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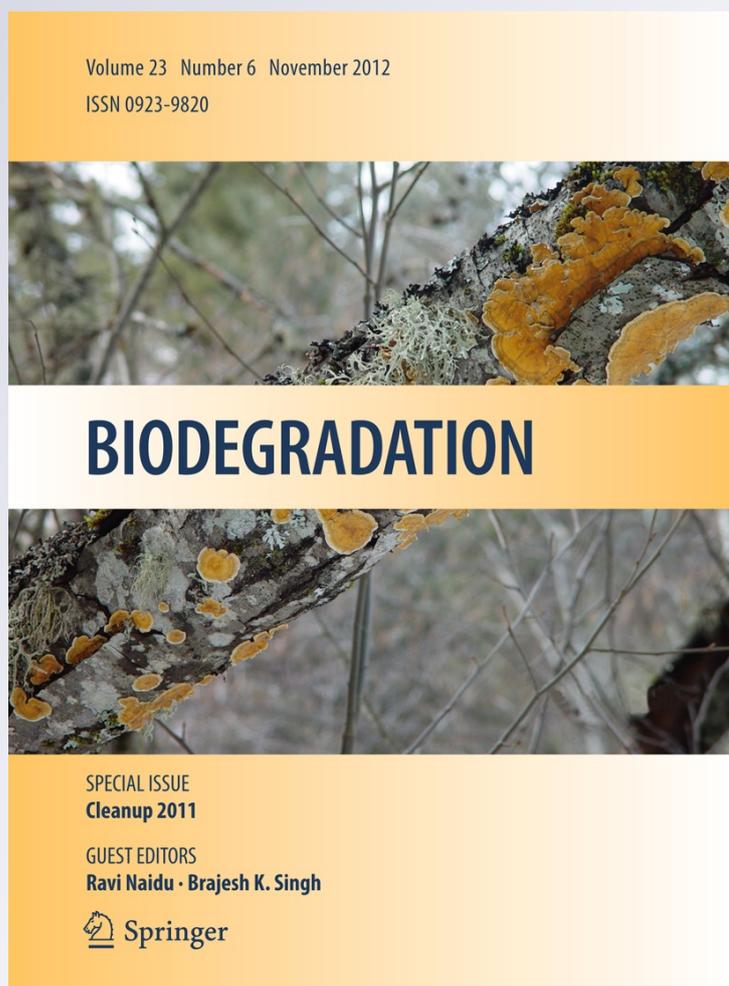
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On-farm biopurification systems for the depuration of pesticide wastewaters: recent biotechnological advances and future perspectives

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Abstract Point source contamination of natural water resources by pesticides constitutes a serious problem and on-farm biopurification systems (BPS) were introduced to resolve it. This paper reviews the processes and parameters controlling BPS depuration efficiency and reports on recent biotechnological advances which have been used for enhancing BPS performance. Biomixture composition and water management are the two factors which either individually or through their interactions control the depuration performance of BPS. Which process (biodegradation or adsorption) will dominate pesticides dissipation in BPS depends on biomixture composition and the physicochemical properties of the pesticides. Biotechnological interventions such as augmentation with pesticide-degrading microbes or pesticide-primed matrices have resulted in enhanced biodegradation

performance of BPS. Despite all these advancement in BPS research, there are still several issues which should be resolved to facilitate their full implementation. Safe handling and disposal of the spent biomixture is a key practical issue which needs further research. The use of BPS for the depuration of wastewaters from post-farm activities such as postharvest treatment of fruits should be a priority research issue considering the lack of alternative treatment systems. However, the key point hampering optimization of BPS is the lack of fundamental knowledge on BPS microbiology. The use of advanced molecular and biochemical methods in BPS would shed light into this issue in the future.

Keywords Pesticides · Biodegradation · Adsorption · Biopurification systems · Biomixture · Bioaugmentation

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Introduction

Pesticide point source contamination has been identified as a significant contributor to the deterioration of the quality of natural water resources (Carter 2000). Previous monitoring studies have demonstrated that point source contamination is responsible for a significant fraction (40–90 %) of the total pesticide load in natural aquifers (Kreuger and Törnqvist 1998; Gerecke et al. 2002; Neumann et al. 2003; Holvoet et al. 2005). Most common on-field point sources of

contamination stem from improper handling, faulty equipment and spillages or leaks during loading, mixing or cleaning of spray equipments. Point source contamination is typically related to high pesticides entries in groundwater which can reach the level of several mg per liter (Helweg et al. 2002). The problem is amplified when operation sites neighbor natural water resources or are directly linked to sewage systems, rivers or streams (Carter 2000; Bach et al. 2005).

Several preventative and mitigation actions have been undertaken to minimize pesticide point source contamination. These include (i) advisory campaigns and stewardship schemes for the application of best management practices during and after pesticide spraying (Higginbotham 2001; Ramwell et al. 2004; Jaeken and Debaer 2005) and (ii) the implementation of advanced decontamination systems using physical, chemical or biological methods (e.g. Sentinel) (Spanoghe et al. 2004). Most of these technologies are cost-inefficient or are associated with elevated technological requirements and as such are not applicable in farmyards. An alternative strategy is the implementation of on-site biopurification systems (BPS). They are designed to collect and decontaminate spraying leftovers derived from filling, emptying, mixing or washing of spraying equipment (Torstensson and Castillo 1997). BPS are excavations or containers filled with a biologically active matrix (usually called biomixture) consisting of soil, lignocellulosic materials and a humified organic substrate mixed at variable volumetric ratios (Castillo et al. 2008). Collection of wastewaters takes place in a concrete area hydraulically linked to the main BPS body (indirect systems) or just above the main body of the systems (direct systems). The first BPS was introduced by Torstensson and Castillo (1997) and it was given the name biobed. Over the years several modifications of the original design have been described to meet regional requirements (Fournier 2004; Pigeon et al. 2005; Spliid et al. 2006; Fait et al. 2007; Boivin and Guine 2011). A comprehensive description of the different designs and practical aspects of on-farm BPS are provided elsewhere (De Wilde et al. 2007; Castillo et al. 2008) and it will not be the focus of this review. Today more than 1500 biobeds are operative in Sweden and similar or modified BPS are established in more than 25 countries globally (Fig. 1). On farm BPS are characterized by low requirements for labor and time, low cost and can

be easily adjusted to local climatic conditions and legislation.

The efficiency of all BPS is based on the capacity of the biomixture to effectively degrade and retain the high pesticide loads discharged during the season. Regional needs have played a decisive role not only in the modification of BPS designs but also in the adjustment of the two main factors controlling their depuration efficiency: biomixture composition and water management. Modifications in these two parameters have enabled the implementation of BPS globally and are expected to promote their use for the decontamination of alternative pesticide-contaminated agricultural wastewaters. Microbial community is the key factor controlling the depuration capacity of BPS and knowledge of microbial dynamics within these systems will allow their effective optimization. Biotechnological augmentation of BPS via inoculation with pesticide-primed material or pesticide-degrading microorganisms has been another alternative for BPS optimization which will be discussed in this article.

The main aims of this review will be (a) to describe the basic processes and main parameters controlling pesticide dissipation in BPS (b) to present biotechnological advancements for ameliorating depuration performance and (c) to highlight future challenges for effective implementation of on-farm BPS.

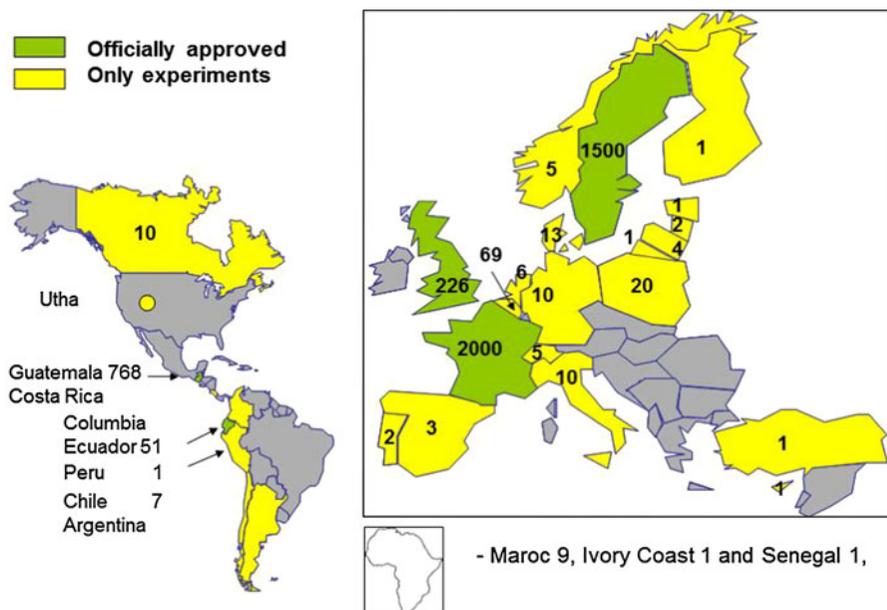
Processes controlling pesticide dissipation in BPS

On-farm BPS are based on a rather simple concept, but their depuration efficiency relies on relatively complex mechanisms and is achieved through a combination of enhanced metabolic activity and extended adsorption onto the biomixture.

Degradation: biotic and/or abiotic?

Degradation is the most significant process controlling pesticide dissipation in BPS. Although microbial degradation is the driving force of these systems, abiotic processes can be also important for certain pesticide—biomixture combinations. Evidence for the significance of biodegradation in pesticide dissipation in BPS substrates was first provided by Fogg et al. (2003) who noticed a significantly faster dissipation of chlorothalonil in BPS substrate which had been sterilized and inoculated with fresh biomixture compared to sterilized

Fig. 1 A world map showing the number and regional distribution of on-farm BPS (adapted by www.biobeds.org after permission by Jens Hunsby)



BPS substrate. On the other hand, the more rapid degradation of terbutylazine consistently observed in peat-biomixtures were associated to abiotic processes favored by the low pH of these substrates (Castillo and Torstensson 2007; Karanasios et al. 2010a)

Characteristics of microbial degradation processes in biomixture

Microbial degradation of pesticides in BPS can be seen as the end result of complex and usually interactive metabolic and co-metabolic functions (Fournier et al. 2000). The relative significance of each process relies upon pesticide characteristics but can be partly regulated by the composition of the biomixture. Higher degradation of certain, mainly persistent, compounds in the presence of straw or other lignocellulosic material is an indicator of co-metabolic transformations where pesticide degradation occurs as a result of microbial feeding on naturally occurring substrates. Pesticides with aromatic structures are susceptible to degradation by extracellular enzymes like phenoloxidases produced by white rot fungi (Castillo et al. 2001). Castillo and Torstensson (2007) reported the co-metabolic degradation of metamitron, chloridazon, isoproturon and linuron in peat-biomixtures which was correlated with high phenoloxidase activity. In contrast, no correlation between phenoxidase activity and pesticide biodegradation was observed in compost-

biomixtures (Karanasios et al. 2010a, b) suggesting the involvement of other broad spectrum enzymes like cytochrome P450 monooxygenases. On the other hand, the rapid degradation and mineralization of pesticides is commonly linked to microbial growth and proliferation where pesticides are utilized as an energy source (Alexander 1981). The repeated application of certain pesticides in BPS could lead to their rapid biodegradation due to the gradual establishment of microbes with highly specialized catabolic capacities. Enhanced microbial degradation is common for certain pesticide groups in soils and is considered a beneficial asset for BPS facilitating the rapid dissipation of pesticides like metalaxyl and iprodione which are prone to microbial adaptation (Vischetti et al. 2008; Karanasios et al. 2012a). Growth-linked degradation of pesticides has been associated with specialized enzymes like esterases or amidases involved in the hydrolysis of organophosphates (Singh 2009), carbamates (Hashimoto et al. 2002) and triazines (Martinez et al. 2001) in soil. However, no such information are available for the degradation of pesticides in BPS and this might be an issue which should be addressed in the future.

Metabolism of pesticides in BPS

Degradation is not always synonymous to detoxification. Thus it is essential to identify the metabolic

pathway of pesticides in BPS and verify their detoxification potential. Only scarce data are available regarding the metabolism of pesticides in BPS. A summary of the metabolic pathways of certain pesticides in BPS are shown in Fig. 2. The metabolism of the organophosphate chlorpyrifos in BPS has been studied the most. Hydrolysis of chlorpyrifos is the initial metabolic step leading to the formation of 3,5,6-trichloropyridinol—(TCP) (Fig. 2a). The latter is known to possess antimicrobial activities and its fate (accumulation vs metabolism) depended on the composition and the maturity of the BPS substrate. The amounts of TCP formed in BPS substrates were three-six fold lower compared to soil which could be attributed to the concurrent formation and degradation of TCP (Kravariti et al. 2010). Further, higher mineralization rates of chlorpyrifos and lower levels of TCP formation were observed in BPS substrates containing garden composts compared to identical substrates with urban compost. This finding was attributed to the higher lignin content of the former compost which favoured the degradation of chlorpyrifos by lignolytic white rot fungi (Coppola et al. 2007). In a recent study, Tortella et al. (2012) showed that the higher the maturity of a peat-biomixture the higher the amounts of TCP accumulating. This was attributed to the increased pH in the mature biomixture which promoted the hydrolysis of chlorpyrifos and the prolific formation of TCP.

The metabolism of the herbicide isoproturon, extensively used in cereals in north Europe, was studied in BPS by von Wirén-Lehr et al. (2001). It was transformed to mono- and di-demethylated derivatives (Fig. 2b) which are common metabolites of the herbicide in soil. The metabolism of glyphosate, a very commonly used herbicide globally, was studied in an established on-farm BPS in Guadeloupe. It was shown that glyphosate was almost completely dissipated with the formation of small amounts of aminomethylphosphonic acid (AMPA) (Fig. 2c) which persisted for at least 6 months (de Roffignac et al. 2007). Metribuzin deamination was the main initial metabolic step of the readily mobile substance metribuzin in BPS, while formation of the diketo derivative was a minor degradation route (Fig. 2d). Both intermediates were further degraded to the desamino-diketo derivative which can be readily degraded in biomixtures (Karanasios 2011).

Metabolic routes and the resulting derivatives can be diversified upon biomixture composition. For

example metabolism of terbuthylazine in compost-biomixtures proceeded mainly via microbial dealkylation to desethyl-terbuthylazine, in contrast to peat-biomixtures where hydroxylation was the main metabolic route (Karanasios 2011) (Fig. 2e). Knowledge of the metabolic route of the pesticides disposed off in a BPS is essential in order to evaluate its performance and plays a decisive role on the further handling of its drainings.

Pesticide adsorption and its interplay with biodegradation in BPS substrates

Adsorption could be seen as a beneficial process which minimises the risk for downward movement of pesticides and provides necessary time for concurrent dissipation processes to take effect in BPS. In an ideal BPS, pesticide adsorption should be extended enough to limit the risk for rapid pesticide loss and protect microflora from pesticide concentration extremes, while a gradual reversibility of the adsorption process would allow a progressive increase in the availability of pesticide residues to degrading enzymes minimizing the risk for residue built up.

However, increasing adsorption could drastically reduce pesticide bioavailability and limit the contribution of biodegradation on the overall pesticide dissipation in BPS. The balance between adsorption and biodegradation in the dissipation of pesticides is largely dependent on pesticide physicochemical properties and the composition of the BPS substrates. This is clearly illustrated in Table 1 where terbuthylazine was more rapidly degraded in biomixture, whereas chlorpyrifos degraded more rapidly in soil (Kravariti et al. 2010). These results demonstrate the dual but contrasting effect of biomixture (organic matter-rich substrate) on degradation which depended on the chemical nature of the pesticide studied: a positive effect towards the less lipophilic terbuthylazine due to increasing biodegradation and a negative effect towards the more lipophilic chlorpyrifos due to increasing adsorption.

Which of the two processes, adsorption or degradation, would dominate the dissipation of pesticides in BPS is a key issue. Prevalence of biodegradation is more desirable since it is expected to result in the irreversible removal of pesticides from the environment. In contrast, adsorption is a fully or partially reversible process which, under certain conditions,

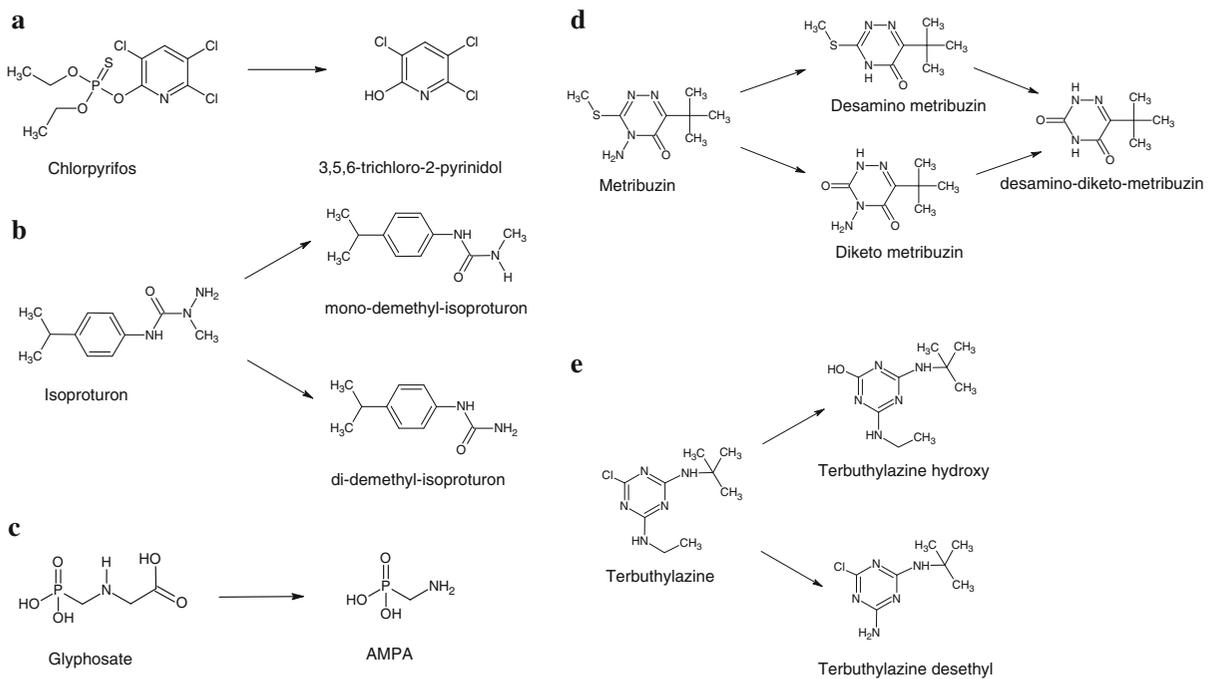


Fig. 2 Metabolic pathways of chlorpyrifos (a), isotropuron (b), glyphosate (c), metribuzin (d) and terbuthylazine (e) in BPS substrates

Table 1 The influence of pesticide properties and BPS substrate composition on the contribution of adsorption or degradation in the dissipation of pesticides in BPS (adapted by Kravariti et al. 2010 after permission)

Pesticide	Substrate	Organic C (%)	pH	$t_{1/2}$ (days)	K_f or K_d (g ml ⁻¹)
Terbuthylazine	Soil	0.9	8.5	231	7
Chlorpyrifos				18	17
Terbuthylazine	Biomixture	6.7	7.0	116	31
Chlorpyrifos				69	746

could lead to the re-release of pesticides in the substrate solution making them available for vertical movement and discharge into natural water resources. The kind of humified material, peat or compost, used in BPS substrates appears to significantly affect the type of process dominating pesticide dissipation. This is clearly illustrated in Fig. 3 where mass balance analysis in a leaching column study showed that the high depuration capacity of peat-biomixtures for terbuthylazine and chlorpyrifos was mostly attributable to its high ability to retain rather than degrade pesticides while the exact opposite was seen for a biomixture where peat had been replaced by a grape marc compost (Karanasios et al. 2012b). It is anticipated that the use of peat-biomixtures under particular conditions could result into the gradual build up of

high pesticide amounts which make its further handling and disposal problematic.

Only a few adsorption studies have been performed in BPS substrates using either the classical batch equilibration method (Karanasios et al. 2010a, b; De Wilde et al. 2009a) or column displacement experiments (De Wilde et al. 2009b, c). Following the former method, pesticides adsorption on biomixtures was adequately described by the freundlich isotherm which suggests modifications in the affinity of pesticide molecules for solid surfaces at increasing pesticide concentrations. Positive correlations between organic carbon content and pesticide adsorption are observed for the more lipophilic molecules. However, normalization of the adsorption coefficients for the organic carbon content of biomixtures does not reduce

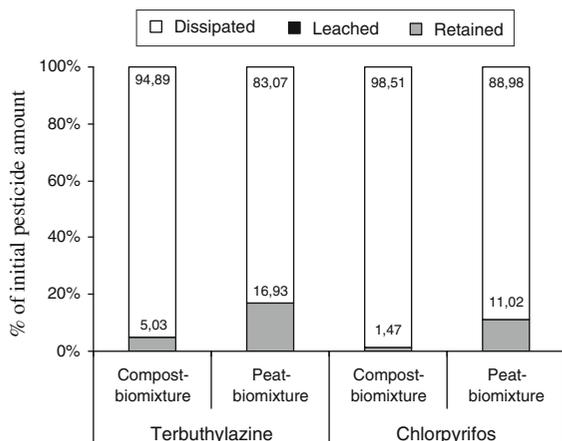


Fig. 3 Mass balance analysis for terbutylazine and chlorpyrifos in a leaching column study revealing the role of humified materials as components of biomixture in the dynamics of pesticide dissipation processes in BPS (adapted by Karanasios et al. 2012b after permission)

the variation in the values of the adsorption coefficients of the more mobile pesticides suggesting that organic matter is not the only factor controlling their adsorption (De Wilde et al. 2009a; Karanasios et al. 2010a). Other factors influencing pesticide adsorption onto BPS substrates include the nature of organic matter, the specific surface area and the particle size of the different components of the biomixture (De Wilde 2009). For instance, in coconut chips which is a relatively coarse material the diffusion of the pesticide molecules onto the specific surface area occurred at a slower rate compared to compost and manure which were the other two components of the biomixture (De Wilde 2009). A significantly higher adsorption of pesticides in BPS substrates over soil is evident in all studies (Karanasios et al. 2010a). Among the different biomixtures tested, peat-biomixtures showed markedly higher adsorption capacity over peat-free biomixtures and this has been linked to the higher aromaticity of the organic matter of peat (De Wilde 2009).

Generally, a positive correlation between adsorption and desorption coefficients has been observed in adsorption/desorption batch equilibrium studies with BPS substrates. These findings suggest that substrates with high adsorption efficiency also have higher strength as lower amounts of the adsorbed pesticides can be re-released back into the aqueous phase (Karanasios et al. 2010a, b). The presence of adjuvants

in pesticide formulations and the simultaneous presence of other pesticides in the biomixture can affect the adsorption behavior of certain pesticides like metalaxyl (De Wilde et al. 2009a).

Factors affecting the performance of BPS

Biomixture composition

The composition of the biomixture is of paramount importance for BPS performance. Its typical composition includes soil, straw and peat with each constituent having a role in BPS function. Soil is the main source of pesticide-degrading microbes. Straw supplies C and energy, promotes the pesticide-degrading activity of lignin-degrading fungi and adds to the adsorption capacity of the biomixture. Peat increases the adsorption capacity of the biomixture, regulates moisture content and decreases pH which promotes fungal ligninolytic activity. The concurrent presence of all three components is essential for maximum degradation capacity (Castillo and Torstensson 2007; Karanasios et al. 2012a). Based on the original peat-biomixture (25 % soil, 25 % peat and 50 % straw) several modifications have been proposed to accommodate regional needs. Considering that the choice of soil does not appear to significantly affect the degradation efficiency of BPS substrates (Fogg et al. 2004a), modifications focused on the complete or partial replacement of the other two components: straw and peat.

Lignocellulosic materials

Straw has been the most popular lignocellulosic material used in BPS. However, the high availability of alternative lignocellulosic materials in different regions, at a reduced or no cost, have led to its potential replacement. The kind of lignocellulosic material utilized depends on the type of crop cultivated in each region. A list of lignocellulosic materials used in BPS is presented in Table 2. Vine branches and pruning residues could be a viable alternative for straw in wine-producing and orchard-dominated regions, respectively (Vischetti et al. 2004; Coppola et al. 2007; Vischetti et al. 2008; Coppola et al. 2011a). Biomixtures based on vine branches showed superior

degradation capacity for various pesticides compared to topsoil and other substrates (Vischetti et al. 2008; Coppola et al. 2011a). Alternative substrates from the wine-producing agro-industry could be grape stalks which significantly promoted the degradation capacity of biomixtures compared to straw and other lignocellulosic materials (Karanasios et al. 2010a).

Citrus peels are produced in large amounts in the coastal areas of the Mediterranean region and were also considered as substitutes of straw. Laboratory studies with biomixtures containing citrus peels showed a retardation of pesticide degradation which was attributed to their high phenolic content known to possess antimicrobial activity (Coppola et al. 2007; Karanasios et al. 2010a). Agricultural residues produced in abundance in areas with arable land like corn cobs and sunflower residues showed equally high dissipation capacity with straw-containing biomixtures (Karanasios et al. 2010a). In tropical areas,

residues from the sugar production industry like bagasse was successfully tested as straw substitute because of their high availability in tropical regions (de Roffignac et al. 2008).

Humified materials

The preferred humified material in original Swedish biobeds was peat. However, the implementation of BPS in other countries made necessary its replacement by alternative materials with the main candidate being agricultural composts. The replacement of peat was necessitated (i) by its generally high cost and low availability in regions like south Europe and (ii) because its use is not conducive with sustainable agricultural practice (Fogg et al. 2003).

Composts and peat differ substantially in physico-chemical characteristics, nutrient availability and

Table 2 Lignocellulosic materials which have been used as components of BPS matrix

Substrates	Pesticides	Reference
Straw; leek residues	Atrazine, carbofuran, simazine, diuron, lenacil, bifenthrin, metalaxyl	Spanoghe et al. (2004)
Bagasse	Glyphosate, malathion, lamda-cyhalothrin	de Roffignac et al. (2008)
Coco chips; straw; willow chopping; straw	Linuron, metalaxyl, isoproturon, bentazone	De Wilde et al. (2009b, c)
Coco chips; straw	Linuron, metalaxyl, isoproturon, bentazone, metamitron	De Wilde et al. (2010c, d)
Straw	Azoxystrobin, bentazone, bromoxynil, ioxynil, dimethoate, diuron, fenpropimorph, fluzazifop- <i>p</i> -butyl, glyphosate, kresoxim methyl, MCPA, mecoprop-P, pirimicarb, propiconazole, propyzamide, prosulfocarb, metamitron, chloridazon, metribuzin, methabenzthiazuron, isoproturon, terbuthylazine, linuron, metalaxyl, isoproturon, pendimethalin, chlorothalonil, epoxiconazole, chlorpyrifos, deltamethrin, cypermethrin, <i>ortho</i> -phenylphenol, thiabendazole, imazalil	von Wirén-Lehr et al. (2001), Fogg et al. (2003, 2004a, b), Spliid et al. (2006), Castillo and Torstensson (2007), De Wilde et al. (2010a), Karanasios et al. (2010b), Kravariti et al. (2010), Karanasios et al. (2012a), Omirou et al. (2012), Tortella et al. (2012)
Vine branches, citrus peels	Chlorpyrifos, metalaxyl, imazamox, bentazone, isoproturon	Vischetti et al. (2004), Coppola et al. (2007), Coppola et al. (2011a)
Corn stovers; corn cobs	Alachlor, acetochlor	Lamar (2001)
Vine braches	Chlorpyrifos, metalaxyl	Vischetti et al. (2008)
Straw; corn cobs; citrus peels; sunflower residues; grape stalks; olive leaves	Chlorpyrifos, indoxacarb, buprofezin, terbuthylazine, metalaxyl, metribuzin, azoxystrobin, iprodione	Karanasios et al. (2010a)
Straw; grape stalks; corn cobs	Chlorpyrifos, terbuthylazine, metribuzin, metalaxyl, iprodione	Karanasios et al. (2012b)

biological activity (Niklasch and Joergensen 2001). Although the properties of individual composts largely depend on composting practices, they are generally characterized by lower C content, higher levels of macronutrients (N, P, K), neutral to basic pH (Zmora-Nahum et al. 2007) and support a metabolically active microbial community. Peat typically has higher water-holding capacity, significantly lower density, acidic pH and it does not generally support a highly active microbial community. These differences can reflect variability in the overall depuration capacity of the biomixtures. The higher C content, the acidic to neutral conditions along with the limited N content of peat-biomixtures generally promote co-metabolic transformations associated with white-rot fungi (Castillo and Torstensson 2007). On the contrary, the neutral/basic pH and the higher N availability of compost-biomixtures generally promote metabolic degradation. As mentioned above, peat shows higher affinity for pesticide adsorption compared to agricultural composts with pesticide adsorption in peat-biomixtures being less reversible (De Wilde et al. 2009a; Karanasios et al. 2010b).

Compost-biomixtures generally show a superior degrading capacity over peat-biomixtures (Karanasios et al. 2010b; Coppola et al. 2011a; Omirou et al. 2012). Agricultural composted materials which have been utilized as replacement of peat in BPS are listed in Table 3. Olive leaves, cotton seed or cotton crop residues composts significantly promoted pesticide degradation compared to peat-biomixtures (Karanasios et al. 2010b). The superior degradation capacity amongst the composts tested was shown by the olive leaves compost (Fig. 4). In a more recent study, Omirou et al. (2012) showed that a biomixture containing a compost of grape seeds and skins rapidly degraded a range of pesticides used in citrus production compared to composted olive or grapevine prunings and grape marc. Pesticide degradation rates in this compost were positively correlated with microbial respiration verifying the microbial nature of its high degradation capacity.

The origin and characteristics of the compost can significantly affect degradation and adsorption behavior. For example garden compost was proven more efficient than urban waste compost in degrading chlorpyrifos and TCP (Coppola et al. 2007). Aging

of compost generally reduces degradation efficiency as compost produced after a 12 month curing period was outperformed by a less mature compost (Monaci et al. 2007).

Water management of BPS

Water management, defined as the frequency and the intensity of wastewater loading on a BPS, has been identified as a key factor controlling the depuration efficiency of on-farm BPS. This was first demonstrated in northern Europe. First studies by Fogg et al. (2004b) showed that the retention of dimethoate and isoproturon in columns packed with a compost-biomixture was clearly reduced at higher water loadings. More recent microcosm (De Wilde et al. 2010c) and macrocosm studies (De Wilde et al. 2010d) showed that water flux strongly affected the retention capacity of peat-biomixtures and suggested that an average water load of 12.5 L m³ of biomixture could be sufficient for effective retention of pesticides by BPS in Belgium. Different water management routines though should be followed in south Europe where dry and warm conditions prevail during the cultivating season, when the higher load on BPS is expected. Recent column studies by Omirou et al. (2012) reported higher pesticide leaching at higher water loadings. Further tests in an on-farm BPS in Cyprus packed with the same substrate showed that under a realistic water loading scheme (6.1 m³ of water during the whole season) the BPS was able to effectively retain and dissipate fully or partially all of the insecticides and fungicides used at pre- and post-harvest level, respectively in citrus production. Concurrent column studies by Karanasios et al. (2012b) demonstrated the significance of the interactions between biomixture composition and water management. The performance of peat- and different compost-biomixtures was tested under two different water management scenarios which differed only in the volume and the frequency of water application: higher water volumes (600 ml) applied on a weekly basis (high volume—low frequency scenario) vs lower water volumes (100–200 ml) applied on a day by day basis (low volume—high frequency scenario). The total amount of pesticide leached was substantially higher at the former scenario. It was also

Table 3 Humified materials which have been used as components of BPS matrix

Substrates	Pesticides	References
<i>Agaricus bisporus</i> SMS compost; olive leaves compost; peat	Dimethoate, indoxacarb, buprofezin, terbuthylazine, metalaxyl, metribuzin, azoxystrobin, iprodione	Karanasios et al. (2012a)
Grape marc compost; olive leaves compost; peat	Terbuthylazine, metalaxyl, metribuzin, chlorpyrifos, iprodione	Karanasios et al. (2012b)
Olive leaves compost; peat	Chlorpyrifos, indoxacarb, buprofezin, terbuthylazine, metalaxyl, metribuzin, azoxystrobin, iprodione	Karanasios et al. (2010a)
<i>Agaricus bisporus</i> SMS compost; olive leaves compost; sea wrack compost; cotton crop residues compost; cotton seeds compost; peat	Dimethoate, indoxacarb, buprofezin, terbuthylazine, metalaxyl, metribuzin, azoxystrobin, iprodione	Karanasios et al. (2010b)
Olive tree prunings-; grape vine prunings-; grape marc-; grape seeds and skins-compost	Deltamethrin, cypermethrin, chlorpyrifos, ortho-phenylphenol, thiabendazole, imazalil	Omirou et al. (2012)
Cotton flower and seed residues compost	Terbuthylazine, chlorpyrifos	Kravariti et al. (2010)
Peat-free compost	Isoproturon, pendimethalin, chlorpyrifos, chlorothalonil, epoxiconazole, dimethoate	Fogg et al. (2003); Fogg et al. (2004a, b)
Garden waste compost; peat	Linuron, isoproturon, metalaxyl, bentazone	De Wilde et al. (2009b, c)
Peat	Azoxystrobin, bentazone, bromoxynil, ioxynil, dimethoate, diuron, fenpropimorph, fluazifop-p-butyl, glyphosate, kresoxim methyl, MCPA, mecoprop-P, pirimicarb, propiconazole, propyzamide, prosulfocarb, metamitron, chloridazon, metribuzin, methabenzthiazuron, isoproturon, terbuthylazine, linuron, metalaxyl, chlorpyrifos	Spliid et al. (2006), Castillo and Torstensson (2007), De Wilde et al. (2010c), De Wilde et al. (2010d), Tortella et al. (2012)
Urban compost; Garden compost; peat	Chlorpyrifos, metalaxyl, isoproturon, bentazon	Vischetti et al. (2004, 2008), Coppola et al. (2007, 2011a)
Prunings compost	Penconazole, dimethomorph, iprovalicarb, metalaxyl, azoxystrobin, fludioxinil, cyprodinil	Monaci et al. (2009)

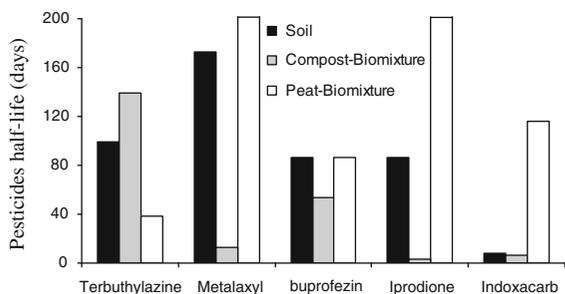


Fig. 4 Half-life values of selected pesticides in soil, a compost- and a peat-biomixture. In the compost-biomixture peat has been replaced by olive leaves compost (adapted by Karanasios et al. 2010b after permission)

apparent that the depuration performance of the substrates tested was affected differently by the different water management scenarios with peat-biomixtures performing better than compost-biomixtures at the high volume—low frequency water management, compared to the superiority of a compost-biomixture at the low volume—high frequency water management scenario. It appears that peat-free BPS could treat large wastewater volumes provided that the frequency and the intensity of water application is modulated to extend the contact period between pesticides and compost-biomixtures, thus exploiting their high biodegradation capacity.

Biotechnological advances for maximizing the biodegradation potential of BPS

Upon disposal onto BPS, pesticides interact with the microbial community. Its response to pesticide loading will depend mostly on pesticide-associated factors and it could be expressed either as a growth and proliferation of pesticide-degrading microorganisms which could utilize the pesticide as energy source or as general reduction of the size and activity of certain or the whole microbial community due to the inherent toxicity of the pesticide.

Regarding the type of microbes involved in pesticide biodegradation in BPS, this mostly relies on the type of biomixture used. The original Swedish peat-biomixture depends largely on the capacity of white rot fungi to co-metabolize the wide range of pesticides disposed off in these systems (Castillo et al. 2001). In contrast, peat-free BPS substrates with neutral to alkaline pH appear to rely mostly on bacterial activity for rapid pesticide degradation (Fournier 2004).

High biodegradation and especially high mineralization capacity is desirable in BPS. Attempts have been made for augmenting their biodegradation capacity via inoculation with pesticide-degrading microorganisms. Initial attempts focused on the inoculation of peat-biomixtures with white rot fungi, favoured in such low-pH and lignocellulose-rich substrates. von Wirén-Lehr et al. (2001) showed that inoculation of biomixtures with *Phanerochaete chrysosporium* accelerated the degradation of isoproturon. Similar studies by Bending et al. (2002) demonstrated the high capacity of *Coriolus versicolor*, *Hypholoma fasciculare* and *Stereum hirsutum* to degrade a wide range of pesticides with varying physicochemical properties. Spent mushroom substrate (SMS) derived from edible mushroom cultivation has also been proposed as a biotechnological amelioration of BPS biodegradation potential (Karanasios et al. 2010a; Rodríguez-Cruz et al. 2012). SMS is composed of pasteurized straw inoculated with the edible white rot fungus *Pleurotus ostreatus* and generally constitutes a good source of nutrients and non-specific extracellular enzymes. This substrate is released at large quantities after completion of mushroom production and can be obtained by all mushroom units at no cost since it is of no use for further mushroom production. Amendment of soil and straw with SMS of *P. ostreatus* at variable volumetric ratios accelerated the degradation of different pesticides (Fig. 5) with degradation rates being

correlated with the proportion of SMS in the biomixture (Karanasios et al. 2010a). Despite their high degradation potential, white rot fungi are weak competitors and could be overwhelmed by moulds and soil bacteria thus limiting their practical use for the biotechnological optimization of BPS. In order to avoid these limitations, Peters (2007) proposed the utilization of their lignolytic enzymes (MnP and laccase) as biocatalysts in BPS. These enzymes have showed a remarkable ability to co-metabolize a wide range of pesticides (Pizzul et al. 2009), however their formulation and in situ application without significant activity loss remains a challenge.

An alternative approach for augmentation of the depuration performance of BPS is the supplementation of biomixtures with pesticide-primed material. This could be soil (Sniegowski et al. 2011a) or spent biomixture (De Wilde et al. 2010b) which have been exposed to selected pesticides and have eventually developed a highly specialized pesticide-degrading microbial community. In comparison with the classical approach of pure culture inoculation, this strategy has several advantages: (1) it is a simple and low cost method which does not require the isolation and cultivation of pesticide-degrading microorganisms, (2) it introduces into the BPS a larger genetic pool and higher diversity of pesticide-degrading microbes, (3) pesticide-degrading microbes are better adapted to in situ conditions.

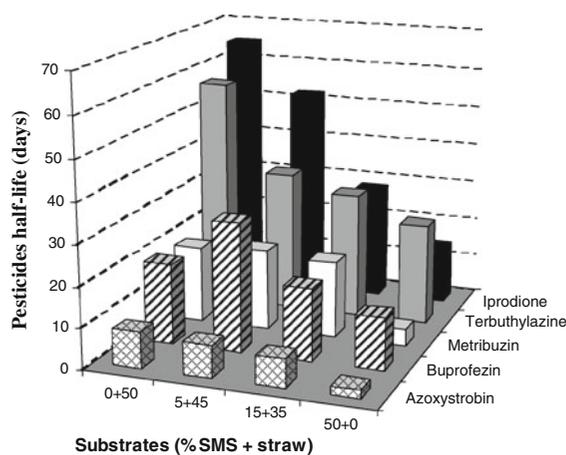


Fig. 5 The calculated half-life ($t_{1/2}$) values of pesticides in biomixtures containing spent mushroom substrate/straw of the edible fungus *Pleurotus ostreatus* mixed with soil at various proportions (0, 5, 15 and 50 %). In all biomixtures the sum of SMS + straw constituted 50 % of the biomixture by volume while the other 50 % was soil (adapted by Karanasios et al. 2010a after permission)

Following this strategy, De Wilde et al. (2010b) showed that augmentation of a fresh biomixture (5 % by weight) with primed-material originated from a BPS heavily exposed to metalaxyl accelerated the degradation of this fungicide and reduced the acclimation phase needed for the onset of degradation. The same approach was not successful for isoproturon where the addition of a primed soil, which however had not been treated with the herbicide for the last 3 years, in fresh biomixture did not accelerate the degradation of the herbicide. Thus careful selection and prior testing of the pesticide-primed material is required for successful bioaugmentation of BPS. Supplementation of fresh biomixture with linuron-primed soil at volumetric proportion between 25 and 50 % resulted in the rapid establishment of a pesticide-mineralizing microbial population (Sniegowski et al. 2011a). Targeted molecular fingerprinting analysis showed the concomitant proliferation of a *Variovorax* sp. phylotype related to a known linuron-degrading strain. Further studies demonstrated that the linuron-mineralization capacity established after amendment with linuron-primed soil was maintained after exposure to multiple stress conditions (cold and drought periods, no pesticide addition, application of pesticide mixtures) which are likely to occur in on-farm BPS (Sniegowski et al. 2011b, 2012).

Despite their high potential, the application of pesticide-primed material for bioaugmentation of BPS has still several limitations: (1) it is mainly applicable as a targeted treatment for accelerating the dissipation of persistent or mobile pesticides which are not effectively retained in BPS; this suggests that this method cannot be used as a holistic approach considering the wide variety of pesticides used in a region during the season; (2) it is only applicable for pesticides with at least fair susceptibility to microbial adaptation such as organophosphate and carbamate insecticides (Karpouzias and Singh 2006); triazine (Houot et al. 2000) and phenylurea herbicides (Walker and Austin 2003); phenylamide fungicides (Droby and Coffey 1991).

Future perspectives

Handling and disposal of the spent BPS substrate

Spent BPS substrates can potentially contain high pesticide residues and could be considered as

hazardous waste which should be treated accordingly. However there seems to be a gap in legislation in terms of appropriate handling of spent BPS substrate even at countries where the use of BPS is officially approved. Possible ways of handling this material include dispersal, landfill disposal or incineration. Some of these techniques can lead to complete depuration but are rather expensive (incineration) or they can not be considered as terminal processes as they include transfer of the contaminant to another environmental medium. A limited number of studies have explored this issue. In a pioneering study Torstensson (2000) showed that an 8-month storage period in a protected area of the farmyard could be enough for the reduction of pesticide residues to levels below the detection limits. A recent study by De Wilde et al. (2010a) showed that tunnel-composting resulted in a substantial dissipation of most pesticides except bifenthrin, while barrel incubation of spent biomixture was less efficient in the removal of pesticides. Higher composting temperatures and thus degradation efficiency can be achieved by the amendment of spent biomixture with fresh material to initiate the composting process, since the largely decomposed spent substrate could not support by itself the composting process. After detoxification, the spent biomixture is usually spread over agricultural fields or fallow land. However, there are still no reports regarding the impact of this practice on the diversity and function of soil microbes, considering their pivotal role as drivers of the main geochemical cycles. This is an area of future research considering the introduction of novel culture-independent molecular tools which advanced our knowledge of the ecology and functions of soil microorganisms.

Use of BPS for the depuration of alternative agro-industrial effluents

So far the use of BPS is restricted to pesticide-contaminated wastewaters produced by on-farm activities. However, alternative agro-industrial activities produce effluents contaminated with high pesticide loads. An example of this is the postharvest treatment of fruits (citrus, apple, pears and bananas) which leads to the production of large wastewater volumes containing high concentrations of fungicides (thiabendazole, imazalil, *ortho*-phenylphenol) and antioxidants (ethoxyquin, diphenylamine) used for the protection

of fruits from deterioration at storage. These wastewaters constitute a serious point source for the contamination of natural water resources (Castillo et al. 2000). This risk has been identified by the European Commission (EC) which has given authorization to these pesticides 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 (GAC) (Garcia Portillo et al. 2004). This system achieved 7000 times reduction in TBZ concentrations (EC 2000). However, the high cost for its construction and maintenance plus its advanced technological requirements for operation precludes its implementation in fruit packaging plants. A simple comparison of the cost of the packing material of these two systems is indicative of their cost-differences: the minimum cost for granular activated carbon is 1.5\$ kg⁻¹ excluding shipment costs, compared to the zero cost of a biomixture composed of soil (no cost), straw or other lignocellulosic materials—remains of local crops (no cost) and compost of agricultural by-products (no cost if produced on site).

BPS might offer an integrated solution for the depuration of both on-farm and postharvest wastewaters produced during fruit production. However, the high wastewater volumes (25–100 m³) produced by the fruit packaging industry within a short period (3–4 months) make necessary the application of adaptations of BPS water management routines and biomixture content to effectively depurate these wastewaters as well. The first step towards the use of BPS for the depuration of such wastewaters was presented by Omirou et al. (2012). They showed that a typical offset type BPS previously used for the depuration of wastewaters produced at on-farm level was able to effectively retain the particularly persistent fungicides used in the local citrus packaging industry (thiabendazole, imazalil, ortho-phenylphenol) (Fig. 6). This was achieved via utilization of a compost-biomixture highly efficient in the degradation of those fungicides and the gradual loading of the total water volume on the BPS in order to maintain high moisture in the core of the BPS during the season.

Getting to know microbial functions and dynamics in BPS

Although it is widely known that the microbial activity is decisive for the depuration activity of BPS, the microbiology of these systems is still a 'black box'. So far most studies have tried to link pesticide dissipation with broad microbial functional measurements like basal respiration (Coppola et al. 2007, 2011a), hydrolytic and phenoloxidase activity (Castillo and Torstensson 2007; Karanasios et al. 2010a, b) with variable results. However, the role of the different microbial components of the BPS microflora on pesticide dissipation and their response to pesticides exposure has been overlooked so far. The introduction of culture-independent methods has significantly advanced our understanding of the ecological role of microbes in terrestrial and aquatic ecosystems (Torsvik and Ovreas 2002). The application of high resolution molecular techniques like—omics, high-throughput sequencing and stable isotope probing in studying the dynamics of microbial communities in BPS would substantially increase our understanding of the microbial processes involved in pesticide dissipation in these systems and facilitate their modification towards an optimized biodepuration performance. A first idea of the application of these tools was given by Coppola et al. (2011b) who showed via molecular fingerprinting and cloning that the regular disposal of fungicides in BPS induced significant but temporal changes in the bacterial and fungal community and provided preliminary evidence for the involvement of yeast and ascomycetes in the degradation of the fungicides tested.

Conclusions

On-farm BPS offer an attractive solution for the prevention of the contamination of natural water resources by pesticides due to improper handling at the farm level. Biomixture composition and water management appear to be the two main parameters which either alone or interactively control the depuration performance of BPS but also the dynamics of processes involved in pesticide dissipation; biodegradation and/or adsorption. Optimization of those parameters is expected to unravel the full potential of BPS and allow their full implementation not only

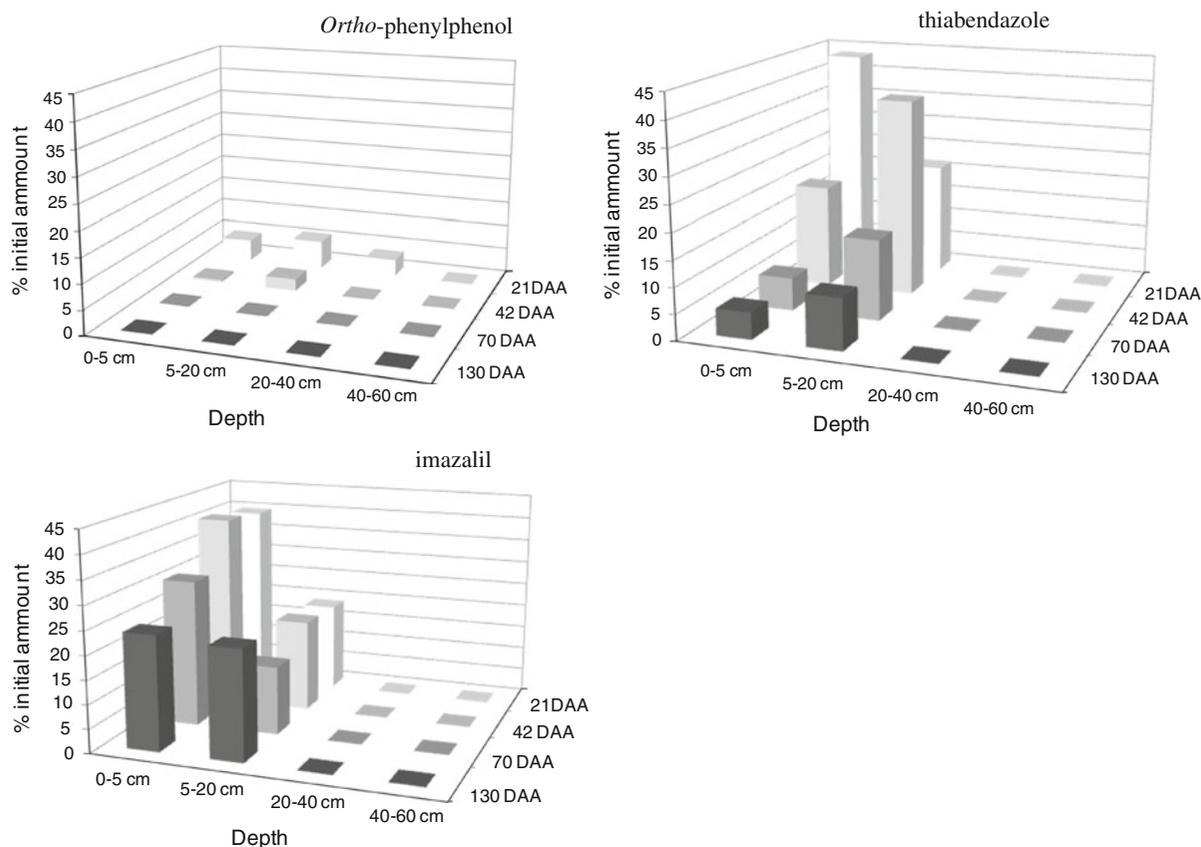


Fig. 6 The distribution of *ortho*-phenylphenol, thiabendazole, imazalil residues (% of applied amount) at different layers (0–5 cm, 5–20 cm, 20–40 cm and 40–60 cm) of the an on-farm

BPS at 21, 42, 70 and 130 days after their application (DAA) (adapted by Omirou et al. 2012 after permission)

for the depuration of wastewaters produced at on-farm level but also for pesticide-contaminated wastewaters produced by other agro-industrial activities like the postharvest treatment of fruits. Such optimization could involve the (a) use of locally produced biomixtures with high degradation capacity in order to minimize the risk for pesticide build-up after prolonged use and (b) implementation of large storage tanks which will allow farmers to regulate wastewater input onto BPS and maintain optimum moisture conditions for maximum biodegradation. However, optimization of the depuration performance of BPS would be difficult without a good understanding of the microbial dynamics within the core of BPS. The application of novel high resolution molecular and biochemical tools could shed light into pesticide-microbe interactions within BPS.

Despite the extensive research advances in BPS during the last 20 years there are still certain

shortcomings which should be the target of future research. These are the lack of well-tested procedures for the safe disposal and handling of spent BPS substrates and the reduced depuration capacity of BPS against mobile or recalcitrant chemicals. Biotechnological augmentation of BPS could provide viable solutions for the latter.

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