Biodepuration of wastewaters from the fruit-packaging industry using Biobeds: Bioaugmentation, risk assessment and optimized management

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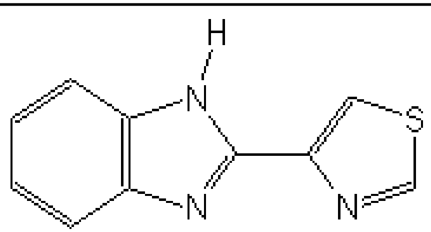
Establishing the problem…

- **Fungicides** and **antioxidants** are applied for the control of postharvest fungal decays by *Penicilium* and physiological disorders (apple scald) in apples, pomes, citrus, oranges

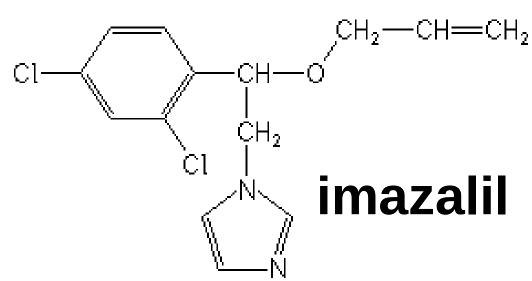

- Production of **large amounts of wastewaters** (10-100 m^3) containing high pesticide loads (10-200 mg/L)

Fungicides used at EU level in fruit packaging plants

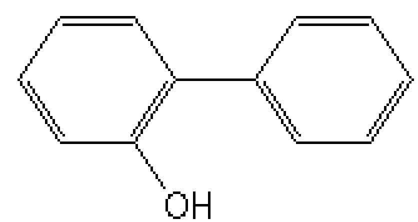
Fungicides	Registration Status	Registration in member states
Imazalil	**Yes** until 31/7/2021	Cyprus, Greece, France, Spain, Italy, Portugal
Thiabendazole	**Yes** until 31/12/2021	Cyprus, France, Spain, Italy, Portugal, Greece
Ortho-phenylphenol	**Yes** until 31/12/2019	Cyprus, Greece, Spain



thiabendazole



imazalil

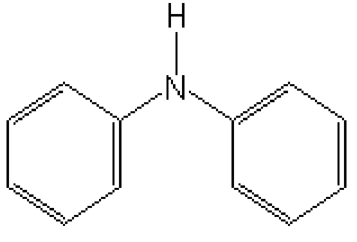


Ortho-phenylphenol

Antioxidants used at EU level in fruit packaging plants

Fungicides	Registration Status	Registration in member states
Ethoxyquin	**Not inclusion in Annex I since 2009**	-
Diphenylamine	**Not inclusion in Annex I since 2009**	Under re-evaluation

diphenylamine

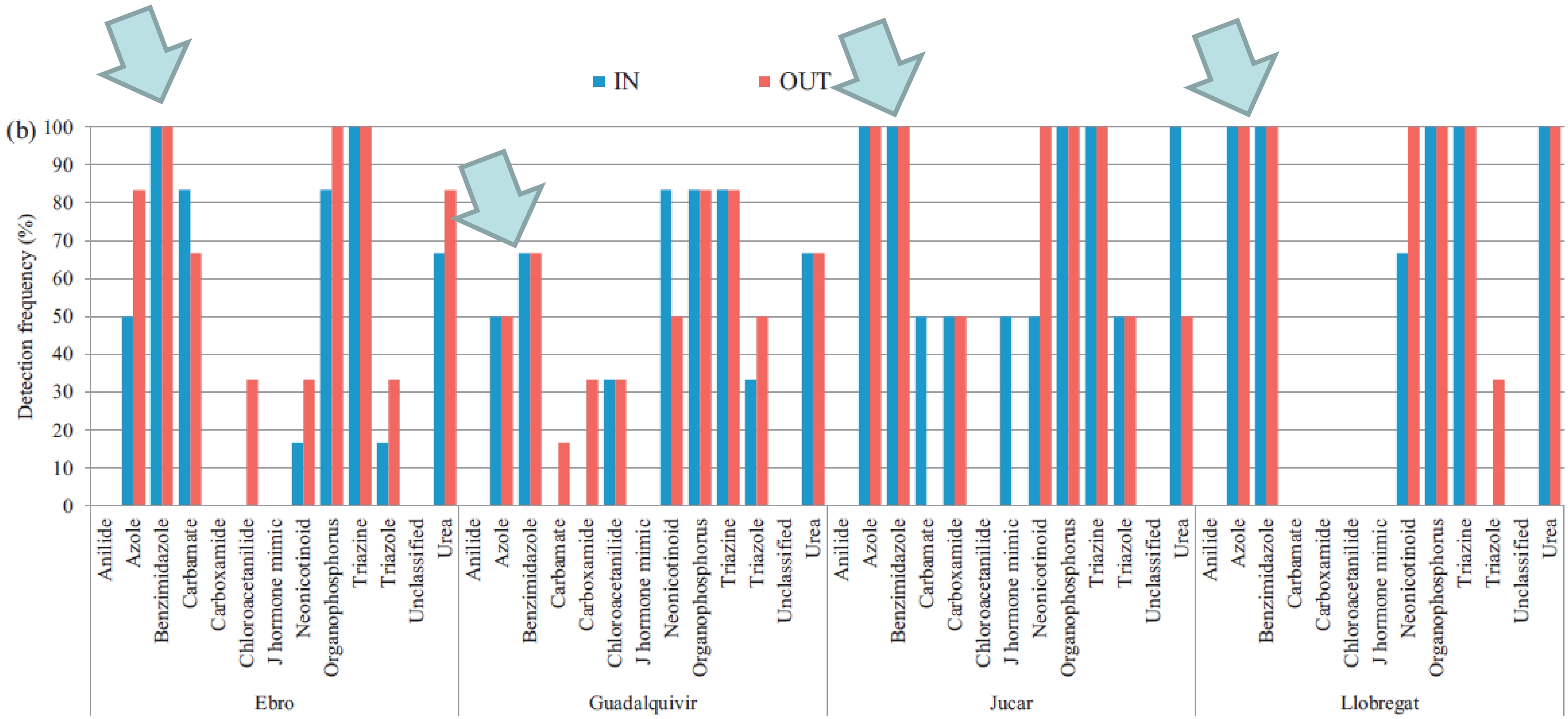


All those pesticides share a common paragraph in their registration documents…..

Member States should pay particular attention ***to ensure that appropriate waste management practices to handle the waste solution remaining after application, including for instance the cleaning water of the drenching system and the discharge of the processing waste are put in place***

How do we handle these wastewaters today?

- Disposal into municipal wastewater treatment plants

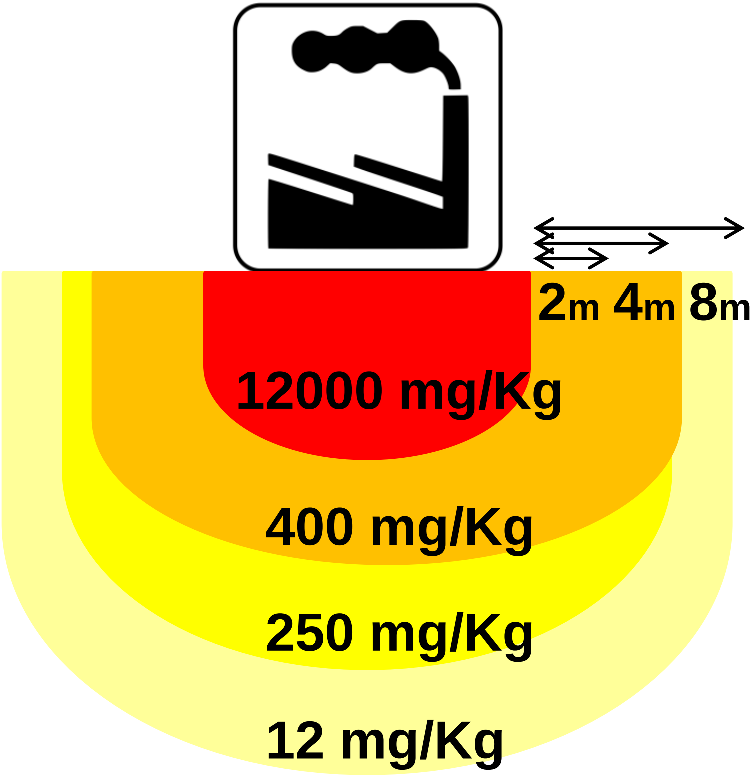


No removal of those pesticides in MWTPs

Campo et al. (2013) J Hazard. Mat. 263P: 146-157

How do we handle these wastewaters today?

- Land spreading on adjacent field sites



Result in the progressive contamination of soil especially for persistent chemicals like thiabendazole

How do we handle these wastewaters today?

- Physicochemical treatment with CONTROL TecEco® (Technidex) based on filtration through activated carbon

- ***Highly efficient for thiabendazole***
- ***Not adopted due to high cost and high engineering needs***

How do we handle these wastewaters today?

- Treatment by companies certified to treat *ex situ* toxic wastes

- ***Unacceptable cost*** (0.70 – 3 € per litre of wastewater (depending on the volume))

So we aim….

- To provide an **effective and economic solution** for the local fruit packaging plants to depurate the wastewaters produced by their phytosanitary activities

Biobeds - Why not?

Biobed Packing Material

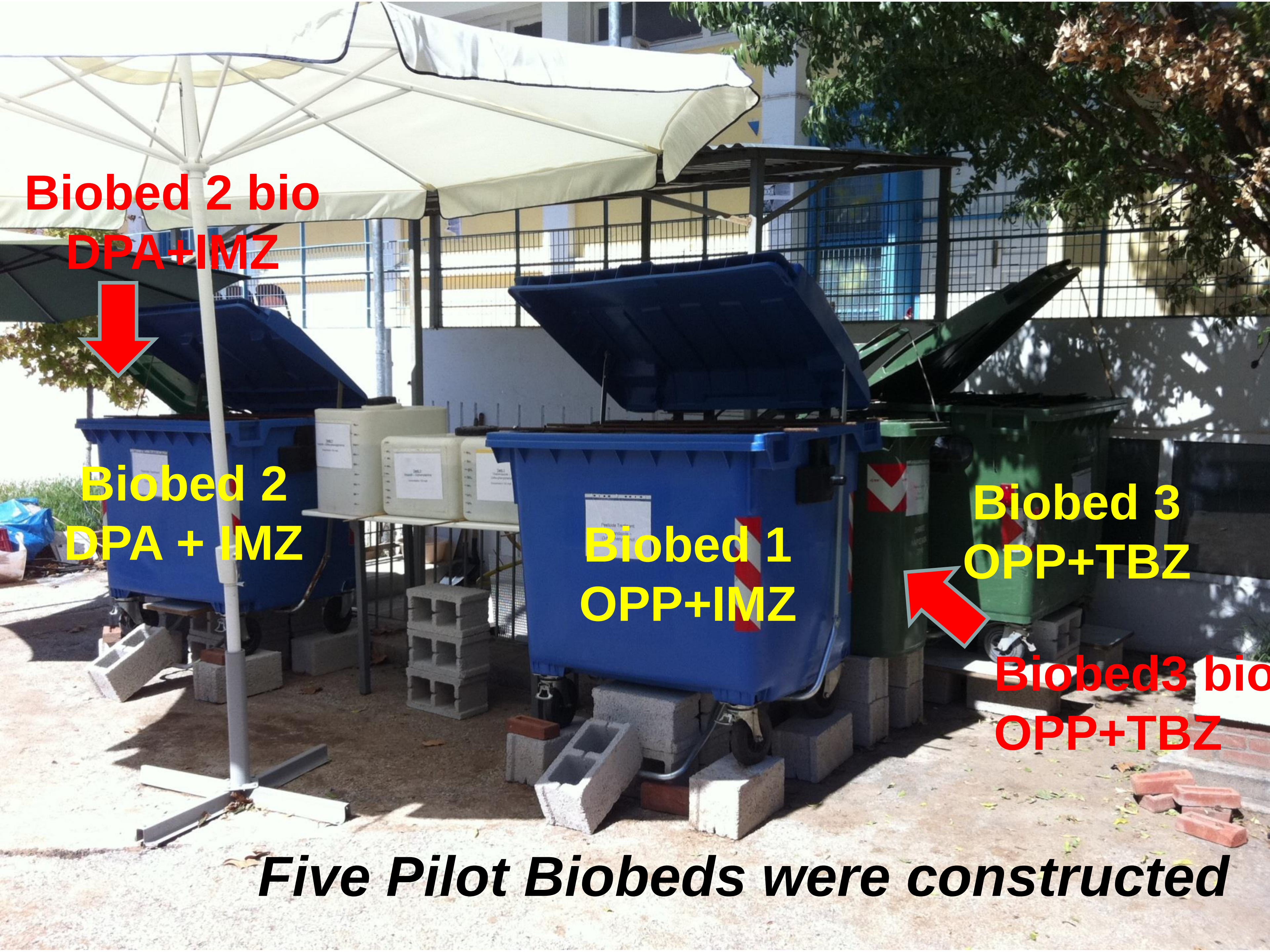
- **Soil**
- **Straw**
- **Spent Mushroom Substrate:** Pasteurized straw colonized by *Pleurotus ostreatus* edible basidiomycetes (good pesticide-degrader) which is considered a waste for mushroom growers after 2-4 harvests and they would like to find a way to get rid off it

Biobeds for treatment of wastewaters from post-harvest activities

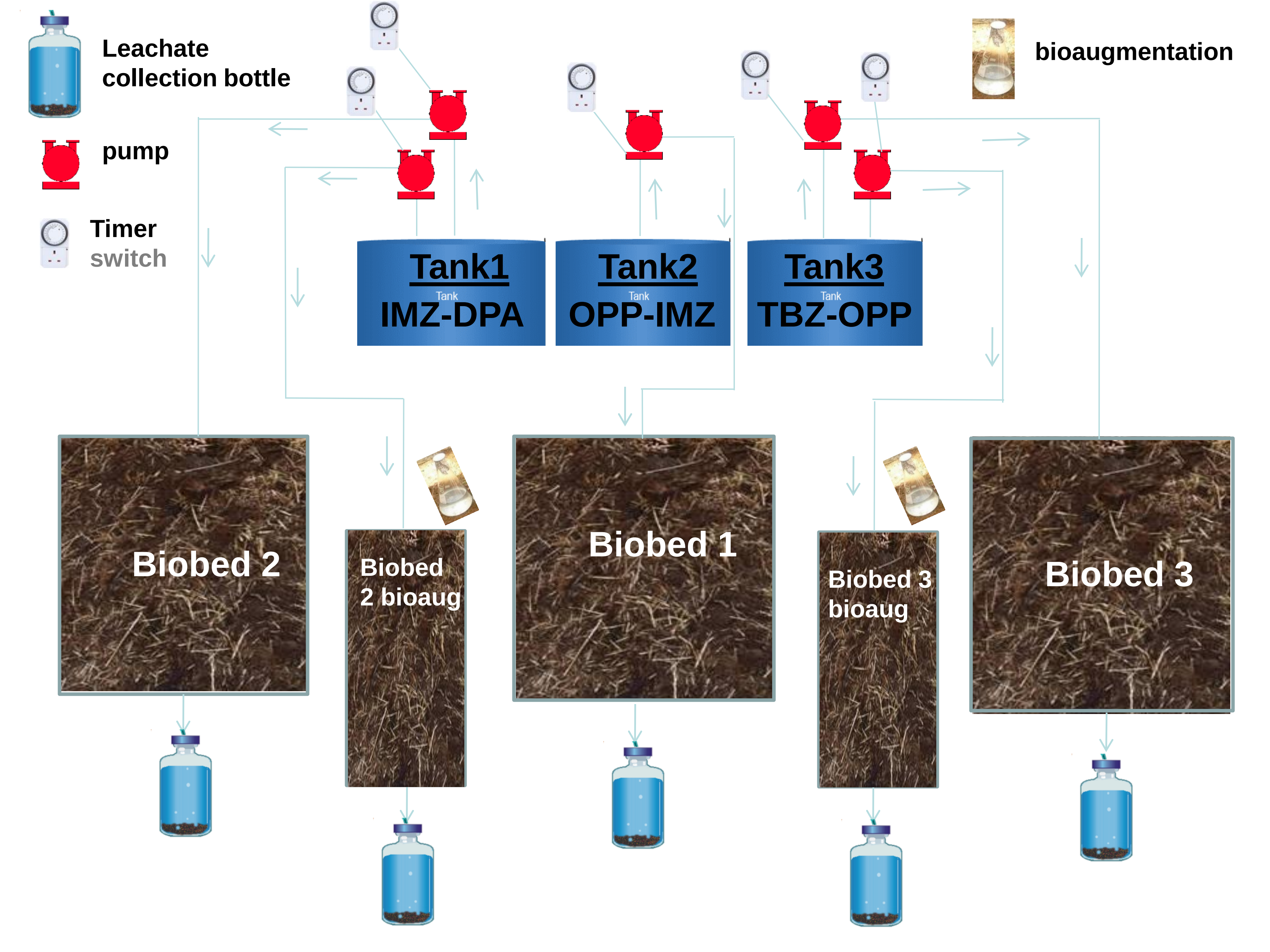
Evaluation of pilot biobed systems

Handling of biobed effluent

Handling of biobed spent packing material

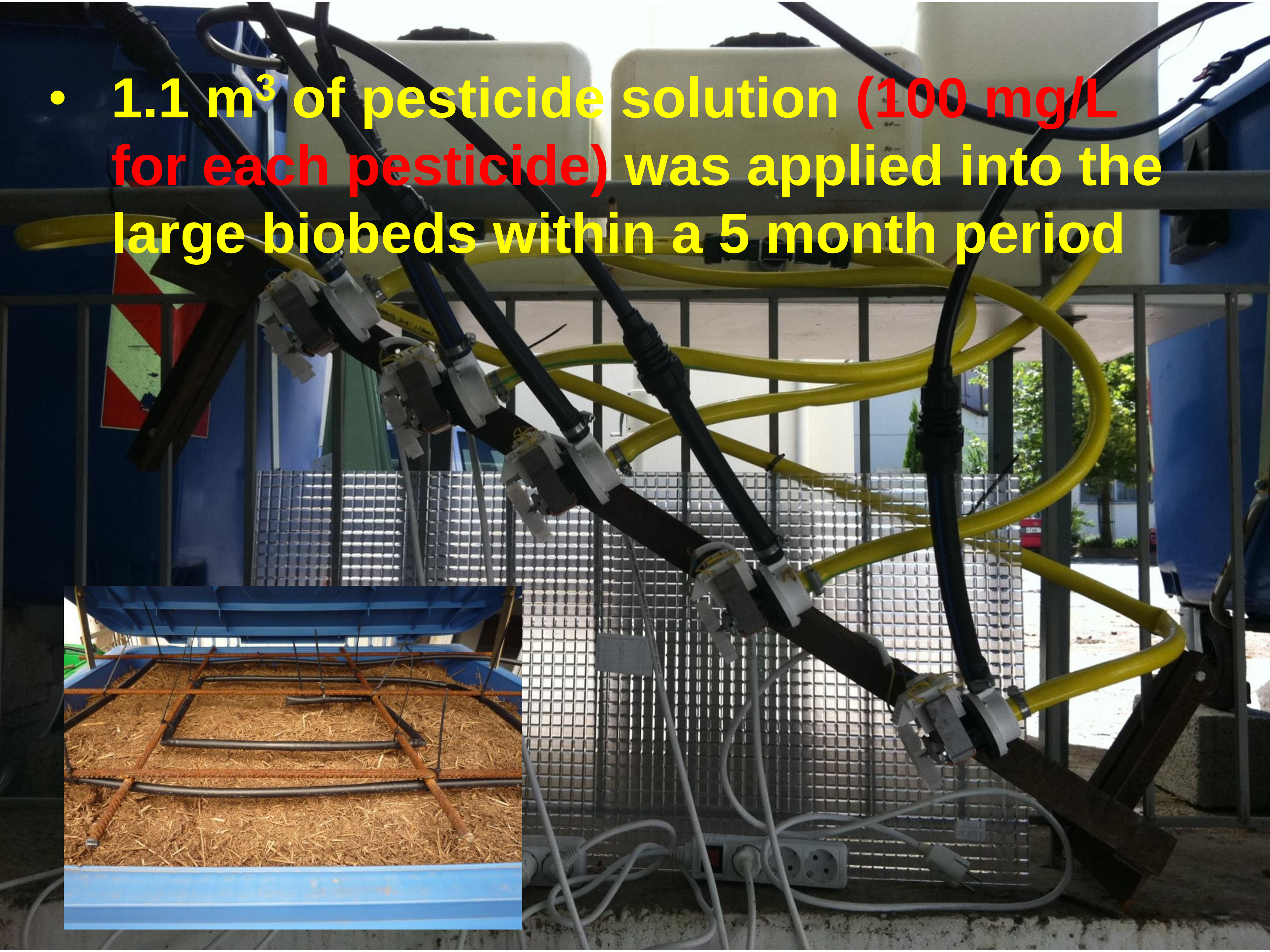


Five Pilot Biobeds were constructed



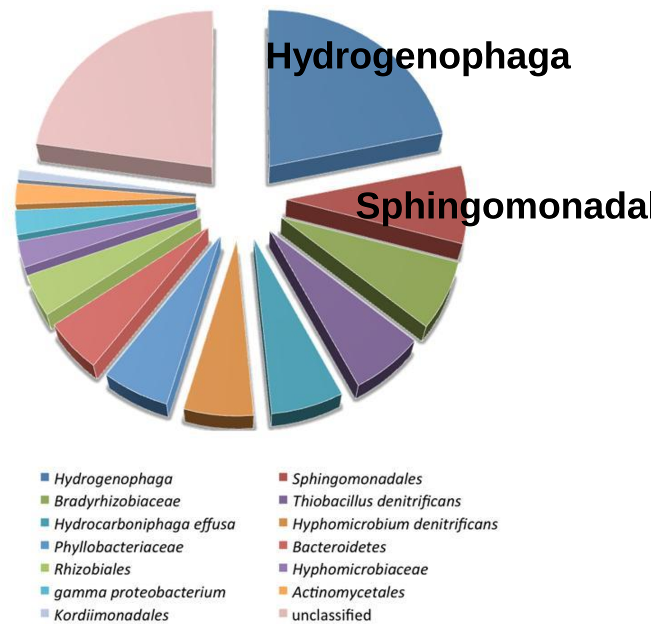
Leachate collection bottle
pump
Timer switch
bioaugmentation
Tank1
Tank
IMZ-DPA
Tank2
Tank
OPP-IMZ
Tank3
Tank
TBZ-OPP
Biobed 2
Biobed 2 bioaug
Biobed 1
Biobed 3 bioaug
Biobed 3

- 1.1 m^3 of pesticide solution (100 mg/L for each pesticide) was applied into the large biobeds within a 5 month period




Leachates collected on a day by day intervals and analyzed via HPLC-UV

Bioaugmentation Procedure



Biobed 3 bio

- **TBZ degrading consortium**
- ***Sphingomonas haloaromaticans*** **(OPP-degrader)**

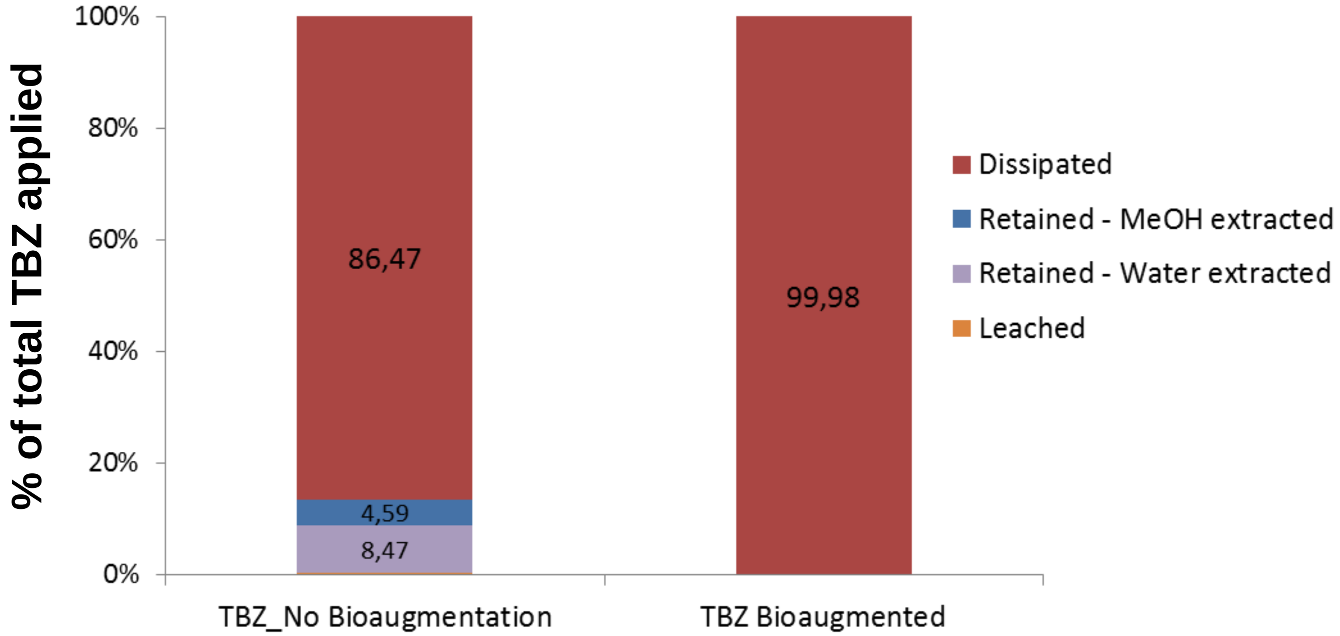
Biobed 2 bio

- ***Pseudomonas putida*** **(DPA-degrader)**

At the end of the treatment period....

- *3 cores from each biobed (layers 0-20, 20-50 and 50-80 cm)*
- *Extraction 3 times with water* ***(readily available for leaching)***
- *Subsequent Extraction with Acetonitrile* ***(less labile for leaching)***

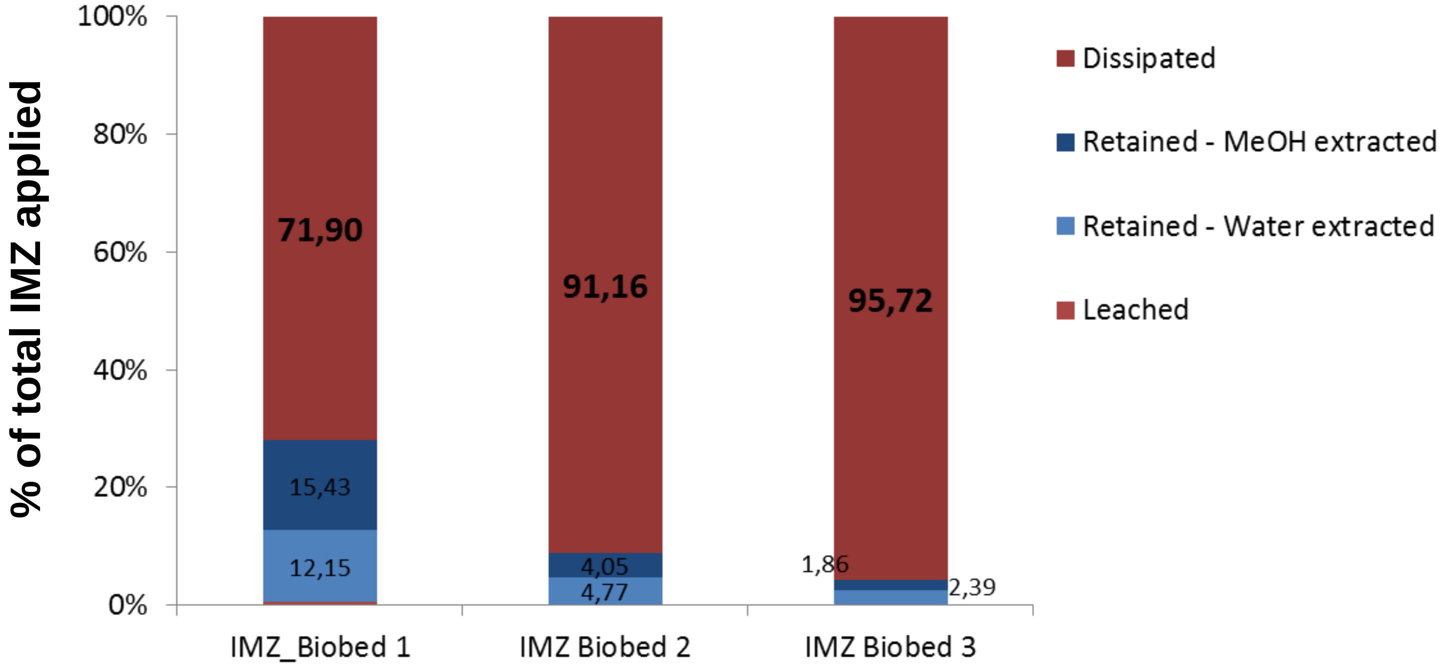
Mass Balance Thiabendazole



TBZ degrading consortium accelerates significantly the dissipation of TBZ in the biobed

Karas et al. (2016) J Hazardous Materials (in press)

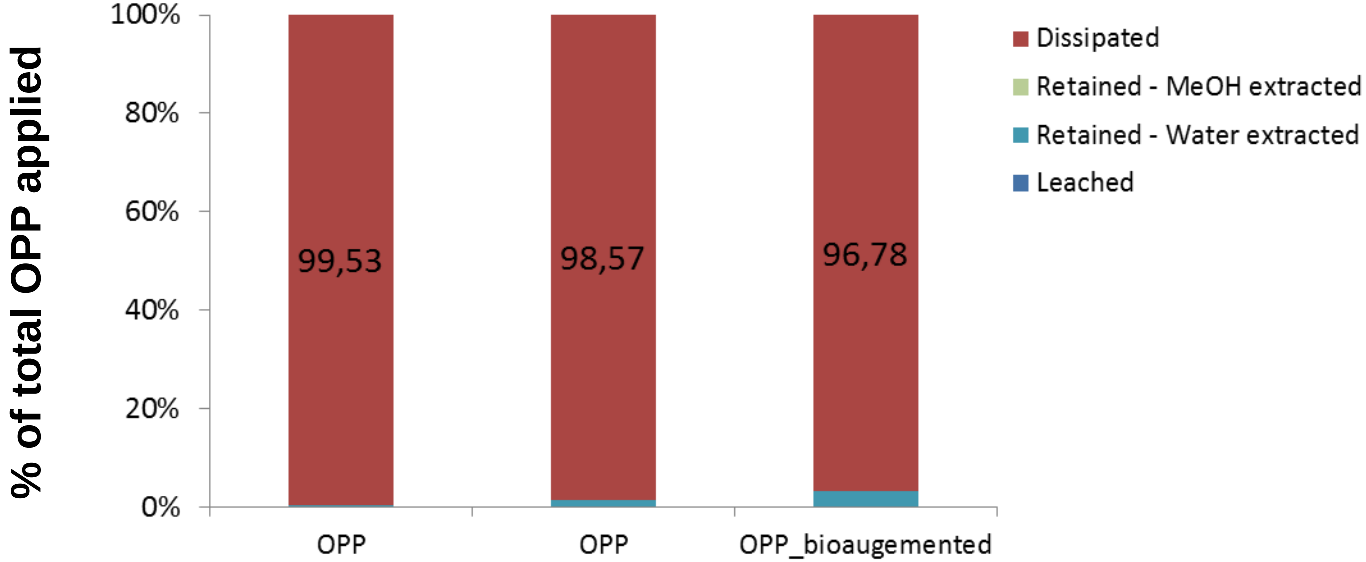
Mass balance Imazalil



- Imazalil is the most persistent chemical from the ones tested (DT_{50}= 44-122 d) but even this is dissipated effectively (72-96% of total applied)

- *99% of the amount retained was in the 0-20 cm layer*

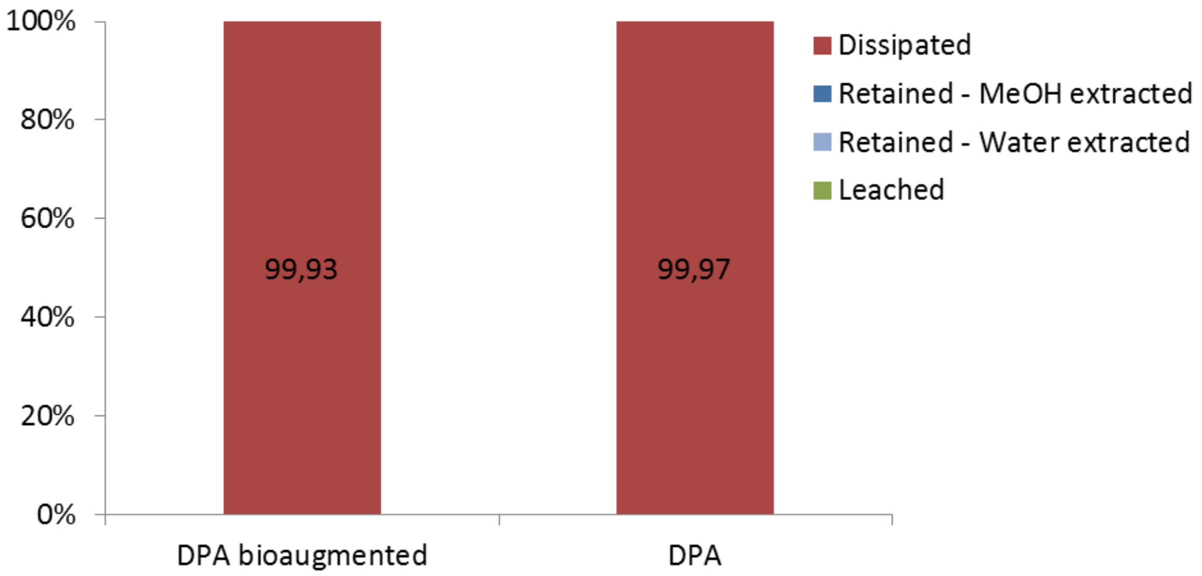
Mass balance *ortho*-phenylphenol



- OPP dissipated and only small amounts were retained in the biobed (water extractable)

- The indigenous microbial community had the capacity to degrade OPP and no bioaugmentation was needed

Mass balance diphenylamine



- DPA dissipated almost totally from biobeds with only small amounts leaching
- The indigenous microbial community had the capacity to degrade OPP and no bioaugmentation was needed

Biobeds for treatment of wastewaters <u>from post-harvest activities</u>

Evaluation of pilot biobed systems

Handling of biobed effluent

Handling of biobed spent packing material

How much pesticide in the biobed-treated effluents?

Pesticides	% Leached	Depuration Efficiency (%)
Thiabendazole	0 and 0.46	>99.5
Imazalil	0.52, 0.02, 0.03	>99.5
Diphenylamine	0.03 and 0.07	>99.9
Ortho-phenylphenol	0.01-0.10	>99.9

Is this acceptable for direct environmental release i.e. land-spreading ?

Risk Assessment

Risk assessment assumptions

- Depuration efficiencies of pilot biobeds per pesticide
- 100 mg L^{-1} of pesticide concentration in wastewaters
- Two Scenarios tested:

  - Pome fruits packaging plants (25 m^3 of wastewater containing DPA + IMZ or TBZ)

  - Citrus fruits packaging plants (42 m^3 of wastewater containing OPP and 11 m^3 of wastewater containing IMZ or TBZ)

- Discharge of treated effluents in a 0.1 ha disposal site

Risk assessment Calculations

- **Predicted Environmental Concentrations in Soil** (PECsoil) calculated with PECsoil calculator
- **Predicted Environmental Concentrations in Water and Sediment** (PECsw or PECsed) calculated with STEP1-2 calculation tool
- **Toxicological endpoints (i.e. Regulatory Acceptable Concentrations, RACs)** for aquatics, earthworms and terrestrial arthropods were taken from pesticide regulatory documents

Exposure

Effects

Risk Assessment Decisions

Trigger for Unacceptable Risk

- PECs / RACs > 1 (Aquatics)
- Toxicity Exposure Ratio (TER) <10 (Earthworms)
- Hazard Quotient (HQ) > 2 (Soil arthropods)

PECs / RACs Aquatics

Pesticides		Acute Toxicity		Chronic Toxicity		
		Invertebrates	Fish	Algae	Fish	Sediment-Dwelling Invertebrates
		Daphnia magna	*Oncorhynchus mykiss*	*Pseudokirchneriella subcapitata*	*Oncorhynchus mykiss*	*Chironomus sp.*
Ortho-phenylphenol	Scenario II	0.041	0.025	0.003	0.278	0.005
Diphenylamine	Scenario I	0.053	0.029	0.021	0.009	n.d.[d]
Imazalil	Scenario I	0.138	0.244	0.108	0.502	0.119
	Scenario II	0.197	0.351	0.156	0.723	0.172
Thiabendazole	Scenario I - Step1	**1.490**	0.920	0.022	**4.217**	0.025
	Scenario I - Step2	0.576	-[a]	-	**1.633**	-
	Mitigation/Refinement	-	-	-	0.817[b] (0.366)[c]	-
	Scenario II - Step1	0.656	0.405	0.010	**1.858**	0.011
	Scenario II - Step2	-	-	-	0.717	-

No unacceptable risk for DPA, OPP, IMZ and TBZ (only in Scenario II)

Unacceptable risk for TBZ in Scenario I which required refinement:

- Bioaugmentation of biobeds (lower TBZ concentrations effluents)
- Disposal of effluents in a 0.2 ha site

Terrestrial organisms

TER<10 **HQ>2**

Pesticides		TER - Earthworms	HQ - Soil-dwelling arthropods
Ortho-phenylphenol	Scenario II	16517	n.d.[a]
Diphenylamine	Scenario I	n.d.[a]	n.d.[a]
Imazalil	Scenario I	4045	0.540
	Scenario II	9310	0.238
Thiabendazole	Scenario I	>335	<0.639
	Scenario II	>743.5	<0.281

No unacceptable risk for earthworms and soil-dwelling arthropods

Karas et al. (2016) J Hazardous Materials (in press)

Biobeds for treatment of wastewaters from post-harvest activities

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Handling of biobed effluent

Handling of biobed spent packing material

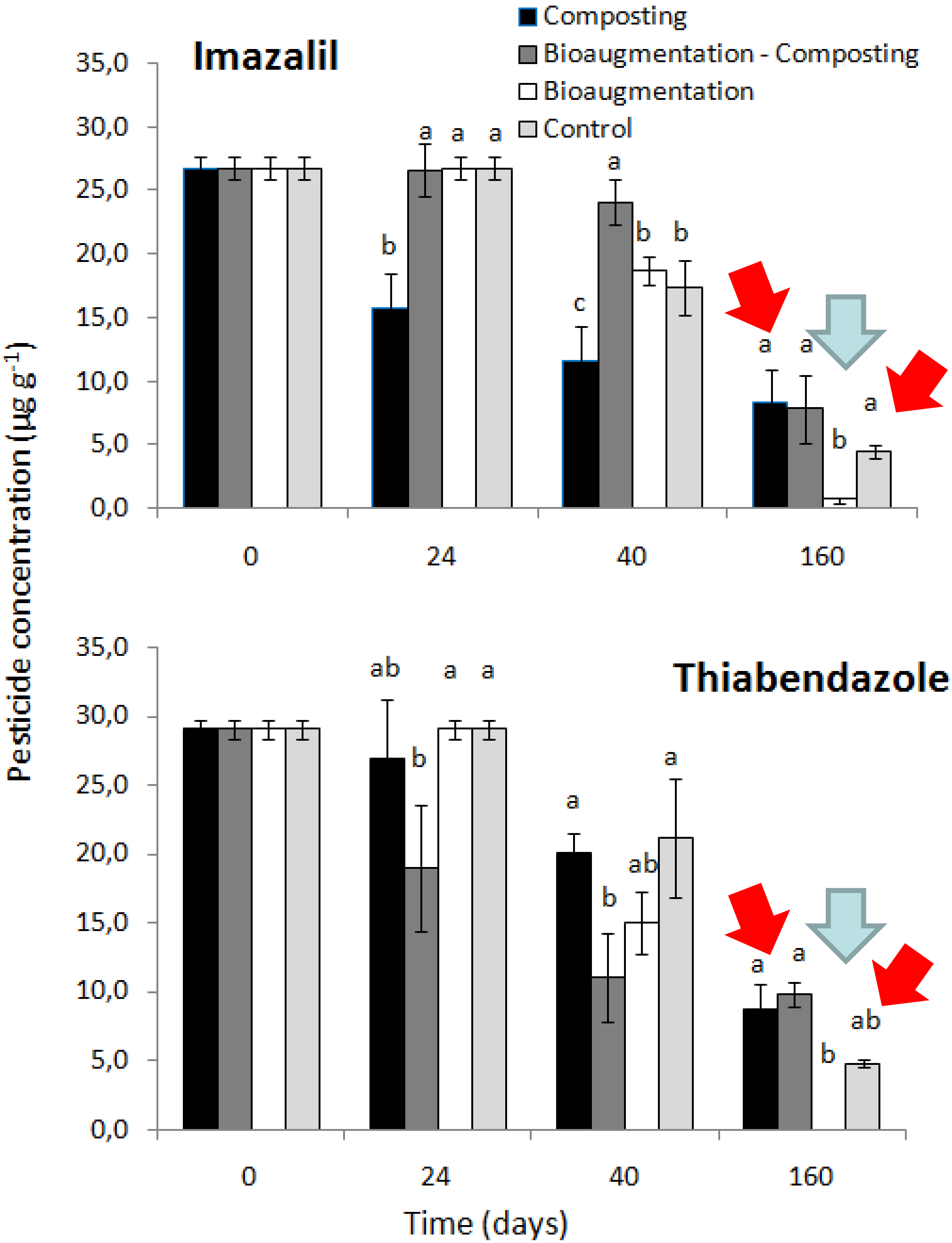
How do we decontaminate the spend biobed packing materia?

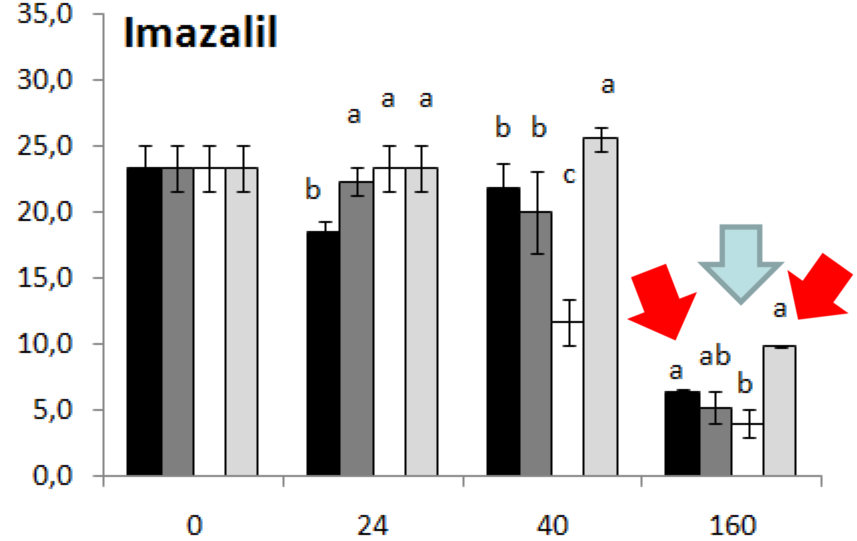
- **<u>Composting</u>** with fresh organic matter (straw + cotton crop residues + N)

- **<u>Storage</u>** at ambient temperature (CONTROL)

- **<u>Bioaugmentation</u>** with pesticide-degrading microbes
  - Mixture (10^6 cells/g) of TBZ - , DPA and OPP-degrading bacteria

- **<u>Bioaugmentation</u>** and **<u>composting</u>**





Bioaugmentation: the most effective decontamination method

<u>Composting</u> or <u>storage at ambient temperature</u> resulted in acceptable levels of decontamination

Karas et al. (2016) J Hazardous Materials (submitted)

Overall…..

- Depuration efficiency of biobeds > 99.5% for all pesticides
- Bioaugmentation for persistent chemicals like TBZ optimizes performance
- Treated effluents could be disposed into soil disposal site in the vast majority of cases without environmental risk
- Bioaugmentation most potent method for the decontamination of the spent biobed packing material. *In the absence of inocula, composting or storage at ambient temperature could achieve effective reduction in pesticide levels in the spent packing material*

Many thanks to....

Panagiotis Karas, PhD student

Dr. Chiara Perruchon

Dr Michalis Omirou
Agricultural Research Institute of Cyprus

Dr Constantinos Ehaliotis
Agricultural University of Athens

Dr Evangelos Karanasios
Benaki Phytopathological Institute

Funding……

ΕΠΙΤΡΟΠΗ ΕΡΕΥΝΩΝ
ΠΑΝΕΠΙΣΤΗΜΙΟΥ ΘΕΣΣΑΛΙΑΣ

European Union
European Social Fund

OPERATIONAL PROGRAMME
EDUCATION AND LIFELONG LEARNING
investing in knowledge society
MINISTRY OF EDUCATION & RELIGIOUS AFFAIRS
MANAGING AUTHORITY
Co-financed by Greece and the European Union

NSRF 2007-2013
programme for development
EUROPEAN SOCIAL FUND

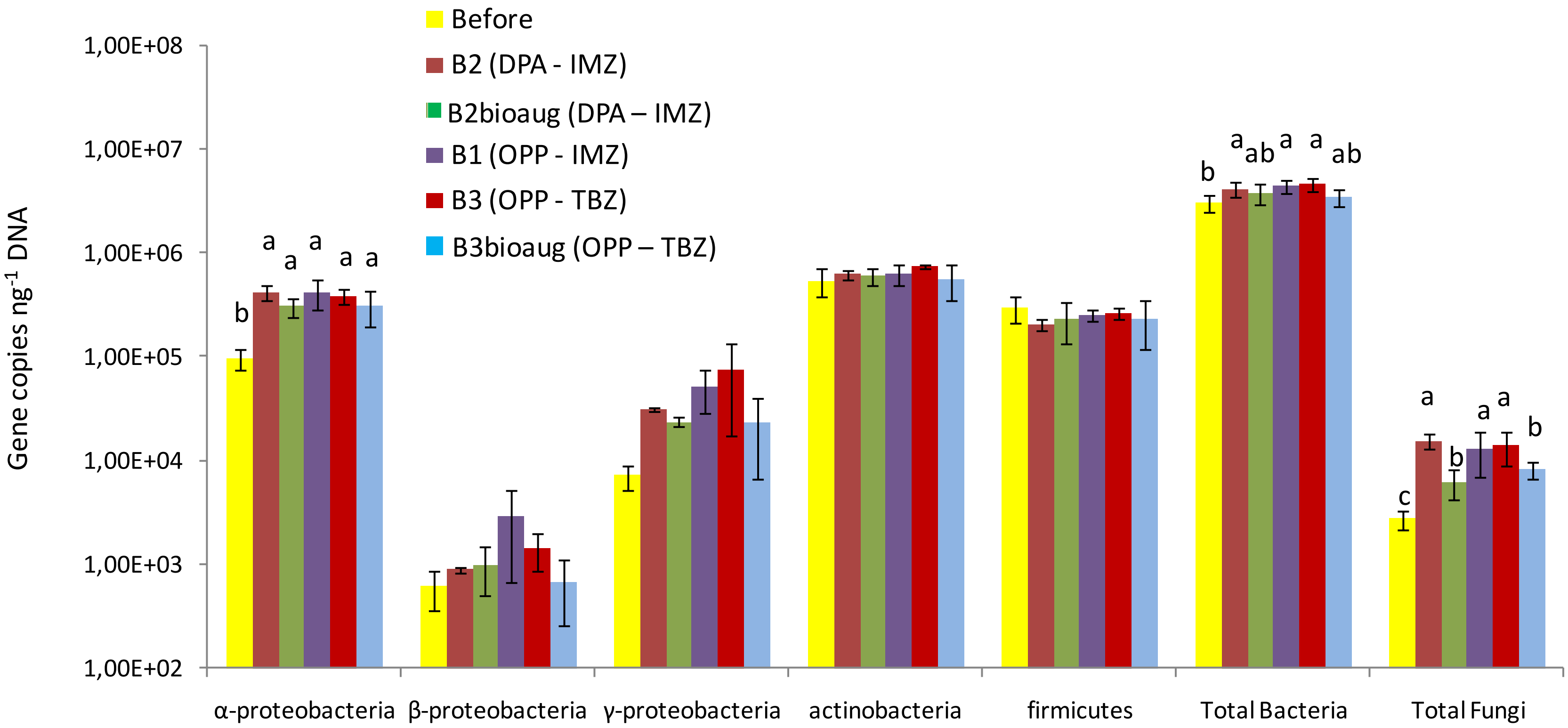
ΔΕΣΜΗ 2008

B&B

Ελληνική Δημοκρατία
ΠΕΡΙΦΕΡΕΙΑ ΘΕΣΣΑΛΙΑΣ

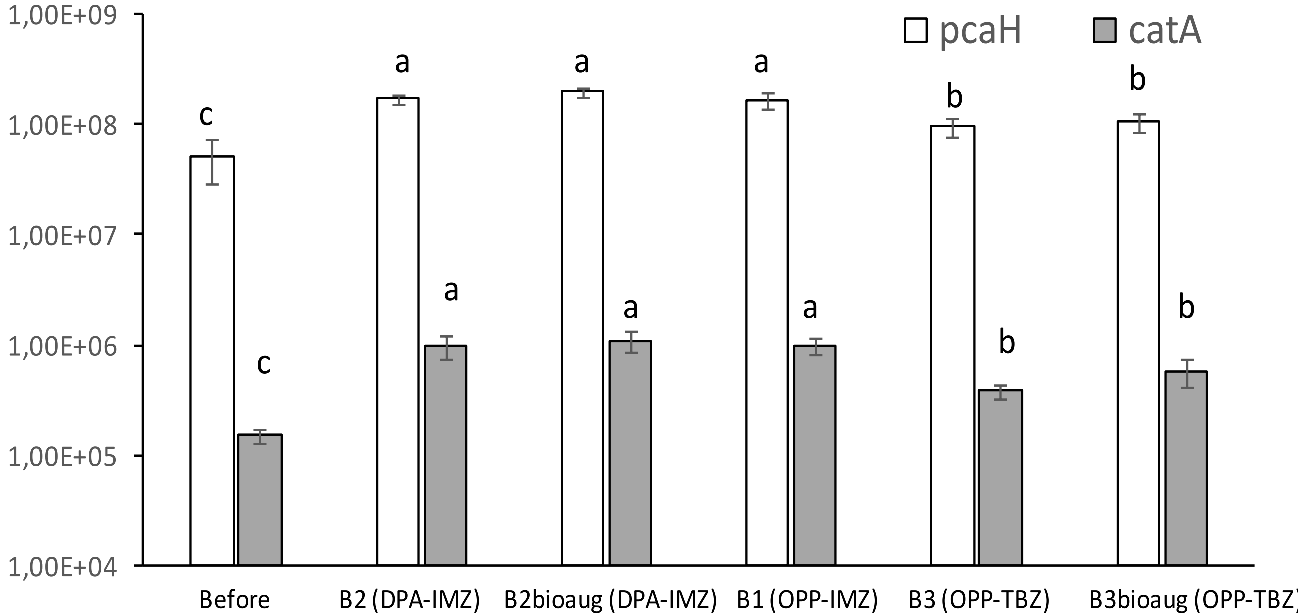
Microbial Community Changes in the Biobed Systems

q-PCR of main bacteria taxa and fungi



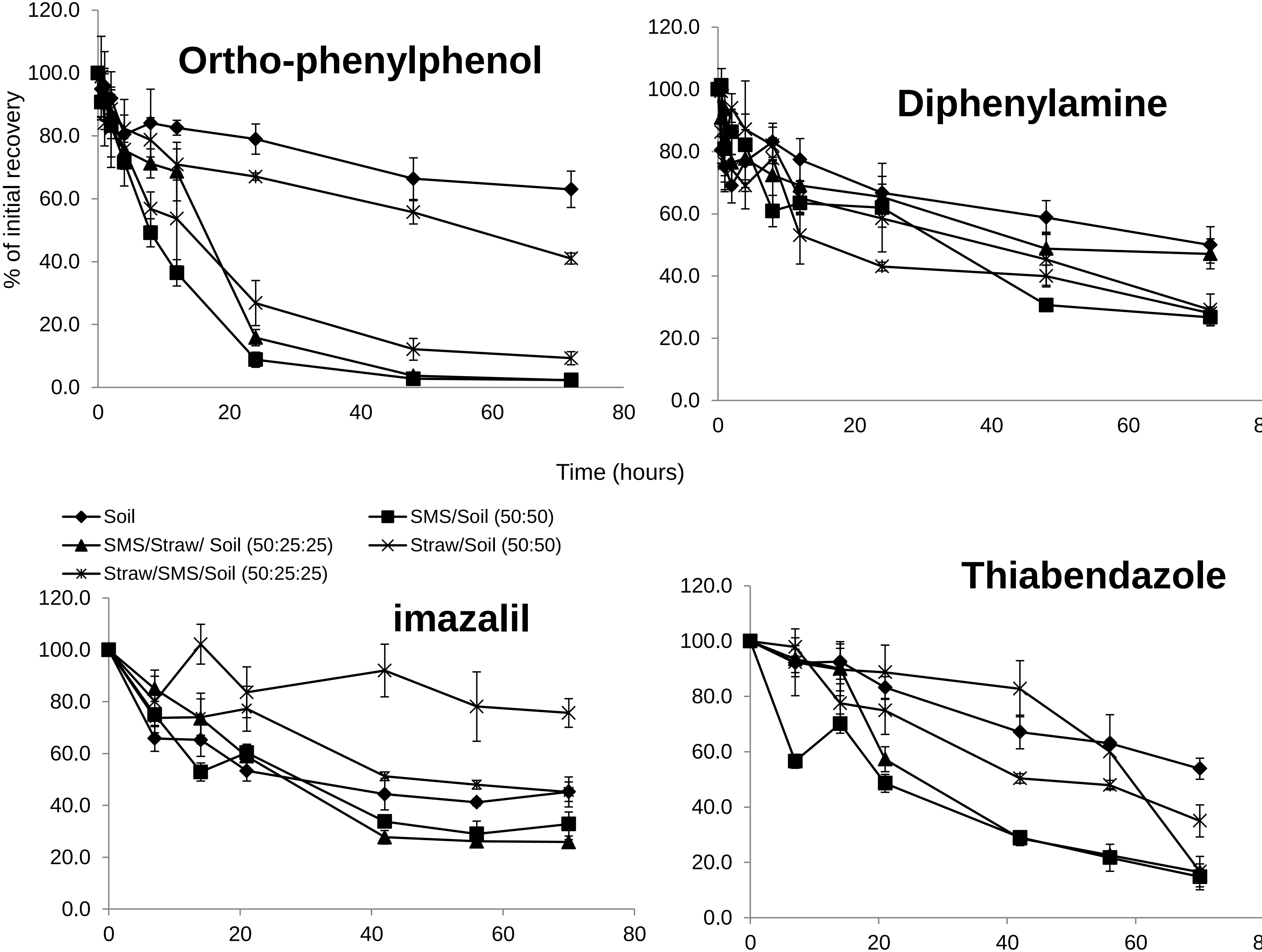
- Dominance of actinobacteria, firmicutes and a-proteobacteria
- Increase in the abundance of a-proteboacteria & fungi at the end of the study

Changes in the abundance of *pcaH* and *catA* genes



Significant increase in the abundance of *catA* & *pcaH* genes in all biobeds regardless of bioaugmentation or not at the end of the study

Hypothesis: General stimulation of the catabolic properties of the biobeds microbial community against aromatic organic compounds



Karas et al. (2015) Sci Total Environ