Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

IN-VIVO ASSESSMENT OF SOIL ENZYMES OF DIFFERENT HERBICIDES IN PADDY CULTIVATED SOILS AND ITS RISK ASSESSMENT

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ABSTRACT

Soil enzyme activities are the conservative communication of the soil community to metabolic desires and obtainable nutrients. The purpose of this research is to evaluate the changes and recovery of soil enzymes activity at regular intervals, after the sequential application of herbicides of Pretilachlor 50% EC, Pyrazosulfuron ethyl 10% WP, Bispyribac sodium 10% SC and Penoxsulam 21.7 SC in transplanted paddy field. Two experimentswith three replications were conducted at experimental farm of IIBAT, Padappai, during Kharif season of 2016 and 2017. The soil samples were collected at the following intervals of 0, 6, 10, 15, 18, 23, 25, 45 and 60 days after treatment for estimation of soil enzymes activity. All the samples were processed and analyzed for the enzyme activity by a validated UV spectrophotometric method. The results exposed that the effects of herbicides on soil enzyme activity was decreased suddenly after application of herbicides and it was slowly increased after three to five days of herbicides application. Herbicide treated plots were comparable to non-herbicide treated plots with respect to selected soil enzymes of Dehydrogenase, Urease and Alkaline Phosphatase activity was concluded that the toxic effects of herbicides in paddy field are usually severe immediately after application. Later on, soil enzyme activities are increased rapidly after three to five days of pre and post emergence application of herbicides. At 45 days after transplanting enzyme activities are shows similar or more compared with control and weedy check.

Keywords: Transplanted Paddy, Herbicides, Sequential applications, Dehydrogenase, Urease, Alkaline Phosphatase

1. INTRODUCTION

Herbicides are used for control of weeds in a crop and often residues of these herbicides or their metabolites are present in soils [1-3]. Most of the herbicides are having limited effects on microbial population related to soil fertility [3, 4]. Soil enzymes are the mediators and promoters of important soil functions. Herbicides are having effects on soil enzyme activities. Enzymes are the main stimulators in human life; similarly in the soil they are having a vital role in maintaining health of soil. A distinctive stability of chemical, physical and biological components subsidise to maintaining soil health.Soil enzyme activities have been suggested as suitable indicators of soil quality because they are a measure of soil microbial activity and are related to the nutrient cycles and transformations [4]. Soil enzyme activities are primary and sensitive indicators to measure the degree of soil degradation in both natural and agro-ecosystems. Since they are sensitive to ecological stress and land management practices these biological parameters have been used to assess soil quality and health as affected by agricultural practices. Enzyme activity is influenced by soil conditions such as organic matter content, moisture, temperature and many other factors like chemical nature of herbicides, concentration used, microbial community structure, type of soil, and soil conditions. Herbicides reaching the soil may disturb microbial metabolism or enzyme activities [5]. Herbicides are extraneous to soil component pools and are expected to affect activities of different soil enzymes and discussed in the following sections. The most valuable single use of soil enzymes is to assess the effects of various inputs on the relative

health of the soil [6], Soil enzyme activities commonly correlated with microbial parameter [7] and have been shown to be sensitive index of long term pesticide effects. Assay of enzyme activities are of great value in screening of the susceptibility of soil processes to agrochemical amendments [8-10].

Dehydrogenase activity is thought to reflect the total scope of activity of soil micro flora and is consequently a good indicator of microbial activity. It may be considered as a valuable parameter for assessing the side effects of herbicide treatments on the soil microbial biomass and can also be used as an indicator of the microbiological redox system. Dehydrogenase is an intracellular enzyme involved in microbial oxygen metabolism. This activity depends on the metabolic state of soil biota and may be a good indicator of soil microbial activity [11].

Urea is one of the main chemical nitrogen fertilizers and its application has been increased. Urea hydrolysis in soil is an enzymatic decomposition practice by the enzyme urease. When applied to soil, urea is hydrolyzed by enzyme urease to NH_4^+ in several days. Urease is an enzyme that catalyzes the hydrolysis of urea in rich in soils. Soil urease is involved in nitrogen mineralization and delivering nitrogen to plants from natural and fertilizer sources. The rate of urea hydrolysis depends on several factors like soil type, organic matter content, soil moisture content, $CaCO_3$ content, temperature and level of alkalinity. Some of these factors are affected the rate of urea hydrolysis in soils [12].

Phosphatases are extra cellular enzymes produced by many soil microorganisms and are responsible for the hydrolysis of organic P compounds to inorganic P. Phosphatases represent a wide range of intracellular as well as soil accumulated activities that catalyze the hydrolysis of both the esters and anhydrides of phosphoric acid. Phosphatase is concentrated in the surface layer and rhizosphere where most of the new and less humidified organic matter is prevailing. Phosphatases play a crucial role in the phosphorous acquisition of plants and microorganisms, and thus in the cycling of P within the soil.

2. MATERIAL AND METHODS

Two field studies were conducted during kharif seasons of 2016 and 2017. Sandy clay soil was used for this experiment. Soil was taken from the cultivated layer (0-20 cm) at our research farm of International Institute of Biotechnology and Toxicology, Padappai. The field experiment in each year was laid out in a randomized block design with three replications. Eight weed control treatments were included with pre and post emergence herbicides for weed control in transplanted rice. Treatments included in the study were T1 - Untreated Control, T2 - Pre-emergence application of Pretilachlor 50% EC @ 750 ml/ha at 3 DAT followed by Bispyribac Sodium 10% SC 200 ml/ha at 15 DAT, T3 - Pre-emergence application of Pretilachlor 50% EC @ 1000 ml/ha at 3 DAT followed by Bispyribac Sodium 10% SC 250 ml/ha at 15 DAT, T4 - Pre-emergence application of Pyrazosulfuron ethyl 10% WP 150 g/ha at 3 DAT followed by Bispyribac Sodium 10% SC 200 ml/ha at 15 DAT, T5 - Pre-emergence application of Pyrazosulfuron ethyl 10% WP 200 g/ha at 3 DAT followed by Bispyribac Sodium 10% SC 250 ml/ha at 15 DAT, T6 - Pre-emergence application of Pretilachlor 50% EC @ 1000 ml/ha at 3 DAT followed by Penoxsulam 21.7 SC 94 ml/ha at 15 DAT, T7 - Pre-emergence application of Pyrazosulfuron ethyl 10% WP 200 g/ha at 3 DAT followed by Penoxsulam 21.7 SC 94 ml/ha at 15 DAT, T8 - Hand Weeding at 25 and 45 DAT.

2.1. Assay of dehydrogenase enzyme activity

One gram of soil sample was taken in 50 ml glass tube. Then 50 mg of $CaCO_3$ was added followed by 2.5 ml of distilled water and 1ml of 3% Triphenyltetrazolium chloride (TTC) was added. Swirled for sufficient minutes and incubated at 37ºC for 24 hours. The red precipitate of the Triphenylformazan (TPF) was dissolved in 10 ml of methanol and the contents were shaken for half an hour, the contents were filtered into 25 ml volumetric flask and the volume was made upto 25 ml with methanol. Intensity of red colour was measured photometrically at 485 nm within one hour [13].

2.2. Assay of Urease enzyme activity

Urease activity in soil was assayed by measuring the ratio of release of NH_4^+ from the hydrolysis of urea [14]. Five gram of soil was taken in a 50 ml volumetric flask, then adding 0.2 ml of toluene and 9 ml THAM buffer, the flask was swirled for a few seconds to mix the contents and 1ml of 0.2M urea solution was added and swirled the flask again for a few seconds. The flask was stoppered and placed in an incubator at 37ºC for two hours. After two hours, the stopper was removed, and around 35 ml of KCl-Ag_2SO_4 solution was added, swirled the flask for a few seconds, and allowed the flask to stand until the contents reached to room temperature (about 5 min). The contents were made up to 50 ml by addition of KCl-

Ag_2SO_4solution; the flask was stoppered and inverted some times to mix the contents. NH_4^+-N was determined in the resulting soil suspension, by pipetting out 20 ml aliquot of the suspension distilling with 0.2 g of MgO for 4 min. Controls also performed by following the assay of urease activity procedure, but for the addition of 1ml of 0.2M urea solution after the addition of KCl-Ag_2SO_4 solution.

2.3. Assay for Alkaline Phosphatase

The procedure followed was [15] for alkaline phosphatases. One gram of soil sample was taken in a cleaned glass tube. Then 0.2 ml of toluene was added followed by 4 ml MUB buffer pH 11.0 and 1 ml of p-nitro phenyl phosphate (only for samples) was added. Glass tubes swirled for few seconds, stoppered and incubated for 1 hour at 37ºC. After incubation, 1 ml of 0.5M $CaCl_2$ $2H_2O$ and 4 ml of 0.5M NaOH was added, swirled and filtered. The intensity of yellow color was measured with spectrophotometer at 420 nm. Controls were run simultaneously following the same procedure except adding 1ml of p-nitro phenyl phosphate (PNP) solution.

2.4. Statistical Analysis

Statistical significance in soil enzymes activities were determined by one-way analysis of variance (ANOVA) followed by Student Newman Keuls (SNK) test. Statistical analyses were performed using IBM SPSS Statistics software (version 26.0).

3. RESULTS AND DISCUSSION

3.1. Dehydrogenase activity (DHA)

Dehydrogenase activity is supposed to reflect the total scope of activity of soil micro flora and is consequently a respectable indicator of microbial activity. It may be considered as a valued parameter for assessing the side effects of herbicide treatments on the soil microbial biomass and can also be used as an indicator of the microbiological redox system. Dehydrogenase is an intracellular enzyme involved in microbial oxygen uptake. This activity hangs on the metabolic state of soil biota and may be a good indicator of soil microbial activity [11].

Table 1: Soil Dehydrogenase Activity (µg TPF produced g^{-1} day^{-1}) as influenced by weed management practices (2016 and 2017 Pooled Data)

S.No	Treatments	0 DAT	6 DAT	10 DAT	15 DAT	18 DAT	23 DAT	25 DAT	45 DAT	60 DAT
T_1	Untreated Control	5.4	5.7	6.6	7.4	7.7	8.6	9.7	10.8	11.2
T_2	Pre-emergence application of **Pretilachlor 50% EC** @ 750 ml/ha at 3 DAT followed by **Bispyribac Sodium 10% SC** 200 ml/ha at 15 DAT	5.4	4.6	5.7	7.2	6.0	7.0	7.6	11.0	11.7
T_3	Pre-emergence application of **Pretilachlor 50% EC** @ 1000 ml/ha at 3 DAT followed by **Bispyribac Sodium 10% SC** 250 ml/ha at 15 DAT	5.4	4.8	5.5	6.9	5.8	6.8	7.3	11.2	12.0
T_4	Pre-emergence application of **Pyrazosulfuron ethyl 10% WP** 150 g/ha at 3 DAT followed by **Bispyribac Sodium 10% SC** 200 ml/ha at 15 DAT	5.4	4.5	5.6	7.0	5.9	7.1	7.5	10.9	11.8
T_5	Pre-emergence application of **Pyrazosulfuron ethyl 10% WP** 200 g/ha at 3 DAT followed by **Bispyribac Sodium 10% SC** 250 ml/ha at 15 DAT	5.4	4.4	5.9	6.8	5.7	6.6	7.0	10.5	11.4
T_6	Pre-emergence application of **Pretilachlor 50% EC** @ 1000 ml/ha at 3 DAT followed by **Penoxsulam 21.7 SC** 94 ml/ha at 15 DAT	5.4	4.2	6.0	7.1	6.0	6.9	7.2	10.4	11.6
T_7	Pre-emergence application of **Pyrazosulfuron ethyl 10% WP** 200 g/ha at 3 DAT followed by **Penoxsulam 21.7 SC** 94 ml/ha at 15 DAT	5.4	4.5	6.1	7.0	5.8	6.8	7.4	10.8	11.0
T_8	Hand Weeding at 25 and 45 DAT	5.4	5.6	6.4	7.4	8.1	9.0	9.5	10.7	11.8
	CD (P=0.05)	**NS**	**0.43**	**0.34**	**0.50**	**0.46**	**0.41**	**0.42**	**0.99**	**0.73**

**- Significantly different from control T1, T8, $p<0.05$, One-way ANOVA post hoc SNK test DAT – Days after transplanting*

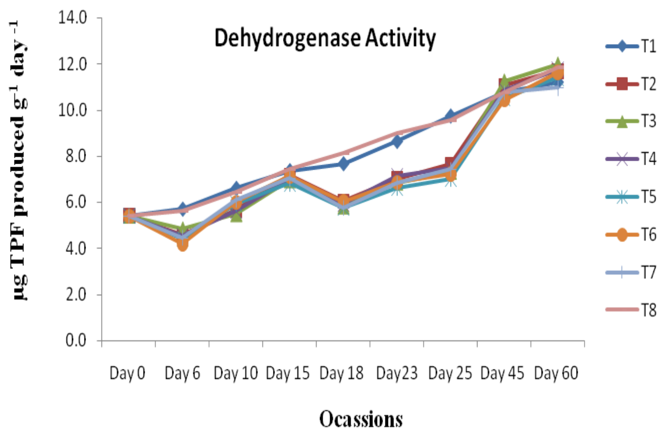

Fig. 1: Dehydrogenase Activity (µg TPF produced g^{-1} day^{-1})

The activity of dehydrogenase (µg TPF produced $g^{-1}day^{-1}$) as influenced by the herbicide treatments is presented in Fig.1 and Table 1. A close perusal of data during 2016 and 2017 indicates that significant difference exist between herbicide treatments. The soil dehydrogenase activity showed decreasing trend up to 3 days after treatment imposition [16] thereafter it showed increasing trend. Dehydrogenase activity levels were found significantly higher in hand weeding and untreated control plots.

At 6 DAT the higher dehydrogenase activity was recorded in untreated control T1 (5.7µg TPF produced g^{-1} day^{-1}), which was on par with hand weeding treatment T8 (5.6 µg TPF produced g^{-1} day^{-1}) except sequence application of herbicide plots. Dehydrogenase activity was recovered and recorded the on par results with untreated control by 15 DAT in sequence application of pretilachlor fb bispyribac sodium (T3) and pyrazosulfuron ethyl fb bispyribac sodium (T5) herbicide treatments.

At 15 DAT post emergence herbicides (Bispyribac & Penoxsulam) was applied. Both the herbicides showed decreased activity of dehydrogenase at 3 days after herbicide application (18 DAT) and later stages DHA increased significantly at 45 and 60 DAT. It is evident from the data that the soil treated with Pyrazosulfuron-ethyl and Bispyribac herbicides had the lower dehydrogenase activity immediately after herbicide application as compared to Pretilachlor and Penoxsulam applied soils but later it increased a recorded higher dehydrogenase activity than untreated control.

Dehydrogenase activity is a sensitive disindicator of the microbial activity response to herbicide inputs. Spandana Bhatt *et al* reported that the application of herbicides to the soil led to a significant drop in DHA with respect to untreated control soil samples. The same results were reported by Wyszkowska [17].

The sequence application of herbicides related to soil enzyme activities decreased with time. This is because of recovery of microbial population & enzyme activities after initial inhibition due to microbial adaptation to these herbicides and their degradation. The increase in soil DHA in herbicides treated soil from 20th day after application might be due to increase in microbial community composition with the capability of utilizing the herbicides as carbon source [18].

3.2. Urease Activity (UA)

Urease activity is a suitable indicator to evaluate the soil pollution situation. As close perusal of data on urease activity (µg of NH_4^+ released g^{-1} soil 2 hr^{-1}) indicated that significant difference exist between herbicide treatments (Table 2 and Fig. 2). Urease activity increased up to 45 DAT thereafter it was decreased.

At 6 DAT, the higher urease activity was recorded in untreated control T1 (55.9), which was highly significant over rest of the treatments. But at 15 DAT, sequence application of pretilachlor fb bispyribac sodium (T3) treated plots were on par with untreated control. At 15 DAT post emergence herbicides (Bispyribac & Penoxsulam) was applied. Both the herbicides showed decreased activity of urease at 3 days after herbicide application (18 DAT) and later stages urease increased significantly at 45 and 60 DAT. It is evident from the data that the soil treated with Pyrazosulfuron-ethyl and Bispyribac herbicides had the lower urease activity immediately after herbicide application as compared to Pretilachlor and Penoxsulam applied soils but later it increased a recorded higher urease activity than untreated control. It may be due to microbial multiplication in increased supply of nutrients available in the form of weeds killed by herbicides [19].The addition of herbicide might have provided substrate of urease in soil only initially which soon thereafter got hydrolyzed to ammonium carbonate thus leading to paucity of the substrate and this might be reason for the decreased urease activity after 45 DAT.

Table 2: Soil Urease Activity (μg of NH_4^+ released g^{-1} soil $2hr^{-1}$) as influenced by weed management practices (2016 and 2017 Pooled Data)

S.No	Treatments	0 DAT	6 DAT	10 DAT	15 DAT	18 DAT	23 DAT	25 DAT	45 DAT	60 DAT
T_1	Untreated Control	45.5	55.9	73.5	75.2	78.6	85.6	88.4	108.3	87.2
T_2	Pre-emergence application of **Pretilachlor 50% EC** @ 750 ml/ha at 3 DAT followed by **Bispyribac Sodium 10% SC** 200 ml/ha at 15 DAT	45.5	40.9	64.2	74.6	65.6	77.5	80.2	92.6	78.2
T_3	Pre-emergence application of **Pretilachlor 50% EC** @ 1000 ml/ha at 3 DAT followed by **Bispyribac Sodium 10% SC** 250 ml/ha at 15 DAT	45.5	42.7	67.5	75.4	66.8	79.2	81.6	90.4	79.8
T_4	Pre-emergence application of **Pyrazosulfuron ethyl 10% WP** 150 g/ha at 3 DAT followed by **Bispyribac Sodium 10% SC** 200 ml/ha at 15 DAT	45.5	38.5	61.8	76.1	68.1	77.4	79.5	88.6	78.4
T_5	Pre-emergence application of **Pyrazosulfuron ethyl 10% WP** 200 g/ha at 3 DAT followed by **Bispyribac Sodium 10% SC** 250 ml/ha at 15 DAT	45.5	39.8	62.6	74.0	64.2	75.2	78.2	89.6	77.8
T_6	Pre-emergence application of **Pretilachlor 50% EC** @ 1000 ml/ha at 3 DAT followed by **Penoxsulam 21.7 SC** 94 ml/ha at 15 DAT	45.5	43.2	69.0	73.8	65.1	74.0	77.3	87.4	78.2
T_7	Pre-emergence application of **Pyrazosulfuron ethyl 10% WP** 200 g/ha at 3 DAT followed by **Penoxsulam 21.7 SC** 94 ml/ha at 15 DAT	45.5	42.6	67.2	73.2	64.5	73.8	76.0	86.0	76.5
T_8	Hand Weeding at 25 and 45 DAT	45.5	55.0	70.0	73.8	77.0	84.2	86.4	112.5	89.8
	CD (P=0.05)	**NS**	**0.22**	**0.13**	**0.04**	**0.18**	**0.15**	**0.14**	**0.34**	**0.17**

**- Significantly different from control T1, T8, $p<0.05$, One-way ANOVA post hoc SNK test*

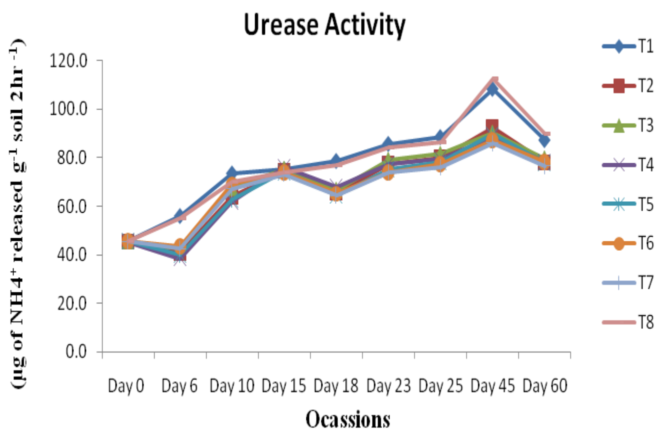

Fig. 2: Urease Activity (μg of NH_4^+ released g^{-1} soil $2hr^{-1}$)

3.3. Alkaline Phosphatase Activity (PA)

Alkaline phosphatase activity (μg of PNP released g^{-1} soil h^{-1}) as influenced by herbicide treatments is presented in Fig.3 and Table 3. The data indicates that statically significant difference exists between sequence applications of herbicide treatments.

Alkaline phosphatase activity increased from 0 DAT to 45 DAT, thereafter the activity decreased at 60 days after transplanting.

At 6 DAT the higher alkaline phosphatase activity was recorded in T1 untreated control (65.9) which was on par with hand weeding treatment T8 (65.4). The enzyme activity was recovered and recorded the on par results with untreated control by 15 DAT in sequence application of pretilachlor (fb) bispyribac sodium (T3) and pyrazosulfuron ethyl fb bispyribac sodium (T5) herbicide treatments.

At 15 DAT post emergence herbicides (Bispyribac&Penoxsulam) was applied. Both the herbicides showed decreased activity of alkaline phosphatase at 3 days after herbicide application (18 DAT) and later stages alkaline phosphatase increased significantly at 45 and 60 DAT. It is evident from the data that the soil treated with Pyrazosulfuron-ethyl and Bispyribac herbicides had the lower alkaline phosphatase activity immediately after herbicide application as compared to Pretilachlor and Penoxsulam applied soils

but later it increased a recorded higher alkaline phosphatase activity than untreated control.

Initial decrease in enzymatic activity might be due to the fact that alkaline phosphatase is of extracellular origin and the expiry of some microorganisms can cause a reduction in the production of elimination of enzymes leading to a reduction in the soil activity [20]. It was observed in this experimentation that the inhibitory effect of herbicides on enzyme activities is short lived and reduced with time. Recovery of enzyme activities after early inhibition might be due to growth of microbial population after adaptation or most probably due to increased availability of nutrients due to degradation of herbicides [21].

Table 3: Soil Alkaline Phosphatase Activity (µg of PNP released g^{-1} soil h^{-1}) as influenced by weed management practices (2016 and 2017 Pooled Data)

S.No	Treatments	0 DAT	6 DAT	10 DAT	15 DAT	18 DAT	23 DAT	25 DAT	45 DAT	60 DAT
T_1	Untreated Control	55.3	65.9	73.6	83.9	91.6	108.3	111.6	155.1	139.0
T_2	Pre-emergence application of **Pretilachlor 50% EC** @ 750 ml/ha at 3 DAT followed by **Bispyribac Sodium 10% SC** 200 ml/ha at 15 DAT	55.3	37.4	66.8	83.3	75.8	110.8	116.4	149.7	134.0
T_3	Pre-emergence application of **Pretilachlor 50% EC** @ 1000 ml/ha at 3 DAT followed by **Bispyribac Sodium 10% SC** 250 ml/ha at 15 DAT	55.3	35.0	67.8	85.0	78.2	108.8	118.2	151.8	134.0
T_4	Pre-emergence application of **Pyrazosulfuron ethyl 10% WP** 150 g/ha at 3 DAT followed by **Bispyribac Sodium 10% SC** 200 ml/ha at 15 DAT	55.3	34.2	67.5	86.6	76.9	107.6	117.6	150.5	135.5
T_5	Pre-emergence application of **Pyrazosulfuron ethyl 10% WP** 200 g/ha at 3 DAT followed by **Bispyribac Sodium 10% SC** 250 ml/ha at 15 DAT	55.3	35.0	68.9	86.3	77.0	104.7	117.3	146.5	134.1
T_6	Pre-emergence application of **Pretilachlor 50% EC** @ 1000 ml/ha at 3 DAT followed by **Penoxsulam 21.7 SC** 94 ml/ha at 15 DAT	55.3	35.8	68.2	85.9	77.1	108.3	116.0	149.1	133.9
T_7	Pre-emergence application of **Pyrazosulfuron ethyl 10% WP** 200 g/ha at 3 DAT followed by **Penoxsulam 21.7 SC** 94 ml/ha at 15 DAT	55.3	37.6	64.9	87.9	75.2	111.3	118.2	145.6	131.5
T_8	Hand Weeding at 25 and 45 DAT	55.3	65.4	73.2	84.7	92.9	110.3	117.7	157.8	141.3
	CD (P=0.05)	**NS**	**0.56**	**0.12**	**0.08**	**0.29**	**0.09**	**0.10**	**0.13**	**0.17**

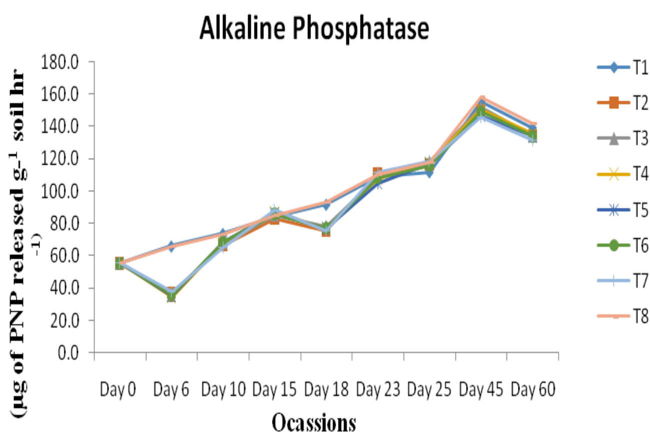

Fig. 3: Alkaline Phosphatase activity (µg of PNP released g^{-1} soil hr^{-1})

4. CONCLUSION

From the present study we concluded that the selected herbicides are normally most severe immediately after application. Later on, microorganisms take part in a degradation process, and then the degraded organic herbicides provide carbon rich substrates which in terms maximize the enzyme activity in the rhizosphere.

5. ACKNOWLEDGEMENTS

The authors wish to acknowledge the Executive Council Members, Management and Scientific Academic Board of IIBAT for providing the facility in timely manner for conducting the experiment.

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