

Impact of pesticides on soil microbiological parameters and possible bioremediation strategies

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Abstract Intensive agriculture is spectacularly successful since last couple of decades due to the inputs viz; fertilizers and pesticides along with high yielding varieties. The mandate for agriculture development was to feed and adequate nutrition supply to the expanding population by side the agriculture would be entering to into new area of commercial and export orientation. The attention of public health and proper utilization natural resources are also the main issues related with agriculture development. Concern for pesticide contamination in the environment in the current context of pesticide use has assumed great importance [1]. The fate of the pesticides in the soil environment in respect of pest control efficacy, non-target organism exposure and offsite mobility has been given due consideration [2]. Kinetics and pathways of degradation depend on abiotic and biotic factors [6], which are specific to a particular pesticide and therefore find preference. Adverse effect of pesticidal chemicals on soil microorganisms [3], may affect soil fertility [4] becomes a foreign chemicals major issue. Soil microorganisms show an early warning about soil disturbances by foreign chemicals than any other parameters.

But the fate and behavior of these chemicals in soil ecosystem is very important since they are degraded by various factors and have the potential to be in the soil, water etc. So it is indispensable to monitor the persistence, degradation of

pesticides in soil and is also necessary to study the effect of pesticide on the soil quality or soil health by in depth studies on soil microbial activity.

The removal of metabolites or degraded products should be removed from soil and it has now a day's primary concern to the environmentalist. Toxicity or the contamination of pesticides can be reduced by the bioremediation process which involves the uses of microbes or plants. Either they degrade or use the pesticides by various co metabolic processes.

Keywords Pesticide · Degradation · Soil Microbial Biomass-C · Soil respiration · FDA · Bioremediation

Introduction

The increasing global population and higher demand of food leads to increasing and sustainability of food production through intensive agriculture, attention of public health and proper utilization natural resources. The improvement of agriculture with advanced agricultural technology to meet this demand, keeping soil in its productive quality plays a dominant role for much of today's productivity.

Concern for pesticide contamination in the environment in the current context of pesticide use has assumed great importance [1]. The fate of the pesticides in the soil environment in respect of pest control efficacy; non-target organism exposure and offsite mobility has become a matter of environmental concern [2] potentially because of the adverse effects of pesticidal chemicals on soil microorganisms [3] may affect soil fertility [4]. An ideal pesticide should be toxic only to the target organism, biodegradable and undesirable residues should not affect nontarget surfaces.

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Due to continuous use of pesticides, appreciable quantities of pesticides and their degraded products may accumulate in the soil ecosystem. Microbes and plants are among the most important biological agents that remove and degrade waste materials to enable their recycling in the environment. Soil microflora, mainly bacteria, fungi, algae and protozoa makes a valuable contribution in making the soil fertile through their primary catabolic role in the degradation of plants and animal residues in the cycling of the organic, inorganic nutrients content of soil. Pesticide that disrupt the activities of the soil microorganisms could be expected to affect the nutritional quality of soils and would therefore, have serious ecological consequences [5].

Pesticide applied in environment are transformed in biological and non biological processes into one or more transformation products. These transformations are carried out by different mechanisms through physical, chemical and biological agents in which microorganisms play a significant role. The transformation mechanism includes oxidation hydrolysis reduction conjugation etc, catalyzed by various types of enzymes resulting in usually less bio active products. The degradation of pesticides in soil systems depends on their chemical and physical properties and how they interact with the biotic and abiotic soil components [6]. Although mechanism of pesticide degradation in soil may be either abiotic or biotic in nature, the latter has received much attention [2]. Therefore in recent times the role of microorganisms in pesticide degradation dealt with utmost sincerity. In recent times attention was directed to study the soil quality aspects. The soil quality is those soil functions that allow soil to accept, store and recycle water, nutrients and energy. Soil quality does not depend just on the physical, physico-chemical and chemical and chemical properties of soil but closely linked to the soil microbiological properties [7]. Microorganisms are vital for soil fertility and for the degradation of organic matter and pollutants in soils. Important fraction of soil quality includes light fraction, macro organic matter, microbial biomass carbon, nitrogen, mineralizable carbon, carbohydrates and enzymes. Microbial biomass in soils is considered as an important attribute of soil quality [8] and it is the main agent that supports the soil function and associated processes involved with the storing and cycling of nutrients and energy and ecosystem functioning. It was hypothesized that the size of the microbial biomass should be a strong predictor of the pesticide degradation capacity of a particular soil [9]. Paul and Voroney (1989) [10] observed that the knowledge of soil microbial biomass carbon could help in understanding how various ecosystem works, since microorganisms form a vital part of the soil food web [11]. For proper appreciation of ecosystem functioning and soil disturbances due to soil management practices, in addition

to microbial biomass carbon, microbial activities must also be determined [12]. Nannipieri *et al.*, (1990) [13] identified and reviewed the methods of studying microbial activities in soil. Soil respiration is an age old and reliable method in this respect. Changes in soil respiration were used as criteria for pesticide toxicity [14]. Anderson and Domesch (1985b) [15] proposed that the ratio of basal soil respiration to microbial biomass, the qCO_2 , is a measure of microbial response to disturbances. The Q_R , which is the ratio between the rate of basal respiration and substrate induced respiration of soil microorganisms, to assess the status of the soil microbial communities. Dick (1994) [16] stressed upon soil enzyme studies as biological/biochemical indicators of soil quality. In general, hydrolytic enzyme is good choices and soil quality indices because organic residue decomposing organisms are probably the major contributors to soil enzyme activity. The hydrolysis of fluorescein diacetate has the potential to broadly represent soil enzyme activities [17] and accumulated biological effects because fluorescence diacetate is hydrolyzed by a number of different enzymes, such as protease, lipase and esterase and its hydrolysis was found among a wide array of the primary decomposers, bacteria and fungi [18].

Considering the transformation of various pesticides by microorganisms and physical forces in the laboratory to yield various transformation products, it seems quite reasonable to expect these compounds to be found in soil, plant and/or water and they may be more or less toxic than the mother one. As a result, the transformation products need thorough toxicological evaluation for assessing the environmental hazards associated with pesticides and some proposal to reduce their toxicological effects.

Factors influencing pesticides degradation in soil

Pesticide structure

The structure of a pesticide molecule determines its physical and chemical properties and inherent biodegradability. The substituents on phenyl ring influences degradation and introduction of polar groups viz; OH, COOH, and NH_2 make the compound susceptible to microbial attack. Halogen or alkyl substituents tend to make the molecule more resistant to biodegradation (Cork and Krueger, 1991). Chlorinated hydrocarbons such as DDT, pentachloro and dieldrin are insoluble in water, sorb tightly to soil and are thus relatively unavailable for biodegradation. The insecticide carbofuran and the herbicide 2, 4-D, which are of different molecular structure, can be degraded in a matter of few days in field soils.

Pesticide concentration

Concentration of pesticide application is an important parameter in determining the rate of biodegradation. The degradation kinetics of many pesticides approaches first order, the rate of degradation decreases roughly in proportion with the residual pesticide concentration [19]. Gupta and Gajbhiye (2002) [20] reported that the half-life of flufenacet in three Indian soils, viz., insptisol, vertisol and utilisol, varied from 10.1 to 31.0 days at low rate ($1.0 \mu\text{g g}^{-1}$ soil) compared to 13.0 to 29.2 days at high rate ($10.0 \mu\text{g g}^{-1}$ soil) of application. Prakash and Suseela Devi (2000) [21] reported the reduced degradation rate of butachlor at higher initial concentrations, which could be attributed to limitation in the number of reaction sites in soils and toxic effect on microorganisms or enzyme inhibition.

Soil types

Soil properties like organic matter, clay content, pH, etc. affect the degradation of pesticides in soil. Therefore, it is important to study the effect of soil types in pesticide degradation. Gold et al. (1996) [22] reported that soil, pH and clay content greatly affect the persistence of bifenthrin, chlorpyrifos, cypermethrin, fenvalerate, permethrin and isofenphos under field conditions. Jones and Ananyeva (2001) [23] reported that the degradation of metalaxyl and propachlor occurred at different rates in different soils. The half-lives in pasture, arable and pine forest soil were 10, 19 and 36 days respectively for metalaxyl and 2.6, 6.1 and 8.2 days for propachlor. The presence of organic matter and clay content might have posed synergistic effect in fluchloralin dissipation.

Soil moisture

Water acts as solvent for pesticide movement and diffusion, and is essential for microbial functioning. Pesticide degradation is slow in dry soils. The rate of pesticide transformation generally increased with water content. However the rate of diffusion of atmospheric oxygen is limited and anaerobic pesticide transformation can prevail over aerobic degradation in paddy soil. Phorate was found to be more persistent in flooded soil than in nonflooded soil [24]. The herbicides atrazine and trifluraline disappeared more rapidly under anaerobic conditions than under aerobic conditions. DDT is fairly stable in aerobic soils, but is degraded rapidly to DDD in submerged soils [19]. Thus, the transformation of pesticides in the submerged soils is different from that of the soils in field moist state.

Temperature

The effect of temperature on pesticide degradation depends on the molecular structure of the pesticide. Temperature affects adsorption by altering the solubility and hydrolysis of pesticides in soil [25, 26]. As adsorption processes are exothermic and desorption processes are endothermic, it is expected that adsorption will reduce with increase in temperature with a corresponding increase in pesticide solubility. Microbial activity is stimulated by increase in temperature and some ecological groups tend to dominate within certain temperature ranges. The maximum growth and activity of microorganisms in soils occur at 25–35°C [27] and the pesticide degradation is optimal at mesophilic temperature range of around 25–40°C [19]. Jitender et al. (1993) [28] conducted laboratory experiments with thio-bencarb and butachlor incubated at 25 and 35°C for 90 days and observed a direct relationship between temperature and pesticide concentration. Lower temperature and higher concentration resulted in greater persistence.

Soil pH

Soil pH may affect pesticide adsorption, abiotic and biotic degradation processes [25]. It influences the sorptive behaviour of pesticide molecule on clay and organic surfaces and thus, the chemical speciation, mobility and bioavailability [29]. For instance, the sorption of prometryn to clay montmorillonite is more at pH 3 than at pH 7 [19]. The effect of soil pH on degradation of a given pesticide depends greatly on whether a compound is susceptible to alkaline or acid catalyzed hydrolysis [26].

Soil salinity

Limited information is available on the degradation of pesticides in saline soils although salinity is a severe problem in many arid, semiarid and coastal regions. Parathion was degraded faster in non saline soil than in saline soils and its stability increased with increasing electrical conductivity [30]. However, reports on the stability of pesticides in estuarine and seawater of varying degrees of salinity are available. A high salt content in seawater may be innocuous or inhibitory to degradation.

Soil organic matter

Soil organic matter can either decrease the microbially mediated pesticide degradation by stimulating pesticide adsorption processes or enhance microbial activity [31] by cometabolism [32, 33]. The addition of organic materials to flooded soils enhanced the bacterial degradation of some

organochlorine insecticides such as BHC, DDT, methoxychlor and heptachlor [34]. Microbial degradation of linuron in nonsterilized soils was stimulated by organic matter amendment [29]. A certain minimum level of organic matter (probably greater than 1.0%) is essential to ensure the presence of an active autochthonous (the indigenous flora and fauna of a region) microbial population that can degrade pesticides [25].

Dissipation of pesticide

Pendimethalin [N-(1-ethylpropyl)-2, 6-dinitro-3, 4-xylidine,] [1], a selective pre-emergent herbicide controlling most annual grasses and certain broad leaf weeds like cotton, paddy, soybean etc. Several soil fungi *Aspergillus flavus*, *A. terreus*, *Fusarium solani*, *F. oxysporum*, *Penicillium citrinum* and *P. simlicissimum* effectively degraded pendimethalin in mineral solution. Degradation of pendimethalin by *F. solani* resulted in the characterization of three metabolites as N-propyl-3-methyl-4-hydroxy-2, 6-dinitroaniline (II), N-(1-ethylpropyl)-2-amino-6-nitro-3, 4-xylidine (III) and 2, 6-dinitro-3, 4-xylidene (IV) [35]. Moreover, pendimethalin at the recommended doses (1kg ai/hac) stimulated root associated nitrogen fixing activity of young barley seedlings in a neutral alluvial loam soil. Isolate of *Azotobacter vinelandii* and *Azospirillum lipoferum* obtained from herbicide treated barley rhizosphere showed in vitro tolerance to high concentration of the herbicide in N-free media. *Azotobacter* isolates utilized pendimethalin as a carbon source to fix, N₂ in pure culture with equal efficiency to that with manitol. In order to identify the other metabolic products made an extensive study on the microbial transformation of pendimethalin by *Azotobacter*

which resulted in the isolation and identification of six metabolites and thus corroborated to the fact that dinitro-aniline herbicides undergo microbial transformation through N-dealkylation, nitro reduction. Although cyclisation is a predominant mechanism in dinitroaniline herbicide degradation unfortunately none of the cyclised products could be isolated (Fig. 1).

Oxadiazon [2-tert-butyl-4-(2, 4-dichloro-5-isopropoxyphenyl)- Δ^2 -1, 3, 4-oxadiazolin-5-one] is a selective pre-emergent soil applied herbicide, was possible to isolate *Fusarium solani* from soil cropped under paddy cultivation with no previous history and which was degraded by co-metabolic process and eleven metabolites were characterized based on IR, H¹NMR and GC-MS data. Only three of them are characterized as M₁[2,4-(dichloroisopropoxy)benzene], M₂[1-(2,4-dichloro-5-isopropoxyphenyl)-1-(methoxycarbonyl)-1,2-trimethyl-acetyl hydrazine], and M₃[1-trimethyl-acetyl-2-(2,4-dichloro-5-isopropoxyphenyl) hydrazine] [36]. These were seemed to be fungal metabolites and the main pathways for degradation was proved to proceed via dechlorination, N-decarboxymethylation, acetylation, C-dealkylation and ring cleavage (Fig. 2).

A laboratory study of Oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenoxy) - 4-(trifluoromethyl) benzene] a member of nitrodiphenyl ether herbicides, revealed that *A. chroococcum* degraded more than 60% of the herbicide in 7 days [37] utilizing it as sole carbon source. Which leading to the formation of couples of metabolites of which two major metabolized were characterized, metabolite I, [N-[4-{2-chloro-4-(trifluoromethyl)-phenoxy}-2-ethoxyphenyl] acetamide] and II, [4-[2-chloro-4-(trifluoromethyl) phenoxy] -2-ethoxy benzene amine] during the microbial decomposition. The major degradative pathways of Oxy-

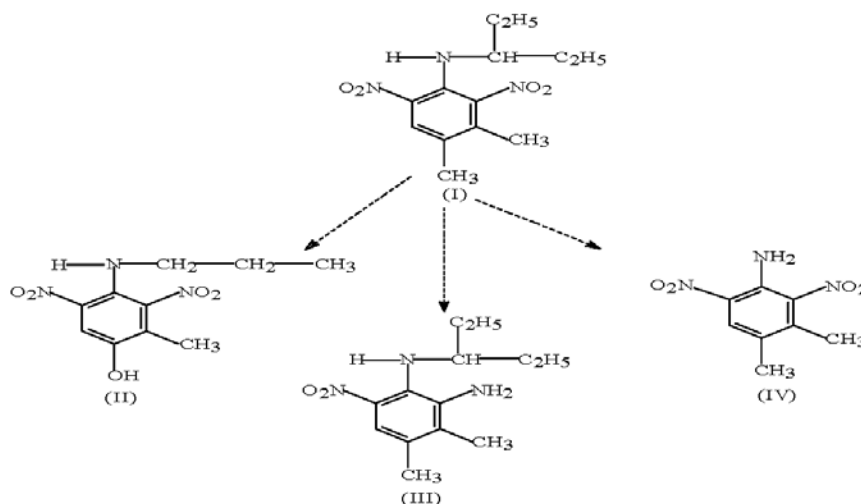


Fig. 1 Hypothetical transformation pathway of Pendimethalin by *F. solani*

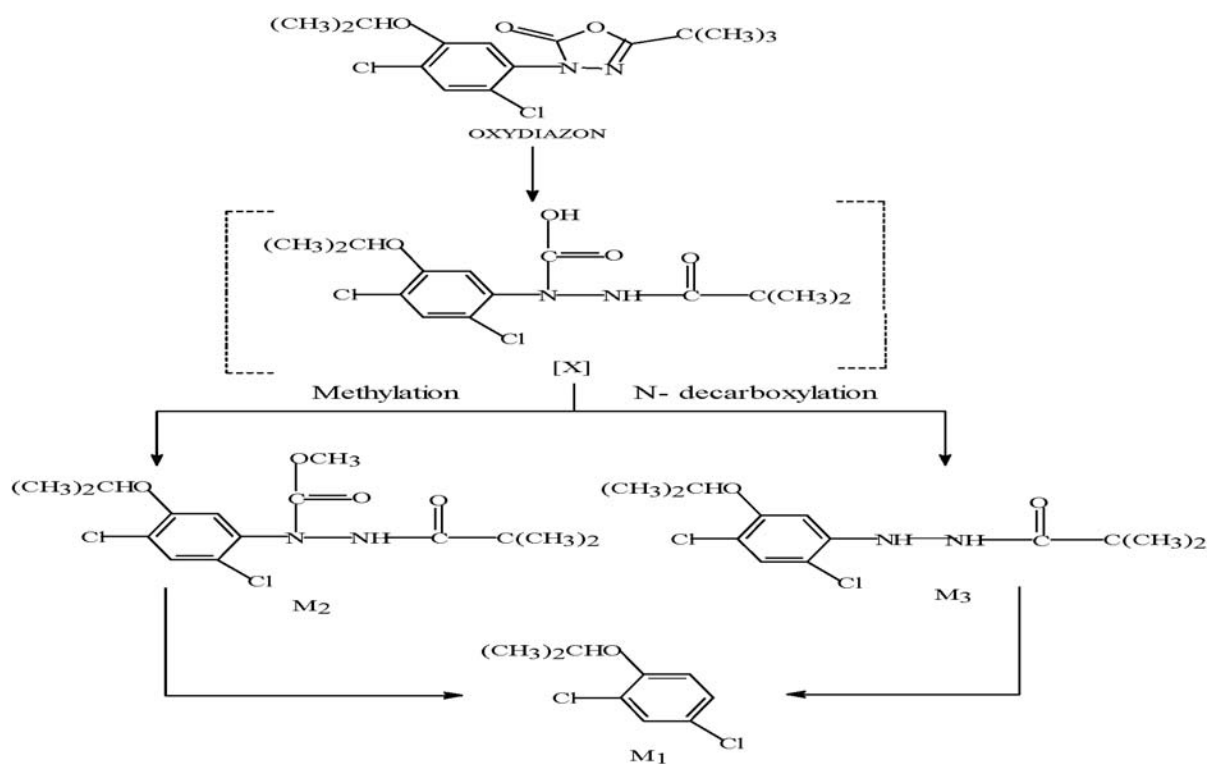


Fig. 2 Hypothetical transformation pathway of Oxadiazon by *F. solani*.

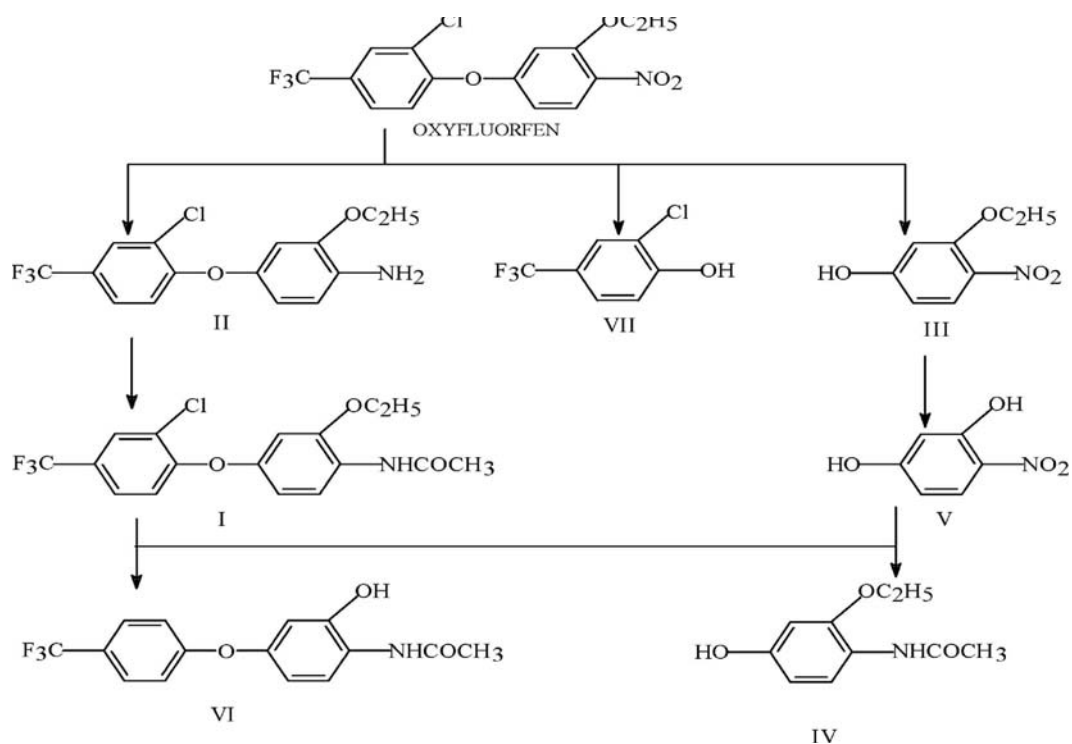


Fig. 3 Hypothetical transformation pathway of Oxyfluorefen by *A. chroococcum* (Beijerinck).

fluorefen involved hydrolysis of ether linkage, nitro group reduction and dechlorination (Fig. 3).

Soil biotic components

The slow degradation of the pesticides under sterilized condition rather than rapid degradation under nonsterilized conditions indicated the role of microbes in pesticide degradation. Numerous workers reported microbial degradation of pesticides in soil [2, 38, 39, 40, 41]. Degradation of phorate [42], metalaxyl [42, 43] and fipronil [1] proceeded more rapidly in nonsterilized than in sterile soils. The breakdown of pesticides in soils is brought about by a variety of biotic mechanisms. The principal route involves the use of pesticides as carbon, energy and nitrogen sources. Microorganisms can also degrade pesticides cometabolically [44].

Effect of pesticide on soil microbiological parameters

Soil microbial biomass

Microbial biomass is defined as the part of organic matter in soil that constitutes living microorganisms smaller than 5–10 cubic micrometers and it is a fraction of soil organic matter that is sensitive to management practices and pollution [45]. Microorganisms include bacteria, actinomycetes, algae, protozoa and micro fauna. Usually, plant roots and faunas larger than 5–10 cubic micrometer such as earth worms, are not included [46]. Microbial biomass being an important attributes of soil quality [47] and is an ecologically important parameter [11]. Several workers have studied the effects of pesticide application using this parameter.

Anderson (1981) [48] conducted experiments with three fungicides, viz., captan, thiram and verdasan at application rates of 5 and 50 $\mu\text{g g}^{-1}$. The 5 $\mu\text{g g}^{-1}$ rate caused 40% decreased in biomass and within 8 days, biomass in captan and thiram amended soils had recovered to that of the controls. Although the fungal to bacterial balance was restored in verdasan-amended soils, biomass recovery was not complete. At 50 $\mu\text{g g}^{-1}$ fungicides caused long-term decreases in the biomass and altered the relative proportions of bacterial to fungal populations. Verdasan had the greatest effect on soil microbial biomass. Initial application of pesticides may decrease the activity of microorganism due to their toxicity as a result MBC is decreased but later he degraded pesticide used by the organisms as their C-source for cell proliferation.

Duah-Yentumi and Johnson (1986) [49] studied the effect of pesticides on soil microflora in arable soils that had received repeated applications of carbofuran and carbosulfan

(insecticides), iprodione and vinclozolin (fungicides), and Methyl Chloro Phenoxy Acetic Acid (MCPA), simazine and paraquat (herbicides). Carbofuran at single and 5-fold application rates did not show any detectable detrimental effects on soil microbial biomass, but single application of carbosulfan caused a significant biomass reduction. There was dramatic reduction in soil microbial biomass following vinclozolin application due to the reduction in fungal biomass. Iprodione showed fluctuating trends in biomass. MCPA and simazine caused no detectable effects on the microflora, but repeated paraquat applications significantly lowered soil microbial biomass, chiefly the fungal biomass. The results indicated that there might be substantially variable effects on soil microbial biomass produced by single or repeated applications of different pesticides.

Wardle and Parkinson (1990b) [50] studied the side effects of glyphosate on the soil microflora by applying a range of concentrations from 0, 2, 20 and 200 $\mu\text{g g}^{-1}$ to incubated soil samples and monitored the changes in various microbial groups over 27 days. Bacterial propagule numbers were temporarily enhanced by 20 and 200 $\mu\text{g g}^{-1}$, while actinomycete and fungal propagule numbers were unaffected by glyphosate.

Wardle and Parkinson (1992) [51] studied the influence of the herbicides 2, 4-D and glyphosate on soil microbial biomass in a field experiment. The results suggested that both the herbicides were capable of reducing microbial biomass within a few days following application but only in plots with weeds present.

Perucci and Scarponi (1994) [52] investigated the effects on imazethapyr, on the soil microbial biomass, Imazethapyr applied in a field at the recommended rate for soybean weeding. In the laboratory experiment, the herbicide was incorporated at the field rate, 10 and 100-fold field rate. In both the field trial and the laboratory experiment, imazethapyr had no adverse effect on the microbial biomass at the field rate. But at 10 and 100-fold rates, the herbicides decreased the soil microbial biomass carbon contents.

Rath *et al.* (1998) [53] observed that the application of 2, 4-Dinitroaniline (2, 4-D) and its analog 2, 4, 5-T at 0.75 $\mu\text{g g}^{-1}$ soil led to a distinct increase in microbial biomass carbon contents over that of untreated soil samples both under flooded and nonflooded conditions, 2-4-D was inhibitory to microorganisms at 7.5 and 15.0 $\mu\text{g g}^{-1}$ soil. Repeated applications of a commercial formulation of hexachlorocyclohexane (HCH) to flooded soil caused marked increase in microbial biomass contents. Technical grade 3-HCH was also stimulatory to microbial biomass content.

Vischetti *et al.* (2000) [54] studied the relationship between the degradation of rimsulfuron and soil microbial biomass carbon in a laboratory incubated clay loam soil

under different conditions and at different initial dosages (field rate, 10 and 100- times the field rate). The authors suggested that rimsulfuron could pose environmental risks in cold and dry climatic conditions. Significant decrease in microbial biomass carbon contents in rimsulfuron treated soils compared to the untreated soil was observed initially, especially at higher temperatures and low moisture levels, but never exceeded 20.3% of that in the control soil. The microbial biomass carbon contents then returned to its initial values at varying times depending on incubation conditions.

Perucci *et al.* (2000) [31] investigated the interactive effects of either rimsulfuron or imazethapyr with organic matter, on some soil biochemical and microbiological properties. The herbicides were applied at field and 10-fold field rates. The effect of both the herbicides on soil microbial biomass was not detectable at the field rates. The higher rates of herbicides application impaired the observed microbial parameters to a greater degree. The detrimental effects reduced by organic amendments.

Haney *et al.* (2002) [55] determined the effect of isopropylamine salt of glyphosate on soil microbial biomass and activity across a range of soils varying in fertility. The herbicide was applied at 234 mg kg⁻¹ soil. Glyphosate significantly stimulated soil microbial biomass and its activity. It appeared to be rapidly degraded by soil microbes regardless of soil type and organic matter content, even at high application rates, without adversely affecting microbial biomass.

In a separate study [56] conducted by clay loam soil from agricultural fields of alluvial (AL) soil (typic udifluent) [Order - Entisol (Ent = Recent = Little profile development, Suborder- Fluvent = alluvial deposits, Fluv = Flood Plain, Great group- Udifluent; Fluvius = River Ud = Humid Climates, Typic = Typical] and coastal saline (CS) soil (typic endoaquept) [Order - Inceptisol (Ept = Inception = Beginning, Embryonic soils with few diagnostic features, Suborder- Aquept = Wet, Aqu = Characteristics associated with wetness, Great group- Endoaquept; Endo = Fully water saturated, Typic = Typical] were investigated for the degradation and effect of pencycuron application at field rate (187.5 g. a.i./ha) (FR), 2-times FR (375.0 g. a.i./ha) (2FR) and 10-times FR (1875.0 g. a.i./ha) (10FR) with and without decomposed cow manure (DCM) on soil microbial variables under laboratory conditions. Pencycuron degraded faster in CS soil and in soil amended with DCM. Pencycuron spiking at FR and 2FR resulted in a short-lived (in case of 10FR slightly longer) and transitory toxic effect on soil microbial biomass-C (MBC), ergosterol content and fluorescein diacetate hydrolyzing activity (FDHA). Amendment of DCM did not seem to have any

counteractive effect of the toxicity of pencycuron on the microbial variables.

Soil Respiration

Active living cells need constant supply of energy, which the heterotrophic microflora derives through organic matter transformation. Under aerobic condition, the end product of the transformation is the evolution of CO₂ and H₂O (respiration). The metabolic activities of soil microorganisms can, therefore, be quantified by measured CO₂ evolution [13]. It can be studied both in unamended and amended soils. Respiration of unamended soil is termed as basal respiration while the substrate induced respiration is the respiration of amended soil. Basal respiration reflects overall, potential microbial activity [57]. Substrate induced respiration is a measure of the total physiologically active part of the soil microflora [58] (Anderson and Domsch, 1978). The combination of the basal and substrate induced respiration represent carbon available index [59]. Measurement of soil respiration is an effective tool to characterize the microbial status of soil and hence bioindicators of soil health or soil quality [60]. Like other metabolic activities it depends on the physiological state of the microbial cells is influenced by several soil factors. Soil respiration was most frequently used for assessment of the side effects of chemicals, such as heavy metals, pesticides etc. [61]. The degree of inhibitory effect depends not only on the intensity of the stress but also on the period of exposure of the microbes to the stress.

Zelles *et al.* (1985) [62] investigated the effects of some herbicides (atrazine, pentachlorophenol, 4-chloroanile and chloroacetamide), fungicides (zineb and captan) and insecticides (lindane and 4-nitrophenol) on soil respiration. Fresh soil was either used immediately or amended with 0.5% alfalfa meal, in order to improve the conditions of the microorganisms. The study was limited to 48 days. Atrazine, lindane and captan caused minor effects. The remaining pesticides induced measurable changes in the behaviour of the microorganisms. Several pesticides in various concentrations stimulated the production of CO₂. Improvement of soil by addition of alfalfa meal promoted the reversibility of the effects by pesticides.

The effects of 2, 4-D, picloram and glyphosate on certain microbial variables were monitored over 27 days in an Alberta agricultural soil [63] at concentrations of 0, 2, 20 and 200 µg g⁻¹ soil. All the herbicides at 200 µg g⁻¹ soil enhanced the basal respirations only for 9 days following applications. Substrate induced respirations were temporarily depressed by 200 µg g⁻¹ picloram and 2, 4-D but briefly enhanced by 200 µg g⁻¹ glyphosate. It was concluded that because changes in microbial variables only occurred at

herbicides concentrations of much higher than that which occurs following field applications, the side effects of these chemicals were probably of little ecological significance.

Tu (1992) [64] conducted laboratory tests with eight herbicides viz., atrazine, butylate, ethalfluralin, imazethapyr, linuron, metazachlor, metribuzin and trifluraline applied to a loamy sand at 10 mg kg⁻¹ soil to determine if these materials caused any serious effect on microbial activities related to soil fertility. Soil respiration increased significantly after 96 hour incubation with atrazine. Results indicated that the herbicidal treatments at the levels tested were not drastic enough to be considered deleterious to soil microbial activities which are important to soil fertility.

Haney and Senseman (2000) [65] applied the isopropylamine salt of glyphosate at 47, 94, 140 and 234 µg g⁻¹ soils in a Weswood silt loam. Glyphosate significantly stimulated soil microbial activity as measured by C and N mineralization but did not affect soil microbial biomass. An increase in C mineralization rate occurred from the first day following glyphosate addition and continued upto 14 days. Glyphosate appeared to be directly and rapidly degraded by microbes, even at high application rates, without adversely affecting microbial activity.

Araújo et al. (2003) [3] studied in vitro, changes in the microbial activity of typical hapludult and hapludox Brazilian soils with applied glyphosate. Glyphosate was applied at a rate of 2.16 µg g⁻¹ soil and microbial activity was measured by soil respiration (evolution of CO₂) over a period of 32 days. The result was an increase of 10–15% in the CO₂ evolved in the presence of glyphosate compared with the same soil, which never received glyphosate.

Ecophysiological parameters

In the assessment of effects of disturbances on soil quality, there has been increasing interest in the development of easily measured bioindicators, which are sensitive to perturbation. The soil microbial biomass and microbial biomass C to organic C ratios both respond readily to soil disturbances and can provide an early warning on the deterioration of soil quality [66, 67]. Anderson and Domsch (1985b) [15] proposed the ratio of soil basal respiration to microbial biomass i.e., microbial metabolic quotient or specific respiration of the biomass (qCO₂) based on Odum's theory of ecosystem succession, as an alternative measure of changes in microbial biomass and its activity in response to disturbances. The respiration rate per unit of biomass is a more sensitive indicator of toxic effects than the respiration rate or the amount of biomass alone [11]. This index supposedly declines during succession and following recovery from disturbance, because 'equilibrium' conditions are ap-

proaches and the soil microflora becomes more efficient at conserving carbon [68]. Thus, qCO₂ is a reliable measure for detection of the effect of xenobiotic compounds on soil microbial biomass carbon. Anderson and Domesch (1990) [69] stated that microbial qCO₂ increases due to disturbances caused by pesticidal chemicals applied, resulting from microbes utilizing large part of their energy budget for cell maintenance. Pal et al., 2005 [56] reported that clay loam soil from agricultural fields of alluvial (AL) soil (typic udifluent) and coastal saline (CS) soil (typic endoaquept) were investigated for the degradation and effect of pencycuron application at field rate (FR), 2-times FR (2FR) and 10-times FR (10FR) with and without decomposed cow manure (DCM) on soil microbial variables under laboratory conditions. The eco-physiological status of the soil microbial communities as expressed by microbial metabolic quotient (qCO₂) and microbial respiration quotient (Q_R) changed, but for a short period, indicating pencycuron induced disturbance. The duration of this disturbance was slightly longer at 10FR. Pencycuron was more toxic to the metabolically activated soil microbial populations, specifically the fungi. It is concluded that side effects of pencycuron at 10FR on the microbial variables studied were only short-lived and probably of little ecological significance.

Perucci and Dumontet (2000) [70] proposed another stress identifying index i.e. specific hydrolytic activity (qFDA) for assessing microbial activity in reply to xenophobic treatments and indicates it a response to disturbance. According to the study, qFDA increases through the incubation period after the application of rimsulfuron and imazethapyr in soil which implies an impairment of the metabolic activity of the soil microflora.

Fluorescein diacetate hydrolyzing activity (FDHA)

Soil enzyme assays may not always reflect the overall microbial activity of soil, because the enzymes are substrate specific [13]. The product of this hydrolysis is fluorescein, which can be quantified by spectrophotometry. The FDHA appears to be widespread among the primary decomposers, bacteria and fungi [18].

The estimation of FDHA is a widely accepted simple method for precision measurement of the total microbial activity in soils [71]. Dumontet *et al.* (1997) [72] suggested that FDHA might be considered as a suitable tool for measuring the early detrimental effect of pesticides on soil microbial biomass, as it is a sensitive and nonspecific test able to depict the hydrolytic activity of soil microbes.

Zelles *et al.* (1985) [62] investigated the effects of herbicides (atrazine, pentachlorophenol, 4-chloroaniline and chloroacetamide), fungicides (zineb and captan) and

insecticides (lindane and 4-nitrophenol) on FDHA in soil unamended and amended with 0.5% alfalfa meal upto 48 days. Atrazine, lindane and captan showed insignificant effect. The remaining pesticides induced measurable changes in the behaviour of microorganisms. While higher concentrations caused reversible or irreversible reductions of FDHA in most cases, small concentrations sometimes produced stimulative effects. Improvement of soil by addition of alfalfa meal promoted the reversibility of effects caused by pesticides.

Perucci and Scarponi (1994) [52] investigated the effects on FDHA with imazethapyr applied in a field trial at the recommended field rate for soybean weeding. In the laboratory experiment, the herbicide was incorporated at the field rate, 10- and 100-fold rate. In both the field trial and the laboratory experiment, the field rate application of imazethapyr had no adverse effects but at 10- and 100-fold rates, the herbicides decreased the FDHA.

Perucci *et al.* (1999) [73] studied the effects of rimsulfuron on FDHA of soil under laboratory conditions. The authors did not observe any detrimental influence on FDHA at the field dose but the effect was prominent at the higher doses. The onset and magnitude of the effect were dependent on temperature and humidity conditions. However, the effects were generally slight and transitory. The findings were discussed in terms of rimsulfuron toxicity to the soil microbial biomass and consequent release of endocellular enzymes from the dead microorganisms.

Araújo *et al.* (2003) [3] studied, in vitro the changes in the microbial activity of typical Hapludult and Hapludox Brazilian soils with applied glyphosate. Glyphosate was applied at the rate of $2.16 \mu\text{g g}^{-1}$ soil and microbial activity was measured by FDHA over a period of 32 days. The results were an increase of 9–19% in Fluorescein diacetate (FDA) hydrolysis in the presence of glyphosate compared to the same soil, which never received glyphosate. Soil, which was exposed to glyphosate for several years, had the positive response in microbial activity.

Correlation of pesticide degradation rate and microbial properties of soil

Microbial processes affect the degradation of most pesticides in soil [74]. Analysis of relationship between ecosystem properties, the size and composition of microbial biomass, and pesticide degradation capacity may be useful for assessment of ecosystem and landscape dynamics of the pesticides. A close positive correlation between soil microbial biomass, soil respiration and the degradation rate constant of metribuzin [75], linuron and glyphosate [14], alachlor [76], 2, 4-Dinitroaniline (2, 4-D) and dicamba [9]

was recorded in agricultural and forest soils. Metalaxyl and propachlor transformation rate constants positively correlated with basal, substrate-induced respiration and physico-chemical (pH, organic C and clay content) properties of soil [23]. In contrast, no correlations were found between microbial biomass and degradation of the pesticides 2, 4-D and atrazine [77, 78]. It was opined that this relationship might be useful for developing approaches for evaluating and predicting the fate of pesticides in different ecosystems [9].

The relationship between rimsulfuron [54, 73], imazamox and benfluralin [79] degradation and microbial biomass content was studied in a laboratory incubated clay loam soil under different conditions of soil moisture, temperatures and also at different initial dosages. The relationship between pesticides degradation and microbial biomass-C content gave parabolic curves ($P < 0.05$ in all cases) under all conditions tested. The authors suggested quadratic equations might be useful in order to deduce the trend of soil microbial biomass in relation to pesticide concentration. From these equations it is possible to observe that the entity of microbial biomass decreases and the trend of the parabolic curves are similar, and independent of initial concentrations. These relationships helped in modeling behaviour of soil microbial biomass after pesticide treatment.

Remediation of contamination by microbes or plants

Environmental contamination caused by pesticides generally falls into two broad categories:

- 1) A diffused low-level contamination from continued use of pesticides in agriculture and remnants of persistent pesticides used in the past.
- 2) Heavy pollution of soil and surface water/ground water in defined areas due to disposal or accidental releases of concentrated pesticide formulations.

Research into decontamination strategies has tended to focus on small areas contaminated with high concentrations of pesticides, especially those compounds that pose an immediate threat to the environment and human health. In contrast, a diffused low-level contamination of the environment has received little attention, except where there has been a legislation to limit the amount of residues in a given commodity, such as foodstuffs, drinking water *etc.*

The natural processes that break down toxic chemicals in the environment have become the focus of much attention to develop safe and environment-friendly deactivation technologies. The processes involved in pesticide biodegradation such as oxidation, hydroxylation, aromatic ring cleavage, hydrolysis, dehalogenation, dealkylation, or conjugate formation, have been well studied in recent

years. This has provided a basis for the targeted use of microbes and plants in enhanced remediation of contaminated sites [80, 81]. Both bioremediation (using microbes) and phytoremediation (using plants) offer the potential for low-cost, low maintenance, environment-friendly and renewable resources for *in situ* remediation of contaminated environments that are far more cost-effective than any *ex-situ* decontamination technique.

This study reviews the transformation of some selected pesticides, microbial contribution to degradation have, been studied in our laboratory during the last few years, at the same time factors influencing the degradation of pesticides in soil, impacts of pesticides on soil microbial biomass, soil ergosterol content, soil respiration, fluorescein diacetate hydrolyzing activity, ecophysiological parameters and the correlation between pesticide transformation, remediation of pesticidal contamination through microbes and plants.

Microbes or Plants?

The choice of using microbes, plants or both in a remediation effort depends on the extent of contamination, nature of the chemicals present, and the amount of source available for decontamination. The decontamination rates achieved by bioremediation technology are generally slower than those achieved by some physical and chemical methods. The fundamental constraint to the success of either of the technologies is the ability of microbes or plants to grow in a system that might be heavily contaminated with organic and inorganic chemicals. The hydrophobic nature of most pesticides presents are the major obstacle in their uptake by microbes or plants. Indeed, the uptake and translocation of highly hydrophobic pesticides through plant roots may only be limited in most species. Thus, whilst plants have been shown to possess useful enzymatic mechanisms to degrade most pesticides [82], their main application in phyto-remediation has been to 'bio-extract' inorganic pollutants, such as toxic heavy metals. There are other constraints to the use of plants alone in remediation; plant growth is dependent on a number of environmental factors, such as availability of nutrients and water, soil type and pH, *etc.* The maximum benefits of phyto-remediation may therefore, be achieved in long-term applications, or when used in conjunction with other immediate remedial actions. Despite such limitations, plants are known to absorb a wide range of air-borne chemicals through the foliage surface. The bio-remediation through the addition of stabilized fraction of organic waste could either lower the microbially mediated pesticide degradation in soil by adsorption process or enhance it through the improvement in microflora metabolic activity [83]. Numerous microbial strains have been shown to degrade

or 'bio-fix' a wide range of environmental chemicals. The bacterial species found to be most useful in bioremediation belong to the genera *Flavobacterium*, *Artlrobacter*, *Azotobacter*, *Burkholderia* and *Pseudomonas*. For example strains of *Pseudomonas* sp. and *Klebsiella pneumoniae* have been shown to possess hydrolyze enzymes that are capable of breaking down s-triazine herbicides, such as atrazine, which because of aqueous solubility and persistence could leach into groundwater. Similarly, a number of enzymes such as oxygenases, hydroxylases, hydrolases and isomerases present in *Pseudomonas* and *Alcaligenes* sp. have been shown to degrade the herbicide 2, 4-D [80]. It was also well established by our experiment that several soil fungi *Aspergillus flavus*, *A. terreus*, *Fusarium solani*, *F. oxysporum*, *Penicillium citrinum* and *P. simlicissimum* effectively degraded pre-emergent dinitroaniline herbicide pendimethalin supplied as sole carbon in mineral solution. Both bacteria and fungi can degrade OP pesticides through hydrolytic cleavage, and pyrethroids (e.g. permethrin) through cleavage of the ester bonds. With the exception of dithio-carbamates, microbial degradation of all types of carbamate pesticides has also been demonstrated; for example, a rapid hydrolysis of carbaryl has been reported due to presence of the enzyme carbaryl esterase in *Pseudomonas* sp. [81]. A few strains of *Pseudomonas* have also been genetically altered to confer ability to degrade recalcitrant chemicals, such as chlorobenzenes that are commonly used in pesticide synthesis [84]. The white-rot fungi *Phanerochaete chrysosporium* have enzymes that not only enable them to degrade lignin and cellulose, but also breakdown many recalcitrant chemicals including halogenated-phenol ring-containing compounds, such as the wood preservative pentachlorophenol, which is a particularly persistent pollutant in industrial wastes from paper and leather tanning industry [85]. Many recalcitrant chemicals are also known to be transformed by microbes to products that are more efficiently absorbed and translocated by plants. Thus, a combination of bio- and phyto-remediation in the immediate vicinity of the plant root mass (rhizosphere) could enhance the degradation process of pesticides. This interaction could be further improved by manipulating microflora in the rhizosphere, for example, through the introduction of known bioremediating species of microbes. The synergy should greatly enhance the overall rate of remediation, especially under conditions that promote the growth of both microbes and plants.

Problems and solutions

There are a number of difficulties that have hindered the full-scale commercial adoption of bio- and phyto-reme-

diation. Tests carried out on the remediating efficacy of microbes or plants under controlled laboratory conditions do not usually simulate true field situations. There is a general lack of reliable techniques to prove efficacy of remediation *in the field*, and ordinary sampling techniques often fail to reveal the real levels of a pollutant in a heterogeneous field. An inherent problem with bioremediation *in soil environments* is the fact that target substances may not be readily available for uniform dispersal, due to low solubility and high binding capacities. This could lead to regions of high concentration of pesticides that often prevent microbial activity, a problem that has proved a major stumbling block in utilization of bioremediation to its maximum potential. The use of mechanical aids, for example ploughing, biological means such as the use of microfauna and macro-invertebrates to disturb the soil matrix can enhance bioremediation *in situ* [86]. The use cell free enzyme preparations to degrade organic pollutants is also gaining popularity, as it is not subject to many of the limitations that are associated with microbial growth under field conditions. One example is the use of aqueous fire-fighting foam containing OP-hydrolase to degrade a number of organo-phosphate (OP) compounds [87]. Bioremediation could also benefit from advances in techniques such as micro-encapsulation for a slow or timed release of bioremediations to overcome problems encountered under field conditions, and to enhance the persistence of microbial or enzymatic preparations to achieve maximum benefits.

Non-domestic landfills and other sites, with a long history of pollutant dumping, are especially problematical from a remediation standpoint. These sites may harbour a wide variety of contaminants, often with low bioavailability, which further declines with time [88]. The present of co-contaminants (e.g. phenols) can result in further complications as they can inhibit the activity of microbial communities present to degrade other pollutants [89]. It is, therefore, desirable to utilize those indigenous and non-indigenous microbes and plants that have diverse degradative properties; and most importantly which would less likely be suppressed by the presence of co-contaminants. Further difficulties may be encountered with long-standing contaminated sites where pesticides may have chemically bound to soil or penetrated deep into the soil subsurface, or even into groundwater. The survival of aerobic microbes under these conditions of low oxygen would be limited, and the use of anaerobic microbial communities could be advantageous. Similar considerations apply to the use of plants. Since the species most suitable for use in phyto-remediation at a particular site would be those able to grow under field conditions. In fact, it has often been demonstrated that the indigenous plants and synergistic communities of microbes

present at a contaminated site are those best suited for remediation purposes. This is due to natural selection over time of those species/ strains that are capable of exploiting the contaminated environments [90]. Thus, research into bio- and phyto-remediation has also been aimed at generating the environmental conditions that give maximal growth of indigenous microbial communities or plants with the ability to remove and/or degrade contaminants *in situ* [89].

Available literature shows a clear dearth of information regarding the fate and effects of pesticides in tropical soils and remediation for their transformed product. Therefore, there is an urgent need to generate regionally specific database for pesticide effects in tropical environments.

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