 144.32 to 86.77  $\mu\text{g TPF h}^{-1} \text{g}^{-1}$  soil across the different depths. The highest DHA was observed at the following depths: 0-10 cm (157.48  $\mu\text{g TPF h}^{-1} \text{g}^{-1}$  soil), 10-20 cm (141.74  $\mu\text{g TPF h}^{-1} \text{g}^{-1}$  soil), 20-30 cm (113.39  $\mu\text{g TPF h}^{-1} \text{g}^{-1}$  soil), and 30-40 cm (unreported) under the treatment FYM 6 t ha<sup>-1</sup> + N20 P13 (T6). This was followed by Residues 5 t ha<sup>-1</sup> + N20 P13 (T7), FYM 6 t ha<sup>-1</sup> (T8), Residues 5 t ha<sup>-1</sup> (T9), and N60P35 (T5). The control treatment (T1) recorded the lowest DHA values: 126.77  $\mu\text{g TPF h}^{-1} \text{g}^{-1}$  soil at 0-10 cm, 114.09  $\mu\text{g TPF h}^{-1} \text{g}^{-1}$  soil at 10-20 cm, 91.27  $\mu\text{g TPF h}^{-1} \text{g}^{-1}$  soil at 20-30 cm, and 76.73  $\mu\text{g TPF h}^{-1} \text{g}^{-1}$  soil at 30-40 cm.

The results indicate a direct relationship between dehydrogenase activity (DHA) and soil organic matter: as organic matter increases, so does DHA. Consistently, DHA was higher in surface soils compared to subsurface soils. Among the treatments, T8 and T9 were similar but not significantly different from each other, while both were significantly different from T6. Treatments T3, T4, and T5 were similar but significantly different from T6, and T6 and T7 were similar but significantly different from T1.

Dehydrogenase enzymes are crucial for the biological oxidation of soil organic matter. They facilitate the removal of hydrogen ions ( $H^+$ ) from organic compounds and transfer them to an electron acceptor, such as  $NAD^+$  or  $FAD^+$ . This process releases energy, which microorganisms use for growth and metabolism. The activity of dehydrogenase enzymes is a key indicator of soil microbial activity and overall soil health. Higher dehydrogenase activity suggests greater microbial activity and enhanced nutrient availability in the soil.

Soil microorganisms break down organic matter into simpler compounds, and dehydrogenase enzymes further process these compounds by transferring hydrogen ions to electron acceptors. This electron transfer generates energy utilized by microorganisms. Dehydrogenase activity typically decreases with soil depth for several reasons. First, the amount of organic matter decreases with increasing depth, limiting the energy and nutrients available to microorganisms and thus reducing dehydrogenase activity. Second, oxygen availability diminishes with depth as oxygen diffusion becomes restricted in denser soil layers, leading to anaerobic conditions that can inhibit the activity of aerobic microorganisms responsible for producing dehydrogenase enzymes.

Similar findings were reported by Bhavani et al. (2017) [7] and Rawat et al. (2021) [8], who observed the impact of organic and inorganic fertilizers on soil enzyme activity in the Malwa Plateau. Aher et al. (2018) [9] noted that organic farming results in 27-102% higher enzyme activity compared to traditional farming. Additionally, Meena et al. (2019) [10] found that soil enzymes, including dehydrogenase, were more active under organic farming conditions.

#### **Urease activity ( $\mu\text{g NH}_4\text{-N g}^{-1}\text{ soil h}^{-1}$ );**

The Urease activity of the soil at different depths i.e. (0-10, 10-20, 20-30, 30-40 cm) and there statistically analysed data with respect to different manures and fertiliser application, including unfertilized control, is shown in Table 1. The results revealed that the Urease activity of surface soil was higher compare to subsurface soil. Urease activity ranged from 145 to 167.02  $\mu\text{g NH}_4\text{-N g}^{-1}\text{ soil h}^{-1}$ , 123.25 to 142.01  $\mu\text{g NH}_4\text{-N g}^{-1}\text{ soil h}^{-1}$ , 98.60 to 113.61  $\mu\text{g NH}_4\text{-N g}^{-1}\text{ soil h}^{-1}$ , and 76.61 to 88.62  $\mu\text{g NH}_4\text{-N g}^{-1}\text{ soil h}^{-1}$  for soil depths of 0-10, 10-20, 20-30, and 30-40 cm, respectively. The Urease activity at 0-10 cm depth was significantly higher under FYM 6 t ha<sup>-1</sup> + N<sub>20</sub> P<sub>13</sub> (T6), which was comparable to Residues 5 t ha<sup>-1</sup> + N<sub>20</sub> P<sub>13</sub> (T7) and FYM 6 t ha<sup>-1</sup> (T8), but significantly superior to all other treatments. All soil depths showed a similar trend urease activity was lowest in the control treatment (145.00  $\mu\text{g NH}_4\text{-N g}^{-1}\text{ soil h}^{-1}$ , 123.25  $\mu\text{g NH}_4\text{-N g}^{-1}\text{ soil h}^{-1}$ , 98.60  $\mu\text{g NH}_4\text{-N g}^{-1}\text{ soil h}^{-1}$  76.91  $\mu\text{g NH}_4\text{-N g}^{-1}\text{ soil h}^{-1}$  at (0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm) depths respectively. Treatments T8 and T9 were similar but not significantly different from each other, and both were significantly different from T6 and T7. Additionally, T2, T3, T4, and T5 were similar but significantly different from T6 and T7, while T6 and T7 were similar but significantly different from the control treatment (T1).

Urease is an enzyme responsible for the hydrolysis of urea. It plays a crucial role in breaking down urea fertilizers, with extracellular urease being stabilized by immobilization on organic and mineral soil colloids,

thereby performing a significant portion of ureolytic activity in the soil. Identifying factors that affect urease efficiency is essential for optimizing its role in soil ecosystems.

Similar results were reported by Kachikerimath and Dhyan Singh (2001)[11] and Thaware et al. (2016), where the application of organic sources such as crop residue stalks and organic manures showed higher urease activity compared to the control. Thaware et al. (2016)[12] found that incorporating 5% soybean straw was significantly superior to other crop residues in terms of urease activity. Rawat et al. (2021)[13] also reported that urease activity was highest with the application of FYM 6 t ha<sup>-1</sup> + N<sub>20</sub> P<sub>13</sub>.

### Assay of Acid and Alkaline Phosphate;

#### Acid phosphate;

Acid phosphate levels were measured in various organic and inorganic treatments at 0-10, 10-20, 20-30, and 30-40 cm soil depths and the results are shown in Table 2. The acid phosphate activity as influenced by application various treatments for various depth is ranged from 257.73 to 384.57 μg PNP g<sup>-1</sup> soil h<sup>-1</sup>, (0-10cm depth) 224.23 to 334.57 μg PNP g<sup>-1</sup> soil h<sup>-1</sup>, (10-20cm depth), 190.59 to 284.39 μg PNP g<sup>-1</sup> soil h<sup>-1</sup>, (20-30 cm depth) 142.95 to 213.29 μg PNP g<sup>-1</sup> soil h<sup>-1</sup>, (30-40 cm depth). The acid phosphate increasing significantly with an increasing level of manures and fertilizers application. Among the manures and fertilizers combinations higher acid phosphate enzyme activity found was in treatment received FYM 6 t ha<sup>-1</sup> + N<sub>20</sub> P<sub>13</sub> (T6) 384.57 μg PNP g<sup>-1</sup> soil h<sup>-1</sup> at (0-10 cm) depth. As compare to manure and as the fertilizers dose are decrease, acid phosphate also decreases significantly followed by residues 5 t ha<sup>-1</sup> + N<sub>20</sub> P<sub>13</sub> (T7) 362.93 and FYM 6 t ha<sup>-1</sup> (T8) 353.50 and residues 5 t ha<sup>-1</sup> (T9) 348.50 μg PNP g<sup>-1</sup> soil h<sup>-1</sup>. Lower acid phosphate was found in control (T1) 257.73 μg PNP g<sup>-1</sup> soil h<sup>-1</sup> at (0-10cm) 224.23 μg PNP g<sup>-1</sup> soil h<sup>-1</sup> (10-20cm), 190.59 μg PNP g<sup>-1</sup> soil h<sup>-1</sup> (20-30cm), 142.95 μg PNP g<sup>-1</sup> soil h<sup>-1</sup> at 30-40cm soil depths as compare to increase fertilizers dose significantly increase acid phosphate 292.83 μg PNP g<sup>-1</sup> soil h<sup>-1</sup> N<sub>20</sub> P<sub>13</sub> (T2), 335.83 μg PNP g<sup>-1</sup> soil h<sup>-1</sup> N<sub>30</sub> P<sub>20</sub> (T3), 344.80 μg PNP g<sup>-1</sup> soil h<sup>-1</sup> N<sub>40</sub> P<sub>26</sub> (T4), 350.17 μg PNP g<sup>-1</sup> soil h<sup>-1</sup> N<sub>60</sub> P<sub>35</sub> (T5) at 0-10cm depth. The treatment T8 and T9 are at par i.e. but not significantly different. But significantly different from T6 and T7 also and the T2, T3, T4 and T5 are as par i.e. but not significantly different whereas significantly different from T6 and T7 also the T6 and T7 are as par i.e. but not significantly different, but significantly different from T1.

Acid phosphate is an enzyme that plays an important role in the cycling of phosphorus in soil. It is secreted by plants and microbes and is involved in the hydrolysis of organic phosphorus compounds to release inorganic phosphate. It is generally observed that acid phosphatase activity decreases with increasing soil depth. This can be attributed to a number of factors, including decreased availability of organic matter, lower microbial activity, and changes in soil pH. Similar results were reported by Rawat *et al.*, (2021)[14] that the urease activity highest in FYM 6 t ha<sup>-1</sup> + N<sub>20</sub> P<sub>13</sub>. The fact that the upper layer had higher acid phosphate values more than the lower layer indicates that acid phosphate decreased as soil depth increased. Also the treatments which include integrated combination of FYM and fertilizers are found higher in Acid phosphate content and followed by treatments which includes chemical combinations of fertilizers.

#### Alkaline phosphate (μg PNP g<sup>-1</sup> soil h<sup>-1</sup>)

Alkaline phosphate levels were measured in different organic and inorganic treatments at 0-10, 10-20, 20-30, and 30-40 cm soil depths and the results are shown in Table 2. The analysis of variance revealed that alkaline phosphate was significantly influenced by long-term use of manure and fertiliser treatments at all stages of the study. The alkaline phosphate study revealed that among treatments, the highest value of alkaline phosphate received with FYM 6 t ha<sup>-1</sup> + N<sub>20</sub> P<sub>13</sub> is 399.97 μg PNP g<sup>-1</sup> soil h<sup>-1</sup>, 327.97 μg PNP g<sup>-1</sup> soil h<sup>-1</sup>, 229.39 μg PNP g<sup>-1</sup> soil h<sup>-1</sup>, and 183.66 μg PNP g<sup>-1</sup> soil h<sup>-1</sup> at 0-10, 10-20, 20-30, and 30-40 cm soil depths, followed by Residues 5 t ha<sup>-1</sup> + N<sub>20</sub> P<sub>13</sub> (T7), 387.43 μg PNP g<sup>-1</sup> soil h<sup>-1</sup>, 317.70 μg PNP g<sup>-1</sup> soil h<sup>-1</sup>, 222.93 μg PNP g<sup>-1</sup> soil h<sup>-1</sup>, 177.91 μg PNP g<sup>-1</sup> soil h<sup>-1</sup> at 0-10, 10-20, 20-30, and 30-40 cm soil depths. Lower alkaline phosphate was found to be under control (T1) at all soil depths. The fact that the upper layer had higher alkaline phosphate values than the lower layer indicates that alkaline phosphate decreased as soil depth increased. The treatment T8 and T9 are at par i.e. but not significantly different. But significantly different from T6 and T7 also and the T3, T4 are as par i.e. but significantly not different whereas significantly different from T6 and T7 also the T6 and T7 are as par i.e. but not significantly different but significantly different from T1.

The term phosphatase refers to a broad class of enzymes that catalyse the hydrolysis of H<sub>3</sub>PO<sub>4</sub> esters and anhydrides, involving five major groups. Phosphatase assay in soil is more important due to its relative importance in soil organic P mineralization and plant nutrition. Only microorganisms produce alkaline phosphatase activity (Jadhao *et al.*, 2014). Alkaline phosphate enzymes are produced by certain soil microbes, and their activity can be influenced by the presence and activity of these microbes. As soil depth increases, conditions can become less favorable for microbial activity, leading to a decrease in enzyme production and activity. Studies by others authors have shown that the increase in alkaline phosphate could be attributed to the use of organic manure, which increases microbial activity. Kanchikerimath and Dhyani Singh *et al.*, (2001) [15] investigated soil organic matter and biological properties changes. With the addition of balanced nutrients and manure, the alkaline phosphatase activities increased significantly. The significantly higher alkaline phosphatase activities in organically treated soil could be attributed to increased microbial activity and diversity of phosphate solubilizing bacteria (Mandal *et al.*, 2007) [16].

#### **Arysulphatase activity (μg PNP g<sup>-1</sup> soil h<sup>-1</sup>);**

Arysulphatase activity levels were measured in different organic and inorganic treatments at 0-10, 10-20, 20-30, and 30-40 cm soil depths and the results are shown in Table 1. The analysis of variance revealed that Arysulphatase activity was significantly influenced by long-term use of manure and fertiliser treatments at all stages of the study. The arysulphatase activity study revealed the highest value of arysulphatase activity received with FYM 6 t ha<sup>-1</sup> + N<sub>20</sub> P<sub>13</sub> (T6) is 40.01 μg PNP g<sup>-1</sup> soil h<sup>-1</sup>, 32.81 μg PNP g<sup>-1</sup> soil h<sup>-1</sup>, 24.60 μg PNP g<sup>-1</sup> soil h<sup>-1</sup>, and 15.99 μg PNP g<sup>-1</sup> soil h<sup>-1</sup>, at 0-10, 10-20, 20-30, and 30-40 cm soil depths, followed by Residues 5 t ha<sup>-1</sup> + N<sub>20</sub> P<sub>13</sub> (T7) and FYM 6 t ha<sup>-1</sup> (T8). Lower arysulphatase activity was found to be under control (T1) 20.47 81 μg PNP g<sup>-1</sup> soil h<sup>-1</sup>, 16.79 81 μg PNP g<sup>-1</sup> soil h<sup>-1</sup>, 12.59 81 μg PNP g<sup>-1</sup> soil h<sup>-1</sup>, 8.18 81 μg PNP g<sup>-1</sup> soil h<sup>-1</sup> at (0-10cm, 10-20cm, 20-30cm, 30-40cm) respective soil depths. The fact that the upper layer had higher acid phosphate values than the lower layer indicates that arysulphatase activity decreased as soil depth increased. Treatment T8, T9 are on par i.e. not significantly different but significantly different from T6 and T1. T3 & T4 and T5 are as per i.e. not significantly different but significantly different from T6 and T1.

There are several reasons Arysulphate enzyme activity might decrease as soil depth increases arysulphate enzyme requires oxygen to function properly. As soil depth increases, the amount of oxygen available for microbial activity decreases due to the limited diffusion of gases in soil. This reduced oxygen availability can lead to a decrease in the activity of arysulphateenzyme. Arysulphate enzyme catalyzes the oxidation of organic sulfur compounds. As soil depth increases, the availability of organic matter decreases, leading to a decrease in substrate availability for Arysulphateenzyme. Soil temperature decreases with increasing soil depth due to the insulating properties of the soil. This decrease in temperature can also lead to a decrease in microbial activity, including the activity of Arysulphateenzyme. Similar studies by others authors have shown that the application of organics in the form of composts resulted in the highest enzyme activity; similar results were obtained by Vong *et al.* 2003 [17] and Thaware *et al.*, 2016 [18] where the application of organic sources such as crop residue stalk and organic manure resulted in the highest enzyme activity than the control.

### Conclusion:

The study revealed significant variations in soil enzymatic activity content across different treatments and depths. The control treatment (T1) exhibited the lowest soil enzymatic activity levels (Dehydrogenase (DHA), Urease enzyme, Acid & alkaline phosphate, Arysulphatase activity) particularly at 30–40 cm depth, whereas treatment T6 (FYM 6 t ha<sup>-1</sup> + N20 P13) demonstrated notable improvements, especially at 0–10 cm depth. The presence of FYM and nutrient supplementation significantly enhanced soil organic carbon content, with treatment T6 showing the highest values. Additionally, the DHA, Urease activity and acid & alkaline phosphate activity was notably higher in treatment T6 compared to the control treatment (T1), indicating the effectiveness of FYM and nutrient supplementation in enhancing soil carbon dynamics. These findings underscore the importance of appropriate soil management practices, such as FYM application and nutrient supplementation, in promoting soil microorganism and improving soil fertility. Further research is acceptable to explore the long-term effects of these treatments on soil enzyme activity and overall soil health.

### References

- Aheret, T., Korkmaz, S., & Gokceoglu, C. (2018). Soil enzyme activity under different agricultural practices. *Agricultural Research*, **9**(2), 124-135.
- Bhavani S, Shaker KC, Jayasree G, Padmaja B. Effect of long-term application of inorganic and organic fertilizers on soil biological properties of rice. *Journal of Pharmacognosy and Phytochemistry*. 2017;**6**(5):1107-1110.
- Cassida LE, Klein DA, Santoro J. Soil dehydrogenase activity. *Soil Science*. **1964**;98:371-376.
- Jadhao, R. S., Patil, S. S., & Singh, A. (2014). Soil enzyme activity and its correlation with soil fertility. *Journal of Soil Science and Plant Nutrition*, **14**(3), 567-578.
- Kiss, S., Dragan-Bularda, M. and Radulescu, D. (1978) Soil polysaccharides: activity and agricultural importance. In: Burns RG (ed.) *Soil Enzymes*. Academic, London, pp 117–147.
- Kachikermath, S. S., & Dhyan Singh. (2001). Soil enzyme activity in relation to soil management practices. *Journal of Agricultural Sciences*, **6**(1), 45-56.

Formatted: Font: (Default) Times New Roman

Comment [mm4]: On references: add or change references using limited 5 years ago

Formatted: Font: (Default) Times New Roman

Formatted: Font: (Default) Times New Roman

- Mandal, A., Patra, A. K., Singh, D., Swarup, A., Masto, R. E. 2007. Effect of long term application of manure and fertilizer on biological activities in soil during crop development stages. *Bioresource Technology*, **98**:3585-3592.
- Meena, B. (2019) Long-term sustaining crop productivity and soil health in maize–chickpea system through integrated nutrient management practices in Vertisols of central India Field Crops Research. **10**.10-12.012.
- Panse, V.G. and Sukhatme, P.V. (1961) Statistical Methods for Agricultural Workers, ICAR, NewDelhi
- Rawat, M. (2021). Impact of organic and inorganic fertilizers on soil enzymatic activities in Typic Haplusterts of Malwa Plateau, M.Sc. Thesis, Rajmata VijayarajeScindia Krishi Vishwa Vidyalaya, Gwalior, pp.- 64-69.
- Singh, J. S., Singh, S. P., & Singh, R. P. (2013). Soil enzyme activity under different land use systems. *Journal of Soil Science and Plant Nutrition*, **13**(4), 123-135.
- Tabatabai, M.A. and Bremner, J.M. (1969). Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Sil Biol. & Biochem.* **1**: 301-307.
- Thaware, V., Singh, S., & Yadav, R. (2016). Soil enzyme activity in relation to soil health and crop productivity. *Soil Biology and Biochemistry*, **98**, 12-20.
- Vong, P. C., Menzies, N. W., & Gill, R. A. (2003). Soil enzyme activities in relation to soil properties and management practices. *Soil Biology and Biochemistry*, **35**(10), 1507-1516.
- Waksman, S. A., & Waksman, J. (1992). Soil enzymes and their role in soil fertility. *Soil Science*, **154**(5), 341-353.

**Table 1. Dehydrogenase activity (DHA) ( $\mu\text{g TPF h}^{-1} \text{g}^{-1}$  soil), Urease activity and Arysulphatase activity as influenced by manures and fertilizers treatments at different depths of soil ;**

| TREATMENT                            | Dehydrogenase activity (DHA) |               |               |              | Urease activity ( $\mu\text{g NH}_4\text{-N g}^{-1}$ soil h <sup>-1</sup> ) |               |               |              | Arysulphatase activity ( $\mu\text{g PNP g}^{-1}$ soil h <sup>-1</sup> ) |              |              |              |
|--------------------------------------|------------------------------|---------------|---------------|--------------|---|---------------|---------------|--------------|--|--------------|--------------|--------------|
|                                      | 0-10 cm                      | 10-20 cm      | 20-30 cm      | 30-40 cm     | 0-10 cm   | 10-20 cm      | 20-30 cm      | 30-40 cm     | 0-10 cm  | 10-20 cm     | 20-30 cm     | 30-40 cm     |
| T1-Control                           | 126.77                       | 114.09        | 91.27         | 76.73        | 145.00  | 123.25        | 98.60         | 76.91        | 20.47  | 16.79        | 12.59        | 8.18         |
| T2- N20 P13                          | 134.02                       | 120.62        | 96.49         | 80.75        | 151.38  | 128.65        | 102.94        | 80.29        | 24.12  | 19.78        | 14.84        | 9.64         |
| T3-N30 P20                           | 144.17                       | 129.75        | 103.80        | 87.11        | 153.05  | 130.09        | 104.07        | 81.18        | 28.14  | 23.08        | 17.31        | 11.25        |
| T4-N40 P26                           | 146.80                       | 132.12        | 105.70        | 88.08        | 155.83  | 132.46        | 105.97        | 82.5         | 29.5   | 24.31        | 18.23        | 11.85        |
| T5-N60 P35                           | 150.22                       | 135.20        | 108.16        | 90.13        | 158.51  | 134.73        | 107.79        | 84.07        | 31.54  | 25.8         | 19.40        | 12.61        |
| T6- FYM 6 t ha <sup>-1</sup> +T2     | <b>157.48</b>                | <b>141.74</b> | <b>113.39</b> | <b>94.49</b> | <b>167.07</b>   | <b>142.01</b> | <b>113.61</b> | <b>88.62</b> | <b>40.01</b>   | <b>32.81</b> | <b>24.60</b> | <b>15.99</b> |
| T7-Residues 5 t ha <sup>-1</sup> +T2 | 152.53                       | 137.28        | 109.82        | 91.52        | 162.07  | 137.76        | 110.21        | 85.96        | 34.47  | 28.27        | 21.20        | 13.78        |
| T8-FYM 6 t ha <sup>-1</sup>          | 145.28                       | 130.76        | 104.60        | 87.17        | 154.44  | 131.28        | 105.02        | 81.92        | 30.42  | 24.94        | 18.71        | 12.16        |
| T9 -Residues 5t ha <sup>-1</sup>     | 141.63                       | 127.47        | 101.98        | 84.98        | 153.13  | 130.16        | 104.13        | 81.22        | 28.17  | 23.10        | 17.32        | 11.26        |
| SEm±                                 | 1.94                         | 0.89          | 0.81          | 0.52         | 1.80  | 1.37          | 1.30          | 1.01         | 0.17   | 0.49         | 0.45         | 0.26         |
| CD at 5%                             | 5.82                         | 2.66          | 2.44          | 1.55         | 5.38  | 4.12          | 3.89          | 3.03         | 0.52   | 1.48         | 1.34         | 0.79         |

**Table 2. Assay of Acid and Alkaline Phosphate ( $\mu\text{g PNP g}^{-1}$  soil h<sup>-1</sup>) activity as influenced by manures and fertilizers treatments at different depths of soil;**

| Assay of Acid and Alkaline Phosphate ( $\mu\text{g PNP g}^{-1}$ soil h <sup>-1</sup> ) |
|--|
|--|

|  | Acid phosphate |               |               |               | Alkaline Phosphate |               |               |               |
|--|----------------|---------------|---------------|---------------|--------------------|---------------|---------------|---------------|
|  | 0-10<br>cm     | 10-20<br>cm   | 20-30<br>cm   | 30-40<br>cm   | 0-10<br>cm         | 10-20<br>cm   | 20-30<br>cm   | 30-40<br>Cm   |
| <b>T1-Control</b>                          | 257.73         | 224.23        | 190.59        | 142.95        | 311.63             | 255.54        | 178.88        | 143.10        |
| <b>T2- N20 P13</b>                         | 292.83         | 254.77        | 216.55        | 162.42        | 325.83             | 267.18        | 187.03        | 149.62        |
| <b>T3-N30 P20</b>                          | 355.83         | 292.18        | 248.35        | 186.26        | 338.80             | 277.82        | 194.47        | 155.58        |
| <b>T4-N40 P26</b>                          | 344.80         | 299.98        | 254.98        | 190.23        | 349.10             | 286.6         | 200.38        | 160.31        |
| <b>T5-N60 P35</b>                          | 350.17         | 304.26        | 256.17        | 191.88        | 385.73             | 316.30        | 221.41        | 176.13        |
| <b>T6- FYM 6 t ha<sup>-1</sup>+T2</b>      | <b>384.57</b>  | <b>334.57</b> | <b>284.39</b> | <b>213.29</b> | <b>399.97</b>      | <b>327.97</b> | <b>229.58</b> | <b>183.66</b> |
| <b>T7-Residues 5 t ha<sup>-1</sup> +T2</b> | 362.93         | 315.75        | 268.39        | 201.29        | 387.43             | 317.70        | 222.39        | 177.91        |
| <b>T8-FYM 6 t ha<sup>-1</sup></b>          | 353.50         | 307.55        | 261.41        | 196.06        | 361.73             | 296.62        | 207.63        | 166.11        |
| <b>T9 –Residues 5t ha<sup>-1</sup></b>     | 348.50         | 303.20        | 257.72        | 193.29        | 358.53             | 294.00        | 205.80        | 164.64        |
| <b>SEm±</b>                                | 1.42           | 1.45          | 1.30          | 1.15          | 2.00               | 1.78          | 1.08          | 1.39          |
| <b>CD at 5%</b>                            | 4.27           | 4.33          | 3.89          | 3.74          | 6.01               | 5.35          | 3.25          | 4.18          |

UNDER PEER REVIEW