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Influence of different abiotic and biotic factors on the metalaxyl and carbofuran dissipation

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HIGHLIGHTS

- ▶ Metalaxyl and carbofuran dissipation are unaffected by soil microbes in short time scales.
- ▶ Metalaxyl and carbofuran dissipation depends on photodegradation and adsorption.
- ▶ Photodegradation has a stronger effect than adsorption.

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ABSTRACT

Metalaxyl and carbofuran dissipation was studied in response to different factors (soil bacterial communities, light irradiation, presence of an inorganic culture medium and presence of soil) and combinations of these factors in short-term experiments (48 h). The soil microbial communities have no effect on metalaxyl or carbofuran dissipation in the time scale employed. Light irradiation and soil promote metalaxyl and carbofuran dissipation by photodegradation and adsorption, respectively. However, photodegradation has a stronger effect on metalaxyl and carbofuran dissipation than the adsorption of the pesticides in the soil. The addition of the culture medium have no direct effect on pesticide dissipation, degradation by microbial communities or adsorption but its presence greatly increased photodegradation.

1. Introduction

The increase of agricultural production during the last century was associated with the use of pesticides to fight against plant diseases. However, only a small amount of an applied pesticide is in contact with the organisms responsible for diseases (Pimentel, 1995), whereas a large amount of the pesticide reaches the soil, causing potential risks to the environment and to human health (EPA, 2009).

Pesticides in the soil can be affected by physical, chemical and biological processes such as adsorption on soil colloids, uptake by plant roots, transport via soluble and particulate runoff, infiltration, volatilisation, and chemical and biological degradation (Arias-Estévez et al., 2008). The dissipation of pesticides from soil solution has a key role in their potential to damage plants and soil macro and microorganisms and to contaminate nearby water sources. The dissipation depends on the pesticide structure and chemical characteristics, the physicochemical soil characteristics, the soil biology and various environmental factors (Arias-Estévez et al., 2008).

Carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate) is a carbamate insecticide that is highly used worldwide. Carbofuran has a high solubility in water (700 mg L^{-1}) (Worthing, 1991), thus it can reach the groundwater via lixiviation and could cause problems relating to human health. Metalaxyl [*N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester] is a systemic fungicide used in many countries to fight against plant diseases caused by fungi such as *Pythium* spp. and *Phytopthora* spp. Its solubility in water (8400 mg L^{-1}) is higher than that of carbofuran; therefore, metalaxyl can also more easily reach the groundwater.

Different metalaxyl and carbofuran dissipation mechanisms were studied. Metalaxyl and carbofuran sorption was studied by many authors, showing that their dissipation from soil solution

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depends mainly on clay and organic matter content (Andrades et al., 2001; Rama Krishna and Philip, 2008; Bermúdez-Couso et al., 2011a, 2011b). Other processes, such as photodegradation and microbial degradation, were also studied (Trabue et al., 2001; Massoud et al., 2008; Baker et al., 2010; Lu et al., 2011; Plangklang and Reungsang, 2012). These processes could have an antagonistic effect. For example, pesticide adsorption on soil colloids removes them from the soil solution, thereby preventing degradation (Krishna and Philip, 2011). The effects of the different processes should be studied to improve the overall dissipation of pesticides from the soil. However, studies comparing all possible dissipation mechanisms and their interactions in short periods of time have not been performed.

The main objective of this study was to evaluate the effects of sorption, photodegradation and microbial degradation and their interactions on metalaxyl and carbofuran dissipation in short periods of time. To this end, experiments have been carried out in water solutions in the absence and presence of soils and in the presence of light or darkness.

2. Materials and methods

2.1. Chemicals

Carbofuran (2,3-dihydro-2-2-dimethyl-7-benzofuranyl-*N*-methylcarbamate) with 99.5% purity was obtained from Dr. Ehrenstorfer Lab. (Augsburg, Germany). Metalaxyl [methyl *N*-methoxyacetyl-*N*-(2,6-dimethyl)-DL-alaninate] with a purity >99.5% was obtained from Sigma–Aldrich (Steinheim, Germany). The chemical structures and selected properties of carbofuran and metalaxyl are shown in Table S1. All organic solvents used for sample preparation were residue analysis grade and were supplied by Panreac (Barcelona, Spain). HPLC grade solvents for HPLC work were also supplied by Panreac.

2.2. Soil samples

The soil samples used were collected in the "A Limia" district (Galicia, NW Iberian Peninsula). This area is devoted to intensive farming of potato and wheat in rotation with other crops. Two samples were collected from the topmost layer (0–20 cm) and were air dried, sieved through a 2 mm mesh and stored in polyethylene bottles until analysis. The soil characteristics were previously described by Bermúdez-Couso et al. (2012). The texture of the soils was clay for Soil 1 and clay loam for Soil 2; both samples were acid (pH 4.5 for Soil 1, pH 4.4 for Soil 2). Soil organic carbon was 22.7% and 2.7% for Soil 1 and for Soil 2, respectively.

2.3. Pesticide toxicity to soil bacteria

The pesticide toxicity to the bacterial community was measured using the leucine (Leu) incorporation technique (Kirchman et al., 1985) to determine the bacterial growth. The bacterial community was extracted from the soil with the homogenisation and centrifugation technique described by Bååth (1994) and Bååth et al. (2001). The soils (2 g, fresh weight) were mixed at maximum intensity with 20 ml distilled water for 3 min using a multivortex shaker. The mixtures were then centrifuged at 1000 g for 10 min to create a bacterial suspension in the supernatant. Aliquots of 1.35 ml were added to microcentrifugation vials. Then, 0.15 ml of different concentrations of either metalaxyl or carbofuran in water was added to each vial. Seven different concentrations of each pesticide were used for each soil type. A control with distilled water substituted for pesticide solution was included for each soil. Next, 2 μL [^{3}H]Leu (37 MBq ml^{-1} and 5.74 TBq mmol^{-1}; Amersham) was added along with unlabeled Leu to each tube, resulting in 275 nM Leu in the bacterial suspensions. After 2 h of incubation, bacterial growth was stopped with 75 μL of 100% trichloroacetic acid. Washing and subsequent measurement of radioactivity were performed as described by Bååth et al. (2001) using an scintillator TriCarb 2810TR. The results for the different pesticide concentrations were standardised with respect to the bacterial growth in the presence of distilled water.

2.4. Pesticide dissipation experiments

The dissipation experiments were performed with metalaxyl (10 mg L^{-1}) and carbofuran (4 mg L^{-1}) solutions at different solution conditions to separate the different pesticide dissipation mechanisms: photodegradation, microbial degradation, chemical degradation and adsorption. The pesticide solutions were made with distilled water and then passed through 0.20 μm pore size cellulose nitrate filters to remove the microorganisms from the solutions. A total of 10 mL of each of these solutions was then mixed with 10 mL of one of three different solutions in the 40 mL EPA vials: (a) distilled filtered water, (b) a soil bacterial suspension extracted according to the methods of Bååth (1994) and Bååth et al. (2001), and (c) suspension with 1 g of soil. Then, the vials were placed in the simulation light cabin equipped with a xenon lamp with an output of 550 W m^{-2} and quartz filters with a cut-off at 285 nm (Atlas Suntest CPS + Atlas, Germany). Two types of experiments were performed for all treatments: (a) in darkness for 48 h and (b) with a cycle of 12 h light and 12 h darkness over 48 h. The temperature during the experiments was kept at 32 ± 2 °C in the light phase and 25 ± 2 °C during the darkness phase. All experiments were performed in triplicate. Subsamples of 0.2 mL were taken at appropriate time intervals, and the metalaxyl or carbofuran concentrations were measured.

Because it had been previously found that the microorganisms' growth could be limited by the absence of any nutrient, the experiments were reproduced but changing the distilled filtered water solution containing the pesticides by a filtered culture medium containing the same pesticide concentrations. The culture medium was made with filtered distilled water and 1.75 mg L^{-1} K_2HPO_4, 0.50 mg L^{-1} KH_2PO_4, 0.58 mg L^{-1} NH_4Cl and 0.25 mg L^{-1} $MgSO_4{\cdot}7H_2O$.

In the experiments with the soil, once the dissipation tests were finished, the pesticides retained in the soil were extracted. The procedure employed was the same for both carbofuran and metalaxyl, except that methanol was used as the solvent for metalaxyl and acetonitrile was the solvent for carbofuran. The procedure was as follows: the vials were opened and incubated at 40 °C to evaporate all the liquid. Once dry, 10 mL of solvent was added to the vials, which were then shaken for 30 min. The vials were placed in a sonicator for 5 min and centrifuged at 2000 rpm for 10 min. After centrifugation, the supernatant was transferred to a 60 mL EPA vial. This procedure was repeated three times. The combined extracts (10 + 10 + 10 mL) were concentrated using a nitrogen evaporator (Caliper LS, Turbovap LV, Caliper, USA). The dried residue was dissolved in 1 mL of distilled water, transferred to a 2 mL vial and then analysed by HPLC-UV. The pesticide extracted by this method is considered the adsorbed pesticide. The concentration of pesticides in soil studied before dissipation experiments were lower than the detection limit (0.1 μmol kg^{-1} and 0.04 μmol kg^{-1} for metalaxyl and carbofuran, respectively).

2.5. Carbofuran and metalaxyl determination

The determination of pesticides was performed using high performance liquid chromatography (HPLC) with a UV–Visible detector from Dionex Corporation (Sunnyvale, EE.UU.), equipped with a P680 quaternary pump, an ASI-100 autosampler, a TCC-10 thermostatic column compartment and a UVD170U detector.