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Persistence of the herbicides florasulam and halauxifen-methyl in alluvial and saline alluvial soils, and their effects on microbial indicators of soil quality

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Highlights

- Florasulam and halauxifen-methyl degraded rapidly in soil with a half-life of less than 18 days.

- Recommended doses of the herbicides did not ultimately impair, respiration or the degradation of or β-glucosidase.

- qCO_2 and qFDHA, indicated that the soil microbial biomass could overcome the stress imposed upon them at the recommended doses.

Abstract

The persistence of florasulam and halauxifen-methyl in combination was investigated in an alluvial (AL) and saline alluvial (SAL) soils under laboratory condition at field recommended (FR) dose (i.e. 12.76 g a.i. ha^{-1}), 10 FR and 0FR (control) throughout the experiment. Effect of these herbicides on soil microbial biomass carbon (MBC), basal soil respiration (BSR), substrate induced soil respiration (SIR), hydrolysis of fluorescein diacetate (FDHA) and β-glucosidase activities was also measured under same conditions. The residues of florasulam and haluaxifen-methyl were extracted with acetonitrile using modified QuChERS method, followed by quantification using liquid chromatography-mass spectrometry (LC-MS/MS). Half-lives of florasulam and halauxifen-methyl were in the range of 12.65–16.82 days and 7.13–10.24 days in AL respectively and the same for SAL was 15.28–17.60 days and 7.25–8.88 days. Herbicide treatment inhibited MBC, BSR, SIR, and microbial metabolic quotient (qCO_2) up to 30 days of incubation at the applied doses in both the soils. At the end of incubation, FR treated soils became statistically at par with control. However, the

FDHA and β-glucosidase activities were affected in both the soils at the applied doses of the herbicide up to 15 days. Thereafter, the FR treated soils were statistically at par with control. Data revealed that, recommended dose of this herbicide formulation did not ultimately impair microbial components of the studied soils.

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Keywords

Florasulam
Halauxifen-methyl
Microbial parameters
Alluvial soil
Saline alluvial soil

1. Introduction

Recent strategies for maximizing agricultural productivity is primarily dependent on the use of high yielding seeds, application of fertilizer and synthetically produced pesticides. Herbicides, in particular, are the important components to protect crops from weed infestation, and those are directly applied to soil. Soil acts as a large sink of applied pesticides where most of the transformation of pesticides occurs. Soil types have a profound influence in this respect. Soils vary with respect to their physical, chemical and microbiological properties. All these properties lead to transformation processes [1]. Of those, microbiological transformations are the predominant [2]. A good pesticide is one that dissipates immediately after performing their assigned role. However, this being rarely the case, they may remain into the soil and affect soil microbial activities [3], [4], resulting in alteration of usual functions of terrestrial ecosystem [5]. Thus, it is prudent to evaluate the fate and impact of new herbicides on soil microorganisms prior to their introduction in a particular soil and climate. Sulfonylurea is a well known herbicide for weed killing through inhibition of acetolactate synthase (ALS). A number of studies related to their impact on non-target sites of these herbicides have been published [6], [7], [8]. To overcome the shortfall of sulfonylurea herbicides [9], after late '90s a new group of ALS inhibitors, triazolopyridine sulfonamide, have been marketed. Florasulam (N-[2,6-difluorophenyl]-8-fluoro-5-methoxy [1,2,4] triazolo [1,5-c] pyrimidine-2-sulfonamide) belongings to this group commonly used as mixtures with other herbicides of different mode of action [9]. Halauxifen-methyl (methyl 4-amino-3-chloro-6-[4-chloro-2-fluoro-3-methoxyphenyl])

pyridine-2-carboxylate) is the first member of a new synthetic auxin herbicides, the arylpicolinates. Together with florasulam it can provide a new option for controlling broadleaf weeds in food and forage crops. It is likely to be introduced within short time in tropical climates like India. Information is available only on the persistence of this herbicide mixture under field condition [10], [11]. It is prudent to understand the fate of these herbicides on their persistence pattern and their impact on microbial and biochemical components in different types of soils.

Microbial biomass carbon (MBC) is an important attribute of soil quality [12], and they give an early warning of soil disturbances due to natural and anthropogenic stresses [13]. The MBC has been used as a tool of determining pesticide toxicity [7], [14], [15]. Both basal and substrate induced soil respirations (BSR and SIR respectively), and enzyme activities are the long standing methods in deciding microbial activities in soil. Changes in soil respirations have been used as an important parameter for pesticide toxicity estimation [16]. The microbial metabolic quotient (qCO$_2$) [17] has been widely used as a measure for detection of the effect of xenobiotic compounds on soil microflora. Effect of pesticides on soil enzyme activity has been recorded [18]. The β-glucosidase is an important enzyme in terrestrial carbon cycle producing glucose, which constitutes important energy source for microbial biomass [19]. Thus determination of β-glucosidase has been suggested as a good indicator of soil quality among other hydrolytic enzyme activities [20] and is not confounded by any soil input [21]. Pesticide influence on β-glucosidase activity has been reported [22], [23]. The fluorescein di-acetate hydrolysis is a broad spectrum enzyme assay because such hydrolysis requires the simultaneous mediation of protease, lipase, and esterase activities [24], [25] and this activity occurs within a numbers of primary decomposers like bacteria and fungi [26]. This enzyme assay is a better choice from soil quality perspective than individual soil enzyme assay [21] and has been used in pesticide toxicity studies.

The aim of this study was to evaluate the persistence behavior of the mixture formulation containing florasulam and halauxifen-methyl in tropical alluvial and saline alluvial soils when applied at FR (field recommended) and 10 FR doses, and to find out the correlation of dynamics of the herbicides with that of microbial components and enzyme activities. To determine the effects on microbial components, MBC, BSR and SIR were measured and microbial metabolic quotient (qCO$_2$) was calculated. Soil enzyme activities (hydrolysis of fluorescein di-acetate and β-glucosidase) were also carried out and specific hydrolytic activity (qFDHA) was evaluated. Such information for this herbicide mixture is lacking.

2. Materials and methods

2.1. Collection of soil samples

The AL (Typic udifluvent) and SAL (Typic endoaquept) were used for this present study. The AL and SAL (0–15 cm) were collected in the month of January, 2014 from the agricultural fields of Bidhan Chandra Krishi Viswavidyalaya Experimental Farm at Mohanpur, India (N 22°56′ E 88°31′) and the Agricultural Experimental Farm of Central Soil Salinity Research Institute, Canning Town, India (N 22°15′ E 88°40′) respectively. The selected sites had no history of pesticide treatment in the recent past. Collected soil samples were sieved (2 mm) and maintained at 60% water-holding capacity of soil by adding the required amount of distilled water. The soil samples were incubated at 28 ± 2 °C in dark for 7 days before the commencement of dynamics of herbicides, microbiological and biochemical analyses. The physico–chemical parameters of the soils were determined by standard procedures [27]using air dried soil samples.

The AL was a clay loam containing 33.2% sand, 30.2% silt and 36.6% clay and the pH, EC_e(dS m^{-1}), organic carbon (g kg^{-1}) and total nitrogen (g kg^{-1}) were 6.85, 0.87, 10.3 and 0.98 respectively. The SAL was also a clay loam containing 25.7% sand, 45.3% silt and 29.0% clay and the pH, EC_e (dS m^{-1}), organic carbon (g kg^{-1}) and total nitrogen (g kg^{-1}) were 7.50, 5.60, 9.3 and 0.91 respectively.

2.2. Experimental plan

Altogether seventy-five pots (twenty-five each for 0FR, FR and 10 FR) each containing 250 g of soil were set up for the persistence study as well as microbiological and biochemical parameters for 5, 10, 15, 30 and 45 days. For persistence study at 0 day another fifteen pots (five each for 0FR, FR and 10FR) were set up and the soil samples were collected 2 h after herbicide treatment. The pots were covered with perforated polypropylenesheets and incubated at 28 ± 2 °C in dark. The formulation (florasulam 10% + halauxifen-methyl 10.4% WG, Dow Agro-Sciences, India) was dissolved in distilled water and applied to soils at FR (12.76 g a.i. ha^{-1}) and 10 FR (127.6 g a.i. ha^{-1}). The final concentration of the applied doses were 5.7 µg kg^{-1} (FR) and 57 µg kg^{-1} (10 FR) obtained assuming bulk density of soil was 1.5 g cm^{-3} and even distribution of applied herbicide. The control (0FR) soils received only equal volumes of distilled water. The 10 FR dose is recommended for ecotoxicological experiments in laboratory incubation study to testify the side effect of pesticide on soil microflora [28], [29]. Desired moisture content of the soil samples was maintained

by adding requisite quantity of distilled water periodically. Five replicate soil samples were drawn at 5, 10, 15, 30 and 45 days for microbiological and biochemical analyses. Persistence study of the herbicides was carried out with five replicate soil samples drawn at 0, 5, 10, 15, 30 and 45 days.

2.3. Residue analysis of florasulam and halauxifen-methyl

The soil sample (10 g) was taken in a 50 mL fluorinated ethylene propylene (FEP) centrifugetube (Nalgene, Rochester, NY). 10 mL milli-Q water was added and samples were acidified with 0.1 mL acetic acid (Merck, India). It was then vortexed for 1 min for proper incorporation of the acidified water into sample matrix. After 15 min, 10 ml acetonitrile (J. T. Baker, HPLCgrade) was added and shaken vigorously for 1 min. Then 6 g $MgSO_4$ and 1.5 g NaCl (SRL, India) was added to it and again vortexes for 2 min followed by 15 min vertical shaking. Then the sample was centrifuged for 5 min at 5000 rpm. 2 mL of the supernatant extract was cleaned up with 25 mg primary secondary amine (PSA: Varian, Harbor City, CA; 40 mm particle size) and 25 mg florisil (60–100 mesh; Acros, Geel, Belgium).
Quantification of florasulam and halauxifen-methyl residue was done by High performance Liquid Chromatography (HPLC) coupled with tandem mass spectrometry. The HPLC separation was performed by Alliance 2695 separation module liquid chromatograph(Waters, Milford, MA, USA) equipped with a quaternary solvent delivery system via auto sampler on a reversed phase Symmetry C_{18} (5 μm; 2.1 × 100 mm) column (Waters, USA). Micromass (Manchester, UK) Quattro Micro triple-quadruple spectrometer equipped with an electrospray source (ESI) was used for detection and quantification. Injection volume was20 μl and the analysis performed with a flow rate of 0.3 ml min^{-1}. The mobile phase was composed of (A) water, 5 mM ammonium acetate and 0.1% acetic acid and (B) methanol, 5 mm ammonium acetate and 0.1% acetic acid. Gradient: 0.0–2.0 min – 5.0% B to 95%B, 2.0–8.0 min; back to the initial condition of 5% B, at 10.0 min, it ends with 5% B. Quantification of the residue was performed in multiple reaction monitoring (MRM) mode. Ion transitions were 359.87 > 128.90, 359.87 > 81.60 for florasulam and 344.82 > 250.10, 344.82 > 285.00 for halauxifen-methyl respectively. Cone voltage for florasulam and halauxifen-methyl was set at 28 V and 42 V respectively.
The capillary voltage and source temperature was maintained at 0.50 kV and 120 °C. Desolvation temperature was 350 °C. Desolvation gas flow and cone flow were 650.0 L/hr. and 25 L/hr. respectively.

2.4. Microbiological assay

The MBC of soils was determined according to Joergensen (1995) [30] and Vance et al. (1987) [31]. Fumigation of soil was carried out by chloroform followed by extraction with 0.5 M K_2SO_4 solution. The extracted aliquot was refluxed with 0.4 N $K_2Cr_2O_7$ and acid mixture (H_2SO_4/H_3PO_4; 3:2). Finally, the residual dichromate was back titrated with standard Mohr's salt solution (0.04 N). In case of SAL, 1.5% silver sulphate was used prior to reflux with acid mixture. MBC of the soils were calculated using the relationship: MBC = 2.64 E_c, where E_c is the difference of K_2SO_4 extractable C between the fumigated and un-fumigated soils.

The BSR and SIR of the soils were analyzed according to Alef (1995) [32]. The estimation of CO_2 evolved during the incubation of soil (at 22 °C for 24 h) in a closed system was done by trapping CO_2 in NaOH solution (0.1 M) in a closed container, followed by back titration with standard HCl solution. For SIR, 0.5% glucose solution was added. Incubation time was 6 h for SIR. Rest of the procedure was same as BSR. Analysis of FDHA of soils was done according to Schnürer and Rosswall (1982) [25]. Fluorescein di-acetate was used as substrate. 1 g moist soil was incubated with sodium phosphate buffer (60 mM; pH 7.6) and fluorescein di-acetate at 24 °C in orbital shaker for 3 h. Absorbance of the released fluorescein was estimated at 490 nm. Calibration curve was prepared with standard fluorescein solution at different concentration level.

Analysis of β-glucosidase activity of soils was measured according to Alef and Nannipieri (1995) [33]. The method is based on the determination of p-nitrophenol (PNP) released after the incubation of soil with p-nitrophenyl-β-glucopyranoside (PNG) for 1 h at 37 °C. Absorbance of the released PNP was measured at 420 nm. Calibration curve was prepared with standard PNP solution at different concentration level.

2.5. Pesticide residue data analysis

Half-life ($T_{1/2}$) values were obtained by fitting first-order kinetics to observe degradation patterns (mean of five replicates) as:

$$C_t = C_0 \times e^{-kt}$$

Where, C_t is chemical concentration (mg kg^{-1}) at time t, C_0 is initial concentration (mg kg^{-1}), and k is the first order rate constant (t^{-1}) independent of C_t and C_0. The $T_{1/2}$ of florasulam and halauxifen-methyl were calculated using Hoskins formula [34].

2.6. Statistical analysis

The recorded data for different microbial and biochemical parameters of soil were statistically analyzed assigning different doses (0FR, FR, 10FR) of herbicide application

as treatment factors. The treatment factor had three levels and the replicate had five levels. Analysis of variance (ANOVA) was carried out by Completely Randomized Design (CRD) using SPSS 16.0 statistical package. The mean values of the treatments were compared by Duncan's multiple range tests at 5% probability level.

3. Result and discussions

3.1. Degradation of florasulam and halauxifen-methyl in soil

Degradation of both florasulam and halauxifen-methyl in formulation (Table 1) followed first order kinetics irrespective of treatments and soil. The degradation kinetics rate constants (k) of florasulam and halauxifen-methyl showed a higher value at 10 FR than FR. The half-life values, calculated from the best fit line of the logarithm of the residual concentration versus incubation period, for florasulam ranged from 12.65 to 16.82 days and that of halauxifen-methyl were 7.13–10.24 days at FR and 10 FR respectively for AL soil. The same for SAL, ranged from 15.28 to 17.50 days and 7.25–8.88 days. Data indicated the less persistent behavior of halauxifen-methyl compared to florasulam irrespective of soil type.

Table 1. Statistical data on the persistence of florasulam and halauxifen-methyl in soil.

Soil type	Name of compound	Treatment	Regression equation	R^2	Half-life ($T_{1/2}$) (day)
Alluvial soil	Florasulam	FR	y = −0.0238x + 2.89	0.9441	12.65
		10 FR	y = −0.0179x + 4.27	0.9596	16.82
	Halauxifen-methyl	FR	y = −0.0422x + 2.88	0.9821	7.13
		10 FR	y = −0.0294x + 3.22	0.9738	10.24
Saline alluvial soil	Florasulam	FR	y = −0.0197x + 2.96	0.9944	15.28
		10 FR	y = −0.0171x + 3.23	0.9822	17.60
	Halauxifen-methyl	FR	y = −0.0415x + 3.01	0.9908	7.25
		10 FR	y = −0.0339x + 3.30	0.9970	8.88

The degradation of pesticide in soil depends on many factors [35] which include type of soil, rate of application [36] and chemical nature of the pesticide [37]. The difference in the half-life values of the two herbicides in two different soils seemed to be related to the chemical nature of the herbicides, and also soil types because other conditions of this study were same. The difference between half-life of the herbicides due to dose variation seemed to be related to the variation in microbial biomass together with their activities at the applied doses irrespective of soil types (Table 2). Variation in half-life of herbicide due to dose variation was already reported by Vischetti et al. (2000) [38].

Table 2. Dynamics of soil microbial biomass, basal and substrate induced respiration and microbial metabolic quotient (qCO_2) in soils treated with florasulam and halauxifen-methyl at different days of incubation.

Days of incubation	Treatment	Microbial biomass carbon (µg C g^{-1}oven dry soil)		Basal soil respiration (µg CO_2–C g^{-1} soil h^{-1})		Substrate induced respiration (µg CO_2–C g^{-1} soil^{-1})		qCO_2 (mg CO_2–C mg^{-1}MBC)	
		AL*	SAL*	AL	SAL	AL	SAL	AL	SAL
5	Control	493a (±2.16)	437.78a (±1.62)	1.32a (±0.01)	1.28a (±0.02)	10.71a (±0.04)	9.08a (±0.02)	2.68c (±0.03)	2.93b (±0.05)
	FR*	343b (±1.93)	368.19b (±4.27)	1.21b (±0.02)	1.22ab (±0.01)	9.65b (±0.04)	8.96b (±0.03)	3.53b (±0.04)	3.20a (±0.04)
	10 FR	212c (±3.11)	346.97c (±3.27)	1.01c (±0.02)	1.12b (±0.13)	8.98c (±0.02)	8.46c (±0.04)	4.72a (±0.13)	3.23a (±0.36)
10	Control	480a (±1.38)	418.76a (±3.60)	1.29a (±0.01)	1.21a (±0.02)	10.59a (±0.04)	9.15a (±0.04)	2.70c (±0.02)	2.89b (±0.07)
	FR	294b (±3.04)	318.79b (±2.44)	1.14b (±0.01)	1.12b (±0.01)	8.69b (±0.05)	8.37b (±0.05)	3.88b (±0.01)	3.51a (±0.07)
	10 FR	176c (±1.48)	277.55c (±2.02)	0.87c (±0.01)	0.98c (±0.07)	7.43c (±0.07)	7.88c (±0.03)	4.93a (±0.13)	3.54a (±0.25)
15	Control	473a (±1.08)	407.88a (±3.34)	1.29a (±0.01)	1.18a (±0.03)	10.48a (±0.04)	9.04a (±0.04)	2.72c (±0.02)	2.89c (±0.09)
	FR	354b (±3.89)	297.07b (±2.40)	1.15b (±0.01)	1.05b (±0.04)	9.67b (±0.04)	7.56b (±0.03)	3.26b (±0.02)	3.55b (±0.12)
	10 FR	305c (±3.52)	228.81c (±1.74)	1.13c (±0.01)	0.90c (±0.04)	8.75c (±0.05)	6.54c (±0.04)	3.70a (±0.07)	3.94a (±0.18)
30	Control	459a (±2.68)	401.77a (±2.84)	1.28a (±0.01)	1.13a (±0.02)	10.52a (±0.05)	9.03a (±0.16)	2.79b (±0.03)	2.81c (±0.05)
	FR	402b (±3.63)	320.77b (±2.71)	1.23b (±0.02)	1.10b (±0.03)	10.09b (±0.04)	7.90b (±0.05)	3.05a (±0.06)	3.42b (±0.11)
	10 FR	383c (±4.04)	246.48c (±2.58)	1.19c (±0.01)	1.03c (±0.05)	9.55c (±0.07)	7.51c (±0.03)	3.10a (±0.03)	4.18a (±0.21)
45	Control	472a (±5.08)	397.66a (±2.28)	1.32a (±0.02)	1.18a (±0.01)	10.44a (±0.03)	8.91a (±0.02)	2.79b (±0.03)	2.97b (±0.02)
	FR	465a (±4.08)	413.75a (±1.54)	1.29a (±0.02)	1.24a (±0.01)	10.41a (±0.07)	8.84a (±0.05)	2.77b (±0.04)	3.00b (±0.02)
	10 FR	415b (±2.88)	367.98b (±1.78)	1.22b (±0.01)	1.17b (±0.01)	10.38b (±0.06)	8.45b (±0.05)	2.94a (±0.04)	3.19a (±0.04)

*AL – Alluvial soil, SAL – Saline alluvial soil; FR – field rate.

Values in the same column in a particular section followed by different lowercase letters are statistically different at $p < 0.05$ by Duncan's multiple range test.

Data in parenthesis represents standard deviation.

3.2. Microbial biomass carbon

Irrespective of the soils, control samples always registered a significant higher value of MBC than the herbicide treated soils (Table 2). For both AL and SAL, gradual decrease in MBC was significant up to 30 days. At 45 days of incubation, FR treated soils became statistically at par with the control.

Microbial biomass is a standardized component of eco-toxicity assessment in OECD (Organization for Economic Co-operation and Development) guidelines for pesticide registration [39]. Microbial biomass is an important attribute of soil quality as it comprised of the living pool of organic matter in soil [40]. Any substantial change in soil management practices can alter the MBC much faster than any physical or physicochemical properties of soil. MBC of the studied soils were carried out by fumigation-extraction method with a slight modification to SAL. Addition of silver sulphate was essential for precipitating out the chloride interference as silver chloride. MBC of control samples for AL accounted higher value than SAL at any day of incubation. This is in well agreement with Tripathi et al. (2001) [41], that the MBC was less in saline soil than the non-saline soil. Gradual decrease of MBC beyond half-life period is quite surprising as the studied herbicides accounts a half-life value only 1.5–2.5 days under field condition [11]. The same depressive effect on MBC after 30 days of incubation was reported for rimsulfuron [6] although its half-life accounted only 3 days under field condition [37]. This may indicate a long term detrimental effect but the authentication awaits field study. Subsequent rise in MBC in FR treated soil is related to the proliferation of surviving microorganisms using the dead cell debris of the killed microorganisms together with the degraded products of the herbicides [42]. A gradual increase in MBC was also observed for 10 FR treated soils after crossing the half-life period. But a significant difference between the FR and 10 FR treated soils may be attributed to the stronger inhibition of microbial populations due to higher application rates or the higher concentration of toxic metabolites. However, at FR the herbicides exerted temporary inhibition on soil microbial biomass.

3.3. Soil respiration and microbial metabolic quotient

The change in BSR and SIR of herbicide treated soils followed the trend of MBC (Table 2). Microbial metabolic quotient i.e., the ratio of BSR to MBC (qCO_2) showed a gradual increase at FR and 10 FR treated soils up to 30 days of incubation. At 45 days

qCO_2 of FR treated soils was statistically at par with the control. The qCO_2 of 10FR treated soils was always higher than 0FR and FR treated soils.

The metabolic activities of soil microorganism can be quantified by measuring CO_2 evolution. The BSR reflects overall potential microbial activity [43]. The SIR is a measure of the total physiologically active part of soil microflora [44]. The combination of BSR and SIR represent carbon availability index [45]. Herbicide induced alteration in soil respiration was also studied by Cycoń et al. (2013) [29]. Amongst the other proposed metabolic quotient, qCO_2 provides a better interpretation of the soil responses to disturbances. The carbon substrates at the disposal of microorganisms serve two purposes – one for cell maintenance and the other for cell proliferation. When microorganisms are under stress they spent more energy for their cell maintenance than proliferation [46]. As a result, they respire more per unit of microbial biomass. This can be detected by qCO_2 values. An increase in qCO_2 can be considered to indicate a detrimental effect of herbicide treatment, which can force microbes to utilize a large part of their energy budget for cell maintenance [47] to overcome the toxic effect of herbicide [48]. Higher qCO_2 value indicates that the microorganisms are under stress [49], [50]. Throughout the incubation period the microbes in 10 FR treated soils were under the influence of stressful effects of herbicides. The stress was short-lived for FR treated soils. Increase in qCO_2 at the beginning of incubation followed by decrease in response to sulfonylurea herbicide was reported by Sofo et al. (2012) [8].

3.4. Fluorescein di-acetate hydrolyzing and β-glucosidase activities

The FDHA and β – glucosidase activity of the treated soils decreased gradually up to 15th days of incubation. At 30 days onwards, the FR treated soils became statistically at par with the control. Even at the end of incubation 10 FR treated soils recorded significantly lower values than the control (Table 3).

Table 3. Dynamics of fluorescein diacetate hydrolyzing activity (FDHA) and β – glucosidase activity in soils treated with florasulam and halauxifen-methyl at different days of incubation.

Days of incubation	Treatment	FDHA (µg fluorescein g^{-1} oven dry soil h^{-1})		β – glucosidase (µg pnp g^{-1} oven dry soil h^{-1})		qFDHA (µg fluorescein – µg C g^{-1} oven dry soil)	
		AL*	SAL*	AL	SAL	AL	SAL
5	Control	44.8a (±1.63)	54.60a (±2.22)	19.3a (±0.14)	15.4a (±0.41)	0.091c (±0.003)	0.125b (±0.008)
	FR*	37.6b (±1.11)	48.31b (±2.53)	18.1b (±0.35)	14.5b (±0.51)	0.110b (±0.003)	0.131a (±0.002)
	10 FR	28.48c	44.05c	16.5c	13.9c	0.134a	0.127b

Days of incubation	Treatment	FDHA (µg fluorescein g⁻¹ oven dry soil h⁻¹)		β – glucosidase (µg pnp g⁻¹ oven dry soil h⁻¹)		qFDHA (µg fluorescein – µg C g⁻¹ oven dry soil)	
		AL*	SAL*	AL	SAL	AL	SAL
		(±2.17)	(±0.78)	(±0.33)	(±0.22)	(±0.010)	(±0.004)
10	Control	44.5a (±1.83)	59.48a (±2.27)	19.5a (±0.13)	15.0a (±0.30)	0.093c (±0.002)	0.142a (±0.003)
	FR	30.5b (±1.28)	47.71b (±3.32)	16.9b (±0.28)	13.8b (±0.43)	0.104b (±0.003)	0.150a (±0.005)
	10 FR	21.6c (±1.52)	37.44c (±2.83)	14.7c (±0.27)	12.4c (±0.20)	0.123a (±0.005)	0.135b (±0.002)
15	Control	43.9a (±1.88)	43.64a (±2.77)	19.3a (±0.30)	15.2a (±0.34)	0.093c (±0.010)	0.107c (±0.004)
	FR	36.2b (±0.71)	39.48b (±0.91)	18.5b (±0.21)	12.1b (±0.27)	0.102b (±0.007)	0.133b (±0.003)
	10 FR	27.7c (±2.26)	30.79c (±1.59)	16.6c (±0.60)	10.2c (±0.30)	0.091c (±0.004)	0.135b (±0.003)
30	Control	46.2a (±1.70)	43.04a (±1.13)	19.4a (±0.34)	14.9a (±0.27)	0.101b (±0.005)	0.107c (±0.003)
	FR	44.4a (±1.34)	40.51a (±0.97)	19.8a (±0.20)	13.9a (±0.34)	0.110b (±0.003)	0.126b (±0.003)
	10 FR	32.7b (±1.45)	32.66b (±1.29)	18.7b (±0.55)	12.3b (±0.23)	0.085c (±0.002)	0.133b (±0.006)
45	Control	46.6a (±1.89)	42.50a (±1.79)	19.5a (±0.42)	14.7a (±0.30)	0.099b (±0.004)	0.107c (±0.010)
	FR	46.2a (±2.01)	41.06a (±2.89)	20.1a (±0.34)	14.1a (±0.16)	0.099b (±0.003)	0.099c (±0.002)
	10 FR	35.8b (±1.63)	37.76b (±3.01)	18.9b (±0.44)	13.1b (±0.25)	0.086c (±0.005)	0.103c (±0.003)

*AL – Alluvial soil, SAL – Saline alluvial soil; FR – field rate.

Values in the same column in a particular section followed by different lowercase letters are statistically different at p < 0.05 by Duncan's multiple range test.

Data in parenthesis represents standard deviation.

Microorganisms are one of the main sources of enzyme in soils. Enzymes in soils are the enzymes produced by active microbial cells, dead microbial cell debris, free enzymes and the enzymes entrapped in the soil particles [51]. The enzyme activities of FR treated soils started increasing at 30 days when MBC was still under inhibition. This dissimilarity effect between MBC and enzyme activities seemed to be related to removal of the inhibitory effect of the herbicides and their degraded products on the enzymes

from dead microbial cells, free and entrapped enzyme molecules present in soil [42]. Bishnu et al. (2012) [42] reported the diminished followed recovered activities for FDHA and β – glucosidase. However, inconsistent trend was also observed for β – glucosidase activity [8]. Generally herbicides are designed to control weed infestation not soil enzymes. Although an array of compounds are used to inhibit enzyme activities, but, their structural complexity makes it unlikely to anticipate direct impact on hydrolase activities measured by using artificial substrates and assay conditions. The predominant mode of inhibition is of indirect type. As the enzymes in soil have rarely been purified, it is hard to differentiate whether the effect is direct or indirect. Indirect inhibition is a clear indication of masking the source of enzyme (microbial activities) and at the same time altered intra and extracellular level. Recovery of soil biological activity after application of xenobiotic compounds makes an ecological approach for the assessment of side effect was proposed by Domsch et al. (1983) [52]. After scrutinizing a large number of case studies, it was proposed that recovery of enzyme activities up to 30 days are normal, 60 days are tolerable and more than 60 days may pose a serious problem [52].

3.5. Specific hydrolytic activity (qFDHA)

The qFDHA indicates FDHA per unit of MBC. This parameter was used to quantify the effect of soil amendments which include herbicide, organic manure etc. on soil microbial activity[6], [53]. Up to 10th day of incubation, the qFDHA of AL treated with FR and 10FR was higher over control (Table 3). Thereafter, there was a reduction in qFDHA in both the treated soils and finally became at par with the control soil at the end of incubation. For SAL, the qFDHA was found to be higher up to 30th day of incubation in case of treated soils. Then it became at par with the control (Table 3).
Present study indicated herbicide combination exerted detrimental influence on FDHA of soils, and the effect was dependent on soil types. The longer detrimental influence on SAL compared to AL seemed to be related to the twin effect of soil salinity and herbicide stress. Higher qFDHA value of herbicide treated soils was related to higher detrimental influence on MBC than FDHA. Actually, detrimental influence of herbicide on MBC was 63.2% and FDHA was 45.9% in case of AL at 10th day of incubation. The same figure for SAL at 30th day of incubation was 25.25% and 6.25% respectively.

4. Conclusions

Soil microbial and biochemical parameters are now considered as the effective indicators of soil quality. The qCO_2 and qFDHA parameters have been used to determine the metabolic stress caused by the pesticide treatment on soil microbial

components. Present study indicated that florasulam and halauxifen-methyl treatments of soils at field rate of application exerted temporary stress on soil MBC, qCO_2 and qFDHA. An inhibitory effect of this combination was also found to be dependent on soil types as alluvial soil varied in comparison to saline alluvial soil.

Acknowledgment

References

[1]

G.D. Bending, S.D. Lincoln, R.N. Edmondson**Spatial variation in the degradation rate of the pesticides isoproturon, azoxystrobin and diflufenican in soil and its relationship with chemical and microbial properties**
Environ. Pollut., 139 (2006), pp. 279-287
ArticleDownload PDFView Record in Scopus

[2]

D.J. Cork, J.P. Krueger**Microbial transformations of herbicides and pesticides**
Adv. Appl. Microbiol., 36 (1991), pp. 1-66
ArticleDownload PDFView Record in Scopus

[3]

M.S. Girvan, J. Bullimore, A.S. Ball, J.N. Pretty, A.M. Osborn**Responses of active bacterial and fungal communities in soils under winter wheat to different fertilizer and pesticide regimens**
Appl. Environ. Microbiol., 70 (2004), pp. 2692-2701
CrossRefView Record in Scopus

[4]

M. Ros, M. Goberna, J.L. Moreno**Molecular and physiological bacteria diversity of a semi-arid soil contaminated with different levels of formulated atrazine**
Appl. Soil Ecol., 34 (2006), pp. 93-102
ArticleDownload PDFView Record in Scopus

[5]

F. García-Orenes, C. Guerrero, A. Roldán, J. Mataix-Solera, A. Cerdá, M. Campoy, R. Zornoza, G .Bárcenas, F. Caravaca**Soil microbial biomass and activity under different agricultural management systems in a semiarid Mediterranean agroecosystem**

Soil Tillage Res., 109 (2010), pp. 110-115

ArticleDownload PDFView Record in Scopus

[6]

P. Perucci, S. Dumontet, S.A. Bufo, A. Mazzatura, C. Casucci**Effects of organic amendment and herbicide treatment on soil microbial biomass**

Biol. Fertil. Soils, 32 (2000), pp. 17-23

CrossRefView Record in Scopus

[7]

L. Radivojević, L. Šantrić, J.G. Umiljendić**Rimsulfuron in soil: effects on microbiological properties under varying soil conditions**

Pestic. Phytomed., 26 (2) (2011), pp. 135-140

CrossRefView Record in Scopus

[8]

A. Sofo, A. Scopa, S. Dumontet, A. Mazzatura, V. Pasquale**Toxic effects of four sulphonylureas herbicides on soil microbial biomass**

J. Environ. Sci. Health., Part B, 47 (2012), pp. 653-659

CrossRefView Record in Scopus

[9]

T.W. Jabusch, R.S. Tjeerdema**Chemistry and fate of triazolopyrimidine sulfonamide herbicides**

Rev. Environ. Contam. Toxicol., 193 (2008), pp. 31-52

CrossRefView Record in Scopus

[10]

Z. Li, W. Guan, H. Hong, Y. Ye, Y. Ma**Determination and study on residue and dissipation of florasulam in wheat and soil under field conditions**

Bull. Env. Contam. Toxicol., 90 (2013), pp. 280-284

CrossRefView Record in Scopus

[11]

S. Mukherjee, A. Goon, B. Ghosh, A. Kundu, K. Chakrabarti, S. Roy, A. Bhattacharyya**Persistence behaviour of a mixed formulation (florasulam 10% + halauxifen methyl 10.4% WG) in wheat**

J. Crop Weed, 10 (2) (2014), pp. 414-418

View Record in Scopus

[12]

S. Tripathi, A. Chakraborty, K. Chakrabarti, B.K. Bandyopadhyay**Enzyme activities and microbial biomass in coastal soils of India**

Soil Biol. Biochem., 39 (2007), pp. 2840-2848

ArticleDownload PDFView Record in Scopus

[13]

D.S. Powlson, P.C. Brookers, B.T. Cristensen**Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation**

Soil Biol. Biochem., 39 (1987), pp. 76-86

View Record in Scopus

[
1
4
]

M. Baboo, M. Pasayat, A. Samal, M. Kujur, J.K. Maharana, A.K. Patel**Effect of four herbicides on soil organic carbon, microbial biomass-c, enzyme activity and microbial populations in agricultural soil**

Int. J. Res. Environ. Sci. Te, 3 (4) (2013), pp. 100-112

View Record in Scopus

[15]

E. Pose-Juan, M.J. Sánchez-Martín, E. Herrero-Hernández, M.S. Rodríguez-Cruz**Application of mesotrione at different doses in an amended soil: dissipation and effect on the soil microbial biomass and activity**

Sci. Total Environ., 536 (1) (2015), pp. 31-38

ArticleDownload PDFView Record in Scopus

[16]

H.R. Gerber, J.P.E. Anderson, B. Bugel-Mongensen, D. Castle, K.H. Domsch, H.P. Mal Komes, D.J.Arnold, H.V.D. Werf, R. Verbeken, J.W. Vonk**Revision of recommended laboratory tests for assessing side effects of pesticides on soil microflora**

Toxicol. Environ. Chem., 30 (1991), pp. 249-269

CrossRefView Record in Scopus

[17]

T.H. Anderson, K.H. Domsch**Determination of eco-physiological maintenance requirements of soil microorganisms in dormant state**

Biol. Fertil. Soils, 1 (1985), pp. 81-89

View Record in Scopus

[18]

F. Sannino, L. Gianfreda**Pesticide influence on soil enzymatic activities**

Chemosphere, 45 (2001), pp. 417-425

ArticleDownload PDFView Record in Scopus

[19]

M.A. Tabatabai**Soil enzymes**

R.W. Weaver, J.S. Angel, P.S. Bottomley (Eds.), Methods of Soil Analysis. Part 2: Microbial and Biochemical Properties, Soil Science Society America, Madison, WI, USA (1994), pp. 775-833

View Record in Scopus

[20]

R.P. Dick, D.P. Breakwell, R.F. Turco**Soil enzyme activities and biodiversity measurements as integrative microbiological indicators**

J.W. Doran, A.J. Jones (Eds.), Methods for Assessing Soil Quality, Special Publication No. 49, Soil Science Society America, Madison, WI, USA (1996), pp. 247-271

View Record in Scopus

[21]

R.P. Dick**Soil enzyme activities as indicators of soil quality**

J.W. Doran, D.C. Coleman, D.F. Bezdicek, B.A. Stewart (Eds.), Defining Soil Quality for Sustainable Environment. Special Publication No. 35, Soil Science Society of America, Inc, Madison, WI (1994), pp. 107-124

View Record in Scopus

[22]

R.M. Niemi, I. Heiskanen, J.H. Ahtiainen, A. Rahkonen, K. Mäntykoski, L. Welling, P. Laitinen, P.R uuttunen**Microbial toxicity and impacts on soil enzyme activities of pesticides used in potato cultivation**

Appl. Soil Ecol., 41 (2009), pp. 293-304

ArticleDownload PDFView Record in Scopus

[23]

M. Tejada**Evolution of soil biological properties after addition of glyphosate, diflufenican and glyphosate plus diflufenican herbicides**

Chemosphere, 76 (2009), pp. 365-373

ArticleDownload PDFView Record in Scopus

[24]

W. Riah, K. Laval, E. Laroche-Ajzenberg, C. Mougin, X. Latour, I. Trinsoutrot-Gattin**Effects of pesticides on soil enzymes: a review**

Environ. Chem. Lett., 12 (2014), pp. 257-273

CrossRefView Record in Scopus

[25]

J. Schnürer, T. Rosswall**Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter**

Appl. Environ. Microbiol., 43 (1982), pp. 1256-1261

View Record in Scopus

[26]

C. Janvier, F. Villeneuve, C. Alabouvette, V. Edel-Hermann, T. Mateille, C. Steinberg**Soil health through soil disease suppression: which strategy from descriptors to indicators?**

Soil Biol. Biochem., 39 (2007), pp. 1-23

ArticleDownload PDFView Record in Scopus

[27]

M.L. Jackson**Soil Chemical Analysis**

Prentice Hall of India, New Delhi (1967)

[28]

L. Sommerville**Perspective on side effect testing**

L. Sommerville, M.P. Greaves (Eds.), Pesticide Effects on Soil Microflora, Taylor and
Francis, London(1987), pp. 5-13

[29]

M. Cycoń, M. Wójcikb, S. Borymskic, Z. Piotrowska-Segetc**Short-term effects of the herbicide
napropamide on the activity and structure of the soil microbial community assessed by
the multi-approach analysis**

Appl. Soil Ecol., 66 (2013), pp. 8-18

ArticleDownload PDFView Record in Scopus

[30]

R.G. Joergensen**Microbial biomass**

K. Alef, P. Nannipieri (Eds.), Methods in Applied Soil Microbiology and
Biochemistry, Academic, London(1995), pp. 382-386

[31]

E.D. Vance, P.C. Brookes, D.S. Jenkinson**An extraction method for measuring soil microbial
biomass-C**

Soil Biol. Biochem., 19 (1987), pp. 703-707

ArticleDownload PDFView Record in Scopus

[32]

K. Alef**Estimation of soil respiration**

K. Alef, P. Nannipieri (Eds.), Methods in Applied Soil Microbiology and
Biochemistry, Academic, London(1995), pp. 215-216

[33]

K. Alef, P. Nannipieri**β-glucosidase activity**

K. Alef, P. Nannipieri (Eds.), Methods in Applied Soil Microbiology and
Biochemistry, Academic, London(1995), pp. 350-352

[34]

W.M. Hoskins**Mathematical treatment of the rate of loss of pesticide residue**

Pl. Prot. Bull., 9 (1961), pp. 163-168

[35]

E. Topp, T. Vallaeys, G. Soulas**Pesticides: microbial degradation and effects on
microorganisms**

J.D. VanElsas, J.T. Trevors, E.M.H. Wellington (Eds.), Modern Soil Microbiology, Marcel
Dekker, New York (1997), pp. 547-575

[36]

E. Schuster, D. Schroder**Side effects of sequentially applied pesticides on non-target soil microorganism: field experiments**
Soil Biol. Biochem., 22 (1990), pp. 367-373
[ArticleDownload PDFView Record in Scopus]

[37]

M. Kah, S. Beulke, C.D. Brown**Factors influencing degradation of pesticides in soil**
J. Agric. Food Chem., 55 (2007), pp. 4487-4492
[CrossRefView Record in Scopus]

[38]

C. Vischetti, P. Perucci, L. Scarponi**Relationship between rimsulfuron degradation and microbial biomass content in a clay loam soil**
Biol. Fertil. Soils, 31 (2000), pp. 310-314
[CrossRefView Record in Scopus]

[39]

J.P.E. Anderson, D. Castle, H. Ehle, D. Eichler, H.T. Lacrman, G. Mass, H.P. Malkomes**Guidelines for the Official Testing of Plant Protection Chemicals**
(second ed.), Biologische Bundesanstalt fur Land-use Forstwirtsshaft, Braunschweig, Germany (1992)
part VI, 1–1, Effects on the activity of soil micro-flora

[40]

F. Gil-Sotres, C. Trasar-Cepedab, M.C. Leirósa, S. Seoanea**Different approaches to evaluating soil quality using biochemical properties**
Soil Biol. Biochem., 37 (2005), pp. 877-887
[ArticleDownload PDFView Record in Scopus]

[41]

S. Tripathi, A. Chakraborty, K. Chakrabarti**Microbial biomass and activities in coastal saline soils of West Bengal**
J. Ind. Soc. Coast. Agr. Res., 19 (2001), pp. 129-133
[CrossRefView Record in Scopus]

[42]

A. Bishnu, A. Chakraborty, K. Chakrabarti, T. Saha**Ethion degradation and its correlation with microbial and biochemical parameters of tea soils**
Biol. Fertil. Soils, 48 (2012), pp. 19-29
[CrossRefView Record in Scopus]

[43]

T.R.G. Gray**Methods for studying the microbial ecology of soil**
R. Grigorova, J.R. Norris (Eds.), Methods in Microbiology, Academic Press, London (1990), pp. 309-342
[ArticleDownload PDFView Record in Scopus]

[44]

J.P.E. Anderson, K.H. Domsch**A physiological method for quantitative measurement of soil microbial biomass in soils**

Soil Biol. Biochem., 10 (1978), pp. 215-221

ArticleDownload PDFView Record in Scopus

[45]

W. Cheng, Q. Zhang, D.C. Coleman, C.R. Caroll, C.A. Hoffmann**Is available carbon limiting microbial respiration in the rhizosphere?**

Spol. Biol. Biochem., 28 (1996), pp. 1283-1288

ArticleDownload PDFView Record in Scopus

[46]

J.L. Moreno, A. Aliaga, S. Navarro, T. Hernández, C. García**Effects of atrazine on microbial activity in semiarid soil**

Appl. Soil Ecol., 35 (2007), pp. 120-127

ArticleDownload PDFView Record in Scopus

[47]

T.H. Anderson, K.H. Domsch**Application of ecophysical quotients (qCO_2 and q^D) on microbial biomass from soils of different cropping histories**

Soil Biol. Biochem., 22 (1990), pp. 251-255

ArticleDownload PDFView Record in Scopus

[48]

E. Gómez, L. Ferreras, L. Lovotti, E. Fernández**Impact of glyphosate on microbial biomass and metabolic activity in a vertic argiudoll from Argentina**

Eur. J. Soil Biol., 45 (2009), pp. 163-167

ArticleDownload PDFView Record in Scopus

[49]

T.H. Anderson, K.H. Domsch**The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils**

Soil Biol. Biochem., 25 (1993), pp. 393-395

ArticleDownload PDFView Record in Scopus

[50]

E.P. Odum**Trends expected in stressed ecosystems**

BioSciences, 35 (1985), pp. 419-422

CrossRef

[51]

M.D. Wallenstein, R.G. Burns**Ecology of extracellular enzyme activities and organic matter degradation in soil: a complex community-driven process**

R.P. Dick (Ed.), Methods of Soil Enzymology, Soil Science Society of America, Madison, Wisconsin, USA(2011), pp. 35-55

View Record in Scopus

[52]

K.H. Domsch, G. Jagnow, T.-H. Anderson**An ecological concept for the assessment of the side-effects of agrochemicals on soil microorganisms**

Residue Rev., 86 (1983), pp. 67-105

[53]

M.A. Sánchez-Monedero, C. Mondini, M.L. Cayuela, A. Roig, M. Contin, M. De Nobili**Fluorescein diacetate hydrolysis, respiration and microbial biomass in freshly amended soils**

Biol. Fertil. Soils, 44 (2008), pp. 885-890

CrossRefView Record in Scopus