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Removal of carbofuran is not affected by co-application of chlorpyrifos in a coconut fiber/compost based biomixture after aging or pre-exposure

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ABSTRACT

Biomixtures constitute the biologically active part of biopurification systems (BPS), which are used to treat pesticide-containing wastewater. The aim of this work was to determine whether co-application of chlorpyrifos (CLP) affects the removal of carbofuran (CFN) (both insecticide/nematicides) in a coconut fiber–compost–soil biomixture (FCS biomixture), after aging or previous exposure to CFN. Removal of CFN and two of its transformation products (3-hydroxycarbofuran and 3-ketocarbofuran) was enhanced in pre-exposed biomixtures in comparison to aged biomixtures. The co-application of CLP did not affect CFN removal, which suggests that CLP does not inhibit microbial populations in charge of CFN transformation. Contrary to the removal behavior, mineralization of radiolabeled ^{14}C-pesticides showed higher mineralization rates of CFN in aged biomixtures (with respect to freshly prepared or pre-exposed biomixtures). In the case of CLP, mineralization was favored in freshly prepared biomixtures, which could be ascribed to high sorption during aging and microbial inhibition by CFN in pre-exposure. Regardless of removal and mineralization results, toxicological assays revealed a steep decrease in the acute toxicity of the matrix on the microcrustacean *Daphnia magna* (over 97%) after 8 days of treatment of individual pesticides or the mixture CFN/CLP. Results suggest that FCS biomixtures are suitable to be used in BPS for the treatment of wastewater in fields where both pesticides are employed.

Introduction

Biopurification systems (BPS) are biological devices developed as a tool to treat pesticide contaminated wastewater produced during agricultural activities (Karanasios et al., 2012). Use of BPS is mainly focused on reducing point source contamination such as accidental spillages during tank filling or mishandling of application residues or cleaning of spraying equipment, where the pesticide concentrations are usually high. BPS employ a biologically active biomixture where pesticides are removed from the wastewater by sorption and/or biodegradation. The biomixture acts as a filter matrix that will receive wastewater of high pesticide concentration during operation (initial operation concentrations in the order of mg/kg in the biomixture). This matrix is constituted by (Castillo et al., 2008): soil (usually pre-exposed to the target pesticide) that provides degrading microbiota, peat (as a humic component) and a lignocellulosic substrate (to promote the growth and activity of ligninolytic fungi, able to nonspecifically oxidize diverse organic pollutants, Asgher et al., 2008). Though peat has been usually employed in

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the first developed biomixture compositions, it has been successfully substituted by compost (Coppola et al., 2007; Kravvariti et al., 2010), which is more easily available at most geographical regions. Similarly, the use of lignocellulosic substrates has varied according to local availability, resulting in the use of alternative wastes such as cane bagasse, wood chips, olive leaves, grape stalks and barley husk (Karanasios et al., 2010; Urrutia et al., 2013; Chin-Pampillo et al., 2015a).

Efficiency of biomixtures is affected by diverse factors including moisture, temperature, composition, pre-exposure and maturity of the biomixture (Castillo et al., 2008). Among these factors, maturity, aging or pre-exposure of the biomixture have been scarcely studied. In this sense, Tortella et al. (2012) described the degradation of CLP at two maturity stages (15 and 30 days) and compared it to the un-aged biomixture, finding removal efficiencies over 50% in every case, however, the accumulation of the transformation product trichloropyridinol (TCP) was higher in the biomixture aged for 30 days. On the other hand, pesticide pre-exposure of the soil employed is suggested before preparation of biomixtures (Sniegowski et al., 2012), nonetheless few studies deal with the effect of the pre-exposure of the whole biomixture already prepared and usually report the outcome after successive pesticide applications (Vischetti et al., 2008; Tortella et al., 2013a, 2013b).

Carbofuran (CFN) is an insecticide that belongs to the group of carbamates, which also presents nematicide and acaricide activities, and poses a threat on non-target organisms due to its high toxicity (EC_{50} 0.0094 mg/L, acute test on *Daphnia magna*, 48 hr) (University of Hertfordshire, 2013), as it has been demonstrated by the occurrence of diverse alterations in aquatic life (Gupta, 1994; Adhikari et al., 2004). This pesticide is currently banned in the EU and the USA, and progressively in other latitudes, however, its use is still permitted in some countries, even though the banning in most of them is a matter of time; therefore while in use, measures to avoid contamination with CFN should be applied. Chlorpyrifos (CLP) is an organophosphate insecticide of worldwide use with a broad spectrum of activity. CLP is considered as a hydrophobic compound ($\log K_{ow}$ = 4.7), with low water solubility (1.4 mg/L) and high affinity to organic matter (K_{oc} = 8.5 L/g), reasons why it shows strong adsorption and reduced bioavailability in soil (Racke, 1993; Racke et al., 1996), in contrast with CFN (K_{oc} = 0.086 L/g, $1/n$ = 0.89) (University of Hertfordshire, 2013). The neurotoxic potential of CLP (EC_{50} 0.0001 mg/L, acute test on *D. magna*, 48 hr) (University of Hertfordshire, 2013) has been reviewed somewhere else (van Wijngaarden et al., 1993; Richardson, 1995; Eaton et al., 2008), and includes deleterious effects on aquatic life. The decrease in microbial abundance and activity has been observed in soil after contamination with CLP, which is usually ascribed to the production of TCP as a result of CLP hydrolysis; this process is considered to result in inhibition of CLP degradation (Racke et al., 1988; Chu et al., 2008). CFN and CLP can be applied to the same crops in tropical regions where CFN is still employed. Given the toxic effect of CLP on microbial communities, degradation of CFN could be affected in co-application of both compounds or in application of CLP in combination with other pesticides such as other carbamates.

The current study aimed to determine the effect of maturity and pre-exposure of a coconut fiber–compost–soil (FCS) biomixture on CFN degradation and mineralization, during single pesticide application and co-application of CLP. This FCS biomixture has been selected among several biomixtures due to its great efficiency to remove CFN (Chin-Pampillo et al., 2015a, 2015b). Additional toxicological assays yielded useful information on the feasible co-application of CFN and CLP into a single biomixture and the potential use of this matrix at environmental level.

1. Materials and methods

1.1. Chemicals and reagents

Commercial formulations of CFN (Furadan®48SC, 48%, W/V) and CLP (Solver®48EC, 48%, W/V) were acquired from a local store. Analytical standards CFN (2,2-dimethyl-2,3-dihydro-1-benzofuran-7-yl methylcarbamate, >99% purity), 3-hydroxycarbofuran (99.5%) and 3-ketocarbofuran (99.5%) were obtained from Chemservice (West Chester, Pennsylvania, USA). Radio-labeled CFN (^{14}C-CFN; [Ring-U-^{14}C]-Carbofuran; 2.89×10^9 Bq/g; radiochemical purity 100%; chemical purity 99.5%) and radio-labeled CLP (^{14}C-CLP; [Ring-2,6-$^{14}C_2$]-chlorpyrifos; 4.38×10^9 Bq/g; radiochemical purity 98.99%; chemical purity 98.34%) were obtained from Izotop (Institute of Isotopes Co., Budapest, Hungary). Carbendazim-d_4 (surrogate standard, 99.0%) and carbofuran-d_3 (internal standard, 99.5%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Potassium hydroxide analytical grade was purchased from Merck (Darmstadt, Germany). Ultima Gold cocktail for Liquid Scintillation Counting was purchased from Perkin Elmer (Waltham, Massachusetts, USA). Solvents and extraction chemicals are listed in Ruíz-Hidalgo et al. (2014).

1.2. Biomixture components and preparation

Clay loam soil (S) (sand 40%, silt 27%, clay 33%) was collected from the upper soil layer (0–20 cm) of an onion field with history of CFN application, in Tierra Blanca, Cartago, Costa Rica. Soil was air-dried and sieved through a 2-mm sieve. Coconut fiber (F) was acquired in a local market and garden compost (C) was collected from a composting station located at Universidad de Costa Rica and sieved as described for the soil after air-drying.

Biomixture was prepared by mixing coconut fiber, compost, and pre-exposed soil at a volumetric proportion of 50:25:25 and moistened to approximately 75% of maximum water-holding capacity (maximum water-holding capacity: 0.77 mL water/g biomixture). The biomixture was separated into three batches and treated as follows: FCS-fresh was used fresh prepared; FCS-pre was pre-exposed to CFN by spiking 25 mg/kg CFN and stored for 10 days (for CFN removal); and FCS-age was stored at 25°C during one month prior to use. In indicated experiments a biomixture pre-exposed and aged for five weeks was also employed (FCS-pre/age).

1.3. Experimental procedures

1.3.1. Degradation experiments

The degradation of CFN and the formation of its transformation products, 3-hydroxycarbofuran and 3-ketocarbofuran

were assayed by weighing 5 g of each biomixture (FCS-pre/age and FCS-age) into 50 mL polypropylene tubes. Each sample was spiked with commercial CFN (20 mg/kg), or a mixture of commercial CFN and CLP (20 mg/kg each) manually homogenized and incubated in the dark at (25 ± 1)°C during 8 days. The concentration of spiking was determined considering the recommended application indications for the formulations Furadan®48SC and Solver®48EC, the potential volume of the wastewaters applied in the biomixture, and the mass of the biomixture in a cylindrical container (200 L). The remaining CFN concentration was determined by sacrificing duplicate systems at times 0 hr, 2 days, 4 days and 8 days as described in Section 1.4.1.

1.3.2. Mineralization studies

The mineralization of ^{14}C-CFN and ^{14}C-CLP was determined through $^{14}CO_2$ production in biometric systems containing $^{14}CO_2$ traps with 10 mL KOH (0.1 mol/L). The biomixture (50 g), was weighed into the biometric flask and spiked with one of the following combinations: commercial CFN (20 mg/kg) and ^{14}C-CFN (3000 dpm/g); commercial CLP (20 mg/kg) and ^{14}C-CLP (3000 dpm/g); commercial CFN and CLP (20 mg/kg each) and ^{14}C-CFN (3000 dpm/g); commercial CFN and CLP (20 mg/kg each) and ^{14}C-CLP (3000 dpm/g). The systems were incubated in the dark at (25 ± 1)°C during 28 days. The KOH in the flasks was withdrawn at selected times and replaced with the same amount of fresh KOH. Activity of ^{14}C in the caught $^{14}CO_2$ produced from the respective mineralized pesticide was analyzed in the KOH samples as described in Section 1.4.2.

1.3.3. Toxicity experiments

Residual toxicity was determined in the FCS-age biomixture by adding either commercial CFN (20 mg/kg), commercial CLP (20 mg/kg) or a mixture of commercial CFN and CLP (20 mg/kg each) to unitary systems containing 80 g of the biomixture. The systems were incubated in the dark at (25 ± 1)°C during 8 days and then were used to perform the acute toxicity test with *D. magna* as described in Section 1.4.3. Biomixtures sacrificed right after spiking (time 0 hr) were used as a baseline to estimate the decrease in the toxicity.

1.4. Analytical procedures

1.4.1. Extraction and analysis of CFN and transformation products

Extraction was carried out as described in Ruíz-Hidalgo et al. (2014). Briefly, before extraction unitary samples (5 g) were spiked with carbendazim-d_4 as surrogate standard and 10 mL water were added. Extraction was performed adding 15 mL acetonitrile acidified with acetic acid (1%, V/V) and 1.55 g sodium acetate anhydrous, 6 g magnesium sulfate anhydrous and 1 g sodium chloride. Samples were shaken during 1 min and centrifuged for 7 min at 4500 r/min; the extracts were cleaned by transferring 3 mL of the supernatant to a 15 mL tube containing 900 mg magnesium sulfate anhydrous, 150 mg bondesil-PSA and 75 mg silica C18, followed by vortexing for 1 min and centrifuging for 7 min at 4500 r/min. Clean extract (1.5 mL) was concentrated to dryness with a nitrogen flow in a bath at 20–30°C; carbofuran-d_3 was added as internal standard and finally the extract was reconstituted in 1.5 mL water acidified with formic acid (0.1%, V/V), filtered (0.45 μm) and placed in HPLC vials. CFN and its transformation products were analyzed by LC–MS/MS using ultra high performance liquid chromatography (UPLC 1290 Infinity LC, Agilent Technologies, CA, USA) coupled to a triple quadrupole mass spectrometer (6460, Agilent Technologies, CA, USA). Chromatographic separation was done at 40°C by injecting 6 μL samples (2 μL loop) in a Poroshell 120 EC-C18 column (100 mm × 2.1 mm i.d., particle size 2.7 μm; Agilent Technologies, CA, USA), and using acidified water (formic acid 0.1% V/V, A) and acidified methanol (formic acid 0.1% V/V, B) as mobile phases. The mobile phase flow was 0.3 mL/min at the following conditions: 30% B for 3 min, followed by a 15 min linear gradient to 100% B, 4 min at 100% B and 0.1 min gradient back to 30% B, followed by 5 min at initial conditions. Selected transitions for the analytes are shown in Table 1. The mass spectrometer employed a jet stream (electrospray) ionization source operating at the following conditions: gas temperature 300°C; gas flow 7 L/min; nebulizer 45 psi; sheath gas temperature 250°C; sheath gas flow 11 L/min; capillary voltage 3500 V (for positive and negative); nozzle voltage 500 V (for positive and negative); heater MS1 and MS2 100°C. Recovery of the method was 91% for CFN, 98% for 3-hydroxycarbofuran and 95% for 3-ketocarbofuran. Limit of detection (LOD) and limit of quantification (LOQ) were 13 and 26 μg/kg for CFN and 3-ketocarbofuran; 16 and 32 μg/kg for 3-hydroxycarbofuran, relative to wet weight of biomixture.

1.4.2. Determination of $^{14}CO_2$ from mineralization assays

Scintillant liquid (10 mL) was added to 2 mL aliquots from the removed KOH solution samples and the ^{14}C activity from the trapped $^{14}CO_2$ was measured using a liquid scintillation counter (LS6000SC, Beckman Instruments Inc., USA). The total cumulative $^{14}CO_2$ activity evolved (mineralized) from the pesticides and the initially added activity of ^{14}C-CFN or ^{14}C-CLP was used to calculate the percentage of ^{14}C-pesticide mineralized. Pesticide mineralization was modeled according to a first order model to determine mineralization rate constants. Mineralization rates were compared by a covariance test performed in the Minitab 16® software (version 16.2.4, Minitab Inc., USA).

1.4.3. Toxicological analysis

An acute test based on *D. magna* immobilization was employed to estimate the residual toxicity in the biomixtures. The test was conducted following the U.S. EPA guidelines (EPA, 2002) and some modifications as described by Ruíz-Hidalgo et al. (2014), using elutriates from the biomixtures at time 0 hr (after pesticide spiking) and after 8 days of treatment, as analytical matrix. Elutriates were obtained according to the protocol EPA-823-B-01-002 (EPA, 2001). Briefly, distilled water (40 mL) were added to samples (10 g) and mechanically shaken for 1 hr followed by centrifugation for 10 min at 3500 r/min; the resulting supernatant was used as elutriate. Daphnids used were cultured in the Laboratory of Ecotoxicological Assays (CICA-University of Costa Rica) and fed with *Pseudokirchneriella subcapitata*. They were kept in moderately hard reconstituted water prepared and supplemented with B12 vitamin complex (EPA, 2002). Appropriate dilutions from elutriates were prepared using moderately hard reconstituted water without B12 vitamin complex. A number of 5 daphnid neonates of less than 24 hr

Table 1 – Selected transitions and other parameters in the detection of CFN and its transformation products using the DMRM method.

Compound	Retention time (min)	Transition	Fragmentor (V)	Collision energy (V)	Type of transition
3-Hydroxycarbofuran	3.60 ± 1.00	238 → 163	72	9	Q
		238 → 107		33	q
3-Ketocarbofuran	6.10 ± 1.00	236 → 161	82	17	Q
		236 → 179		9	q
Carbofuran	7.92 ± 1.00	222 → 165	82	9	Q
		222 → 123		21	q
Carbendazim-d_4	1.45 ± 1.00	196 → 164	102	17	Q
		196 → 136		34	q
Carbofuran-d_3	7.92 ± 1.00	225 → 165	86	9	Q
		225 → 123		21	q

Q: quantification transition; q: qualifier transition; CFN: carbofuran; DMRM: dynamic multiple reaction monitoring.

were placed in a 25 mL glass vial and then exposed to 10 mL of proper dilutions from the elutriates. The acute tests were performed in triplicates in darkness at (23 ± 1)°C. EC_{50}, the concentration producing 50% of immobilization in the daphnids, was determined using the binomial probability test on the TOXCALC — Toxicity Data Analysis Software (version 3.0, Tidepool Scientific Software, USA). Toxicity results were expressed as toxicity units (TU), calculated according to the expression: $TU = (EC_{50})^{-1} \times 100$.

2. Results and discussion

The capacity of CFN removal in a coconut fiber–compost–soil (FCS) biomixture under different preparation conditions, and during co-application of CLP was tested. The capability of this FCS biomixture (aged during one month) to rapidly eliminate CFN was previously demonstrated (Chin-Pampillo et al., 2015a), resulting in a low half-life of 2.5 days. The use of coconut fiber as an easily available lignocellulosic material, responds to the adaptation of biomixtures to different geographical regions (Urrutia et al., 2013). Similarly, compost emerges as a promising substitute for peat, as compost-based biomixtures have shown comparable or even better performance than the traditionally used peat-based biomixtures (Coppola et al., 2007, 2011; Vischetti et al., 2008; Chin-Pampillo et al., 2015a).

2.1. Degradation of CFN and transformation products

The degradation profiles of CFN in FCS-pre/age and FCS-age biomixtures are presented in Fig. 1a. Chemical hydrolysis of CFN may occur at basic pH values (Morales et al., 2012), however the FCS biomixture has a pH value of 6.7, low enough to assure the stability of CFN and to ascribe its removal to other processes, mostly microbial degradation. After 8 days biomixtures removed CFN from 19.8 and 17.2 mg/kg to 0.8 and 2.8 mg/kg, a reduction ranging from 95.6% to 83.7% for FCS-pre/age and FCS-age, respectively. During co-application of CLP, CFN removal was 89.5% (FCS-age) and 96.3% (FCS-pre/age). Despite showing similar final removal values, the degradation of CFN was more accelerated in the pre-exposed and aged biomixtures, compared to the aged matrix; in this respect FCS-pre/age showed an estimated half-life of 1.2 days, while this parameter was almost 4-fold higher in the FCS-age biomixture (4.7 days). This effect of pre-exposure, reported by the term "enhanced or accelerated biodegradation of pesticides" described in soils (Arbeli and Fuentes, 2007) and in particular for CFN (Felsot et al., 1981; Karpouzas et al., 1999), seems to occur also in the biomixture according to our results.

The degradation process did not produce accumulation of the metabolite 3-hydroxycarbofuran (Fig. 1b), as it completely disappeared with respect to the initial concentrations (64 to 74 μg/kg); on the contrary, the accumulation of 3-ketocarbofuran was slightly observed in the FCS-age but not in the FCS-pre/aged biomixture, which is also coincident with the enhanced biodegradation of carbofuran described in soils (Fig. 1c). Similar FCS biomixtures did not accumulate any of these transformation products (Chin-Pampillo et al., 2015a, 2015b), thus showing the same behavior as the FCS-pre/age matrix. Accumulation of 3-hydroxycarbofuran and 3-ketocarbofuran is highly undesirable, as they present similar toxicity features to CFN itself (Gupta, 1994); the chemical nature of these products makes them more hydrophilic than CFN, and therefore they can be more bioavailable to affect non-target organisms. Remarkably, the removal trend of CFN degradation and metabolite production during co-application of CLP showed no difference with respect to the application of CFN alone, as described below.

These results suggest that previous exposure of the biomixture to CFN (and not only pre-exposure of the soil employed in its preparation) improves the capacity of the biomixture to degrade this pesticide and reduces its persistence as well as the accumulation of some of the major toxic transformation products. It has been demonstrated that the aging process is an important issue affecting the capability of degradation of biomixtures (Castillo et al., 2008; Fernandez-Alberti et al., 2012; Tortella et al., 2012); in the present case the aging of the biomixture slowed the degrading process and at least temporarily, resulted in the accumulation of one major transformation product (3-ketocarbofuran).

The results exhibited an advantageous effect of the pre-exposure of the biomixture to CFN. This finding correlates to the enhancing effect in the capacity of degradation by pre-exposure reported in several works after successive applications of the pesticides metalaxyl, carbendazim and atrazine (Vischetti et al., 2008; Tortella et al., 2013a, 2013b). On the contrary, Fogg et al. (2003) described a decrease in the degradation rate after repeated applications of a pesticide mixture in a topsoil–compost–wheat straw biomixture, which

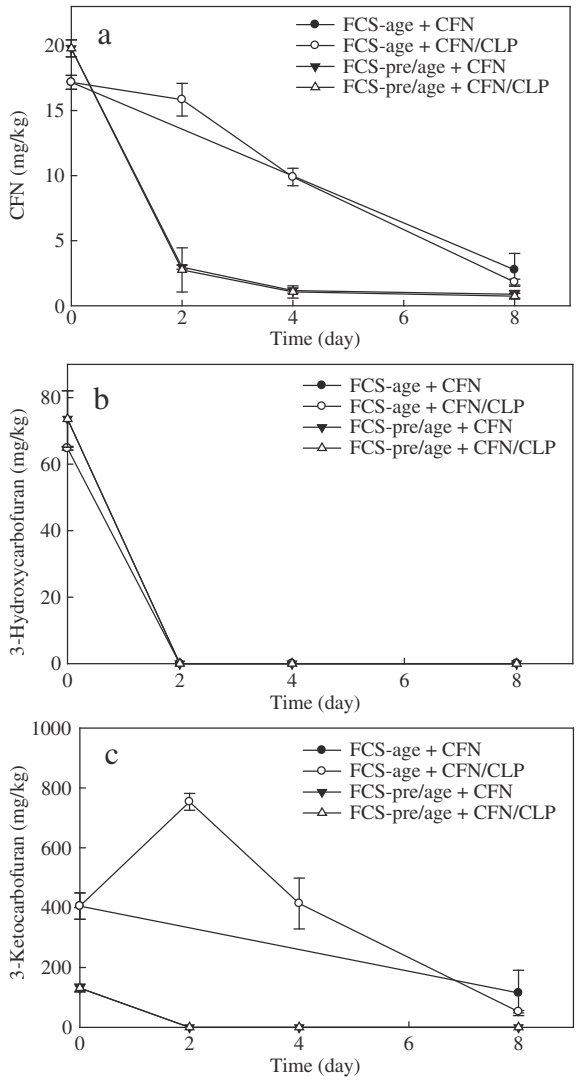


Fig. 1 – Removal profile of CFN in a coconut fiber–compost–soil biomixture (a) and accumulation of transformation products 3-hydroxycarbofuran (b) and 3-ketocarbofuran (c). Each value is the mean of duplicates with error bars representing the standard deviation of the mean. CFN: carbofuran.

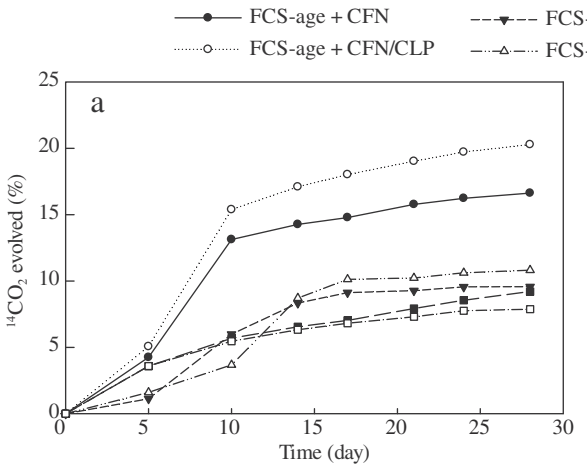
was tentatively ascribed to the toxicity of the pesticides over the microbial community.

The co-application of CLP did not reduce the capacity of the biomixture to remove CFN, which suggests that microbial activity related with CFN degradation in the biomixture was not affected by CLP or its transformation products, particularly TCP, widely described in soil and biomixture-like matrices (Racke et al., 1988; Coppola et al., 2007), and very likely produced in this case. Kadian et al. (2012) found an inhibitory effect of the application of CLP over the microbial activity in soil, however they found that the addition of organic amendments helped to enhance the microbial activity after several days. In addition, the antimicrobial properties of TCP have been reported (Feng et al., 1997; Singh et al., 2003) and its formation has been described in biomixtures regardless of their maturity (up to 30 days) (Tortella et al., 2012). In this context the co-application of other pesticides with CLP has resulted in increased inhibition of microbial communities in soil (Chu et al., 2008). Considering this condition, the results demonstrate that degradation of CFN in FCS during CLP co-application is apparently unaffected. In a similar approach, the degradation of CLP in soil was not altered in the presence of the pesticide chlorothalonil (Chu et al., 2008); on the contrary, Fogg et al. (2003) described slower degradation when several pesticides (isoproturon, pendimethalin, chlorpyrifos, chlorothalonil, epoxiconazole and dimethoate) were applied as a mixture than when applied alone in a biomixture. Overall results indicate that the FCS biomixture can be potentially employed for the simultaneous treatment of CFN and CLP containing wastewater in crop-fields where both pesticides are applied. Given the cross-degradation that occurs among carbamates (Racke and Coats, 1988; Morel-Chevillet et al., 1996), this finding suggests that a similar trend could take place in the co-application of CLP with other carbamates.

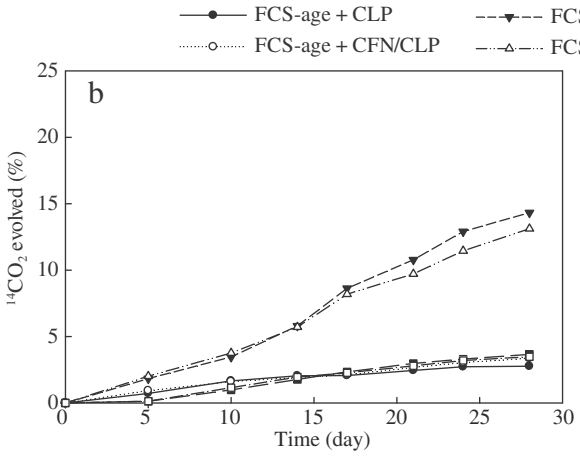
2.2. Mineralization of CFN and CLP

In order to estimate the complete oxidation of CFN to CO_2 and water, mineralization assays with ^{14}C-CFN and ^{14}C-CLP were performed, as shown in Fig. 2 (both pesticides were radiolabeled at the ring moiety). Regardless of CLP co-application, the FCS-fresh and FCS-pre showed similar behavior with respect to the mineralization of CFN (Fig. 2), achieving mineralization rate constants between 0.0027 and 0.0046 day^{-1}. Nonetheless, in FCS-age biomixtures significantly higher CFN mineralization was achieved ($p < 0.05$). Interestingly, in this case CFN mineralization rate was apparently higher (though not statistically significant; $p = 0.104$) during co-application of CLP (0.0081 day^{-1} and 21% mineralized in 28 days) than in single CFN application (0.0065 day^{-1} and 16% mineralized in 28 days). Mineralization of CLP is shown in Fig. 2b, where it can be observed that highest mineralization took place in the FCS-fresh biomixtures ($p < 0.05$), regardless of the presence of CFN (nearly 14% after 28 days and rate constants of 0.0059 and 0.0051 day^{-1}, during application of solely CLP and co-application, respectively). In the meantime, CLP mineralization in FCS-pre and FCS-age was rather low and similar ($p = 0.206$), slightly over 2% in 28 days and with k values ranging from 0.0015 to 0.0010 day^{-1}. The mineralization rate here achieved for CLP (~0.51% day^{-1} in FCS-fresh) was slightly higher than those obtained by Coppola et al. (2007) in biomixtures made with garden or urban compost and citrus peel or straw (between 0.11% day^{-1} and 0.40% day^{-1}, pH between 5.66 and 8.18; 20°C, 60% water holding capacity). Nonetheless, mineralization achieved in this work is lower than reported in several tropical soils (pH 4.8–5.6; 25°C, 22%–33% gravimetric water content) (Chai et al., 2013).

The results suggest that maturity of the biomixture enhances its capability to mineralize CFN and CFN co-applied with CLP, however pre-exposure to CFN seemed to inhibit that effect. This observation is interesting, considering that the removal of CFN is enhanced by pre-exposure, as it was described above. The faster removal but slower mineralization in pre-exposed biomixtures suggests that a metabolic pathway that does not take into account the ring breakdown in CFN (as evaluated in the mineralization assays) is taking place at a higher extent in these biomixtures. On the contrary, in the aged biomixtures it seems that ring breakdown occurs faster than in pre-exposed



Biomixture	Pesticides applied	Mineralization rate constant k (day^{-1})
FCS-fresh	CFN, ^{14}C-CFN	0.0038 ± 0.0008 a
FCS-fresh	CFN/CLP, ^{14}C-CFN	0.0046 ± 0.0007 a
FCS-age	CFN, ^{14}C-CFN	0.0065 ± 0.0012 b
FCS-age	CFN/CLP, ^{14}C-CFN	0.0081 ± 0.0014 b
FCS-pre	CFN, ^{14}C-CFN	0.0032 ± 0.0004 a
FCS-pre	CFN/CLP, ^{14}C-CFN	0.0027 ± 0.0004 a



Biomixture	Pesticides applied	Mineralization rate constant k (day^{-1})
FCS-fresh	CLP, ^{14}C-CLP	0.0059 ± 0.0002 a
FCS-fresh	CFN/CLP, ^{14}C-CLP	0.00514 ± 0.00007 a
FCS-age	CLP, ^{14}C-CLP	0.0010 ± 0.0001 b
FCS-age	CFN/CLP, ^{14}C-CLP	0.00118 ± 0.00006 b
FCS-pre	CLP, ^{14}C-CLP	0.00149 ± 0.00009 b
FCS-pre	CFN/CLP, ^{14}C-CLP	0.00140 ± 0.00009 b

Fig. 2 – Mineralization of ^{14}C-CFN (a) and ^{14}C-CLP (b) represented as percentage of $^{14}CO_2$ evolved from coconut fiber–compost–soil biomixtures. Each value is the mean of three replicates. The tables show the mineralization rate constants; values with the same letter are not statistically different ($p > 0.05$). CFN: carbofuran.

biomixtures (although other transformations in the CFN structure cannot be discarded in the former). A possible reason for this could be that a first exposure to CFN selectively affects microbial populations capable to breakdown the ring moiety, but not microorganisms that carry out the first transformation reactions at the carbamate moiety; this is partially expected considering the higher tolerance towards the xenobiotic shown by degrading microbiota; however this hypothesis should be studied further by molecular studies of microbial populations, and the determination of other potential transformation products or mineralization assays employing CFN radiolabeled at other positions such as in the carbamate ester functional group.

With respect to CLP, the highest mineralization capacity was observed in the fresh biomixtures ($p < 0.05$), showing that an aging process has a negative effect over the mineralization of this pesticide. CLP presents high adsorption affinity to organic matter (Racke et al., 1990; Kravvariti et al., 2010) and a high correlation between the sorption capacity of the pesticide and the lignin content was described by Rodríguez-Cruz et al. (2009). The relatively high lignin content in the FCS biomixture (around 12%) and the activation of sorption sites by the degradation of organic matter of biomixture during aging (Castillo et al., 2008) might support the sorption of CLP to the matrix and explain the observed trends. Increase in the sorption capacity could result in a decrease in the bioavailability of CLP and consequently in the ability of microorganisms to mineralize this pesticide.

2.3. Decrease in the toxicity on D. magna

Toxicity analyses were performed in elutriates from biomixtures in order to estimate the effects of potential lixiviates derived from the biomixtures on biota. This is an indicator of the environmental applicability of the biomixtures in the co-application of CFN and CLP. The results regarding to residual toxicity of the elutriates from aged biomixtures are shown in Table 2. The biomixture did not present intrinsic toxicity, resulting in 0 TU for blank (not spiked) biomixtures at times 0 hr and after 8 days. The toxicity of the biomixture immediately after application of pesticides (time 0 hr) was high, particularly in the co-application CFN/CLP compared to the toxicity of the pesticides applied separately (more than double toxicity, around 485 TU in the co-application), which suggests that

Table 2 – Toxicity of elutriates from biomixtures at times 0 hr and 8 days as determined with an acute test on *D. magna* after 24 and 48 hr of exposure.

Biomixture	Toxicity of the elutriate at time 0 hr (TU)		Toxicity of elutriate at time 8 days (TU)	
	24 hr	48 hr	24 hr	48 hr
Blank (FCS)	0	0	0	0
FCS-age + CFN	118 (64–128)[a]	240 (128–256)	0	0
FCS-age + CLP	79 (64–128)	181 (128–256)	1.1 (1.0–1.3)	3.6 (2.0–4.0)
FCS-age + CFN/CLP	217 (128–256)	485 (256–513)	1.8 (1.0–2.0)	2.7 (2.0–4.0)

TU: toxicity units; FCS: coconut fiber, compost, soil biomixture; CFN: carbofuran; CLP: chlorpyrifos.
[a] The 95% confidence limits are shown in parentheses.

co-application enhances the toxic effect of these pesticides on the daphnids. After 8 days of treatment the acute toxicity on *D. magna* decreased in every case, reaching complete elimination during the treatment of solely CFN, and almost completely for CLP (97.1% decrease) and the mixture CFN/CLP (99.4% decrease). As mentioned above, in the case of CLP (and at a lesser extent for CFN) part of the detoxification could be ascribed to sorption in the matrix which reduces its bioavailability and therefore its extraction during elutriate preparation; nonetheless, sorption capacity is a desirable feature in biomixtures (Karanasios et al., 2012), as it reduces the production of pesticide-rich lixiviates, and favors the retention of more recalcitrant pesticides that require longer biodegradation treatments.

It is important to note that residual toxicity of the biomixture significantly decreased or was totally eliminated in individual application of both pesticides or in co-application, despite differences in the removal of CFN and mineralization of CFN and CLP. It is clear that a complete toxicity evaluation would require a more extensive use of biomarkers from other trophic levels; nonetheless, these findings demonstrate at least an apparent effectiveness of the FCS biomixture and its potential use in BPS to prevent the environmental impact of these pesticides on aquatic life.

3. Conclusions

The FCS biomixture is a suitable matrix for the removal of pesticides. Despite being design for CFN elimination, this biomixture was also capable to mineralize CLP alone and in co-application CFN/CLP. As co-application of CLP does not inhibit CFN elimination, the biomixture could be employed in fields where both pesticides are applied. Pre-exposure and aging of the biomixture accelerated the CFN removal and elimination of transformation products with respect to only aged biomixtures; however, in terms of mineralization, aging showed a better performance, maybe due to the inhibition by pre-exposure of some microbial populations in charge of metabolizing intermediate products from the pesticides. Results could be an indicator of the efficiency of the biomixture for other combinations of pesticides. The marked reduction in the acute residual toxicity of the matrix over *D. magna*, even in co-application of CFN/CLP, supports the environmental friendliness of the biomixture to be used in BPS.

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REFERENCES

Adhikari, S., Sarkar, B., Chatterjee, A., Mahapatra, C., Ayyappan, S., 2004. Effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recovery in a freshwater teleost, *Labeo rohita* (Hamilton). Ecotoxicol. Environ. Saf. 58, 220–226.

Arbeli, Z., Fuentes, C.L., 2007. Accelerated biodegradation of pesticides: An overview of the phenomenon, its basis and possible solutions; and a discussion on the tropical dimension. Crop. Prot. 26, 1733–1746.

Asgher, M., Bhati, H.N., Ashraf, M., Legge, R.L., 2008. Recent developments in biodegradation of industrial pollutants by white rot fungi and their enzyme system. Biodegradation 19, 771–783.

Castillo, M.P., Torstensson, L., Stenström, J., 2008. Biobeds for environmental protection from pesticide use — A review. J. Agric. Food Chem. 56, 6206–6219.

Chai, L.K., Wong, M.H., Bruun Hansen, H.C., 2013. Degradation of chlorpyrifos in humid tropical soils. J. Environ. Manag. 125, 28–32.

Chin-Pampillo, J.S., Ruiz-Hidalgo, K., Masís-Mora, M., Carazo-Rojas, E., Rodríguez-Rodríguez, C.E., 2015a. Adaptation of biomixtures for carbofuran degradation in on-farm biopurification systems in tropical regions. Environ. Sci. Pollut. Res. 22, 9839–9848.

Chin-Pampillo, J.S., Ruiz-Hidalgo, K., Masís-Mora, M., Carazo, R.E., Rodríguez-Rodríguez, C.E., 2015b. Design of an optimized biomixture for the degradation of carbofuran based on pesticide removal and toxicity reduction of the matrix. Environ. Sci. Pollut. Res. 22, 19184–19193.

Chu, X., Fang, H., Pan, X., Wang, X., Shan, M., Feng, B., et al., 2008. Degradation of chlorpyrifos alone and in combination with chlorothalonil and their effects on soil microbial populations. J. Environ. Sci. 20, 464–469.

Coppola, L., Castillo, M.d.P., Monaci, E., Vischetti, C., 2007. Adaptation of the biobed composition for chlorpyrifos degradation to Southern Europe conditions. J. Agric. Food Chem. 55, 396–401.

Coppola, L., Castillo, M.P., Vischetti, C., 2011. Degradation of isoproturon and bentazone in peat- and compost-based biomixtures. Pest Manag. Sci. 67, 107–113.
Eaton, D.L., Daroff, R.B., Autrup, H., Bridges, J., Buffler, P., Costa, L.G., et al., 2008. Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. Crit. Rev. Toxicol. S2, 1–125.
EPA, 2001. EPA-823-B-01-002. Methods for collection, storage and manipulation of sediments for chemical and toxicological analyses. Technical Manual Office of Water, Washington, DC (4305).
EPA, 2002. EPA-821-R-02-012. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Office of Water, Washington, DC (4303 T).
Felsot, A.S., Maddox, J.V., Bruce, W., 1981. Enhanced microbial degradation of carbofuran in soils with histories of furadan use. Bull. Environ. Contam. Toxicol. 26, 781–788.
Feng, Y.G., Racke, K.D., Bollag, J., 1997. Isolation and characterization of a chlorinated-pyridinol-degrading bacterium. Appl. Environ. Microbiol. 63, 4096–4098.
Fernandez-Alberti, S., Rubilar, O., Tortella, G.R., Diez, M.C., 2012. Chlorprifos degradation in a biomix: Effect of pre-incubation and water holding capacity. Soil Sci. Plant Nutr. 12, 785–799.
Fogg, P., Boxall, A.B.A., Walker, A., Jukes, A.A., 2003. Pesticide degradation in a "biobed" composting substrate. Pest Manag. Sci. 59, 527–537.
Gupta, R.C., 1994. Carbofuran toxicity. J. Toxic. Environ. Health A 43, 383–418.
Kadian, N., Malik, A., Satya, S., Dureja, P., 2012. Effect of organic amendments on microbial activity in chlorpyrifos contaminated soil. J. Environ. Manag. 95, S199–S202.
Karanasios, E., Tsiropoulos, N.G., Karpouzas, D.G., Menkissoglu-Spiroudi, U., 2010. Novel biomixtures based on local Mediterranean lignocellulosic materials: Evaluation for use in biobed systems. Chemosphere 80, 914–921.
Karanasios, E., Tsiropoulos, N.G., Karpouzas, D.G., 2012. On-farm biopurification systems for the depuration of pesticide wastewaters: Recent biotechnological advances and future perspectives. Biodegradation 23, 787–802.
Karpouzas, D.G., Walker, A., Froud-Williams, R.J., Drennan, D.S.H., 1999. Evidence for the enhanced biodegradation of ethoprophos and carbofuran in soils from Greece and the UK. Pestic. Sci. 55, 301–311.
Kravvariti, K., Tsiropoulos, N.G., Karpouzas, D.G., 2010. Degradation and adsorption of terbuthylazine and chlorpyrifos in biobed biomixtures from composted cotton crop residues. Pest Manag. Sci. 66, 1122–1128.
Morales, J., Manso, J.A., Cid, A., Mejuto, J.C., 2012. Degradation of carbofuran and carbofuran-derivatives in presence of humic substances under basic conditions. Chemosphere 89, 1267–1271.
Morel-Chevillet, C., Parekh, N.S., Pautrel, D., Fourier, J., 1996. Cross enhancement of carbofuran biodegradation in soil samples previously treated with carbamate pesticides. Soil Biol. Biochem. 28, 1767–1776.
Racke, K.D., 1993. Environmental fate of chlorpyrifos. Rev. Environ. Contam. Toxicol. 131, 1–150.
Racke, K.D., Coats, J.R., 1988. Enhanced degradation and comparative fate of carbamate insecticides in soil. J. Agric. Food Chem. 36, 193–199.
Racke, K.D., Coats, J.R., Titus, K.P., 1988. Degradation of chlorpyrifos and its hydrolysis product 3,5,6-trichloro-2-pyridinol, in soil. J. Environ. Sci. Health B 23, 527–539.
Racke, K.D., Laskowski, D.A., Schultz, M.R., 1990. Resistance of chlorpyrifos to enhanced biodegradation in soil. J. Agric. Food Chem. 38, 1430–1436.
Racke, K.D., Steele, K.P., Yoder, R.N., Dick, W.A., Avidov, E., 1996. Factors affecting the hydrolytic degradation of chlorpyirifos in soil. J. Agric. Food Chem. 44, 1582–1592.
Richardson, R.J., 1995. Assessment of the neurotoxic potential of chlorpyrifos relative to other organophosphorus compounds: A critical review of the literature. J. Toxicol. Environ. Health 44, 135–165.
Rodríguez-Cruz, M.S., Valderrabano, M., del Hoyo, C., Sánchez-Martín, M.J., 2009. Physicochemical study of the sorption of pesticides by wood components. J. Environ. Qual. 38, 719–728.
Ruíz-Hidalgo, K., Chin-Pampillo, J.S., Masís-Mora, M., Carazo, E., Rodríguez-Rodríguez, C.E., 2014. Degradation of carbofuran by *Trametes versicolor* in rice husk as a potential lignocellulosic substrate for biomixtures: From mineralization to toxicity reduction. Process Biochem. 49, 2266–2271.
Singh, B.K., Walker, A., Morgan, J.A.W., Wright, D.J., 2003. Effects of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos-degrading bacterium. Appl. Environ. Microbiol. 69, 5198–5206.
Sniegowski, K., Bers, K., Ryckeboer, J., Jaeken, P., Spanohe, P., Springael, D., 2012. Minimal pesticide-primed soil inoculum density to secure maximum pesticide degradation efficiency in on-farm biopurification systems. Chemosphere 88, 1114–1118.
Tortella, G.R., Rubilar, O., Castillo, M.d.P., Cea, M., Mella-Herrera, R., Diez, M.C., 2012. Chlorpyrifos degradation in a biomixture of biobed at different maturity stages. Chemosphere 88, 224–228.
Tortella, G.R., Mella-Herrera, R.A., Sousa, D.Z., Rubilar, O., Acuña, J.J., Briceño, G., et al., 2013a. Atrazine dissipation and its impact on the microbial communities and community level physiological profiles in a microcosm simulating the biomixture of on-farm biopurification system. J. Hazard. Mater. 260, 459–467.
Tortella, G.R., Mella-Herrera, R.A., Sousa, D.Z., Rubilar, O., Briceño, G., Parra, L., et al., 2013b. Carbendazim dissipation in the biomixture of on-farm biopurification systems and its effect on microbial communities. Chemosphere 93, 1084–1093.
University of Hertfordshire, 2013. The Pesticide Properties DataBase (PPDB) developed by the Agriculture & Environment Research Unit (AERU). University of Hertfordshire, Hatfield, UK.
Urrutia, C., Rubilar, O., Tortella, G.R., Diez, M.C., 2013. Degradation of pesticide mixture on modified matrix of a biopurification system with alternatives lignocellulosic wastes. Chemosphere 92, 1361–1366.
van Wijngaarden, R., Leeuwangh, P., Lucassen, W.G.H., Romijn, K., Ronday, R., van der Velde, R., et al., 1993. Acute toxicity of chlorpyrifos to fish, newt, and aquatic invertebrates. Bull. Environ. Contam. Toxicol. 51, 716–723.
Vischetti, C., Monaci, E., Cardinalli, A., Casucci, C., Perucci, P., 2008. The effect of initial concentration, co-application and repeated applications on pesticides degradation in a biobed mixture. Chemosphere 72, 1739–1743.