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Herbicides impact on Fe and Mn reduction and dehydrogenase activity in an agricultural soil

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ABSTRACT

Agricultural use of herbicides has often resulted in contamination of the soil ecosystem, by direct or indirect action, after short, average or long period of time. Under soil submergence a number of transformations in the soil environment are generated due to the effects of hypoxia and anoxia that seriously affect the rhizosphere of the crop particularly the nutrient dynamics. Various assessment techniques are utilized for quantifying the response of reducing soil conditions on nutrient transport and soil enzymatic responses. However, the impact of herbicides under soil submergence poses a challenge to compare its effect on Fe and Mn transformation and dehydrogenase activity. To test this hypothesis, a laboratory study was conducted to assess the effect of herbicide glyphosate, paraquat and pendimethalin on Fe and Mn reduction and soil dehydrogenase activity; used as a measure of the metabolic activity of microorganisms, under submerged soil conditions. Glyphosate at field application dose ($0.90\ \mu g g^{-1}$) and double the field application dose ($1.80\ \mu g g^{-1}$) inhibited the Fe and Mn reduction from 5.18 to 14.35% and stimulated the soil dehydrogenase activity from 11.64 to 43.12%. However, both inhibition and stimulation effect on Fe & Mn reduction and soil dehydrogenase activity was resulted from the application of herbicides paraquat and pendimethalin at their field ($0.45\ \mu g g^{-1}$) and double the field application dose (). The present investigation concluded a stimulation as well as inhibition impact on Fe and Mn reduction and dehydrogenase activity.

Keywords: *Dehydrogenase activity, Fe and Mn reduction, herbicide*

Submerging or water logging the soil creates conditions markedly different from those of a well-drained soil. As long as oxygen is present in the soil, other oxidized compounds of the soil are relatively safe for biological and chemical reduction. After oxygen is disappeared from a waterlogged soil, the need of electron acceptors by facultative anaerobic and true anaerobic organisms resulted in the reduction of several oxidized components like, the reduction of the oxidized inorganic ions like manganese and ferric ions (Ponnamperuma, 1972). So, under waterlogged paddy fields, due to high solubility of Fe^{+2} and Mn^{+2} toxicity symptoms are observed.

Sources of enzymes in soils are primarily the microbial biomass originating from plant and animal residues. These enzymes are protein in nature with catalytic properties owing to their power of specific activation that can cause biochemical reactions to proceed at faster rates (Tabatabai, 1994). It is one of the main components in participating to and assuring the correct and integrated sequence of all the biochemical routes (viz. hydrolysis, oxidation, reduction, etc.), present in soil biogeochemical cycles (Shaffer, 1994). Soil dehydrogenase activity is closely related to soil fertility properties such as nutrient status, pH, temperature and moisture (Baligar and Wright 1991).

However, these activities are sometimes inhibited and/or stimulated because of the presence or absence of inhibitors or activators like herbicide molecules. But few studies have demonstrated that the application of herbicides influence the biodynamic in soil like dynamics of iron and manganese reduction and microbial and enzymatic activities (Wardle and Parkinson, 1992; Tu, 1994; Min *et al*. 2001) under submerged soil condition. Herbicide application can affect the availability of soil macro as well as micro nutrients (Singh, 2014); reduces the soil nutrient availability for uptake by plants (Sebamio *et al*. 2012) and affects soil microbial biomass carbon and carbon mineralization (Kumar *et al*., 2012).

Although soil appears to be a system biologically in equilibrium each disturbance modifies the activity of the microflora and consequently the soils fertility. The impact of these chemicals on soil system has become a matter of interest of contemporary research. Once herbicides come in contact with the soil they can alters the catalytic characteristics in the soil environment substantially by interacting directly or indirectly with enzymes hence the reduction of iron (Fe) and manganese (Mn) may occur.

In spite of the numerous efforts aimed at understanding a possible cause-and-effect relationship between herbicides and enzyme activity and its related biochemical processes, knowledge of this topic particularly in the Indian subcontinent is still lacking,

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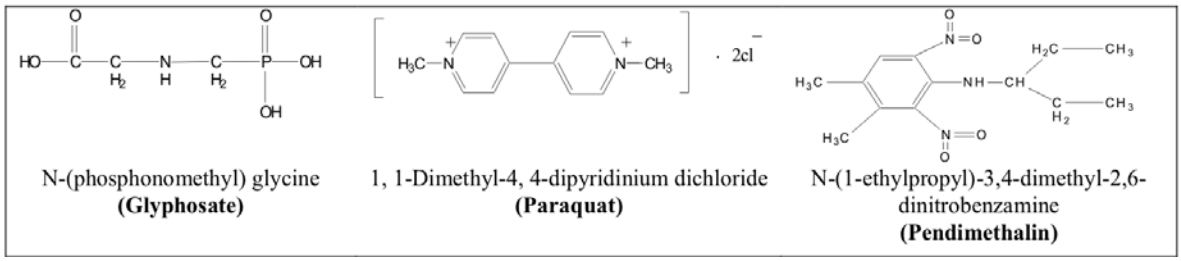
because of the heterogeneity of enzymatic activities in soils. Hence, an innovative methodology has been adopted for the study of the interactions of diversed polar herbicides (glyphosate, paraquat and pendimethalin) with the Fe and Mn reduction and dehydrogenase activity in a submerged soil.

MATERIALS AND METHODS

The experiment was conducted by taking pure analytical grade of glyphosate [N-(phosphonomethyl) glycine], paraquat (1,1-Dimethyl-4,4-dipyridybniumdichloride) and pendimethalin[N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzamine] from their respective manufacturing companies. The herbicides were applied both at their field application and double the field application doses. The field application dose of the herbicides are at 2.0 kg a.i ha^{-1} (0.90 $\mu g\, g^{-1}$) , 1.0 kg a.i. ha^{-1} (0.45) and 1.0 kg a.i. ha^{-1} (0.45) respectively.



An alluvial soil (*typic Haplusteps*) was collected from the agricultural research farm, Banaras Hindu University, Varanasi, India. (25°20¢N and 83°00¢E, MSL-200 meters). Soil was then air dried, ground with a wooden mortar and pestle, sieved (2 mm) and stored in plastic containers. Physico-chemical properties of the processed soil samples were determined by the standard procedures (Kanwar and Chopra 1988) with a pH of 7.7, organic carbon content (g kg^{-1}) of 4.6, specific conductance of 0.39 dS/m, exchangeable Fe (mg kg^{-1}) of 6.54 and exchangeable Mn (mg kg^{-1}) of 13.15.

To restore the normal soil biological activity, the air dried soil sample was pre-incubated after adjusting soil water content to 60 per cent water holding capacity. Before submergence, herbicides glyphosate, paraquat and pendimethalin (in acetone) were added at three rates like control (no herbicides), field application dose and double the field application dose. After 30 minutes, the soil was homogenized for thorough mixing of the herbicide molecules. For stimulating the submerged soil system in the laboratory for the assessment of Fe and Mn reduction, 10g pre-incubated herbicides treated air dried processed soil was placed in test tubes (150 × 20 mm) and 12.5ml of distilled water was added to provide a standing water column of about 50 mm over the surface of the soil. Soil samples were then incubated at room temperature (25 ± 3°C) for a period of 42.

At 7, 14, 21, 28 and 42 days after incubation under submerged condition, iron (Fe^{2+}) and manganese (Mn^{2+}) in each of the three soil replicates were extracted by shaking the soils in each tube with 100 mL of 1M sodium acetate (pH adjusted to 2.8) for 1h as described by Howler and Bouldin (1971). The soil suspension was filtered and the concentration of Fe^{2+} in the filtrate was determined using o-phenanthroline described by Murti *et al.* (1966). For estimation of soluble Mn^{+2} in the filtrate, a suitable aliquat was taken and determined directly by Thermo elemental type SOLAAR S4 Atomic Absorption Spectrometer (Page *et al*. 1992). Measurements were made using the hollow cathode lamps for Mn at the proper slit width (0.2 nm) and wavelength (279.5nm) were adjusted and other AAS conditions employed in these determinations are summarized in table 2. The flame type used for all elements was air-acetylene. Working solutions were prepared by dilution just before the use of standard solutions for atomic absorption spectroscopy. The means of three separate readings for each solution were used to calculate the concentrations. Proper quality assurance procedures and precautions were carried out to ensure dependability of the results. Samples Reagent blank determinations were used to correct the instrument readings. A recovery study of the analytical procedure was carried out by spiking and homogenizing several already analyzed samples with varied amounts of standard solutions of the metals.

Soil dehydrogenase activity in triplicate from the submerged soil samples were assayed by Spectrophotometric method using 2, 3, 5-triphenyl tetrazolium chloride (TTC) reduction method (Casida et al. 1964). To the submerged herbicides treated soils, 0.2 mL of 3 per cent triphenyl tetrazollium chloride (TTC) solution was added as a substrate at the end of desired incubation period. After thorough mixing, the contents were incubated at 28 ± 0.5°C temperature for one day (24h). After 24 h, 10 mL of methanol was added

and was shaken vigorously. After 6h, the supernatant liquid was taken for estimation in spectrophotometer at 485 nm and the activity of the enzyme was expressed as . The spectrophotometric measurements were carried out using an ELICO UV/visible double beam Spectrophotometer SL-164 with 1 cm matched quartz cells. Double distilled deionized water was used to prepare all solutions.

Data were statistically analysed using SAS software. Analysis of variance (ANOVA) was used to detect the treatment effects on measured variables. Least square difference (LSD) values were calculated to test the significance of treatment difference and LSD values were evaluated at the 5 per cent level of significance of measured Fe and Mn reduction and dehydrogenase activities (Panse and Sukhatme 1985).

RESULTS AND DISCUSSION

Microbial functional diversity in soil is related both to the rate of substrate utilization and to the utilization of specific substrate. Microbial Fe (III) reduction accounts for most of the Fe (III) reduction in many anoxic soils and aquatic sediments. Non-enzymatic processes such as reduction of Fe (III) by organic compounds and sulphide are generally of minor significance. Mn (IV) may reduced by nonenzymatic processes, but enzymatic Mn(IV) reduction does predominate in waterlogged environment (Lovely, 1995). It is also reported that submerging a soil will decrease the redox potential (however, application of pesticides will retard the decrease in redox potential of flooded soil that affects the reduction of Fe and Mn (Bhattacharya *et al*., 1996). But in the present study both inhibition and stimulation is noticed to reduction of Fe and Mn and also to dehydrogenase activity during the entire incubation study which might be because of the change in redox potential.

Effect of pure formulation of glyphosate at 0.90 (R1) and 1.80 (R2) added to the submerged alluvial soil to assess the Fe & Mn reduction and soil dehydrogenase activity was studied (Fig. 1). Under non flooded conditions, pesticides seldom affect microorganisms and their activities when applied at field rates. Soil treated with herbicide glyphosate retained the original yellow brown colour even after 42 days of flooding. However, the soil without herbicide treatment started to turn grey colour after 7 days of flooding (Ponnamperuma, 1972). This indicated glyphosate application slowed or inhibited the reduction of Fe^{3+} and Mn^{4+} to Fe^{2+} and Mn^{2+} under flooding situation. Submerging soil without glyphosate application over a period of 42 days revealed a significant increase in Fe^{2+}, soluble Mn^{2+} and soil dehydrogenase activity () which might be due to reduction in redox potential (Ponnamperuma, 1972). Application of glyphosate at 0.90 soil and above significantly reduced or inhibited the Fe^{2+}, soluble Mn^{2+} concentration but stimulated the soil dehydrogenase activity. The extent of inhibition varied with the

Table 1. Particulars of herbicides used

Technical	Trade Name	Chemical name and formula	AI	Mode of action mammals)	LD 50[a] (mg kg^{-1}	Field recommended dose	Sources
Glyphosate	No Weed	N-(phosphonomethyl) glycine $C_3H_8NO_5P$	WSC	Nonselective-systemic herbicides	5600mg kg^{-1} (Champaign, 1994)	2.0 kg a.i./ha (0.90 µg g^{-1}) (Gupta, 2010)	Dhanuka Agrotech Ltd., Gurgaon, Haryana, India
Paraquat	Ozone	1, 1-Dimethyl-4, 4-dipyridinium dichloride $C_{12}H_{14}Cl_2N_2$	SL	Nonselective-contact herbicide	10 to 150 mg kg^{-1} (Stevens and Sumner, 1991)	1.0 kg a.i. ha^{-1} (0.45 µg g^{-1}) (Gupta, 2010)	Dhanuka Agrotech Ltd., Gurgaon, Haryana, India
Pendimethalin	Dhanutop	N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzamine $C_{13}H_9N_3O_5$	EC	Selective herbicide	5000 mg kg^{-1} (Champaign 1994)	1.0 kg a.i. ha^{-1} (0.45 µg g^{-1}) (Gupta, 2010)	Dhanuka Agrotech Ltd., Gurgaon, Haryana, India

Note: AI- Active ingredient, WSC- Water Soluble Concentrate, SL- Soluble Liquid, EC- Emulsifiable Concentrate
[a] lethal dose of active ingredient causing death to 50% of population after exposure during a single dose application (Tomlin, 1997).

Table 2. Standard conditions used in determination of manganese and their detection limits using Atomic Absorption Spectrometer

Element	Wavelength (nm)	Slit width (nm)	Standards (mg L^{-1})	Fuel flow rate (L min^{-1})	Detection limit (ppm)	Measurement time	Flame type	Replicates
Mn	279.5	0.2	0.1,0.25,0.5	0.9	0.002	4s	Air – C_2H_2	3

concentration of herbicide application. Inhibition of Fe and Mn reduction to glyphosate application may be because of inhibition of microorganisms participating in these reduction reactions that might be due to a decline in the redox potential (Bhattacharya *et al*. 1996) and a decrease in microbial populations with a reduced microbial functionality in soil due to application of pesticide (Nannipieri *et al*., 2002).

Application of paraquat at 0.45 soil inhibited the Fe^{2+} concentration after 14 days of submergence (Fig. 2). Whereas increasing the level of paraquat to 0.90 soil the Fe^{2+} concentration was less inhibited. Mn^{2+} concentration was inhibited significantly irrespective of the levels of paraquat application after 14 days of submergence. Both inhibiting and stimulating effect on dehydrogenase activities was observed in the submerged soil with paraquat irrespective of the application and the effect was not significant at 42 days of submergence. Application of pendimethalin at 0.45 soil and 0.90 soil had a significant inhibition effect on Fe and Mn reduction after 14 days of submergence (Fig. 3). No significant variation was noticed to the levels or concentrations of pendimethalin application. Dehydrogenase activity was initially stimulated followed by an inhibition and another stimulated at 42 days of submergence.

Soil reduction enhances the availability of Fe and Mn to the soil solution that facilitates Fe toxicity in highly reducing soil. So far as the Fe and Mn reduction under submergence is concerned, the effect of glyphosate in inhibiting soil reduction for over 42 days of incubation after submerging may be beneficial over paraquat and pendimethalin application in the efficient management of Fe and Mn nutrients to growing crops in soils studied.

Therefore, dehydrogenase activity reflects the metabolic activity of the soil (Salazar *et al*. 2011). As, soil enzymes are both extracellular and intracellular in nature the stimulation of dehydrogenase activity by glyphosate was probably due to release of more enzymes from the dead and inhibited anaerobic microorganisms. This result revealed an inversely relationship to Fe and Mn reduction and dehydrogenase activity under flooded soil to glyphosate application. However both inhibiting and/or stimulating impact of paraquat and pendimethalin application was noticed on soil dehydrogenase activity irrespective of its concentrations or levels. This revealed a non significant relationship to Fe and Mn reduction and soil dehydrogenase activity under submerged soil condition.

Although recent studies have demonstrated the importance of microbial Fe (III) and Mn (IV) reduction and have identified dehydrogenase activity which may serve as models for this metabolism, very little information about the biochemistry of this processes are available. However, a chain of chemical reaction is initiated upon flooding a soil leading to reduction of redox potential (Eh, mV). These reactions affect physical, chemical and biological processes that have significant implications for soil submergence and plant growth (Gambrell *et al*., 1991). Physical processes include restriction of atmospheric gas diffusion in the soil leading to depletion of soil oxygen and accumulation of carbon dioxide. Shortly after flooding, the limited supply of oxygen in soil pore spaces is depleted rapidly by roots, microorganisms, and soil reductants. This process leads to oxygen depletion and reduction in soil oxidation reduction potential (Eh) followed by a chain of soil chemical changes. Hence, further studies on the biochemistry and microbial ecology of Fe and Mn reduction and the dehydrogenase activity in response to glyphosate, paraquat and pendimethalin applications under soil submergence would enhance our understanding of the factors controlling the rate and extent of inhibition/stimulation of this important process.

Glyphosate at field application dose and double the field application dose inhibited the Fe and Mn reduction and stimulated the soil dehydrogenase activity. Whereas, both inhibition and stimulation effect on Fe & Mn reduction and soil dehydrogenase activity was resulted from the application of herbicides paraquat and pendimethalin at their field and double the field application dose. However, considering the interrelationship between soil-plant-water system prevailing in the natural field condition, there might be a significant difference in laboratory results and field results. From the result it is revealed that dehydrogenase activity is apparently not very much affected by the application of herbicides. Moreover, the inhibition in reduction of Fe and Mn under submergence can reduce Fe and Mn toxicity under acidic soil reaction. Therefore, for reaching a more scientific conclusion for the effect of herbicides on Fe and Mn reduction and soil dehydrogenase activity, further detail studies under field condition is needed.

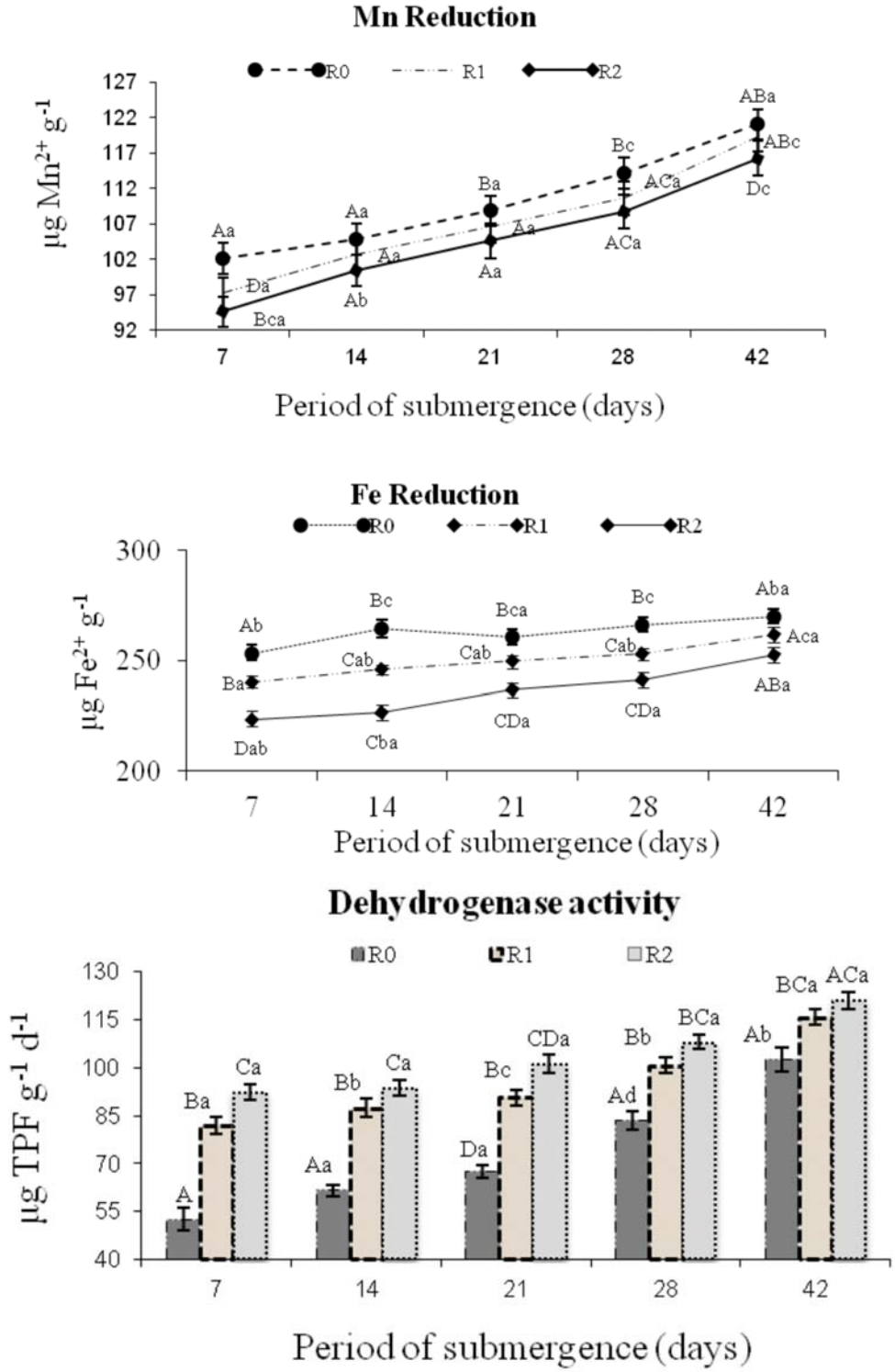


Fig. 1: Effect of glyphosate on Fe and Mn reduction and dehydrogenase activity in soil

T0: No pendimethalin; T1: pendimethalin at 0.90 $\mu g g^{-1}$; T2: pendimethalin at 1.80
(Means with same case letter are not significantly different in the treatments.)

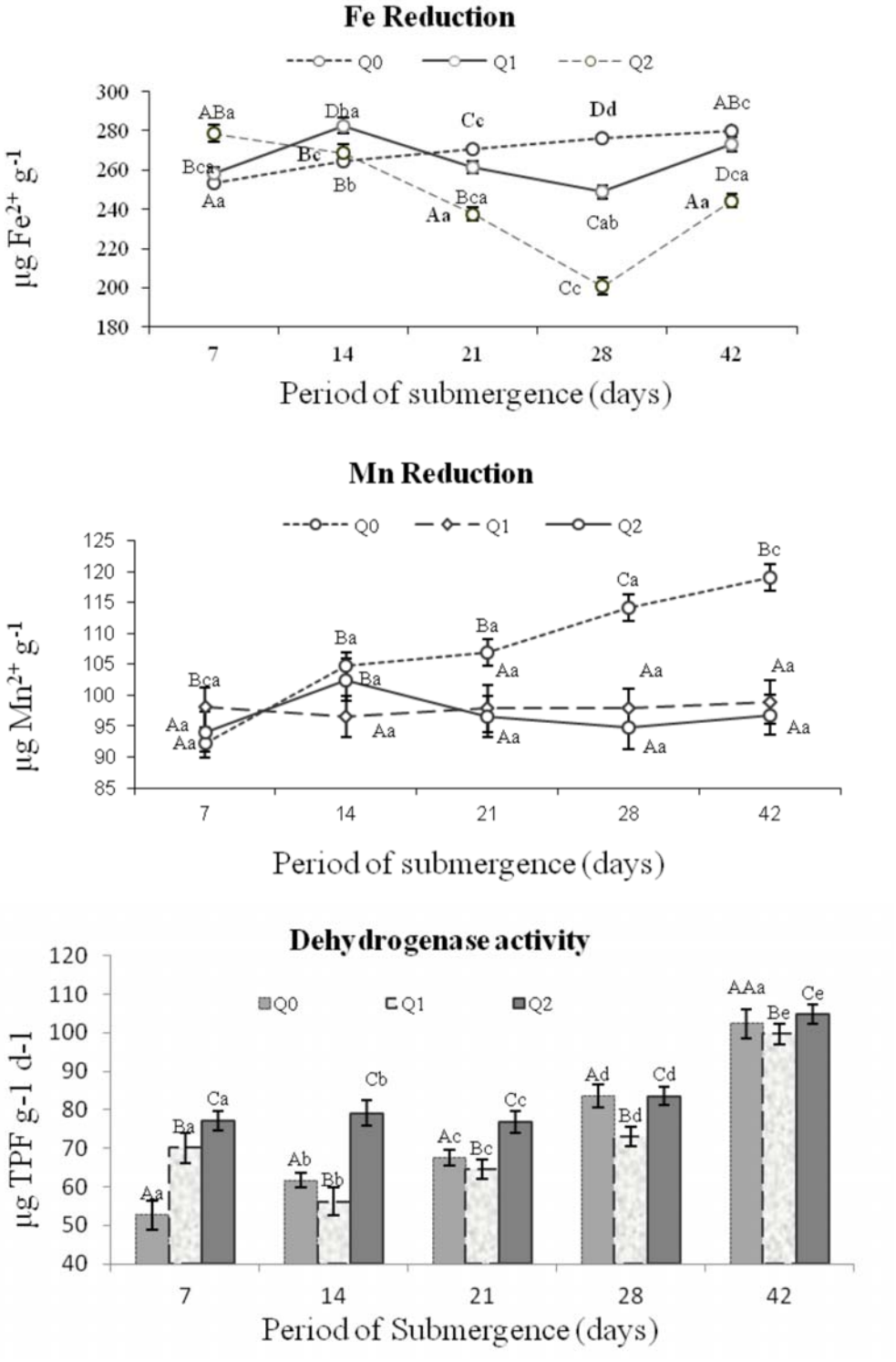


Fig. 2: Effect of Paraquat on Fe and Mn reduction and dehydrogenase activity in soil

Q0: No paraquat; Q1: paraquat at 0.90 $\mu g g^{-1}$*; Q2: paraquat at 1.80*
(Means with same case letter are not significantly different in the treatments.)

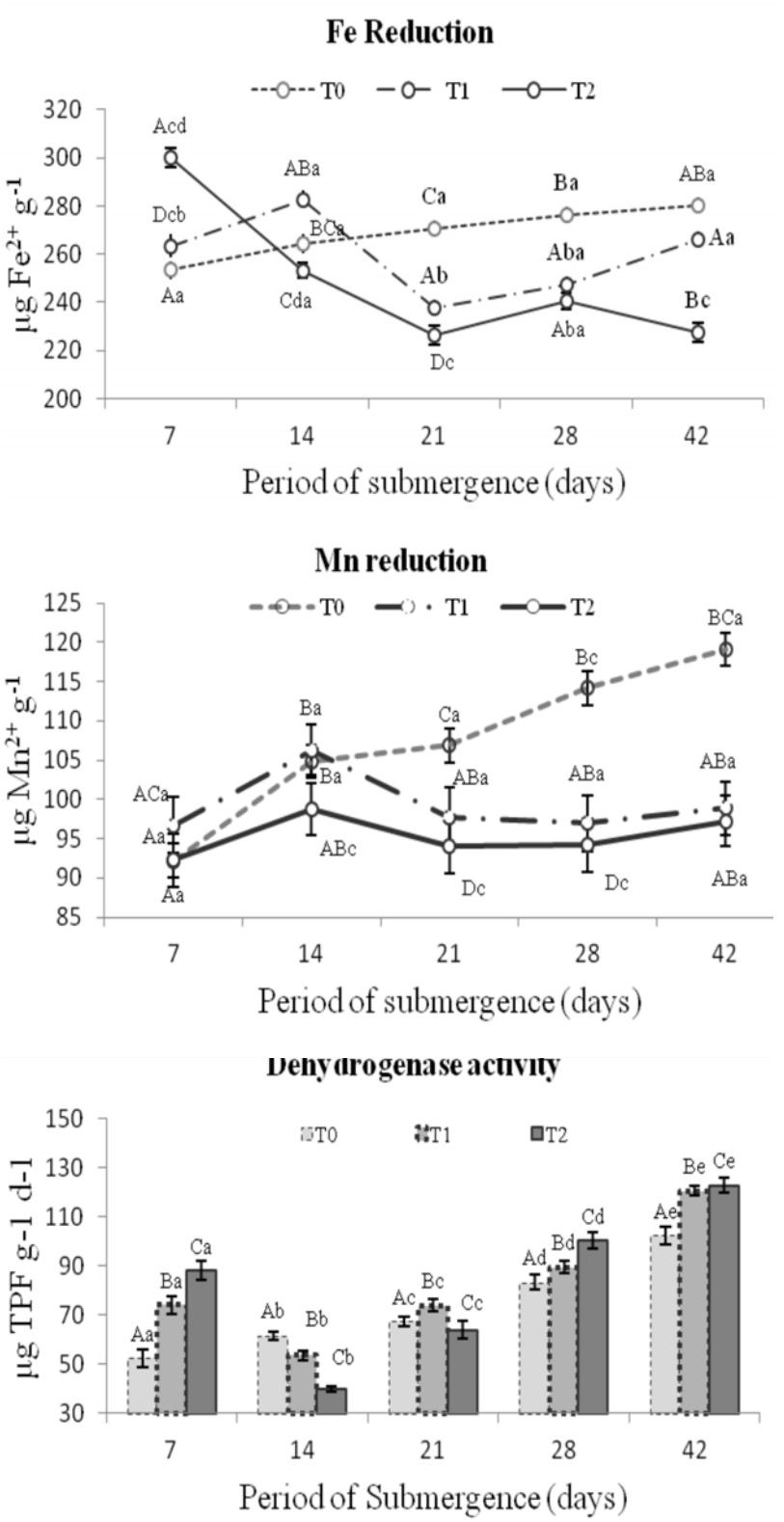


Fig. 3: Effect of Pendimethalin on Fe and Mn reduction and dehydrogenase activity in soil

T0: No pendimethalin; T1: pendimethalin at 0.90 $\mu g g^{-1}$*; T2: pendimethalin at 1.80*
(Means with same case letter are not significantly different in the treatments.)

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