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Degradation and Effects of Pesticides on Soil Microbiological Parameters-A Review*

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Abstract: Intensive use of agrochemicals mainly pesticides in natural environments have become a major concern and have stimulated scientists to develop reliable methods to assess the potential for the transformations of these anthropogenic chemicals. These informations are very much essential for modeling the fate and effects of these chemicals in the environment. This article is a review of the information available in the literature highlighting the various soil properties, which will influence the degradation of pesticides. The extent of biodegradability depends upon the chemical structure of the pesticides. Soil physico-chemical properties influence the transformation of pesticides to a great extent. Soil microbial components largely govern pesticide degradation in soil. Maintenance of soil quality constitutes considerable intervention in the research agenda. Soil microbial biomass and its activities are important attributes of soil quality. The impact of pesticides on the soil microbial biomass, ergosterol content, respiration and fluorescein diacetate hydrolyzing activity as available in the literatures is reviewed here with especial emphasis. Most of the pesticides at field application rate did not impair the soil biological parameters. At higher rates of application the suppressive effects were transitory. Studies revealed positive correlation between pesticide transformation rate, soil physico-chemical and biological properties. Ecophysiological parameters, like the ratio of basal soil respiration to microbial biomass (qCO_2), are helpful to detect the stress on microorganisms due to pesticides. Most of the studies were conducted under laboratory conditions. Field studies are conspicuously lacking in the world literature. Future research need is projected.

Key words: Pesticide, microbial biomass, ergosterol, microbial activities, ecophysiological parameters

Introduction

Modern agriculture is readily associated with the use of different chemical inputs. Variant classes of pesticides are used in managing different groups of pests to maximize crop production and meet the demands for higher supplies of food of the fast-growing human population. Accurate agricultural pesticide use statistics are much harder to obtain for many of the developing countries that comprise the bulk of the tropical landmass than in the well characterized North Europe and Japanese markets.

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Regarding the most common pesticides used in the tropics, there are many similarities to pesticides used in temperate areas. Based on the information on the fate of the pesticides in temperate soils and climates, many of the insecticides find their way into tropical agriculture. However, tropical soils and climates are different from that in the temperate regions. Hence it is prudent to study the fate of pesticides in the areas where these are to be applied.

Concern for pesticide contamination in the environment in the current context of pesticide use has assumed great importance (Zhu *et al*., 2004). 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 (Hafez and Thiemann, 2003) potentially because of the adverse effects of pesticidal chemicals on soil microorganisms (Araújo *et al*., 2003), which in turn may affect soil fertility (Schuster and Schröder, 1990). An ideal pesticide should be toxic only to the target organism, biodegradable and should not leach into ground water. Unfortunately, this is rarely the case and the widespread use of pesticides in modern agriculture is of concern (Johnsen *et al*., 2001). The vast majority of investigations regarding fate of pesticides in soil were conducted in soils under temperate conditions. But tropical countries make substantial use of pesticides for control of agricultural and other pests. Given the concerns related to both pest control efficacy and environmental risk, it is perhaps surprising that more attention was not focused on pesticides fate in tropical soils.

Due to the continuous use of pesticides in agriculture, appreciable quantities of pesticides and their degraded products may accumulate in the ecosystem leading to serious problem to man and the environment. Therefore, it is essential to study the residue and degradation pattern of pesticides in crops, soils and water systematically in order to generate meaningful data from the point of view of plant protection, public health and environmental safety. The degradation of pesticides in soil and their effect on microorganisms should be studied so that their use can be properly regulated (Lynch, 1995).

Pesticides, which enter the soil environment, are subject to a variety of degradative processes. The overall degradation of a pesticide from soil results from a combination of mechanisms such as microbial degradation, chemical hydrolysis, photolysis, volatility, leaching and surface runoff. The degree to which each mechanism will contribute to the overall degradation of the pesticide is in turn dependent on the physicochemical properties of the pesticide (e.g., water solubility, sorptive affinity), characteristics of the soil (e.g., pH, organic matter content, microbial biomass, redox status), environmental conditions (e.g., temperature, moisture) and management practices (e.g., application rate, formulation type). Within each of these variables there are complex interactions and interdependencies, which are difficult to quantify *in situ*. Degradation of pesticides in soils was mostly studied under aerobic conditions. Rice is the principal cereal crop grown in the tropics during the monsoon season under flooded anaerobic condition. Thus, there is need to investigate the fate of pesticides under anaerobic conditions due to general lack of such information. Soil properties potentially influence the behavior of pesticides in soils. 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 (Beigel *et al*., 1999; Sannino *et al*., 1999). Thus, pesticide behaviour should be studied in different types of soils including problem soils. Although mechanism of pesticide degradation in soil may be either abiotic or biotic in nature, the latter has received much attention (Hafez and Thiemann, 2003).

Within the recent years much attention was directed to study the soil quality aspects. Soil quality does not depend just on the physical, physico-chemical and chemical properties of soil but closely linked to the soil microbiological properties (Elliot *et al*., 1996). Microorganisms are vital for soil fertility and for the degradation of organic matter and pollutants in soils. Microbial biomass in soil is considered as an important attribute of soil quality (Doran and Parkin, 1994). It serves as a measure of potential biological activity and its dynamic changes would help in understanding the processes involved in nutrient cycling and ecosystem functioning (Rath *et al*., 1998). Because of concerns about the environment, the side effects of pesticides on soil microorganisms were studied extensively (Greaves *et al*., 1976; Anderson, 1978; Greaves, 1982; Gerbar *et al*., 1989) but no single test was sufficient for side effect evaluation (Johnen and Drew, 1978; Atlas *et al*., 1978). As the productivity and health of agricultural systems depend on the functional processes (e.g., nutrient cycling) carried out by the microbial communities in soil, most of the studies on the effects of pesticides were based on the biochemical aspects of microbial functions in soil, such as microbial biomass, soil respiration and so forth (Katayama and Fujie, 2000). Paul and Voroney (1989) observed that the knowledge of soil microbial biomass carbon could help in understanding how various ecosystems work, since microorganisms form a vital part of the soil food web (Beelen and Doelman, 1997). However, estimation of microbial biomass does not clearly differentiate between microbial components, i.e., bacteria, fungi, protozoa etc. Soil fungi often make up at least 75-90% of the soil microbial biomass and together with bacteria, are responsible for about 90% of total energy flux of organic matter decomposition in soil (Paul and Clark, 1996). Ergosterol, predominantly occurring in the phospholipid bilayer of the fungal cell membrane, is endogenous almost exclusively to fungi and so may be a useful index of fungal biomass (Hart and Brookes, 1996a). 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 (Brookes *et al*., 1987).

It is well known that pesticides affect nontarget microorganisms in soil. For the detection of side effects of pesticides in soil, methods are needed which should be able to indicate changes in the activity of soil microorganisms at a pesticide application level as low as possible. Furthermore, suitable techniques should be nonspecific, i.e., they should relate to a comprehensive range of microorganisms, rapid and easy to perform (Zelles *et al*., 1985). Nannipieri *et al*. (1990) 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 (Torstensson and Stenstrom, 1986; Gerbar *et al*., 1989). Anderson and Domsch (1985b) proposed that the ratio of basal soil respiration to microbial biomass, the qCO_2, is a measure of microbial response to disturbances. Ananyeva *et al*. (1997) used 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. Other widely used methods are soil enzyme assays. Dick (1994) stressed upon soil enzyme studies as biological/biochemical indicators of soil quality. In general, hydrolytic enzymes are good choices as soil quality indices because organic residue decomposing organisms are probably the major contributors to soil enzyme activity (Dick *et al*., 1996). The hydrolysis of fluorescein diacetate has the potential to broadly represent soil enzyme activities (Schnürer and Rosswall, 1982) and accumulated biological effects because fluorescein 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 (Lundgren, 1981). Perucci *et al*. (2000) suggested that fluorescein diacetate hydrolyzing activity could be considered as a suitable tool for measuring the early detrimental effect of pesticides on soil microbial biomass.

This study reviews the 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 and above microbial parameters.

Factors Influencing Pesticide Degradation in Soil

Pesticides in the soil environment are subject to a variety of degradative processes. The overall degradation of a pesticide from soil results from a combination of loss mechanisms. The degree to which each mechanism will contribute to the overall loss of the pesticide is in turn dependent on the physico-chemical properties of the pesticides, characteristics of the soils, environmental conditions and management practices. The studies in respect of the salient factors that determine the degradation of pesticides are presented.

Pesticide Structure

The structure of a pesticide molecule determines its physical and chemical properties and inherent biodegradability. The introduction of substituents on a benzene ring influences its degradation. Minor alterations in structure frequently cause a drastic change in the susceptibility of a compound to biotransformations. Introduction of polar groups such as OH, COOH and NH_2 may provide the microbial system, a site of attack. Halogen or alkyl substituents tend to make the molecule more resistant to biodegradation (Cork and Krueger, 1991). Chlorinated hydrocarbons such as DDT, pentalene 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. Minor differences in the position or nature of substituents in pesticides of the same class can influence the rate of degradation (Topp *et al.*, 1997).

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 (Topp *et al.*, 1997). Gupta and Gajbhiye (2002) reported that the half-life of flufenacet in three Indian soils, viz., inseptisol, vertisol and ultisol, varied from 10.1 to 31.0 days at low rate (1.0 $\mu g\ g^{-1}$ soil) compared to 13.0 to 29.2 days at high rate (10.0 $\mu g\ g^{-1}$ soil) of application. Prakash and Suseela Devi (2000) reported the reduced degradation rate of butachlor at higher initial concentrations, which could be attributed to limitation in the number of reaction sites in soil and toxic effect on microorganisms or enzyme inhibition. Yu *et al.* (2003) reported that the half-lives of butachlor in non-rhizosphere, wheat rhizosphere and inoculated rhizosphere soils ranged from (6.3 to 18.0) days at 1.0 mg kg^{-1} (2.9 to 19.9) days at 10.0 mg kg^{-1} and (10.8 to 23.2) days at 100.0 mg kg^{-1} indicating the degradation of butachlor to be dependent on application rate and soil type.

Pesticide Solubility

Pesticides with low water solubility tend to be more resistant to microbial degradation than compounds of higher water solubility. Microorganisms can use only the dissolved fraction of the compound in soil solution. Therefore, the rate of dissolution of pesticides would govern the rate of their biodegradation (Cork and Krueger, 1991).

Soil Types

Soil properties like organic matter, clay content, pH etc. affect the degradation of pesticides in soil (Gupta and Gajbhiye, 2002). The role of the soil in pesticide biodegradation is critical because it provides the environment for degradative microorganisms. Soil particles can sorb pesticides, regulating their bioavailability and influencing their persistence. Both content and type of clay and organic matter are important soil parameters, which influence the activity of pesticide degrading microorganisms. Therefore, it is important to study the effect of soil types in pesticide degradation. Gold *et al.* (1996) reported that soil pH and clay content greatly affected the persistence of bifenthrin, chlorpyriphos, cypermethrine, fenvelerate, permethrin and isofenphos under field conditions. The half-lives of rimsulfuron under field conditions varied within a wider range of 5.6 days in a sandy clay loam soil in the United States (Schneiders *et al.*, 1993) and of 120 days in a light sandy soil in Denmark (Reinke *et al.*, 1991). Jones and Ananyeva (2001) reported that the degradation of metalaxyl and propachlor occurred at different rates in different soils. The half-lives in pasture, arable and pine forest soils were 10, 19 and 36 days for metalaxyl and 2.6, 6.1 and 8.2 days for propachlor. Gupta and Gajbhiye (2002) stated that the degradation of flufenacet was greatly influenced by soil types and the half-life values varied from 10.1 to 22.3 days in an inseptisol, 10.5 to 24.1 days in a ultisol and 29.2 to 31.0 days in a vertisol. Hafez and Thiemann (2003) mentioned that the degradations of imidacloprid and diazinon were faster in the silty loam soil followed by sandy loam and sandy soil. Degradation of pencycuron was soil dependent (Pal *et al.*, 2005b). Pencycuron degraded faster in coastal saline soil than alluvial soil and in soil amended with decomposed cow manure whereas microbial mediated degradation of pencycuron was more in alluvial soil than in coastal saline soil.

Soil Moisture

Water acts as solvent for pesticides movement and diffusion and is essential for microbial functioning. Pesticide degradation is slow in dry soils. The rate of pesticide transformation generally increases with water content. In very wet soils such as rice paddies, the rate of diffusion of atmospheric oxygen into the soil is limited and anaerobic pesticide transformation can prevail over aerobic degradation. Poor oxygen transfer at high moisture content can, however, accelerate or retard the degradation of pesticides. Phorate was more persistent in flooded soil than in nonflooded soil (Walter-Echols and Liechtenstein, 1978). The herbicides atrazine and trifluraline disappeared more rapidly under anaerobic conditions than under aerobic conditions. The insecticide γ-BHC persists for several years in aerobic soils, but it is biodegraded partly in submerged soils and a high content of organic matter hastens the biodegradation (Ponnamperuma, 1972). DDT is fairly stable in aerobic soils, but is degraded rapidly to DDD in submerged soils (Topp *et al.*, 1997). The alteration in the oxidation state is important in microbes-pesticides interaction because the oxidized or reduced forms of pesticides often determine their toxicity in the environment through adsorption, solubility etc. (Hicks *et al.*, 1990) and also the microbial activity under submerged condition. Thus, the transformation of pesticides in the submerged soils is different from that of the soils in field moist state. In contrast, Baskaran *et al.* (1999) reported that soil moisture content had no effect on the degradation of imidacloprid and bifenthrin. Racke *et al.* (1994) also found similar type of behaviour while studying the degradation of chlorpyriphos. Schneiders *et al.* (1993) reported that the half-lives of rimsulfuron were 24.5 and 22.5 days under anaerobic and aerobic conditions respectively in a sandy loam soil. Pencycuron degraded rapidly in aerobic soil compared the submerged soil (Pal *et al.*, 2005b).

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 (Burns, 1975, Racke *et al.*, 1997). 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. Vischetti *et al.* (1995) found that the half-lives of rimsulfuron ranged from 14.8 days at 10°C to 3.5 days at 25°C in a clay loam soil incubated at 75% humidity. Microbial activity is stimulated by increase in temperature and some ecological groups tend to dominate within certain temperature ranges. Perucci *et al.* (1999) studied the effect of rimsulfuron on the growth and activity of microbial biomass under laboratory conditions at varying conditions of temperature in a silty clay loam soil. The onset and magnitude of the effects were temperature dependent and generally slight and transitory. Rimsulfuron hydrolyses rapidly in soil under conditions of high temperature (Vischetti *et al.*, 2000). The maximum growth and activity of microorganisms in soils occur at 25-35°C (Alexander, 1977) and the pesticide degradation is optimal at mesophillic temperature range of around 25-40°C (Topp *et al.*, 1997). Jitender *et al.* (1993) conducted laboratory experiments with thiobencarb 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. Getzin (1981) observed that the half-lives of chlorpyriphos ranged from >20 to 1 day over the temperature range of 5 to 45°C, respectively. Zhu *et al.* (2004) reported faster degradation of fipronil at 35°C than at 25°C in nonsterile clay loam soil.

Soil pH

Soil pH may affect pesticide adsorption, abiotic and biotic degradation processes (Burns, 1975). It influences the sorptive behavior of pesticide molecules on clay and organic surfaces and thus, the chemical speciation, mobility and bioavailability (Hicks *et al.*, 1990). For instance, the sorption of prometryn to clay montmorillonite is more at pH 3 than at pH 7 (Topp *et al.*, 1997). 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 (Racke *et al.*, 1997). Rimsulfuron hydrolyses rapidly in soil under conditions of high temperature (Vischetti *et al.*, 2000).

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 nonsaline soil than in saline soils and its stability increased with increasing electrical conductivity (Reddy and Sethunathan, 1985). 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 (Walker, 1976) or inhibitory to degradation (Weber, 1976; Kodama and Kuwatsuka, 1980). Degradation of pencycuron was less in the coastal saline soil compared to the alluvial soil (Pal *et al.*, 2005b).

Soil Organic Matter

The presence of organic matter may alter the behaviour of pesticides in soils. Soil organic matter can either decrease the microbially mediated pesticide degradation by stimulating pesticide adsorption processes or enhance microbial activity (Perucci *et al.*, 2000) by cometabolism (Walker, 1975; Nair and Schnoor, 1994; Thom *et al.*, 1997). The addition of organic materials to flooded soils enhanced the bacterial degradation of some organochlorine insecticides such as BHC, DDT, methoxychlor and heptachlor (Yoshida, 1978). Microbial degradation of linuron (Hicks *et al.*, 1990) and pencycuron (Pal *et al.*, 2005b) in nonsterile soils was stimulated by organic matter amendment. A certain minimum level of organic matter (probably greater than 1.0%) is essential to ensure the presence of an active autochthonous microbial population that can degrade pesticides (Burns, 1975). The species diversity arising from such situation may increase the presence of sufficient number of enzyme systems that are able to attack pesticide molecules (Farmer and Morrison, 1964; Butcher *et al*., 1969).

Soil Biotic Components

The degradation of the pesticides under nonsterile but not under sterile condition or rapid degradation under nonsterile conditions indicated the role of microbes in pesticide degradation. Numerous workers reported microbial degradation of pesticides in soil (Adhya *et al*., 1987; Banerjee *et al*., 1999; Karpouzas *et al*., 1999; Sukul and Spiteller, 2001; Hafez and Thiemann, 2003). Degradation of phorate (Bailey and Coffey, 1985), metalaxyl (Bailey and Coffey, 1985; Droby and Coffey, 1991; Sukul and Spiteller, 2001), fipronil (Zhu *et al*., 2004) and pencycuron (Pal *et al*., 2005b) proceeded more rapidly in nonsterile 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 (Burns and Edwards, 1980). Sukul and Spiteller (2001) reported that the half-life values of metalaxyl were in the range of 36 to 73 and 232 to 602 days in nonsterile and sterile soils respectively, indicating the microbial role in degradation. Hafez and Thiemann (2003) reported the half-lives of imidacloprid and diazinon was in the range of 6.93 to 28.88 and 3.63 to 22.36 weeks in sterile and nonsterile soils respectively. Zhu *et al*. (2004) concluded that the soil microbes predominantly influenced the degradation of fipronil in a nonsterile clay loam soil. The half-live values under nonsterile condition were 9.72 and 8.78 days and 33.51 and 32.07 days under sterile condition at 25 and 35°C, respectively.

For the introduction of new pesticide, it is worthwhile to study its degradation including the persistence of metabolites formed in different agroclimatic situations.

Effects of Pesticides on Soil Microbiological Parameters

Pesticides may alter the soil microbial population, both qualitatively and quantitatively, in several ways. The most obvious effect is that of the direct toxicity of applied pesticide to the susceptible microbial species (Matsumara and Boush, 1971). Other microorganisms become resistant to the pesticide and can increase their biomass because of decreased competition. The potential inhibitory effect of a pesticide was assessed by various parameters or assays such as soil microbial biomass, enumeration of different microbial populations, measurement of functional or specific soil enzyme activity etc. (Topp *et al*., 1997). Current microbial studies rely much on soil microbial biomass than the numbers of soil microorganisms. Conventional microbial methods estimate only a small fraction of total microbial population, as many of these are non-culturable in artificial media and under laboratory conditions (Trevors, 1998).

Soil Microbial Biomass

Microbial biomass is defined as the part of organic matter in soil that constitutes living microorganisms smaller than 5-10 cubic micrometer. Microorganisms include bacteria, actinomycetes, algae, protozoa and micro fauna. Usually, plant roots and faunas larger than 5-10 cubic micrometer such as earthworms, are not included (Sparling, 1985). Microbial biomass being an important attributes of soil quality (Doran and Parkin, 1994) and is an ecologically important parameter (Beelen and Doelman, 1997). Several workers have studied the effects of pesticides application using this parameter.

Two fungicides, viz., captan and thiram caused significant decreases in the microbial biomass (Anderson, 1981). Within 8 days, microbial biomass of captan and thiram amended soils returned to the level of control soil. At higher rates fungicides caused long-term decreases in the biomass. Pencycuron resulted in a short-lived and transitory toxic effect on soil microbial biomass (Pal *et al*., 2005a).

Effects of repeated applications of carbofuran and carbosulfan (insecticides), iprodione and vinclozolin (fungicides) and MCPA, simazine and paraquat (herbicides) on soil microbial biomass were investigated in arable soils (Duah-Yentumi and Johnson, 1986). Carbofuran did not show any detectable detrimental effect on soil microbial biomass, but repeated application of carbofuran significantly reduced microbial biomass. There was dramatic reduction in soil microbial biomass following vinclozolin application and this was due to a reduction in fungal biomass; iprodione showed less obvious biomass trends. MCPA and simazine caused no detectable effects on the microflora, but repeated paraquat application 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.

Side effects of four herbicides, three fungicides, one insecticide and one growth regulator were studied on the soil microflora for two year in a field experiment (Schuster and Schröder, 1990). Successive application of the pesticides caused only slight and short-lived side effects on the soil microbial biomass depending on weather conditions.

Glyphosate temporarily enhanced bacterial propagule numbers, while actinomycete and fungal propagule numbers were unaffected in incubated soil samples over 27 days (Wardle and Parkinson, 1990b). Glyphosate and 2,4-D were applied at field application rates to tilled field plots in a mixed cropping area in South Central Alberta for 45 days (Wardle and Parkinson, 1991). Glyphosate did not influence any of the microbial parameters tested. Addition of 2,4-D significantly influenced the microbial parameters investigated, but the effects were transient, being detectable only within the first 1-5 days of addition. The effects of 2,4-D addition on the microbial parameters tested, even when significant, were typically small and probably of little ecological consequence especially when spatial and temporal variations in these parameters were taken into account. In another study the same authors observed that 2,4-D and glyphosate increased microbial biomass in field plots with weeds present (Wardle and Parkinson, 1992). Isopropylamine salt of glyphosate stimulated soil microbial biomass across a range of soils varying in fertility (Haney *et al*., 2002). It appeared that soil microbes degraded glyphosate rapidly even at high application rate regardless of soil type or organic matter content.

Imazethapyr, in both laboratory and field trials, did not affect microbial biomass at the field rate. But at higher application rates it inhibited microbial biomass (Perucci and Scarponi, 1994).

Effects of 19 years of cumulative annual field application of five pesticides (benomyl, chlorfenvinphos, aldicarb, triadimefon and glyphosate), applied at or slightly above the recommended rates in 25 combinations, on soil microbial biomass were investigated (Hart and Brookes, 1996b). The addition of aldicarb reduced soil microbial biomass and the effect appeared to be persistent. The continuous use of these pesticides, either singly or in combination, had no measurable long-term harmful effects on the soil microbial biomass.

Application of 2,4-D and its analog 2,4,5-T at 0.75 $\mu g\ g^{-1}$ in soils led to a distinct increase in microbial biomass C content over that of untreated soil samples both under flooded and nonflooded conditions. 2,4-D was inhibitory to microorganisms at higher rates of application to soil. Repeated application of a commercial formulation of hexachlorocyclohexane (HCH) to flooded soil caused a marked increase in microbial biomass content. Technical grade γ-HCH was also stimulatory to microbial biomass content (Rath *et al*., 1998).

Rimsulfuron adversely affected the microbiological processes but not at field dose under laboratory conditions, at varying conditions of temperature and humidity in a silty clay loam soil (Perucci *et al*., 1999). Temperature and humidity conditions exerted slight and transitory effects. In another study rimsulfuron or imazethapyr, with sludge compost did not impair soil microbial biomass at field dose but not at higher rates (Perucci *et al*., 2000). Organic amendments in conjunction with the herbicides reduced the detrimental effect on soil microbial biomass. However, based on laboratory studies rimsulfuron could pose environmental risks (Vischetti *et al*., 2000) as microbial biomass decreased compared to untreated soil.

The relationship between the soil microbial biomass content and the persistence of imazamox and benfluralin in three different soils, incubated under different laboratory conditions was determined (Vischetti *et al*., 2002). Microbial biomass decreased compared to untreated soil. The microbial biomass returned to the initial values at varying times depending on incubation conditions.

The literature revealed that several studies on the impact of different pesticides, more particularly the herbicides, were done mostly under laboratory conditions. Laboratory studies seldom reflect field conditions as several factors, which might influence are not taken into account. Effect of pesticides on microbial biomass in problem soils and also under waterlogged conditions such as those in rice paddies, was grossly ignored. Pesticides, when applied in field rate, do not impair microbial biomass. Moreover, microbial biomass studies do not reflect the impact of pesticides on different microbial components and communities.

Soil Ergosterol

Ergosterol is endogenous almost exclusively to fungi, with certain green microalgae and protozoa being the only non-fungal sources and so may be a useful index of fungal presence (Newell, 1992). The $\delta^{5,7}$-diene double bonding of ergosterol gives it a unique pattern of ultraviolet absorption, with a maximum at 282 nm, which makes it readily distinguishable from the major sterols of animals, vascular plants and non-fungal microorganisms (Newell, 1992).

Grant and West (1987), West *et al*. (1987), Davis and Lamar (1992), Djajakirana *et al*. (1996) and Stahl and Parkin (1996) measured the fungal biomass by ergosterol estimation.

With the increasing use and the wide spectrum activity of fungicide, it is important that they be fully assessed for side effects on soil microorganisms, particularly the fungi. Peacock and Goosey (1989), Guan *et al*. (1992) and Kwok and Loeffler (1993) used this concept to study the effect of fungicides *in vitro*.

Hart and Brookes (1996a) investigated the effects of two ergosterol biosynthesis-inhibiting fungicides, epoxiconazole (at concentrations equivalent to 2- and 20-fold field rate) and triadimefon (field rate and 10-fold field rate) and of straw amendment on ergosterol in a sandy loam soil. Both the concentrations of the fungicides decreased soil ergosterol content by about 30% after 7 days of incubation in the unamended soils. However, the values returned to control thereafter. After amendment of the soil with wheat straw, the inhibition of ergosterol biosynthesis reappeared, ranging from 4 to 34%. Epoxiconazole had greater effect than triadimefon. The inhibition was also transient, but was longer lasting than in the unamended soil. Results indicated that fungicides were more active against newly synthesized microorganisms than against the original population. The toxicity of pencycuron was more on the newly generated soil microflora stimulated by the addition of decomposed cow manure (Pal *et al*., 2005a).

The authors opined that soil ergosterol content was a more sensitive measure of pesticide side effect than microbial biomass C and could be a useful method in this respect.

Microbial Activity

Measurement of microbial biomass and ergosterol does not reflect the metabolic state of the microorganisms. Along with microbial biomass, microbial activities should also be measured for proper appreciation of ecosystem functioning and soil disturbances due to perturbations (Brookes *et al*., 1987).

Soil Respiration

Active living cells need constant supply of energy, which the heterotrophic microflora derive through organic matter transformation. Under aerobic condition, the end product of the transformation is the evolution of CO_2 and H_2O (respiration). The metabolic activities of soil microorganisms can, therefore, be quantified by measuring CO_2 evolution (Nannipieri *et al*., 1990). Soil respiration is one of the oldest and still the most frequently used parameter for quantifying microbial activity in soil. 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 (Gray, 1990). Substrate induced respiration is a measure of the total physiologically active part of the soil microflora (Anderson and Domsch, 1978). The combination of the basal and substrate induced respiration represent carbon availability index (Cheng *et al*. 1996). Both the methods were commonly applied to characterize the microbial status of soil and hence bioindicators of soil health or soil quality (Gregorich *et al*. 1994; Pankhurst *et al*., 1995). Like other metabolic activities it depends on the physiological state of the microbial cells and 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. (Alef, 1995). 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.

The effects of 2,4-D, picloram and glyphosate on soil respiration were monitored (Wardle and Parkinson, 1990a) over 27 days to Alberta agricultural soil at concentrations of 0, 2, 20 and 200 $\mu g\ g^{-1}$. All the herbicides at only 200 $\mu g\ g^{-1}$ enhanced basal soil respiration only upto 9 days following application. Substrate induced respiration was temporarily depressed by picloram and 2,4-D and briefly enhanced by glyphosate applied at 200 $\mu g\ g^{-1}$ concentrations. The side effects of these chemicals were probably of little ecological significance. In another study the effects of glyphosate and 2,4-D on soil respiration was investigated (Wardle and Parkinson, 1991) in a mixed cropped area in South Central Alberta for 45 days. Glyphosate had no adverse influence on basal respiration, substrate induced respiration, basal : substrate induced respiration and bacteria: fungi ratio. 2,4-D had transient effects within the first 1-5 days of application on the microbial variables investigated and therefore of little ecological significance. Glyphosate treated Hapludult and Hapludox Brazilian soils, *in vitro*, over a period of 32 days increased soil respiration compared to the control (Araújo *et al*., 2003). Soil, which had been exposed to glyphosate for several years, had the strongest response on microbial activity. Glyphosate showed some irregular stimulations and retardations of the respiration rates of soil microbiota (Haney and Senseman, 2000). High doses suppressed respiration (Roslycky, 1982).

The effects of some herbicides (atrazine, pentachlorophenol, 4-Chloroaniline and chloroacetamide), fungicides (zineb and captan), insecticides (lindane and 4-Notrophenol) and bactericides ($HgCl_2$) on soil respiration upto 48 days were investigated (Zelles *et al*., 1985). Atrazine, lindane and captan had inconsiderable effects on soil respiration. The other pesticides changed the behavior of microorganisms considerably. Addition of alfalfa meal promoted the reversibility of the effects caused by pesticides.

Laboratory tests were conducted to determine any serious effects of the herbicides atrazine, butylate, imazethapyr, linuron, metazachlor, metribuzin and trifluraline on soil respiration (Tu, 1992). Atrazine significantly increased the soil respiration after 96 h of incubation. The herbicides when applied at the tested rates, did not seriously affect the soil respiration.

Soil respiration of two non-flooded soils treated with bensulfuron-methyl at field and 10-fold field rates after 1 and 4 weeks of incubation under laboratory conditions was not influenced (Gigliotti *et al.*, 1998).

Ecophysiological Quotients (qCO_2 and Q_R)

For better appreciation of metabolic status of microorganisms due to stress, there is increasing interest in the clubbing of two parameters into one, such as the ratio of soil basal respiration to microbial biomass (i.e., microbial metabolic quotient or specific respiration of the biomass, qCO_2) based on Odum's theory of ecosystem succession. This was proposed as an alternative measure of changes in the physiological status of the microbial community and its activity in response to disturbance (Anderson and Domsch, 1985b, 1990). A close relationship was found between pesticide degradation rate constant and microbial metabolic quotient in various soil ecosystems (Jones and Ananyeva, 2001). Microbial qCO_2 increases due to disturbances caused by pesticidal chemicals applied, resulting from microbes utilizing large part of their energy budget for maintenance than cell synthesis (Anderson and Domsch, 1990). The soils amended with organic matter, in general, registered lower qCO_2 values than the unamended soils, indicating a better utilization of the substrate for cell growth than cell maintenance (Perucci *et al.*, 2000). The potential stress imposed by pesticide application is likely determined not only by the chemical characteristics of the stressor but also by the abundance of native microbial community (Jones and Ananyeva, 2001). The duration and magnitude of the response to pesticide induced stress or disturbance should be compared to that of naturally occurring stressors such as moisture (Domsch, 1984). The respiration per unit of biomass is a more sensitive indicator of toxic effects than the respiration rate or the amount of biomass alone (Beelen and Doelman, 1997). However, the results in this respect should be addressed cautiously.

The ratio of basal soil respiration to substrate-induced respiration (Q_R) was used to assess the effects of various perturbations in soil ecosystems (Anderson and Domsch, 1985a; Insam and Domsch, 1988). Basal soil respiration is generally attributed mostly to the metabolically dormant population and that of substrate-induced respiration to the metabolically activated population (Ohya *et al.*, 1988). Pencycuron application at field rate had no detrimental effect on the ecophysiological parameters but not at higher rates (Pal *et al.*, 2005a). Inspite of the utility of Q_R, very few reports (Wardle and Parkinson, 1990b, 1991; Haney and Senseman, 2000) in this respect are available.

FDA Hydrolyzing Activity (FDHA)

Soil enzyme assays may not always reflect the overall microbial activity of soil, because the enzymes are substrate specific (Nannipieri *et al.*, 1990). In this respect, the measurement of Fluorescein Diacetate Hydrolyzing Activity (FDHA) is a promising method of determining overall soil microbial activity (Dick, 1994). Fluorescein diacetate is hydrolyzed by a number of different enzymes, such as protease, lipase and esterase. 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 (Lundgren, 1981).

The estimation of FDHA is a widely accepted simple method for precision measurement of the total microbial activity in soils (Adam and Duncan, 2001). Dumontet *et al.* (1997) 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.

Pesticides induced measurable changes in the behavior of microorganisms and lower rate of application sometimes produced stimulative effects on FDHA while higher concentrations caused mostly reversible or irreversible reductions (Zelles *et al*., 1985). Improvement of soil by addition of alfalfa meal promoted the reversibility of effects caused by pesticides. Imazethapyr, under field and laboratory studies, applied at field rate application had no adverse effect on FDHA but not at the higher rates (Haney *et al*., 2002). Rimsulfuron in a laboratory experiment had slight and transitory detrimental influence on FDHA at higher doses but not at field dose(Perucci *et al*., 1999). Glyphosate, *in vitro*, in typical Brazilian soils increase FDA hydrolysis compared with the same soil, which has never received Glyphosate (Araújo *et al*., 2003) Pencycuron resulted in a short-lived and transitory toxic effect on fluorescein diacetate hydrolyzing activity(Pal *et al*., 2005a).

Correlation of Pesticide Transformation Rate with Soil Microbiological Properties

Microbial processes affect the degradation of most pesticides in soil (Alexander, 1994). 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 (Moorman and Harper, 1989), linuron and glyphosate (Torstenssen and Stenstrom, 1986), alachlor (Walker *et al*., 1992), 2,4-D and dicamba (Voos and Groffman, 1997) 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 (Jones and Ananyeva, 2001). In contrast, no correlations were found between microbial biomass and degradation of the pesticides 2,4-D and atrazine (Entry *et al*., 1994; Entry and Emmingham, 1996; Ghani *et al*., 1996). It was opined that this relationship might be useful for developing approaches for evaluating and predicting the fate of pesticides in different ecosystems (Voos and Groffman, 1997).

The relationship between rimsulfuron (Perucci *et al*., 1999; Vischetti *et al*., 2000), imazamox and benfluralin (Vischetti *et al*., 2002) 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 pesticide 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.

Conclusions

Available literature shows a clear dearth of information regarding the fate and effects of pesticides in tropical soils. Furthermore, field validation studies are extremely limited. Therefore, there is an urgent need to generate regionally specific database for pesticide effects in tropical environments.

Projection of Future Work

Several parameters as commonly used in environmental microbiology, have been studied as tests of pesticide toxicity. Most of the studies showed that pesticides at recommended field rate did not impair microbial biomass and its activities. However, microbial community structure may be markedly changed, even if the overall microbial activity remains unaffected by the pesticide. Bacterial population may be increased compared to fungal population and vice-versa. Some microorganism may be suppressed and others may proliferate in the vacant ecological niches. Work in this respect is lacking. Bacterial biomass could be measured by the analysis of muramic acid (Zelles, 1995) and fungal biomass can easily be measured by the ergosterol control (Djajakirana *et al*., 1996).

Thus, risk assessment should include the measure of microbial diversity. Work in this respect has just been initiated. Protocol for such diversity studies are now available (Trevors, 1998; Johnsen *et al*., 2001).

There is an urgent need for standardization of handling soil samples for these toxicity studies. It is now well known that soil microbial community structure in field soils is different from that of the soil samples used in the laboratory.

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