3rd European Biobed Workshop

Università Cattolica del Sacro Cuore (UCSC),

Piacenza, Italy

August 31st - September 1st, 2010.

3rd European Biobed Workshop Program

		31th August
Time	**Name**	
11:00		Registration
12:15		Lunch
13:00	Husby, J.	*Introduction of the workshop*
13:10	Capri, E.	*Welcome*
		Session: Degradation of pesticides in biopurification systems (Chair: MP Castillo)
13:20	Pizzul, L.	Studies on the degradation of glyphosate by ligninolytic enzymes
13:40	Coppola, L.	Fungicdes degradation in an organic biomixture: impact on microbial diversity
14:00	De Wilde, T.	Sorption and degradation of pesticides in biopurification systems
14:20	Diez, C.	Chlorpyrifos and atrazine degradation in a biomix of biobed with soil derived from volcanic ashes. Biological and physico-chemical aspects
14:40	Ngombe, D.L.	Carbon dioxide emission as an indicator of 2,4-D degradation in biobeds
		Session: Composition of the biomix (Chair: D Karpouzas)
15:00	Karanasios, E.	Implementation of biobeds in Greece: Where do we stand?
15:20	Eklo, O.M.	Composition of biomix and effects on pesticide leaching tested by sorption and column experiments
15:40	Omirou, M.	The implementation of biobeds in Cyprus
16:00		*Coffee+poster*
		Session: Efficiency of the system and its use in practice (Chair: E. Nilsson)
16:40	Ferrari, F.	The new Biomassbed developed within the Life project ArtWET
17:00	Monaci, E.	Bio-cleaning of contaminated water by fungicides applied in vineyard
17:20	Hendrickx, N.	The BIOREM project, results from practice.
17:40	Felgentreu, D.	Experiences with biobeds after several years of use in Germany
18:00	De Werd, R.	Biofilters in the Netherlands: results of on-farm testing and opportunities for implementation
18:20	Bozdogan, A.M.	Studies on biobed in Turkey
19:30		*Dinner*

1th September		
Session: Approved systems/ Construction and dimensions of the system (Chair: NH Spliid)		
8:00	Spliid, N.H.	New closed biobed with recirculation and evaporation for use under colder climates
8:20	Lemborgne, M.	Héliosec® a simple system for waste water management.
8:40	Wolf, T.	Biobed hydrology – observations during initial year of field scale studies in Canada
9:00	Mestdagh, I.	Bioremediation systems in Belgium: state of the art
9:20	Darmedru, J.Y.	Phytobac®, french experience
Session: Final discussion		
9:40	Final discussion	
10:20	Coffee	
11:00	Closure	

Participants

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Abstracts

The Biobed Workshops and Biobeds in the World

Husby, J.

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The biobed idea started in Sweden in the beginning of 1990s. The farmer Gören Olson was worried that his pesticides from spill when filling the sprayer could end up in the neighbours' wells. With the knowledge that pesticides normally are broken down in the top soil he created a place to park the sprayer on a pit with mixture of topsoil, straw and peat underneath - a design by Lenart Torstenson and Maria Del Pilar Castilo from the Swedish Agriculture University. This idea gave inspiration to other researchers in a number of countries.

In 2004 my colleague Christian Guyot, who is involved in Phytobac development, told me about the work in France. Other colleagues could tell about different research programs going on in their countries. Based on that the idea of a workshop came up. The first European workshop was held in Sweden in 2004, arranged together with Eskil Nilson from Visavi, Sweden.

In 2007 the second European Biobed Workshop was part of a project under EU Clean Region. The workshop was held in Gent, Belgium, and Niels Henrik Spliid at the University of Aarhus, Denmark was the organizer together with Tineke De Wilde at the University of Gent.

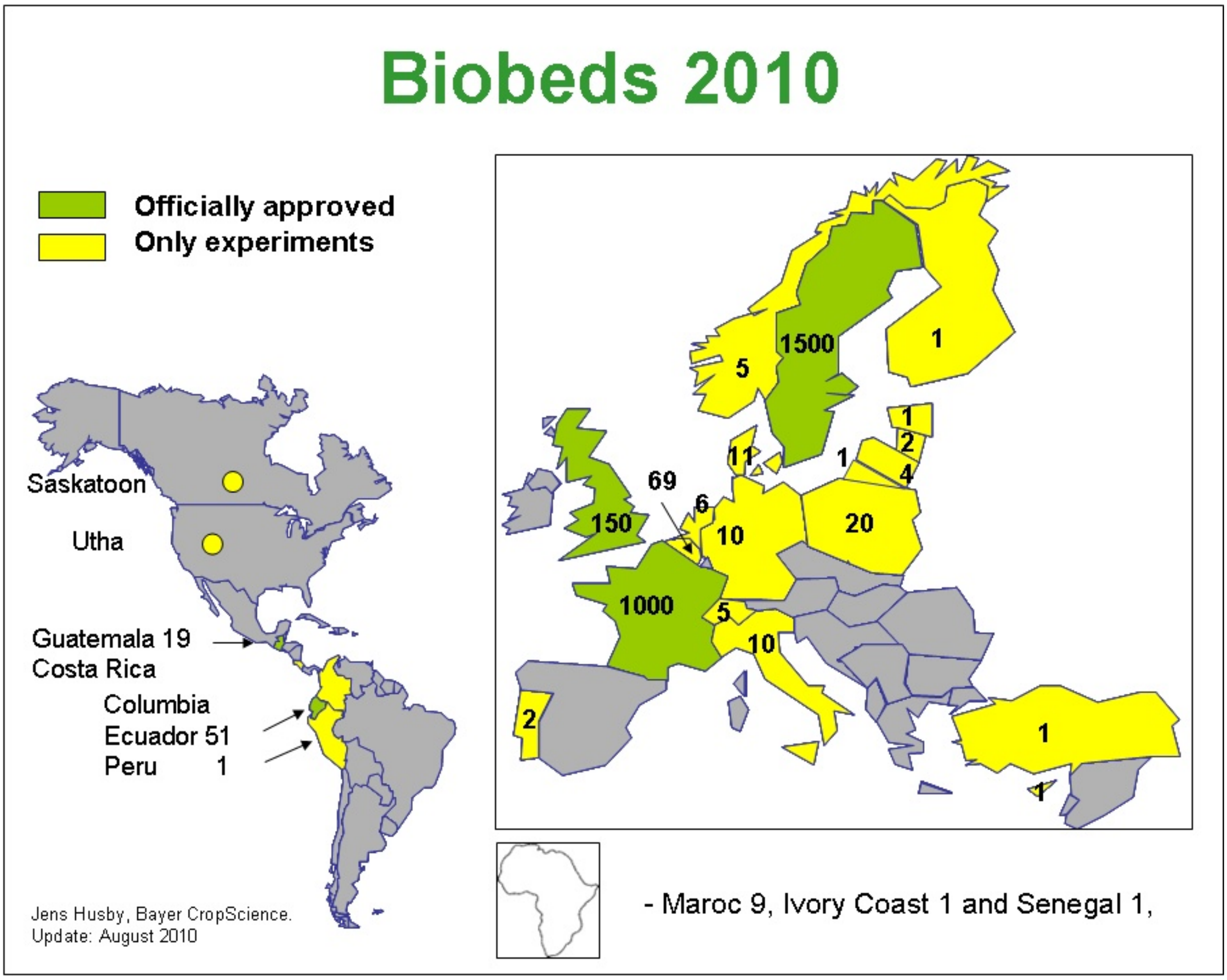
Already at the second workshop there was an interest to arranging a third one. A small survey here in 2010 among the researchers working within the area of bioremediation systems showed that there was again a lot of new research. Ettore Capri at the Univrsità Cattolica del Sacro Cuore, Italy has now organized the workshop while Tineke DeWilde has taken care of abstracts and presentations.

Bayer CropScience took the initiative to and fully funded the first workshop and has also funded what was necessary to make the 2 next workshops possible.

If we look at the number of participant at the 3 workshops in 2004, 2007 and 2010 there have been 14, 41, 45 participants from 6, 12, 16 countries.

An estimate from people involved in bioremediation systems shows that the numbers are growing in the world. In UK the numbers of biobeds grew from 2007 to 2010 from 75 to 150 and in France the figures were 500 to 1000. While Sweden, France, UK and Belgium have the largest number of systems, other countries are mostly at the research stage. The estimation for the total number of bioremediation systems in the world is about 2800 in 2010, but most of them are in the four countries mentioned above.

It is the hope with these workshops that information exchange will inspire to more research and creation of bioremediation systems which can be part of preventing point pollution in connection with the use of pesticides.



An estimate of biobeds and other bioremediation systems in the world.

Studies on the degradation of glyphosate by ligninolytic enzymes

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The ability of the fungal lignin-degrading systems to degrade pesticides has been the basis for the design of biobeds. The effect of laccases and peroxidases on degradation of, for example, bentazon, isoproturon, metribuzin and methoxychlor (1-3, 5) and pesticide metabolites, e.g., 3,5,6-trichloro-2-pyridinol (TCP), a product of chlorpyrifos degradation (4) has been reported. However, and despite of its extensive use, the degradation of the herbicide glyphosate by ligninolytic enzymes had not been explored.

We tested the ability of manganese peroxidase and laccase to degrade glyphosate in the presence of different mediators under *in vitro* conditions. We obtained complete transformation of the herbicide, with formation of the metabolite AMPA (6). To better understand the degradation process, we then focused on the effect of different factors (pH, temperature, buffer composition, presence of mediators) on the extent of the transformation of glyphosate and the metabolite AMPA and on the activity of laccase. These results are discussed.

1. Castillo MdP, Ander P, Stenström J, Torstensson L (2000) Degradation of the herbicide bentazon as related to enzyme production by *Phanerochaete chrysosporium* in two solid substrate fermentation systems. World Journal of Microbiology and Biotechnology 16:289-295
2. Castillo MdP, Torstensson L (2007) Effect of biobed composition, moisture, and temperature on the degradation of pesticides. J Agr Food Chem 55:5725-5733
3. Castillo MdP, von Wirén-Lehr S, Scheunert I, Torstensson L (2001) Degradation of isoproturon by the white rot fungus *Phanerochaete chrysosporium*. Biol Fert Soils 33:521-528
4. Coppola L, Castillo MdP, Monaci E, Vischetti C (2007) Adaptation of the biobed composition for chlorpyrifos degradation to Southern Europe conditions. J Agric Food Chem 55:396-401
5. Hirai H, Nakanishi S, Nishida T (2004) Oxidative dechlorination of methoxychlor by ligninolytic enzymes from white-rot fungi. Chemosphere 55:641-645
6. Pizzul L, Castillo M, Stenström J (2009) Degradation of glyphosate and other pesticides by ligninolytic enzymes. Biodegradation 20:751-759

Fungicides degradation in an organic biomixture: impact on microbial diversity

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There is little understanding on pesticides application and how presence and activity of microbial biomass influence biodegradation rate of pesticides. The current state of knowledge is derived primarily from a cultural study on individual microorganisms degrading individual pesticides, but the degradation of pesticides generally involves biochemical conversion of pesticides by microbial consortia.

The aim of the present study was to evaluate the efficiency of an organic biomixture, used as filter in a biobed, in the degradation of fungicides generally used to control pests in vineyards and to assess their effects on the microbial community.

A laboratory experiment was carried out using a biomixture made up of pruning residues composted for 5 years and straw. Commercial formulates of dimetomorph, penconazole, azoxystrobin, metalaxyl, fludioxonil and cyprodinil were mixed and downloaded onto the biomixture following concentrations and time schedule of treatments for vineyard in Italy. Residues were analyzed for a 112-day period. Moreover, the evolution of the microbial community was monitored by using Denaturing Gradient Gel Electrophoresis (DGGE). This molecular approach was useful to describe the genotypic diversity of complex culturable and unculturable microbial community after the addition of the fungicides.

Results from this study showed that the biomix had a good capability of degrading pesticides. Indeed, at the end of the experiment, the concentration of most of the pesticides was close to complete degradation. The applied fungicides caused an evident reduction in microbial diversity, even if there was a selection of strains able to degrade recalcitrant fungicides, such as penconazole and fludioxonil. At the end of degradation process, no significant changes in the composition of microbial community were seen.

Sorption and degradation of pesticides in biopurification systems

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In order to optimize the efficiency of biopurification systems, the fate of pesticides and the contribution of degradation and retention process needs to be well characterized. The intention was to unravel sorption and degradation processes on an increasing spatial scale. The first part of this research, which focused on the sorption of pesticides with varying phyisico-chemical characteristics, was performed under static conditions. This means that transport of pesticides was not taken into account. Pesticides with a high organic carbon partitioning coefficient (K_{oc}) (*e.g.* linuron) showed a faster sorption, compared to the more mobile pesticides (*e.g.* bentazone). The organic matter content appeared to play a minor role in the sorption kinetics of the pesticides. Structural differences between the substratums were probably the reason for varying sorption kinetics of a pesticide. For example, sorption of pesticides to compost (fine) was in most cases faster than to coco chips (coarse). Simulations performed with HYDRUS-1D, showed that leaching of pesticides with a high mobility is more liable to nonequilibrium sorption. The second objective proposed in this optic, was to determine which organic substratums show the highest sorption capacity. Quantification of the sorption capacity with the Freundlich equation led to the following ranking with increasing sorption capacity: sandy loam soil < willow chopping, cow manure < straw, coco chips, compost < peat mix. However, in the selection of the substratums to be used in a biopurification system, the first criteria should be the function the substratum will fulfill in the system, followed by its sorption capacity.

The second part of this research was performed under dynamic conditions, thus incorporating transport of pesticides. Experiments were performed in small or large scale columns. This experimental set-up aims at studying sorption and degradation processes at the same time. The first question posed in this regard, was how pesticides will behave in different matrix compositions. Sorption and degradation characteristics of linuron, bentazone, metalaxyl and isoproturon were quantified using inverse modeling techniques present in the transport model HYDRUS-1D. The sorption strength of the different pesticides to the organic matrix, increased with decreasing mobility of the pesticides, which validated the results obtained under static conditions. The major difference between static and dynamic conditions was that a higher sorption capacity was observed in batch experiments. This led to the assumption that sorption coefficients obtained in batch experiments are not suitable for describing solute transport at the column or field scale. Concerning increasing degradability of the pesticides, pesticides could be ranked as follows linuron > metalaxyl-isoproturon > bentazone. Delayed degradation could be observed for some pesticides in the micro- and macrocosms. The time period with little or no degradation has been designated as acclimation period or lag time and could be fitted by implementing the Monod kinetics into HYDRUS-1D. Finally, concerning the composition of the mixture, it could be concluded that the addition of cow manure stimulated degradation of certain pesticides, and that a decrease in the soil fraction did not reduce the efficiency of the system.

Another observation from the previous column experiments indicated that organic matrix from a well established biopurification system could be used as inoculation source for a new biopurification system, as this matrix probably gained an increased ability to degrade the applied pesticides. To validate the hypothesis that an inoculation with pesticide-primed material enhances degradation of pesticides, column experiments were set-up where metalaxyl or isoproturon primed material was included in the matrix and transport of both pesticides was followed-up. This hypothesis was valid for degradation of metalaxyl in the presence of the metalaxyl-primed soil, but could not be verified in the case of isoproturon.

The last part of the studies performed under dynamic conditions were carried out to gain some knowledge on the influence of a variable flux on pesticide degradation and retention. The flux, which consists of a hydraulic and chemical fraction, may vary in a biopurification system. To ensure proper working of the system when changes occur in the flux, column experiments were performed on a micro and macro scale at three fluxes to observe changes in degradation and retention. In microcosms, where a higher hydraulic and chemical load was applied compared to the macrocosms, sorption of intermediate mobile pesticides decreased considerably with increasing flux. This is in contrast to the macrocosms, where the three applied fluxes were lower and did not induce breakthrough of most of the applied pesticides. However, for the most mobile pesticide, it could be concluded that a higher flux influenced degradation negatively.

Chlorpyrifos and atrazine degradation in a biomix of biobed with soil derived from volcanic ashes. Biological and physic-chemical aspects.

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Many pesticides used in protection of crops, weeds and diseases could be released into the environment due to a bad agricultural practice with a consequent risk to humans, flora and fauna. The adsorption and mobility of organic pesticides in soils depend on the ionic or neutral character of the molecule, on its water solubility and its polarity as well as on the content and nature of the colloidal fraction of the soil, clay and organic matter [1]. These contaminants could be removed using a biobed system based on the adsorption and degradation potential of the components of the biobed [2].

The study is associated with national projects (FONDECYT and FONDEF) focused to fruit producers. We evaluate the effect of allophanic top soil (Andisol) as a component of the biomix of the biobed on sorption and degradation capacity of chlorpyrifos and atrazine, two pesticides widely used in Chile. The allophanic top soil used was an Andisol, located in Southern Chile (sampled from 0-20 cm depth). The soil has pH 5.9, organic matter 14.6%, nitrogen 0.72%, sand 16.1%, silt 58.2% and clay 25.7%. The biomix was composed by a mixture of top soil (25%), commercial peat (25%) and straw (50%).

The biomix was pre-incubated for 0, 15 and 30 day at 20 ± 1 °C before to be contaminated with chlorpyrifos and atrazine (160, 320 and 480 mg kg^{-1}). During the incubation period, CO_2 evolution, phenol oxidase activity (MnP), fluorescein diacetate hydrolysis (FDA), residual concentration of pesticides and its degradation products were periodically evaluated.

Assays of degradation with different allophanic soil pH values (4.7, 6.6 and 8.0) were performed in batch conditions. Besides, assays of lixiviation, simulating three rainfall conditions (low, medium and high), were performed in columns in continuous conditions. The sorption studies were conducted with both substrates (top soil and biomix) with chlorpyrifos (10 to 80 mg L^{-1}) and atrazine (5 to 40 mg L^{-1}) using $CaCl_2$ 0.1 mol L^{-1} as background electrolyte solution. After equilibrium the pesticides concentration in the supernatant was determined by HPLC. The isotherms were fitted using the Freundlich sorption model.

The results of this study demonstrate that pre-incubation time of the biomix with allophanic soil between 0-30 days had no major effect on the chlorpyrifos and atrazine degradation. High concentration of the pesticides (>90%) can be degraded in the biomix using allophonic top soil. Degradation products (TCP, DIA, DEA and HA) were formed during the degradation of chlorpyrifos and atrazine in the biomix, but they were also degraded by this system. The FDA was affected mainly by the presence of chlorpyrifos more that for the presence of atrazina, the activity dehidrogenada was affected by both pesticides, whereas CO_2 evolution was stimulated by the presence of both pesticides. On the other hand, the phenoloxidase activity was affected by the pH of the soil more than for the presence of the pesticides, being a minor in the biomix with alkaline soil.

Respect to sorption, marked differences were observed between both pesticides due to the high differences in chemical properties of the pesticides (Table 1). These differences can be explained by differences in hydrophobicity and reactivity of the molecules and suggests sorption of both compounds was due to interactions with mineral/oxide surfaces and soil organic matter [3]. Partitioning into soil organic matter is thought to be the main mechanism for sorption of wide range of pesticides; however these results are dependent of the K_{ow} constant of each compound.

Table 1-Characteristics of the pesticides and Freundlich parameters (K_f, 1/n) for soil (a) and biomix (b).

Pesticide	Koc	DT_{50} Soils (days)	Water Solubility (mg L^{-1})	Mobility Class	K_f	1/n
Chlorpyrifos	6000	7-15	1.4	Non mobile	[a]2685 [b]2798	[a]0.40 [b]0.47
Atrazine	40-155	35-50	33	Moderately mobile	[a]6.47 [b]5.76	[a]0.78 [b]0.95

The lixiviation study confirms the low mobility of atrazine. This pesticide is retained (>80%) in the first 10 cm of the column, and was not detected in the lixiviate after 5 month of columns of operation.

References:

[1] Shawhney, B.L. and Brown, K. 1989. Reactions and movement of organic chemicals in soils. Soil Sci. Soc. Am. Inc. Madison, WI, USA, 474 pp.

[2] Castillo, M., and Tortensson, L. 2007. Effect of biobed composition, moisture, and temperature on the degradation of pesticides. J. Agric. Food Chem. 55: 5725-5733.

[3] Cea, Mara, J.C. Seaman, Alejandra A. Jara, M.L. Mora, M.C. Diez. 2005. Describing chlorophenol sorption on variable-charge soil using the triple-layer model. J Colloid Interf. Sci. 292 (1):171-178.

Acknowledgements: Investigation financed by FONDECYT 1090678 and FONDEF D09R1006 projects.

Carbon dioxide emission as an indicator of 2,4-D degradation in biobeds.

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The objective of this study is to quantify the breakdown of pesticides in biobeds and to understand the microbial dynamics associated with this breakdown under Canadian climatic conditions. Initial studies showed that the half-life of the herbicide 2,4-D was reduced five-fold in a biobed substrate compared to topsoil. Laboratory studies showed that CO_2 evolution lagged several days after initial addition of 2,4-D, but then spiked sharply above background levels for several days before returning to normal levels. Subsequent additions of 2,4-D to the same substrate resulted in an immediate release of CO_2 in a similar spike, suggesting a build-up of degrading microbes after the first addition. The quantity of CO_2 evolved from the biobed substrate increased with the quantity of herbicide applied. In soil, an overall increase in CO_2 evolution was also observed after 2,4-D addition, but evolution did not increase sharply at the time of application. Microbial biomass measurements are being conducted to relate CO_2 evolution to microbial activity.

Implementation of biobeds in Greece: Where do we stand?

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The success of biobeds in northern Europe has stimulated research in southern European countries like Greece where climatic and environmental conditions, agronomic and plant protection practices strongly differ. The main component of a biobed, the biomixture has been acknowledged as a major factor controlling the efficiency of the biobed. Thus at the first step of our research we focused on identifying various by-products of the local agricultural practice (either raw or composted) which could be used as alternatives to peat or even straw, the two main components of the original Swedish biomixture. A series of degradation and adsorption studies suggested that 1) that the simultaneous presence of all three components (lignocellulosic material- a humified carbon source - soil) is a prerequisite for enhanced biobed efficiency as the absence of straw or compost in the final mixture leads to lower pesticide degradation rates 2) a biomixture of composted olive leaves (instead of peat): straw:soil (25:50:25) (BX1) showed a higher degrading potential compared to peat-based biomixtures (OBX) 3) substitution of straw in BX1 by grape stalks further accelerated its degrading potential while other lignocellulosic materials like corn stalks and sunflower residues could perform equally well with straw. Adsorption studies revealed that OBX showed a higher averaged ability to retain pesticides, attributed to its higher organic C content, followed by compost-containing biomixtures and soil. Composted materials showed a large variation in their physicochemical properties and microbial characteristics, compared to peat, which are reflected in considerable differences in the degradation and adsorption capacity of the corresponding biomixtures. Generally, composts with higher organic C and sufficient N content can be used successfully as components of biomixtures. Biotechnological amelioration of biobed systems in Greece have been also considered with the use of spent mushroom substrate as biomixture constituent (see other presentation) or bioaugmentation of these systems with pesticide-degrading microorganisms like white rot fungi or bacteria. The second step in the implementation of biobeds in Greece included the evaluation of the selected biomixture in column studies under variable hydraulic loadings and pesticide applications. At the most intense water loading scenario the columns packed with the peat biomixture showed the highest ability to retain pesticides, followed by compost containing biomixtures while the higher leaching was evident in the soil columns. The completion of the column studies will lead us to the third and final step of biobeds implementation in Greece: the construction and assessment of pilot on-farm biobed systems in selected regions in Greece depending on the main cropping priorities (corn/cotton - cultivated area; vine-cultivated area; fruit/olives – cultivating area).

Acknowledgements: Evangelos Karanasios is financially supported by the State Scholarship Foundation of Greece. This work was partially funded by the Institute of Research Promotion of Cyprus through the project: BIOBEDS

Composition of biomix and effects on pesticide leaching tested by sorption and column experiments.

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This study provides results from sorption properties of possible biomixes to be used as filter materials in setup to prevent leaching of pesticides from point-sources. The experiments included fungicides (fenhexamid, kresoxim-methyl, metalaxyl), insecticides (pirimicarb, pyraclostrobin, boscalid, dimethoate) and herbicides (MCPA, metribuzin and bentazone) all relatively mobile and some also relatively persistent. The concentration of the pesticide applied in the test corresponded to the pesticide content outside the sprayer after use. The materials tested were granulated peat, compost, granulated active carbon, rice husk ashes, and micro silica. The purpose of the selected materials was the following: Granulated peat was used to sorb oil spills from the engines, rice husk ash should sorb the pesticides and the compost should act like a degrading agent. Active carbon should serve like a reference material, and micro silica could be a potential sorptive material. Introductory sorption tests with pesticides and the selected materials were conducted following simplified OECD guidelines. The sorption tests were followed by tests with columns packed with layers of the different materials separate or in mixture starting with short columns (50 cm) increasing the length to 100cm. Increasing the length of the columns, the compost layer caused clogging. The compost was mixed with granulated peat to get better hydrological conditions, and the rice husk ash was mixed with peat in different ratios to get a better dispersion and sorption of the pesticides.

The sorption experiment showed 100 % sorption of all pesticides tested with rice husk ashes, while active carbon sorbed more than 95 % of the substances. At least 70 % of each of the pesticides was sorbed to granulated peat. Compost differed much in sorption properties from pesticide to pesticide. The micro silica seemed not to be suitable. The reference column with 20 cm of active carbon had no leaching of pesticides. The other columns with compost (20 cm in the bottom and active carbon mixed with granulated peat in the top layer) gave 0.1 % leaching from each of the pesticides added. Columns with peat mixed with rice husk ash leached less than 10 % of the applied. By increasing the length of the columns to 100 cm the reference substance active carbon still kept all the pesticides in the columns. Rice husk ash in mixture with peat sorbed more than 97 % of all the substances except for MCPA and bentazone which was not affected by the column material.

The implementation of biobeds in Cyprus

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Public health and environmental protection is of great importance, thus appropriate methods should be developed for the prevention of pesticide contamination from point sources. A possible and effective measure is the installation of a “biobed” which is an on-farm simple bio-filtration system designed to receive and dissipate pesticides contained in wastewaters. Biobeds are pits on the ground (1 m deep, 20-35 m^2 surface) which are filled with a bioorganic mixture (biomixture) comprised commonly of soil, straw and compost or turf at various volumetric portions. The choice of the biomixture is of prime importance and depends on the availability of organic substrates available in each region. Thus, the first step was to select appropriate biomixtures for the biobed system in Cyprus. The ability of different compost-derived biomixtures to degrade pesticides that are mainly applied in the citrus production such as chlorpyrifos, deltamethrin, a-cypermethrin, ortho-phenyl phenol (OPP), thiabendazole (TBZ) and imazalil was initially investigated. The biomixtures tested were prepared by mixing top-soil (25% v), straw (50% v) and various composted materials from the local agriculture (25% v) including: olive-tree prunings (**b1**), grape-vine prunings (**b2**), grape marc (**b3**), winery by-products **(b4**). In addition top-soil, straw and composted winery wastes (**b5**) were mixed in the volumetric proportions of 1:1:2 as another biomixture type. A mixture of soil with straw at volumetric proportions of 3:1 (**b6**) and top-soil singly (**b7**) were also included for comparison purposes. Pesticides were applied as two mixtures a) pre-harvest mixture (chlorpyrifos, deltamethrin, a-cypermethrin) and b) postharvest mixture (OPP, TBZ, imazalil). Adsorption of pesticides was also tested in the same biomixtures. The dissipation pattern of all pesticides was best described by first order kinetics and was significantly influenced by the type of composted material used with imazalil being the most persistence chemical in all biomixtures. In general compost-biomixtures were more efficient in pesticide sorption compared to soil or soil/straw mixtures. Biomixture b4 showed the highest degradation and sorption capacity for the pesticides tested. Therefore these by-products will be further examined for their ability to dissipate pesticides in an offset-type field biobed which is currently under construction in the experimental station of the Agricultural Research Institute in Cyprus.

The new Biomassbed developed within the Life project ArtWET

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Although plant protection products are already regulated in Europe under Directive 91/414/EEC, there is increasing concern about the pollution of ground and surface water caused by point sources of pesticides, such as tank filling, spillages, faulty equipment, washing, waste disposal, and direct contamination taken into account by the Water Framework Directive 2002/60/EC and the Directive about the Sustainable use of Pesticides 2009/128/EC. One tool for the reduction of pesticide point source contamination the Biomassbed, developed in 2003 in Italy. The biomassbed is a biological system where chemicals are bound and biologically degraded. It is an offset lined system where wastewater containing pesticide residues leach, by pumping, through a biomix reactor.

From 2007 until now, within the Life Project ArtWET, this system was improved and three new Biomassbed plants were installed in Italy: two are working in vineyard farms, while the third is a modular prototype placed at the Campus of Catholic University of Piacenza.

The actual design of the Biomassbed is a modular versatile system that includes a (1) bioreactor working on line with inert substrate allowing biofilm growth and the biomix, (2) a module for separation of suspended matter from water, a module designed for photodegradation.

The advantages given consists in a more efficient system of simple maintenance, which allows a mitigation of waste water containing pesticide residues on the average of two order of magnitudes in concentration for the major part of the tested compounds, mainly insecticides and fungicides, only if Biomassbed is designed and utilised properly.

Anyway, the diffusion of such tools should take care of: (1) it is necessary to plan transparent procedures approved by national and local authorities; (2) it will be necessary to avoid the Home-made systems, and the re-production of foreigner tools not validated at local agronomic, climatic and social conditions; (3) the installation costs should be, at least, partially sustained by public founding.

*Acknowledgements**:** This research are done thanks to the project LIFE 06 ENV/F/000133.*

Bio-cleaning of contaminated water by fungicides applied in vineyard

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Biological systems are nowadays being developed all over the EU countries to protect water-bodies from pesticides contamination at farm level. These systems rely on organic substrates for sorption and degradation of pesticides.

Viticulture is one of the most important agricultural practice in Mediterranean area and it requires intensive use of fungicides, some of which, recalcitrant to degradation and often detected in stream water nearby vineyards. BiomassBed is an indirect system designed to collect accidental pesticides losses and water remnant of tank sprayers. This system was installed in vineyard and tested in bio-cleaning of fungicides contaminated water during two years of field experiment. At the end of each treatment, wastewater of sprayers tank containing mixtures of cymoxanil, dimethomorph, metalaxyl, folpet, penconazole, cyprodinil, fludioxonil, azoxystrobin, mandipropamid, pyraclostrobin at different concentrations were discharged into the BiomassBed and repeatedly re-circulated throughout an organic biomass of pruning residues and straw for fungicides adsorption and biodegradation.

Water collected was sampled and analysed for fungicide residues and organic biomass analysed to asses fungicides dissipation.

Fungicides were removed from water in a range of 92.4-100% of initial concentration. Metalaxyl was the less retained and probably desorbed during recirculation of water. However, metalaxyl residues dissipated in 70 days in collected stagnant water. A contribution of abiotic and biotic factors to the disappearance of metalaxyl in water has been hypothesised.

Penconazole has showed the tendency to accumulate in the organic biomass before starting to degrade. Among all fungicides penconazole was the most recalcitrant active ingredient despite the low concentration discharged.

The BIOREM project, results from practice.

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Monitoring of pesticide concentrations in surface waters show that in all the Flemish watercourses, active ingredients are present at low to high concentrations. International studies assign 40 to 90 % of this pollution to point source contamination. The filling and cleaning process of sprayers represents a major part of this point sources. On farm biopurification of this waste water, contaminated with pesticides, could be an efficient aid in diminishing the residual load on surface waters. In that respect, a bioremediation system was optimized to implement as an efficient and easy-to-use on farm system. At the end of the track, the biofilter consisted of a buffer tank, a tower of tree filter units, an evaporation system containing vegetation and an effluent collection tank. Retention and degradation of reference pesticides was investigated on small scale as well as on practical scale (see abstract De Wilde, T.) in the presence or absence of pesticide primed materials, which are materials with a history of pesticide contamination. For mobile pesticides like bentazon that is barely retained on the matrix, no clear difference between the primed and non-primed systems was perceptible. On the contrary, isoproturon showed better retention on the pesticide-primed matrix. Consequently, microbial pesticide degradation and mineralization capacity of both systems was tested. The biofilter inoculated with pesticide-primed material shows an initial higher degradation and mineralization capacity for linuron, isoproturon, metalaxyl and metamitron then the biofilter inoculated with non-primed material, and in addition reaches its maximum capacity much faster. Besides the filter efficiency, an important issue for legislation in Flanders is the reduction of the effluent volume. Therefore, an evaporation system was optimized by using sedge and wilow as vegetation, because these plants show high persistency and evapotranspiration capacity. These experimental data were further validated in practice by sampling a biofilter system on an arable farm. During the years, adaptations to the system as a consequence of the research performed, resulted in an increased mean retention from 95.59 % to 99.47 %. Whereas at the beginning of the project, only 47.07 % of the active ingredients had a retention higher than 99 %, 90 % of all active ingredients showed more than 99 % retention in the season of 2009. In conclusion, experimental data from pesticide-primed bioremediation systems can be extrapolated to on farm systems and are promising tools in reducing the pesticide load of surface waters originating from point sources.

Experiences with biobeds after several years of use in Germany

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Pesticides play an important role in the production of food on the field and in the greenhouses, for example in the cultivation of wine, fruit and vegetable.

Used according to label instructions the application of pesticides poses a minimal risk to the environment. However, routine monitoring of surface water and groundwater has shown that contamination with pesticides occurs (Federal Environment Agency: Report 2004). Recording and evaluating ground water contaminations caused by point sources of pollution - Detailing the requirements prescribed by the EC Water Framework Directive

The ISO Norm 22368-2004 Crop protection equipment [Test methods for the evaluation of cleaning systems, Part 1: Internal cleaning of complete sprayers and Part 2: External cleaning of sprayers] specifies tests for determining the performance of the rinsing systems fitted onto sprayers used in crop protection for the internal cleaning of the complete sprayer, including the tank and two test methods for evaluating the performance of cleaning systems fitted onto sprayers used in crop protection for the removal of deposits on the external surfaces of the sprayer.

The purpose of the tests is to provide sprayer designers with information on contamination of the sprayer and permit the comparison of different attachments or adjustments in relation to external cleaning (Test A), and to allow the performance of different cleaning devices to be determined (Test B). Residue measurements at different devices revealed that there are between 0.1% and 1.0% of the initial concentration in the equipment and 0.02% to 0.5% of the initial concentration on the equipment.

The plant protection act in Germany regulates that the tank container (with technically conditioned eluates) has to be filled with clear water again and the waste water has to be sprayed on a cultural or agricultural area. Fruit and wine farmers as well as greenhouse producers frequently do not possess such „compensation areas “.They must clean the sprayer equipment on their own yard.

To a high extent this is the cause for point source contamination of water in agriculture. Point source entries occur when residual liquids of plant protection products flow either directly into the groundwater or via a surface water passage. These pesticide entries are usually associated with the filling and cleaning of field sprayers or leakages of wastewater tanks.

To prevent the risk of pesticide leaching into surface water and groundwater the biobeds seem to be a good alternative.

The results of so far five years of research show that biobeds are able to inactivate, adsorb, reduce or microbially mineralize pesticide residues in leachates. The activity of the biobeds was not affected by repeated applications of tank mixtures containing herbicides, fungicides and insecticides (normal field application rate). The best results with regard to the microbial decomposition of the pesticides were achieved with repeated application of the leachates on the biobed surface.

Detailed information about the remaining pesticide concentrations in the biomix is not yet available, because the investigations are not completed. First results show, that fate and behaviour of high concentrations of relatively complex mixtures of pesticides in the biomix, which was filled into several biobeds, were similar.

The pesticide residues in the biomix matrix may cause problems when it is necessary to change the biobed fillings after long-term use. Therefore we recommend composting of the replaced biobed mixture for at least one year.

Looking at the leachates most of the pesticides were not detectable or their concentrations were less than 0.1 µg/l at the end of each year of investigation and after five years of use of the biobeds as well. Only the herbicides diuron (in every year) and isoproturon (in 2006 and 2009) were measured in concentrations between 0.1 µg/l and 0.4 µg/l.

Water management is a critical point in the use of the biobeds and has to be observed. With too little humidity the activity of the microorganism decreases particularly in the upper layers. After heavy rain the biobeds may overflow or anaerobic conditions may occur.

The overall results of the five years of research permit the conclusion, that biobeds are a useful tool to reduce the concentrations of pesticides in surface waters.

We would like to provide recommendations on how biobeds should be operated under German climatic conditions.

Biofilters in the Netherlands: results of on-farm testing and opportunities for implementation

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The high density of watercourses in the Netherlands enlarges the risks of point source pollution of surface water with plant protection products (PPP's) in agricultural and horticultural areas. Biopurification may be a method to reduce the risk of several point sources in a practical way. Based on positive results in Belgium and other surrounding countries, several on farm tests and demonstrations of biopurification have been carried out in the Netherlands since 2008 and are still ongoing. The goal of these tests and demonstrations is to determine the effectiveness of biopurification systems under Dutch circumstances and to make potential users aware of its possibilities. A secondary goal is to facilitate the discussion between policy makers and the agribusiness on the implementation of biopurification in regulations and in practice.

The on-farm tests took place at experimental and at commercial farms: a flower bulb farm, a fruit growing farm and at the yard of a contract sprayer working in arable farming and bulb production. A system comparable to the Phytobac© and four so called 'biofilters' were built. These biopurification systems were used for the treatment of contaminated water from filling and cleaning areas for sprayers and other machinery used to apply PPP's. Furthermore leachate from composting flower bulb waste and condensation water from flower bulb storage cells, in which ppp's were applied, were used as influent.

In most cases, the concentrations of PPP in the influent were reduced at least 99%.. As observed in other studies the purification was less effective for bentazon (88%) compared to other herbicides applied. At the other locations in the Netherlands the effectiveness for most of a wide range of PPP's was also close to 100%. The treatment was less effective for carbendazim, thiofanate-methyl and kresoxim-methyl. Relatively high (up to 70 mg/L) concentrations of herbicides, due to spray liquid remnants emitted during internal cleaning, seemed not to decrease the effectiveness in the on farm tests.

The Dutch government is interested in the possibilities of biopurification. A stakeholders committee including government and agribusiness has proposed to approve biopurification as a suitable method for cleaning water from external cleaning of sprayers in case this cleaning takes place frequently on the farmyard. If approved by the involved ministry, further conditions need to be defined for effective setup and use of biopurification and the disposal of effluent and filter substrate.

Studies on biobed in Turkey

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In Turkey, the first biobed research project was started on July 2007. This research project was funded by TUBITAK - The Scientific and Technical Research Council of Turkey, TOVAG 107O215. The specific aim of this study was to investigate the degradability of mixtures of pesticides in the biobed mixture. Biobed mixture was 50% straw, 25% soil and 25% peat. Soil and biobed mixture samples were taken at 0-15, 15-30 and 30-50 cm. Soil and biobed mixture samples were removed for each chemical treatment at 7, 14, 21, 28, 35, 42, 49, 56, 63 and 70 days after treatment (DAT). Malathion, dichlorvos and fenthion active substance were used for the degradation studies. These active substances degradation studies were determined by GC-NPD (gas chromatograph equipped with nitrogen-phosphorus detector). The samples were extracted by SPE (solid phase extraction) method.

In the result of this study, it was concluded that biobed should be used to reduce pesticide-contaminated waters during filling, mixing, and cleaning of sprayers in Turkey.

In 2010, our biobed research team started two new projects about using of organic materials in Turkey. One of them is usage of cotton seed and, the other is usage of sludge from wastewater treatment. These projects are to be funded by Cukurova University Research Project Unit.

New closed biobed with recirculation and evaporation for use under colder climates

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From April 2010 new regulations from the EPA in Denmark prescribe filling and cleaning of sprayers in the treated field or on impermeable surfaces with collection of the waste water. Biobeds with a clay layer or an open bottom are not allowed, based on the results from our previous studies[1], which showed a relatively high risk of leaching of the most polar pesticides. In the former studies the tested biobed was even in the summer time drowned in water from rain when the bottom valve was closed. The experiences have shown that biobeds used under climates like in Denmark require special precautions to avoiding problems with surplus water.

The Danish producer of concrete panels, Hans Christensen, Karrebæksminde, has developed a new biobed system with a closed bottom. The water is collected from the bottom and recirculated to the biobed. The water is distributed to the platform by drip pipes and the evaporation mainly takes place from the concrete.

A new study running in 2010 and 2011 financed by Bayer Crop Science will elucidate the potential for evaporation of surplus water and demonstrate the degradation potential for 21 different pesticides applied to the biobed. 5 gram of each pesticide will be applied to the biobed in the spring and autumn 2010 and in the spring 2011. Water samples will be analyzed from the collection tank. The biobed mixture, which consists of 50% chopped wheat straw, 25% sphagnum and 25% topsoil from the area as prescribed for the Swedish biobeds[2], will be analyzed as well.

Furthermore, three other biobeds of the same type and in use on farms will be analyzed. Samples from the water tank and from the biobed mixture will be taken in spring and autumn 2010 and 2011. The three biobeds represent a): Large-scale farming (same place as the research biobed), b): Orchard farming and c): Mixed use and general cleaning of machinery on the biobed.

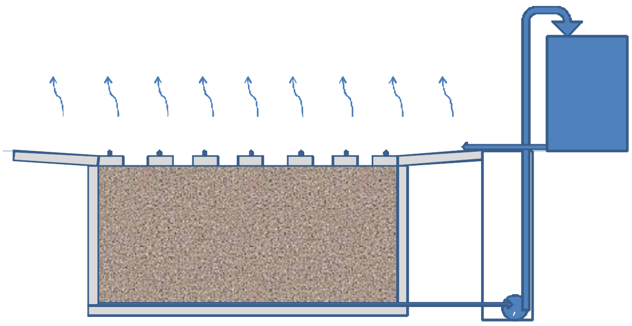	
Biobed: Closed system with recirculation and evaporation from the concrete.	The biobed with pallet containers to collect the water.

Table 1. List of compounds included in the study.

Active ingredient	Typ*)e	Product	Company
Bentazone, 480 gL^{-1}	H	Fighter 480	BASF
Bromoxynil, 200 gL^{-1}	H	Oxitril CM	Bayer CropScience
Aclonifen, 600 gL^{-1}	H	Fenix	Bayer CropScience
Fluazifop-P-butyl 250 gL^{-1}	H	Fusilade X-tra	Syngenta AG
Diflufenican, 500 gL^{-1}	H	DFF	Bayer CropScience
Iodosulfuron methyl, natrium, 50 gkg^{-1}	H	Hussar	Bayer CropScience
MCPA, 750 gL^{-1}	H	M-750	Klarsø & Co.
Metamitron, 700 gkg^{-1}	H	Goltix WG	Makhteshim
Pendimethalin, 400 gL^{-1}	H	Stomp	BASF
Propyzamide, 500 gL^{-1}	H	Kerb 500 SC	Dow
Prosulfocarb, 800 gL^{-1}	H	Boxer EC	Syngenta
Terbuthylazine, 500 gL^{-1}	H	Inter-Terbuthylazin	Inter Trade
Azoxystrobin, 250 gL^{-1}	F	Amistar	Syngenta
Kresoxim-methyl, 500 gkg^{-1}	F	Candit	BASF
Prothioconazole, 250 gL^{-1}	F	Proline EC 250	Bayer CropScience
Epoxiconazole, 125 gL^{-1}	F	Opus	BASF
Fenpropidin, 750 gL^{-1}	F	Tern	Makhteshim
Dimethoate, 500 gL^{-1}	I	Danadim Progress	Cheminova
Pirimicarb, 500 gkg^{-1}	I	Pirimor G	Syngenta

*): H: Herbicide, F: Fungicide, I: Insecticide.

The list of compounds includes very mobile pesticides such as bentazone and less mobile pesticides such as bromoxynil. Some of the pesticides are expected to degrade easily, for instance MCPA, while other compounds with sorptive properties are expected to degrade slowly.

The study will be reported at the end of 2011 and will give important information on the possibilities of handling surplus water in biobeds under colder climates and the potential for degradation of pesticides when the percolated water is circulated through the biobed.

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Héliosec® a simple system for waste water management

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When the Water Framework Directive (more formally the directive 2000/60/EC) was finally adopted by European Union (EU) member states, it committed the EU to achieving and maintaining good water quality in all of its bodies of water. This regulatory restriction, however, was turned into an opportunity by Syngenta France when effluent management became a priority for growers across the region.

Syngenta has tested and developed a concept based on the natural dehydration. The folder has been submitted to the French environment ministry. This device Héliosec®, the software and the approach are now officially recognized by French authorities.

The Héliosec® device

Héliosec® is delivered as a kit to the farmers. This kit contains a polyethylene tank with a double wall and a protective frame construction that is totally secure. This means it does not leak and neither child nor animal can intrude. A thin liner is installed in the tank and at the end will help to collect the dry residue in safe conditions. A specific gauge has been specifically designed. It easily helps to know the level of waste water in the tank as often as necessary. Héliosec® is patented.

The Héliosec® software.

Specific software has been developed to help growers to evaluate their waste water production and to minimize them. A calculation tool helps to do simulations of the level evolution during the time. A check list helps also to install the Héliosec® according "Good Agricultural Practices". Legal information are also given. Then, with the simulation tool, the size and the number of Héliosec® needed are estimated. The software is built as an interactive tool showing immediately to the farmers the impact of their practices modifications. A memo is given to the growers.

The Héliosec® final dry residue.

The final dry residue is collected with the liner and is disposed through a specialized destruction plant.

Héliosec® distribution

In France, distributors of agrochemical products which are engaged is sustainable agriculture are trained to use the software. They also sell the Héliosec® to the farmers.

Biobed hydrology – observations during initial year of field scale studies in Canada

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To comply with local laws, biobeds established in Saskatchewan, Canada in 2009 were lined with an impermeable geo-membrane to prevent possible leaching of pesticide to groundwater. Lysimeters were installed to remove excess moisture, and moisture status was measured at various depths throughout the season. Grass cover was established on the biomix surface. Unusually heavy rainfalls saturated the biomix early in the season, resulting in large amounts of water pumped from the lysimeters. The moisture status of the biobeds eventually returned to below field capacity, and later in the season, dried out to enough for cracks to form in the biomix. The management of water status and preferential flow therefore became a preoccupation in the first year of operation. An above-ground biobed installation remained drier. A sharing of expertise from other researchers is sought so that these conditions can be better managed in the future.

Bioremediation systems in Belgium: state of the art

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Bioremediation systems are used to treat waste water from the spraying process which is contaminated with PPPs. In Belgium, two types are installed namely biofilter and phytobac. However the construction and legislation of those two systems differs between the two regions, Flanders and Walloon. This is seen in the number of installation and also in the location (farm, research centre) where the systems are installed.

Until now, in Flanders 8 biofilters and 8 phytobacs are installed. More than half of them are installed at a research centre. In the Walloon area, around 50 biofilters are already installed at farm locations, even one biofilter is used by a community for their maintenance of streets, parks, …. In Flanders, bioremediation systems are not allowed while in the Walloon area they are tolerated. This results in a different policy between the two regions. In Flanders, farmers can not install bioremediation systems except for demonstration reasons. But also the construction differs between the regions. In Flanders, 'closed' systems are used. This means that no leakage can occur to the environment. The waste water is mainly evaporated after treatment by micro organisms. The organic material in the system is composed to have a long live time as it is not allowed to put this material onto the field after use. In the Walloon area, leakage after treatment is allowed. Therefore, less organic material is necessary and it is replaced more often. Organic material which is not used any more, can be put on the field (1 m^3 per ha).

In this presentation, the state of the art of bioremediation systems in Belgium is illustrated by practical examples. How do we advise the farmers which system they need to install, which parameters are taken into account, what is the price, …? The examples are a biofilter system in Flanders and in the Walloon area and a phytobac. Each system is connected to a certain farm type and size. Also the behaviour of the farmers is taken into account when installing bioremediation systems.

Finally, for contract sprayers another treatment system for waste water is advised. This physico-chemical system, named Sentinel, is not limited by volumes of waste water, neither by the chemical load of the waste water. This system is ideal for contract sprayers which can have high volumes and/or more concentrated waste water compared to the average farmer.

A comparison between the three systems shows that for each type of farm, an ideal systems can be created.

Phytobac®, french experience

Darmedru, J.

Environment consultant

Inspired by the swedish concept of biobed, Phytobac® has been developped by Bayer CropScience since 1996. After some trials the first was built in 1998 near Troyes. Quickly farmers perceived the benefits of Phytobac® for management of liquid waste, simple, biological working. Bayer CropScience got informations, training for benefits of Phytobac®, how to use and build it. And got partners, Biotisa and Hermex, to manufacture Phytobac® ready to use kits, increasing quality and security. At present there are one thousand of Phytobac® and « lits biologiques », for crop and non crop, as individual and collective.

MY WORKSHOP NOTES ………..