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Metsulfuron-methyl Herbicide on Dehydrogenase and Acid Phosphatase Enzyme Activity on Three Different Soils

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Abstract

An experiment was conducted during the year 2012–13 to investigate the effects of metsulfuron-methyl herbicide on dehydrogenase and acid phosphatase activity on three diverse soils at two doses (4 and 6 g a.i. ha^{-1}). Different soils exhibited different rates of recovery of soil dehydrogenase and acid phosphatase activity in a period of 15 days after application. Initial dehydrogenase value 1.26, 1.17 and 1.11 reached to final 1.28, 1.14 and 1.03 at lower dose; initial dehydrogenase value 1.26, 1.17 and 1.11 reached to final 1.24, 1.09 and 0.97 at higher, respectively for entisol, mollisol and inceptisol. Similarly, Initial acid phosphate value 1.30, 1.28 and 1.31 reached to final 1.26, 1.28 and 1.26 at lower dose; initial acid phosphate value 1.30, 1.28 and 1.31 reached to final 1.26, 1.22 and 1.25 at higher, respectively for entisol, mollisol and inceptisol. In this study metsulfuron-methyl exerted an initial adverse effect on both enzyme activity but dehydrogenase activity restored in due course of time in each soil. To prevent adverse effects especially in Inceptisol a higher recommended dose of 6 g a.i. ha^{-1} should be avoided. Recovery was faster in all the three types of soil at the lower dose. Lower recommended dose of 4 g a.i. ha^{-1} of metsulfuron-methyl would not significantly affect Entisol and Mollisol while in Inceptisol the recovery process was slower and can be accelerated by the addition of organic matter to the soil. At a higher dose of 6 g a.i. ha^{-1}, the dehydrogenase activity recovery rate in all the soils types was slow.

Keywords: Enzymatic activity, entisol, inceptisol, mollisol, metsulfuron-methyl

1. Introduction

Pesticides undergo different dissipation processes in soil like microbial degradation, chemical hydrolysis, photolysis, volatilization, leaching and surface runoff (Das and Mukherjee, 2011). The contribution made by each of these dissipation processes towards the overall dissipation depends upon the nature of soil, pesticides and other environmental factor (Das and Mukherjee, 2012). Hence, it is important to avoid serious injury to the soil microflora, whose functions are vital in maintaining the soil fertility (Das and Mukherjee, 2014). Metsulfuron-methyl is a residual sulfonylurea herbicide used as a selective pre and post emergence for control of broadleaf weeds and some annual grasses. It is a systemic compound with foliar and soil activity and works rapidly after it is taken up by the plant. Its mode of action is by inhibiting cell division in shoots and roots of the plant, and it is biologically active at low dose. Soil dehydrogenase activity varies significantly with type and concentration of pesticides[1]. Inhibition of dehydrogenase by quinalphos and stimulation of dehydrogenase by chlorpyrifos has been reported by (Menon et al., 2005; Klose et al., 2006) reported that soil fumigation reduced dehydrogenase by 35% over a period of 90 days. Metalaxyl application initially increased and then decreased dehydrogenase in fungicide treated soil (Sukul, 2006). Singh and Kumar (2008) revealed that acetamiprid increased dehydrogenase upto 22% after the first application. Soils with different physicochemical properties are likely to have different soil microbial populations in terms of their activity, number and composition. Hence, each soil type will respond differently to the addition of any potentially toxic compound. Any factor that affects soil microbial population dynamics will alter soil enzyme activity (Das and Mukherjee, 2012).

Among soil enzymatic activities, dehydrogenase and phosphatase are the most commonly measured activities in soils and therefore proposed by many authors as potential indicators of soil state (Floch et al., 2011). There is also evidence that soil enzymes may provide valuable general information on transformation of pesticides in soils (Hussain et al., 2009). The majority of herbicides are either neutral toward this activity or they inhibit it (Jastrzebska, 2011). Herbicides, except butachlor (Xia et al., 2011), have a repressive effect on dehydrogenase activity, whatever conditions of application, including dose and soil pH (Sebiomo et al., 2012).

Though several studies have documented the effects of various herbicides application on soil microbial and enzyme activities (Goyal et al., 1993; Dinesh et al., 2000) no information is available on the role of metsulfuron-methyl herbicides soil enzyme activity under the conditions of North-western Indian Himalayas. Hence, in this study three physico-chemically different soils were used to assess the effect of metsulfuron-methyl herbicides at two different doses used in agriculture by conducting dehydrogenase and acid phosphatase activity test during 2013–2013 at ICAR-VPKAS, Almora, Uttarakhand.

2. Materials and Methods

The soils used in this experiment were entisol, mollisol and inceptisol order varying in physico-chemical properties. The experiment was conducted during the year 2012–13 at the experimental farm of Vivekananda Institute of Hill Agriculture, located in the Indian Himalayan region at Hawalbagh (29°36'N and 79°40'E with 1250 m amsl), in the state of Uttarakhand, India. Commercial grade metsulfuron-methyl was used in this experiment at two different application rates (4 and 6 g a.i. ha^{-1}). Soil samples were collected in polythene bags, air dried in shade, ground, sieved through a 2 mm sieve and stored at room temperature. The soils belonged to Entisol, Mollisol and Inceptisol. All the soils belonging to three different orders were maintained at field capacity and incubated at 30±1 °C for a period of 15 days for revival of inherent soil microbial activity. Commercial formulation of the herbicide metsulfuron-methyl 20% WP was added to each soil at 4 and 6 g a.i. ha^{-1}. Different physico-chemical properties were analysed with standard method (Jackson, 1967). Organic carbon measured by using the Walkley and Black method (1965), clay, sand and silt measured by employing the Bouyoucos hygrometer (Bouyoucos and Cook, 1967). The electrical conductivity was measured using conductivity meter. Soil pH (1:2.5), CEC [by 1 M NH_4OAc (pH 7) extraction], Olsen P (by 0.5 M $NaHCO_3$ extraction), sulfate [by 0.04 M $Ca(H_2PO_4)_2$ extraction], were measured according to Black (1965). Acid oxalate-extractable Fe and aluminum (Al), and exchangeable potassium (K), calcium (Ca), magnesium (Mg), and sodium (Na) were determined for the initial soil samples by the standard methods of Blakemore et al. (1987). The properties of the collected soils from three different orders are given in Table 1. The lower dose (4 g a.i.) and higher dose (6 g a.i.) has been mentioned as LD and HD.

Soil was fortified at two level viz., 4 g a.i. and 6 g a.i. with the herbicide having three replications for each level of fortification. 10 g of soil was transferred in beakers and period of exposure was 15 days. Beakers in triplicate were withdrawn at 0, 1, 3, 5, 7, 10 and 15 days interval along with control sample. Soil dehydrogenase activity in triplicates of each treatment was assayed by the Klein et al. (1971), reduction method using 2, 3, 5-Triphenyl Tetrazolium Chloride as reducing agent. From the 10 g of soil from each beaker 5 g of soil sample were mixed with 50 mg of $CaCO_3$ and 1ml of 3% (w/v) 2, 3, 5-Triphenyltetrazolium Chloride (TTC) and again incubated for 24 h at 37±1 °C. Dehydrogenase enzyme converts TTC to 2, 3, 5-Triphenyl Formazan (TPF). The TPF formed was extracted with acetone (3×15 ml), the extracts were filtered through Whatman Number 1 and absorption was measured at 487 nm with double beam spectrophotometer. Effect of metsulfuron-methyl on dehydrogenase activity was calculated relative to the control. The data obtained from this experiment were subjected to statistical analysis using SAS 9.3 version. For calculating two-way ANOVA, three different soils types and incubation time was taken as the factors. Acid phosphatase activity was assayed using 1 g soil (wet equivalent), 4 ml of 0.1 M modified universal buffer (pH 6.5), and 1 ml of 25 mM p-nitrophenyl phosphate. After incubation for 1 h at 37±1 °C the enzyme reaction was stopped by adding 4 ml of 0.5 M NaOH and 1 ml of 0.5 M $CaCl_2$ to prevent dispersion of humic substances. After centrifugation at 4000 rpm for 10 min, the absorbance was measured in the supernatant at 400 nm; acid phosphatase activity was expressed as μg p-nitrophenol released g^{-1} soil h^{-1}.

Table 1: Physico-chemical properties of three different soil types

Physico-chemical properties	Soil order		
	Entisol	Mollisol	Inceptisol
pH	6.4	6.9	6.7
Sand, 0.02–2 mm (%)	65.1	58.3	61.7
Silt, 0.002–0.2 mm (%)	15.6	14.7	16.9
Clay, <0.002 mm (%)	19.3	27.0	21.4
Organic carbon (%)	0.63	0.69	0.64
CEC (meq 100 g^{-1})	11.13	10.94	11.67
Surface area (m^2 g^{-1})	51.3	45.1	48.67
EC (mS m^{-1})	0.29	0.31	0.33
Specific gravity	2.11	2.15	2.08
Olsen P (mg kg^{-1})	9.38	9.16	10.48
SO_4 (mg kg^{-1})	11.16	12.37	11.68
Acid oxalate Al%	0.21%	0.22%	0.26
Acid oxalate Fe%	0.03%	0.06%	0.05
K (cmol Kg^{-1})	1.85	1.82	1.73
Ca (cmol Kg^{-1})	3.49	4.45	3.94
Mg (cmol Kg^{-1})	1.23	1.21	1.27
Na (cmol Kg^{-1})	0.11	0.19	0.17

3. Results and Discussion

Effect of lower and higher dose of metsulfuron-methyl herbicide in three different soil types on their dehydrogenase activity has been presented in Table 2. Results of this experiment indicated that there was an adverse effect of metsulfuron-methyl herbicide on dehydrogenase activity initially at both the dose. With the increase in incubation period there was change in recovery of dehydrogenase activity in all the soils and the dehydrogenase activity recovery rates varied in all the three different soil types. Slower recovery at the higher recommended dose of the herbicide was observed. The herbicide does not have the same impact on the dehydrogenase activity for the three soil types and dehydrogenase activity depends on the incubation period as the p value for factor A (soil) is<0.0001. At a lower recommended dose of 4 g a.i. ha^{-1} the recovery rates in all the soils types was variable. In all the soils, a decrease in dehydrogenase activity was observed till the 15th d of the incubation period at higher dose. But, for entisol dehydrogen activity increased till the 15th day only at lower dose. The initial (0 day) value 1.26, 1.17 and 1.11 reached to final 1.25, 1.14 and 1.03 at lower dose on 15th day for entisol, mollisol and inceptisol, respectively. In Entisol, dehydrogenase activity decreased till 5th day (1.17) and thereafter increased upto 15th days (1.25). For mollisol dehydrogenase activity increased upto 3rd day (1.23) and there after decreased. Again, for inceptisol dehydrogenase activity decreased upto 1st day (1.08), then increased upto 5th day (1.14) and thereby decreased upto 15 day (1.03). A better response of the activity to the lower dose was observed in all the soils. This indicating that dose of the herbicides may changes the toxicity in the soils. Higher dose adversely affect soil dehydrogenase activity more as compare to lower dose. Besides soil varying soil order also contributed wide difference in dehydrogenase activity which may be due to difference in physico-chemicals properties of the soil order. Maarit Niemi et al. (2009) showed that stimulation or decreased depended on the enzyme, the herbicide, its concentration and duration of the exposure. Also, Dutta et al. (2010) studied the effects of chlorpyrifos on the Fluorescein Diacetate Hydrolysing Activity (FDA) and showed that this enzyme activity was not affected by chlorpyrifos at field rate, but at higher dosage significantly decreased was observed.

Table 2: Effect of lower and higher dose metsulfuron-methyl in three different soil types on their dehydrogenase activity (in logarithm of soil dehydrogenase activity)

Incubation time (Days)	Soil order					
	Entisol		Mollisol		Inceptisol	
	LD	HD	LD	HD	LD	HD
0	1.26±0.01	1.26±0.02	1.17±0.04	1.17±0.01	1.11±0.03	1.11±0.02
1	1.23±0.03	1.25±0.01	1.19±0.01	1.18±0.03	1.08±0.02	1.13±0.01
3	1.19±0.01	1.19±0.01	1.23±0.02	1.19±0.03	1.13±0.02	1.09±0.01
5	1.17±0.02	1.21±0.02	1.19±0.02	1.25±0.02	1.14±0.03	1.03±0.04
7	1.25±0.04	1.22±0.03	1.16±0.03	1.12±0.01	1.06±0.01	1.01±0.04
10	1.27±0.02	1.23±0.01	1.15±0.01	1.11±0.02	1.05±0.02	0.99±0.03
15	1.25±0.03	1.24±0.04	1.14±0.02	1.09±0.01	1.03±0.04	0.97±0.04

For lower dose (4 g a.i.)	df	F	p-level
Factor A (Soil)	2	1009	<0.0001
Factor B (Incubation period)	5	45	<0.0001
Interaction (A×B)	10	37	<0.0001

For higher dose (6 g a.i.)	df	F	p-level
Factor A (Soil)	2	1591	<0.0001
Factor B (Incubation period)	5	127	<0.0001
Interaction (A×B)	10	59	<0.0001

*LD=Lower dose (4 g a.i.); HD=Higher dose (6 g a.i.); data are presented as mean±SD of three replicates

At a higher dose of 6 g a.i. ha^{-1}, the dehydrogenase activity recovery rate in all the soils types was slow. The initial (0 day) value 1.26, 1.17 and 1.11 reached to final 1.24, 1.09 and 0.97 at higher dose on 15th day for entisol, mollisol and inceptisol, respectively. Likewise, in a study conducted by Fang et al. (2009) an increase in the inhibitory effect of chlorpyrifos on soil microbes with increasing pesticide concentrations was observed. In Entisol dehydrogenase activity decreased upto 3rd day (1.19) and thereafter increased upto 15th day (1.24). In Mollisol dehydrogenase activity increased upto 5th days (1.25) and thereafter decreased upto 15th day (1.09). But in Inceptisol slightly different trends was observed. In Inceptisol, dehydrogenase activity increased at 1st day (1.13) and then decreased slowly upto 15th days (0.97). At higher dose the pattern of changes of dehydrogen activity with time changes significantly and this is only due to varying in physicochemical properties of different soil. Similar report has been suggested by Mukherjee et al. (2016) in case of flubendiamide.

In case of acid phosphatase, initial acid phosphate value 1.30, 1.28 and 1.31 reached to final 1.26, 1.28 and 1.26 at lower dose respectively for entisol, mollisol and inceptisol. In all the three soil type, a decrease in acid phosphatase activity was observed till the 15th d of the incubation period. But for mollisol at lower dose there was no change in acid phosphatase activity. Slower recovery at the higher dose of the herbicide was observed. At lower dose (4 g a.i. ha^{-1}) the recovery rates in all the soils types was also variable. In entisol acid phosphatase activity increased upto 3rd day (1.32) and there after decrease upto 15th day (1.26). But, in case of mollisol acid phosphatase activity decreased up to 5th day (1.24) and thereafter increased up to 15th day (1.28). For inceptisol acid phosphatase activity increased up to 3rd day (1.31) and there after decreased continuously up to 15th day. Similar trend has also been observed by Das et al. (2015).

Initial acid phosphate value 1.30, 1.28 and 1.31 reached to final 1.26, 1.22 and 1.25 at higher dose, respectively for entisol, mollisol and inceptisol. At higher dose (6 g a.i. ha^{-1}), for entisol, acid phosphatase activity increased up to 3rd day (1.35) and thereafter decreased up to 15th day (1.26). But, for mollisol acid phosphatase activity decreased up to 3rd day (1.25), then on 5th (1.26) day increased and again decreased from 7th day (1.24) to 15th day (1.22). Similarly for inceptisol acid phosphatase activity decrease up to 3rd day (1.30) then increase in 5th day (1.31) and again decrease from 5th day (1.31) up to 15th day (1.25). Similar observations were also made by Pandey and Singh (2004); Singh and Singh (2005a, b); Tejada et al. (2011) who reported that dehydrogenase activities were inhibited by insecticides. Inhibition of dehydrogenase activity by chlorpyrifos and quinalphos was also reported by Menon et al. (2005). In the other hand some studies showed dehydrogenase activity increasing after pesticides application (Fragoeiro and Magan, 2008; Singh and Kumar, 2008) (Table 3).

This study shows that significant difference in enzymatic activity in three different soil order i.e. entisol, mollisol and inceptisol is due to variation in physico-chemical properties

Table 3: Effect of lower and higher dose metsulfuron-methyl in three different soil types on their acid phosphatase activity (in logarithm of soil phosphatase activity)

Incubation time (Days)	Soil order					
	Entisol		Mollisol		Inceptisol	
	LD	HD	LD	HD	LD	HD
0	1.30±0.02	1.30±0.01	1.28±0.02	1.28±0.03	1.31±0.03	1.31±0.03
1	1.31±0.01	1.32±0.03	1.27±0.03	1.26±0.01	1.30±0.02	1.30±0.01
3	1.32±0.01	1.35±0.03	1.25±0.02	1.25±0.01	1.31±0.01	1.30±0.02
5	1.29±0.02	1.31±0.03	1.24±0.01	1.26±0.02	1.29±0.04	1.31±0.03
7	1.27±0.3	1.28±0.02	1.26±0.03	1.24±0.02	1.27±0.01	1.27±0.04
10	1.26±0.4	1.28±0.01	1.27±0.04	1.23±0.03	1.28±0.03	1.26±0.02
15	1.26±0.2	1.26±0.02	1.28±0.03	1.22±0.01	1.26±0.02	1.25±0.04

For lower dose (4 g a.i.)	df	F	*p*-level
Factor A (Soil)	2	1246	<0.0001
Factor B (Incubation period)	5	61	<0.0001
Interaction (A×B)	10	37	<0.0001

For higher dose (6 g a.i.)	df	F	*p*-level
Factor A (Soil)	2	1579	<0.0001
Factor B (Incubation period)	5	76	<0.0001
Interaction (A×B)	10	43	<0.0001

*LD=Lower dose (4 g a.i.); HD=Higher dose (6 g a.i.); data are presented as mean±SD of three replicates as well as the differences in potential microbes which plays a significant role.

4. Conclusion

The herbicide metsulfuron methyl exhibits different behavior under different soil type due to varying in physicochemical properties of the soil order. These leads to changes in both the enzyme *viz.*, dehydrogenase and phosphatase activity in all the soil type. At lower dose enzymes were less affected than higher dose due to toxic effect at higher dose. The herbicide affected more for both the enzyme in inceptisol than mollisol and entisol at both doses.

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