Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

ISSN: 0972-6268	Vol. 10	No. 4	pp. 511--516	2011

Original Research Paper

Mechanism of Phytoremediation: Study of uptake and metabolism of Methyl Parathion and *p*-Nitrophenol in Maize

Nayeem Ullah Khan and Bhavya Varma
Fr. Cecil J. Saldanha Centre for Experimental Research in Bioscience, St. Joseph's College Research Centre, 46 Langford Road, Bangalore-560027, Karnataka, India

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 14/6/2011
Accepted: 26/8/2011

Key Words:
Phytoremediation
Methyl parathion
p-nitrophenol
Hydroquinone

ABSTRACT

Phytoremediation is a green technology, where plants are used to remove contaminants (organic or inorganic) from soil. This is the first report of using an indigenous plant for the purpose of phytoremediation. Maize, a crop plant has been used for this purpose where uptake of methyl parathion by the plant has been shown to occur 80%. Average uptake of *p*-nitrophenol is 64%. Streptomycin behaves like organic matter and affects the enzyme *p*-nitrophenol 4-hydroxylase both in the root and shoot. The enzyme has been demonstrated to occur in a plant for the first time. Influence of soil organisms is both positive and negative in the uptake of organophosphate. The results obtained were confirmed by HPLC. Methyl parathion hydrolyses to *p*-nitrophenol, which is further metabolized to hydroquinone with nitrite release. Maize showed an uptake of 31.29% of methyl parathion in unsterilized soil. In the presence of streptomycin and in unsterilized soil uptake of hydroquinone is 99.78% and 98.36% respectively, while in sterilized soil there was no degradation.

INTRODUCTION

Methyl parathion (MP) is an organophosphate insecticide and acaricide widely used throughout the world since its introduction in 1952 (Barr et al. 2002). Methyl parathion has been used primarily for the control of boll weevils on cotton. Methyl parathion enters the environment through spraying on farm crops. It breaks down quickly to *p*-nitrophenol (PNP) and then hydroquinone (HQ) by interacting with water/bacteria/sunlight. Methyl parathion degrades rapidly in seawater, and lakes and rivers. In water, methyl parathion is subjected to photolysis, with a half life of 8 days during the summer and 38 days in winter (EHC 145 1992). Methyl parathion contamination measurements in water have been done (Albins et al. 1986, Ferrand et al. 1992, Nwankoala & Osibanjo 1992). Methyl parathion sticks to soil particles and does not move from the soil to groundwater. Methyl parathion degrades more rapidly in anaerobic soils than in aerobic soils (Adhya et al. 1981).

p-nitrophenol is also an insecticide and contaminates groundwater. It is a potent uncoupler of oxidative and photophosphorylation (Zayer & Kocher 1988), and it is a major environmental pollutant (EPA 1990). It has carcinogenic, mutagenic and toxic properties (Parries 1980). *p*-nitrophenol is an indicator and acts as a marker for the presence of methyl parathion. Unlike methyl parathion, *p*-nitrophenol does not stick to soil and it has contaminated groundwater in USA (EPA 1990).

p-nitrophenol hydrolyses into hydroquinone by releasing nitrite. Hydroquinone occurs in the environment as a result of man-made processes, as well as in natural products from plants and animals. Because of its physical and chemical properties, hydroquinone will be distributed mainly to the water component when released into the environment. It degrades as a result of both photochemical and biological processes; consequently, it does not persist in the environment. Bioaccumulation has not been observed (WHO 1996). Hydroquinone is extensively used as a reducing agent, photographic developer, an antioxidant for many oxidizable products, stabilizer or polymerizing inhibitor for certain materials that polymerize in the presence of free radicals, and as a chemical intermediate for the production of antioxidants and antiozonants, agrochemicals and polymers (WHO 1996). It is a skin lightening agent, and is used in cosmetics, hair dyes and medicine preparation. Hence, it is not an environmental hazard.

Methyl parathion and *p*-nitrophenol are toxic to insects and crustaceans and moderately toxic to mammals. In humans, exposure to high levels of methyl parathion for a short period causes dizziness, loss of consciousness, death confusion, headaches, difficulty in breathing, vomiting, etc. The EPA and IARC have determined that methyl parathion is not classifiable to human carcinogens. The growing plant or active animal can metabolize residues much more rapidly than a comparatively static system such as soil, where the residues become tightly adsorbed onto various soil fractions. The persistence of pesticides in soil is influenced by the type of soil to which they are applied, and particularly by soil

characteristics such as particle size, mineral and organic content and hydrogen ion concentration. Their residual life also depends upon the biological activity in the soil, since the breakdown patterns of many pesticides is mediated by enzymes (Edwards 1975). Enzymes are of biological origin but some can also exist for considerable periods outside living organisms. They may be released from dying organisms, microorganisms, roots of plants or in excreta from soil animals, and still remain active. It is not known that how extensive is the breakdown of pesticides by free soil enzymes, but there is evidence that microorganisms do not account for more than 40% of the breakdown of persistent pesticides. Soil organic matter is extremely important in adsorbing pesticides, and many workers who have demonstrated that the uptake of pesticides by plants is inversely related to the amount of organic matter in soil (Edwards 1975). In the present study detoxification of soil by uptake mechanism is studied in detail. The conversion procedure of *p*-nitrophenol to hydroquinone was monitored using HPLC and UV-spectrophotometer. In the process of degradation from *p*-nitrophenol to hydroquinone, nitrite (NO_2^-) is released which is estimated by *N*-(1-naphthyl)-ethylenediamine dihydrochloride (NEDA) method (Nagaraja et al. 2001). The experimental soil was tested for its organic matter, because these pesticides bind to organic matter. In the uptake experiments, the insecticide *p*-nitrophenol and metabolite hydroquinone enter the plant system and these may also disappear inside the plant due to the enzyme activity. The aim of the study is to demonstrate the presence of the enzyme system that metabolize *p*-nitrophenol to hydroquinone. For detoxification to occur in the plant via enzymes, entry of pesticide into plant must occur. Hence, another aim of the present research is to study the uptake of pesticides as xenobiotics.

MATERIALS AND METHODS

Soil preparation: Red lateritic garden soil was used for uptake studies. The types of soil used were unsterilized soil, sterilized soil, soil spiked with 1 g of streptomycin (SM) per 400 g of soil and soil with organic matter (vermicompost in 1:1 mixture). The soil was spiked with 60 mL of 10 mM chemicals (methylparathion, *p*-nitrophenol and hydroquinone) mixed well in polypropylene pots.

Intact-plants experiments: Maize was selected for the uptake experiment where their roots were intact. The seeds were sown in pots (5 seeds/pot). After plants were grown and 15-day old, they were transferred to pesticide treated soil for the uptake experiments. The uptake experiments lasted for five days. The pots had a drainage hole to drain the excess water. Every pot with plants received 10 mL water everyday. Excess water was collected in a plastic bowl kept below the pot and was added back sometime in the evening to the respective pots.

Equipment: The instrument was HPLC model UV-2075, PU-2080, LC-Net ADC II, Rheodyne injecter-7725, the analytical column used was a stainless-steel C_{18} column. Mobile phase was acetonitrile: distilled water: phosphoric acid (50:50:0.1) (Kulakarni & Chaudhari 2006). Twenty four hours after spiking the soil with compounds HPLC measurements of soil solutions were done. The soil solutions were prepared from each pot as follows: soil (1 g) from a depth of 1 cm was taken and filtered through non-absorbent cotton and acidified by 6 N HCl, ether dried and dissolved in methanol.

OD measurements of soil solution: The zero day and 5th day OD measurements of soil solution were made. The soil solutions were prepared from each pot as follows: soil (1 g) from a depth of 1 cm was taken and diluted with 15 mL water, filtered and used for OD measurement in a Shimadzu UV-visible spectrophotometer. Standard solutions of uptake chemicals used were prepared to determine their l_{max}. Methyl parathion was prepared in 0.1 N sodium hydroxide. The solution was dark yellow. This was diluted with 0.1 N sodium hydroxide and if any turbidity persisted, few drops of methanol were added to clear it. The clear solution was made up to 1 mM concentration by distilled water. One mM solution each of *p*-nitrophenol and hydroquinone were made in water.

Nitrite estimation from soil: Locally available maize seeds were sown in pots (5 seeds/pot). 15-day old plants were transferred to pesticide treated soil for the nitrite estimation. One gramme of soil was added to 15 mL distilled water and 1 mL of the soil solution was taken and diluted to 50 mL with distilled water and 2 mL *N*-(1-naphthyl)-ethylenediamine dihydrochloride reagent was added and finally optical density was taken at 540 nm.

Assay of *p*-nitrophenol 4-hydroxylase: The enzyme was assayed from maize plant as follows: From the 15-day old plants 5 g plant tissue (leaf and root) was weighed and ground in a porcelain mortar with 15 mL tris-HCl buffer, pH 7.2. The slurry was strained through a cheese cloth and extract was centrifuged at 3000 rpm for 15 min. The supernatant was collected and used as the enzyme source. Two mL of enzyme was taken into a test tube to which 1 mL dithiotritol (40 mM), 1 mL dimethyltetrahydropteridine (1.8 mM) and 1 mL *p*-nitrophenol (8 mM) solution was added and incubated at 30°C for 2 h. The reaction was terminated by adding 1 mL 6 N HCl.

From the above solution, 1 mL sample was taken in a test tube to which 2 mL *N*-(1-naphthyl)-ethylenediamine dihydrochloride reagent and 7 mL distilled water were added

and OD was taken at 540 nm. Alternatively the enzyme was assayed after stopping reaction by estimating the product hydroquinone at 300 nm spectrophotometrically (Imrana 2008).

RESULTS

Pesticides can be taken up from sprays to the foliage, stem, seeds and fruits or through roots. Roots absorb lipid-soluble pesticides much less readily than water-soluble ones. Rhizofiltration hypothesis can be tested for methyl parathion, *p*-nitrophenol and hydroquinone. The rooted plant maize showed uptake of methyl parathion above 79% in unautoclaved and sterilized soil spiked with streptomycin. The uptake of *p*-nitrophenol in unsterilized soil is 64.13% addition of streptomycin to this soil lowers its value slightly (62.6%), while in sterilized soil with streptomycin there is a stimulation of *p*-nitrophenol uptake (65.5%) (Table 1) in soil alone, there is no degradation of the three compounds which were so readily taken up by the maize plant. In maize leaves, metabolism of methyl parathion has been reported earlier where it was completely metabolized within 4 days when applied to corn leaves (Howard 1989). The persistence of pesticide in soil is markedly influenced by the types of soil and its organic content. Their residual life also depends upon the biological activity of the soil. The persistence of pesticide methyl parathion, *p*-nitrophenol and hydroquinone was tested with organic matter (vermin compost) and streptomycin. In the presence of organic matter, uptake of the pesticide is decreased. It is of interest to know whether organic matter can bind methyl parathion, *p*-nitrophenol and hydroquinone. Uptake of methyl parathion is high in sterilized soil (65.79%) and hydroquinone is very less (10.53%) (Table 2). Hydroquinone seems to be the end product of methyl parathion metabolism in some soil microorganisms.

The data of Table 1 was obtained by UV-spectrophotometer for a period of 5 days, whereas the experiments using HPLC were completed within a period of 24 h. Maize plants are efficient absorbers and metabolisers of hydroquinone and showed less uptake of methyl parathion (Fig. 1 & Fig. 2). It appears that streptomycin is either binding or degrading methyl parathion. Degradation of *p*-nitrophenol in sterilized soil in presence of streptomycin affects binding of methyl parathion to streptomycin. This is evident by nitrite release experiments (Table 3).

Methyl parathion was not detected in the maize plant in HPLC experiments. Probably it is hydrolysed immediately to *p*-nitrophenol. Hence, *p*-nitrophenol uptake only has been studied. Enzymes mediate degradation pathway of

Table 1: Effect of SM on MP, PNP and HQ uptake in maize. OD of sample solutions taken from soil as described in text. Fifteen day old maize plants as described in Materials and Method were used. The OD for MP, PNP and HQ were taken at 400 nm, 400 nm and 300 nm respectively. OD is represented as mean ± SD.

Treatment	Zero h OD ± SD			24 h OD ± SD			Percentage %		
	MP	PNP	HQ	MP	PNP	HQ	MP	PNP	HQ
Unsterilized soil	2.14± 0.017	3.036± 0.1686	3.073±0.177	0.258±0.002	1.089±0.0005	0.441±0.0001	88.03	64.13	85.60
Unsterilized soil+SM	0.97± 0.009	2.007± 0.0012	2.782±0.0181	0.324±0.0031	0.75±0.01	0.119±0.0041	66.80	62.63	95.72
Sterilized soil+SM	2.13±0.006	2.738± 0.0335	2.405±0.0301	0.387±0.0031	0.944±0.0029	0.194±0.0002	81.84	65.52	91.93

Table 2: Effect of organic matter on MP and its metabolites. Uptake of compounds by maize plant in soils with various treatments. Mean of 0 and 24 h OD of the soil solutions as recorded at 400 nm, 400 nm and 300 nm respectively for MP, PNP and HQ. OD is represented as mean± SD. All other experimental conditions are as in Table 1.

Treatment	Zero h OD ± SD			24 h OD ± SD			Percentage %		
	MP	PNP	HQ	MP	PNP	HQ	MP	PNP	HQ
Unsterilized soil	1.67 ± 0.0084	1.42 ± 0.0371	1.24 ± 0.0148	0.695 ± 0.001	0.960 ± 0.02	0.473 ± 0.002	58.55	32.39	61.85
Unsterilized soil+SM	1.66 ± 0.0126	1.32 ± 0.0287	1.333 ± 0.0042	1.15 ± 0.05	1.721 ± 0.122	0.813 ± 0.086	30.84	-	39.01
Sterilized soil+SM	1.88 ± 0.012	1.54 ± 0.0403	2.544 ± 0.0318	0.643 ± 0.065	1.244 ± 0.215	2.276 ± 0.241	65.79	19.22	10.53

Table 3: Nitrite release from MP and PNP under various treatments. All experimental data presented here is as described under Table 1 and 2.

Treatment	Zero h OD ± SD		24 h OD±SD		Percentage %	
	MP	PNP	MP	PNP	MP	PNP
Unsterilized soil	0.054 ± 0.0006	0.074 ± 0.0003	0.047 ± 0.0012	0.057 ± 0.0057	12.96	23.59
Unsterilized soil+SM	0.042 ± 0.0006	0.075 ± 0.0009	0.045 ± 0.0017	0.049 ± 0.0016	-	34.92
Sterilized soil+SM	0.042 ± 0.0009	0.044 ± 0.0008	0.040 ± 0.0001	0.024	4.69	15.40

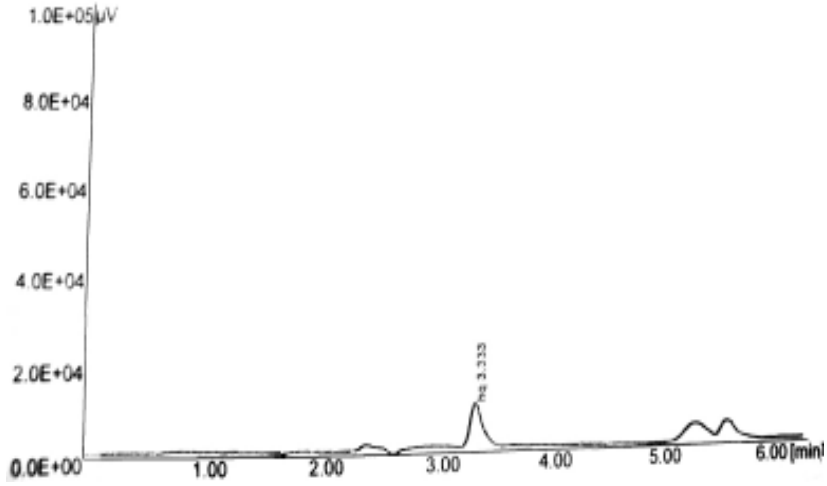

Fig. 1: HPLC separation profile of hydroquinone in maize extracted from unsterilized soil (24 hr result). HPLC conditions column-C_{18}, mobile phase-acetonitrile: distilled water: phosphoric acid (50:50:0.1), flow rate of 1mL per minute and detected wavelength 225 nm.

p-nitrophenol. Nitrite release is stimulated in presence of streptomycin with respect to leaf enzyme as well as root extract (Fig. 3). Streptomycin is enhancing the degradation of hydroquinone in leaf extract. Almost there is no degradation of hydroquinone in absence of streptomycin. Root extract strongly inhibits production of hydroquinone in presence of streptomycin (Fig. 4).

DISCUSSION

In the present investigation, the role of maize plant and the role of bacteria in uptake/degradation of pesticide has been studied. It has also been suggested that plants, which are able to survive high concentrations of pesticide mixtures, can contribute to pesticide waste degradation in soil as the result of intense microbial activity in the root zone or rhizosphere (Anderson et al. 1994). In these experiments, the effects of root and rhizosphere have been established by addition of an antibiotic (streptomycin). In streptomycin spiked unsterilized soil, uptake of methyl parathion is 66.8% and *p*-nitrophenol is 62.63% (Table 1). In our experiments uptake or degradation cannot be distinguished. Plants can also contribute to the removal of pesticide through plant tissues (Cunningham et al. 1997). Plants and their attendant rhizosphere microbes often show ability to transform pesticide through a mechanism called *ex planta* phytoremediation (Sandowsky 1999). Organic matter influences uptake of xenobiotics by binding to it; all pesticide uptake studies using streptomycin and soils rich in organic matter should take this binding effect into consideration (Table 3). The crop plant maize is consumed by humans and hence, any pesticide should be tested for its persistence in the plant from safety point of view. To be doubly sure, the uptake study results of UV-Spectrophotometer were confirmed using HPLC (Fig. 1, Fig. 2).

Nitrite release from *p*-nitrophenol is augmented in presence of streptomycin when crude enzyme preparation of leaf is used and similar results were obtained with crude preparations of root (Fig. 3). Streptomycin stimulates degradation of hydroquinone in leaf extract, while in root extract, it strongly inhibits hydroquinone appearance in crude enzyme preparation (Fig. 4). *p*-nitrophenol is a priority environmental pollutant, occurring in industrial effluents and in soils as a hydrolytic product of parathion or methyl parathion (Munnecke 1976, Keith & Telliard 1979). Several aerobic pure cultures of bacteria belonging to species of *Flavobacterium*, *Pseudomonas*, *Moraxella*, *Nocardia* and *Arthrobacter* metabolize *p*-nitrophenol with the removal of the nitro group as nitrite (Kadiyala & Spain 1998). Two alternative pathways that convert *p*-nitrophenol to maleylacetate have been elucidated for aerobic *p*-nitrophenol degradation (Spain 1995). The first pathway is more common in Gram-negative isolates and results in the formation of hydroquinone from *p*-nitrophenol, probably via 1,4-benzoquinone, with concomitant nitrite release. In the second catabolic pathway, an *Arthrobacter* sp. hydroxylates *p*-nitrophenol to produce either 4-nitrocatechol or 4-nitroresorcinol. Subsequent oxidative removal of the nitro group yields 1, 2, 4-trihydroxybenzene (THB) with concomitant release of nitrite. While a complete pathway for *p*-nitrophenol degradation via hydroquinone has been described in detail, the initial steps in the pathway involving conversion of *p*-nitrophenol to THB are not fully understood. A similar situation seems to exists in maize probably there is a difference in pathway of degradation of *p*-nitrophenol

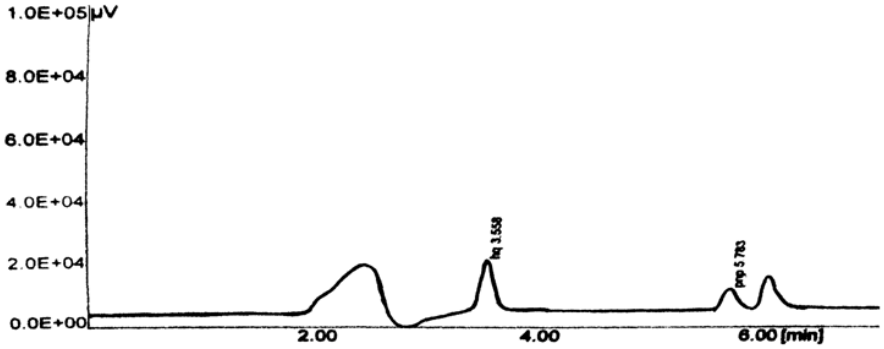

Fig. 2: HPLC separation profile of methyl parathion in maize extracted from unsterilized soil (24 hr result). All experimental conditions are described as in Fig. 1.

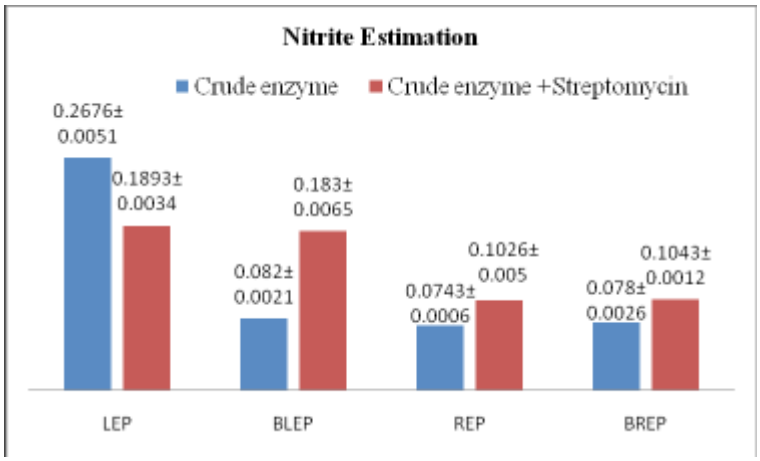

Fig. 3: Nitrite estimation: Effect of SM on enzyme activity. Nitrite is estimated by as described in text (OD 540 nm); the values are mean ± SD. The reaction mixture contains 51 µg/0.5 mL of protein in leaf and 50 µg/0.5 mL of protein in root as the enzyme source.

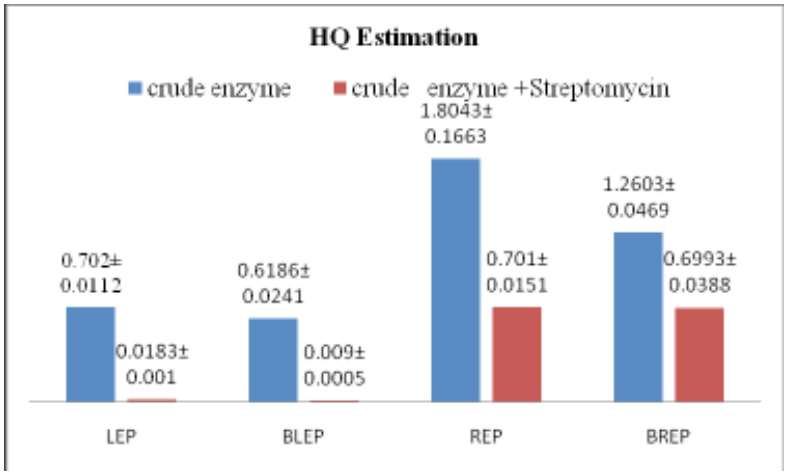

Fig. 4: Effect of SM on HQ formation catalyzed by the enzyme. HQ in leaf extract estimated as OD at 300 nm; the values are mean ± SD.

in leaf and root. However, confusion still persists as to the exact pathway followed in root and leaf. The end products of both pathways are either hydroquinone or 1, 2, 4-trihydrobenzene (THB); both these compounds end up as maleylacetate. Thus, any *p*-nitrophenol taken up by the plant from the soil will ultimately be metabolized by maize.

Whether a compound enters a plant or not depends on its hydrophobicity. This is indicated by Log K_{ow} values of the compounds; the critical value is 2.1; at the values less than this the compounds are less hydrophobic and will not pass through lipid membrane associated with epidermal layers of the roots. Compounds showing a greater value of log K_{ow} than the critical value of 2.1 are hydrophobic and bind to the membrane and do not enter the roots (Burken & Schnoor 1996). The log K_{ow} value of methyl parathion is 3.80, which is greater than log K_{ow} of 2.1 (Imrana 2008). Hence, the roots form an effective barrier for uptake of methyl parathion. Thus, rhizofiltration is an effective mechanism for preventing entry of methyl parathion into maize plants. *p*-nitrophenol, which enters the plant escaping rhizofiltration method either from the soil or from the leaves, will be metabolized at the points of its entry.

CONCLUSION

In the degradation pathway of methyl parathion, nitrite is released from *p*-nitrophenol forming hydroquinone. The nitrite release experiment was completed within a period of 24 h. Soil microorganisms are degrading methyl parathion and releasing nitrite, but the presence of streptomycin in soil inhibits nitrite release. In sterilized soil with streptomycin the degradation of methyl parathion compared to *p*-nitrophenol. Further clarifications of whether there is degradation or uptake in these experiments, can be decided by studying the metabolism of these compounds by crude enzyme preparation. This may also clarify the exact role of soil microorganisms and rhizosphere microorganisms in uptake, degradation and persistence of pesticides in rhizofiltration.

ACKNOWLEDGEMENTS

The authors wish to thank Principal, Fr. Ambrose Pinto of St. Joseph's College, Bangalore for providing laboratory facilities.

REFERENCES

Adhya, T. K., Sudhakar, B. and Sethunathan, N. 1981. Hydrolysis of selected organophosphate insecticides by two bacterial isolates from flooded soil. J. Appl. Bacteriol., 50: 167-172.

Albins, T. A., Pomonis, P. J. and Sdoukos, A. Th. 1986. Seasonal fluctuations of organochlorine and triazines pesticides in the aquatic system of Ioannina basin. Sci. Total Environ., 58: 243-253.

Anderson, T.A., Kruger, E.L. and Coats, J.R. 1994. Enhanced degradation of a mixture of three herbicides in the rhizosphere of an herbicide-tolerant plant. Chemosphere, 28: 1551-1557.

Barr, D.B., Turner, W.E., DiPietro, E., McClure, P.C., Baker, S.E., Barr J.R., Gehle, K., Grissom, R.E., Jr. Bravo, R., Driskell, W.J., Patterson, D.G. Jr., Hill, R.H. Jr., Needham, L.L., Pirkle, J.L. and Sampson, E.J. 2002. National Center for Environmental Health Measurement of *p*-nitrophenol in the urine of residents whose homes were contaminated with methyl parathion. Environ. Health Perspect., 110(6): 1085-1091.

Burken, J.G. and Schnoor, J.L. 1996. Phytoremediation: Plant uptake of atrazine and role of root exudates. ASCE J. Environ. Engg., 122: 958-963.

Cunningham, S.D., Shann, J.R., Crowley, D.E. and Anderson, T.A. 1997. Phytoremediation of contaminated water and soil. In: Kruger, E.L., Anderson, T.A. and Coats, J.R. (Ed.), Phytoremediation of Soil and Water Contaminants. American Chemical Society, Washington, pp. 2-17.

Edwards, C.A. 1975. Factors that affect the persistence of pesticides in plants and soils. pp. 39-56. http://old.iupac.org/publications/pac/1975/p.

EHC 1992. Methyl parathion. www.inchem.org/documents/ehc/ehc/ehc145. Environmental Health Criteria, 145, Geneva.

EPA 1976. Frequency of organic compounds identified in water. EPA-600/4-76-062, Environmental Protection Agency, Athens, U.S.

FAO/WHO 1996. Pesticide Residues in Food-1995 Evaluations. Part II-Toxicological and Environmental. Geneva, World Health Organization, Joint FAO/WHO Meeting on Pesticide Residues (WHO/PCS/96.48).

Ferrando, M.D., Alarcon, V., Fernadez-Casalderray, A., Gamon, M. and Andreu-Moliner, E. 1992. Persistence of some pesticides in the aquatic environment. Bull. Environ. Contam. Toxicol., 48: 747-755.

Howard, P.H. 1989. Pesticides. In: Handbook of Environmental Fate and Exposure Data for Organic Chemicals. 3. Chelsea MI, Lewis Publishers.

Imrana, N. 2008. Phytoremediation of Pesticide Polluted Agricultural Soils. Ph.D. Thesis, Bangalore University, India.

Kadiyala, V. and Spain, J. 1998. A two-component monooxygenase catalyzes both the hydroxylation of *p*-nitrophenol and the oxidative release of nitrite from 4-nitrocatechol in *Bacillus sphaericus* JS905. Appl. Environ. Microbiol., 64: 2479-2484.

Keith, L.H. and Telliard, W.A. 1979. Perspective view. Environ. Sci. Technol., 13: 416-423.

Kulkarni, M. and Chaudhari, A. 2006. Efficient *Pseudomonas putida* for degradation of *p*-nitrophenol. Indian J. Biotechnol., 5: 411-415.

Munnecke, D. M. 1976. Enzymatic hydrolysis of organophosphate insecticides, a possible pesticide disposal method. Appl. Environ. Microbiol., 32: 7-13.

Nagaraja, P., Shivaswamy, M. and Kumar, H. 2001. Highly sensitive N-(1-napthyl) ethylene diamine method for the spectrophotometric determination of trace amounts of nitrite in various water samples. Intern. J. Environ. Anal. Chem., 80: 39-48.

Nwankwoala, A.U. and Osibanjo, O. 1992. Baseline levels of selected organochlorine pesticide residues in surface waters in Ibadan (Nigeria) by electron capture detection. Sci. Total Environ., 119: 179-190.

Parris, G.E. 1980. Environmental and metabolic transformation of primary aromatic amines and related compounds. Residue Rev., 76: 1-30.

Sandowsky, M.J. 1999. Phytoremediation: Past promises and future and future practices. In: Bell, C.R., Brylinsky, M. and Jhonson, P. (Ed.), Plant-Microbe Interaction. Atlantic Canada Society for Microbial Ecology, Canada.

Spain, J.C. 1995. Bacterial degradation of nitroaromatic compounds under aerobic conditions. In: Spain, J.C. (Ed.) Biodegradation of nitroaromatic compounds. Plenum Press, New York, 49: 19-35.

EPA 1990. National Pesticide Survey: 4-Nitrophenol. Office of Water, Office of Pesticides and Toxic Substances. pmep.cce.cornell.edu/profiles, Environmental Protection Agency, US., Washington, DC.

Zeyer, J. and Kocher, H.P. 1988. Purification and characterization of a bacterial nitrophenol oxygenase which converts orthonitrophenol to catechol and nitrite. J. Bacteriol., 170: 1789-1794.