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Response of Soil Microbial Communities to the Selective Herbicides: A Microcosm Approach

Aabid Hussain Lone*, K.P. Raverkar, Navneet Pareek and Ramesh Chandra

Department of Soil Science, G.B. Pant University of Agriculture and Technology, Pantnagar U. S. Nagar Uttarakhand - 263 145, India.

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The impact of six different herbicides representing several chemical families on soil microbial community was investigated using laboratory microcosm approach. The herbicides consisted of isoproturon, metribuzin, clodinafop propargyl, atlantis (mesosulfuron methyl + idosulfuron methyl sodium) and sulfosulfuron applied at normal agricultural rates. The sixth herbicide namely UPH-110 (Clodinafop propargyl 12% + Metribuzin 42% WG) was tested at four different application rates. The impact was assessed on various soil biological health indicators viz, microbial biomass carbon, soil respiration, dehydrogenase and phosphatase activities. The response of microbial community was mixed. Overall among the various methodological approaches adopted, dehydrogenase activity was most sensitive and microbial biomass carbon the least under the existing conditions. Interestingly, the sensitivity of acid phosphatase towards the applied herbicides was more than alkaline phosphatase. Influence whether positive or negative was however only transitory except UPH-110 @ 1000 g ha^{-1} in case of soil respiration. The influence of UPH-110 was dose dependent. Significant toxic impact was mostly observed at higher concentrations (600 and 1000 g ha^{-1}). The magnitude of hazard and duration of toxicity increased as the dose increased.

Key words*:* Herbicide, Microbial biomass carbon, Microbial activity, Phosphatase activity, Soil Respiration, Dehydrogenase activity.

Soil is the essence of life. The increasing awareness towards soil shows that it is critically important component of the earth's biosphere, functioning not only in the production of food and fibre but also in the ecosystem functioning and maintenance of local, regional and global environmental quality[1] (Glanz, 1995). Growing concern for preserving soil as a natural resource has prompted researchers over the past years to focus on studying the changes occurring in soil or those that may occur in future under certain harmful environmental factors, pesticides being an important one. Positive, negative or neutral effects of pesticides on soil microbiological properties have all been observed. Pesticides have adverse impact upon soil microbial population[2] (Khalid *et al*., 2001), microbial diversity and activities[3] (Wang *et al*., 2006). The change in the soil microflora has been listed as one of the possible causes of productivity decline[4] (Reichardt *et al.*, 1998). The applied pesticides also reduce soil enzymatic activities that act as a ''biological index'' of soil fertility and biological processes in the soil environment[5] (Antonious, 2003) and biological nitrogen fixation in legumes. On the other hand, it has also been noted that certain soil microbes are capable of causing degradation of pesticides.

* To whom all correspondence should be addressed.
E-mail: aabidlone08@gmail.com

Pesticidal residues are generally degraded and degradation products are assimilated by soil microorganisms[6] (Bhuyan *et al*., 1993) resulting in increased population sizes and activities of microorganisms which in turn influences the transformations of plant nutrient elements in soil[7] (Jana *et al*., 1998).

Side-effects of herbicides on soil microbial populations can be studied on a short or a long-term basis. However, according to Haney, *et al*. (2000)[8] experiments conducted on a short-term basis may provide a more realistic evaluation of the effect of herbicides on soil microorganisms. Most studies about herbicide effects on soil microbes have focussed on one or two herbicides at a time[9,10] (Olivier *et al*., 2010; Valiolahpor *et al*., 2011). While results of these studies indicate that herbicides applied at recommended rates generally do not have significant effects on soil microorganisms, however evaluating only a few herbicides at a time limits comparison amongst herbicides on their relative effects on soil microbial ecology. The objective of present study was to evaluate under laboratory microcosm conditions, the response of soil microbial communities towards six different herbicides, namely isoproturon, metribuzin, clodinafop propargyl, atlantis, sulfosulfuron and UPH-110 representing several chemical families, modes of action and different soil residual properties. Various parameters used to evaluate microbial response were: microbial biomass carbon (MBC), soil respiration, dehydrogenase activity and phosphatase activity.

MATERIALS AND METHODS

The experiment was laid out in Completely Randomised Design (CRD) with three replications. The treatment details, methods, procedures and techniques adopted during the course of investigation are as follows:

Treatment details

A total of 10 treatments were examined in the present study. The first five treatments were applied at normal agricultural rate while UPH-110 was tested at four different concentrations (Table 1).

Microbiological and biochemical assays

Microbial biomass carbon was determined using fumigation extraction method[11] (Jenkinson, 1988). The total phosphatase activity (acid and alkaline phosphatses) was determined by the method given by Tabatabai and Bremner (1969)[12]. *P*- nitrophenol phosphate tetrahydrate solutions of pH 6.5 and pH 11 were used for the assay of acid and alkaline phosphatase, respectively. Dehydrogenase activity was determined as per the method given by Casida *et al*. (1964)[13]. Soil respiration was measured using the method proposed by Anderson (1982)[14]. CO_2-evolution was estimated at 3rd, 7th, 15th, 30th and 60th day during the 60-day incubation period. Respiration rate was expressed as the average daily rate (mg CO_2 kg^{-1} of air-dried soil day^{-1}).

Soil sampling and processing

The soil used for experiment was procured from Norman E. Borlaug Crop Research Centre of G.B. Pant University of Agriculture and Technology, Pantnagar from 0-15cm layer of a field that had received no pesticides in the recent past. The soil was sandy loam in texture, neutral in pH, high in organic carbon, medium in N and K and low in P. It was thoroughly homogenised and passed through 2 mm sieve. Microcosms were prepared with 130 g soil samples (oven dry weight basis) placed in sterile conical flasks of 500 ml capacity. In order to adjust the moisture content to field capacity, the water holding capacity (WHC) of soil was determined and moisture content was adjusted accordingly using sterile ultrapure water. Three replications were maintained for each treatment. Soil samples were stabilised by keeping in dark for one week before exposing them to the treatments. The conditioning period of one week allowed the soil to establish a steady-state level of microbial activity. Subsequently the samples were treated with herbicides as per treatment details. The conversion of the field rate application to 'µg' of xenobiotic compound per kg of soil was done, assuming the even distribution of herbicides in 0–15 cm layer and a bulk density of about 1.5 Mg m^{-3}. Herbicides were added to the soil as water solutions and moisture content of the soil was adjusted to field capacity (40% WHC). Control flasks received sterile water only. The mouth of flasks was loosely capped with the help of rubber corks to avoid excessive accumulation of CO_2 in the head space. The flasks were periodically weighed and compensation for any sort of moisture loss was made as and when required. All the flasks

were incubated at 28±2°C in dark. The sets of samples were collected for analysis at 1st, 3rd, 7th, 15th, 30th, 45th and 60th day after the herbicide application and stored at 4°C in deep freezer until analysis.

Statistical analysis

Statistical analysis was performed using STPR-2 software. Data was analyzed using a one way analysis of variance (ANOVA). Values were considered to be significantly different at a 95% confidence level ($P \leq 0.05$).

RESULTS

Microbial Biomass Carbon

The sensitivity of microbial biomass carbon (MBC) to the applied herbicides was low (Table 2). At doses corresponding to normal agricultural rates (FR), a statistically significant increase in the biomass synthesis was witnessed only in case of clodinafap and for the remaining four treatments; the alterations brought about were statistically non-significant. With regard to UPH-110, the two higher concentrations (T_8 and T_9) showed a significant inhibition in MBC synthesis at different time periods. For T_8, the depression was noticeable at 3rd and 7th day and for T_9, the significant inhibitory effect prevailed for one month. The magnitude of inhibition escalated with increase in concentration.

Phosphatase activity

None of the herbicides except clodinafop had a negative impact on alkaline phosphatase activity (Table 3). At FR, the influence of isoproturon and two sulfonylurea herbicides was insignificant throughout, but metribuzin and clodinafop showed a significant stimulatory and inhibitory impact respectively. The effects however in both the cases were transitory and lasted only for initial 3 and 7 days respectively in case of clodinafop and metribuzin. Regarding UPH-110, the influence of two lower concentrations (T_6 and T_7) was statistically non significant but the higher concentrations (T_8 and T_9) markedly activated the alkaline phosphatase activity. The stimulated enzyme activity was significant however only up to 15th day in both the cases. Highest activity at all the initial four stages was found in T_9 treated microcosms.

The response trend towards the applied herbicide followed by acid phosphatase was more or less opposite to that of alkaline phosphatase (Table 3). Moreover under the existing conditions, it was found to be more sensitive to herbicides as significant changes were observed in case of T_1 and T_7 unlike alkaline phosphatase. At FR; isoproturon and clodinafop stimulated the activity up to 7th while metribuzin inhibited it up to 15th day.

Table 1. Details of the treatments employed in present investigation

Code	Treatments	Application rates		Chemical family of herbicide
		µg kg^{-1} oven dry soil	Equivalent dose (g ha^{-1})	
T_1	Isoproturon 75% WP	595	1333	Phenylureas
T_2	Metribuzin 70%WP	134	300	Triazines
T_3	Clodinofop propargyl 15%WP	179	400	Aryloxyphen-oxypropionates
T_4	Atlantis(MesosulfuronMethyl 3% + Idosulfuron Methyl Sodium 0.6%WG).	179	400	Sulfonylureas
T_5	Sulfosulfuron 75%W	15	33.33	Sulfonylureas
T_6	UPH-110 (Clodinafop propargyl 12% + Metribuzin 42% WG))	179	400	Aryloxyphen-oxypropionates & Triazines
T_7	UPH-110	223	500	-do-
T_8	UPH-110	268	600	-do-
T_9	UPH-110	446	1000	-do-
T_{10}	Control	-	-	-

The effect of two sulfonylurea herbicides was however statistically neutral. In case of UPH-110, with the exception of T_6, a significant reduction was observed for the remaining three doses at one stage or the other. The reduction in case of T_7 was notable only at day 3. For T_8 and T_9, the duration of inhibitory effect was 7 and 15 days respectively. The highest reduction was invariably recorded for T_9.

Microbial activity

Microbial activity was measured in terms of dehydrogenase activity and soil respiration. Variable effects on dehydrogenase activity were observed at FR (Table 4a). A marked decrease in the activity over varying periods was noticed due to isoproturon (7 days), metribuzin (15 days) and atlantis (3 days). Clodinafop stimulated the dehydrogenase activity up to one week. The impact of sulfosulfuron was non-significant throughout. UPH-110 at lowest concentration (T_6) was non-significant but the higher concentrations displayed a significant inhibitory effect. The influence lasted up to 7th, 15th and 30th day respectively for T_7, T_8, T_9. The highest reduction was registered at the concentration of 1000 g ha^{-1} (T_9) of UPH-110.

The effect of herbicides on respiration is depicted in Table 4 (b). Sulfonylurea herbicides didn't affect the evolution of CO_2 significantly. On the contrary it was significantly bolstered during the first week by clodinafop. A significant decline in CO_2 flush was noticed at 3rd and 7th day in the microcosms having received isoproturon and metribuzin. Throughout the study UPH-110 at two lower rates (T_6 and T_7) didn't show any significant influence. However, T_8 (up to 7th day) and T_9 (throughout the study) significantly lowered the microbial activity. Even at the final day (60th day), the rate of CO_2 production was significantly lesser

Table 2. Microbial biomass carbon (µg g^{-1} soil) and total population (×10^7cfu g^{-1} soil) as influenced due to various herbicides at different

Code	Treatment details	Periods						
		Days of sampling						
		1st (S1)	3rd (S2)	7th (S3)	15th (S4)	30th (S5)	45th (S6)	60th (S7)
		Microbial biomass carbon (µg g^{-1} soil)						
T1	Isoproturon 75% WP	156.05	155.96	148.75	143.72	135.60	123.32	115.31
T2	Metribuzin 70%WP	158.34	157.43	152.02	147.65	138.80	125.73	113.18
T3	Clodinofop Propargyl 15%WP	168.29	167.20	159.95	144.21	138.05	122.81	115.39
T4	Atlantis	155.10	151.49	149.27	147.44	135.11	121.60	117.20
T5	Sulfosulfuron 75%WG	153.74	152.20	146.57	141.17	132.68	124.09	118.25
T6	UPH-110@ 400 g ha-	158.46	154.38	146.24	144.02	133.45	122.79	116.37
T7	UPH-110@ 500 g ha^{-1}	157.63	150.47	148.99	142.25	136.30	125.13	117.12
T8	UPH-110@ 600 g ha^{-1}	152.95	145.18	137.45	142.51	132.55	122.76	116.43
T9	UPH-110@ 1000 g ha^{-1}	148.41	142.04	133.56	135.07	128.62	121.57	114.84
T10	Control	156.13	153.84	149.20	144.33	135.44	124.53	115.93
	LSD P≤0.05	5.19	5.09	4.00	4.72	4.68	5.48	5.28
		Total population (×10^7cfu g^{-1} soil)						
T1	Isoproturon 75% WP	8.96	8.84	8.3	7.14	6.95	5.96	5.09
T2	Metribuzin 70%WP	7.9	7.85	7.8	7.31	6.7	5.74	5.04
T3	Clodinofop Propargyl 15%WP	9.11	9.02	7.84	7.49	6.74	5.74	5.00
T4	Atlantis	8.64	8.63	8.00	7.36	6.94	5.87	5.23
T5	Sulfosulfuron 75%WG	8.66	8.57	7.98	7.42	6.76	5.78	5.27
T6	UPH-110@ 400 g ha-	8.54	8.49	8	7.2	6.72	5.94	5.18
T7	UPH-110@ 500 g ha^{-1}	8.74	8.49	7.97	7.31	6.69	5.87	5.00
T8	UPH-110@ 600 g ha^{-1}	8.57	7.97	7.19	7.3	6.75	5.88	4.97
T9	UPH-110@ 1000 g ha^{-1}	7.76	7.62	6.83	6.5	6.73	5.9	5.19
T10	Control	8.73	8.54	7.96	7.3	6.77	5.84	5.14
	LSD P≤0.05	0.26	0.34	0.37	0.36	0.27	0.28	0.25

Table 3. Alkaline and acid phosphatase activity in soil as influenced by various herbicides at different time periods

Code	Treatment details	Days of sampling						
		1st (S1)	3rd (S2)	7th (S3)	15th (S4)	30th (S5)	45th (S6)	60th (S7)
		Microbial biomass carbon (µg g^{-1} soil)						
TT1	Isoproturon 75% WP	18.48	18.27	18.90	16.71	15.74	13.91	11.14
T2	Metribuzin 70%WP	23.18	22.32	20.72	15.82	18.08	14.56	12.81
T3	Clodinofop Propargyl 15%WP	16.24	17.40	18.15	16.80	16.08	12.98	13.16
T4	Atlantis	17.28	18.85	18.55	17.73	16.29	13.72	11.56
T5	Sulfosulfuron 75%WG	18.64	19.78	18.72	15.67	15.88	13.14	12.65
T6	UPH-110@ 400 g ha-	20.24	19.73	18.74	16.37	17.33	13.75	11.82
T7	UPH-110@ 500 g ha^{-1}	19.45	20.47	19.18	17.17	16.40	13.62	12.46
T8	UPH-110@ 600 g ha^{-1}	22.88	21.82	20.66	19.57	15.55	14.06	13.08
T9	UPH-110@ 1000 g ha^{-1}	23.10	22.87	21.55	20.16	18.05	15.10	11.43
T10	Control	19.18	19.42	18.68	17.15	15.57	13.94	12.05
	LSD P≤0.05	2.896	1.91	1.76	2.30	2.72	2.35	2.44
	Acid phosphatase activity (µg *p*-nitrophenol h^{-1} g^{-1} soil)							
T1	Isoproturon 75% WP	14.11	13.67	12.18	13.01	15.14	17.12	18.76
T2	Metribuzin 70%WP	9.33	9.29	9.16	10.26	14.25	16.73	17.85
T3	Clodinofop Propargyl 15%WP	14.28	14.02	11.67	13.90	15.76	15.73	20.04
T4	Atlantis	11.16	11.68	11.14	12.94	15.72	17.61	19.40
T5	Sulfosulfuron 75%WG	11.87	11.26	10.08	13.28	15.06	16.18	19.81
T6	UPH-110@ 400 g ha-	12.42	11.11	11.13	12.80	14.96	15.89	18.17
T7	UPH-110@ 500 g ha^{-1}	11.01	9.71	10.15	12.40	15.41	16.68	18.89
T8	UPH-110@ 600 g ha^{-1}	9.19	9.29	9.21	11.80	16.16	17.81	20.28
T9	UPH-110@ 1000 g ha^{-1}	8.71	8.09	8.71	9.21	14.36	18.03	17.97
T10	Control	11.65	11.54	10.97	12.50	15.46	17.13	19.03
	LSD P≤0.05	1.90	1.83	1.73	1.98	2.04	2.42	2.49

Table 4(a). Dehydrogenase activity (µg TPF 24 $hrs^{-1}g^{-1}$ soil) in soil as influenced by various herbicides at different time periods

Code	Treatment details	Days of sampling						
		1st (S1)	3rd (S2)	7th (S3)	15th (S4)	30th (S5)	45th (S6)	60th (S7)
		Dehydrogenase activity (µg TPF 24 $hrs^{-1}g^{-1}$ soil)						
T1	Isoproturon 75% WP	126.15	125.37	118.20	116.22	103.34	92.27	74.99
T2	Metribuzin 70%WP	130.02	123.94	117.65	110.85	103.29	90.60	77.63
T3	Clodinofop Propargyl 15%WP	147.86	142.64	128.86	114.05	102.32	92.00	76.84
T4	Atlantis	130.23	126.54	120.55	112.96	104.40	92.13	75.82
T5	Sulfosulfuron 75%WG	135.83	132.19	121.30	115.17	103.37	91.06	76.33
T6	UPH-110@ 400 g ha-	136.00	128.48	122.55	118.44	105.07	91.87	77.54
T7	UPH-110@ 500 g ha^{-1}	135.74	125.32	118.41	117.40	101.13	92.11	75.63
T8	UPH-110@ 600 g ha^{-1}	130.73	122.93	117.11	110.87	103.22	89.36	78.30
T9	UPH-110@ 1000 g ha^{-1}	128.80	121.79	113.37	108.76	96.94	88.72	77.02
T10	Control	136.34	131.53	123.10	115.64	103.49	91.69	76.63
	LSD P≤0.05	4.231	4.483	4.399	3.948	3.677	4.713	3.989

than control. Further the cumulative CO_2 production at the end of the incubation period was statistically different from control only in case of T_9.

DISCUSSION

Undisturbed microbial biomass carbon levels due to a phenylurea herbicide diuron at field

Table 4(b). Effect of various herbicides on Respiration rate (mg CO_2 kg^{-1} soil day^{-1}) at different time periods

Treatment details		Days of sampling					
		3rd day	7th day	15th day	30th day	60th day	Cumulative
		Respiration rate (mg CO_2 kg^{-1} soil day^{-1})					
T1	Isoproturon 75% WP	14.19	14.31	11.34	10.19	7.81	577.62
T2	Metribuzin 70%WP	15.44	14.80	10.79	10.39	7.80	581.55
T3	ClodinofopPropargyl 15%WP	18.66	16.80	11.15	10.12	7.86	600.17
T4	Atlantis	16.79	15.86	11.08	9.88	7.80	584.55
T5	Sulfosulfuron 75%WG	17.59	16.01	11.41	10.04	7.95	597.19
T6	UPH-110@ 400 g ha^{-}	16.18	15.75	10.99	10.32	7.87	590.36
T7	UPH-110@ 500 g ha^{-1}	14.79	15.52	11.07	10.34	7.92	587.67
T8	UPH-110@ 600 g ha^{-1}	14.10	14.77	10.79	9.92	7.88	572.93
T9	UPH-110@ 1000 g ha^{-1}	12.87	12.75	10.04	9.75	7.67	546.19
T10	Control	16.95	15.82	11.12	10.15	7.90	592.21
	LSD P≤0.05	1.24	0.88	0.52	0.32	0.12	19.77

rate were reported by Mariusz and Zofia (2009)[15]. Metribuzin, a triazine herbicide also didn't alter MBC significantly at field rate (Cesare *et al*. 2002)[16]. The significant favourable effect of clodinafop on microbial biomass extended however only for one week. Increase in MBC synthesis due to clodinafop coincided with enhancement in total population (Table 2). This proliferation of total microbial population could explain the observed results. A number of researchers have demonstrated that sulfonylurea herbicides at field rate do not affect microbial biomass carbon[10,17] (Valiolahpor *et al*. 2011, El-Ghamry *et al*. 2002). Decline in MBC synthesis with increased dose of UPH-110 (Table 2) clearly demonstrates the overall toxicity of the product to soil microflora at higher rates. A part of the reason in our opinion about less sensitivity of this parameter (MBC) towards the applied herbicides could be the method employed in its measurement and metabolic diversity in different microbial groups. Chander *et al*. (2001)[18] reported that Substrate Induced Respiration (SIR) biomass proved to be a more sensitive parameter in polluted soils than microbial biomass estimated by either fumigation extraction or fumigation incubation methods. Probably the glucose-responsive and more active part of the microbial community, determined by the SIR biomass, is more sensitive to pollutants than the total microbial biomass, as measured biochemically (Hoper, 2006)[19.] Zabaloy *et al*. (2008)[20] noticed stimulation in MBC due to glyphosate using SIR biomass method. While no response in MBC estimated by fumigation incubation method was observed at equivalent rates of the same herbicide [08,21](Haney *et al*. 2000; Accinelli *et al*. 2002).

To the best of our knowledge, very little work has been done so far to ascertain the impact of herbicides on soil phosphatase activity. Shaffer (1993)[22] underlined that confusing and somewhat contradictory results are often reported about the action of one pesticide on the activity of certain enzyme. In our study we observed that isoproturon stimulated the activity of acid phosphatase but kept the alkaline phosphatase activity unchanged (Table 3). The results are in partial agreement with the findings of Nowak *et al*. (2006) [23]. They reported stimulation in the activity of not only acid but also alkaline phosphatase when the soil was treated with isoproturon. Herbicide simazine that belongs to same chemical family as metribuzin was found to reduce acid phosphatase activity. This is in accordance with our results concerning metribuzin. We didn't notice any significant effect of either of two sulfonylurea herbicides used in our study on the activity of acid or alkaline phosphatase. Latha and Gopal (2010)[24] had obtained similar results about alkaline phosphates due to a sulfonylurea herbicide pyrazosulfuron ethyl. We relate the effect of clodinafop on acid phosphatase activity as observed in our study to the increase in microbial growth. The stressed microbial growth as indicated by population count and MBC (Table 2) is the most probable reason in our opinion for the hazardous effect of metribuzin and UPH-110 (at higher concentrations) on acid phosphatase activity. Our

results showed that alkaline phosphatase activity was affected positively by metribuzin and UPH-110 @ 600 and 1000 g ha^{-1}. This is probably because the synthesis of this enzyme is activated under stress conditions. Ozkanca and Flint (1997)[25] remarked that the synthesis of this enzyme can be activated under conditions where the cell is unable to grow. Lim *et al.* (1996)[26] reported that alkaline phosphatase can be produced in response to several different stresses and may be an important component of the bacterial global stress response network. They further argued that alkaline phosphatase could be involved in the survival of bacteria under stress conditions either through its role as a phosphate scavenger or through a non-specific function oriented towards an increase in cell permeability by the action of porin proteins co-synthesized with this enzyme. Earlier Omar and Abdel-Sater (2001)[27] showed promotion of alkaline phosphatase activity and inhibition of acid phosphatase activity under the situation when the herbicides had significantly lowered the bacterial and actinomycetes growth. Perucci *et al*. (2000)[28] also observed stimulation in alkaline phosphatase activity under conditions when the MBC was detrimentally decreased by herbicidal action but curiously enough acid phosphatase activity was also activated under these stressful conditions. We further observed that acid phosphatase was more sensitive to the applied herbicides than alkaline phosphatase for the reasons unknown.

In general, reports on the impact of herbicides on microbial activity often show considerable variation depending upon type of herbicide and application rates, soil properties and incubation conditions. In the present investigation, only temporary effects of herbicides on microbial activity in most of the cases were observed, which mostly recovered within few days or weeks (except UPH-110 at 1000 g ha^{-1}). We observed that isoproturon at field rate exerted transient inhibitory effects on microbial activity (both DHA and respiration). Likewise Alexandre and Claudio (2001)[29]showed reduction in microbial activity at field rate due to herbicide diuron. An investigation by Sebiomo *et al.* (2011)[30] showed reduction in DHA due to atrazine at field rate. Radivojevic *et al.* (2003)[31] reported that soil respiration was negatively affected by metribuzin under laboratory conditions at normal application rates. These findings are in agreement with our observations concerning metribuzin. The stimulatory nature of clodinafop was very evident in both the parameters of microbial activity measurement, Tables 4(a) & 4(b). We suggest that increase in respiration and DHA could be ascribed to the growth in clodinafop degrading organisms as indicated by high microbial population and biomass (Table 2). Araujo *et al.* (2003)[32] also observed an increase in CO_2 evolution due to glyphosate compared with the same soil that never received glyphosate. The contrasting nature of herbicides about their impact on soil microbial activity can largely be related to differences in soil type and/or may also be due to the variable effects of these agrochemicals on the cellular metabolism of respective groups of soil microflora. Certain microorganisms are able to use the applied herbicides as a source of energy and nutrients to multiply, that causes an increase in their metabolism and respiratory activity. On the other hand, there are some agrochemicals, which are not utilizable by soil microorganisms and they may negatively affect the metabolic activity and integrity of microbial cells, which results in the decrease of soil respiration activity[33] (Das *et al.* 2005). The literature available shows that sulfonylurea herbicides don't affect microbial activity at concentrations corresponding to field rate. Radivojevic *et al.* (2011)[34] reported that rimsulfuron decreased dehydrogenase activity at 10 FR and that changes brought about at field rate were below detection limits. Zabaloy and Gomez (2008)[35] didn't observe any change in CO_2 evolution when metsulfuron–methyl was applied to soil at the doses ranging from 0.1 to 10 mg kg^{-1}. Ismail *et al.* (1996)[36] also showed a decrease in soil respiration with metsulfuron–methyl at 10 FR and no effect at FR. Our observations about sulfosulfuron are in agreement with these findings. The results of atlantis (mesosulfulfuron methyl + idosufuron sodium) also agree with these findings so far as the CO_2 evolution is concerned but reduction in dehydrogenase activity was significant at field rate during the first two samplings which is contrary to the available findings. The trend that microbial activity decreased at higher rates and also prolonged the duration of hazard in case of UPH-110 is supported by the report of Luigi *et al.* (1996)[37]. They reported that respiration was inhibited by 40% after four

weeks of incubation by the highest employed concentration of bentazon. At 30 weeks after incubation it was less inhibited (by 15%) with the field rate (10 ppm) and by 25% with 100 ppm bentazon. In this study we could observe significant response in case of atlantis and also significant difference in CO_2 evolution even at 60th day was seen due to UPH-110 (1000g ha^{-1}) unlike other parameters. From these findings we suggest that under the existing environmental conditions, soil microbial activity was a most sensitive parameter to study the short term response of soil microbes to the applied herbicides. Haney *et al*. (2000)[08] and Cesare *et al*. (2002)[16] also rated microbial activity more sensitive than MBC in the evaluation of short term soil microbial response to herbicides. Moreover in our study, it was also observed that the two methods employed for measuring microbial activity were more or less equivalent in consequences (with few exceptions) and confirmed the results of each other.

CONCLUSION

The results clearly show that the herbicides differ in terms of their impact on soil microbial community. Differences also exist in the sensitivity of various parameters used to study microbial response to herbicides. Microbial activity measured in terms of dehydrogenase activity and soil respiration was most sensitive under the existing conditions. Interestingly enough, acid phosphatase activity also was more sensitive than alkaline phosphatase activity. Herbicidal influence is also determined by the employed dose. The duration as well as magnitude of toxicity increases as the dose increases.

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