

Fate, bioavailability and bioremediation of fenamiphos and its metabolites in soil

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Abstract

Fenamiphos (0-ethyl-0(3-methyl-4-methylthiophenyl)-isopropylamido-phosphate), an organophosphorous pesticide, is widely used as a systemic and contact insecticide against soil nematodes in turf and horticultural crops around the world including Australia. This pesticide has been classified as highly toxic by the US EPA. There are reports on contamination of water bodies with fenamiphos and its metabolites due to their high mobility in soil. The data available on the environmental fate of fenamiphos is limited given that the previously published research focus has been temperate soils. This research focuses on (a) the fate of fenamiphos and its metabolites in soils from Australia and Ecuador, (b) the toxicity of fenamiphos and its metabolites to biota including algae, *Daphnia* and earthworms (c) the bioavailability of fenamiphos and its metabolites in long term contaminated soils and (d) the bioremediation of fenamiphos and its toxic metabolites by a novel bacterium.

The persistence of fenamiphos in five soils collected from different geographical regions such as Australia and Ecuador under 3 temperature regimes (18, 25 and 37⁰C) simulating typical environmental conditions was studied. The effect of soil properties (soil pH, temperature and microbial biomass) on the degradation of fenamiphos was determined. The rate of degradation increased with increase in temperature. Fenamiphos degradation was higher at 37°C than at 25 °C and 18°C (except under alkaline pH). The degradation pathway differed in different soils. Fenamiphos sulfoxide (FSO) was identified as the major degradation product in all the soils. Fenamiphos sulfone (FSO₂) and the corresponding phenols: fenamiphos phenol (FP), fenamiphos sulfoxide phenol (FSOP) and fenamiphos sulfone phenol (FSO₂P) were also detected. The degradation of fenamiphos was faster in the alkaline soils, followed by neutral and acidic soils. It was observed that in some cases both,

the pH and temperature had influence on the degradation of the pesticide as for alkaline pH and high temperature. Under sterile conditions, the dissipation of the pesticide was slower than in the non sterile soils suggesting microbial role in the pesticide degradation.

The sorption of fenamiphos and its major degradation products fenamiphos sulfoxide (FSO) and fenamiphos sulfone (FSO₂) were determined in thirteen contrasting soils collected from Australia and Ecuador. The sorption coefficients (K_d) exhibited a wide range of variation from 2.48 to 14.94 L/Kg for fenamiphos; from 0 to 7.42 L/Kg for FSO and from 0 to 9.49 L/Kg for FSO₂. The sorption affinity of the three compounds for all soils tested was as follows: fenamiphos > fenamiphos sulfone > fenamiphos sulfoxide. The sorption was influenced by various factors such as clay content, pH and organic matter content. The data obtained are very interesting as those show that the sorption of fenamiphos and its metabolites in some soils is very low, and in one case is non existent for the metabolites. This is of particular concern as due to its low sorption coefficient, the compound could easily migrate and contaminate water bodies.

The acute toxicity of fenamiphos and its metabolites to the aquatic alga *Pseudokirchneriella subcapitata* and the terrestrial alga *Chlorococcum* sp. showed that the toxicity followed the order: fenamiphos phenol > fenamiphos sulfone phenol > fenamiphos sulfoxide phenol > fenamiphos. The oxidation products of fenamiphos, FSO and FSO₂ were not toxic to both algal species up to 100 mg/L. Both algae were able to transform fenamiphos, FSO and FSO₂, while the phenols were found to be stable in the incubation media. Bioaccumulation of both fenamiphos and its metabolites was observed in the case of *Chlorococcum* sp. while only metabolites were accumulated in *P. subcapitata*.

The degradation of fenamiphos by different species of five green algae and five cyanobacteria was studied. All the species tested were able to transform fenamiphos to its primary oxidation product fenamiphos sulfoxide (FSO) while majority of these cultures were able to hydrolyze FSO to fenomiphos sulfoxide phenol (FSOP). Fenamiphos sulfone phenol (FSO₂P), FSOP and FSO were detected in the culture extracts of these algae and cyanobacteria.

The acute toxicity of fenamiphos and its metabolites to a cladoceran, *Daphnia carinata*, was studied in both cladoceran culture medium and natural water collected from a local river. The toxicity followed the order: Fenamiphos > fenamiphos sulfone > fenamiphos sulfoxide. The hydrolysis products of fenamiphos, FSO and FSO₂ (F phenol, FSO phenol and FSO₂ phenol) were not toxic to *D. carinata* up to 500 µg/L water, suggesting hydrolysis reaction leads to detoxification. The toxicity to *D. carinata* in river water was lower due to the degradation of the pesticide by microorganism under natural conditions.

Acute toxicity of fenamiphos to earthworms was studied in three different soils. The LC₅₀ of fenamiphos to earthworm corresponded to 175 mg/ kg. Residues of fenamiphos caused a reduction in the biomass of worms, especially the ones exposed to the pesticide in the acidic soil as the pesticide showed to be more persistent in this soil. *In vitro* experiments suggest that fenamiphos is biotransformed in the earthworms principally to its oxide.

A novel bacterium able to hydrolyze fenamiphos was isolated from soil and identified as *Microbacterium* sp. Fenamiphos disappeared rapidly in the medium inoculated with *Microbacterium* sp. (added at 10 mg/L) and reached to undetectable levels within 24 h after inoculation, with accumulation of its hydrolysis product fenamiphos phenol. The cell- free extract of *Microbacterium* sp. was highly effective in hydrolyzing fenamiphos to its phenol. After 30 minutes of inoculation, almost all the added fenamiphos (10 mg/L) was hydrolysed by the bacterium with concomitant production of fenamiphos phenol. *Microbacterium* sp. was tested for its ability to hydrolyze other related organophosphorus compounds. This bacterium was not able to hydrolyze coumaphos or methyl parathion.

The potential of *Microbacterium* sp. to remediate fenamiphos and its oxides in three long term (aged) contaminated soils was evaluated. This study clearly demonstrated the ability of *Microbacterium* sp. to detoxify fenamiphos and its toxic oxides (including non water desorbable fraction) in long-term contaminated soils. A sensitive bioassay using *Daphnia* sp. confirmed there was no residual toxicity left following bioremediation of fenamiphos and its toxic oxides by

Microbacterium sp. Further, the ability of this bacterium to survive and proliferate in the contaminated soils as demonstrated by a real time PCR assay shows the potential of this bacterium as an ideal bioremediation agent for fenamiphos or its oxides contaminated sites.