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Herbicidal effect on the bio-indicators of soil health- A review

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Abstract: Soil microbial population, earth worms in soil, soil enzyme activity and organ carbon content in soil are considered as the bio indicators of soil health. They are used as indicators of soil health because of their active role in soil organic matter production, decomposition of xenobiotics and cycling of nutrients, ease of measurement and rapid response to changes in management practices. The assessment of soil health can be used to develop more sustainable crop production system. A number of herbicides have been introduced as pre and post emergence weed killer. The impact of herbicides on soil health depends on the soil type, type and concentration of herbicide used, sensitivity to non-target organisms and environmental conditions. The review elaborates the impact of herbicidal application on the biological indicators of soil health.

Keywords: Enzyme activity in soil, Earth worm population in soil, Herbicides, Soil microbial population, Soil organic carbon content

INTRODUCTION

Soil is a living dynamic system, its physical, chemical and biological condition influences food production, environmental efficiency and global balance (Doran and Zeiss, 2000). Soil quality is defined as the capacity of the soil to function within the ecosystem boundaries to sustain biological productivity, maintain environmental quality and promote plant and animal health (SSSA, 1997). When the biological processes proceeds rapidly without any interference then the soil is biologically active or in good health (Schaller, 2009). Soil microbial biomass, soil enzyme activity, earth worm population in soil and organic carbon content in soil are used as the indicators of soil health (Killham, 2002). They are used as bio indicators due to their relationship to soil biology, ease of measurement and rapid response to the changes in land management practices (Bandick and Dick, 1999). They also promptly respond to environmental changes and adequately reflect biological changes induced by pollution and contamination (Baćmaga *et al*., 2014; Cycoń *et al*., 2012; Panettieri *et al*., 2013). Changes in microbial composition and function will directly influence the rate of carbon and nutrient cycling in soil (Zak *et al*., 2003). Soil enzymes play a major role in the biochemical functioning of soils *viz*., soil organic matter production, the decomposition of xenobiotics, and the cycling of nutrients such as carbon (glucosidase), nitrogen (urease and protease), and phosphorus (phosphatase). Earthworms are also involved in the recycling of carbon and nitrogen in the soil by shredding the organic residues and stimulating the microbial decomposition.

In modern agriculture herbicides are considered to be the most effective and economic practice to control weeds for maximum production and productivity. New generation herbicides are characterized by high biological activity and selectivity, but inappropriate and continuous use may lead to adverse environmental effects (Morgante *et al*., 2012; Ayansina and Amusan 2013; Bai *et al*., 2013 and Baćmaga *et al*., 2014). Kucharski and Wyszkowska (2008) reported that their effect on soil environment depends mainly on the type of active substance, application rates, oxidation-reduction potential of soil, physicochemical properties of soil *etc*. The application of herbicides may cause significant changes in the soil microbial and earth worm population and enzyme activities in soil and thereby influencing the ecological balance of the soil. Changes in the soil environment caused by herbicides can be assessed, by analyzing the response of microorganisms, enzymatic activity and earth worm population to these xenobiotic substances. The consequence occurred due to herbicidal application on the biological indicators of soil health is reviewed to develop a sustainable crop production practice.

Effect of herbicides on soil microorganisms: Soil microorganisms play an important link in the soil-plant-herbicide-fauna-man relationship as they take part in the degradation of herbicides (Milosevic and Goveda-

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rica, 2002). Schloter *et al*. (2003) reported that, soil bacteria, actinomycetes, fungi, algae, protozoa, earthworms and some nematodes take part in various biochemical processes leading to the release of nutrients to the plants and are considered as the indicators of soil quality and health. These organisms have a vital role in maintaining the soil productivity; their number, activity and diversity may serve as the biological indicators of soil fertility (Rezende *et al*., 2004; Blagodatskaya and Kuzyakov, 2013).

Herbicides can cause both qualitative and quantitative changes in the soil microbial population (Saeki and Toyota, 2004). Herbicides not only affect the target weed but also affect the soil microorganism by altering the metabolic activities (Singh and Walker, 2006) and physiological and biochemical behavior (Hussain *et al*., 2009). Effect of herbicides on soil microbial population affects the rate of decomposition of celluloses and lignin in soil ecosystem (Osono and Takeda, 2007; Osono *et al*., 2008). Change in soil microflora has been considered as one of the possible reasons for the decline in rice cropping systems (Reichardt *et al*., 1998). Chauhan *et al*. (2006) reported that healthy population of microorganism can stabilize the ecological system in soil. Hence any changes in the population of microorganism will affect the ability of the soil to regenerate nutrients to support plant growth. The increased dependence of both pre-emergence and post-emergence herbicides for weed control in rice has led to concern about their toxicological behavior in rice field environment (Latha and Gopal, 2010).

Sensitivity to a given herbicide varies greatly among the different microbial species and strains. Stimulatory or depressive effect of herbicides on the microbial population may depend on the toxicity of applied herbicide (Abdel- Mallek *et a*l., 1994), type, concentration and mode of applied herbicide, environmental conditions, group of microorganisms, bioavailability and persistence (Zain *et al*., 2013). Soil properties like soil pH, organic matter, soil texture, inorganic nutrients present in the soil, soil temperature and soil moisture affects the soil microbial population and persistence of herbicides in the soil.

Effect of herbicides on bacterial population in soil: Total bacterial population in soil is an indicative of qualitative changes due to herbicide application. Adverse to no effect or stimulatory effect of herbicides on soil bacterial population was reported by several research workers (Mukhopadhyay 1980; Balasubramanian and Sankaran, 2001; Devi *et al*., 2008; Sebiomo *et al*., 2011) Consequent to herbicide application under field condition, an initial depressive effect in bacterial population for a short period followed by an increase in total bacterial number was observed, implying that initial depression could be due to the adverse impact on susceptible strains and subsequent increase could be due to the increase in the growth rate of resistant strains (Barman and Varshney, 2008). Chowdhury *et al.* (2008) opined that a decrease in activity of bacteria was observed immediately after the herbicide application due to their toxicity but later they degraded in the soil, the degraded products would be used by the bacteria that need carbon and nitrogen for cell proliferation. Breugelmans *et al*. (2007) reported that the herbicide, linuron was easily degraded by the bacteria, *Variovorax sp*. WDL1, *Comamonas testosteroni* WDL7 and *Hyphomicrobium sulfonivorans WDL6* and utilized carbon as energy source. The herbicide successor 550 SE at optimal dose (4 dm^3 ha^{-1}) increased the population of spore forming oligotrophic bacteria, organotrophic bacteria and *Azotobacter* in soil (Tomkiel *et al*., 2014). 2, 4-D exerted a negative influence on soil bacteria up to 15 days after spraying, while the influence was positive on fungal colonies. With advancement of time, the bacterial population also increased, suggesting the dissipation of the herbicide (Devi *et al*., 2008). Singh and Singh (2009) reported that on the day of herbicide spray, the viable count of bacteria was highest in weedy check and hand weeding treatment compared to herbicide treated plots *viz*., alachlor at 1.25 kg ha^{-1}, fluchloralin at 0.675 kg ha^{-1}, trifluralin at 0.75 kg ha^{-1}, pendimethalin at 0.75 kg ha^{-1} and oxyfluorfen at 0.25 kg ha^{-1} but at 20 days after spray the bacterial population in the herbicide treatments were at par with hand weeding treatment. After 8 days of application, a rapid increase in bacterial population was observed in plots treated with pendimethalin, oxyfluorfen and pretilachlor (Trimurthulu *et al*., 2015). No adverse impact on the population of bacteria was observed following the application of herbicide mixtures *viz*., bispyribac sodium + metamifop and penoxsulam + cyhalofop butyl at 15 days after sowing (DAS) in direct seeded puddled rice (Raj, 2016).

Effect of herbicides on fungal population in soil: Soil fungi widely distributed in the uppermost layer of soil is the dominant organism among the soil microbial group (Chauhan *et al*., 2006). Due to their ability to breakdown complex substances including herbicides, they are known to be extremely adaptable in different environment (Das *et al*., 2006). Fungi are most tolerant to unfavourable environmental conditions (Tomkiel *et al*., 2014). Herbicidal effect on fungal growth is highly specific with respect to herbicide type, dose, microbial and environmental condition (Hattori, 1973). Zain *et al*. (2013) reported that in sandy clay soil having pH of 4.1, the growth of *Mucor sp*. was inhibited more by herbicides, paraquat, glufosinate-ammonium and metsulfuron methyl than that of *Aspergillus sp*. and *Penicillium sp.* at two times their recommended doses. Glufosinate ammonium strongly inhibits the growth of *Trichoderma harziamu* and *Trichoderma longipilus* (Ahmad and Malloch, 1995) and *Magnaporthe grisea* and *Cochliobolus miyabeanus* (Ahn, 2008), where-as the glyphosate showed moderate growth inhibition effects on fungal species (Malik *et al.,* 1989). *Aspergillus sp.* and *Penicillium sp.* have been reported as the potential degraders of herbicides (Romero *et al*., 2009). Glyphosate, an organophosphorus compound is used a source of P, C and N by fungi (Van Eerd *et al*., 2003), resulting in an increase in fungal count (Ratcliff *et al*., 2006). Significant (P = 0.01) decline in fungal population was observed due to atrazine application (Sebiomo *et al*., 2011). Fungal count showed an increasing trend from 7^{th} to 28^{th} day of treatment of butachlor, pyrazosulfuron and glyphosate (Baboo *et al.*, 2013). In direct seeded rice, significantly higher fungal population was observed in the herbicide treatments *viz*., pendimethalin 0.75, butachlor 1.50, thiobencarb 1.50, anilofos 0.375, pretilachlor 0.75, oxadiargyl 0.09 and pyrazosulfuron-ethyl 0.015 kg ha^{-1} applied as pre-emergence and each followed by bispyribac 0.025 kg ha^{-1} at 30 days after sowing compared to control, at all stages of observation indicating the utilization of herbicides as source of C during the degradation process (Kaur *et al*., 2014).

Effect of herbicides on actinomycetes population in soil: Actinomycetes are also able to metabolize the xenobiotic compounds and utilize these compounds as source of energy. Actinomycetes degrade recalcitrant like lignocelluloses and other polymers in soil (Crawford, 1978; Jarerat and Tokiwa, 2001). They enhance the growth of the plants and protect the plants from phytopathogens by releasing enzymes and antibiotics into the rhizosphere soil (Doumbou *et al*., 2001). In glyphosate treated soil, increase in actinomycetes population was observed with time (Araujo *et al*., 2003). Martinez *et al*. (2008) reported that the herbicide, sulfentrazone stimulated the growth of actinomycetes in soil. Application of imazamox and benfluralin resulted in 25 to 64 per cent decline in actinomycetes population (Vischetti *et al*., 2004). Raj (2016) reported a reduction in the population of actinomycetes in the rhizosphere soil at 15 days after the application of bispyribac sodium + metamifop and penoxsulam + cyhalofop butyl, due to the tremendous increase in bacterial population. Pal *et al*. (2013) also made similar observation that reduction in actinomycetes population following the herbicide application might be due to the toxic effect of herbicide applied or due to the competitive influence of various microorganisms on the population of actinomycetes in the rhizosphere soil. Long term application of buctril super (bromoxynil) herbicide in wheat field, decreased the actinomycetes population by 29 per cent (Abbas *et al*., 2015). Sebiomo *et al*. (2011) observed a substantial decrease in actinomycetes population following the application of herbicides, paraquat, glyphosate and atrazine. No change in the population of actinomycetes was observed by the application of metsulfuron- methyl herbicide (He *et al*., 2006). Dayaram (2013) and Sasna (2014) also made similar observation that actinomycetes population in the herbicide treated plots did not vary much compared to pre-treatment count.

Effect of herbicides on soil enzyme activity: Soil enzyme activity is used as a good biological indicator of soil biogeochemical processes because of its involvement in organic matter decomposition (Sinsabaugh *et al*., 1991), organic matter formation, soil organic matter stabilization, catalyzing several reactions necessary for the life process of the microorganisms and recycling of nutrients (Dick *et al*., 1994). They are easy to measure and respond rapidly to changes in land management (Dick, 1997). Since they are sensitive to agrochemicals, they are the good markers for measuring the degree of pollution (Kuperman and Carreiro, 1997). Assay of soil enzymes can be used as good indicators of soil quality and health (Schloter *et al*., 2003) and may provide useful information on microbial activity in the soil (Andreoni *et al*., 2004). Due to greater microbial activity and release of root exudates and enzymes to the rhizosphere, enzyme activities are higher in the rhizosphere soil than in bulk soil (George *et al*., 2005; Villanyi *et al*., 2006). Herbicides can cause both qualitative and quantitative changes in soil enzyme activity (Sebiomo *et al*., 2011; Xia *et al*., 2012).

Effect of herbicides on Dehydrogenase enzyme activity: Dehydrogenase enzyme activity is soil is used as an indicator of biological activity in soil. It is an indicator of overall microbial activity, because it is an intracellular enzyme in all living microbial cells (Quilchano and Maranon, 2002; Stepniewska and Wolinska, 2005). It plays a major role in the biological oxidation of soil organic matter by transferring protons and electrons from substrates to acceptors (Sebiomo *et al*., 2011). Since these processes takes pace during the respiration pathway of microorganisms, it may give indications of the potential of the soil to support biochemical processes which are essential for maintaining soil fertility.

Dehydrogenase enzyme activity in soil is often used as the measure of any disruption caused by pesticides, trace elements or management practices to the soil (Reddy and Faza, 1989; Wilke, 1991; Frank and Malkomes, 1993). It can also be used as a parameter for assessing the side effects of herbicide treatments on the soil microbial biomass (Sebiomo *et al*., 2011). Dehydrogenase activity is usually higher under flooded than unflooded conditions, as most of the microorganisms responsible for dehydrogenase activity belong to obligate anaerobes (Baruah and Mishra, 1984; Tiwari *et al*., 1989; Makoi and Ndakidemi, 2008; Wolinska and Stepniewska, 2012).

The highest activity of dehydrogenase was observed at lower doses of pesticides, and the lowest activity at higher doses of pesticides (Baruah and Mishra, 1986). Hang *et al*. (2002) reported that the dehydrogenase enzyme activities were higher in soil samples treated with herbicides; the higher the concentration of butachlor, higher the dehydrogenase activity. Sebiomo *et al*. (2011) observed that application of atrazine, prime extra (a combination of atrazine and metolachlor) and glyphosate increased the dehydrogenase activity from 2nd to 6th week of application. Compared to control, dehydrogenase activity was significantly higher in field treated with butachlor and cyhalofop butyl each @ 1 kg ha^{-1} at 30, 45 and 60 days after transplanting (DAT) (Vandana *et al*., 2012). Application of pendimethalin and oxyflourfen @ 1 kg ha^{-1} and 0.1 kg ha^{-1} respectively along with one inter cultivation at 30 DAS and one hand weeding at 45 DAS recorded higher dehydrogenase activity at 20 and 40 DAS in maize (Nadiger *et al*., 2013). Based on the field experiments conducted at Thrissur, Kerala, Shitha *et al*. (2015) reported that dehydrogenase activity in soil was unaffected by the application of Round up and Glycel @ 6 and 12 mL L^{-1}. Combined application of bromoxynil + prosulfuron @ 1 mg kg^{-1} caused 74 per cent inhibition in dehydrogenase activity as compared to control (Pampulha and Oliveira, 2006). Similarly, Stepniewska *et al*. (2007) reported that application of fonofos @ 1.0 mg kg^{-1} caused 5 to 21 per cent decrease in dehydrogenase activity; however, 10 times higher concentration of the herbicide resulted in 17 to 44 per cent decrease in dehydrogenase activity compared to control.

Effect of herbicides on Urease enzyme activity in soil: Urease, an extracellular enzyme plays a major role in the hydrolysis of urea to NH_3 and CO_2. Urease is a constitutive enzyme found in a large number of microorganisms, especially in ureolytic bacteria and fungi (Bremner and Mulvaney, 1978). Its activity in soil is correlated with soil organic matter content (Beri *et al*., 1978). Aparna (2000) reported that, the higher availability of substrate nitrogen and other nutrients which promoted the urease activity. The amount of urease enzyme indicates the biological activity of soil (Reddy *et al*., 2011). Urease activity in soil depends on the microbial community, physical and chemical properties of the soil, particularly soil pH and temperature (Corstanje *et al*., 2007; Yang *et al*., 2006). Pal *et al*. (2013) reported a positive correlation between urease activity and microbial population in the soil. Urease enzyme is highly sensitive and is a useful indicator to evaluate the soil pollution (Srinivasulu and Rangaswamy, 2014).

Wang *et al*. (2007) reported that butachlor at higher concentrations (50 mg kg^{-1} and 100 mg kg^{-1}) inhibited the urease activity in soil. Inhibitory effect of higher doses of herbicide on urease enzyme activity decreased with time due to irreversible adsorption of herbicides on to the soil colloids, their partial degradation and or stabilization of microbial population in soil with time (Rao *et al*., 2012). Manual weeding and chemical control of weeds influence the urease activity in soil. Sole application of UPH-203 (Clodinofop propargyl) or in combination with Na-acifluorfen 10 % SL recorded better urease activity than control (Pal *et al*., 2013). The herbicide metribuzin (triazine herbicide) stimulates the activity of urease enzyme in soil (Santric *et al*., 2008). Urease activity in pyrazosulfuron treated soil at 25 g ha^{-1} showed an increasing trend from 7th day to 28th day of incubation (Baboo *et al*., 2013). Under unflooded condition, urease activity was consistently inhibited by pesticide treatments, whereas under flooded conditions all the treatments recorded higher urease activity (Rasool *et al*., 2014). Up to 13.6 per cent increase in urease enzyme activity was noticed when the herbicide Successor T 550 SE (pethoxamid + terbuthylazine) was applied at optimal dose to 40 fold of the recommended dose (Tomkiel *et al*., 2014).

Effect of herbicides on protease enzyme activity in soil: The breakdown of proteinaceous compounds in soil to simpler nitrogenous compounds is brought about by the protease enzyme in soil. The amount of this extracellular enzyme is indicative of the biological capacity of soil (Burns, 1982). The protease enzyme plays a major role in N mineralization and regulates the amount of N available for plant growth (Stevenson, 1986). NH_4-N accumulation in soil organic matter (Sardans and Penuelas, 2005; Tischer, 2005), the presence of proteolytic bacteria and proteinaceous substrate availability influences the protease enzyme activity in soil (Sardans *et al.*, 2008; Anjaneyulu *et al.*, 2011; Subrahmanyam *et al.*, 2011).

Both biotic and abiotic factors affect the protease activity in soil (Makoi and Ndakidemi, 2008). Protease enzyme activity is significantly affected by the type of herbicide, concentration of the herbicide and incubation period. The lowest activity of protease was observed in butachlor treated plot compared to 2, 4-DEE, pretilachlor and pyrazosulfuron ethyl at field rates of 1.0, 0.75, 0.3 and 0.025 g ha^{-1} and at 2, 5, 10 and 100 times field rate (Latha and Gopal, 2010). The protease activity in soil treated with butachlor, pyrazosulfuron and glyphosate showed an increasing trend from 7th to 28th day of incubation (Baboo *et al*., 2013). Rasool *et al*. (2014) reported that, the protease activity was stimulated initially by butachlor application but decreased towards the end of the experiment under unflooded condition, but under flooded condition, the effect was stimulatory. The herbicide nicosulfuron had a stimulating effect on the protease enzyme in loamy and sandy loam soil (Santric *et al*., 2014). Herbicide mixtures, bispyribac sodium + metamifop (60, 70, 80 and 90 g ha^{-1}) and penoxsulam + cyhalofop butyl (120, 125, 130 and 135 g ha^{-1}) recorded comparable or significantly higher values of protease enzyme activity at 30 DAS (15 days after herbicide application), 60 DAS (45 days after herbicide application) and at harvest stage as compared to weedy check or hand weeding twice (Raj, 2016).

Effect of herbicides on β glucosidase enzyme activity in soil: β glucosidase enzyme plays a major role in the transformation or decomposition of organic matter in soil. Both fungi and bacteria secrete this extracellular enzyme which constitutes an important part of the soil matrix as abiotic enzyme (Sinsabaugh and Moorhead, 1994). β glucosidase enzymes releases low molecular sugars from organic matter, the important energy sources of microorganisms (Tabatabai, 1994; Bandick and Dick, 1999). It is a soil quality indicator and gives the reflection of past biological activity and the capacity of soil to stabilize the soil organic matter and can be used to detect the management effect on soil (Bandick and Dick, 1999; Ndiaye *et al*., 2000). Depending on the nature and concentration of herbicide, incubation period and soil condition, application of herbicide influence the β glucosidase activity in soil (Hussain *et al*., 2009).

Soil treated with butachlor and pretilachlor recorded higher levels of β glucosidase activity (Saha *et al*. 2012). Sofo *et al*. (2012) reported that application of triasulfuron at ten-fold the field rate increased the β glucosidase activity in soil. Significant increase in β glucosidase activity in soil (5.6 to 29.4 per cent) was observed at 7 to 14 days after treatment with two highest concentrations (3.0 and 30.0 mg) of nicosulfuron, a sulfonyl urea herbicide (Santric *et al*., 2014). Application of carfentrazone ethyl at optimal dose increased the activity of β glucosidase in soil (Tomkiel *et al*., 2014). Latha and Gopal (2010) pointed out that, when pyrazosulfuron, butachlor and pretilachlor were applied at 100 times field rate the β glucosidase activity was inhibited by 16.21, 21.32 and 10.09 per cent, respectively over control, whereas when applied at field rate, inhibition of β glucosidase activity was only 5.64, 7.47 and 3.59 per cent, respectively over control.

Effect of herbicides on Acid phosphatase activity in soil: Acid phosphatase is an extracellular enzyme produced by many soil microorganisms and it plays a major role in the hydrolysis of organic P to inorganic P. It can be a good indicator of organic phosphorus mineralization and biological activity of soil (Dick and Tabatabai, 1993). Acid phosphatase enzyme is present in all microorganisms and increase in acid phosphatase activity is mainly due to increase in bacterial biomass (Rao *et al*., 2012). Phosphatase activity is highly correlated with organic matter content of the soil (Jordan and Kremer, 1994; Aon and Colaneri, 2001). Acid phosphatase enzyme plays a major role in the P cycling in the soil and P acquisition by plants and microorganisms (Schneider *et al*., 2001). Phosphatase enzyme is mainly concentrated in the surface soil layer and rhizosphere soil (Tarafdar *et al*., 2001).

The factors that influence the rate of synthesis, release and stability of phosphatase enzymes in soil are soil pH (Tabatabai, 1994; Martinez and Tabatabai, 2000), management practices (Wright and Reddy, 2001; Ndakidemi, 2006), crop and species (Ndakidemi, 2006) and soil microbial community (Renella *et al*., 2006; Renella *et al*., 2007).

Manual weeding and chemical weed control significantly influence the acid phosphatase activity in soil. Bacmaga *et al*. (2012) reported that, the herbicide Aurora 40 WG (carfentrazone-ethyl) had no negative effect on acid phosphatase activity in soil. Rao *et al*. (2012) stated that, lowest concentration of oxadiargyl *i.e*., 0.75 kg ha^{-1} recorded the highest phosphatase activity, whereas highest concentration of oxadiargyl (1.5 kg ha^{-1}) recorded the lowest phosphatase activity. Reduction in acid phosphatase activity with herbicide application was reported by several workers (Sukul, 2006; Yu *et al*., 2006; Jastrzebska and Kucharski, 2007). According to Majumdar *et al*. (2010), the weedy check and hand weeding treatments recorded significantly higher acid phosphatase activity than herbicide treatments. It was also pointed out that compared to initial status; herbicide application reduced the acid phosphatase activity by 16.7 to 27.7 per cent at 7 days after herbicide application.

Effect of herbicides on soil organic matter: Soil organic carbon constitute 58 per cent of the soil organic matter (Bianchi *et al*., 2008), and it is an indicator of soil quality (Adeboye and Bala, 2011). It is the important constituent of soil as it provides energy to the microorganisms and release nutrients to the plants through mineralization process (Abbas *et al*., 2015).

Fate of herbicide in the soil is greatly affected by the presence of organic matter by aiding their disappearance (Ayansina and Oso, 2006). Decline in organic carbon content due to long term application of bromoxynil was reported by Abbas *et al*. (2015) and atrazine and metolaclor by Ayansina and Oso (2006). To overcome the injurious impact of herbicides, microbes rapidly decompose the organic matter for the energy resulting in loss of organic carbon in the form of CO_2 might be the reason for the decline in organic carbon content. Decline in enzyme activity and organic carbon content in soil due to herbicide application was reported by Niemi *et al*. (2009). Baboo *et al*. (2013) reported significant reduction in organic carbon level in soil after the application of herbicide. Root exudates and hormones are liberated in to the rhizosphere which increases the organic carbon in the soil. So the death of weeds due to herbicide application results in decline in organic carbon in the soil (Bhattacharyya *et al*., 2013). Mishra *et al*. (2013) revealed that significant quantity of organic matter is accumulated in weedy check and hand weeded conditions compared to herbicides. Following the application of bromoxynil in wheat field for period of 10 years, a reduction of 28.57 and 21.56 per cent in total organic carbon content was observed in Shah Sadar Din and Shadan Lund, two different locations of study (Abbas *et al*., 2015).

The herbicides, pendimethalin, oxyfluorfen and pretilachlor increased the organic carbon content in soil. Presence of herbicides in the rhizosphere of plant influenced the physiological activities of the host plant root system which led to the release of more quanta of exudates and indirectly resulted in higher level of organic carbon in the rhizosphere soil (Trimurthulu *et al.* 2015).

Effect of herbicides on earth worm population in soil: Earthworms play a major role in soil quality by shredding residues, stimulating microbial activity and decomposition, improving soil fertility and soil physical properties *viz*., soil aggregation and infiltration. Since they play a major role in the recycling of carbon and nitrogen in the ecosystem, they are used as bio indicators of soil fertility (Callahan, 1988). Earthworms can also be used as biomarkers for toxicity and bioaccumulation assessment (Nusetti *et al*., 1999; Gobi *et al*., 2004).

Several workers reported that herbicides have adverse effect on the survival of earthworms, as well as its growth and reproduction (Helling *et al*., 2000; Zhou *et al*., 2007; Correia and Moreira, 2010).

Some studies revealed that herbicides are harmless to earthworms. Studies conducted by Monsanto researchers reported that no adverse effects were observed when earth worms were exposed to glyphosate residues in soil at rates equal to or greater than labelled rates (Giesy *et al*., 2000). Application of simazine has no toxic effect on earthworms (Lydy and Link, 2003). Isoproturon, the most widely used herbicide in wheat did not cause any lethal effect on earthworms (*Lumbricus terrestris* L.) even applied at a high concentration of 1.4 g kg^{-1} of soil (Mosleh *et al*., 2003). Mele and Carter (1999) reported that herbicide application had no influence on earthworm species richness. Yadav (2006) reported no significant reduction in the earthworm population as compared to the initial status in the pyrazosulfuron treated plots after harvest. Glyphosate application had no adverse impact on the growth, behavior and mortality of the earthworm, *Pheretima carnosus* (Kaneda *et al*., 2009). Correia and Moreira (2010) revealed that earthworms exposed to soil spiked with glyphosate were all alive throughout the study period. Oluah *et al*. (2010) reported that, the mortality of earthworm, *Nsukkadrilus mbae* ranged from 37.8 to 80. 5 per cent when exposed to atrazine. Singh and Singh (2015), pointed out that the toxicity of 2, 4-D on earthworm, *Eutyphoeus waltoni* was both time and dose dependent. Shitha *et al.* (2015) revealed that either round up or glycel had no negative effect on the multiplication of earthworms. Post emergence application of herbicide mixtures, did not cause any reduction in the number of earth worms present in soil (Raj, 2016).

Conclusion

Environmental safety of herbicides can be determined by assessing the biological indicators of soil health *viz*., microbial and earth worm population in soil, soil enzyme activity and soil organic carbon content in soil because they take part in various biological processes taking place in the soil and respond to even a minute changes in the land management practices . When herbicides are applied to soil, they may increase or decrease the soil microbial and earth worm population, earth worm activity in soil and soil organic carbon content. Bu the effect will depend mainly on the type of active substance present, application rates, oxidation-reduction potential of soil and physicochemical properties of soil. The adverse impact to environment usually occurs when the herbicides are applied at high dose rate and used indiscriminately. An ideal herbicide is one which provides good season long weed control effect and disintegrates before the crop season without leaving any toxic residue in soil. Most of the field study results revealed that herbicides at recommended dose pose minimum adverse impact to the environment, since most of them have no to stimulatory effect on soil bacteria, fungi, actinomycetes, soil enzymatic activity and earth worm population in soil. Compared to pre-emergence herbicides, post emergence herbicides had no inhibitory effect on soil health under field conditions. Though an initial depression in the population of microorganism and enzyme activity immediately after the application of herbicides, they will restore to the normal value with in a short period. The depression is only transitional. Decline in organic carbon content following herbicide application due to death of weed was reported by some researchers. While others reported that herbicide application enhanced the organic carbon content in the rhizosphere soil due to the release of more amounts of hormones and root exudates from the host plant root system. Soil enzymatic activity was also found to be stimulated by the application of certain herbicides, while some herbicides had inhibitory effect. Field study results revealed that following herbicide application no significant reduction in the earth worm population was observed as compared to the initial status. In short the studies on the effect of herbicides on the biological indicators of soil health revealed the environmental safety of the applied herbicide.

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