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Exploring the potential of biobeds for the depuration of pesticide-contaminated wastewaters from the citrus production chain: Laboratory, column and field studies

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ABSTRACT

The high wastewater volumes produced during citrus production at pre- and post-harvest level presents serious pesticide point-source pollution for groundwater bodies. Biobeds are used for preventing such point-source pollution occurring at farm level. We explored the potential of biobeds for the depuration of wastewaters produced through the citrus production chain following a lab-to-field experimentation. The dissipation of pesticides used pre- or post-harvest was studied in compost-based biomixtures, soil, and a straw-soil mixture. A biomixture of composted grape seeds and skins (GSS-1) showed the highest dissipation capacity. In subsequent column studies, GSS-1 restricted pesticides leaching even at the highest water load (462 L m^{-3}). *Ortho*-phenylphenol was the most mobile compound. Studies in an on-farm biobed filled with GSS-1 showed that pesticides were fully retained and partially or fully dissipated. Overall biobeds could be a valuable solution for the depuration of wastewaters produced at pre- and post-harvest level by citrus fruit industries.

1. Introduction

Citrus production constitutes one of the largest agro-industries in the Mediterranean region. It involves the application of high pesticide loads at both pre- and post-harvest level. The high frequency of spraying events performed on citrus orchards to prevent infestations by pests and diseases increases the risk for point source contamination due to accidental spillages during spraying preparation or *via* environmental release of spraying leftovers or rinsates (De Wilde et al., 2010a). Biobeds have been effectively used since 1993 for the depuration of such wastewaters produced by inappropriate on-farm activities (Torstensson and Castillo, 1997; De Wilde et al., 2007).

Moreover, at postharvest level, citrus fruits are subjected to fungicide treatments which lead to the production of large volumes of wastewaters containing high concentrations of the fungicides thiabendazole (TBZ), imazalil (IMZ) and *ortho*-phenylphenol – Na (SOPP). These wastewaters constitute a serious point source for the contamination of natural water resources. Previous monitoring studies reported the frequent detection of TBZ and IMZ in surface water systems of Costa Rica which was attributed to the presence of fruit packaging plants adjacent to the river systems monitored (Castillo et al., 2000). The risk for point source contamination by the postharvest activities of the citrus production industry has been identified by the European Commission (EC) which has given authorization to these fungicides under the clause *that appropriate waste management practices to handle the waste solution remaining after application, including for instance the cleaning water of the drenching system and the discharge of the processing waste are put in place* (EC, 2001, 2010). Despite that, the only depuration system currently available is based on pesticide adsorption onto granular activated carbon (Garcia Portillo et al., 2004). Although this system achieved 7000 times reduction in TBZ concentrations its cost is prohibitive (EC, 2000). Biobeds might offer an integrated solution for the depuration of both on-farm and postharvest wastewaters produced during citrus production. However, primary adaptations of their water management routines and biomixture content are necessary to optimize their performance.

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The composition of the biobeds filtering substrate has been identified as a key factor controlling their depuration efficiency (Castillo and Torstensson, 2007; De Wilde et al., 2007). It is usually a mixture of soil, lignocellulosic materials and humified organic substrates at variable volumetric ratios (Castillo et al., 2008). Different lignocellulosic materials have been utilized with straw being the most popular (Fogg et al., 2003; Castillo and Torstensson, 2007), while grape stalks or cotton crop residues were equally effective in the degradation of pesticides (Karanasios et al., 2010b). Humified substrates like peat have been extensively used in Swedish biobeds, but its use in south Europe was precluded due to low availability, high cost and most importantly due to its limited sustainability as a renewable substrate. Previous studies have demonstrated the degradation superiority of composts over peat in biobed substrates (Coppola et al., 2011; Karanasios et al., 2010a).

Water loading has been identified as the other most important factor controlling the depuration performance of on farm biobeds. Indeed, Fogg et al. (2004a) demonstrated, via a series of leaching column studies, the importance of water loading on biobeds performance. More recent microcosm (De Wilde et al., 2010a) and macrocosm studies (De Wilde et al., 2010b) showed that water flux strongly affected the retention capacity of peat-based biomixtures and suggested that an average water load of 12.5 L m^{-3} of biomixture could be sufficient for effective retention of pesticides by biofilters in Belgium. Similar data are not available in south Europe where the entirely different climatic conditions (low precipitation, particularly warm summer conditions, high surface evaporation) and the reliance on compost-based biomixtures with lower water holding capacity compared to peat would require different water management regimes. Indeed, previous column and full-scale studies employed in south Europe with compost-based biomixtures although showed good retention and depuration capacity they did not address the influence of water load on biobeds performance (Vischetti et al., 2004; Fait et al., 2007). The frequent spraying events in citrus orchards combined with the postharvest-treatment of citrus fruits results in the year long production of large wastewater volumes whose storage and subsequent disposal should be controlled to maintain optimum water conditions within the biobeds profile and secure high depuration efficiency.

Although laboratory and semi-field studies provide an indication of the depuration potential of a particular biobed system, full-scale evaluation is an essential step before implementation. Therefore the main aim of this study was to develop and evaluate biobeds for their ability to depurate pesticide-containing wastewaters produced during the entire citrus production process. The impact of biomixture composition and water loading on biobed performance were assessed at laboratory and bench scale conditions respectively. Their results were utilized for the optimization of the performance of a full-scale offset biobed system, but also for the interpretation of these performance results.

2. Experimental section

2.1. Chemicals

Analytical standards of chlorpyrifos (CHL), cypermethrin (CYP), deltamethrin (DEL), imazalil (IMZ) and thiabendazole (TBZ) were purchased from Dr Ehrenstofer GmbH (>99% purity), while *ortho*-phenylphenol sodium salt (SOPP, 99%) was obtained from Sigma–Aldrich. For pesticide residue analysis, stock solutions (1000 mg L^{-1}) from the above analytical standards were prepared in acetone.

2.2. Biomixtures preparation

Agricultural composts instead of peat were used in the biomixtures tested. Composts derived from olive tree prunings (OTP), grape vine prunings (GVP), grape marc (GM) and winery by-products (seeds and skins) (GSS) were prepared in the composting facilities of the Agricultural Research Institute of Cyprus. All these materials were selected as they are cheap and readily available in the agricultural sector in the Mediterranean region. Pruning type materials (OTP and GVP) were shredded and then composted in 1 m^3 bins for four months. Grape marc and winery by-products (seeds and skins) were collected from a local winery; air dried for three days, shredded (grape marc) and composted as described above. Upon completion of composting, the composts were air dried, passed through a 4-mm mesh sieve and stored until use in the dark at room temperature.

All biomixtures tested were prepared by mixing topsoil, compost and straw at a volumetric ratio of 1:1:2 with the exception of GSS-2 which consisted of topsoil, GSS and straw at a volumetric ratio of 1:2:1. The detailed composition and physicochemical properties of the substrates studied are shown in Table 1.

2.3. Laboratory dissipation study

Commercial formulations of the insecticides CHL (Cyren 48EC), DEL (Decis 25EC) and CYP (Cypermethrin 25EC) and of the fungicides IMZ (Magnate, 50EC), TBZ (Tecto 500SC) and SOPP (Alrikvon, 20EC) were used for the preparation of aqueous pesticide solutions (Table 2). Their dissipation was studied in groups based on their use: a) field-applied insecticides (CHL, DEL and CYP); b) post-harvest applied fungicides (SOPP, IMZ and TBZ) considering that the pesticides of each group will be almost concurrently disposed off in the biobeds at different periods of the year. An aqueous mixed stock solution (150 mg L^{-1}) was prepared for each pesticide group.

For the determination of pesticide dissipation, two bulk samples (600 g) from each of the six biomixtures tested were prepared. Two soil bulk samples were also included in the study for comparison purposes. Each of the bulk samples was split into 21 sub-samples (25 g) which were placed into 250 ml conical flasks. The first set of sub-samples received an appropriate volume of the fungicides (SOPP, IMZ and TBZ) aqueous stock solution to give a final concentration of 35 mg kg^{-1} substrate, while the second set of sub-samples received an appropriate volume of the insecticides (CHL, DEL and CYP) stock solution to give a final concentration of 20 mg kg^{-1} substrate. After pesticide application the moisture content of the biomixtures was adjusted to 60% of their water holding capacity and the samples were incubated in the dark at 25 °C for 100 d. Immediately after application (0 d) and 5, 10, 25, 45, 70 and 100 days later three subsamples from each different biomixture were removed from the incubator and stored at −20 °C until extraction and analysis.

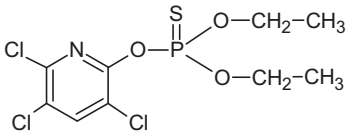
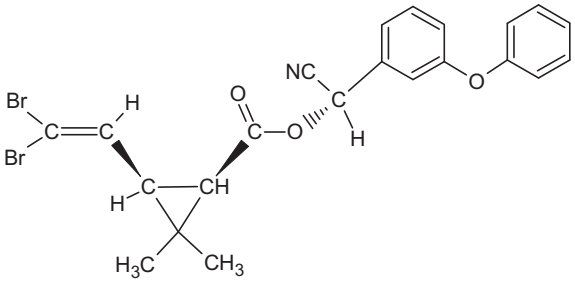
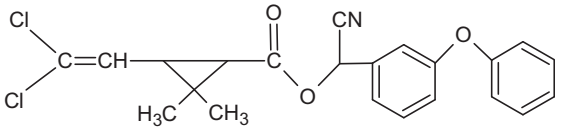
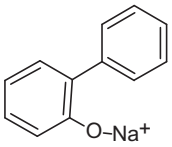
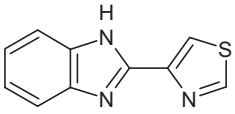
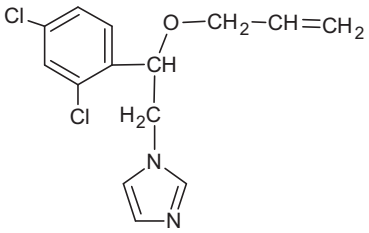
2.4. Microbial respiration

The microbial respiration in the different biomixtures was determined by measuring CO_2–C evolution at 1, 2, 4, 7, 10 and 14 days after application (DAA) using the alkali trap method as described by Ntougias et al. (2006). Microbial respiration was determined both in pesticide-treated and corresponding untreated controls.

Table 1
The composition and the physicochemical properties of the substrates tested.

Biomixture	% Content by volume			Physicochemical Properties								Relative bulk density (g mL^{-3})
	Soil	Compost	Straw	pH	Total C (%)	Total N (%)	C/N ratio	K exchangeable (mg kg^{-1})	P-Olsen (mg kg^{-1})	Total S (%)	Total Ca (%)	
OTP	25	25	50	6.8	5.29	0.61	8.67	459	136	0.23	1.26	0.24
GVP	25	25	50	7.2	4.95	0.65	7.61	376	117	0.25	1.15	0.15
GM	25	25	50	6.2	5.75	0.72	7.98	980	191	0.14	1.14	0.60
GSS-1	25	25	50	7.1	6.83	0.74	9.22	546	91	0.11	1.29	0.58
GSS-2	25	50	25	6.8	16.54	1.30	12.72	1989	324	0.15	1.17	0.58
SS	75	–	25	6.4	10.19	0.27	37.74	242	109	0.08	1.32	–
S	100	–	–	7.9	0.65	0.054	12.03	123	18	0.02	1.11	–

Table 2
Chemical formulae and selected chemical and environmental fate parameters (Koc and water solubility) of the pesticides studied.

Active ingredient	Chemical formula	Koc[a] (L kg^{-1})	Water solubility (mg L^{-1})
Chlorpyrifos (CHL)		8151	1.1
Deltamethrin (DEL)		10,240,000	0.0002
Cypermethrin (CYP)		156,250	0.009
Ortho-phenylphenol − Na (SOPP)		347	15,000
Thiabendazole (TBZ)		7344	30
Imazalil (IMZ)		4753	184

[a] Data obtained from the Pesticide Properties Database (PPDB) run by the University of Hertfordshire in collaboration with other partners.

2.5. Leaching column study

A leaching column study was conducted to evaluate the impact of water loadings on the capacity of biomixtures to retain pesticides. Eighteen leaching columns (PVC tubing, i.d. 12 cm × 110 cm length) were prepared. A 2 cm zone at the bottom of each column was filled with washed gravel (25 mm diameter). The first nine columns were filled with GSS-1 whereas the remaining nine columns were filled with a soil/perlite mixture (25:75 by volume). The latter was included as a 'control' treatment where straw and compost contained in GSS-1 was replaced by an inert material (perlite). The base of each column was connected to a 5 L glass beaker *via* a glass funnel. The amounts of fungicides applied were estimated based on their average amounts contained in the wastewaters produced by post-harvest treatments (16 kg SOPP, 7 kg IMZ and 7 kg TBZ/season for a medium size plant). According to the current practice, citrus industry wastes are disposed in a 1000 m^2 of uncultivated land, resulting to soil concentrations of *ca.* 50, 20 and 20 mg kg^{-1} for SOPP, TBZ and IMZ, respectively. Thus aqueous solution of fungicides was applied at the top of each column resulting in final loads of 250, 100 and 100 mg for SOPP, IMZ and TBZ respectively. Regarding insecticides, their applied amounts, were estimated based on a scenario assuming a 1-ha citrus orchard sprayed twice (500 L spraying tank) with the selected pesticides and 2 × 50 L of spraying remnants are disposed in a biobed. These spraying remnants contain in total 75 g of CHL, 25 g of DEL and 25 g of CYP. In order to test a worst case scenario the pesticide amounts applied in the columns were doubled considering that other sources (spillage of formulated pesticide; external sprayer washing) might add to the total pesticide amount ending in the biobed. Thus, each column received 144, 90 and 90 mg of CHL, DEL and CYP respectively.

Three different watering schemes were tested: low (L, 161 L m^{-3} equivalent to a volume of 1.93 m^3 in a 12 m^2 and 1 m deep biobed), medium (M, 242 L m^{-3} equivalent to a volume of 2.90 m^3 in biobed) and high water load (H, 463 L m^{-3} equivalent to a volume of 5.6 m^3 in biobed) representing disposal of wastewaters produced from respective small, medium and large citrus production enterprises. In accordance, the first three columns from each substrate (biomixture vs soil/perlite) were irrigated at 2, 6, 12, 24 and 48 DAA resulting in a total water load of 463 L m^{-3} (H). The second set of columns was irrigated at 2, 12, 24 and 48 DAA corresponding to a total water load of 242 L m^{-3} (M). The final set of columns was irrigated at 2, 24 and 48 DAA corresponding to a total water load of 161 L m^{-3} (L). Leachate samples were collected after each irrigation event and pesticides residues were monitored as described below.

2.6. Construction and evaluation of full-scale biobed system

The performance of the most effective biomixture and the high water loading scenario (worst case) as determined in the previous experiments were further evaluated on a full scale offset type biobed. This was constructed as described before (ADAS, 2006) at the experimental station of the Agricultural Research Institute of Cyprus at Zygi (Fig. 1). This design was selected since it is the most appropriate for handling large wastewater volumes (Castillo et al., 2008), which are produced during citrus production and processing throughout the year. Initially, a 12 $m^2 \times 1.2$ m deep pit was excavated and its bottom was covered with a waterproof soil membrane. A 10-cm layer of gravel (10 cm deep) was placed on top of the membrane and a drainage tube was installed at the lowest end of the pit to collect the draining water from the bottom of the biobed. A 5 cm layer of sand was placed on top of the gravel and the drainage tube, and the pit was subsequently filled with GSS-1. A bunded (100 mm high) concrete pesticide handling area (20 m^2) on the side of the biobed was constructed with a slight slope (1%) toward a side drain where the wastewaters are collected temporarily before being pumped into a 5 m^3 storage tank. Wastewaters are then applied onto the biobed surface via drip irrigation.

The performance of the on-farm biobed was assessed after an artificial application of the insecticides and fungicides used before. Appropriate volumes of pesticides formulation were dissolved in 500 L of water and applied on the biobed via drip irrigation (flow rate of 3.6 L h^{-1}). In total, the biomixture received 15 g CHL, 5 g DEL, 5 g CYP, 75 g SOPP, 25 g IMZ and 25 mg TBZ. The distribution of pesticide residues in the biobed body and their concentration in the leachate were monitored at 21, 42, 70 and 130 DAA. Biomixture samples from the body of the biobed were collected using a 60 cm long soil core sampler. Five samples were randomly collected from the whole surface of the biobed at each sampling date. After sampling, cores were removed and divided into four sections (0–5, 5–20, 20–40 and 40–60 cm). The core samples derived from each horizon level were pooled together, and three sub-samples were further analyzed. In contrast to biobed systems in northern Europe, where high precipitation might saturate them, extra irrigation with 150 L was performed twice a week to maintain moisture during the warm summer season to 60–70% of the water holding capacity. In total, 6.07 m^3 of water was applied on the biobed surface (equivalent to 505.8 mm precipitation) during the study period. This slightly exceeds the H water scenario of the column study (463 mm plus an assumed average precipitation of 42 mm resulting in 505 mm or 6.03 m^3 for the whole biobed).

2.7. Pesticide residue analysis

Biomixture samples (25 g) were extracted with 50 ml of an ethyl acetate: acetone mixture (70:30 v:v) in a horizontal shaker for 90 min at 200 rpm. The extract was passed through a Whatman 42 filter paper and the filtrate was concentrated to dryness under vacuum. Pesticide residues were re-dissolved in 5 ml acetone and stored at −20 °C until analysis. Leachate samples (50 ml) were extracted three times with 50 ml of dichloromethane for 1 h on a horizontal shaker at 100 rpm. The organic phases from the three extraction steps were combined and were dried over anhydrous sodium sulfate. The extract was evaporated to dryness using rotary evaporator at 40 °C, pesticide residues were re-dissolved in 5 ml acetone and stored at −20 °C until analysis.

The residue levels of all studied pesticides in the biomixture and leachate samples were determined via GC-ECD analyses performed on a fused silica capillary column Restec RTX − 5 (30.0 m × 0,32 mm id × 1.00 μm film thickness). Nitrogen was used as a carrier and a makeup gas at 3.3 and 60 mL min^{-1} respectively. The injector and detector were operated at 250 °C and 325 °C, respectively. The oven temperature was initially set to 80 °C for 2 min, raised to 200 °C (20 °C min^{-1}) where it was held for 8 min, and then raised to 285 °C (10 °C min^{-1}) where it was held for 14 min. The presence of pesticides was confirmed in a GC-MS (Agilent 5890, MSD) equipped with an HP-5MS (30.0 m × 0.25 mm id × 0.25 μm film thickness) column. He was used as the carrier gas at 1.0 mL min^{-1}. The injector and detector were operated at 250 and 280 °C, respectively, while the oven temperature program was as described for GC-ECD. Satisfactory recoveries were obtained for all substrate − pesticide combinations (>80%) with relative standard deviation never exceeding 20%.

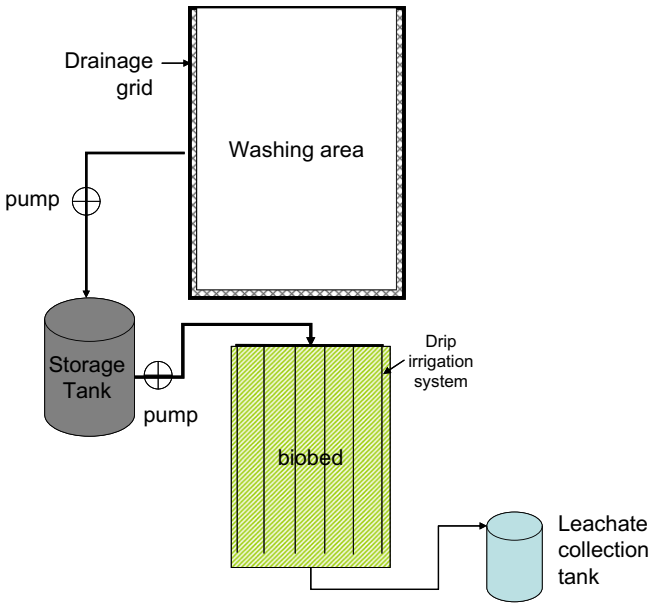


Fig. 1. A schematic representation of the offset type full scale biobed system evaluated in the study.

2.8. Data analysis

Pesticide dissipation data were described by first order kinetics (FOK) or by the hockey-stick model and half-lives ($t_{1/2}$) were estimated accordingly. In all cases, the FOCUS workgroup guidance was followed for the selection of the most appropriate kinetic model (FOCUS, 2006). The χ^2 test was used to evaluate the quality of the fit of each model to the dissipation data. Parameters of the kinetics models as well as the correlation coefficient (R^2) were estimated by least-squares regression using StatSoft, Inc. (2004), STATISTICA version 7 (www.statsoft.com). Correlations between pesticide dissipation rate and microbial respiration of the biomixtures were determined by the Pearson's correlation coefficient (r).

3. Results and discussion

3.1. Laboratory dissipation studies

The dissipation of all pesticides in the different biomixtures was adequately described by FOK with the only exception of IMZ whose dissipation was biphasic and was adequately described only by the hockey-stick model as dictated by the high r^2 and the low χ^2 (<15%) values (Table 3). The biphasic nature of IMZ dissipation has been also reported in previous regulatory studies (EFSA, 2010).

Pesticide dissipation rates varied greatly among the different substrates tested although some clear trends could be identified. Firstly, the most persistent compound was the fungicide TBZ with $t_{1/2}$ values ranging from 26 d in the GSS-1 and GSS-2 to 77.8 and 89.5 d in soil and SS respectively (Table 3). This is in line with the long persistence of TBZ in laboratory dissipation studies (EC, 2001). Secondly, the dissipation of all pesticides was substantially faster in biomixtures compared to soil and SS mixture (Table 3). The only exception was IMZ which dissipated faster in soil and SS compared to the majority of biomixtures. This is in line with previous reports showing the superiority of biomixtures over soil regarding pesticide dissipation (Fogg et al., 2003; Karanasios et al., 2012). The positive effect of compost on the dissipation of pesticides in the biomixtures compared to SS could be attributed to the increasing contribution of compost-derived metabolically active microorganisms. This could be particularly true for the organophosphate CHL (Singh and Walker, 2006) and the pyrethroids DEL and CYP (Maloney et al., 1988; Grant et al., 2002) which are prone to microbial hydrolysis of their ester bond. The involvement of microbes in the faster degradation of insecticides in biomixtures is further supported by the significant correlation observed between microbial respiration (Supplementary data 1) and their dissipation rates in all compost-biomixtures (Table 4). Similarly, Castillo and Torstensson (2007) found a positive correlation between pesticide dissipation and microbial respiration in peat-biomixtures. All the above suggest that compost provides the appropriate genetic pool for enhancing pesticide dissipation.

Among the biomixtures tested, GSS-1 was the most effective in the dissipation of all pesticides tested except IMZ which was degraded at faster rates in SS compared to GSS-1 (Table 3). The next most efficient biomixture in pesticide dissipation was GSS-2 which

Table 3
Half life ($t_{1/2}$), correlation coefficient (R^2) and χ^2 error estimated for the dissipation of the insecticides CHL, DEL, CYP and the fungicides SOPP, IMZ, TBZ in the different biomixtures. The first-order kinetic (FOK) model adequately described the dissipation patterns of all pesticides with the exception of IMZ where only the biphasic Hockey-Stick model provided adequate fit to the measured data.

Substrates[a]	Pesticides		
	R^2	$t_{1/2}$	χ^2
Chlorpyrifos (CHL)			
OTP	0.91	19.5	4.1
GVP	0.96	33.5	2.4
GM	0.87	41.7	3.4
GSS-1	0.88	12.5	7.0
GSS-2	0.91	19.4	3.1
SS	0.97	54.2	11.6
S	0.97	49.9	10.8
Deltamethrin (DEL)			
OTP	0.92	27.4	3.2
GVP	0.96	37.2	7.8
GM	0.95	35.9	2.2
GSS-1	0.91	22.3	3.6
GSS-2	0.96	30.1	1.7
SS	0.87	46.5	8.2
S	0.97	53.3	4.4
Cypermethrin (CYP)			
OTP	0.97	22.8	7.9
GVP	0.91	23.7	6.9
GM	0.95	36.4	5.1
GSS-1	0.94	14.8	4.2
GSS-2	0.98	18.7	11.2
SS	0.93	63.1	12.1
S	0.85	77.0	4.2
Ortho-phenylphenol − Na (SOPP)			
OTP	0.93	33.0	2.1
GVP	0.93	21.1	1.8
GM	0.94	19.5	4.6
GSS-1	0.96	4.9	7.2
GSS-2	0.94	13.1	9.7
SS	0.98	31.1	4.1
S	0.92	43.3	3.6
Thiabendazole (TBZ)			
OTP	0.82	57.4	2.6
GVP	0.90	28.8	3.4
GM	0.91	40.8	1.4
GSS-1	0.93	26.7	5.9
GSS-2	0.98	26.2	10.1
SS	0.98	89.5	3.2
S	0.87	77.8	9.1
Imazalil (IMZ)			
OTP	0.71	48.8	8.3
GVP	0.69	15.5	10.4
GM	0.90	36.8	9.6
GSS-1	0.87	34.4	8.2
GSS-2	0.88	31.7	6.4
SS	0.91	19.2	7.2
S	0.87	28.6	11.4

[a] OTP: olive tree prunings compost biomixture, GVP: Grape vine prunings compost biomixture, GM: grape mark compost biomixture, GSS-1 and GSS-2: grape stalks and seeds compost based biomixture comprising 25 and 50% compost proportion respectively, SS: straw and soil mixture, S: soil.

consisted of the same components as GSS-1 but at different ratios. It is possible that the higher proportion of straw in the latter (50% over 25%) enhanced the microbial activity and dissipation of pesticides. This is in agreement with Castillo and Torstensson (2007) who found a significant positive correlation between straw content and degradation capacity in peat-based biomixtures. The microbial nature of the rapid dissipation observed in GSS-1 is further supported by the significant positive correlation between pesticide dissipation rates and microbial respiration, which was evident for all pesticides in GSS-1 and GSS-2 only (Table 4). Our data suggest that GSS-1 was the most effective biomixture

Table 4
Correlation (r) between microbial respiration and pesticide dissipation rate in biomixtures and soil. The dissipation rates were estimated using the FOK model with the exception of IMZ where the Hockey-Stick model was used.

Substrates	Pesticides					
	CHL	DEL	CYP	SOPP	IMZ	TBZ
OTP	0.65***[a]	0.71**	0.56**	0.58	0.57	0.54
GVP	0.78*	0.85*	0.71**	0.69*	0.24	0.28
GM	0.68**	0.58**	0.38*	0.64*	0.18	0.16
GSS-1	0.84**	0.78***	0.89**	0.84**	0.48*	0.52*
GSS-2	0.74**	0.67*	0.77**	0.73**	0.71***	0.31*
SS	0.56*	0.52*	0.31	0.48*	0.16	0.29
S	0.12	0.28	0.31	0.57	0.11	0.14

[a] Symbols ***, ** and * correspond to significant correlation $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively.

regarding pesticide dissipation and it was further tested for its ability to retain pesticides in leaching column studies.

3.2. Leaching column studies

The amount of pesticides leached was significantly influenced by the type of pesticide ($p < 0.05$) and by water loading ($p < 0.05$). SOPP was the most mobile pesticide with 5.6–8.1% of its applied amount detected in the leachate of the biomixture columns (Table 5). CHL, IMZ, TBZ and particularly the pyrethroids CYP and DEL showed limited leaching (<0.11% and <0.003 respectively in all cases). The high relative leaching of SOPP could be attributed to its highest water solubility (Table 2) and low adsorption affinity (Table 2) which facilitates its rapid vertical transport.

Regarding the temporal basis of pesticides leaching, a rapid breakthrough of all pesticides was noticed at the first irrigation event, 2 DAA (Figs. 2 and 3). This was particularly true for SOPP with more than 80% of its overall leached amount detected at 2 DAA. This rapid SOPP breakthrough could be attributed to the short time interval (48 h) between pesticide application and the first irrigation event which did not allow for the establishment of an equilibration between the adsorbed and dissolved fraction of the pesticide in the columns and limited the possibility for pesticide dissipation. Previous studies with SOPP showed a rapid dissipation and an increasing adsorption with time in soil with no equilibrium established within 168 h (EFSA, 2008). In addition, the limited time interval between pesticide application and the first water loading (2 days) has probably drastically reduced the contact time between pesticide and organic surfaces that may be required for maximum microbial degradation. This is especially true for GSS-1 which has a high dissipation capacity as illustrated in the laboratory study.

The amount of pesticides detected in the leachate gradually decreased and no pesticide residues were detected in the leachate from 24 d onwards (Figs. 2 and 3). This could be attributed to a combination of gradual dissipation and increasing adsorption with time which reduce the available pesticide fraction for leaching. Our data suggest that for biomixtures characterized by high dissipation capacity (GSS-1) water management regimes, which maximize the time interval between pesticide disposal and the next water addition should be applied to maximize the potential for pesticide biodegradation before their vertical transport.

Overall, the highest the water load the highest the amount of pesticide detected in the leachate. This was more clearly illustrated for SOPP with 8.1, 6.1 and 5.6% of the initially applied amount obtained in the leachate from the biomixture columns which were treated with the H, M and L water loading scenario respectively (Table 5). This is in agreement with previous studies which also showed a substantial decrease in pesticide leaching in biomixture-filled columns at reduced water loadings (Fogg et al., 2004a; De

Table 5
The amount of pesticides (as % of the total amount applied) detected in the leachates of the columns packed with biomixture (GSS-1) or soil/perlite mixture.

Water loading ($L\,m^{-3}$)	GSS-1						Soil:Perlite (3:1 v:v)					
	CHL	DEL	CYP	SOPP	IMZ	TBZ	CHL	DEL	CYP	SOPP	IMZ	TBZ
463 (H)	0.11	0.003	0.003	8.1	0.1	0.07	0.40	0.002	0.003	27.6	0.4	0.4
242 (M)	0.09	0.001	0.001	6.1	0.1	0.08	0.33	0.001	0.001	23.9	0.4	0.3
161 (L)	0.05	0.002	nd[a]	5.6	0.09	0.11	0.18	nd	nd	19.7	0.1	0.2

[a] nd: not detected.

Wilde et al., 2010a,b). The replacement of compost and straw with perlite in the soil columns resulted in a significant increase in the amount of pesticides leaching further stressing the beneficial effect of these two substrates in enhancing the dissipation capacity of biomixtures (Table 5). The results of the current study demonstrated that the leaching of the pesticides tested is negligible with the notable exception of SOPP and that the GSS-1 was the best candidate to be used in an on-farm system.

3.3. On farm biobed system evaluation

The temporal distribution of pesticide residues in the profile of the on-farm biobed system is shown in Figs. 4 and 5. Overall, none

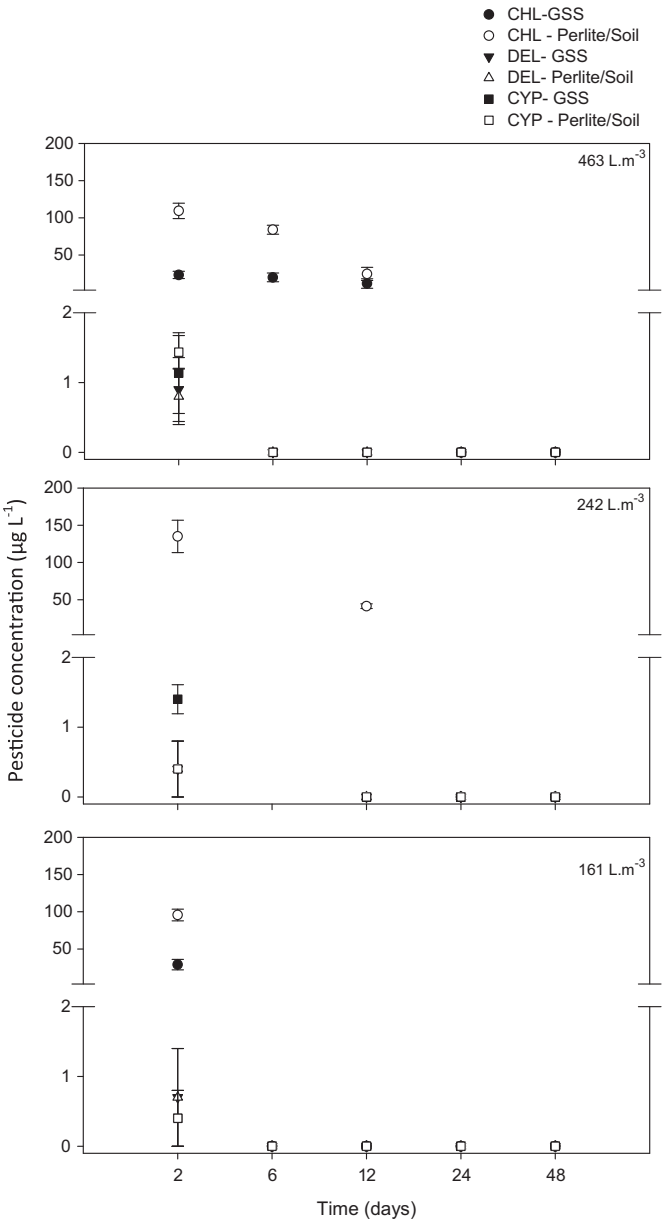


Fig. 2. The concentration ($\mu g\,L^{-1}$) of insecticides chlorpyrifos (CHL), deltamethrin (DEL) and cypermethrin (CYP) in the leachate collected from the columns packed with either GSS-1 (closed symbols) or soil:perlite mixture (open symbols) at three different water loading schemes (463, 242 and 161 $L\,m^{-3}$).

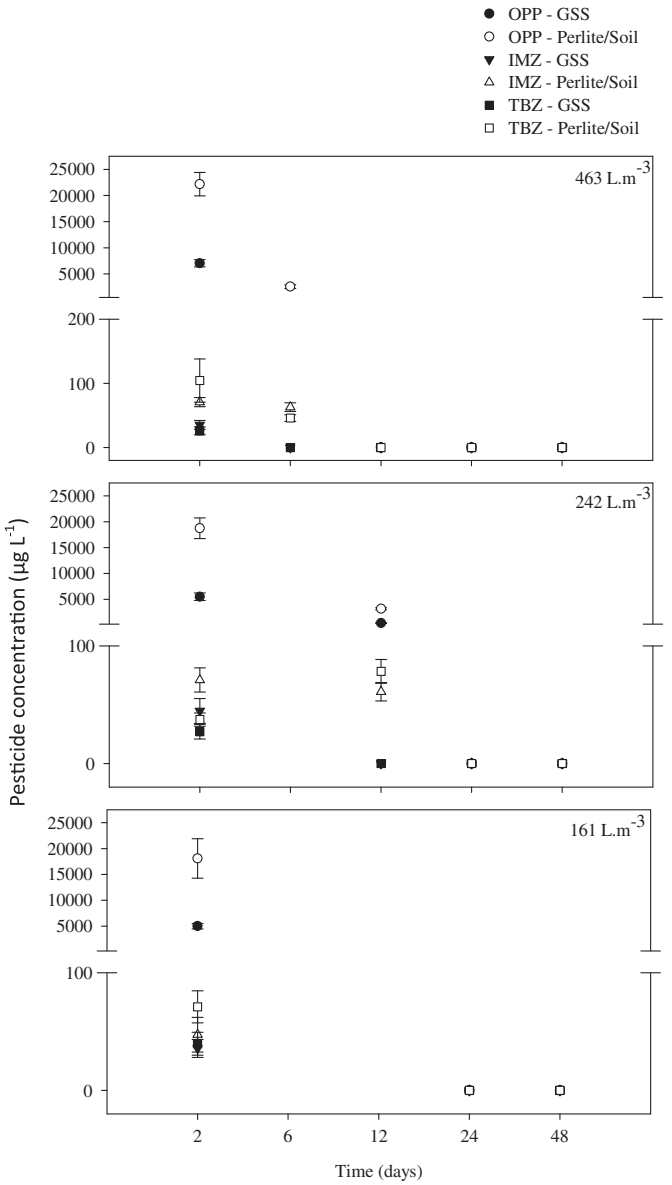


Fig. 3. The concentration ($\mu g\,L^{-1}$) of fungicides *ortho*-phenylphenol – Na (SOPP), thiabendazole (TBZ) and imazalil (IMZ) in the leachate collected from the columns packed with either GSS-1 (closed symbols) or soil:perlite mixture (open symbols) at three different water loading schemes (463, 242 and 161 $L\,m^{-3}$).

of the pesticides applied on the biobed system was detected at the 40–60 cm horizon throughout the experiment.

CHL was the most mobile insecticide with its residues detected at the 20–40 cm horizon (Fig. 4). A rapid loss of CHL was observed during the first 21 DAA with more than 80% of the intended application dose being dissipated. This is in agreement with the dissipation $t_{1/2}$ of 12.5 days obtained for CHL in GSS-1 in the laboratory study (Table 3). From 21 days onwards, CHL residues declined gradually to undetectable levels at the surface layer, while its residues were still detectable at 20–40 cm and especially at the 5–20 layer (Fig. 4). Our results are in line with previous findings which reported that CHL residues were restricted at the top 20 cm in a biomixture-packed column (Fogg et al., 2004b).

The pyrethroids DEL and CYP were immobile with their residues restricted in the upper layer of the biobed (0–5 cm), while trace concentrations only were also measured in the 5–20 cm layer up to 42 DAA (Fig. 4). This is in line with the column leaching study where pyrethroids showed the lowest leaching potential from the

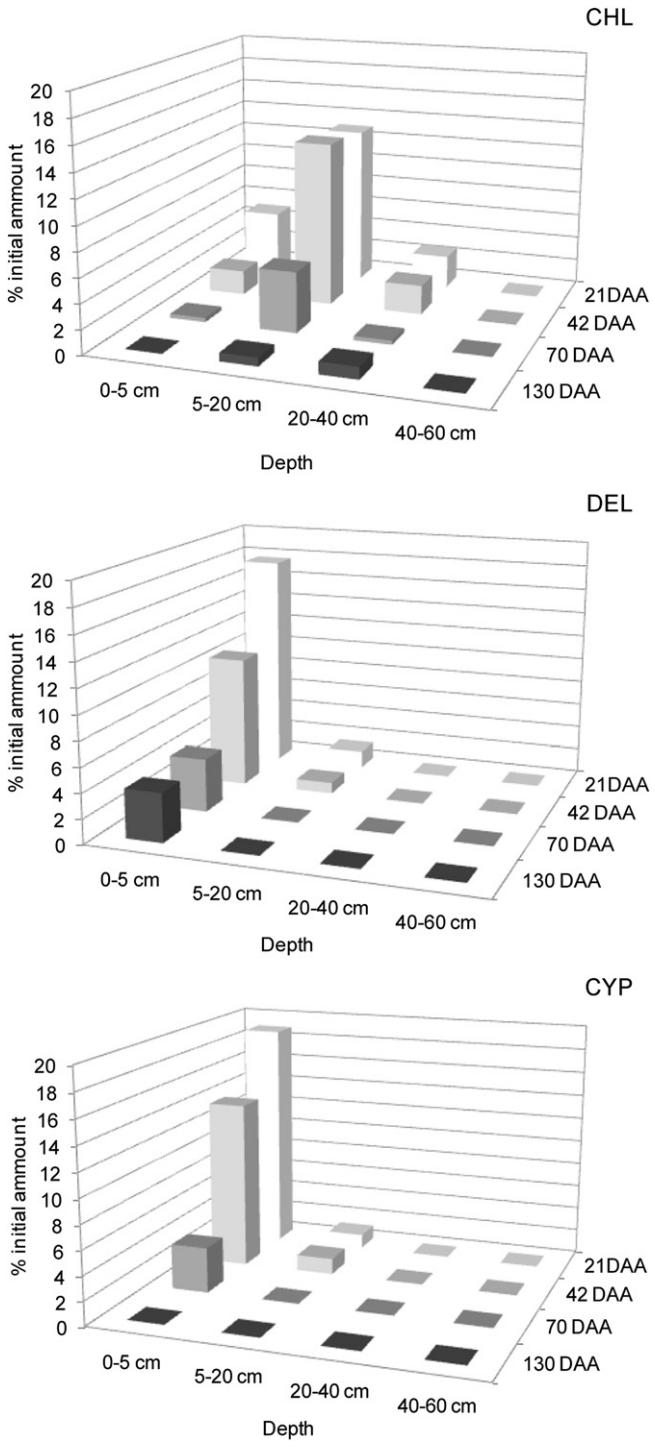


Fig. 4. The distribution of chlorpyrifos (CHL), deltamethrin (DEL) and cypermethrin (CYP) residues (% of applied amount) at different layers (0–5 cm, 5–20 cm, 20–40 cm and 40–60 cm) of the on-farm biobed 21, 42, 70 and 130 days after their application.

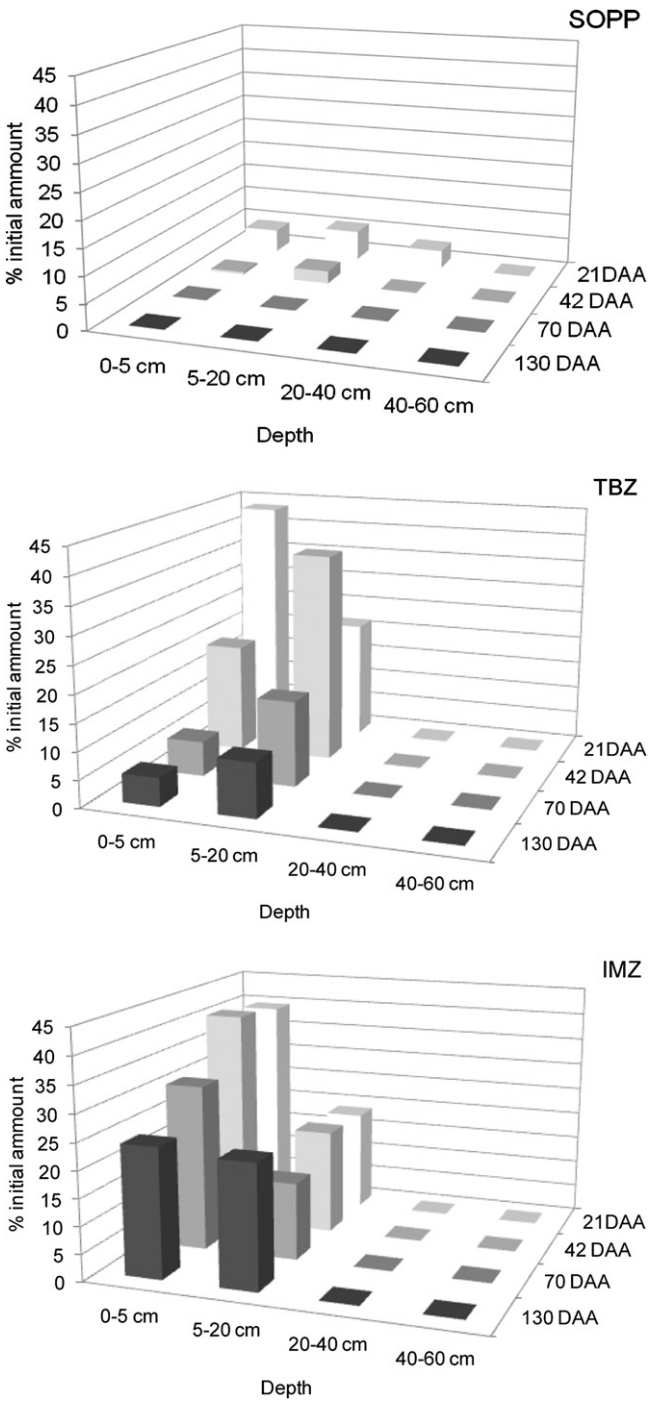


Fig. 5. The distribution of *ortho*-phenylphenol – Na (SOPP) imazalil (IMZ), thiabendazole (TBZ) and residues (% of applied amount) at different layers (0–5 cm, 5–20 cm, 20–40 cm and 40–60 cm) of the on-farm biobed 21, 42, 70 and 130 days after their application.

pesticides tested. Several previous studies have also indicated the low mobility of these pesticides in soil (EC, 2002; Selim and Zhu, 2002; Fenoll et al., 2011) which has been attributed to their high lipophilicity and high adsorption affinity onto the organic matter (Demoute, 1989; Khan et al., 1993; Jin and Webster, 1998; Laskowski, 2002). For both pyrethroids more than 80% of the intended application dose had dissipated during the first 21 days and their residues gradually declined thereafter. Previous studies by Munoz-Leoz et al. (2009) also noticed an initial rapid dissipation phase for DEL which was followed by a gradual reduction in the dissipation rate. This was attributed to increasing adsorption of DEL with time which limits its bioavailability and prevents its biodegradation.

SOPP residues were low but nearly equally distributed in the first 40 cm of the biobed profile at 21 DAA (Fig. 5). SOPP was particularly mobile with >3% of the initial amount detected at the 20–40 cm layer 21 DAA. These results are in accordance with the high mobility and leaching of SOPP observed in the column study. Dissipation of SOPP was rapid with less than 13% of the initial intended dose remaining in the biobed 21 DAA and no residues of SOPP were detected from 70 days onwards. This is in line with the rapid dissipation of SOPP in the GSS-1 in the lab study ($t_{1/2} = 4.9$ d).

Fungicides TBZ and IMZ were detected throughout the experiment at the top 20 cm of the biobed suggesting limited mobility (Fig. 5). Previous studies in soil have also stressed the limited mobility of TBZ (Solel et al., 1979; EC, 2001) and IMZ (EPA, 2003). Their limited mobility is probably attributed to their increased adsorption onto organic matter. Regarding their dissipation, less than 50% of the intended applied dose of the fungicides had dissipated during the first 21 days. Thereafter, a gradual dissipation of TBZ was evident at the top 20 cm of the biobed although 15% of the initially applied amount was still present at the top biobed layer (0–20 cm) at the final sampling day (130 DAA). IMZ was the most persistent compound with more than 40% of its initially applied amount still detected at the 0–20 cm layer 130 DAA. The long persistence of TBZ and mostly of IMZ is in agreement with the results of the laboratory dissipation study (Table 3). Previous regulatory studies for TBZ showed field dissipation $t_{1/2}$ in soil of more 2 years (EC, 2001), while for IMZ similar regulatory studies provided $t_{1/2}$ values ranging from 44 to 128 days (EPA, 2003). The dissipation patterns of TBZ and IMZ in the on-farm biobed system suggest a reduced persistence compared to the soil studies mentioned above and stress the dissipation efficiency of biobed systems for such persistent chemicals.

No pesticide residues were detected in the leachate collected during the experimental period. This is not surprising considering that none of the pesticides tested migrated beyond the top 40 cm of the profile of the on-farm biobed system. The non polar nature and the increasing adsorption affinity of CHL, DEL, CYP, IMZ and TBZ could explain their retention in the biobed system, whereas the more water soluble SOPP dissipated rapidly in the on-farm biobed thus precluding its vertical transport. Overall, all pesticides tested were either partially or fully dissipated in the on farm biobed system stressing the increasing dissipation and depuration capacity of the biobed. The only exception was IMZ which persisted in the surface layers of the biobed. This might result in a build up of its residues with continuous disposal of IMZ in biobeds. Continuation of monitoring of pesticide levels in the on-farm biobed and in its leachates will provide further insights regarding long-term sequential loadings.

4. Conclusions

A laboratory-to-field experimental approach was followed to evaluate and optimize biobed systems for the depuration of wastewaters produced by both pre- and post-harvest pesticide treatments during citrus production. Initial laboratory studies showed that biomixtures based on composted grape seeds and skins had the highest dissipation efficiency for all of the pesticides tested. The significant correlation of microbial respiration with pesticide dissipation rates in the CSS biomixtures stresses the key role of microbes on the dissipation efficiency of biomixtures. Further evaluation in leaching column studies demonstrated the superiority of GSS-1 over soil in retaining most of the pesticides tested with the exception of the rather polar SOPP. Increasing water loadings resulted in an increase of leaching for most of the pesticides studied suggesting that proper water management is essential to minimize pesticides leaching through biobeds. The laboratory and column study findings were further verified in an offset type on-farm biobed which dissipated and retained the pesticides amounts applied. Overall, our study demonstrated that biobeds could be used for the depuration of pesticide-contaminated wastewaters produced during citrus production at both pre- and at post-harvest level, using low cost materials. Simple lab and column experiments may provide valuable information of the efficiency of biomixtures and optimal loading management respectively.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.envpol.2012.03.001.

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