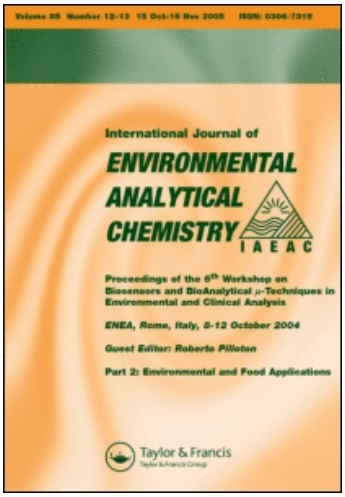

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Biochemical parameter changes in urban-waste compost used as biofilter for pesticide decontamination

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Biochemical parameter changes in urban-waste compost used as biofilter for pesticide decontamination

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Urban-waste compost (UWC) can be used as a biofilter filling to reduce the effects of pesticide spills. Here, water that was contaminated by three different pesticides, the insecticide chlorpyrifos (Chl), the fungicide metalaxyl (Meta) and the herbicide glyphosate (Gly), was percolated through 2 kg of UWC material. The pesticide residues in the leached water and the modifications induced in some of the UWC biochemical and microbiological parameters (including microbial biomass carbon (MBC) and nitrogen (MBN), and fluorescein diacetate (FDA) hydrolysis, alkaline monophosphatase (AMP) and dehydrogenase (DH) activities) were investigated over 2 months of incubation at 20°C. The UWC showed a good retention capacity towards the three pesticides tested, with the highest efficiency for Gly. Chl caused an initial detrimental effect on the MBC content and a decrease in the FDA hydrolysis capacity, while Meta and Gly increased the MBC content throughout the incubation. The results demonstrate that UWC can be successfully used as a biofilter to reduce pesticide spills and to clean up water contaminated with pesticides. The evaluation of the modifications induced on the UWC MBC and MBN, and FDA hydrolysis, AMP and DH activities suggest different biodegradation potentials of the UWC micro-organisms *vs.* the three pesticides studied.

Keywords: Urban waste compost; Pesticides; Microbial biomass; Enzyme activities

1. Introduction

Point pollution with pesticides and inadvertent spills during pesticide handling on farms are particularly dangerous both for the environment and for human health. Although the environmental protection is regulated by European registration legislation (91/414/EEC), there remains a lack of specific legislative tools to prevent these kinds of contamination. Recently, Capri *et al.* [1] demonstrated that only a relatively small proportion of the pesticides used to protect crops in the field reach the surface

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and ground waters through percolation, runoff, drainage, and drift. In contrast, the main contamination derives from bad agronomic practices in pesticide storage and handling, and in the management of spill zones [2, 3]. A widespread application of decontamination techniques at the farm level could improve the protection of aquatic ecosystems, and at the same time reduce water-treatment costs. To help to protect water resources from pesticide pollution, Torstensson *et al*. [4] set up the 'biobed' concept, describing a system that uses organic materials to adsorb and degrade pesticides during all of the operations proceeding and following field applications. The top layer of the biobed should be a soil that is rich in humus, to efficiently adsorb pesticides and to support the metabolic activity of the micro-organisms that are responsible for pesticide biodegradation. For practical reasons, the biobed filling should be adapted to organic wastes that are available in different geographic areas; in Sweden and the UK, for instance, the use of specific organic materials, such as peat [4], is justified by low costs and wide availability. We have been investigating the development of biobeds that are adapted to Mediterranean conditions, and thus that use organic materials that come from the common agricultural practices in this area, such as vine-branch and citrus pulp, in a mixture with urban-waste compost (UWC). Such fillings allow the recycling of the percolating water that is polluted by pesticides [5]. Here, we have carried out laboratory tests to investigate the depurative actions of this UWC to determine if it can be used as a biofilter in a biobed system, and to evaluate the modifications induced in the UWC microflora by the adsorbed pesticides. The pesticides tested were: metalaxyl (Meta, fungicide), chlorpyrifos (Chl, insecticide), and glyphosate (Gly, herbicide), which were chosen because they are widely used in agriculture under Mediterranean conditions.

2. Experimental

2.1 *Compost and pesticides*

The UWC material was kindly supplied by GESENU SpA (Perugia, Italy). The general UWC properties, determined according to Page *et al*. [6], are reported in table 1. The pesticides used were analytical standards of chlorpyrifos (Chl) (*O*,*O*-diethyl,*O*-3,5,6-trichloro-2-pyridyl phosphorothioate), metalaxyl (Meta) (methyl *N*-(methoxyacetyl)-N-(2,6-xylyl)-DL-alaninate) and glyphosate (Gly) (*N*-(phosphonomethyl)glycine). These analytical standards were supplied by Lab. Ehrenstorfer-Schafers (Augsburg, Germany).

Table 1. Physico-chemical properties of the urban waste compost.

Humidity (%)	36.4
pH	7.7
Organic carbon (%)	29.7
Organic nitrogen (%)	1.77
Total nitrogen (%)	1.80
C/N	16.7
P (Olsen) (%)	0.88
Salinity (meq/100 g)	26.6
Total Cu ($mg\,kg^{-1}$)	97.0
Total Zn ($mg\,kg^{-1}$)	303.0

2.2 *Experimental procedures*

The experimental procedures were carried out under laboratory conditions. Each of 81 PVC columns (diameter, 16 cm; height, 80 cm) was filled with 2 kg of UWC (height, 30 cm) at a 60% water holding capacity (WHC). A plastic net was placed at the bottom of each column to allow the drainage of the water. Twenty-seven of the PVC columns (three replicates for each pesticide, for each sampling time) were used as controls and treated with 4 L of deionized water twice a day to determine the effects of water leaching on the biochemical and microbial parameters studied. The other 54 columns were subdivided into six groups of nine columns each (three replicates for each pesticide, for each sampling time) and treated twice a day with 4 L of deionized water containing Chl, Meta and Gly at the field doses (D) of 0.75 g L^{-1}, 1 g L^{-1} and 7 g L^{-1}, respectively, and at fourfold field doses (4D) of 3 g L^{-1}, 4 g L^{-1} and 28 g L^{-1}, respectively. The leached water was collected from each column after each leaching event and analysed to determine the pesticide residues. The modifications of the UWC microbiological and biochemical parameters induced by the pesticides were evaluated considering the time when the pesticide completely disappeared from the drainage water as the starting time (T0), and at T1 and T2, representing 1 and 2 months from T0, respectively. The temperature was kept at 20°C during the entire experimental period, and the columns were constantly insufflated with air to prevent any anoxic mesocosm developing.

2.3 *Pesticide determinations*

Chl and Meta concentrations in the drainage water were determined according to Vischetti *et al.* [5]. Briefly, 100 mL sub-samples were partitioned in $CHCl_3$ (100 mL × 2), and the samples were evaporated to dryness, rinsed with 1 mL CH_3OH, and analysed by HPLC using a C18 inertsil column (25 cm × 4.6 mm), a UV detector at a 230 nm wavelength, a mobile phase of CH_3CN/H_2O (70/30), and a flow rate of 0.7 mL min^{-1}. Under these conditions, the retention times were 5.2 min for Meta and 12 min for Chl. The limit of detection (LOD) was 10 ng for both pesticides, and the limit of quantification (LOQ), determined at a signal-to-noise ratio of 2, was 2 μg L^{-1} for both of the pesticides.

The Gly concentrations in the drainage water were determined according to Glass [7]. Briefly, Gly was initially partitioned and rinsed as for Chl and Meta. The Gly analysis was also performed by HPLC, after the derivatization of the herbicide with 9-fluorenylmethyl chloroformate (FMOCCl), with fluorescence detection, an amine phase column (15 cm × 4.6 mm), a mobile phase of CH_3CN/0.1 M KH_2PO_4 (85/15), and a flow rate of 1.5 mL min^{-1}. Under these conditions, the retention time was 4.5 min, and the LOQ was 12 μg L^{-1}.

2.4 *Determination of microbiological and biochemical parameters*

The hydrolysis rates of fluorescein diacetate (FDA) were estimated using the method described by Schnurer and Rosswall [8], as modified by Perucci *et al.* [9]. Briefly, 0.1 g of sample was added to 10 mL of FDA solution, which was obtained by diluting the FDA stock solution (2 mg mL^{-1} in acetone) in phosphate buffer (pH 7.6), and incubated at 37°C for 1 h. After this incubation, the reaction was stopped by adding

10 mL acetone, and the mixture was centrifuged at 5000 rpm. FDA-hydrolysis activity was expressed as μg of FDA hydrolysed 0.1 g^{-1} d.m.h^{-1}.

Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined using the fumigation extraction method [10]. Alkaline monophosphatase (AMP) (EC 3.1.3.1) activity was assayed according to Tabatabai [11], with slight modifications. This involves the determination of *p*-nitrophenol released after 1 h of incubation at 37°C of: 0.1 g of organic matter, 0.2 mL of toluene, 4 mL of modified universal buffer (MUB), pH 11, and 1 mL of 0.025 M *p*-nitrophenyl-phosphate in MUB buffer. The release of *p*-nitrophenol was detected using a spectrophotometer at 420 nm. The AMP activity was expressed as μg of *p*-nitrophenol 0.1 g^{-1}d.m.h^{-1}. Dehydrogenase [DH] activity was evaluated according to von Mersi and Schinner [12], with slight modifications. Briefly, 0.1 g of organic matter was added to 1.5 mL of 1 M Tris-buffer, pH 7, and a 2 mL aqueous solution of iodonitrotetrazolium chloride (INT); this was incubated at 40°C for 2 h. After the incubation, 10 mL of extracting solution (*N*,*N*-dimethylformamide and ethanol, 1 : 1) was added and the mixture shaken for 1 h. The released iodonitrotetrazolium-formazane (INTF) was measured spectrophotometrically at 464 nm. The DH activity was expressed as nmoles of 0.1 g of INTF per 2 h.

2.5 *Statistical analysis*

The SYSTAT programme was used for the analysis of variance and Duncan's range test on the means. Each value presented is the mean of three replications.

3. Results and discussion

The recoveries of all three of the pesticides from the water samples were always satisfactory, and ranged from 85.7 ± 1.7% for Gly at the D dose to 98.2 ± 1.3% for Meta at the 4D dose.

The depurative efficiency of the UWC is clearly demonstrated in table 2, where the percentages of the three pesticides in the drainage water from the two leaching events per day are given.

Table 2. Pesticide residues (% of initial levels) in the drainage water from the leaching events (twice daily) (mean ± standard deviation of three replications).[a]

Leaching event (no.)	Chl		Meta		Gly	
	D	4D	D	4D	D	4D
1	5.3 ± 0.8	7.8 ± 1.3	35.5 ± 1.4	47.5 ± 2.3	< LOQ	< LOQ
2	< LOQ	5.7 ± 0.8	17.5 ± 0.9	30.4 ± 1.5		
3	< LOQ	< LOQ	10.4 ± 1.1	15.7 ± 1.7		
4			7.3 ± 0.5	10.5 ± 1.1		
5			1.1 ± 0.2	8.2 ± 1.8		
6			< LOQ	3.3 ± 1.4		
7			< LOQ	< LOQ		

[a] Chl: chlorpyrifos; Meta: metalaxyl; Gly: glyphosate; D: field dose; 4D: fourfold field dose (see text).

The Gly concentration was already lower than LOQ at the first leaching event at both doses, the Chl concentration was lower than LOQ at the second leaching event for the D dose and at the third for the 4D dose, while the disappearance of Meta was slightly longer, since its concentration was lower than LOQ at the sixth leaching event for the D dose and at the seventh event for the 4D dose. This thus demonstrates the very good efficiency of UWC when used as a biofilter. These results are also in agreement with other reports: Vischetti *et al*. [5] showed that biofilters made up of a mixture of compost and organic materials from agriculture (citrus peel or vine branches) were depurative for waters contaminated by Chl and Meta, with a rapid disappearance of Chl from the water already at the first leaching event, and the disappearance of Meta between the fourth and the sixth leaching events; Roy *et al*. [13] reported that Gly remained in the first 15 cm of the upper organic layer of a forest soil for a period of 2 years under external weather conditions, showing the strong retention capacity of the organic matter of the soil for this pesticide.

The changes in the MBC and MBN contents and the levels of FDA hydrolysis are given in figures 1, 2, and 3, respectively.

There were significant ($p < 0.05$) decreases in MBC at both of the doses for Chl at time T0; the inhibitory effect disappeared for the D dose, but it remained for the 4D dose until the end of the incubations. According to Vischetti *et al*. [14], the toxic effects of a pesticide on soil microbial biomass are more pronounced at the initial contact between the pesticide and the microflora, and this generally persists until the pesticide has reached 50% of its initial concentration. Meta showed significant increases ($p < 0.05$) in MBC at the 4D dose, while Gly showed a significant ($p < 0.05$) increase in MBC for both doses throughout the entire incubation period. These findings suggest the use by some of the micro-organism species of both Meta and Gly as carbon sources.

Significant ($p < 0.05$) increases in the MBN (see figure 2) were generally observed at the D dose at all three sampling times, except for Meta at T0 and T1, where the MBN remained almost the same as the control. The soil MBC : MBN ratio varied as a consequence of the changes in the MBC and MBN values; this suggests a modification of the species composition of the microbial biomass [9].

In starting from the hypothesis of Swisher and Carroll [15], Perucci [16] revealed a correlation between the rate of FDA hydrolysis and the microbial biomass content. Dumontet *et al*. [17] suggested that FDA hydrolysis can be used as a useful tool to evaluate the initial effects of xenobiotics on soil microbial biomass, since FDA can be hydrolysed by a great number of extracellular enzymes, such as proteases, lipases, and esterases. Therefore, the determination of the global hydrolytic capacity in FDA hydrolysis assays appears to be a good biological test for the evaluation of the direct and/or indirect effects on UWC biochemistry that are due to organic xenobiotics, such as pesticides.

The Chl showed a significant ($p < 0.05$) inhibition of FDA hydrolysis, which, at T0, resulted in 25.7% and 36.0% decreases for the D and 4D doses, respectively, compared with the control samples (figure 3). This inhibition remained for the entire incubation period, suggesting that Chl exerted a direct inhibitory effect on the micro-organisms producing the hydrolytic enzymes. The persistence of this effect also suggests a slow capacity of UWC for the degradation of Chl. A significant ($p < 0.05$) increase in the FDA hydrolysis capacity was observed for Gly and Meta at time T0 for the 4D dose. These results suggest a toxic effect exerted by the two pesticides on some of the species of micro-organisms; as a consequence of the death and cell lysis of those

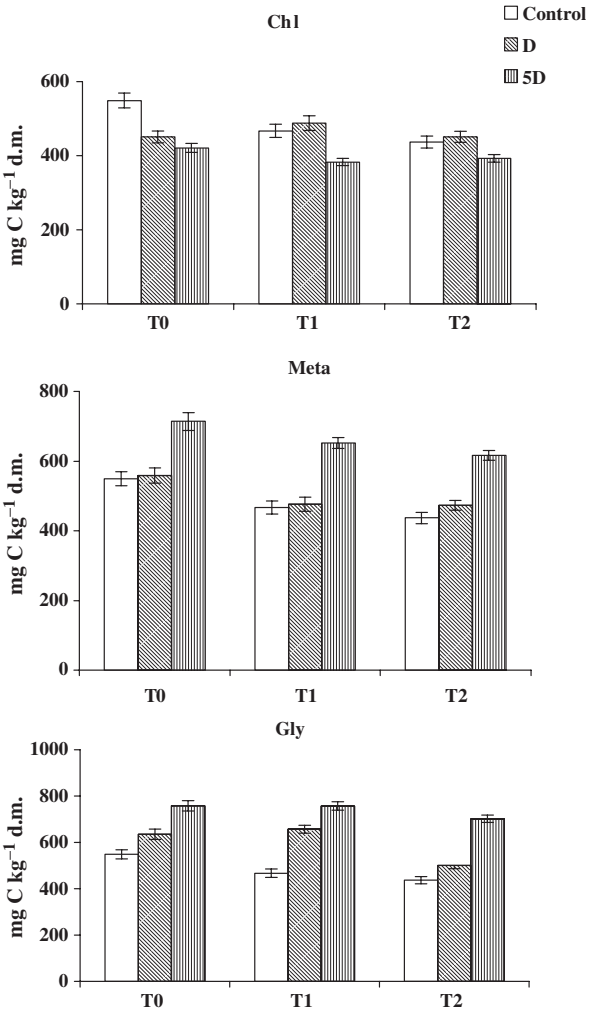

Figure 1. Microbial biomass-C contents of the UWC samples for the three pesticides at the three experimental times (bars represent the standard deviation of three replicates).

sensitive species, hydrolytic endocellular enzymes can be released in the microenvironment, causing a sudden increase in the FDA hydrolysis capacity. At time T1, these new enzymes cannot be protected against the proteases present and can be used as a carbon source by the other micro-organisms, thus explaining the decreases observed.

According to Perucci *et al.* [9], a better interpretation of the FDA hydrolysis data can be obtained if the FDA hydrolytic activity is expressed as a percentage of the FDA hydrolysed per unit of MBC (see table 3). In this way, a new index was obtained: the specific hydrolytic capacity (qFDA), where an increase in qFDA reveals an impairment of the metabolic activity of the substrate microflora.

The qFDA values for Meta and Gly were significantly ($p < 0.05$) lower than those of the controls over the entire incubation time. These findings suggest the ability of the major fraction of the UWC microflora to cleave the Meta and Gly molecules for energy purposes (as a carbon source), since the FDA hydrolytic capacities were similar

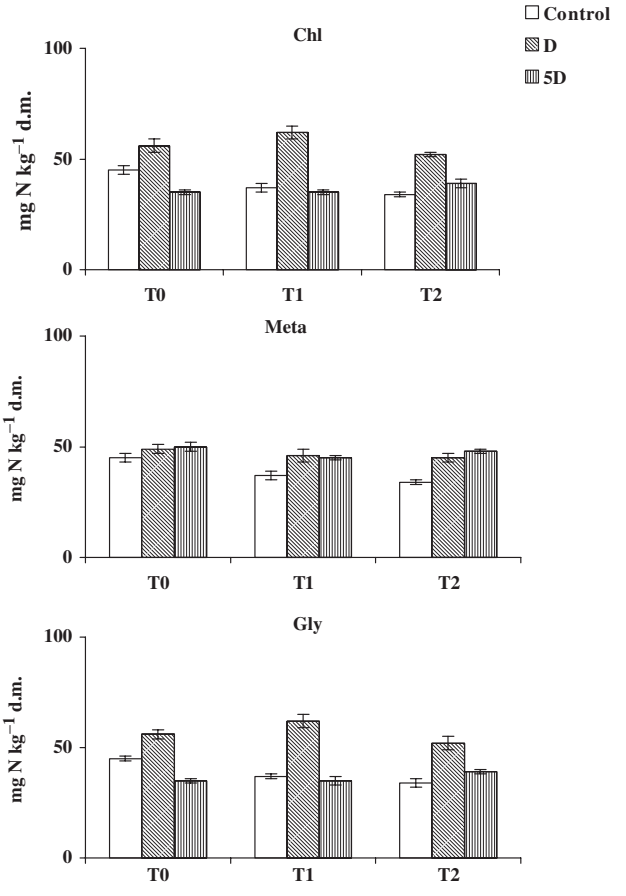

Figure 2. Microbial biomass-N contents of the UWC samples for the three pesticides at the three experimental times (bars represent the standard deviation of three replicates).

to the controls, while the MBC values were increased by the presence of the two pesticides, particularly at the higher dose.

In contrast, the lower qFDA values for Chl will have come from a worsening of the FDA hydrolytic activity in addition to the lower MBC values, indicating a toxic effect of this pesticide on the biological activities of the UWC micro-organisms.

Two specific enzyme activities were tested: alkaline monophosphatase (AMP) and dehydrogenase (DH). The DHs are intracellular enzymes and they are related to the oxidative-reductive potential of the mesocosm studied.

The DH activities (measured as INTF levels; see section 2 and figure 4) were always significantly ($p < 0.05$) lower than the control values and were proportional to the doses added for all of the pesticides tested.

The AMP activities (figure 5) have been used as a synthetic index for the evaluation of the effects of xenobiotic compounds, such as pesticides, on the overall microbial catalytic activity in soil [18]. The AMP activities were also negatively affected during the entire incubation time, and particularly at the highest dose added for all of the pesticides. This behaviour suggests a direct inhibiting interference by the active ingredients and/or their metabolites on the enzyme biochimism.

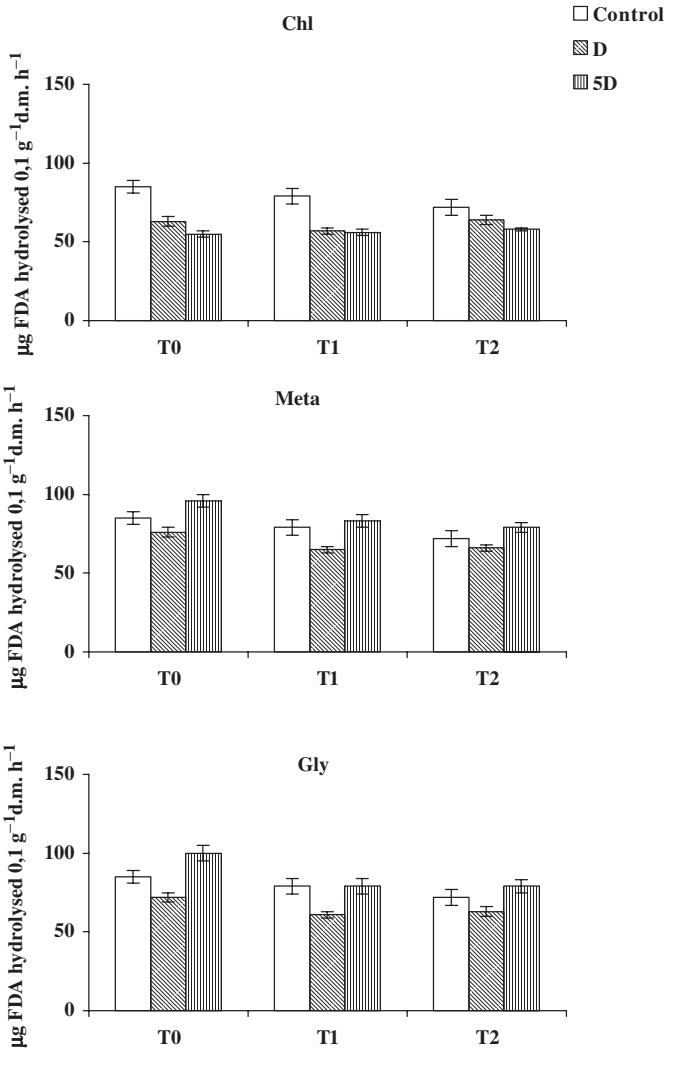

Figure 3. FDA hydrolysis activities of the UWC samples for the three pesticides at the three experimental times (bars represent the standard deviation of three replicates).

Table 3. Percentages of FDA hydrolysed per unit of MBC (qFDA).[a]

	T0	T1	T2
Control	15.5a	16.8a	16.4a
Chl (D)	14.0b	11.7b	14.2b
Chl (4D)	13.0c	14.6c	14.8b
Gly (D)	11.3d	9.3d	12.5c
Gly (4D)	13.2c	10.5d	11.2c
Meta (D)	13.6c	13.6e	14.0b
Meta (4D)	13.5c	12.7e	12.8c

[a] Values in the same column and at the same time followed by different letters are statistically different at $p < 0.05$ ($n = 3$). T0: starting time; T1 and T2: 1 and 2 months from T0, respectively. All other abbreviations as for table 2.

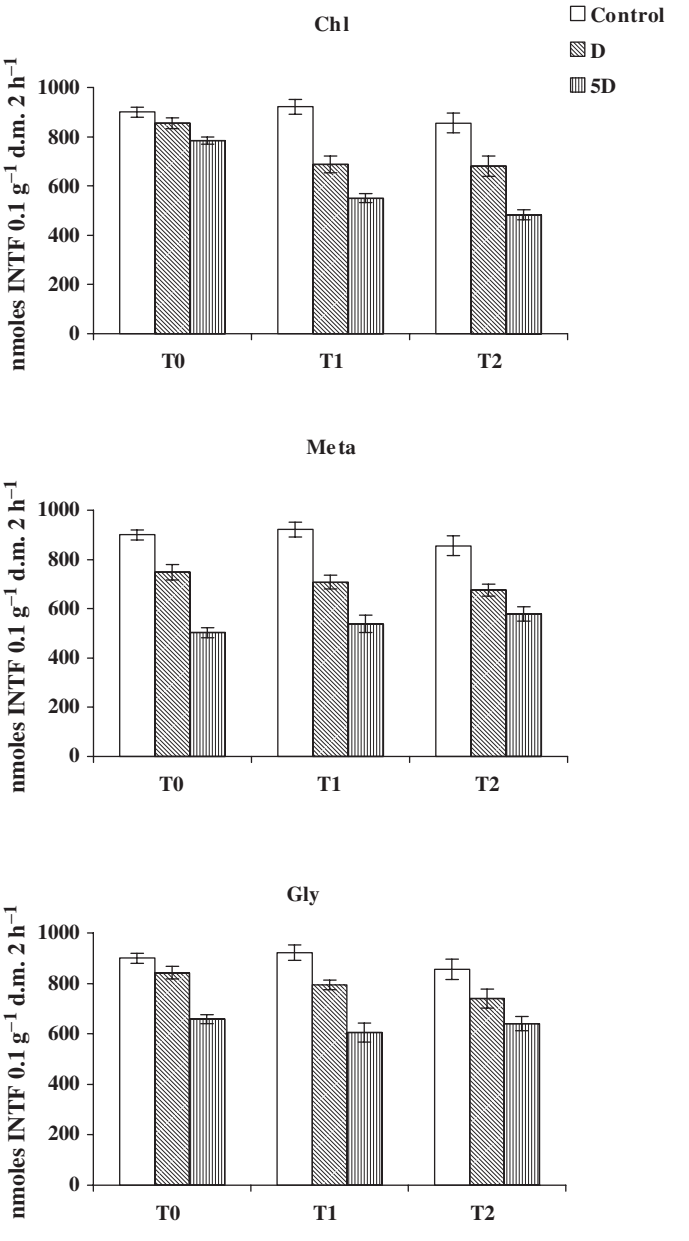

Figure 4. Dehydrogenase activities of the UWC samples for the three pesticides at the three experimental times (bars represent the standard deviation of three replicates).

4. Conclusions

The results of the present study show that UWC can indeed be used successfully used as a biofilter for the pesticide decontamination of water, due to its ability to adsorb and degrade pesticides. The modifications induced by the Meta and Gly pesticides to the

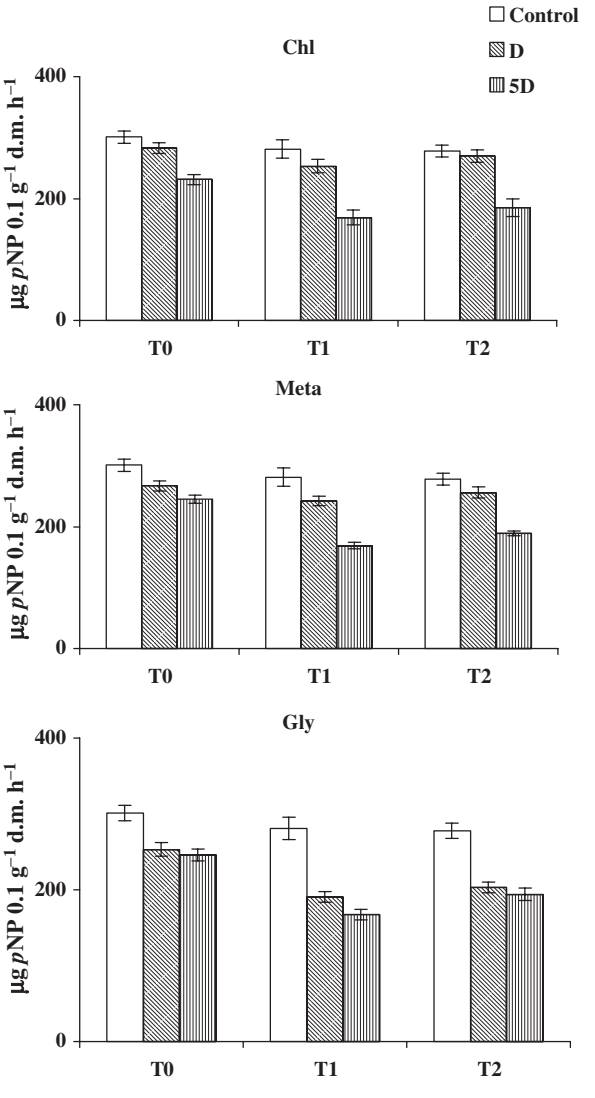

Figure 5. Alkaline monophosphatase activities of the UWC samples for the three pesticides at the three experimental times (bars represent the standard deviation of three replicates).

UWC microbial biomass content, the FDA hydrolysis capacity, and the enzyme activities tested were slight and transitory, while the changes induced on these biochemical parameters by Chl were more evident and long-lasting.

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References

[1] E. Capri, L. Padovani, M. Trevisan. *La Previsione della Contaminazione delle Acque Sotterranee da Prodotto Fitosanitari*, Pitagora Editrice, Bologna (1999).
[2] P.J. Mason, I.D.L. Foster, A.D. Carter, A. Walker, S. Higginbotham, R.L. Jones, I.A.J. Hardy. Relative importance of point source contamination of surface waters: river cherwell catchment monitoring study, paper presented at XI Pesticide Chemistry Conference, Cremona, I, 11–15 September (1999).
[3] M. Bach. Reduction of pesticide contamination at the catchment level, paper presented at the Warwick Conference, Agriculture & the Environment – Challenges and Conflicts for the New Millenium, Wolverhampton, UK, 14–16 April (1999).
[4] L. Torstensson, H. Brown, G.W. Cussans, M.D. Devine, S.O. Duke, C. Fernandez-Quintanilla, A. Helweg, R.E. Labrada, M. Landes, P. Kudsk, J.C. Streibig. Herbicide in the environment, paper presented at the International Weed Control Congress, Copenhagen, 25–28 June (1996).
[5] C. Vischetti, E. Capri, M. Trevisan, C. Casucci, P. Perucci. *Chemosphere*, **55**, 823 (2004).
[6] A.L. Page, R.H. Miller, D.R. Keeney. *Methods of Soil Analysis*, Agronomy Monograph Part 2 ASA and SSSA, 2nd Edn, Madison, WI (1982).
[7] R.L. Glass. *J. Agric. Food Chem.*, **31**, 280 (1983).
[8] J. Schnurer, T. Rosswall. *Appl. Environ. Microbiol.*, **43**, 1256 (1982).
[9] P. Perucci, S. Dumontet, S.A. Bufo, A. Mazzatura, C. Casucci. *Biol. Fertil. Soils*, **32**, 17 (2000).
[10] G.B. Sparling, A. West. *Soil Biol. Biochem.*, **20**, 337 (1988).
[11] M.A. Tabatabai. In A.L. Page *et al.* (Eds), *Methods of Soil Analysis*, pp. 903–947, ASA and SSSA, Madison, WI (1982).
[12] W. von Mersi, F. Schinner. *Biol. Fertil. Soils*, **11**, 216 (1991).
[13] D.N. Roy, S.K. Konar, S. Banerjee, D.A. Charles, D.G. Thompson, R. Prasad. *J. Agric. Food Chem.*, **37**, 437 (1989).
[14] C. Vischetti, C. Casucci, P. Perucci. *Biol. Fertil. Soils*, **35**, 13 (2002).
[15] R. Swisher, G.C. Carroll. *Microbiol. Ecol.*, **6**, 217 (1982).
[16] P. Perucci. *Biol. Fertil. Soils*, **14**, 54 (1992).
[17] S. Dumontet, P. Perucci, S.A. Bufo. Les amendements organiques et traittements avec les herbicides. Influence sur la biomasse microbienne du sol, paper presented at the XXVII Congres du Group Francais des Pesticides, Orleans, France (1997).
[18] S. Dumontet, P. Perucci, A. Scopa, A. Ricciardi. *Soil Sci.*, **1**, 193 (1993).