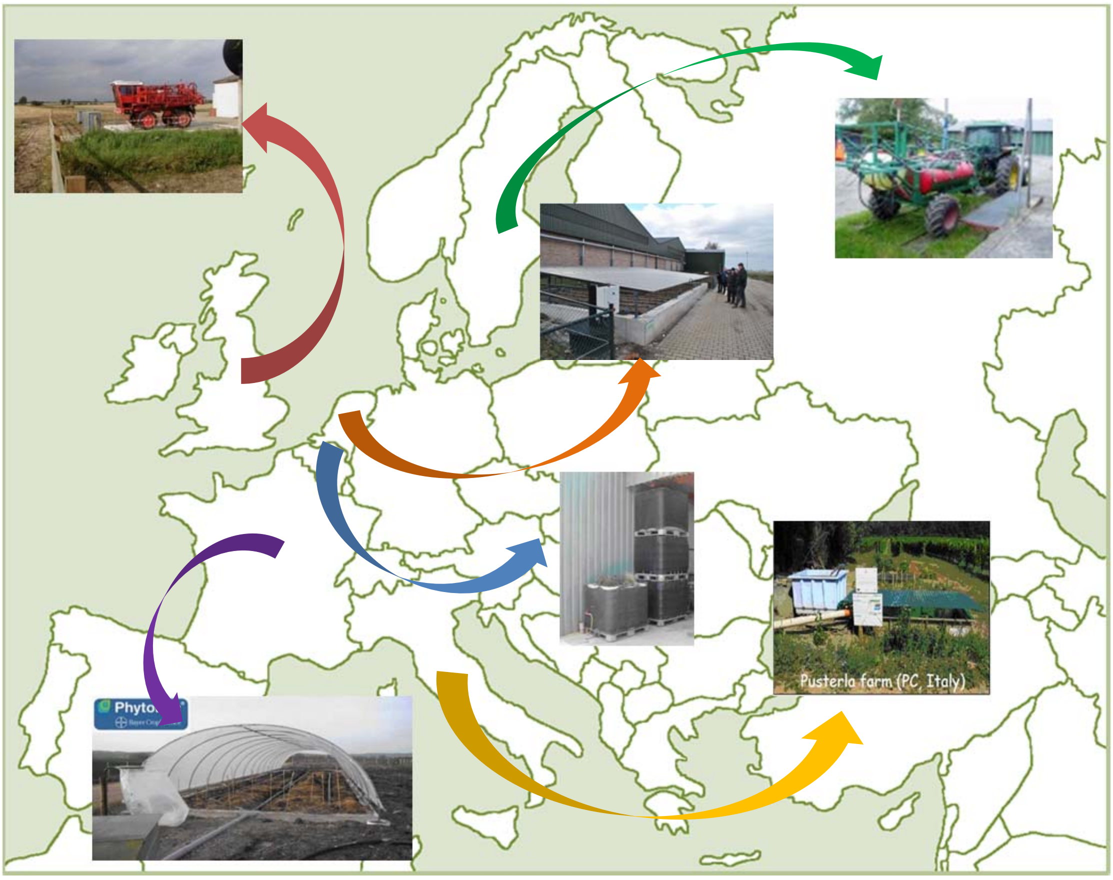
4th European Biobed Workshop



Applied Plant Research (PPO), Wageningen UR

Wageningen, Netherlands

March 20th – 21st 2013

WAGENINGEN UR For quality of life

JTI – Swedish Institute of Agricultural and Environmental Engineering

Bayer CropScience

4th European Biobed Workshop Program

20 March, 2013		
Time	*Name*	
09:00		Registration
09:15	de Werd, R.	Welcome
09:20	Husby, J.	The Biobed Workshop and Biobeds in the World
Session 1: Biological activity and degradation of pesticides in biomixtures		
Chair: Esperanza Romero		
09:30	Mukherjee, S.	Effect of novel biofilter material on microbial respiration
09:50	Elgueta, S.	Fungal pellets for bioaugmentation of a biomixture of biobed
10:10	Springael, D.	Microbiomics, metabolomics and mobilomics suggest a high level of genetic adaptation towards pesticide biodegradation in on farm biopurification systems
10:30	Gebler, L.	Ecotoxicological evaluation of different biobed substrates in Brazil: preliminary results
10:50	Diez, M.C.	Biopurification system for pesticides degradation: an integral study
Session 2: Biomixture composition and degradation of pesticides		
Chair: Rik de Werd		
11:10	Romero, E.	Vermicomposts as components of biomixtures to prevent the punctual contamination of pesticides. Persistence and enzyme activities
11:30	Dakhel, N.	Efficiency of several biomix to retain and degrade pesticides in biobeds under Swiss pedo-climatic conditions
11:50	Gao, W.	Application of Biobeds in China
12:10	Bozdogan, A.M.	Determination of the efficiency of wastewater sludge in biomix
12:30		***LUNCH***
Session 3: Biopurification systems in practice: developments and new applications		
Chair: Dimitrios Karpouzas		
13:30	Husby, J.	A new project studies the degradation of pesticides and evaporation of water in a Biotisa Phytobac® system modified for evaporation of water under North European conditions
13:45	Svensson, S.A.	Could biobeds be of any use in greenhouse production?
14:05	Doruchowski, G.	VERTIBAC - Bioremediation system for neutralisation of pesticides in liquid remnants
14:25	Karpouzas, D.	The use of biobeds for the depuration of wastewaters from the fruit packaging industry – Turning from on-farm to post-farm applications
14:45	Catroux, G.	How to improve Biobed efficacy?
15:00	Oudin, E.	Use of pesticides at the farm level: Biobed in a more complete device
15:15		***COFFEE***
Session 4: Current situation of biopurification systems by country		
Chair: Jens Husby		
15:45	de Werd, R.	Biofilter & Fytobac systems in the Netherlands. Testing, demonstration & implementation
16:05	Basford, B.	Bioremediation systems in the UK. Current position, systems description, regulation and future possibilities
16:25	Pizzul, L.	Biobeds in Sweden. State of the art
16:45		Final discussion
18:30		***DINNER***

21 March, 2013	
Study visit to a biobed at the Verhoeven firma in Erp	
08:30	The bus leaves from the ReeHorst hotel to Erp
09:30	Arrival to Erp
11:30	Departure from Erp
12:30	***LUNCH in the little Dutch town of Haarzuilens***
15:00	Arrival to the Schiphol airport

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Abstracts

The Biobed Workshop and Biobeds in the World

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The 4th European Biobed Workshop in Wageningen, Netherlands takes place 3 years after the last workshop in Piacenza. After the Piacenza workshop we made a survey asking people how often they thought a biobed workshop should take place and the majority thought that every second year would be good.

The European Biobed Workshop was postponed because a Latin American Biobed Workshop came up and that took some effort and time to arrange. The Latin American Biobed Workshop was held in Chile and had representatives from most of Latin America.

The enthusiasm was high at the workshop and it was decided to make the next workshops in Guatemala in 2014 and in Brazil in 2016.

At this 4th workshop it will be a pleasure to welcome participants from outside Europa as people from Canada, USA, Brazil, Chile, China and Singapore will be joining the workshop.

Biobeds in the world

The latest country to allow biobeds is Denmark. In the end of 2012 an updated "deed for washing places" connected to the law for plant protection products now allows the use of biobeds. But there is still an unsolved problem as it is not yet allowed to spread used substrate (biomix) on farmland after composting. This means that for the time being the number of biobeds in Denmark will not grow as it has been the case in other countries where the use of biobeds are allowed and where it is possible to spray used substrate in the fields.

The numbers of biobeds in the world have increased since the last workshop in 2010. From 2010 to 2012 The Netherlands have gone from 12 to 30 biobeds, England from 150 to 360 and France from 1000 to 3000. It is not only in Europe the numbers have increased as Morocco has gone from 18 to 27 biobeds and Guatemala from 19 to 1500.

A lot of countries have started to do research on the biobed issue with Spain being the latest country in Europe, but also North- Middle- and South Asia have started up research in this area.

Used substrate

Allowance to spread used substrate can be a problem in some countries. Even though there have been done some research on the residues after composting the substrate it seems not to be sufficient documentation for some authorities – Denmark mentioned. Here it is necessary to clarify what kind of research is needed.

www.biobeds.org

All presentations from the European Biobed Workshops can be found on www.biobeds.org. The webmaster of the biobed homepage has experienced that not only researchers look at this webpage, but also students find material here. Therefore it will be useful if people working with biobeds send information to the webmaster in order to create more interest in the biobed issue.

This workshop

The organizers of this workshop are: Rik de Werd, Wageningen UR, Dr. Maria del Pilar Castillo and Dr. Leticia Pizzul from the Swedish Institute of Agricultural and Environmental Engineering, Henk

Messelink and Antoinette Tijdeman from Bayer CropScience, Netherlands and Jens Husby from Bayer CropScience, Nordic.

Food, auditorium, abstract book and other costs have been sponsored by Bayer CropScience.

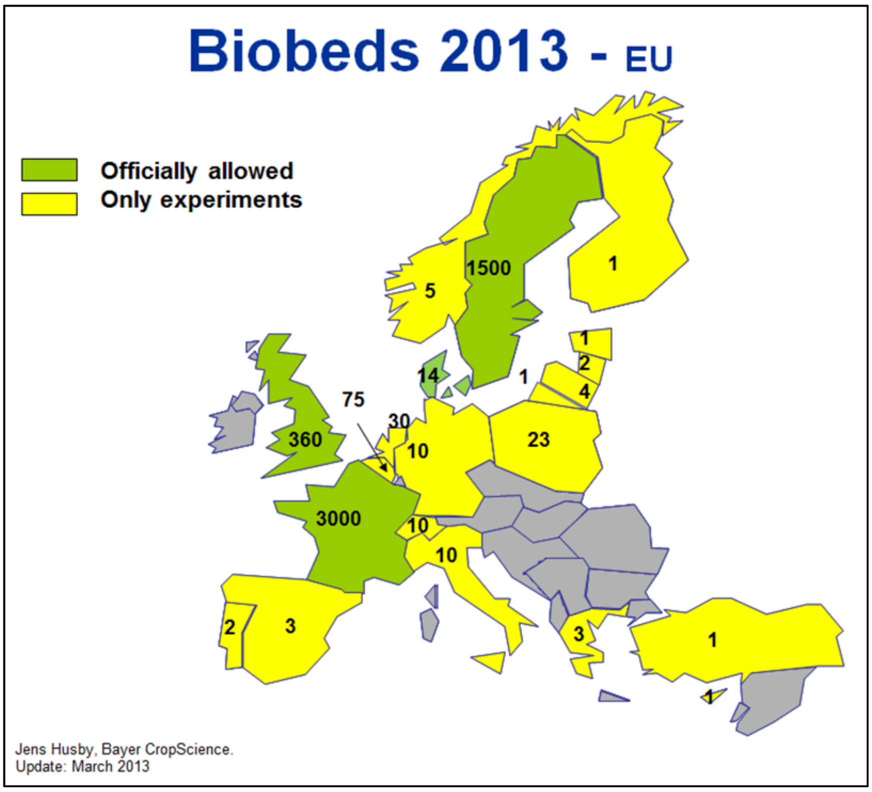

__Figure 1__. An estimate of biobeds and other bioremediation systems in Europe.

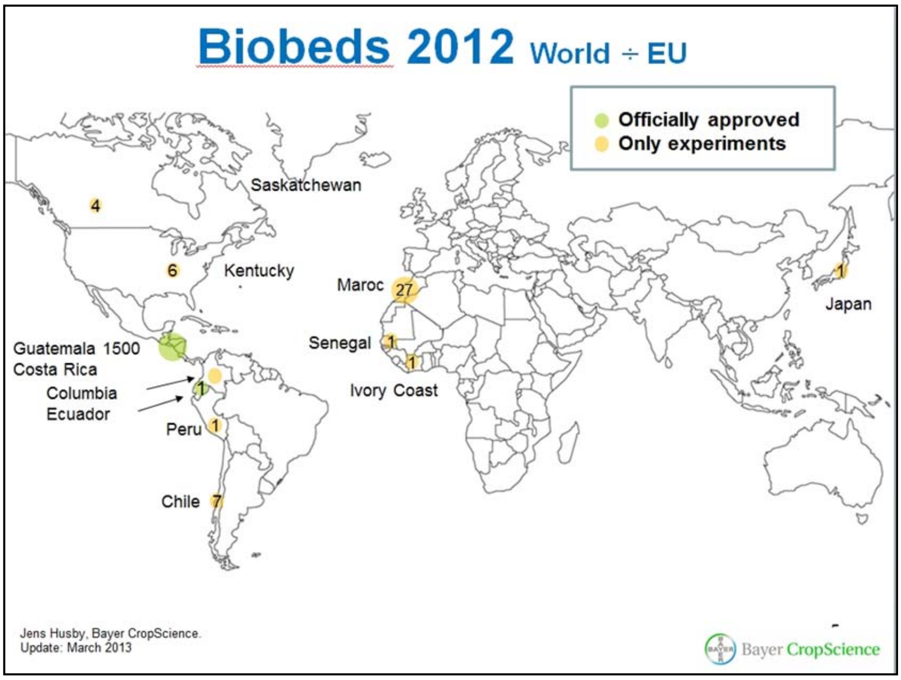

***Figure 2.** An estimate of biobeds and other bioremediation systems in the world ÷ Europe.*

Effect of novel biofilter material on microbial respiration*

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To overcome the problem of point sources pollutions on farms stemming from improper handling, spillages and leakages during filling and cleaning of spraying equipment, an environmental friendly and low-cost technology filter system is under development. A suitable ratio of biomixtures (soil, biochar and digestate) will be used in the final technical set-up of biofilters. Therefore different fundamental processes like microbial respiration, sorption-desorption, degradation, and transport /retardation behavior of three radiolabelled pesticides (Bentazone, Boscalid and Pyrimethanil) will be investigated. Respirometric measurements of microbial activity revealed that CO_2 evolution was significantly suppressed after the addition of biochar. The exact driving mechanism for this suppression is still under investigation. In this presentation, I will focus on the impact of the above mentioned organic amendments on soil microbial respiratory activity.

* The title implies the fact that a study has been conducted to investigate the impact or effect of improved biofilter materials (char and digestate) on microbial respiration (the metabolic activities of soil microorganisms which can be measured by CO_2 production and/or O_2 consumption).

Fungal pellets for bioaugmentation of a biomixture of biobed

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Biobeds provide a matrix that absorbs and facilitates biodegradation of pesticides due to microbial activity, especially by white-rot fungi (WRF). Although the specific role of enzymes in pesticide degradation has not been established yet, evidence suggests that lignin degrading enzyme complex is responsible at least in part for the degrading capabilities of these fungi. Therefore, WRF can be used for bioaugmentation to improve biological activities and biodegradation of organic contaminants in the soil. The aim of this study is to evaluate the effect of different fungal pellets of white-rot fungi in a biomixture contaminated with atrazine.

Fungal pellets of *Anthracophyllum discolor, Stereum hirsutum* RU-104*, Inonotus sp, Stereum hirsutum* Sp1*,* and *Trametes versicolor* were formulated including lignocellulosic supports and oligosaccharides. The biomixture was prepared by mixing an allophonic top soil (Andisol), peat, wheat straw in a volumetric proportion of 1:1:2 and was inoculated with fungal pellets (10% w/w). The biomixture was contaminated with 80 mg Kg^{-1} of atrazine. The concentration of atrazine was measured by HPLC. After 30 days of incubation at 20 ºC, the ligninolytic activity, fluorescein diacetate activity (FDA) and respiratory activity were studied. The biodegradation of atrazine was 100% for *Sterum hirsutum Ru-104*, being (15%) than the control (biomixture non-inoculated with fungal pellets). At the 30^{th} day the FDA activity was similar for all supports; respiration and total ligninolytic activity were higher for *Stereum hirsutum Sp1* and *Inonotus sp*. In conclusion, fungal pellets improve biological activities and degradation in biomixtures contaminated with atrazine.

Acknowledgments

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Microbiomics, metabolomics and mobilomics suggest a high level of genetic adaptation towards pesticide biodegradation in on farm biopurification systems

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Biopurification systems (BPSs) are increasingly being used at farms to treat pesticide-contaminated wastewater in order to mitigate pesticide pollution of water bodies. BPSs act as biofilters in which the contaminated water is sprayed onto a solid matrix (referred to as biomix) composed of waste materials like straw, peat and soil and in which the pesticides are removed by sorption and biodegradation processes. Plasmids and other mobile genetic elements (MGEs) collectively referred to as the mobilome, have been proposed to be important mediators in the genetic adaptation of bacteria towards pollutant biodegradation by controlling the interbacterial exchange of genetic material to generate new catabolic pathways.

On farm BPSs receive continuous loads of pesticides at relatively high concentrations during a substantial time period of the year, thus perceiving strong and long-term selective pressures for evolution and growth of pesticide degrading bacteria. It was hypothesized that due to this selective pressure, specific MGEs like IncP1 plasmids and IS1071 elements that have been often associated with organic xenobiotic degradation, will be enriched in the bacterial community of a BPS and carry genes for pesticide catabolism. Quantification of molecular markers for both mobilome and catabolic genes performed on biomix samples of as well microcosm BPS as BPS systems in operation at farms showed that this was indeed the case. The high prevalence of catabolic markers and MGE markers could be linked with high prevalence of catabolic capacities for pesticide biodegradation and haloaromatic degradation. Moreover, a novel metagenomic approach that selectively amplifies accessory genes of IncP1 plasmids and IS1071 based composite transposon structures by long range PCR was developed and successfully applied on DNA extracted from the biomix material of a BPS in operation. Sequencing of the amplified DNA revealed a total of more than 300 kb of unique DNA that was highly enriched in diverse coding sequences for organic xenobiotic catabolism including dioxygenases and dehalogenases but also many others. Also, sequencing of plasmid DNA directly extracted from the biomix revealed remarkable plasmid diversity. Our data show the extensive catabolic potential of microbiota in a BPS at the genetic level and suggest that the mobilome is an important mediator in shaping this genetic content. The results add to remove uncertainties about the biological aspect of pesticide contaminated wastewater treatment in on farm BPSs.

Acknowledgements

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Ecotoxicological evaluation of different biobed substrates in Brazil: preliminary results

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Biobeds research in Brazil has been conducted since 2011, through a research project funded by Research National Council (CNPq), which seeks to elucidate if there are differences on results based on the environmental conditions in Sweden and Brazil plus an adaptive testing of new alternative substrates.

Due to the lack of analytical support to evaluate chemical degradation for this project, it was chosen to do the ecotoxicological monitoring of reactors through bioindicators (behaviour of earthworms and microbial response). The tests followed the methodology proposed by Monteiro and Frighetto (2000).

The reactors were structured in two crates 3x9 spaces (27 cells), each with a PVC pipe (100 mm diameter) filled with the chosen substrates, with free drainage and a height of 1.5 m, prepared around 30 days before the beginning of the experiment.

Treatments were arranged in blocks of 3x3 (9 cells), each one following these settings: S - Orchard soil collected at 10 cm depth; SS - mixture of soil and debris from pruning peach orchard milled and semi-composted for 3 months; STP - Swedish standard substrate, with wheat straw, peat moss and orchard soil, tested at 0 to 50 cm, 50 to 100 cm and 100 to 150 cm height.

On each block was applied in a single dose, a load of one the following pesticides in their commercial undiluted form (simulating an accidental spill): a) 10 mL of insecticide Lorsban 480 BR© (Chlorpyrifos 480 g L^{-1}), b) 50 mL Finale© herbicide (200 g L^{-1} glufosinate), c) 50 mL mixture of pesticides (Finale© 40 mL and 10 mL Lorsban BR 480©).

The initial collection time (T0) was performed 48 hours after the contamination with the pesticides. Further evaluations follow a period of 90 days between each sample collection, always performing the analysis for each of the three depths tested.

Preliminary results of microbial activity (comparison of evaluations taken at T0 and T1), show the increase of activity (in the order of 100% in most cases), with lower response in the middle region of the reactors (between 0.5 and 1.0 m depth), probably due to low aeration, proposition based on the response of the reactor BM-SPT-T1, filled with standard substrate for Swedish biobeds, which allows aeration even at high depth. The results can be seen in figure 1.

The response of toxicological testing has demonstrated worms with unusual behaviour with regard to the escape of animals to out of contaminated samples, as occurred during the analysis at T0. At this initial stage, the worms were mostly found in non-contaminated samples of soil and of substrate, with little mortality over the surface. However at T1, it was found that most of the test population was dead, at the surface, or alive, buried in the sample of contaminated soil. This behaviour was not expected, but may be linked to the pursuit by the animals for food, in this case, debris generated by intensity of microbial activity in these portions of the soil. At same time, it showed that the worms were not sensing these levels of insecticide, not running away from these plots.

The death on the surface, which occurred mainly in samples contaminated with chlorpyrifos, could be explained by a repellence pulse, where the victims may have been sensing the chronicle poisoning by toxic gases only when they were too weak to escape. The poison was volatilized from soil contaminated by the insecticide, once the smell was still easily identified in the air.

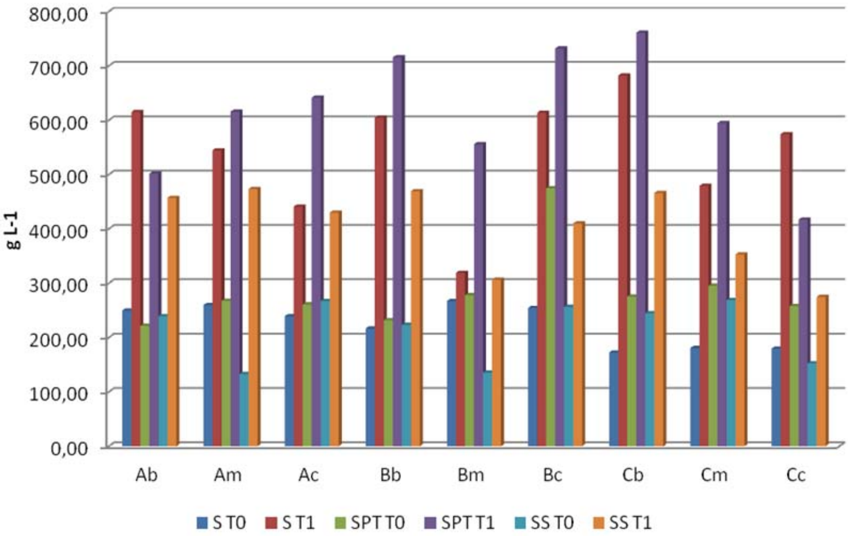

Figure 1. *Microbiological activity by fluorescein acetate, were A= contamination with chlorpyrifos, B= contamination with glufosinate, C= mixture of pesticides; b=bottom of reactor, m=medium of reactor, c=top of reactor; S= Soil, SPT=Swedish substrate, SS=50% soil+50% branches of fruit trees shredded and composted; T0=2 days after pesticide application, T1=3 months after T0.*

At the plots that had only the presence of herbicide, no adverse effect on the test population was noted, and worms were found alive and mostly buried in the contaminated sample under analysis. However, there were greater presences of live animals buried at the plots contaminated with the pesticide mixture than those at plots only with insecticide, but less than those at plots with only herbicide. It may be suggested that there was a possible synergism between worms and the herbicide that protected them of the effect of insecticide. It must be better explained in the future.

The preliminary results indicate that the Swedish substrate showed better responses for microbial activity, indicating greater efficiency degradation of pesticides in this substrate. The behaviour of these bioindicators should be analysed along 2013, in the next sequence of samplings and analysis.

References

Monteiro, R.T.R.and Frighetto, R.T.S. Determinação da umidade, pH e capacidade de retenção de água no solo. In: Frighetto, R.T.S. and Valarini, P.J. (Ed.). Indicadores biológicos e bioquímicos da qualidade do solo. Jaguariúna: Embrapa Meio Ambiente, 2000. p. 37-44.

Biopurification system for pesticides degradation: an integral study

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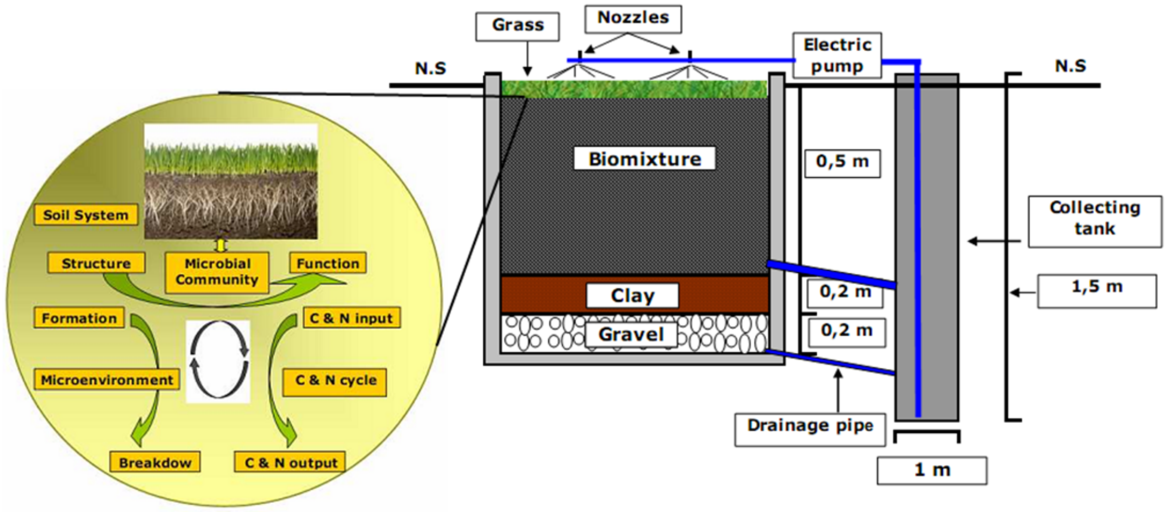
Biobeds are a biological system originated in Sweden as a response to minimize environmental contamination from pesticide manipulation, especially when filling the spraying equipment, a typical point source of contamination. Biobeds are based on the adsorption and degradation potential of organic biomixtures composed of top soil, peat, and straw and covered with grass. Biobeds with some modifications can be used for treating pesticide waste and washings, providing a matrix to absorb the pesticides and facilitate their biodegradation. These systems can be self-built and managed by farmers for their specific farm situation. However, incorrect dimensioning and management of these systems can seriously affect their efficiency.

Biobeds have been successfully implemented for point-source pesticides contamination treatment (which represents small volumes of liquid), generally with high concentration and complex mixtures of pesticides. However, not high volumes of contaminated water with pesticides can be treated in this system due to Swedish biobed has generally no impermeable synthetic layer that isolates it from the ground (unlined biobed). Therefore, it is necessary the implementation and optimization of a technology (lined biobed for example) that can treat high volumes of contaminated water with pesticides with an adequate recirculation system.

On the other hand it is known that the grass layer on top of biobed keeps a good moisture balance by evaporating excess water and preventing the top layer from drying out. Also, the root system can improve soil conditions for microorganisms, which are responsible for the degradation of organic pollutants. However, contaminated water directly onto a biobed may cause phytotoxicity of the grass cover if not sufficiently diluted. In this way the incorporation of tolerant-pesticides plant species to cover the biobed may help to reduce the volume of water by evaporation or absorption at the same time that the rhizosphere improve biomix condition for pesticides degradation. In this way, there it is known that pesticide metabolism of plants and microbial has fundamental similarities and differences, but there is a little known about how these metabolic pathways interact with each other to produce pesticide biotransformation and mineralization.

We hypothesize that the presence of pesticide-tolerant plant species together with adequate operational conditions in the biopurification system (lined biobed), would improve the interactions between microorganisms and biomix, improving the water consumption, resulting in more efficient pesticides degradation.

In our project, we will study the global effect of plant-microorganisms-biomix interactions and operational conditions of lined biobed on pesticides degradation. We will evaluate biomix of biobed prepared with 3 types of soil (sandy soil, red clay soil and organic soil). We will select vegetal forage species tolerant to high levels of pesticides; we will study microbial diversity and resistant-bacteria involved in pesticides degradation in a microcosm study; at the same time, we will evaluate operational conditions such as different feeding rate and strategy of feed (continuous or intermittent) and different depth of the biobeds on pesticides degradation; finally, we will integrate the mayor metabolic pathways associated with pesticide degradation in the biopurification system.



Acknowledgements

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Vermicomposts as components of biomixtures to prevent the punctual contamination of pesticides. Persistence and enzyme activities

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In Spain it is crucial to establish low-cost remediation systems to control the punctual contamination by the use of pesticides in the crop systems. For this reason, the aim of this study was to investigate the potential application of different vermicomposts obtained from agroindustrial wastes related to the olive-oil, wine or horticultural activities, as peat substitutes, in biopurification systems to avoid unwanted risks of pesticides. Five enzyme activities related to the C, N and P cycles were measured to check the pesticide impact on the microbial functions in the different biomixtures trials.

Three vermicomposts obtained from wet olive cake, vine-shoot and biosolid vinasse and refused tomato fruit which are some of the most abundant organic wastes generated in olive orchards (O), vineyards (W) and greenhouse (G) crop systems or in their respective agroindustries were selected as components of the biomixtures. These stabilised substrates were assayed alone in some biomixtures (OV, WV, GV) or mixed respectively with chopped olive pruning, vine-shoot and tomato plant-wastes as textured materials (OM, WM, GM). A peat biomixture (P, peat + barley straw) that often has been used for this purpose was used as referent in each case (OP, WP, GP). The biomixtures were composed of 25% alkaline silty clay loam soil (organic carbon content 23 g kg-1), 50% vermicompost or peat and 25 % of the textured materials except those assayed with vermicompost alone that has 75% of the vermicomposts. Triplicate samples (80 g) of each biomixture were contaminated with 4ml from a pesticide standard solution in acetone containing a mixture of the most common pesticides applied in olive orchards (O): dimetoate, diuron, imidacloprid, oxyfluorfen and tebuconazol; vineyards (W): imidacloprid, metalaxyl, oxyfluorfen and tebuconazol and horticultural greenhouses conditions (G): acetamiprid, imidacloprid, metalaxyl and tebuconazol. The biomixtures were air dried to eliminate the solvent, homogenized, moistened at 80 % of the field capacity and incubated at 20ºC for 0, 3, 7, 14, 28, 42, 84 and 140 days. Water was added when necessary. After incubation, subsamples were extracted twice in an ultrasonic bath with 30 ml of acetone and centrifuged. The collected extracts were mixed, evaporated, dissolved in acetonitrile (10ml), diluted and filtered by 0.2µm before being analysed by HPLC with DAD. Other three subsamples per sample were used to quantify the dehydrogenase, β-glucosidase, urease, acid phosphatase and ortho-diphenol oxidase activities.

The pesticide extraction efficiency in G, V and O biomixtures was higher than 91%, 82% and 86%, respectively. The pesticide degradation kinetics mainly follows first-order kinetic reaction (SFO) or biexponential equations (DFOP) with the exception of particular biomixtures where imidacloprid, oxyfluorfen and tebuconazol follows Hockey stick (HSM) or compartiment (FOMC) models. The quickest degradation rate corresponds to the herbicide dimetoate in the olive biomixtures with DT50 and DT90 values of 4 and 14 d for OV and 9 and 29 d for OM and 17 and 58 d for OP, respectively, which indicate a total degradation of this insecticide during the incubation period in the three olive biomixtures. Diuron, imidacloprid and oxyfluorfen have a greater persistence than dimetoate but the most persistent was tebuconazol. In the vineyard biomixtures, the fungicide metalaxyl was in the peat biomixture (WP) 2 and 7 times more persistent than in WV and WM, respectively. In greenhouse biomixtures, acetamiprid was the least persistent with DT50 and DT90 values < 30 d and <100 d, respectively being totally degraded during the incubation period. However, metalaxil was more persistent in GV and GM (DT50 68 and 78d, respectively) than when it was applied in the vineyard pesticide mixture (DT50 < 37d) but in the peat-biomixture GP the opposite occurs. It can be disregarded that the peat-biomixtures have lower pHs than the vermicompost-biomixtures which especially affects the persistence of the insecticide acetamiprid and the fungicide metalaxil which have very low pKa. Tebuconazol was degraded more significantly in the greenhouse mixtures (DT50 < 94 d) in respect to the others. It was observed that the pesticides were degraded in vermicompost biomixtures quicker than in the peat biomixtures. In addition, it was also observed that the addition of the textured materials contributed to increase the degradation time of the pesticides (OM>OV, VM>VV and (GM>GV).

The lower persistence of dimetoate, metalaxil acetamiprid and diuron compared to imidacloprid, tebuconazol and oxyfluorfen can not only be related to its high solubility but also to their chemical structures. The persistence can also be related to sorption capability of the different organic matrices studied. It was described that the sorption of imidacloprid and diuron was directly related to the lignin content of the organic matrices (Romero et al., 2006). Imidacloprid sorption increased on organic matrices when pH decreased (Cox et al., 1998).

The different enzyme activities studied were lower in the greenhouse and peat biomixtures respect to the olive and vineyard vermicompost biomixtures. However, the pesticide impact on the microbial functions in these biomixtures estimated from the relative activity of the treated biomixtures respect to the untreated control revealed similar o lower activities in all the systems due to the pesticide application.

Acknowledgements

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Efficiency of several biomix to retain and degrade pesticides in biobeds under Swiss pedo-climatic conditions

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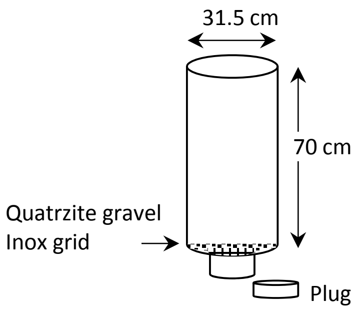
In Switzerland, biobeds are not widely used to clean up wastewater contaminated with pesticides. However, the interest about them is growing considerably and several systems have been built over the last years. There is no consensus yet regarding the nature of the biomix that should be used to fill up biobeds. The aim of this study was to test the efficiency of different substrates (1) to retain pesticides and (2) to degrade them.

For these purposes, 15 small biobeds (70 L) were constructed in PVC (Fig 1).



Biomix	Soil		Straw	Compost C1	Compost C2
	Type	%	%	%	%
S1-70/30	S1	70	30	-	-
S2-65/35	S2	65	35	-	-
S2-50/50	S2	50	50	-	-
S2-	S2	25	50	25	-
S2-	S2	25	50	-	25

Figure 1. *Biobeds design*

Table 1. *Composition of biomix (% in volume)*

Two agricultural soils (S1 and S2: 21% and 28% clay) from the region of Geneva and two industrial composts were tested (C1 and C2: 1 and 3 months composting). Five different biomix were tested (Table 1). Three Biobeds were filled with 50 L of each biomix and regularly watered during two months before pesticide application. The biobeds were closed at their bottoms and treated with 10 L tap water containing 2 herbicides (diuron, terbuthylazin) and three fungicides (fludioxonil, cyprodinil and trifloxystrobin) at concentrations ranging from 33 to 50 mg L^{-1} (approximately 10 times what is expected after the cleaning operations of a sprayer). After 16 h, plugs were removed and water was allowed to drain off and collected. An aliquot of the leachate was sampled and analysed through LC-MS/MS. This operation was repeated two times at three weeks interval at same volumes and concentrations of fungicides. Biobeds were left at outdoor temperature and protected from rain. Four months after the last treatment, biomix were sampled with an Edelman auger in the biobeds and frozen before analysis. Between 1 and 2 kg of biomix were also removed, watered during two months and analysed approximately 1 year after the last treatment. Pesticides were analysed in biomix (4 and 13 months after last treatment) with HPLC-UV-DAD after extraction with methanol (16 h).

Retention of pesticides is good in all biomix and ranging from 85 to 99.5 % (Fig. 2). However, pesticide concentrations in water after clean-up are comprised between 0.1 and 5.1 mg L^{-1}, which is above legal limits for discharge in natural water bodies. As expected, hydrophobic molecules (trifloxistrobin) are more retained than more hydrophilic ones (diuron). Biomix containing compost were the most efficient to retain pesticides.

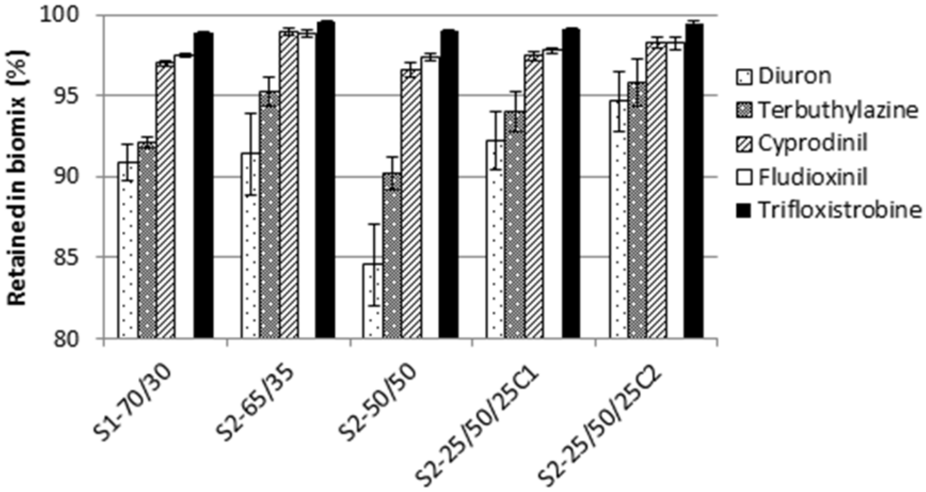


Figure 2. *Retention of pesticides in different biomix*

Analyses of the biomix 4 and 13 months after last treatment showed that some pesticides are well degraded (diuron, trifloxistrobin), whereas others are relatively stable (terbuthylazin, fludioxonil) (Fig. 3).

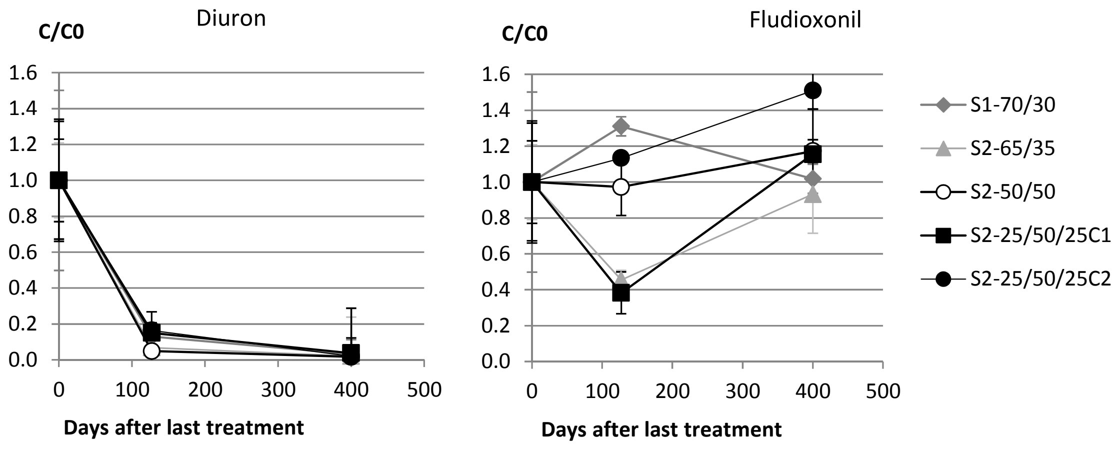


Figure 3. *Evolution of diuron and fludioxonil in biomix (C0= initial concentration)*

It is usually recommended to spread used biomix on agricultural land after a composting step. To avoid potential leaching of pesticides and to fulfill Swiss legal requirements, further investigations are necessary to better understand the fate of pesticides in biomix.

Acknowledgments

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Application of Biobeds in China

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China is one of the largest agriculture countries in the world. The intensification and modernization of farming is developing rapidly, and the utilization of pesticides is increasing accordingly. As a simple and effective technique to minimize environmental contamination from pesticide use, especially typical point source of contamination, biobed has been used in many countries, and its application in China is promising too.

By collaborating with JTI- Swedish Institute of Agricultural and Environmental Engineering, Sweden, our group was engaged in the laboratory evaluations of biobeds application to vegetable farming as the start point. For the consideration of using local resources, we studied different biomixture materials including wheat/maize straw, peat/mushroom residue and top soil in degradation of chlorothalonil and imidacloprid, which are widely used in Chinese vegetable farming.

It was found that different biomixture compositions had different effect on the degradation of the two pesticides analysed. In the degradation of chlorothalonil, wheat straw biomixture was better than the maize straw biomixture; on the other hand, mushroom residues or peat in biomixture made little difference. In the degradation of imidacloprid, peat had significant beneficial effect. The results show that substitution of peat with local mushroom residue will be applicable for the biobeds system set-up in North China.

DETERMINATION OF THE EFFICIENCY OF WASTEWATER SLUDGE IN BIOMIX*

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The aim of this study was to compare of efficiencies on adsorption and degradation of pesticide of wastewater sludge and peat in biobed. In this research; wastewater sludge was used in biobed instead of peat which is expensive in Turkey. In trials, biomixtures were used as below:

A (25% wastewater sludge + 25% farm-soil + 50% straw),

B (25% peat + 25% farm-soil + 50% straw), and

C (100% farm-soil) biomixes were used.

Fenthion a.i. (active ingredient) was used as pesticide, and 100 ml pesticide was applied to each biomixture. Three replicates were carried out for each experiment. Pesticide was analyzed by gas chromatograph equipped with nitrogen-phosphorus detector (GC-NPD). Solid phase extraction method was used in analysis. The average pesticide residue and degradation in biomixture is given in Table 1.

Table 1. Average pesticide residue and degradation

Biomix	Average Pesticide Residue (ppm)				
	0*	3	6	9	12
A	946.35	615.09	440.65	249.04	187.37
B	259.39	208.76	68.97	64.42	43.96
C	75.13	62.50	42.58	35.14	27.10

0*: Trial day.

As seen in Table 1, the highest pesticide residue was obtained in A biomix, 946.35 ppm, and the lowest pesticide residue was observed in C, 75.13 ppm at trial day. In statistical analysis, there was significantly difference between biomixtures. Pesticide residue in A biomix was 3.7 times higher than B biomix which is the standard biomixture in the world. Equations of biomixtures are given in Table 2.

As seen in Table 2, there was no statistical difference on degredation of pesticide between biomixtures. DT_{50} and DT_{90} values in biomixtures was approximetaly similar.

Table 2. Equations, DT_{50} and DT_{90} of biomixtures

Biomixture	Equation	R^2	C_{A0} (mg/kg)	DT_{50} (day)	k_{1deg} (-)	DT_{90} (day)	k_{2deg} (-)
A	$C_A = 945.12\ e^{-0.1381t}$	0.99	946.35	5.0	0.139	16.7	0.138
B	$C_A = 260.23\ e^{-0.1575t}$	0.91	259.39	4.4	0.158	14.6	0.158
C	$C_A = 76.399\ e^{-0.0872t}$	0.99	75.13	8.1	0.086	26.6	0.087

C_A : Residue (mg/kg)

t : Time (day)

R^2 : Coefficient of determination

DT_{50} : The time required for the pesticide concentration to decline to 50% of the amount at application (day),

DT_{90} : The time required for the pesticide concentration to decline to 90% of the amount at application (day).

k_1, k_2 : Decline constant ($days^{-1}$)

C_{A0} : Initial Residue (mg/kg)

Consequently, in this study, there was statistical difference between pesticides residues in biomixture. Yet, there was no statistical difference between degradation of pesticide in biomixture. It was concluded that wastewater sludge can be used instead of peat in biobed which is used on reducing of pesticide contaminated waters during filling, mixing, and cleaning of sprayers in Turkey.

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A new project studies the degradation of pesticides and evaporation of water in a Biotisa Phytobac® system modified for evaporation of water under North European conditions

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Due to new regulations in Denmark waste water and rainwater from sites where sprayers are filled or cleaned have to be collected. For farms with manure tanks it is not a problem, but for plant production farms and other users of pesticides it might be time consuming to collect pesticide waste water and bring it to the field.

A new project financed by Bayer Crop Science has combined a Biotisa Phytobac® system with a washing area and recirculation of the water. The aim of the project is, under Nordic conditions, to measure pesticide degradation in the substrate when it is taken out in compost and evaporation from the substrate and in the washing area.

During filling and cleaning of sprayers water is pumped into the Phytobac buffer tank. Afterwards rain water from the washing area is pumped into the rainwater container and recirculated to the washing area for evaporation. Concurrently water from the Phytobac buffer Biotisa tank is circulated through the two tanks with substrate for adsorption and degradation of the pesticide residues.

After establishment of the plant in spring 2012 the first application of 20 different pesticides was carried out 8th June 2012. A second application of the same pesticides was carried out 27th September 2012. Samples of water and substrate were taken for pesticide residue analysis. Meteorological data including precipitation were collected and the water balances for the two water circulation systems were calculated. During winter time from November to April the washing area is covered and the system is switched off.

At present there are only preliminary data available. For most of the pesticides the dissipation rate in the buffer tank was relatively fast. As expected the degradation rate in the substrate is to a very high degree dependent of the intrinsic properties of the different pesticides. Analyses of the water from the rain water container show that it is very important that the washing area and the sink and pipes are efficiently cleaned before the valve is switched from the Phytobac buffer tank to the rain water tanks in order to keep the pesticide concentration low.

The water balance results from the first season seem to be promising and it should be possible to run the system without problems with surplus water, which it is necessary to transport to the field.

The project will run during the next years. In the spring substrate material will be stacked to compost and the pesticide content will be measured to check how long it takes for the tested pesticides to get down to an acceptable level in order to spread it on the fields.

There will also be looked for accelerated degradation in the substrate and the compost. More application of pesticides to the system will be carried out.

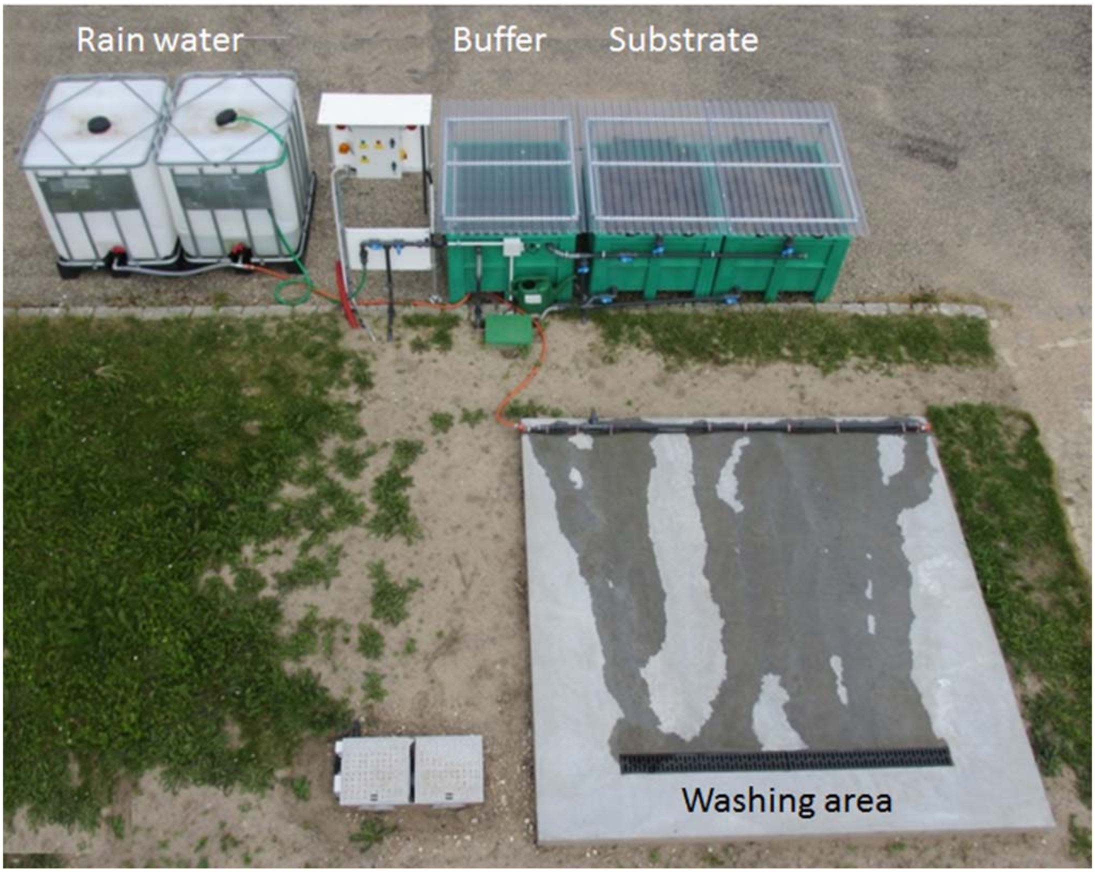

Figure 1. *Modified Biotisa Phytobac® system*

Could biobeds be of any use in greenhouse production?

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Horticultural production in greenhouses is intensive, and in most cases, an all year-round activity. The use of pesticides in greenhouses was earlier regarded, formally at least, as if greenhouses were closed systems, isolated from surrounding soil, air, and water. Therefore, most studies conducted in the past did not include parameters outside of greenhouses. Several studies conducted recently included pesticide residues in waterways downstream greenhouses (Kreuger et al., 2009; Roseth & Haarstad, 2010).

These studies, together with other investigations (i.e. EFSA; 2012), made it clear that greenhouses also contribute to soil and water pollution. However, it is still not clear whether point or diffuse sources dominate the greenhouse pollution. Variations noted in Europe, such as different constructions, water sources, production systems, etc., make it difficult to generalize the source of pollution associated with greenhouses.

The first preliminary results from pesticide handling in greenhouses were presented in 2007 at the 2nd European Biobed Workshop in Ghent. The objective was to transform identified shortcomings into improvements; to reduce the risk of accidents and minimize the consequences, as much as possible, when applied in growers' situations. All operations, except spraying, were studied. A general risk investigation was also carried out (Löfkvist et al., 2009).

The following improvements for handling plant protection products in greenhouses were suggested by Löfkvist & Svensson (2012):

- **Filling station**: A filling/mixing/cleaning station was developed and tested by growers and advisers, suggesting further improvements. It was important to facilitate the handling work and, by that, reduce the temptation of handling pesticides at inexpedient places.
- **Central position of filling station**: We suggest a filling station, combined with a storage facility, at a central position of the greenhouse plan, to avoid risky transports.
- **Mobile filling station**: For larger crop operations, where several spray tanks are filled during one spray application, we suggest sprayer available on the sprayer to prevent and/or collect accidental spills.
- **Improved sprayer design**: Sprayer manufacturers should include a protective cover of electrical engines when they design the equipment to make the sprayer safe to clean.
- **Avoiding floor drains**: We strongly advise that users should avoid all measuring, mixing and cleaning operations close to sinks and floor drains that are connected to ditches or sewage systems.

This is a first step, to avoid undesirable consequences resulting from accidents, bad planning and faulty equipment. However, this will not be enough to prevent other sources of leakage, resulting from the spray operation, from adding pesticides to irrigation water, from condensation water from glass surfaces, etc.

Several projects, initiated by the Federation of Swedish Farmers (LRF), have been granted to a project group with members from the Swedish Institute of Agricultural and Environmental

Engineering (JTI), Swedish University of Agricultural Sciences (SLU), as well as private advisory companies.

The objective of these projects is to develop knowledge on different pesticide leakage sources and flows in greenhouses and nurseries, as well as suggest methods and systems to avoid different leakages.

In one project, pesticide residues are measured in different depths of the greenhouse ground, to understand the risk for downwards leakage. Another project arranges grower courses, to inform and stimulate the use of recirculating irrigation systems, as well as measures to avoid accidental spills. There are other projects, to develop new and innovative mitigation methods, based on the high adsorptive capacity of biochar.

Biobeds or similar systems are rare in the greenhouses. In the first project related to pesticide handling, mentioned above, we advised designated areas for filling, cleaning and parking to be connected either to a biobed or a separate container (for later safe discharge). We see problems still to be solved for the conventional use of biobeds in covered crop production. How could the new and improved understanding and knowledge of biobeds, bioreactors, biopurification systems, bioremediation equipment, etc., be implemented in the greenhouse context?

Some issues must be discussed:

- Indoor biobeds are regarded as breeding places for pests and should be avoided.
- Most greenhouses are in use all year around. Outdoor biobeds in cold climates would suffer from low temperatures. A few outdoor biobeds with electrical heating have been introduced.
- Most greenhouse floors are biologically inactive (sand/gravel/concrete). Parts of the spray deposits on these surfaces. It would be desirable if these losses could be eliminated or neutralized.
- It is important to find suitable procedures/working routines, where use of biobeds is involved in a logical manner, as opposed to a stand-alone component.
- Depending on raw water quality, pesticide use, irrigation principles, etc., large volumes of polluted water from irrigation systems or from filter cleaning must be discharged.

The finished projects were financed through Department of Agrosystems and Centre for Chemical Pesticides (both SLU), the Swedish Board of Agriculture and the Swedish Farmers' Foundation for Agricultural Research (SLF). On-going projects are financed by the Swedish Environmental Protection Agency, SLF, the Swedish Board of Agriculture and Tillväxt Trädgård (cooperation Academy – Industry).

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VERTIBAC - Bioremediation system for neutralisation of pesticides in liquid remnants

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Introduction

The Water Framework Directive 2000/60/EC (WFD) and the Sustainable Use Directive 2009/128/EC put an obligation on Member States to implement measures that can reduce the risk of water contamination by pesticides from point and diffuse sources. The post treatment activities such as cleaning of sprayers, and management of contaminated water after cleaning pose particular risk of point source pollution. This risk can be mitigated by enhancing the farm infrastructure and implementing safe procedures of sprayer cleaning and liquid remnants management. Bioremediation, where cometabolic processes are used to degrade active ingredients of pesticides by enzymes produced during microbial metabolism in soil-type substrate, was found to be very effective [Zablotowicz et al., 1998; Bending et al., 2006]. The systems like Biobed, Phytobac or Biofilter, are offered for installation in farms as a safe and practical way to neutralize the contaminated liquid remnants [De Wilde et al., 2007].

In the countries or regions with a high number of small fruit growing farms, where PPPs are used intensively, and hence sprayers are cleaned frequently the high density of potential point sources pose particularly high risk of water contamination. On the other hand these small farms have limited space in the farmyard and limited investment potential which prevents from establishment of big and costly bioremediation systems. The objective of this work was to develop a compact and economic bioremediation stand to neutralize liquid remnants from sprayer cleaning on small and medium sized farms, and to evaluate the pesticide degradation and water evaporation capacity of the system.

Materials and Methods

The air-assisted fruit-crop sprayer was externally cleaned with low- and high-pressure methods, according to the procedure of the ISO standard 22368-2:2004, after application of fluorescent dye (BSF) in order to evaluate the amount of substance washed off the sprayer and volume of water used.

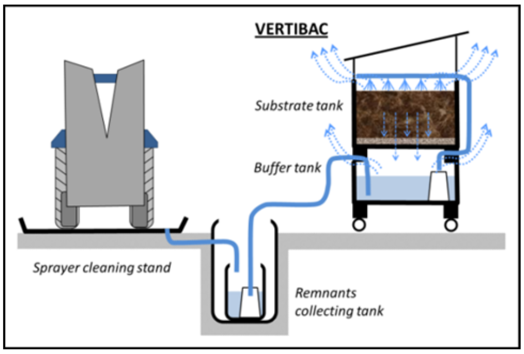

Figure 1. *Vertibac – a compact bioremediation stand for neutralization of liquid remnants.*

A compact bioremediation stand VERTIBAC (Fig. 1), consisting of the lower buffer tank for liquid remnants and effluents (max 400 l), and the upper tank with a classic substrate composition (500 l): straw, soil and peat (vol: 50%/25%/25%), was developed to neutralize contaminated water after sprayer cleaning. The stand was loaded with pesticides over the season 2012, on dates and frequency simulating very intensive programme of apple orchard protection. The samples of substrate were taken to evaluate the effectiveness of pesticide degradation. The volume of liquid remnant inputs and effluents was measured to evaluate the water evaporation capacity of the system.

Results and Conclusions

During the external cleaning of the sprayer (17 l of water), followed by rinsing the surface of cleaning stand (10 l of water), in average 0.55% and 0.78% of the applied product dose was washed off with low- and high-pressure method respectively. In order to test the Vertibac stand in the worst case scenario it was assumed that the liquid remnants contain 1% of the applied dose and that the sprayer is used at 5 ha of orchard. This resulted in pesticide input into the stand amounting 5% of the recommended dose in 27 l of water at each time following from the simulated programme of orchard protection (every 5 to 15 days). The analysis of pesticide residue in substrate was carried out from April to October 2012. The measurements of water evaporation showed that in average the total daily evaporation rate was 3,8 l in May and 8,3 l in August. The total evaporation over 5 months (May-September) was over 1000 l of water. By October 1 most of pesticides degraded almost completely (93,7 – 100%), and only the moderately persistent ones degraded in 88% due to late application and short time for biodegradation. The compact and simple VERTIBAC stand is intended to be used at small and medium horticultural farms, where it is efficient enough to manage the liquid remnants produced after frequent PPP applications.

Acknowledgements

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The use of biobeds for the depuration of wastewaters from the fruit packaging industry – Turning from *on-farm* to *post-farm* applications

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Fruit production is one of the most dynamic agricultural sectors in EU. Upon their harvest fruits are subjected to postharvest treatments with fungicides (thiabendazole, imazalil, 2-phenylphenol) and antioxidants (diphenylamine) to be protected during storage from fungal infestations and the appearance of apple scald. These applications results in the production of large wastewater volumes (10-50 m^3) with high pesticide concentrations (100-200 mg/L) whose direct discharge into natural resources constitutes a serious environmental concern considering the long soil persistence of thiabendazole and imazalil, and the high toxicity of 2-phenylphenol, diphenylamine and thiabendazole to aquatics. The risk associated with the direct environmental release of these wastewaters has been identified by the EC, which has granted authorization for use of these pesticides under the clause that *Member States should ensure that appropriate waste management practices to handle the waste solution remaining after application are put in place*. Despite that no treatment systems are available at EU level and thus urgent action is needed. We have explored the possibility of using modified biobed systems for the depuration of wastewaters from the fruit packaging industry. The design of the biobed systems was modified in order to accommodate the particular characteristics of the fruit packaging industry: large wastewater volumes produced within a short time period (3-4 months) and high pesticide loads.

Preliminary laboratory studies indicated that a biomixture consisted of soil – straw – spent mushroom substrate (25 – 25 – 50% by vol.) stimulated the degradation of the pesticides used in fruit packaging plants. Subsequent leaching column studies showed that spent mushroom substrate either alone or in a mixture with soil and straw was able to restrict the leaching of 2-phenylphenol and imazalil. Similar results were obtained in laboratory and column leaching studies performed in Cyprus with a different biomixture (soil 25% – straw 50%– compost of grape seeds and skins 25% by vol.) (Omirou et al. 2012). On-going studies in pilot biobeds in Greece are assessing their depuration capacity and explore the possibility of bioaugmenting the depuration capacity of these systems via inoculation with bacteria able to specifically degrade diphenylamine, 2-phenylphenol and thiabendazole. In addition, recently completed studies in a full-scale biobed in Cyprus showed that thiabendazole, imazalil and 2-phenylphenol were fully retained and partially or fully dissipated providing first evidence for the potential of biobeds to offer a viable solution for the depuration of pesticide-contaminated wastewaters produced from a dynamic agroindustrial sector.

References

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How to improve Biobed efficacy?

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Biobeds are devices used to treat effluents containing pesticides, produced during washing and rinsing of farm sprayers.

Biobeds are:

(1) able to evaporate the water from the effluents (and not the pesticides!),

(2) able to fix also temporarily the pesticides

(3) able to degrade the pesticides through the action of soil microorganisms from the substrate.

We will discuss some possibilities to improve practically these three functions of the Biobeds, like:

- a better use of pesticides fixation on the substrate
- the regulation of substrate moisture to optimize the microbial degradation of pesticides
- a better microbial adaptation in the substrate to degrade pesticides
- the increase of evaporation

We will discuss also other practical points which are generally not taken into account even if they should pose problems, like:

(1) the risks of limited contaminations in the separator network between pesticides effluents and waters not containing pesticides.

(2) the presence of solids in the effluents (soil constituents with or without pesticides).

Use of pesticides at the farm level: Biobed in a more complete device

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Biobeds are a part of a more complete device used at the farm level to store and use pesticides and to treat their residues after crop treatments.

To install successfully a Biobed, its necessary to consider the others parts of the complete device including the pesticide storage depot, the sprayer filling equipment, the filling and washing area, the collect of effluents and the separator of hydrocarbons.

Using examples of specific equipment, we will discuss the necessity when a Biobed is installed, to have before a complete analysis of the project in order to solve more easily the problems encountered.

Biofilter & Fytobac systems in the Netherlands. Testing, demonstration & implementation

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Applied Plant Research tested Biofilter and Fytobac systems under practical conditions at seven locations. Main goals were: (1) gaining more results, (2) experience, (3) and demonstrating the possibilities of on-farm bioremediation. Waste water was collected from spraying cleaning and filling. Not all water was evaporated by the Phytobac and Biofilter systems. Therefore treated water could be sampled and analysed. The purification efficacy was calculated for the active ingredients, which gave the highest risk in case the waste water would have been discharged to surface water. At each location, for most chemical active ingredients the concentration was at least 99% lower after bioremediation. The average efficacy was 95%. At several locations, a few substances with lower efficacy were present. Analysis of these exceptions did not reveal a relation between the lower efficacy's and particular substances or substance properties. The farmers and contract sprayer who used the installations were satisfied with the system's functioning. Most important aspects for improvement for the systems are prevention of clogging and increasing the evaporation capacity.

Bioremediation has been implemented in Dutch regulation as of January 2013 as an option to manage waste water, resulting from sprayer cleanings. However, if internal cleaning is done in the field and external cleaning is not carried out more than twice a year at the farm yard, there is no obligation for specific facilities at the farm yard.

Numerous presentations, demonstrations and publications by Applied Plant Research and other stakeholders have raised the awareness of the risk of point source pollution. Ready to use Fytobac systems have become available in the Netherlands. The number of farmers and contract sprayers applying bioremediation is growing slowly but surely.

Bioremediation systems in the UK. Current position, systems description, regulation and future possibilities

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Bioremediation has been studied in the UK since 1998 with major research by Government Agencies included from 2000 - 2008. Regulation regarding water supply in UK is managed by the Government's Environment Agency which has approved biobeds in 2007 and biofilters since 2008 as Best Practice. However adoption by farmers in UK has been slow with only 250 biobeds installed to 2013. This is perhaps due to the necessity to have lined and therefore pumped biobeds, the regulatory needs associated with installation, routines of operation, cost and mistrust of changing regulations.

Water management in UK is divided into river catchment areas which in some cases has identified high levels of pesticides in surface and groundwaters with respect to the EU drinking water standard. In these Catchment 'Sensitive' Areas biobeds and biofilters have been and are promoted which may attract capital grants to encourage adoption. In addition some Water Supply Companies have promoted Best Practice on farms with pesticide handling areas and bioremediation. These companies have demonstrated their very high costs of removing pesticides from water by treatment or dilution in the supply operation and now support some farm demonstration biobeds and biofilters thus encouraging adoption. Farmers operating on large scale or with contractor services are showing considerable interest in roofed bunded sprayer filling areas thus producing very low annual amounts of potentially contaminated water.

Future interest in evaporative systems with and without bioremediation is expected with limited monitoring/research of these systems being planned in 2013 season.

Biobeds in Sweden. State of the art

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The first biobed was built in Skåne in 1993. Since then biobeds have been the choice of many Swedish farmers for a safe handling of pesticides. This project intended to perform an inventory of the biobeds in Sweden with the final objective to identify problem areas that can be the basis for research applications and issue of new recommendations. A total of 104 anonymous questionnaires were filled in by the farmers attending the pesticide-spraying authorization courses during 2010, 2011 and 2012. The questions covered different aspects from location and size of the annual sprayed area to details about construction and maintenance of the biobed.

The results of the survey showed that biobeds are seen as a handy and good system to handle pesticides and rather easy and cheap to build and environmentally safe. While most of the biobeds are run as expected, in some of them we identified an incorrect preparation and management of the biomixture and the grass layer, and an unintended use and lack of maintenance. Although farmers expressed satisfaction with the available guidance, in some cases the instructions for building and maintenance were not properly followed. Therefore we need to be aware of the situation and take the necessary measures to avoid potential problems, which may be for example: a) changes in how information is communicated and distributed, b) incorporation of other type of biobed adjusted to the farmer's needs and c) to evaluate the retention and degradation capacity of the biobeds that are not properly managed in order to determine whether there is a risk of pollution.

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My workshop notes……………………