



Pesticides effect on soil microbial ecology and enzyme activity- An overview

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Abstract: In modern agriculture, chemical pesticides are frequently used in agricultural fields to increase crop production. Besides combating insect pests, these insecticides also affect the activity and population of beneficial soil microbial communities. Chemical pesticides upset the activities of soil microbes and thus may affect the nutritional quality of soils. This results in serious ecological consequences. Soil microbes had different response to different pesticides. Soil microbial biomass that plays an important role in the soil ecosystem where they have crucial role in nutrient cycling. It has been reported that field application of glyphosate increased microbial biomass carbon by 17% and microbial biomass nitrogen by 76% in nine soils at 14 days after treatment. The soil microbial biomass C increased significantly upto 30 days in chlorpyrifos as well as cartap hydrochloride treated soil, but thereafter decreased progressively with time. Soil nematodes, earthworms and protozoa are affected by field application rates of the fungicide fenpropimorph and other herbicides. Thus, there is need to assess the effect of indiscriminate use of pesticides on soil microorganisms, affecting microbial activity and soil fertility.

Keywords: Herbicides, Insecticides, Microbial biomass, Mycorrhiza, Soil enzymatic activity

INTRODUCTION

Pesticides are the important agrochemicals used in agricultural system for prevention of crops from pests. Pesticides are often applied several times during one crop season and a part always reaches the soil. The wide use of pesticides has created numerous problems, including the pollution of the environment. The influence of pesticides on soil microorganisms is dependent on physical, chemical and biochemical conditions, in addition to nature and concentration of the pesticides (Aurelia, 2009; Sethi *et al.*, 2013). In many studies it was demonstrated that microorganisms are capable to grow in the presence of several commercial pesticides. Catabolism and detoxification metabolism occur when soil microorganism uses the pesticide as a carbon and energy source. Most pesticides work by poisoning pests. A systemic pesticide moves inside a plant following absorption by the plant. With insecticides and most fungicides, this movement is usually upward (through xylem) and outward (Shinde *et al.*, 2015). Systemic insecticides, which poison pollen and nectar in the flower may kill bees and other needed pollinators. Subclasses of pesticides include herbicides, insecticides, fungicides, rodenticides, pediculicides and biocides (Stoytcheva, 2011). Pesticides would pollute air, soil and water resources, contaminate the food chain and disrupt ecosystem balance. For example, high concentrations of pesticides in soil may influence processes such as plant growth and the activity and diversity of biotic populations. Continuous use of pes-

ticides may accumulate appreciable quantities of pesticides and their degradation products in the soil ecosystem (Kumar *et al.*, 2012). Pesticides that disrupt the activities of soil microbes may affect the nutritional quality of soils, resulting in serious ecological consequences (Handa *et al.*, 1999).

The application of pesticides starts from the pre-sowing stage of crop growth. The indiscriminate use of pesticides disturbs the soil environment by affecting flora and fauna including microflora of soil, and also the physico-chemical properties of the soil like pH, salinity, alkalinity leading to infertility of soil (Sarnaik *et al.*, 2006). When pesticides are applied, the possibilities exist that these chemicals may have certain effects on non-target organisms, including soil micro organisms. Pesticides in the soil affect the non-target and beneficial micro-organisms (Bhuyan *et al.*, 1992) and their activities which are essential for maintaining soil fertility (Schuster and Schroder, 1990). The microbial biomass plays an important role in the soil ecosystem where they fulfill a crucial role in nutrient cycling and decomposition (De-Lorenzo *et al.*, 2001). Modern agriculture worldwide uses a variety of pesticides including insecticides, nematicides, herbicides and fungicides to optimize crop production. However, continuous application of pesticides may result in soil pollution threatening processes driven by soil micro organisms and thereby, affecting soil fertility (Lopez *et al.*, 2002; Cycon *et al.*, 2006).

With increased pesticides use, questions on potential effects regarding public health and the environment

has developed. Many of the studies only examined initial effects of pesticides in laboratory microcosms, rather than in integrated system over long periods and at larger scales. Soil is not only a medium for agricultural production, but is often viewed as a filter and processor for xenobiotics; and how a soil is managed can determine its ability to function in this capacity (Locke and Zablotowicz, 2004).

Much of the research on herbicides effects on soil biota has been done overseas. It was reported that increase in reliance on herbicides in zero-till systems in Australia has no doubt had impacts on soil biota. The application of chlorosulfuron increased root disease by *Rhizoctonia solani* but had no effect on take-all levels (Rovira and McDonald, 1986). Glyphosate and chlorosulfuron have each been associated with increased levels of Pythium root rot in barley seedlings (Blowes, 1987), and take-all fungus (Mekwatanakarn and Sivasithamparam, 1987). If a fungicide improves crop growth though eliminating foliar or soil borne diseases, than increased organic matter in the system will generally boost microbial activity. Fungicides may however have direct effects on the non target organisms particularly these saprobic and symbiotic soil-borne fungi. Benomyl for instance is particularly toxic to mycorrhizal fungi (Smith *et al.*, 2000) which could have implications for the nutrition of the plants. Foliar – applied sprays that miss the target, leaves and spray drift could also cause undesired/unintentional impacts on soil biota. Glyphosate has stimulated populations of fungi and actinomycetes with general increase in over all microbial activity even though bacterial populations were reduced (Araujo *et al.*, 2003). Cypermethrin and monocrotophos had adverse effects on soil bacteria while fenvalerate had very low effect on the soil microbes (Rangaswamy and Venkateswarlu, 1992; Ajaz *et al.*, 2005).

The toxicity level of a pesticide depends on the deadlines of the chemical, the dose, the length of exposure, and the route of entry or absorption by the body (Kumar, 2015). Pesticide degradation in soil genetically results in a reduction in toxicity, however some pesticides have breakdown products (metabolites) that are more toxic than the parent compound. Pesticides are classified according to their potential toxicity to humans and other animals and organisms, as restricted use and general use (NRCS, 1998; Damalas and Eleftherohorinos, 2011).

Pesticides effects on soil microbial biomass: Measurement of total soil microbial biomass (typically measured as carbon or nitrogen in biomass) is an extremely useful tool for interpreting soil biological quality. Specific soil microflora constituents can be ascertained based on abundance of specific cellular components. Microbial biomass is a part of organic matter in soil that constitutes living microorganisms smaller than 5-10 cubic micrometers and it is the fraction of soil organic matter that is sensitive to management prac-

tices and pollution (Powlson, 1994). Soil microbial biomass is an important attribute of soil quality as well as crop ecology (Doran and Parkin, 1994; Beelen and Doelman, 1997). Microbial biomass is a standardized component of ecotoxicity assessment in OECD guidelines for pesticides registration.

Glyphosate use with in North and South America has increased dramatically with use of transgenic herbicide-resistant crops, thus effects on soil ecological processes are of concern. Haney *et al.* (2000) observed no effect of glyphosate on soil microbial biomass 3 days after application. However, Haney *et al.* (2002), demonstrated that field application rates of the isopropylamine glyphosate salt increased microbial biomass carbon (17%) and microbial biomass nitrogen (76%) in nine soils at 14 days after treatment. Hart and Brookes (1996) showed that glyphosate increased microbial biomass carbon (16%) 56 days after an autumn field application. In field trials, normal and tenfold field application rates of rimsulfuron had no effect on biomass carbon. However, under laboratory conditions, doses 10 and 100X field application rates elicited reduced soil biomass (Perucci and Scarponi, 1996).

Soil microbial biomass measurements has been reported to give an early indication of long-term changes in soil organic matter content, long before such changes could be measured by conventional techniques (Hart and Brookes, 1996). Microorganisms form a vital part of the soil food web, therefore microbial biomass is considered to be a measure of potential microbiological and ecosystem functioning (Rath *et al.*, 1998). However for proper understanding of ecosystem functioning and determining soil disturbances because of various agricultural management practices, microbial activities must also be determined (Nannipieri *et al.*, 2003) along with microbial activity in soil has been reported as a criteria for evaluating pesticide toxicity (Jones and Ananyeva, 2001).

Chemical pesticides had made a great contribution to the fight against pests and diseases. However, their widespread and long-term use resulted in insecticide resistance and biomagnifications of insecticides, which in turn resulted in restrictions on their export. Problems, like soil and water contamination and dramatic increase of the harmful residues in many primary and derived agricultural products arose, which endangered both the general environment and human health. Microbial and biochemical parameters of soil are choice indicators of soil quality evaluations (Winding *et al.*, 2005) because of their early responses to soil disturbances than those of the physical and chemical parameters. The study was therefore initiated to evaluate the effect of pesticides and biopesticides on soil microbial biomass carbon in soil at controlled laboratory conditions. Piotrowska-Seget *et al.* (2008) performed a laboratory study to assess the impact of applying successive doses of oxytetracycline (bactericide) or Captan (fungicide) on microbial biomass and activity.

They reported that both oxytetracycline and Captan significantly decreased the numbers of culturable bacteria, although total bacterial biomass was not affected. The study indicated that oxytetracycline or Captan application may negatively affect non-target soil microorganisms and their activities. The soil microbial biomass C increased significantly upto 30 days in chlorpyrifos as well as cartap hydrochloride treated soil, but thereafter decreased progressively with time (Kumar *et al.*, 2012). Comparing the pesticides (Cypermethrin, Malathion, Victor, Monocil and Taffor) and biopesticides Sethi and Gupta (2013) reported in Victor treated soil, a drastic decrease in microbial biomass C was observed as compared to others. However, MBC content increased with time in biopesticide treated soil.

Pesticides effects on soil fauna: Soil fauna (e.g. earthworms, nematodes, microarthropods, protozoans) are important in organic matter (OM) transformations and soil structure formation, and are useful bio-indicators to study Xenobiotic ecotoxicity in soil. Effects of long-term benomyl application in a tall grass prairie were assessed on nematode populations (Smith *et al.*, 2000). Benomyl had no significant effect on herbivores, but significantly reduced certain fungal feeders (Tylenchidae) by 13% and predatory nematodes (Dorylamidae) by 33%. Soil nematodes, earthworms and protozoa are affected by field application rates of the fungicide fenpropimorph and other herbicides. Application of fungicide, carbendazim resulted in reduction of various species of soil fungi extensively in first 20 days (Aggarwal *et al.*, 2005). It was reported that Prosulfuron, an herbicide inhibited N₂O and NO production by the bacteria (Kinney *et al.* 2005). They also reported that Mancozeb and chlorothalonil inhibited N₂O and NO production. Gupta (1994) reported reductions of soil protozoa due to recommended rates of 2,4-D, simazine, diuron, monuron, cotoron. However, increase in protozoa attributed to stimulation of bacteria and fungal populations as herbicide is decomposed. Diazinon decreased protozoan populations (Ingham and Coleman, 1984). Dodd and Jeffries (1989) reported decrease in mycorrhizal fungi when the application of herbicides was done. Earthworms were also affected in general by application of herbicides. Mele and Carter (1999) however reported no effect on earthworms in top 10 cm soil layer. Fraser *et al.* (1994) found that earthworms were extremely sensitive to organophosphate and carbamates and less sensitive to organochlorines. Copper oxychloride used as fungicide in orchards is found to be very toxic for earthworms (Lee, 1985). Pandey and Singh (2004) reported that application of chlorpyrifos reduced bacterial population while there was significant increase in fungal population.

Pesticides effects on Mycorrhiza: Symbiosis of mycorrhizal fungi with plants is another mutualistic plant-microbial association. Mycorrhizal roots have an al-

tered morphology that enhances nutrient and water uptake. Fumigants used as nematicides and fungicides can profoundly influence mycorrhizal establishment. Mycorrhizal fungi can be relatively susceptible to certain fungicides – particularly when the fungicide is applied to the seed or into the soil; while other fungicides can stimulate mycorrhizal growth. Most foliar fungicide sprays (except those that are systemic – e.g. bayleton, benomyl, captan, etc), when applied properly and at recommended rates generally do not affect Mycorrhiza – as the Mycorrhiza in soil do not come in contact with the fungicide. For example, in potatoes, no effect has been observed on Mycorrhiza in programs that have included fungicides Maxim & Amistar, Metham Sodium (100L/acre on loamy soil, 300L/acre on sand). Bravo and Mancozeb (Manebe) – has shown variable results – usually compatible as a foliar – but poor as a seed treatment. Insecticides – Nema-cur has no effect on Mycorrhiza, chlorpyrifos has no effect at low rate, but tends to suppress at high rate; whereas Phenylpyrazole inhibits Mycorrhiza (Ayman *et al.*, 1996; Storer, 2012).

Pesticides effects on soil fertility: Some pesticides developed to boost crop yields could affect the soil fertility. In soil, the native and applied nutrients are transformed or mineralized for plant availability mediated through soil microbes. Common pesticides block the chemical signals that allow nitrogen-fixing bacteria to function. Over time, soils surrounding treated plants can become low in nitrogen compounds, so more fertilizer is needed to produce the same yield (Fox *et al.*, 2007).

Sustainable agricultural practices often use crop rotation: growing a different crop in the same soil each year. Alternating crops, such as beans or clover – with crops like wheat, that don't fix nitrogen, enables soils to enhance nitrogen levels routinely. Leguminous plants contain root nodules that use soil bacteria to fix nitrogen, a process that converts atmospheric nitrogen into useful compounds like ammonia (Kumar, 2015). Several common pesticides were tested on leguminous alfalfa plants, relying on the plants' nitrogen-fixing bacteria to provide the nutrients. The insecticides methyl parathion (not used in the UK, but widely used throughout the world, and registered in at least 38 countries) and DDT (which was banned by the World Health Organization for almost 30 years, before being reinstated in 2006 as an effective intervention against malaria) showed a decrease in crop yield of about 20 per cent. Treatment with pentachlorophenol, showed a decrease in crop yield of over 80 per cent (Fox *et al.*, 2007).

The insecticides DDT, methyl parathion, and especially pentachlorophenol have been shown to interfere with legume-rhizobium chemical signaling. Reduction of this symbiotic chemical signaling results in reduced nitrogen fixation and thus reduces crop yields (Rockets, 2007). Root nodule formation in these plants

Table 1. Effect of pesticides on enzyme activity in soil.

Enzyme activity	Effect	References
Nitrogenase	It is observed that the decrease in total nitrogenase activity (measured from pots sown with <i>Pisum sativum</i> plants) with the application of herbicides. Repeated applications of pesticides significantly stimulated rhizosphere-associated nitrogenase activity.	Singh and Wright (1999) Kanungo <i>et al.</i> (1995) Patnaik <i>et al.</i> (1995, 1996).
Dehydrogenase	It is reported that the effect of organophosphate insecticide (quinalphos) on dehydrogenase activity (DHA) in soil and observed 30% ($p < 0.05$) inhibition in DHA after 15 days.	Mayanglambam <i>et al.</i> (2005)
Urease	Increase or decrease in urease activity following various pesticide applications.	Antonious (2003); Chen <i>et al.</i> (2001); Ingram <i>et al.</i> (2005); Nowak <i>et al.</i> (2004)

saves the world economy \$10 billion in synthetic nitrogen fertilizer every year (Fox *et al.*, 2007; Kumar, 2015).

Effect of pesticides on soil enzyme: Soil contains free enzymes, immobilized extracellular enzymes, and enzymes within microbial cells (Mayanglambam *et al.*, 2005). They are indicator of biological equilibrium (Frankenberger and Tabatabai, 1991), fertility (Schuster and Schroeder, 1990; Nannipieri, 1994; Antonious, 2003), quality (Dick, 1994; Bucket and Dick, 1998), and changes in the biological status of soil due to pollution (Nannipieri *et al.*, 1990; Nannipieri and Bollag, 1991; Schaffer, 1993; Trasar-Cepeda *et al.*, 2000).

The role of soil enzymes and their activities are defined by their relationships with soil and other environmental factors (e.g., acid rain, heavy metals, pesticides, and other industrial chemicals) that affect their activities (Burns, 1982). Pesticides reaching the soil may disturb local metabolism or enzymatic activities (Topp *et al.*, 1997; Engelen *et al.*, 1998; Liu *et al.*, 2008). Negative impact of pesticides on soil enzymes like hydrolases, oxidoreductases and dehydrogenase activities has been widely reported in the literature (Perucci and Scarponi, 1994; Ismail *et al.*, 1998; Monkiedje and Spittler, 2002; Menon *et al.*, 2005). The impact of pesticide (insecticide, herbicide and fungicide) application on the health of paddy field soil showed a decrease in soil dehydrogenase activity with increased pesticide concentrations and toxicity increased in the order insecticide > fungicide > herbicide (Subhani *et al.*, 2002).

Nitrogenase is the enzyme used by organisms to fix atmospheric nitrogen gas (N_2). Application of pesticides affects the efficiency and activity of nitrogenase enzyme. Singh and Wright (1999) observed a decrease in total nitrogenase activity (measured from pots sown with *Pisum sativum* plants) with the application of herbicides (Table 1). Adverse effects of pesticides have also been reported on nitrogenase activities of N_2 -fixing bacteria, purple nonsulfur bacteria, methylotrophic bacteria, and cyanobacteria (Chalam *et al.*, 1997; Martinez Toledo *et al.*, 1998; Hammouda, 1999; Durska, 2004). In contrary, repeated applications of pesticides significantly stimulated rhizosphere-associated nitrogenase activity (Kanungo *et al.*, 1995; Patnaik *et al.*, 1995, 1996).

al., 1995, 1996).

Dehydrogenases occur intracellularly in all living microbial cells and it is linked with microbial respiratory processes (Bolton *et al.*, 1985). Dehydrogenase activity in soil is an indicator of overall microbial activity of soils. Mayanglambam *et al.* (2005) studied the effect of organophosphate insecticide (quinalphos) on dehydrogenase activity (DHA) in soil and observed 30% ($p < 0.05$) inhibition in DHA after 15 days. DHA was recovered after 90 days of treatment which may be due to adaptation of soil microbes to counter the effect of chemical stress in hostile conditions.

Hydrolases are of particular importance on account of their role in the soil nitrogen, phosphorus, carbon, and sulfur cycles (Megharaj *et al.*, 1999). Urease is an enzyme that catalyzes the hydrolysis of urea into CO_2 and NH_3 and is a key component in the nitrogen cycle in soils. Urease activity is found in a large number of soil bacteria and fungi (Sarathchandra *et al.*, 1984). Phosphatase is an exocellular enzyme produced by many soil microorganisms and is responsible for the hydrolysis of organic P compounds to inorganic P (Monkiedje *et al.*, 2002). Several researchers have shown either unchanged, increase or decrease in urease activity following various pesticide applications (Antonious, 2003; Chen *et al.*, 2001; Ingram *et al.*, 2005; Nowak *et al.*, 2004).

Effect of pesticides on carbon and nitrogen mineralization: Carbon mineralization is an important parameter for assessing side effects of pesticides (Sommerville, 1987; Alef, 1995). Haney and Senseman (2000) observed that herbicide treatment significantly stimulated C and N mineralization in soil. Chen *et al.* (2001) compared the effect of benomyl, captan and chlorothalonil on soil nitrogen dynamics in laboratory incubations with or without additions of organic materials. Both nitrogen mineralization and nitrification rates were influenced by all fungicides, with captan eliciting the greatest influence on mineralization rates. Captan increased soil NH_4-N , whereas benomyl or chlorothalonil had little impact. Martinez-Toledo *et al.* (1998) showed that captan (2-10 kg/ha) inhibited nitrifying bacteria in four soils (50-90%) during a 30 day study. Applying bensulfuron at normal field rates had no effect on nitrification, whereas cinosulfuron transiently inhibited nitrification after 1 week, but had

no effect after 4 weeks. Nitrogen mineralization processes, such as ammonification and nitrification, are also affected by the application of pesticides, with the former being inhibited less because it is carried out by a vast diversity of microflora. Odokuma and Osuagwu (2004) have shown that the organochlorine pesticides Lindane and dieldrin were more toxic than the organophosphate pesticides pirimphos methyl and malathion to *Nitrosomonas*, *Nitrobacter* and *Thiobacillus*.

Pesticide degrading microbial populations: It is well documented that with certain pesticides, repeated applications can promote microbial populations capable of selectively degrading that pesticide. The capability of a soil for accelerated degradation might limit the use of that pesticide or related pesticides to control a particular pest. Bacterial isolates can degrade various organophosphorus pesticides (Kanekar *et al.*, 2004). *Pseudomonas spp.*A1113 uses dimethoate and parathion as sole source of carbon and hence grows on the minimal medium and can be used for decontamination of pesticide polluted areas (Shinde *et al.*, 2015).

Conclusion

Chemical pesticides had made a great contribution to the fight against pests and diseases. Soil contamination and dramatic increase of the harmful residues in many primary and derived agricultural products arose, which endangered both the general environment and human health. Microbial and biochemical parameters of soil are choice indicators of soil quality evaluations. Pesticides in the soil affect the non-target and beneficial micro-organisms and their activities which are essential for maintaining soil fertility. Soil bacteria, fungi and almost all flora and fauna populations were reported to reduce and also soil microbial activity, biomass C and N mineralization got affected due to use of chemical pesticides. Judicious use of chemicals and use of bio-pesticides needs to be promoted to save soil health vis-à-vis human health.

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