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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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 31/12/2015	Cyprus, France, Spain, Italy, Portugal
Ortho-phenylphenol	**Yes** until 31/12/2019	Cyprus, Greece, Spain

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**	120 days authorization was granted in Greece, Spain, Portugal

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

<u>Member States should pay particular attention</u> 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. Prevention of any accidental spillage of treatment solution. Member States permitting the release of waste water into the sewage system shall ensure that a local risk assessment is carried out

How do we handle these wastewaters today?

- Direct Disposal into creeks, sewage treatment plants, evaporation ponds

- Land spreading on adjacent field sites
- Physicochemical treatment with CONTROL TEC ECO® based on filtration through activated carbon (***Unacceptably High Cost***)

So we aim….

- To provide a **viable, effective and economic solution** for the local fruit packaging plants to decontaminate the wastewaters produced by their phytosanitary activities

First problem: wastewater volumes..

- Large fruit-packaging plants producing 50 -100 m^3 or more wastewaters

  More engineering based bioreactor system is needed

- Small to medium size fruit packaging plants producing 10-50 m^3 of wastewaters

  Biobeds

Bioreactor treatment of wastewaters

- **Development of microbial inocula for rapid degradation of the pesticides contained in wastewaters**
  - Two thiabendazole-degrading consortia composed of *Pseudomons, Sphingomonas*, other *a-* and *b-proteobacteria*
  - *Pseudomonas monteilli* strain degrading diphenylamine
  - *Pseudomonas stutzeri* and *Sphingomonas haloaromaticans* degrading ortho-phenylphenol

Biobeds for treatment of wastewaters from post-harvest activities

Optimization Steps

Selection of biomixture showing high degradation potential for the given pesticides

↓

Water management (frequency/rate of wastewater discharge on the biobed)

↓

Evaluation of pilot biobed systems

↓

Handling of spent biobed substrate
Handling of biobed effluent

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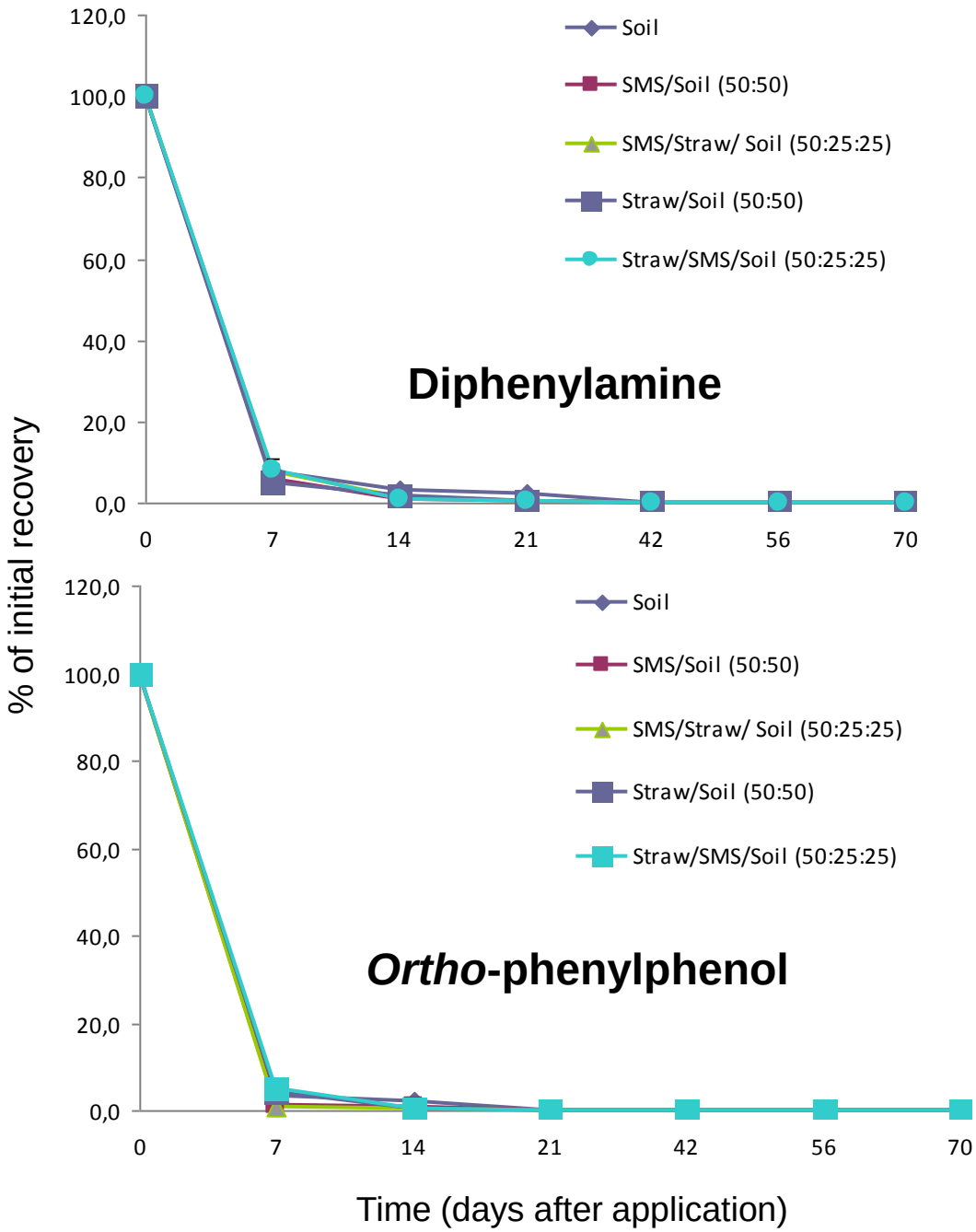
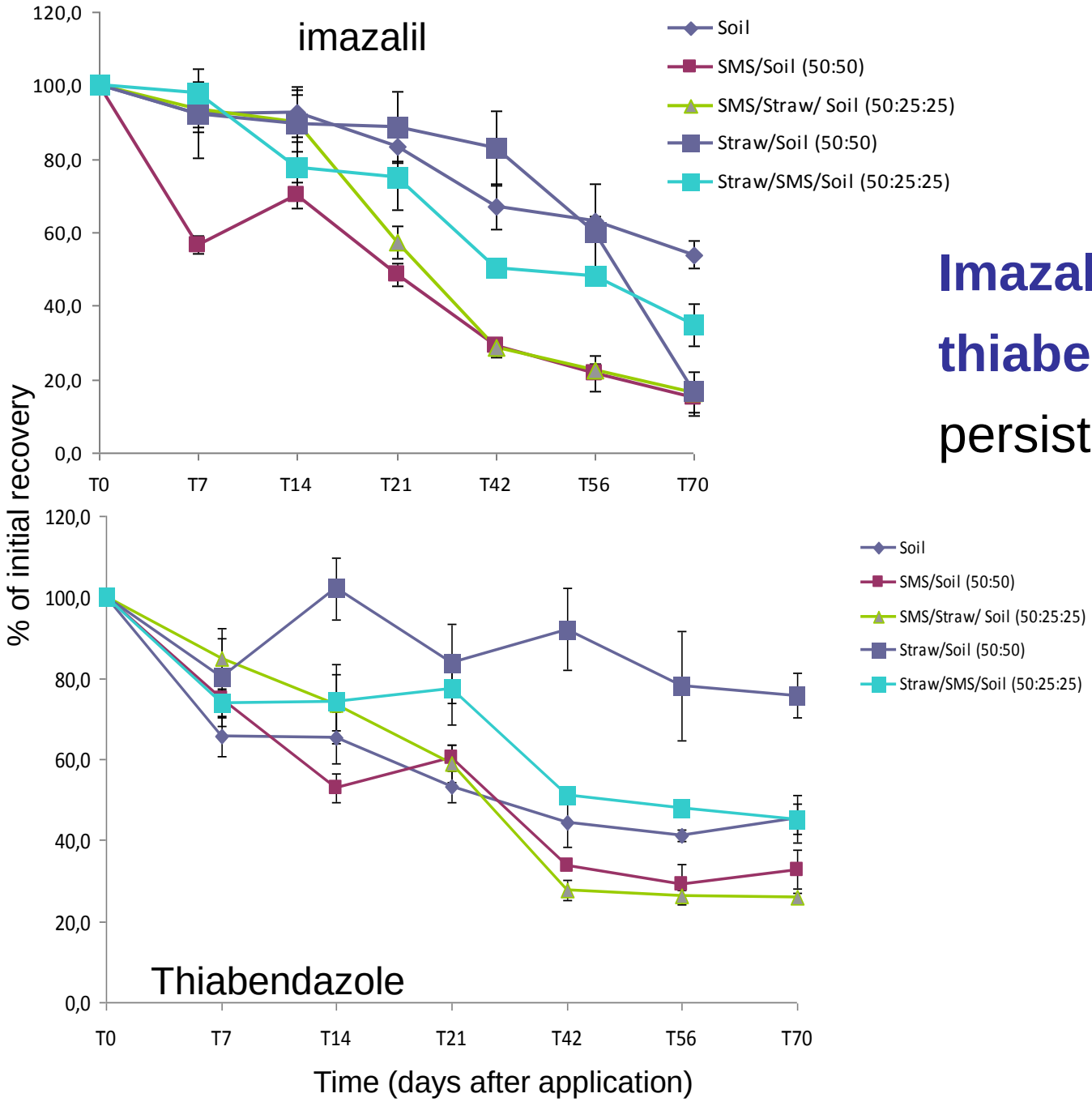
Biomixture Composition

- **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



Ortho-phenylphenol and **diphenylamine** are not persistent chemicals

Karas P., PhD



Imazalil and **thiabendazole** more persistent

Karas P., PhD

DT50 (days) of the given pesticides

Substrates	OPP	DPA	IMZ	TBZ
Soil	1,5	1,9	79,3	53,4
SMS/Soil (50:50)	1,1	1,7	24,2	33,6
SMS/Straw/ Soil (50:25:25)	**1,0**	1,9	26	28,3
Straw/Soil (50:50)	1,5	1,6	55,4	236,5
Straw/SMS/Soil (50:25:25)	1,6	1,9	46	60,4

OPP: ortho-phenylphenol, **DPA:** diphenylamine,

IMZ: Imazalil, **TBZ:** Thiabendazole

Karas P., PhD

Degradation of fungicides in alternative biomixtures used in Cyprus

Substrates	R^2	DT50	χ^2
Ortho-phenylphenol – Na (SOPP)			
OTP	0.93	33.0	2.1
GVP	0.93	21.1	1.8
GM	0.94	19.5	4.6
GSS-1	0.96	4.9	7.2
GSS-2	0.94	13.1	9.7
SS	0.98	31.1	4.1
S	0.92	43.3	3.6
Thiabendazole (TBZ)			
OTP	0.82	57.4	2.6
GVP	0.90	28.8	3.4
GM	0.91	40.8	1.4
GSS-1	0.93	26.7	5.9
GSS-2	0.98	26.2	10.1
SS	0.98	89.5	3.2
S	0.87	77.8	9.1
Imazalil (IMZ)			
OTP	0.71	48.8	8.3
GVP	0.69	15.5	10.4
GM	0.90	36.8	9.6
GSS-1	0.87	34.4	8.2
GSS-2	0.88	31.7	6.4
SS	0.91	19.2	7.2
S	0.87	28.6	11.4

GSS1

- Soil (25%)
- Compost from grape marc and grape stalks (25%)
- Straw (50%)

S: Soil

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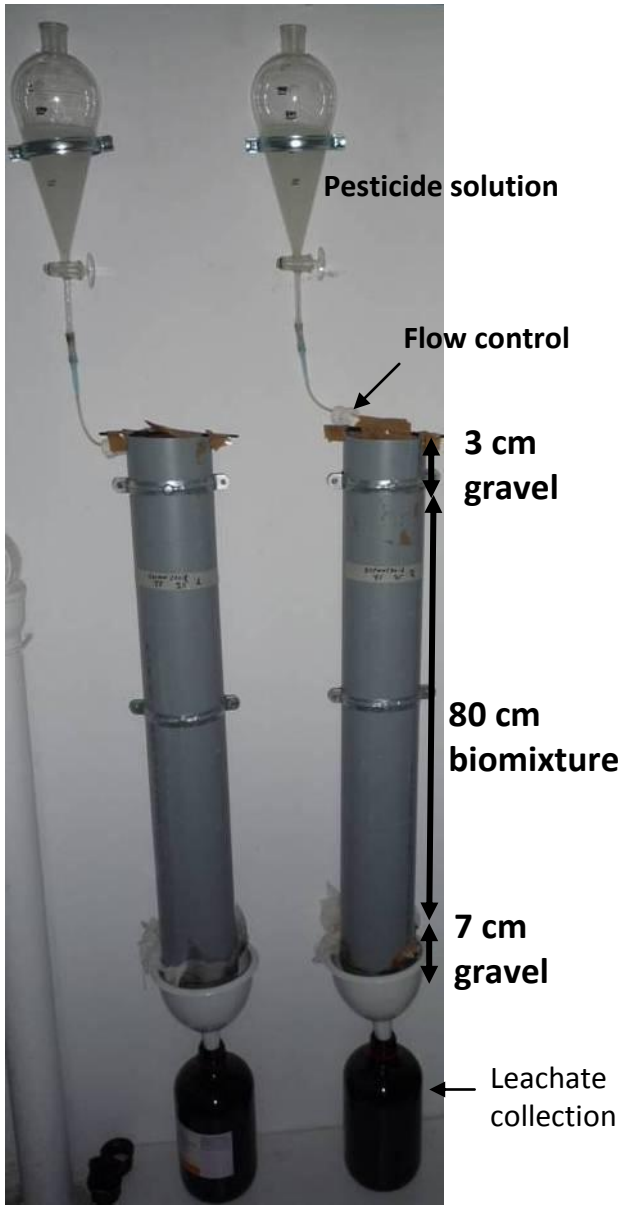
The % of the pesticides leached from columns which received different water loads of wastewaters

Water loading ($L\,m^{-3}$)	biobed substrate		
	SOPP	IMZ	TBZ
463 (H)	8.1	0.1	0.07
242 (M)	6.1	0.1	0.08
161 (L)	5.6	0.09	0.11

Water loading ($L\,m^{-3}$)	Soil: Perlite (1:3 v/v)		
	SOPP	IMZ	TBZ
463 (H)	27.6	0.4	0.4
242 (M)	23.9	0.4	0.3
161 (L)	19.7	0.1	0.2



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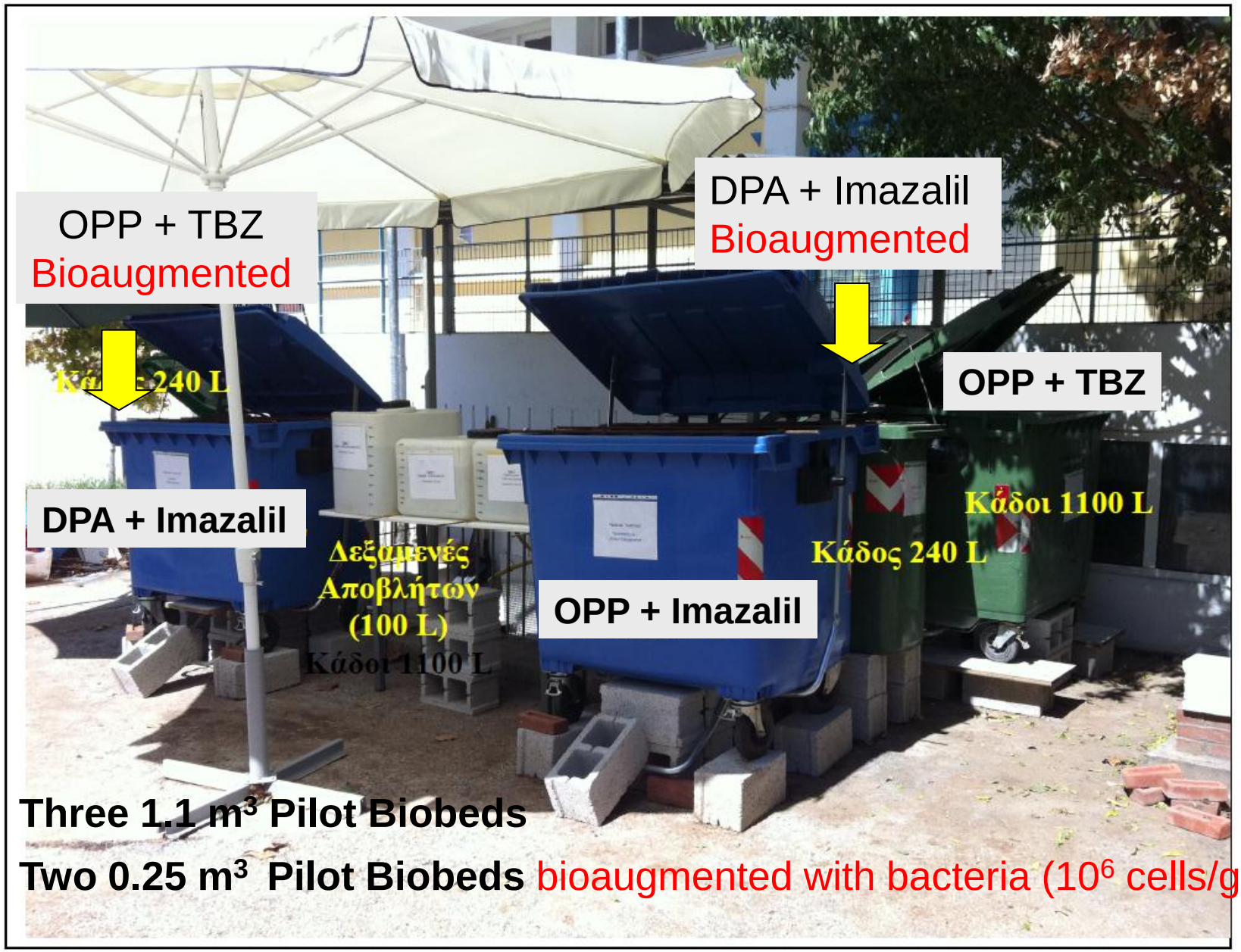


Application of 1.1 m^3 wastewater (3 x 10 min/d) containing 100 mg/L of each pesticide in a period of 5 months (Oct 2012 – Feb 2013)

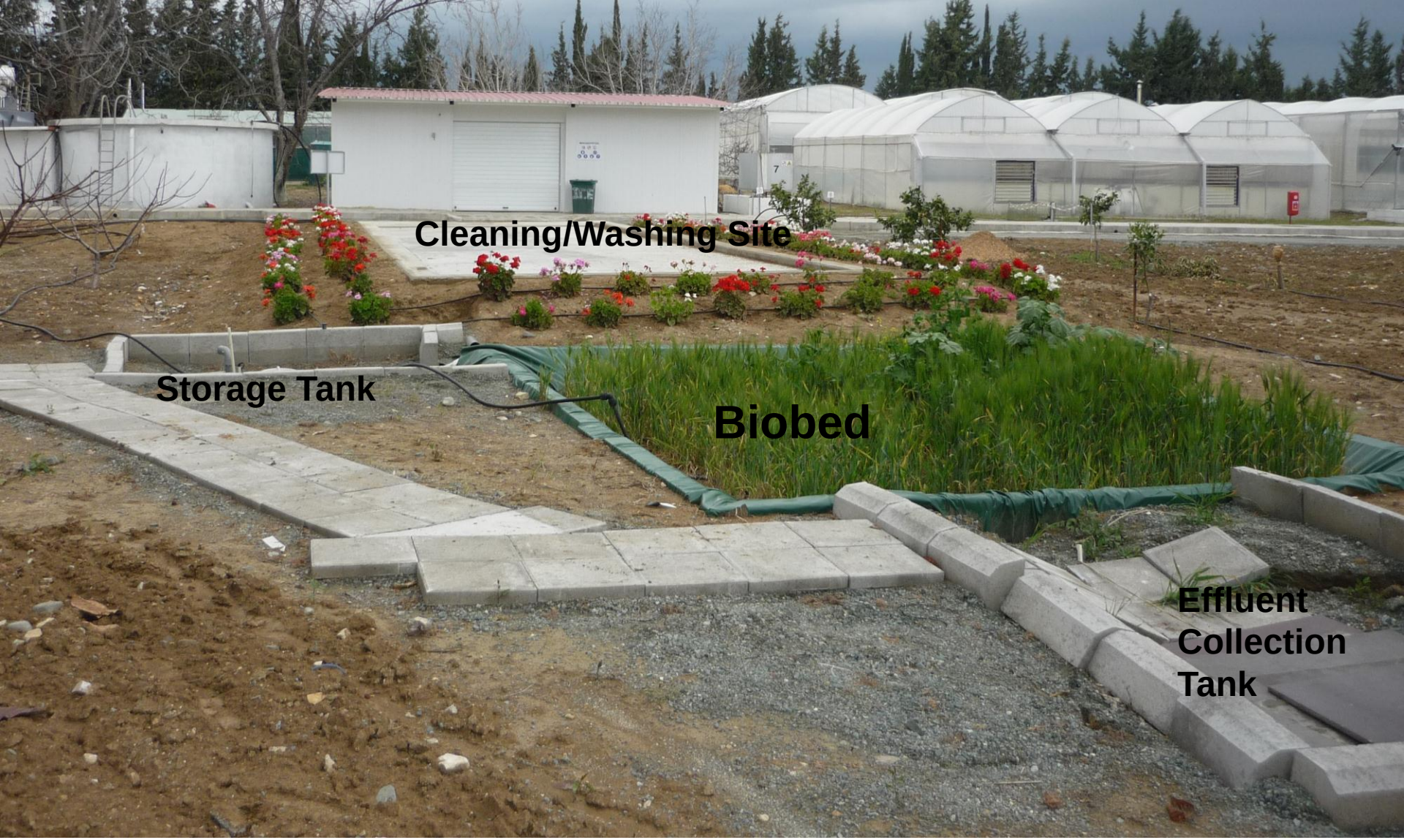
Biomixture

- Soil (25%)
- Straw (25%)
- Spend Mushroom Substrate (50%)

Evaluation of Depuration Efficiency

- Daily measurements of DO & temperature
- Regular measurement of pesticide concentration in the inflowing wastewater and in the efflux of the biobed
- Distribution of pesticides in biobed profile at the end of the treatment
- Microbial measurements before and after treatment in the biobed material (PLFAs, qPCR for functional genes)

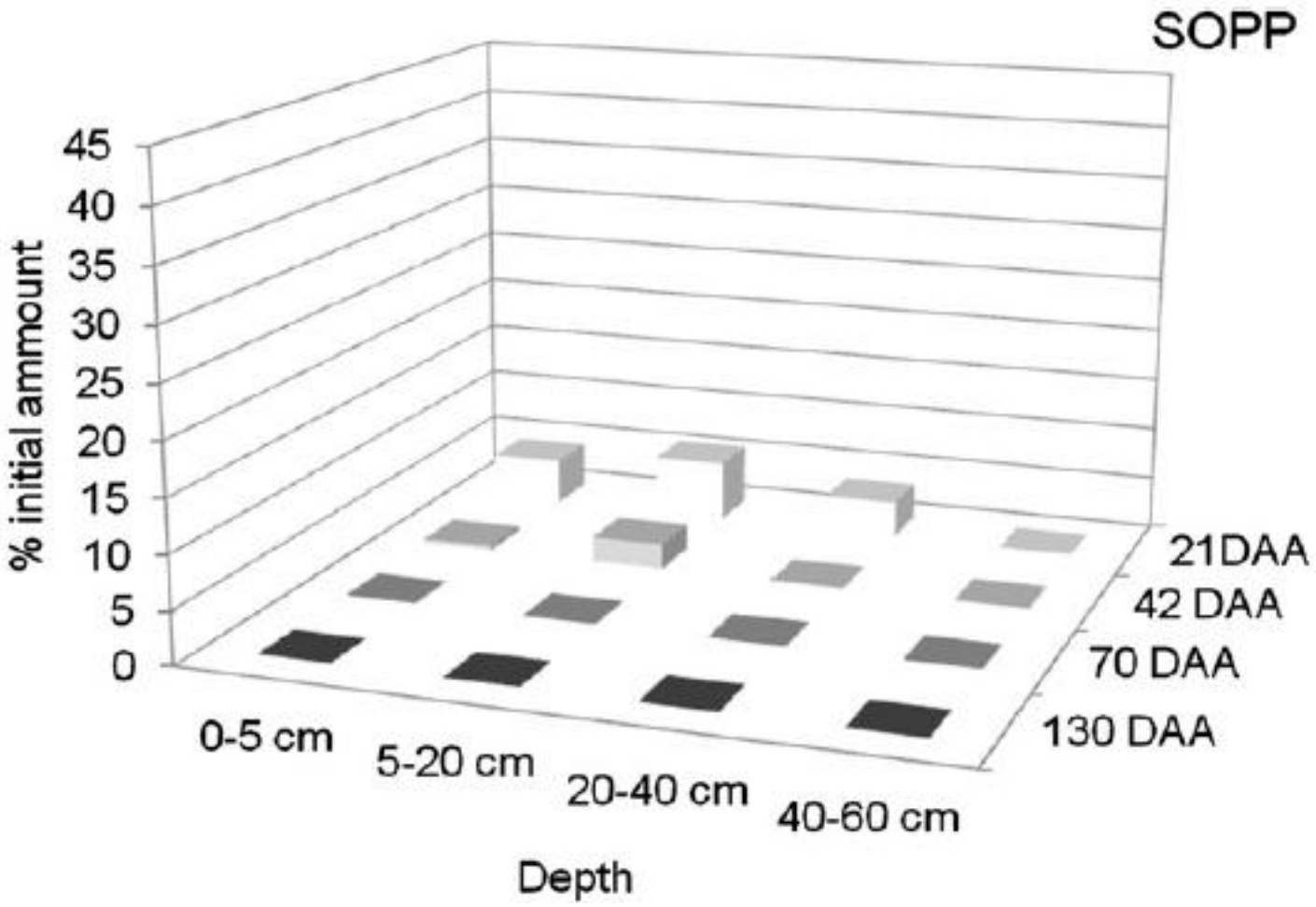
Mass balance analysis



Offset type BIOBED in Cyprus used for the treatment of both on-farm and post-farm wastewaters from citrus production

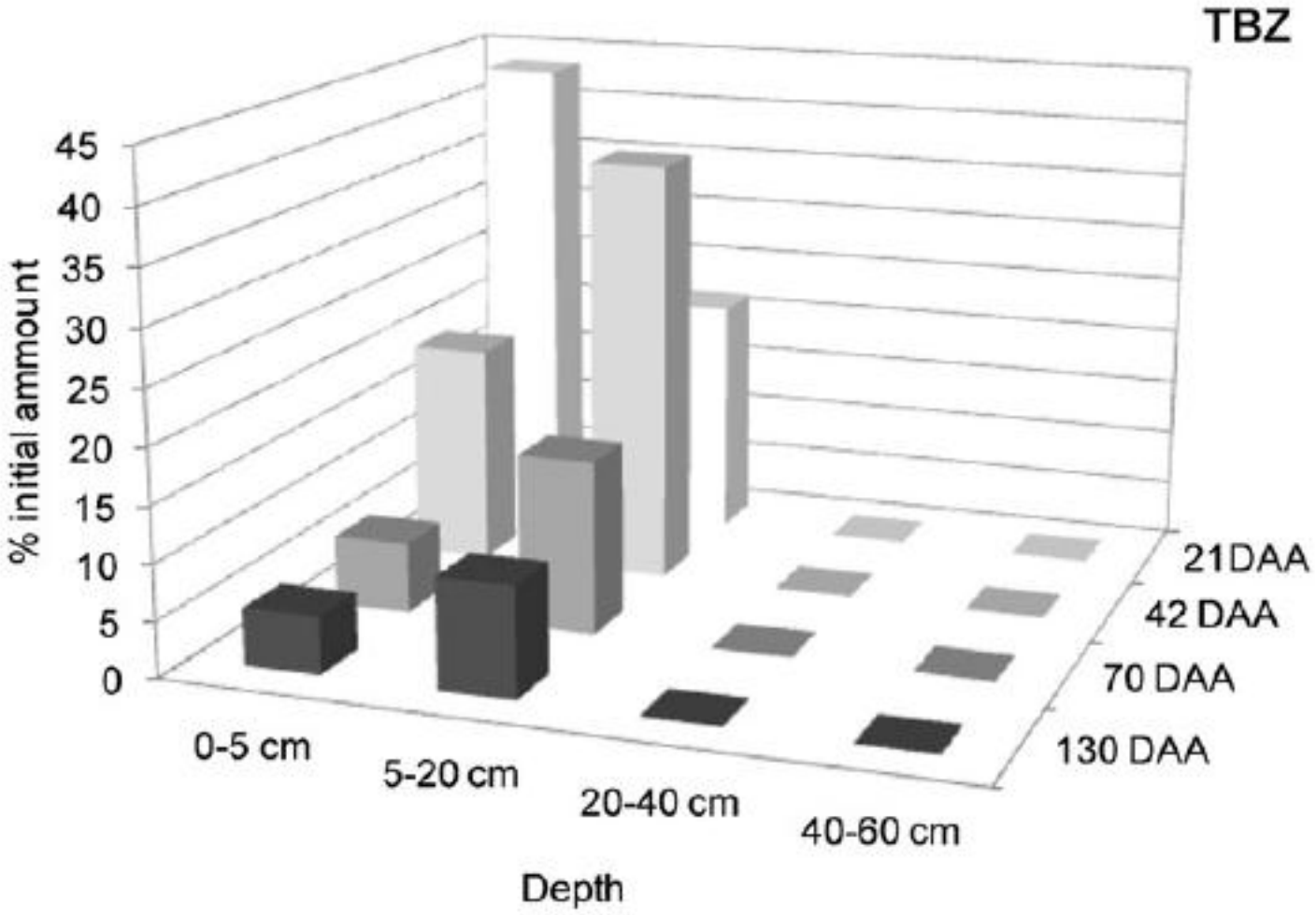
Omirou et al. Environmental Pollution (2012)

Dissipation of ortho-phenylphenol in biobed Cyprus



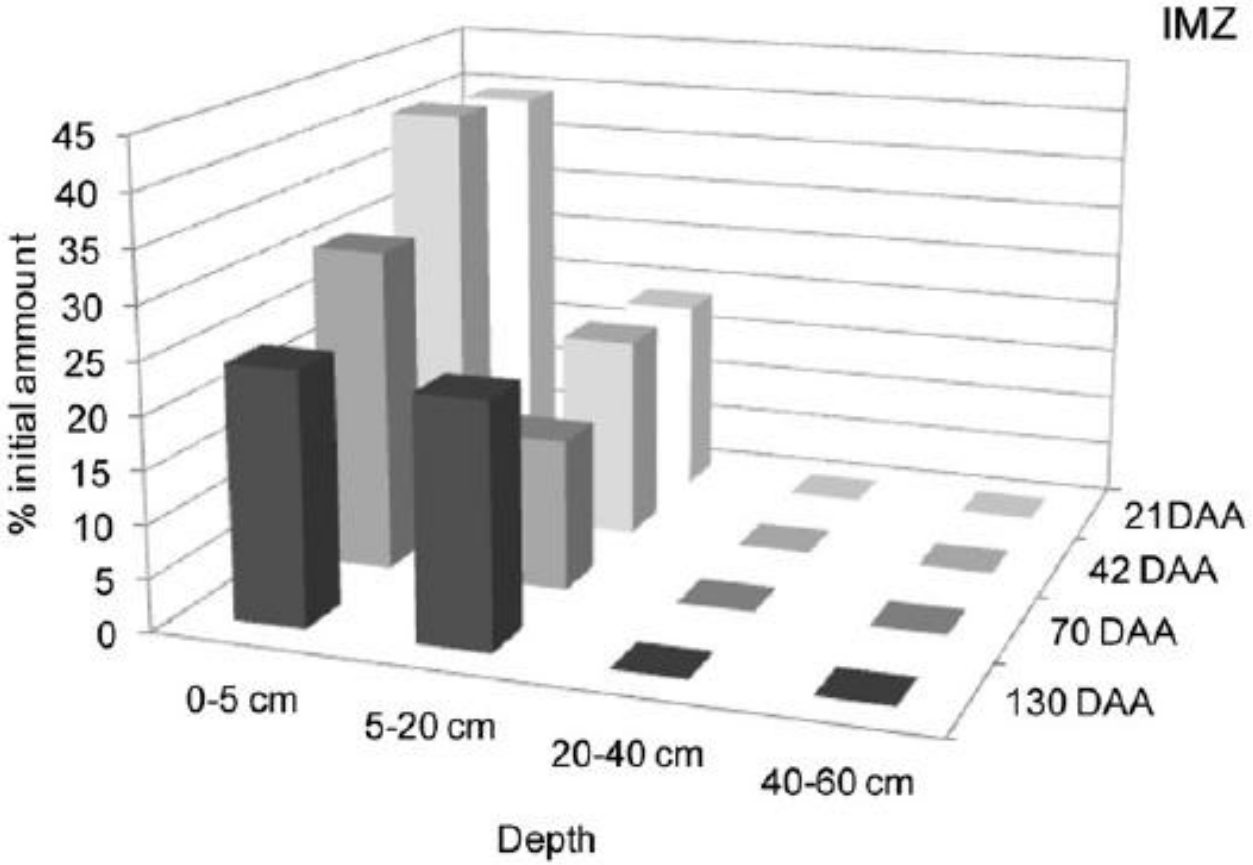
0% of OPP in the leachate

Dissipation of thiabendazole in biobed Cyprus



0% of thiabendazole in leachate

Dissipation of imazalil in Biobed Cyprus



0% of imazalil in the leachate

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How do we treat this waste?

- **<u>Composting</u>** with fresh organic matter (straw + manure)

- **<u>Storage</u>** at standard temperature and moisture

- **<u>Bioaugmentation</u>** with pesticide-degrading microbes

- **<u>Bioaugmentation</u>** (10 days) and **<u>composting</u>**

In progress…….

Cost of a Biobed for use in Fruit Packaging Plants

- Biobeds: **6000-7500 €**

  - Storage Tank (10-25 m^3**):** 1400-3300 €

  - **Plastic membrane for water proofing:** 300-900 €

  - **Cost of dig up and preparation of the site:** 1000 €

  - **Pumps (2):** 500 €

  - **Tubing:** 500 €

  - Small storage tank (max 5 m^3**):** 800 €

  - Plastic for covering the biobed during heavy rainfall events: 100 €

  - Various extra costs: 200 €

- Cost of treatment by subcontractor:**17500 € annually**

  0.70 – 3 € (depending on the volume)

  For a medium size plant producing 25 m^3 **wastewaters = 17500 €/year**

Biobeds use for on-farm activities……

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University of Thessaly

?

Dr Evangelos Karanasios
Benaki Phytopathological Institute

Publications related to biobeds work by our group

1. Kravariti K., Tsiropoulos N., Karpouzas D.G., (2010) Terbuthylazine and chlorpyrifos fate in various biomix substrates originated from composted cotton crop residues. *Pest Manag. Sci.* 66: 1122-1128.
2. Karanasios E, … Karpouzas D.G., (2010) Novel biomixtures based on local Mediterranean ligninocellulosic materials: evaluation for use in biobeds. *Chemosphere* 80 (8): 914-921.
3. Karanasios, E., Tsiropoulos, N**.,** Karpouzas, D.G., Ehaliotis C., (2010) Degradation and adsorption of pesticides in compost-based biomixtures as potential substrates for biobeds in south Europe. *Journal of Agricultural and Food Chemistry* 58 (16): 9147-9156.
4. Karas P., …… Karpouzas DG., (2011) Potential for bioremediation of agro-industrial effluents with high loads of pesticides by selected fungi. *Biodegradation* 22: 215-228.
5. Karanasios E., Karpouzas D.G., … (2012) Key parameters and pesticide practices controlling pesticide degradation efficiency of biobed substrates. *J. Environ. Sci. Health PartB* 47: 589-598
6. Karanasios E. … Karpouzas D.G., (2012) On-farm biopurification systems for the depuration of pesticide-wastewaters: recent advances & future perspectives. *Biodegradation* 23:787-802
7. Rayu S., Karpouzas D.G., Singh B.K. (2012) Emerging technologies in bioremediation: constraints and opportunities. *Biodegradation* 23(6): 917-926
8. Karanasios E., ….. Karpouzas D.G., Tsiropoulos N. (2012) Optimization of water management and biomixture composition for maximizing the pesticide depuration of peat-free biobed systems. *Journal of Environmental Quality* 41(6): 1787-1795
9. Omirou M, ……. Ehaliotis C., Karpouzas D.G., (2012) Exploring the potential of biobeds for the depuration of pesticide-contaminated wastewaters from the citrus production industry: laboratory, column and field studies. *Environmental Pollution* 166: 31-39
10. Marinozzi M, ……., Karpouzas D.G., … (2013) The dissipation of three fungicides in a biobed organic substrate and their impact on the structure and activity of the microbial community. *Environ. Sci. Poll. Res. (in press, DOI 10.1007/s11356-012-1165-9)*
11. Karanasios E., Karpouzas D.G., Tsiropoulos N., (2013) Quantitative and qualitative differences in the metabolism of pesticides in biobed substrates and soil. *Chemosphere* (accepted)