Bull. Environ. Contam. Toxicol. (2004) 72:600–606

DOI: 10.1007/s00128-004-0286-3

Environmental Contamination and Toxicology

Effects of Pesticides and Their Hydrolysates on Catalase Activity in Soil

L. Shiyin,[1] N. Lixiao,[2] P. Panying,[1] S. Cheng,[2] W. Liansheng[2]

[1] School of Chemistry and Environmental Science, Nanjing Normal University, Nanjing 210097, People's Republic of China
[2] State Key Laboratory of Pollution Control and Resources Reuse, School of the Environment, Nanjing University, Nanjing 210093, People's Republic of China

Received: 15 February 2003/Accepted: 5 January 2004

Many enzymes are produced by the microorganisms in soil, and some of them are involved in C, N and P circles in soil. Their catalytic efficiency may be strongly influenced by the environment surrounding the enzymes. As reviewed by Gianfreda and Bollag (1996), natural and anthropogenic factors may affect directly or indirectly the activities of enzymes in soil. Among anthropogenic factors, pesticides are of primary importance because they can be directly applied to soil or transferred by other ways. Pesticides, usually extraneous to soil component pools, were expected to affect the behavior of enzymes (Bollag and Liu, 1990). The activity of soil-enzymes had been considered as eco-toxicological indicators to assess the situation of contaminated soil by pesticides and the possible risk of ecosystem (Dick, 1995).

Catalase (Hydrogen-peroxide oxidoreductase, EC 1.11.1.6.) is an intracellular enzyme found in all aerobic bacteria and most facultative anaerobes, but absent from obligate anaerobes (Weetall et al., 1965; Trevors, 1984; Alef and Nannipieri, 1995). Catalase activity in soil is considered as a sensitive indicator of aerobic microorganisms' activity and is related to both the number of aerobic microorganisms and soil fertility (Garcia and Hernandez, 1997; Pankhurst et al., 1996).

Organophosphorus pesticides and pyrethroids are used widely in China. Most of them are esters of organic acids. It is well known that pesticides tend to be hydrolyzed in natural systems, especially in the presence of feasible pH value, metal oxides (Feng and Pehkonen, 1998) and dissolved metal ions (Stone and Torrents, 1995; Smolen and Stone, 1997). Degradation of pesticides has been attributed to both chemical and microbiological pathways (Cowart et al., 1971; Seiber and Markle, 1972; Walker, 1976; Racke, 1993). The persistence and toxicity of these compounds and their degradation products have become a serious environmental concern as well as a public health priority (Juarez and Sanchez, 1989; Fernandez-Casalderrey et al., 1992). Many investigations were devoted to study the effect of various pesticides on activities of enzymes in soils from

Correspondence to: L. Shiyin

Table 1. Main parameters of soil collected from Purple Mountain.

Parameter	Value
pH (H_2O)	7.02
Sand (%)	63.20
Silit (%)	18.6
O.C. (%)	1.64
Clay (%)	18.2
C.E.C. (cmol Kg^{-1})	9.27
Texture	Silty sand

different origins. As to this topic, many reports were reviewed and summarized by Shaffer (1993). Once pesticides enter into soil environment, metabolites and intermediates could be found when complex chemical and biological reactions are carried out. However, few studies were performed to understand the influences of pesticides and their metabolites on the activities of enzymes in soil. The objective of this study was to characterize the effects of fenvalerate, chlorpyrifos and their hydrolysates on catalase activity in soil in order to understand the possible ecological risk of metabolites of pesticides where pesticides were used.

MATERIALS AND METHODS

Soil without adding pesticides was collected nearby the Purple Mountain in Nanjing. The cultivated soil was collected (surface below 2-10 centimeters), air-dried, passed through a 2 mm stainless steel screen and stored at 4℃ prior to experiment. The pH was determined in a distilled water soil suspension (1:1 /V:V), other physico-chemical properties were determined according to Nelson and Sommers (1982) (see Table 1).

Fenvalerate was provided from the Pesticides Factory of Nanjing in China (≥ 91% a.i.). Chlorpyrifos was purchased from Chem Service (99.2% a.i.). The other chemicals were reagent grade. Hydrolysates were prepared by adding sodium hydroxide (0.01mol/L, 5ml) to solutions containing quantitative pesticide and placed in dark for seven days. The pH of solution containing hydrolysates of pesticides was adjusted with hydrochloric acid (0.01mol/L, 5mL) to neutrality before adding to soil.

Soil 1400.0g was equally divided into seven portions. Different doses of pesticides (0, 1, 10, 40, 80ppm) were mixed with soil. The hydrolysis products pesticides of 40 and 80ppm were also prepared as above. Water holding capacity of soil was adjusted to 60% of the maximum, and the samples were incubated at ambient temperature. The catalase activity of exposed soils was determined periodically according to previous works (Johnson and Temple, 1964; Roberge, 1978). This method is based on the recovery rates of H_2O_2, and the residual H_2O_2 is determined by titration with $KMnO_4$ in the presence of H_2SO_4. The catalase activity was related to blank soil. All data are the average of three replications.

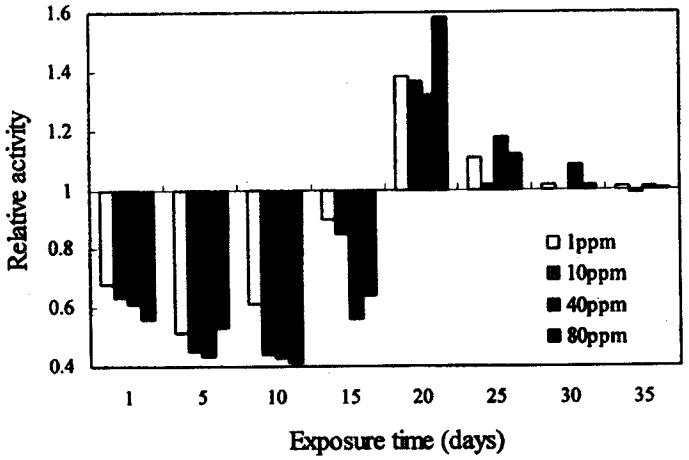

Figure 1. Exposure time versus relative catalase activity in exposed soil at different doses of fenvalerate.

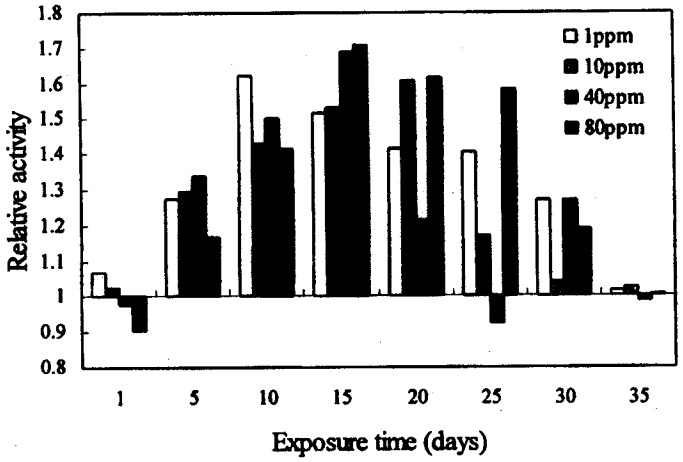

Figure 2. Exposure time versus relative catalase activity in exposed soil at different doses of chlorpyrifos.

RESULTS AND DISCUSSION

The effects of different doses of fenvalerate on catalase activity in soil are depicted in Fig.1. Catalase activities were inhibited when fenvalerate was added to soil in 15 days, and then the activities began to be stimulated. Maximal inhibition occurred at the 10th day when the concentration of fenvalerate was 80ppm. The stimulation reached the maximal when the level of fenvalerate was 80ppm at about the 20th day. After 30 days, catalase activities in exposed soils recovered from stimulative effect and reached the level of catalase activity of the blank soil.

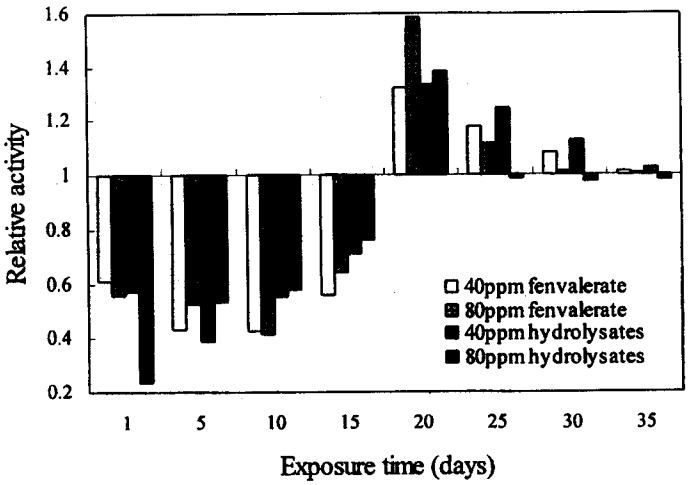

Figure 3. Exposure time versus relative catalase activity in exposed soil at different doses of fenvalerate and fenvalerate's hydrolysates.

As to chlorpyrifos, catalase activity was stimulated at lower level of chlorpyrifos (1ppm), and inhibited at higher concentrations of chlorpyrifos (10~80ppm) after 1-day incubation (Fig. 2). With exposure going on, catalase activities in soil were stimulated strongly. The stimulated extent was various with the different doses and incubation times. Maximal stimulation occurred at the 15th day and 80ppm of chlorpyrifos. The time of maximal stimulation effect was prolonged with the increase of concentration of chlorpyrifos. From the point of eco-toxicology in soil, it is obvious that higher concentration of fenvalerate and chlorpyrifos can alter the environment of soil and affect the growth of plants. Soil environment may take place a great change at early stage when pesticides enter into the soil by agricultural practices or other applications.

Fig. 3 indicated the influences of fenvalerate and its hydrolysis products on catalase activity in soil. Compared to the same level of fenvalerate, fenvalerate's hydrolysates had stronger inhibitory effect on catalase activity at the initial stage of exposure (about 1-5 days). Maximal inhibition of hydrolysates appeared at 1st day at 80ppm of hydrolysates. It was at the 15th day when 80ppm of fenvalerate reached maximal inhibition. After 15-day incubation, the stimulations took place, and the maximal stimulations of both Fenvalerate and its hydrolysates appeared at the 20th day when their concentrations were 80ppm. However, after the 20 days, 80ppm of hydrolysates began to slightly inhibit the catalase activity.

Effects of chlorpyrifos and its hydrolysis products of different doses on catalase activity related to blank sample in soil was illustrated in Fig. 4. Catalase activities were inhibited clearly by chlorpyrifos and its hydrolysates when added to soil for 1 day and the inhibition of the hydrolysates was greater than that of the same

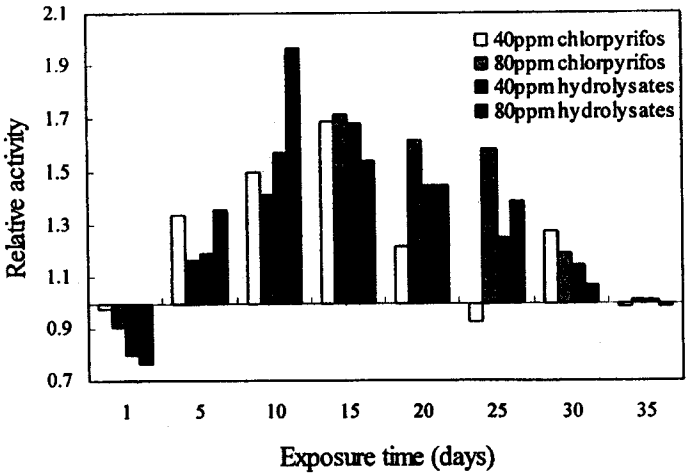

Figure 4. Exposure time versus relative catalase activity in exposed soil at different doses of chlorpyrifos and chlorpyrifos' hydrolysates.

levels of chlorpyrifos, which can be attributed to the formation of new compounds because of hydrolysis of chlorpyrifos and the hydrolysates were more active than chlorpyrifos. After 1 day, the chlorpyrifos and its hydrolysates began to stimulate catalase activities. 80ppm of hydrolysates produced the greatest stimulation at the 10th day and then the activities began to decrease with the increasing of the incubation time. At the 35th day of incubation, catalase activities stimulated by 40ppm of chlorpyrifos and 80ppm of hydrolysates were close to the samples of blank soil. The results showed that catalase activity increased when the organic matters added to soil stimulated the synthesis of enzyme (Garcia et al., 2000). It may be explained by the improved soil aeration in the organic amended soils as a consequence of an increase in soil porosity (Giusquiani et al., 1995).

In conclusion, it is possible to affect microorganism activity in soil at early stage of exposure when fenvalerate, chlorpyrifos are used by agricultural practices or other applications. The influences of fenvalerate, chlorpyrifos and their hydrolysates on catalase activity in soil are various at the their different levels at different incubation times. Catalase activities in soil at different doses of pesticides and their hydrolysates will recover from stimulative effect, and reach to the level of catalase activity of the blank soil after about 35 days.

Acknowledgments. This work is supported by the state natural science foundation under grant 29877010.

REFERENCES

Alef K, Nannipieri P (1995) Enzyme activities. In: Alef K, Nannipien, P (Eds.), Methods in Applied Soil Microbiology and Biochemistry. Academic Press,

London, pp. 311-373.
Bollag JM, Liu SY (1990) Biological transformation processes of pesticides. In: Cheng HH (Ed.), Pesticides in the Soil Environment: Processes, Impacts, and Modeling. Soil Science Society of America, Madison, WI, USA, pp. 169-211.
Cowart RP, Bonner FL, Epp EA (1971) Rate of hydrolysis of seven organicphosphate pesticides. Bull Environ Contam Toxicol 6: 231-234.
Dick R (1995) Soil enzyme activities as indicators of soil quality. In: Doran JW, Coleman DC, Bezdicek DF, Stewart BA (Eds.), Defining soil quality for a sustainable environment. Soil Science Society of America, Madison, WI, USA, pp. 107-124.
Feng H and Pehkonen (1998) Hydrolysis of phorate using simulated environmental conditions: rates, mechanisms, and product analysi. J Agric Food Chem 46: 1192-1199.
Fernandez-Casalderrey A, Ferrando MD, Andreu-Moliner E (1992) Endosulfan and diazinon toxicity to the freshwater rotifer brachionus clayciflorous. J. Environ. Sci. Health: Pestic Food Contam Agric Wastes B27: 155-164.
Garcia C, Hernandez T (1997) Biological and biochemical indicators in derelict soils subject to erosion. Soil Biol Biochem 29: 171-177.
García GJC, Plaza P, Soler-Rovira AP (2000) Long-term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass. Soil Biol Biochem 32: 1907-1913.
Gianfreda L, Bollag JM (1996) Influence of natural and anthropogenic factors on enzyme activity in soil. In: Stotzky G, Bollag JM (Eds.), Soil Biochemistry, Vol. 9 Marcel Dekker, New York, pp. 123-193.
Giusquiani PL, Pagliai M, Gigliotti G, Businelli D, Benetti A (1995) Urban waste compost: effects on physical, chemical and biochemical soil properties. J Environ Qual 24: 175-182.
Johnson JL, Temple KL (1964) Some variables affecting the measurement of 'catalase activity' in soil. Soil Sci Soci America Proce 28: 207-209.
Juarez SLM, Scanchez J (1989) Toxicity of the organophosphorus insecticide metamidophos (O, S-Dimethyl phosphoramidothioate) to larvae of the freshwater prawn macrobrachium rosenbergii (De Man) and the blue shrimp penaeus stylirostris. Bull Environ Contam Toxicol 43, 302-308.
Nelson DW, Sommers LE (1982) Total carbon, organic carbon and organic matter. In: Page AL, Miller RM, Keeny DR (Eds.), Methods of soil analysis. Madison, USA, Part 2, pp. 539-580.
Pankhurst CE, Rogers S, Vadakattu VRSG (1996) Microbial parameters for monitoring soil pollution. In: Lynch JM Wiseman A(Eds.), Environmental biomonitoring: the biotechnology ecotoxicology interface. OECD/CUP, pp. 46-69. Cambridge, UK.
Racke KD (1993) Environmental fate of chlorpyrifos. Rev Environ Contam Toxicol 131: 1-150.
Roberge MR (1978) Methodology of enzymes determination and extraction. In: Burns RG (Ed.), Soil Enzymes. Academic Press, New York, pp. 341-373.

Seiber JN, Markle JA (1972) Hydrolysis and solvent partition of phosdrin and related organophosphates. Bull Environ Contam Toxicol 7: 72-74.

Shaffer A (1993) Pesticide effects on enzyme activuties in the soil ecosystem. In: Stotzky G, Bollag JM (Eds.), Soil Biochemistry, vol. 8. Marcel Dekker, New York, pp. 273-340.

Smolen JM, Stone AT (1997) Divalent metal ion-catalyzed hydrolysis of phosphorothionate ester pesticides and their corresponding oxonates. Environ Sci Technol 31: 1664-1673.

Stone AT, Torrents A (1995) The role of dissolved metals and metal-containing surfaces in catalyzing the hydrolysis of organic pollutants. In: Huang PM (Ed.), Environmental Impact of Soil Component Interactions. Lewis Publishers, Chelsea, MI, pp. 275-297.

Trevors JT (1984) Rapid gas chromatographic method to measure H2O2 oxidoreductase (catalase) activity in soil. Soil Biol Biochem 16: 525-526.

Walker WW (1976) Chemical and microbiological degradation of malathion and patathion in an eatuarine enviroment. J Environ Qual 15: 210-215.

Weetal HH, Weliky N, Vango SP (1965) Detection of microorganisms in soil by their catalytic activity. Nature 206: 1019-1021.