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Microbial activity and plant nutrients transformation as influenced by herbicides application in soil

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ABSTRACT

Several environmental factors affect the soil ecology. Use of herbicides in rice has often resulted in contamination of the soil ecosystem, by direct or indirect action, after short, average or long period of time. To test this hypothesis, a laboratory study was conducted to assess the effect of herbicide glyphosate, paraquat and pendimethalin on reduction of plant nutrients (Fe, Mn), soil microbial biomass carbon and soil dehydrogenase activity under the submerged soil condition. Glyphosate at field application dose (0.90 μg g^{-1}) and double field application dose (1.80 μg g^{-1}) inhibited in reduction of Fe and Mn 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 reduction in plant nutrient (Fe, Mn), soil microbial biomass carbon (MBC) and soil dehydrogenase activity was resulted from application of herbicides paraquat and pendimethalin at their field (0.45 μg g^{-1}) and double field application dose (0.90 μg g^{-1}).

Key words: *Herbicides, Fe, Mn, dehydrogenase activity, MBC, rice, soil*

INTRODUCTION

Environmental factors like soil moisture, temperature, oxidation-reduction, pH, organic matter content and pesticide use have much impact on soil rhizopheric zone (Gu et al., 2009). Submerging a soil creates conditions markedly different from those of a well-drained soil. As long as oxygen is present in 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 the 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 and also originate 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. 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. Soil dehydrogenase activity is closely related to soil fertility properties such as nutrient status, pH, temperature and moisture. However, these activities sometimes inhibited and/or stimulated because of the presence or absence of inhibitors or activators like herbicide molecules. But few studies have demonstrated that application of herbicides influence biodynamic in soil like dynamics of iron and manganese reduction and microbial and enzymatic activities (Min et al., 2001) under the submerged soil condition. Pesticides in general and herbicide application in particular, has evident effect on the availability of soil macro as well as micro nutrients (Singh, 2014); ability of herbicides to chelate with soil minerals, reduces soil nutrient availability for uptake by plants

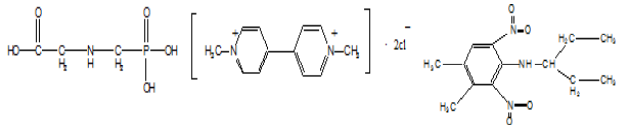
(Sebamio et al., 2012); affects soil microbial biomass carbon and carbon mineralization (Nayak et al., 2012, Kumar et al., 2012).

Soil appears to be a system biologically in equilibrium but this equilibrium is a precarious one and each disturbance in the soil environment because of regularly receive of a very wide range of chemicals *viz.,* pesticides application in agriculture, modifies the activity of the microflora and consequently soils fertility. Impact of these chemicals on soil system has become a matter of interest of contemporary research. Herbicides used in agricultural systems mainly controls the weeds and are applied to soil ecosystem. Once these group of chemicals come in contact with the soil it can alters the catalytic characteristics in soil environment substantially by interacting directly / indirectly with enzyme molecules and the reduction of iron (Fe) and manganese (Mn).

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

MATERIALS AND METHODS

The present laboratory incubation study 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 (0.90 µg g^{-1}) , 1.0 kg a.i./ha (0.45 µg g^{-1}) and 1.0 kg a.i./ha (0.45 µg g^{-1}), respectively.



N-(phosphonomethyl) glycine **(Glyphosate)**

1, 1-Dimethyl-4, 4-dipyridinium dichloride **(Paraquat)**

N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzamine **(Pendimethalin)**

An alluvial soil (typichaplusteps) was collected from Agricultural Research Farm, Banaras Hindu University, Varanasi, India. (25° 20' N and 83° 00' E, MSL-200 m) where rice was cultivated for the last 15 years. The soil was then air dried, grinded with a wooden mortar and pestle, sieved (2 mm) and stored in plastic containers. The physico-chemical propertiesof the processed soil samples were determined by the standard procedures (Kanwar and Chopra, 1988). Its properties were; pH 7.7, organic carbon (g/kg) 4.6, specific conductance 0.39 dS/m, exchangeable Fe (mg/kg) 6.54 and exchangeable Mn (mg/kg) 13.15.

To restore normal soil biological activity, air dried soil sample was pre-incubated after adjusting soil water content to 60 percent 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 field application dose. After 30 minutes, soil was homogenized for thorough mixing of the herbicide molecules. For stimulating submerged soil system in the laboratory for assessment of Fe and Mn reduction, 10g pre-incubated herbicides treated air dried processed soil was placed in test tubes (150mm × 20mm) and 12.5ml of distilled water was added to provide a standing water column of about 50 mm over the surface of the soil. The soil samples were then incubated at room temperature (25±3°C) for a period of 42 days.

At 7, 14, 21, 28 and 42 days after incubation under submerged condition, iron (Fe^{2+}) and soluble manganese (Mn^{2+}) in each of the three soil replicates were extracted by shaking the soils in each tube with 100 mL of 1.0 M sodium acetate (pH adjusted to 2.8) for 1.0 hr as described by Howler and Bouldin (1971). Soil suspension was filtered and the concentration of Fe^{2+} in filtrate was determined using o-phenanthroline described by Murti et al. (1966). For estimation of soluble Mn^{2+} in the filtrate, a suitable aliquot was taken and determined directly by Thermo elemental type SOLAAR S4 atomic absorption spectrometer (Page et al., 1982). Measurements were made using the hollow cathode lamps for Mn at the proper slit width and wavelength were adjusted and other AAS conditions employed in these determinations are summarized in table 2. 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 were generally carefully handled to avoid contamination. Glassware was properly cleaned, and reagents were of analytical grade. Double distilled deionized water was used throughout the study. Reagents 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 submerged herbicides treated soils, 0.2 mL of 3% triphenyltetrazollium 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, clan pink colour supernatant liquid was taken for estimation in spectrophotometer at 485 nm (blue filter) and activity of the enzyme was expressed as μg TPF g^{-1} soil day^{-1}. The spectrophotometric measurements were carried out using an Elico UV/visible double beam spectrophotometer SL-164 with 1 cm matched quartz cells. All chemicals were of analytical reagent grade. The double distilled deionized water was used to prepare all solutions. Freshly prepared solutions were always employed. Microbial biomass carbon (MBC) at different incubation stages were determined as described by Pelczar et al. (1978)

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

RESULTS AND DISCUSSION

Microbial functional diversity in soil is related both to rate of substrate utilization and to the presence and absence of utilization of specific substrate. Microbial Fe^{+3} reduction accounts for most of Fe^{+3} reduction in many anoxic soils and aquatic sediments. Non-enzymatic processes such as reduction of Fe^{+3} by organic compounds and sulphide are generally of minor significance. Mn^{+4} may reduced by nonenzymatic processes, but enzymatic Mn^{+4} reduction does predominate in some environment, *viz.,* waterlogged condition.

Effect of glyphosate at 0.90 μg g^{-1} (R1) and 1.80 μg g^{-1} (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 (Tu and Miles, 1976). But, the soil treated with herbicide glyphosate

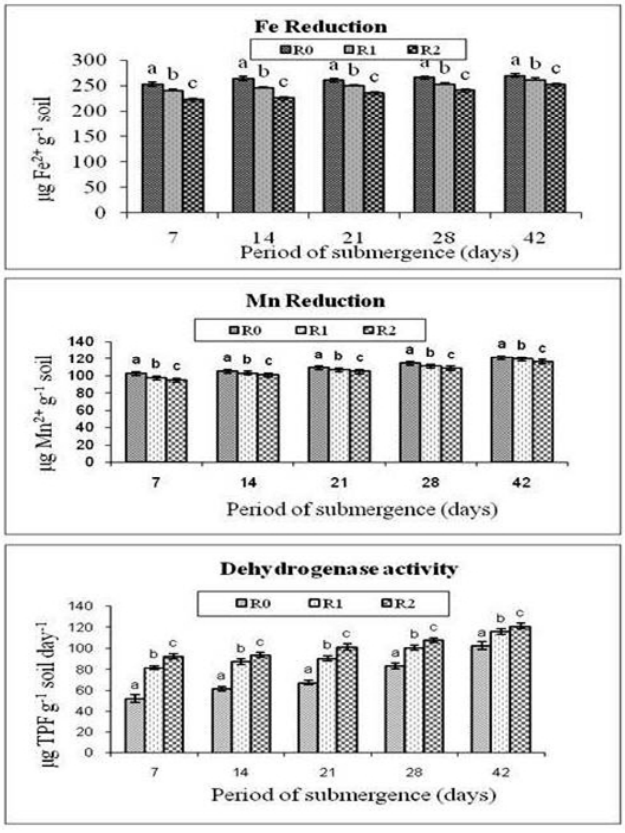


Fig 1. Effect of herbicide Glyphosate on Fe and Mn reduction and Dehydrogenase activity in soil. [R0: No glyphocate; R1: glyphosate @ 0.90 μg g^{-1}; R2: glyphosate @ 1.80 μg g^{-1}].

Table 1. Particulars of herbicides used.

Technical	Trade Name	Chemical name and formula	AI	Mode of action	LD 50[a](mg kg^{-1} mammals)	Field recommended dose	Sources
Glyphosate	No Weed	N-(phosphonomethyl) glycine $C_3H_8NO_5P$	WSC	Nonselective-systemic herbicides	5600mg/kg (Champaign,1994)	2.0 kg a.i./ha (0.90 mg g^{-1})(Gupta, 2010)	DhanukaAgrotech Ltd., Gurgaon, Haryana, India
Paraquat	Ozone	1, 1-Dimethyl-4, 4-dipyridinium dichloride $C_{12}H_{14}Cl_2N_2$	SL	Nonselective-contact herbicide	110 to 150 mg/kg (Stevens and Sumner, 1991)	1.0 kg a.i./ha (0.45 mg g^{-1}) (Gupta, 2010)	DhanukaAgrotech 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 (Champaign, 1994)	1.0 kg a.i./ha (0.45 mg g^{-1}) (Gupta, 2010)	DhanukaAgrotech Ltd., Gurgaon, Haryana, India

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).

retained 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 that herbicide glyphosate application retarded or inhibited the reduction of Fe^{3+} and Mn^{4+} to Fe^{2+} and Mn^{2+} under flooding situation. Submerging the soil without glyphosate application over a period of 42 days revealed a significant increase in Fe^{2+}, soluble Mn^{2+} and soil dehydrogenase activity (µg TPF formed g^{-1} soil day^{-1}), which might be due to reduction in redox potential (Ponnamperuma, 1972). Application of herbicide glyphosate at 0.90 µg g^{-1} soil significantly reduced or inhibited the Fe^{2+}, soluble Mn^{2+} concentration but stimulated the soil dehydrogenase activity (µg TPF formed g^{-1} soil day^{-1}). Interestingly, increasing level of herbicides to double (1.80 µg g^{-1}), rate of inhibition and stimulation was also varied significantly. So the extent of inhibition varied with the concentration of herbicide application. Inhibition of Fe and Mn reduction to herbicide glyphosate application may be because of inhibition of microorganisms participating in these reduction reactions that might be due to decline in redox potenatial (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 µg g^{-1} soil inhibited the Fe2+ concentration after 14 days of submergence (Fig. 2). Whereas increasing level of paraquat to 0.90 µg g^{-1} soil the Fe^{2+} concentration was less inhibited. But Mn^{2+} concentration was inhibited significantly irrespective of the levels of paraquat application after 14 days of submergence. However, both inhibiting and stimulating effect on dehydrogenase activities (µg TPF formed g^{-1} soil day^{-1}) was observed in submerged soil by the herbicide paraquat irrespective of the doses of application and effect was invisible at 42 days of submergence. Application of herbicide pendimethalin at 0.45 µg g^{-1} soil and 0.90 µg g^{-1} soil had a significant inhibition effect on Fe and Mn reduction after 14 days of submergence (Fig. 3). Soil reduction enhances availability of Fe and Mn to rice plant that facilitates Fe toxicity and Zn deficiency in highly reducing soil. So far as Fe and Mn reduction under submergence is concerned, effect of glyphosate in inhibiting soil reduction for over 42 days of incubation

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)	Fuel flow rate (L/min)	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

after submerging may be beneficial over paraquat and pendimethalin application in the efficient management of Fe and Mn nutrients to rice grown soils.

Many of the dehydrogenase enzymes are anaerobic in nature so, it is involved in the reduction of iron and manganese in the flooded soil. Interestingly in this experiment dehydrogenase activity (µg TPF formed g^{-1} soil day^{-1}) was stimulated initially followed by an inhibition and again stimulated at 42 days of submergence. Therefore, dehydrogenase activity reflects the metabolic activity of the soil (Salazar et al., 2011). As, soil enzymes are both extracellular and intracellular in nature (they are released from the living and dead microorganisms), the stimulation of dehydrogenase activity (in term of µg TPF formed g^{-1} soil day^{-1}) by herbicide glyphosate was probably due to release of more enzymes form dead and inhibited anaerobic microorganisms. This result revealed an

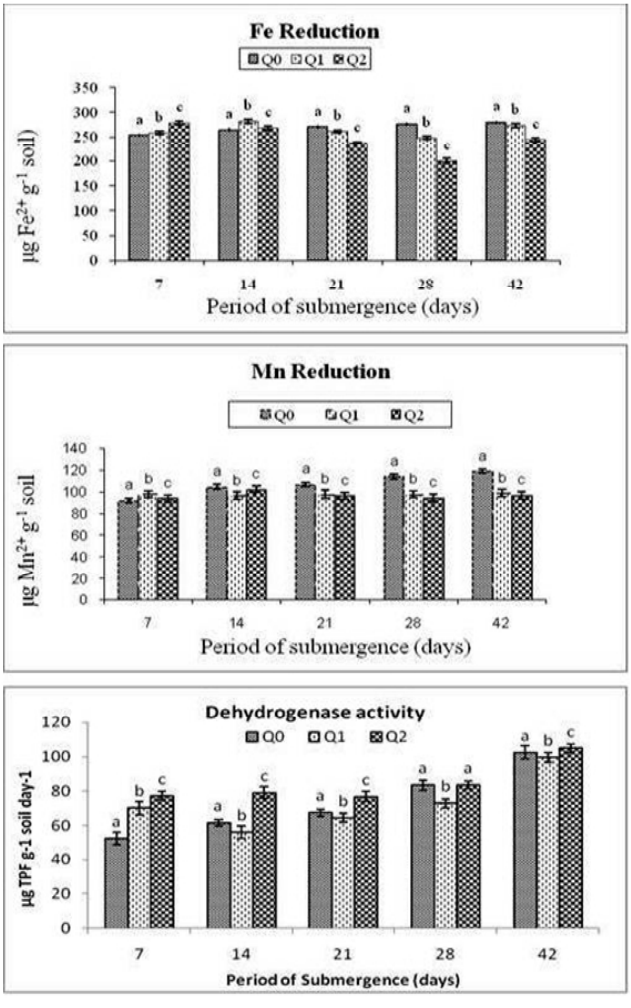


Fig. 2. Effect of Herbicide Paraquat on Fe and Mn reduction and Dehydrogenase activity in soil. [Q0: No paraquat; Q1: paraquat @ 0.90 µg g^{-1}; Q2: paraquat @ 1.80 µg g^{-1}].

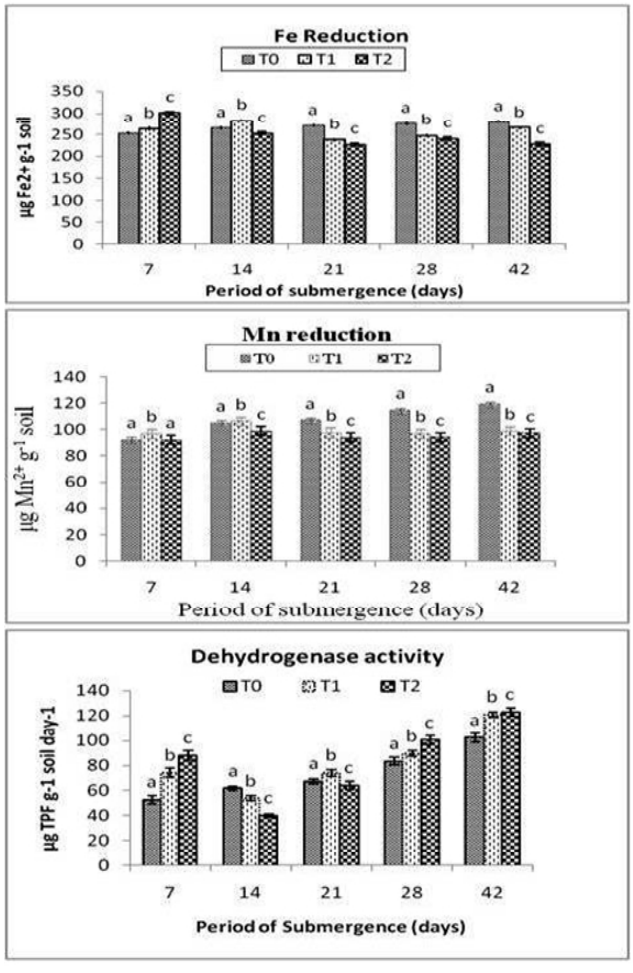


Fig. 3. Effect of herbicide Pendimethalin on Fe and Mn reduction and Dehydrogenase activity in soil. T0: No pendimethalin; T1: pendimethalin @ 0.90 µg g^{-1}; T2: pendimethalin @ 1.80 µg g^{-1}.

inversely relationship to Fe and Mn reduction and dehydrogenase activity under flooded soil to glyphosate application. Howeverboth inhibiting and/or stimulating impact of herbicide 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.

Influence of herbicides application on MBC was studied by incubating the rhizosphere soil samples collected from rice field within 24 hours of collection. Microbial biomass carbon content was stimulated upto 28 days of incubation where as at 42 days of incubation the impact was inhibited (Fig. 4). MBC content was varied between 121.1 to 192.45 mgg^{-1} in glyphosate treated soils. However the numerical value of MBC varies from 156.08 to 192.4 mgg^{-1} in paraquat and 152.59 to 192.4 mgg^{-1} in pendimethalin treated soils respectively. The highest stimulation was recorded at 28 days after incubation whereas lowest value was obtained at 42 days of incubation (Panda et al., 2017). Stimulation effect was more pronounced in paraquat and pendimethalin treated soil than glyphosate treated soil.

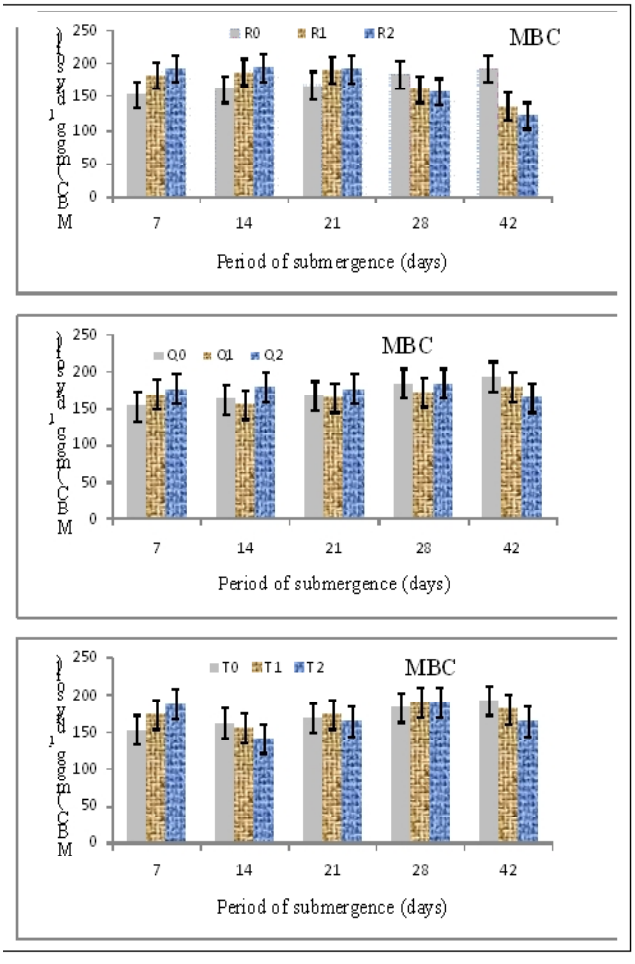


Fig. 4. Effect of Herbicide glyphosate, paraquat and Pendimethalin on microbial biomass carbon in soil. R = Glyphosate, Q = Paraquat and T = Pendimethalin.

Although recent studies have demonstrated importance of microbial Fe^{+3} and Mn^{+4} reduction, microbial biomass carbon and have identified dehydrogenase activity, which may serve as models for this metabolism, very little information about the biochemistry of this processes are available. It is not clear whether Fe & Mn reducing microorganisms those are available in pure culture representative of the important Fe and Mn reducers in soils. Further studies on biochemistry and microbial ecology of iron and manganese reduction and dehydrogenase activity in response to these herbicides glyphosate, paraquat and Pendimethalin applications 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 and Mn reduction, microbial biomass carbon 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 interrelationship between soil-plant-water system prevailing in natural field condition, there might be a significant difference in laboratory results and field results. Therefore, for reaching a more scientific conclusion for effect of herbicides on Fe and Mn reduction, microbial biomass carbon and soil dehydrogenase activity, further detail studies under field condition is needed.

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