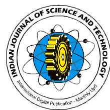
Indian Journal of Science and Technology



Vol. 4 No. 12 (Dec 2011) ISSN: 0974- 6846

Phytoremediation using an indigenous crop plant (wheat): the uptake of methyl parathion and metabolism of *p*-nitrophenol

Nayeem Ullah Khan[1], Bhavya Varma[1], Nazeeb Imrana[1] and Paddu Krishnappa Shetty[2]

Fr. Cecil J. Saldanha Centre for Experimental Research in Bioscience, St. Joseph's College Research Center, Post Box. 27094, 46, Langford Road, Bangalore - 560027, India[1]
School of Natural Science and Engineering, National Institute of Advanced Studies Indian Institute of Science Campus, Bangalore- 560012, India[2]
nayeem65@hotmail.com; bhavya.varma542@gmail.com; nazeebimrana@yahoo.co.in;
pkshetty17@gmail.com[2]; pks@nias.iisc.ernet.in[2]

Abstract

Phytoremediation is an emerging technology for degradation of organophosphate pesticides. Rhizofiltration, soil microorganisms and rhizosphere play an important role in phytoremediation. For this purpose, experiments were carried out to analyze the pesticide uptake by plants and residue in the soil under controlled and experimental conditions. Organophosphate pesticide binds to both organic matter and antibiotic streptomycin. Streptomycin inhibits the activity of the enzyme (*p*-nitrophenol 4-hydroxylase) extracted from root and shoot. The results obtained from UV-visible spectrophotometer were confirmed by HPLC analysis. Wheat plants enhanced uptake/degradation of methyl parathion, *p*-nitrophenol and hydroquinone in unsterilized soil by 64.85%, 94.7% and 55.8% respectively. Methyl parathion hydrolyzes to *p*-nitrophenol, which is further metabolized to hydroquinone with nitrite release. The enzyme *p*-nitrophenol 4-hydroxylase is active as evidenced by release of nitrite by leaf and root extracts and also by the appearance of hydroquinone in the reaction mixture.

Keywords: Phytoremediation, Streptomycin, methyl parathion, *p*-nitrophenol, hydroquinone, pesticide degradation.

Introduction

Phytoremediation is the method of choice for remediating pesticide-contaminated soils. Phytoremediation technology has already undergone successful pilot study for mitigation of organophosphate pesticide, chloropyrifos (Moore *et al.*, 2002). Plants are not passive targets for their associate organisms but rather actively affect the structure of rhizosphere communities by releasing attractants and repellents from their roots in assisting cometabolism of xenobiotics in the soil (Elizabeth *et al.*, 1998).

Persistence in the environment is a matter of concern because the pesticide is a hazardous substance and hence there is a need for continued research on this subject which is in the interest of public health and safety. Persistence is directly related to the uptake by plants (non-target organisms) and half-life of the hazardous pesticide in the environment. Pioneering work has been done on methyl parathion (MP) persistence where the pesticide was shown to be adsorbed to the soil particles (UNEP, 2005). Since methyl parathion is readily adsorbed to the soil, it has been presumed not to contaminate the ground water (Howard, 1989; USDA, 1990). When the compound is applied as a pesticide, it takes several months to degrade in the soil. It is also important to note that when large concentration of methyl parathion reaches the soil, as in an accidental spill, the degradation will occur only after many years. Degradation is faster in the presence of sediments; it is faster in fresh water than in salt water (Howard, 1989). It is clear from the literature cited that some work has been done on the uptake as well as persistence of methyl parathion and its degradation product *p*-nitrophenol (PNP).

p-nitrophenol was also selected for the study because nitrophenolic compounds accumulate in soil as a result of hydrolysis of several organophosphate pesticides such as parathion and methyl parathion (Adriana, 2004). *p*-nitrophenol is also an insecticide and contaminates ground water as mentioned earlier. It is a potent uncoupler of oxidative and photo-phosphorylation (Zayer & Kocher, 1988). It is a major environmental pollutant (EPA, 1990). It has carcinogenic, mutagenic and toxic properties (Parris, 1980). It is persistent in the environment probably due to the presence of nitro group conferring resistance to biodegradation in soil (Alexander, 1994; Esteve-Nunez *et al.*, 2001). Hydroquinone (HQ) was also included in the uptake studies, since it is the degradation product of methyl parathion and parathion (Adriana, 2004). Micro-organisms that degrade organic pollutants in culture sometimes may fail to function when inoculated into natural environments because the pollution levels in nature may be too low to support that growth or because the organisms themselves may be susceptible to toxins or predators in the environment, may use other organic compounds in preference to the pollutant or may be they are unable to move through soil to sites containing the chemicals (Rebecca *et al.*, 1985; Baudoin *et al.*, 2003). It is for these reasons phytoremediation is preferred over bioremediation by cultured micro-organisms (*ex situ* bioremediation). The research presented describes experiments with rooted and rootless plants to establish role of roots in keeping xenobiotics away from plants (rhizofiltration); experiments

Indian Journal of Science and Technology Vol. 4 No. 12 (Dec 2011) ISSN: 0974- 6846

were planned with sterilized, unsterilized and streptomycin- spiked soil to establish the role of soil micro-organisms and rhizosphere in remediation, where the role of enzyme system (*p*-nitrophenol 4-hydroxylase) from a eukaryote (wheat) was also investigated.

Materials and methods

Soil preparation

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

Rootless plant experiments

Tecoma stans L a shrub growing at St.Joseph's College campus, Bangalore, India and *Amaranthus spinosus* L, a herb growing as a weed at the vacant sites of Bangalore, were the two plants used for rootless experiments. Twigs of *Tecoma stans* L were a meter in length with 12 leaves. For the purpose of comparison 12 leaves were maintained even on the cut-twigs of *Amaranthus spinosus.* The experimental branches were cut under water.

The cut branches of the above plants were placed in the soils. The cut-twigs were placed in pots, the depth was sufficient to support the branches, so that they were held vertically. To avoid evaporation, pot surface was covered with polythene sheath. The pots had a drainage hole, to drain the excess water. Every pot with cut-twig received 10 ml water every day. Any excess water was collected in a plastic bowl kept below the pot and was added back sometime in the evening to the respective pots.

Intact-plants experiments

Wheat (*Triticum vulgare*) was selected for the uptake experiment; whole plant with roots was used. 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. Plants were maintained in a manner similar to rootless plants as described earlier.

OD measurements of soil solution

The zero day and 5th day OD measurements of soil solution were done. 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 λ_{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, of *p*-nitrophenol and hydroquinone were made in water.

HPLC measurements of soil solution

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), flow rate was 1 ml per min and monitored at A_{225} (Kulakarni & Chaudhari, 2006). Twenty-four hours after spiking the soil with compounds, HPLC measurements of soil solution were done. The soil solutions were prepared from each pot as follows: soil (1g) 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.

Nitrite estimation

Locally available wheat seeds were sown in pots (5 seeds/pot). When plants were 15-day old, plants were transferred to pesticide-treated soil for the nitrite estimation experiment. One gram of soil was suspended in 15 ml distilled water, from this 1 ml sample was taken and diluted to 50 ml with distilled water and 2 ml N- (1-naphthyl)-ethylenediamine dihydrochloride reagent was added and OD was taken at 540 nm (Nagaraja *et al.,* 2001).

Assay of p-nitrophenol 4-hydroxylase

The enzyme was assayed 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 in a test tube, to this 1 ml dithiotritol (40 mM), 1ml dimethyltetrahydropteridine (1.8 mM) and 1 ml *p*-nitrophenol (40 μM) solution, were added and incubated at 30 ^{o}C for 2 h. The reaction was terminated by adding 1ml 6 N HCl. From the above solution 1ml-sample was taken in a test tube, to this 2 ml N-(1-naphthyl)-ethylenediamine dihydrochloride reagent and 7 ml distilled water was 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). Protein was estimated in crude enzyme mixture by dye binding assay (Bradford, 1976).

Results

Role of roots in phytoremediation and effect of organic matter on the uptake of pesticides

Rhizofiltration is the mechanism for phytoremediation. Roots are barriers to xenobiotics and prevent the chemicals from entering the plants. Rhizofiltration hypothesis is tested using methyl parathion, *p*-nitrophenol and hydroquinone. Methyl parathion in unsterilized soil gets degraded near the root (rhizosphere) and the uptake or degradation is up to 72%; this is the average of 6, 12 and 24 hours with 0 hour as the base line for percentage calculation. The latter

Indian Journal of Science and Technology Vol. 4 No. 12 (Dec 2011) ISSN: 0974- 6846

Fig.1.Effect of sterilized soil and SM on methylparathion, p-nitrophenol and hydroquinone uptake in wheat.24 h OD at 400, 400 and 300 nm was recorded and represented as mean. The values given are significant at P < 0.05; n = 10.

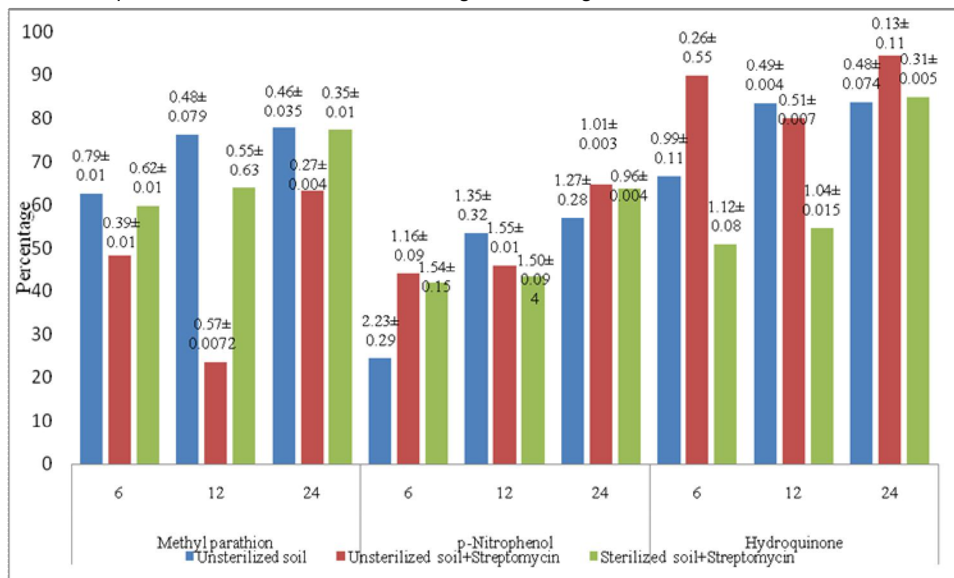


Table1. Uptake of Pesticides by 12-leaved twigs. Mean of four 0-day OD determination; 5th day value of OD represents one single determination and represents true mean (μ); t-values were calculated for three degrees of freedom by the formula t = μ × √n / SD and data were analyzed by using this formula, the comparison was between 0 day and 5th day OD and all the paired values were significantly different at P < 0.05.

Treatment	0 day OD± SD	5th day OD		Uptake (%)	
		Tecoma	*Amaranthus*	*Tecoma*	*Amaranthus*
HQ (P)	0.62±0.005	0.106	0.381	83	39.2
HQ (A)		0.222	0.386	64	38.4
PNP (P)	0.73±0.025	0.091	0.073	87.6	90
PNP (A)		0.344	0.127	53	82
MP (P)	0.8±0.23	0.057	0.051	93.5	94.2
MP (A)		0.097	0.140	89	84.2

Table 2. Uptake of compounds by wheat plants in soils with various treatments. Mean of 24 h OD of the soil suspension as recorded at 400, 400 and 300 nm for MP, PNP and HQ respectively

Treatment	Wheat 24 h OD		
Soil (control)	0.521 ± 0.018	1.345 ± 0.012	0.333 ± 0.005
Soil + manure (1:1)	0.455 ± 0.012*	1.029 ±0.003*	2.371 ± 0.019
Soil + SM (control)	0.225 ± 0.025	1.212 ± 0.104	0.051 ± 0.102
Soil (A) + manure (1:1) + SM	0.890 ± 0.0033	1.350 ±0.0116	0.852 ± 0.0152
Soil + SM (control)	0.335 ± 0.07	0.627 ± 0.185	0.178 ± 0.153
Soil (A) + manure (1:1)	0.587 ± 0.0128	1.987 ±0.0182	0.507 ± 0.0167

Not significant at P < 0.05; A in parentheses indicates autoclaved soil

method of disposal is called co-metabolism hypothesis which can be tested by adding streptomycin to the soil.

This antibiotic greatly reduces the uptake of methyl parathion. In sterilized soil with streptomycin, and methyl parathion, the percentage is further reduced to 67%; this perhaps is due to the binding of streptomycin to methyl parathion. In unsterilized soil with streptomycin, there is also a reduction of uptake to 45% and this may be due to streptomycin-insensitive organisms degrading methyl parathion. There is no significant difference between uptake of *p*-nitrophenol in presence or absence of microorganisms or streptomycin in the soil. This may be because of inhibitory effect of streptomycin on the growth of microorganisms. If any uptake has taken place, it is due to the action of *p*-nitrophenol- insensitive microorganisms which are also insensitive to streptomycin. Streptomycin insensitive organisms help in the uptake of hydroquinone to some extent, as evidenced by 10% difference between unsterilized soil and streptomycin (Fig.1).

The uptake of methyl parathion goes up to 90% for rootless plants (cut twigs of *Tecoma stans* L & *Amaranthus spinosus* L) (Table.1). Another way by which xenobiotics are disposed off from the soil is by metabolism of soil micro-organisms and root-associated rhizosphere micro-organisms. This means there is a significant influence of the rhizosphere micro-organisms. However, it is of interest to know that organic matter can bind methyl parathion, *p*-nitrophenol and hydroquinone (Table.2). Streptomycin too behaves like organic matter and binds these compounds (Table.2). Hydroquinone is the end product of methyl parathion metabolism in some soil micro-organisms (Kulkarni & Chaudhrai, 2006) Roots seem to have an inhibitory effect on the uptake of xenobiotics. Experiments on rootless plants prove that sterilizing reduces uptake of xenobiotics. This establishes clearly the role of soil microorganisms in metabolism and mineralization of methyl parathion, *p*-nitrophenol and hydroquinone (Table.1).

Analysis of pesticide residues in the crop plant wheat is important from safety point of view and hence the results were confirmed using HPLC. The data of Fig.1 was obtained by UV-visible spectrophotometer for a period of 5 days whereas the experiments using HPLC were completed within a period of 24 h. The two additional compounds *p*-nitrophenol and hydroquinone were tested for their uptake by rooted plant wheat. There is an uptake of hydroquinone by wheat plant (55.8%, Fig. 2) Wheat on the other hand shows an uptake of *p*-nitrophenol to the

extent of 94.7% (Fig. 3). No peaks were detected for all the three compounds in soil spiked with streptomycin. We conclude streptomycin binds all the three compounds. However wheat seems to take up the streptomycin - bound hydroquinone (wheat, 55.79%).

Fig.2.HQ extracted from wheat grown in unsterilized soil (24 h); column C_{18}; mobile phase: acetonitrile: distilled water: phosphoric acid (50:50:0.1); flow rate of 1ml per min monitored by 225 nm wavelength

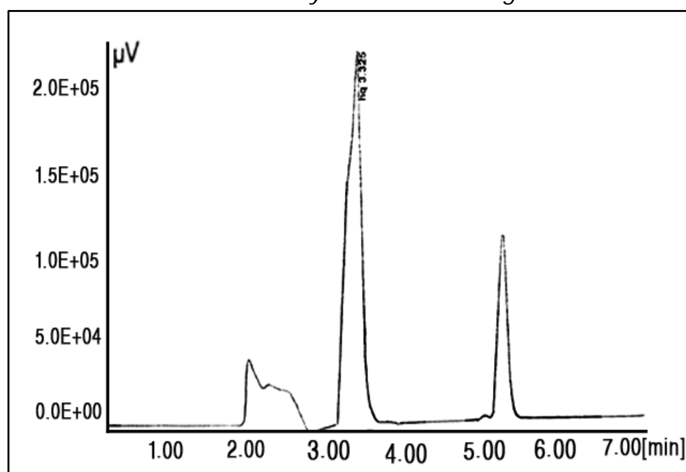
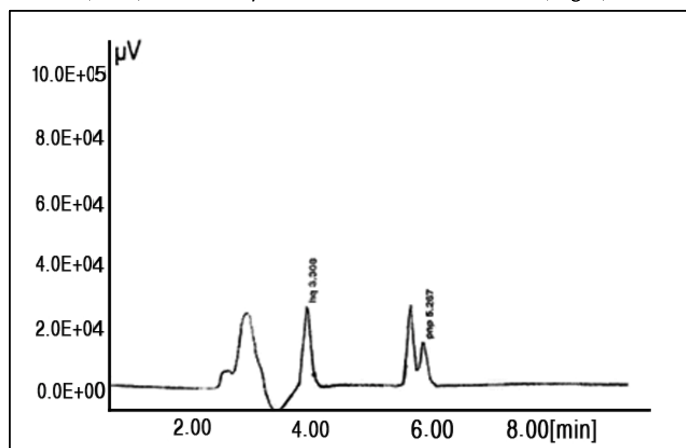


Fig.3. PNP extracted from wheat grown in unsterilized soil (24 h). Other experimental conditions as in (Fig.2)



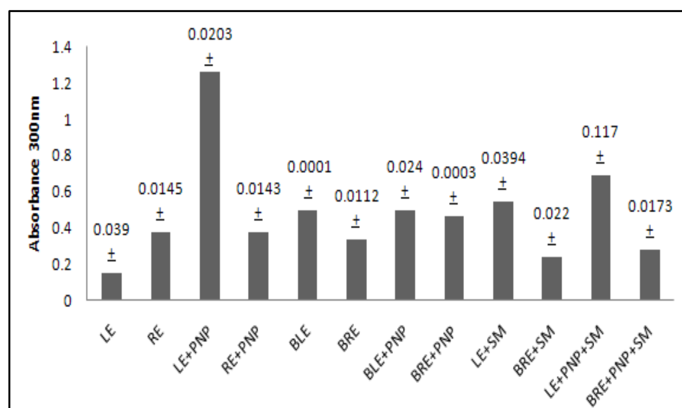
Effect of soil and rhizosphere microorganisms on nitrite release with methyl parathion and p-nitrophenol as substrates

p-Nitrophenol converts into final compound hydroquinone by releasing nitrite. The nitrite release experiment was completed within a period of 24 h. Nitrite release was tested from methyl parathion and *p*-nitrophenol in rooted crop plant wheat. Methyl parathion metabolism is faster in wheat (40.8%) This metabolism comes down drastically in presence of streptomycin in the soil as indicated by very little or no release of nitrite. *p*-Nitrophenol metabolism is also faster in wheat (46.43%) compared to metabolism of methyl parathion and this can be inferred by nitrite release which is further confirmed with enzyme assay. The data concerning nitrite release in the soil are not shown but the percentages of nitrite release are given in parentheses in the text above.

Fate of Methyl parathion and p-nitrophenol in wheat plant

Methyl parathion was not detected in the wheat plant. Probably it is hydrolyzed immediately to *p*-nitrophenol. Hence *p*-nitrophenol uptake only has been studied by wheat plant (Fig.3). Metabolism of *p*-nitrophenol in wheat plant results in formation of hydroquinone with nitrite release (Fig.4). Nitrite release has also been noticed in root extract (RE) (data not shown). Similarly hydroquinone formation has been demonstrated in root using boiled root extract (BRE) as control (P<0.05). Leaf extract (LE) has also *p*-nitrophenol 4-hydroxylase activity (Fig. 4, P < 0.05). Interestingly streptomycin seems to inhibit *p*-nitrophenol 4-hydroxylase. This has been demonstrated by monitoring hydroquinone formation which happens to a lesser extent in presence of streptomycin (Fig. 4, P < 0.05). The enzyme assay gives a qualitatively positive test for nitrite release but turbidity makes it difficult to assay the enzyme by monitoring nitrite at 540 nm. Quantitatively consistent results were obtained if the enzyme is assayed by following hydroquinone formation at 300 nm (Fig. 4). Boiling activates root extract *p*-nitrophenol 4-hydroxylase activity (Fig. 4, P < 0.05). Like leaf extract enzyme, this enzyme is also inhibited by streptomycin (Fig. 4, P < 0.05).

Fig.4. SD is given above each bar. Each bar represents OD at 300 nm. The reaction mixture contains 101 μg/ml of protein in leaf and 103 μg/ml of protein in root as the enzyme source. Student's t-test has been done for a pair of comparable values and provided in the text. RE, root extract; BRE, boiled root extract; for expansions, other abbreviations refer. Fig.5.



Discussion

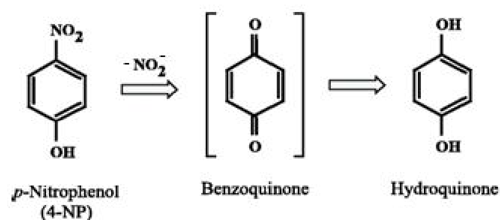
Phytoremediation is a promising technology for cleanup of hazardous organic and inorganic pollutants, which include agro-chemicals (Anderson *et al.*, 1994; Hoagland *et al.*, 1997; Kruger *et al.*, 1997) polycyclic aromatic hydrocarbons (April & Sims, 1990; Reilley *et al.*, 1996) and polychlorinated biphenyls (Brazil *et al.*, 1995; Donnely & Fletcher, 1995) It has also been suggested that plants that 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 our experiments these effects of root and rhizosphere have been established by addition of antibiotic streptomycin. In presence of streptomycin only 46% of the methyl parathion is taken up or degraded. In our experiments uptake or degradation cannot be distinguished with respect to xenobiotic in soil. Rooted - wheat shows degradation or uptake to the extent of 67% which is slightly increased in unsterilized soil with streptomycin. It means the rhizosphere of wheat and soil micro-organisms are positively interacting for the uptake of compounds from the soil (Fig.1). The rhizosphere effect is due to coincidental metabolism by microbial population (co-metabolism; Coats, 1993). Plants can also contribute to the removal of pesticide through plant tissue (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). Earlier experiments on phytoremediation of lindane were unsuccessful (Li, 1994) The attempts were made using ryegrass. It is of interest to know that root exudates actively or passively release a range of organic compounds such as sugars, amino acids, aromatic and organic acids which have a stimulatory effect on the growth of rhizosphere microbes (Sims, 1990; Anderson *et al.,* 1994; April & Rentz *et al.,* 2005). Since organic matter influences uptake of xenobiotics by binding to it, all uptake studies using streptomycin and soils rich in organic matter should take this binding effect into consideration.

Wheat effectively takes up methyl parathion as earlier reported for barley (*Hordeum vulgare*) (Lichtenstein *et al.,* 1977). For maize leaves metabolism of methyl parathion has been reported (Howard, 1989). It has a short half-life of 1 h on cotton leaves (Hayes & Laws, 1990). Methyl parathion was completely metabolized within 4 days when applied to corn leaves (Howard, 1989). There was up to 90% uptake of this compound by rootless *Tecoma stans* L and *Amaranthus spinosus* L (Table 1) when compared to rooted plants; which show *ex planta* phytoremediation, where rhizofiltration keeps out xenobiotics. It is likely that methyl parathion is metabolized by *Tecoma stans* L since it has aromatic ring-cleaving enzymes (Mohan, 1977). The uptake of this compound is 90% for *Tecoma stans* L and *Amaranthus spinosus* L *Tecoma stans* L cut-twigs are equally effective in removing *p*-nitrophenol from the soil. Hydroquinone is also taken up although less effectively by cut-twigs of *Tecoma stans* L. and *Amaranthus spinosus* L (Table 1).

Rhizofiltration is taking place in the rooted plants and this is evident when their uptake is compared with rootless *Tecoma stans* L and *Amaranthus spinosus* L (Table 1). The probable mechanism of rhizofilteration is as follows. For uptake a log K_{ow} value of 2.1 seems to be critical and this was established by Briggs *et al.* in the year 1982. A structure activity relationship (SAR) was established for the uptake of compounds and this was termed transpiration stream concentration factor (TSCF) which related to transpiration rate of plant showing uptake (Shone & Wood, 1972). It reaches a maximum value of 0.8 at a corresponding log K_{ow} of 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 roots form an effective barrier for uptake of methyl parathion and it is quite likely that the methyl parathion is metabolized by soil micro-organisms. This becomes apparent when we compare the uptake of this compound in sterilized and unsterilized soil (Fig.1). ^{14}C - ring-labeled atrazine has been shown to be metabolized to radioactive $^{14}CO_2$ by poplar trees (Burken *et al.,* 1997 & 1998). It is likely that *Tecoma* possesses ring-cleaving enzymes. Metabolism of aromatic compound has been reported in *Tecoma* leaves (Mohan, 1977). Uptake of atrazine by poplar trees has been shown to be stimulated by root exudates (Burken *et al.,* 1997). Burken *et al.* (1997) have established a model for uptake and metabolism of organic compounds by hybrid poplar trees.

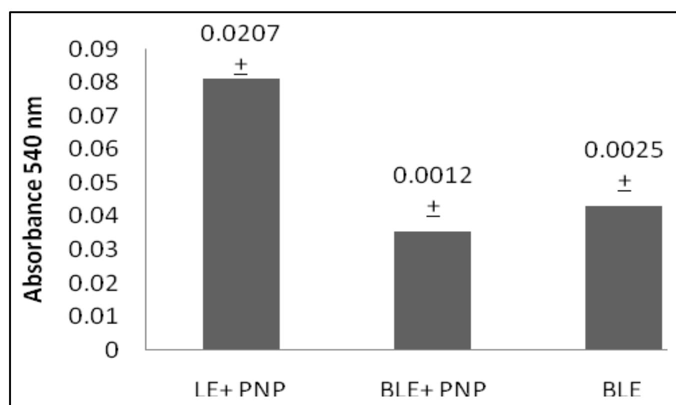
HPLC experiments and OD records confirm the earlier findings (Imrana, 2008) about uptake of methyl parathion in wheat. In the soil spiked with methyl parathion, peak corresponding to this compound is not observed in soil samples on HPLC analysis (Fig.2&3). This may be due to binding of methyl parathion to organic matter (Fuhermann & Lichenstein, 1978). Present results confirm their earlier findings with barley and earthworm where methyl parathion residues were found bound to organic matter of the soil as well as biota representing barley and earth worm (Briggs *et al.*, 1982). Based on the results obtained with nitrite release experiment (result section) the following scheme is proposed:



It is clear from the results that streptomycin slows down uptake or metabolism of *p*-nitrophenol and methyl parathion. Streptomycin inhibits the enzyme *p*-nitrophenol 4-hydroxylase of the root (Fig.4) and also the leaf enzyme (Fig.4). The root enzyme was assayed by hydroquinone formation since nitrite release did not give consistent results, however the enzyme has been assayed by nitrite release in experiments with bacteria (Zayer & Kocher, 1988). The difference between root and leaf hydroxylase

is the heat activation which is confined to the root enzyme (Fig.5). The enzyme *p*-nitrophenol 4-hydroxylase and some of its important properties including its assay have been described for the first time. The enzyme was known to be present in soil bacteria and was inferred by isolation of metabolites (Pakala *et al.,* 2006). The same metabolic route for methyl parathion has been described for the first time here in wheat. These studies declare wheat grown in methyl parathion and *p*-nitrophenol contaminated soils to be completely safe for human consumption. This paper is the first comprehensive study of phytoremediation by any indigenous plant. Part of the work (enzyme assay) resulted in filing of a patent (Khan *et al.,* 2011).

Fig.5. SD is given above every bar. The first pair of values are significant at P < 0.05. Each bar represents OD at 540 nm. LE, leaf extract; BLE, boiled leaf extract

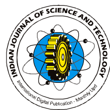


Acknowledgements

The authors wish to thank Principal, Fr. Ambrose Pinto of St Joseph's College, Bangalore, India for providing laboratory facilities. The grants from UGC (MRP 2011) to Khan NU (PI) are gratefully acknowledged.

References

1. Adriana ZDC (2004) Bioremediation of the organophosphate methyl parathion using genetically engineered and native organisms. *Biological & Agricual. Engg. Texas A & M University.* pp: 112.
2. Alexander M (1994) Effect of nature on biodegradation. *Academic Press, San Diego, California,* US. pp: 159-76.
3. Anderson TA, Kruger EL and Coats JR (1994) Enhanced degradation of a mixture of three herbicides in the rhizosphere of an herbicide-tolerant plant. *Chemosphere*. 28, 1551-1557.
4. April W and Sims RC (1990) Evaluation and use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. *Chemosphere*. 20, 253-265.
5. Baudoin E, Benizri E and Guckert A (2003) Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. *Soil. Biol. Biochem.* 35, 1183-1192.
6. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*. 72, 248-54.
7. Brazil GM, Kenefick L, Callanan M, Haro A, de Lorenzo V, Dowling DN, and O'Gara F (1995) Construction of a rhizosphere psuedomonad with potential to degrade polychlorinated biphenyls and detection of bph gene expression in the rhizosphere. *Appl. Environ. Microbiol.* 61, 1946-1952.
8. Briggs GG, Bromilow RH and Evans AA (1982) Relationship between lipophilicity and root uptake translocation of non-ionized chemical by barley. *Pestic. Sci*. 13, 495-504.
9. Burken JG and Schnoor JL (1996) Phytoremediation: Plant uptake of atrazine and role of root exudates. ASCE. *J. Environ. Engg*. 122, 958-963.
10. Burken JG and Schnoor JL (1997) Uptake and metabolism of atrazine by poplar trees. *Environ. Sci. Technol*. 31, 1399-1406.
11. Burken JG and Schnoor JL (1998) Predictive relationships for uptake of organic contaminants by hybrid poplar trees. *Environ. Sci. Technol*. 32, 3379-3385.
12. Coats JR (1993) What happens to degradable pesticides? *Chemtech*. 23, 25-29.
13. Cunningham SD, Shann JR, Crowley DE and Anderson TA (1997) Phytoremediation of contaminated water and soil. In: Phytoremediation of Soil & Water Contaminants Kruger EL, Anderson TA & Coats JR (eds.). 664, 2-17. *Am. Chem. Soc.*, Washington, DC.
14. Donnely PK and Fletcher JA (1995) PCB Metabolism by ectomycorrhizal fungi. *Environ. Contam. Toxicol.* 54, 507-13.
15. Elizabeth M (1998) Estabrook Yoder I. Plant-plant communications: rhizosphere signaling between parasitic angiosperms and their hosts. *Plant Physiol.* 116, 1-7.
16. Esteve-Nunez A, Caballero A and Ramos JL (2001) Biological degradation of 2, 4, 6-trinitrotoluene. *Microbiol. Mol. Biol. Rev.* 65, 335-352.
17. Fuhermann TW and Lichtenstein EP (1978) Release of soil bound methyl [^{14}C] parathion residues and their uptake by earthworms and oat plants. *J. Agri. Food Chem*. 26, 605-610.
18. Hayes WJ and Laws ER (1990) Classes of pesticide. In: Handbook of pesticide toxicology. Academic Press, NY. 3, 230-257.
19. Hoagland RE, Zablotowicz RM and Locke MA (1997) An integrated phytoremediation strategy for chloraacetamide herbicide in soil. In: Phytoremediation of Soil & Water Contaminants Kruger EL, Anderson TA & Coats JR (eds.). *Am. Chem. Soc.* Washington, DC. 664, 92-105.
20. Howard PH (1989) Pesticides. In: *Handbook of environmental fate and exposure data for organic chemicals*. Lewis Publishers, Chelsea, MI, USA.



21. Imrana N (2008) Phytoremediation of pesticide polluted agricultural soils. Dept. Botany. Bangalore University. pp: 201.
22. Khan NU, Varma B and Reddy B (2011) A simple method to screen plants for the phytoremediation of *p*-nitrophenol contaminated substrata. *Reference number* 5958/ RQ - CHE/ 2011; *Application number* 2572/ CHE/ 2011 (dated 27.07.2011). *Patent office, Chennai, India*
23. Kruger EL, Anhalt JC, Sorenson D, Nelson B and Chouchy AL (1997) Atrazine degradation in pesticide contaminated soil: Phytoremediation potential. In: Phytoremediation of soil & water contaminants. Kruger EL, Anderson TA & Coats JR (eds.). *Am. Chem. Soc.* Washington, DC. 664, 54-64.
24. Kulakarni M and Chaudhari A (2006) Efficient pseudomonas putida for degradation of *p*- nitrophenol. *Indian J. Biotechnol.* 5, 411- 415.
25. Li H, Guangyao S, Wentao S and Ouyong X (1994) Uptake of trifluralin and lindane from water by ryegrass. *Chemosphere.* 23, 25-29.
26. Lichtenstein EP, Katan KP and Anderegg J (1977) Binding of persistent and nonpersistent ^{14}C- labeled insecticides in an agricultural soil. *J. Agri. Food Chem.* 25, 43- 47.
27. Mohan VP (1977) Metabolism of Protocatechuic acid in Tecoma stans L. Dept. Biochem. IISc. pp: 110.
28. Moore MT, Schulz R, Cooper CM, Smith JR and Rodgers Jr JH (2002) Mitigation of chlorpyrifos runoff constructed wetland. *Chemosphere.* 46, 827-35.
29. 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.
30. Pakala PB, Gorla P, Pinjari AB, Krovidi RK, Baru R, Yanamandra M, Merrick M and Siddavattam D (2006) Biodegradation of methyl parathion and *p*-nitrophenol: evidence for the presence of a *p*-nitrophenol 2-hydroxylase in a gram negative *Serratia* sp. strain DS001. *Appl. Microbiol. Biotechnol.* doi.10.1007/s00253-006-0595-z.
31. Parris GE (1980) Environmental and metabolic transformation of primary aromatic amines and related compounds. *Residue Rev.* 76, 1-30.
32. Rebacca M, Goldstein Lawrence M and Mallory Alexander M (1985) Reasons for possible failure of inoculation to enhance biodegradation. *Appl. Environ. Microbiol.* 25, 212-219.
33. Reilley KA, Banks MK and Sehwab AP (1996) Organic chemical in the environment: Dissipitation of polycyclic aromatic hydrocarbons in the rhizosphere. *J. Environ. Qual.* 25, 212-219.
34. Rentz A, Alvarez PJJ, Schnoor JL and Benzol (2005) (a) pyrene co-metabolism in the presence of plant root extracts and exudates: Implication for phytoremediation. *Environ. Pollut.* 136, 477-484.
35. Sandowsky MJ (1999) Phytoremediation: Past promises and future and future practices. In: Plant-microbe interaction. Bell CR, Brylinsky M & Jhonson P (eds.) Atlantic Canada Soc. Microbial Ecology. Canada.
36. Shone MTG and Wood AV (1972) Factors affecting absorption and translocation of simazine by barley. *J. Exp. Bot.* 23, 141-151.
37. United Nations Environmental Programme (UNEP) (2005) Inclusion of chemicals in Annex III of the Rotterdam Convention: review of notifications of final regulatory actions to ban or severely restrict a chemical: methyl parathion. Geneva, Brazil.
38. US Department of Agricultural, Soil Conservation Service (USDA) (1990) The British Crop Protection Council. Croydon, England.
39. US Environmental protection agency (USEPA) (1990) National pesticide survey: 4- Nitrophenol. Office of water, office of pesticides and toxic substances, US, Washington, DC.
40. Zeyer J and Kocher HP (1988) Purification and characterization of a bacterial nitrophenol oxygenase which converts orthonitrophenol to catechol and nitrite. *J. Bacteriol.* 170, 1789-94.