



3^{er} European Biobed Workshop



**CHLORPYRIFOS AND ATRAZINE DEGRADATION IN A BIOMIX OF
BIOBED WITH SOIL DERIVED FROM VOLCANIC ASHES.
BIOLOGICAL AND PHYSICO-CHEMICAL ASPECTS.**

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GENERAL ASPECTS

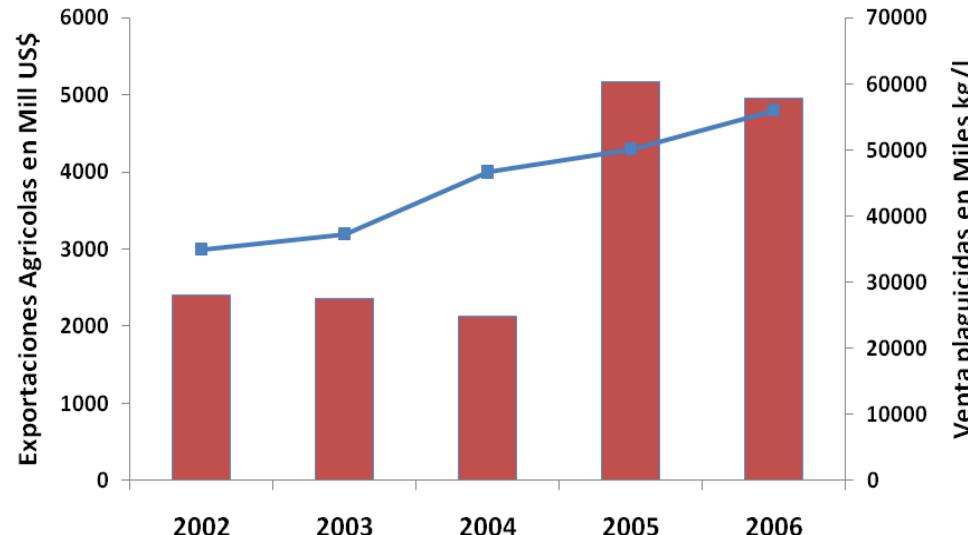
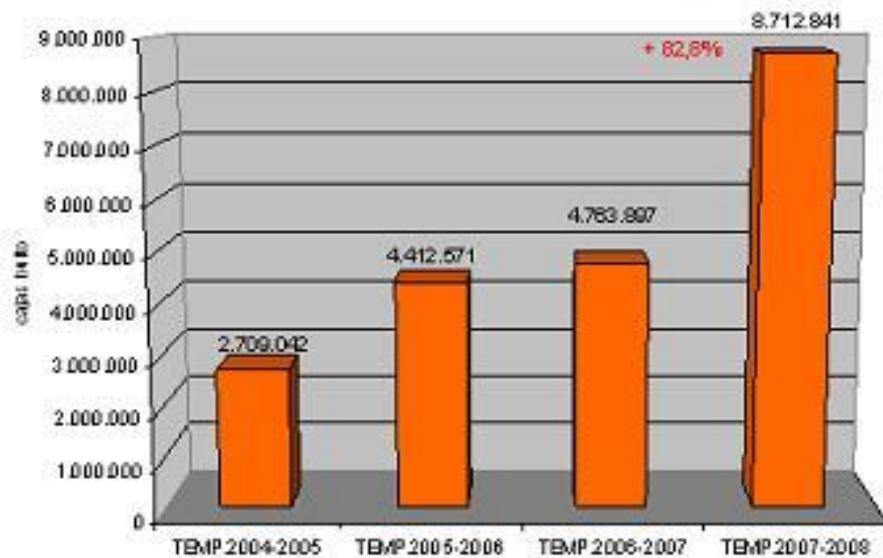
- Chilean Legislation only considers the triple washing for equipment and deposits of pesticides as part of Good Agricultural Practices



- In Chile, several pesticides are used for different proposals and are permitted by the legislation currently (SAG) www.sag.cl
- In Chile precedents do not exist in relation to the origin of the pollution by pesticides (point sources or diffuse contamination).
- No biobeds are installed in Chile yet



**Evolución de las exportaciones de Cerezas chilenas,
en las últimas cuatro temporadas**
(Fuente: Asoex)



**Sales of pesticides and exports of
agricultural products**



PESTICIDE CONTAMINATION Point Source Contamination



Unsatisfactory management of pesticides



Surface waters



Groundwater



Soils



PESTICIDES DETECTED IN SOME PLACES IN CHILE



Place	Detected pesticides	Levels	Sample	Ref.
Aconcagua River	Lindane, diazinon, oxifluorphen, dicofol, azinfos-methyl, metabenzotiazuron, lenacil , diflubenzuron, atrazine	0.1-1.12 µg/L	Water	Baéz et al. (1996)
Traiguen River	Simazine, 2,4-D, pichloram, hexazinone, carbendazim	0.2-9.7 µg/L	Water	Palma et al. (2004)
San Pedro lake Lleu-Lleu, Icalma	Heptachlor, aldrin, endrin, pp - DDT, pp'-DDE, pp'-DDD	1.68-0.89 ng/g	Sediments	Barra et al. (2001)
Chillan River	Heptaclor, endrin, endosulfan, metoxichlor	35-58 ng/L	Water	Cooman et al. (2005)
Limari River basin	2,4 D, Aldicarb, atrazine + N-dealkyl metabolites, captan, carbofuran, chlorothalonil, cyanazine, dimethoate, diclofop-methyl, parathion, pentachlorophenol, simazine, trifluraline	1-0.05 µg/L	Water	DGA (2004)
Nuble Region	Aldrin, DDT, Dieldrin		Soil	Henriquez et al. (2006)

HOW WE APPROACHED THE TOPIC ? – NATIONAL SUPPORT

FONDECYT

Oriented Towards
Scientific Research

Biomix optimization

Soil type influence

Rainfall conditions

Bioaugmentation

Biostimulation

Scientific information

Doctoral theses



FONDEF

Oriented Towards
Farmers

Operational Guide

Pesticide analysis service

Technical service

Biobed pilot installation

Technicianl training

Workshops

Scientific information

Technical information

Academic exchanges

**National and International
Networks**

EXPLORA

Oriented towards
Secondary School
Students

**On-site demonstration
Units**

Student training

Family education



FACTORS AFFECTING PESTICIDES DEGRADATION

Components of the biomix



Types of soil

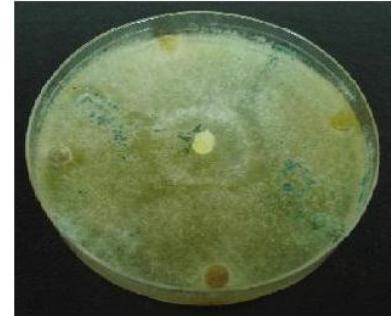
Peat availability

Residues availability

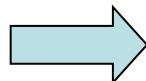
Biochemical properties

Organic matter and nutrients content

Microorganisms competition



Pollutants Characteristics



Water solubility

Org. carbon partition coefficient (K_{oc})

Chemical structure.

ANDISOL SOIL: RELEVANCE IN THE BIOMIX



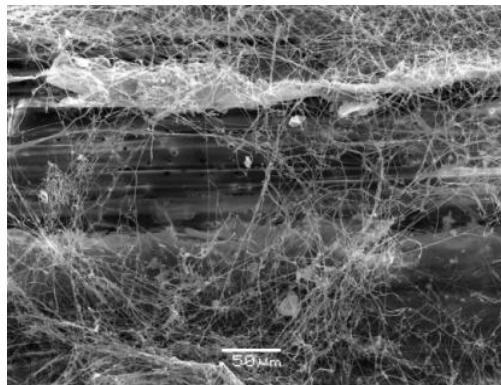
Zone	pH	Organic Matter (%)
North	7.4-8.6	1.2-2.8
Centre	6-7	3-6
South	5-6	8-15

Parameter	Unit	Dept		
		0 - 20 cm	20 - 40 cm	40 - 60 cm
Specific Area	m ² /g	176-223		
pH (w)		5.9	6.4	6.8
Sand	%	16.1	nd	nd
Silt	%	58.2	nd	nd
Clay	%	25.7	Nd	nd
Organic Matter	%	14.6	7.9	4.5
Al ₂ O ₃	%	18.6	20.8	21.6
Fe ₂ O ₃	%	10.5	11.7	12.4
SiO ₂	%	41.6	43.7	45.0

MnP PRODUCTION BY *A. DISCOLOR*

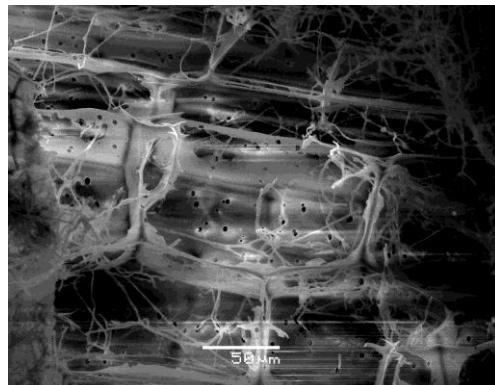
Kirk Medium	MnP Activity (U/L)	Proteins (mg/L)
Without substrate	130	6
+ Wheat straw (a)	165	30
+ Wheat grain (b)	1041	111

7 Days



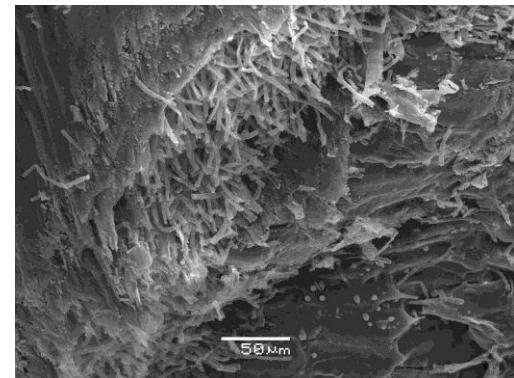
(a)

120 Days



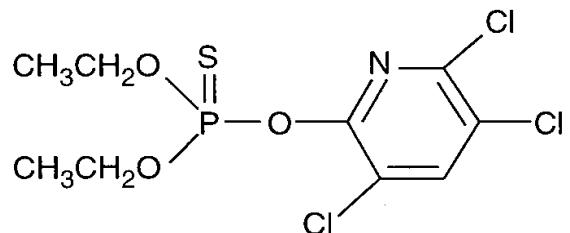
(a)

7 Days

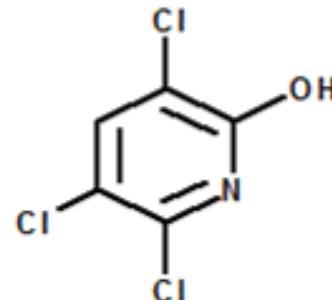


(b)

CHLORPYRIFOS AND METABOLITE CHARACTERISTICS



Principal metabolite



Chlorpyrifos

O,O-Diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate

TCP

3,5,6-trichloro-2-pyridinol

Water Solubility (mg/L)

Chlorpyrifos

TCP

1.4

80.9

Adsorption Coefficient (Koc)

6000

149

Aerobic Soil Half-life (Days)

7-15

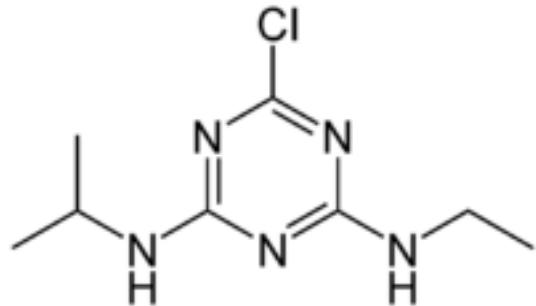
52

Gus index (leaching potential)

0.15 (low)

2.9 (high)

ATRAZINE CHARACTERISTICS



Degradation products

deisopropylatrazine (**DIA**),
deethylatrazine (**DEA**)
hydroxiatrazine (**HA**)

Atrazine

Water Solubility (mg/L)

33

Adsorption Coefficient (Koc)

40-155

Aerobic Soil Half-life (Days)

35-50

Gus index (leaching potential)

3.3 (low)

SOME ASSAYS

Pesticides, Biomix and Top soil

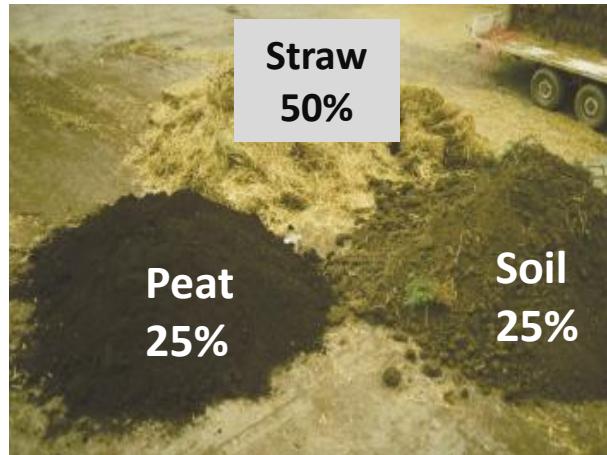
Chlorpyrifos and Atrazine

100, 200 and 300 times field dose

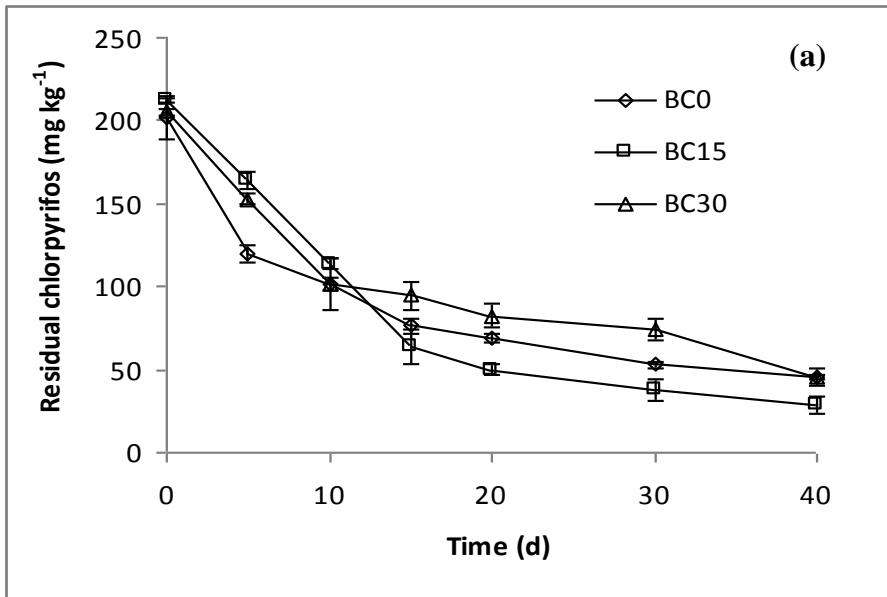
Andisol (0 to 20 cm depth)



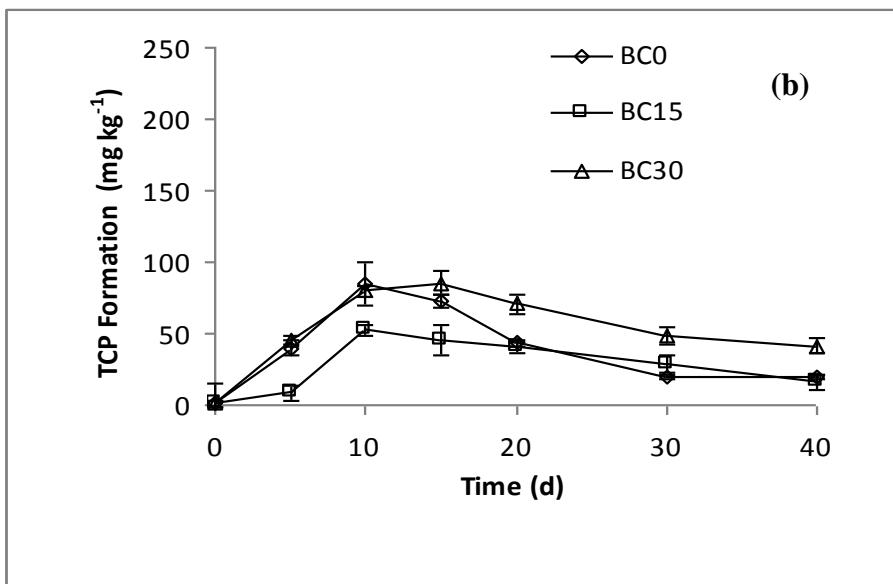
Components of biomix

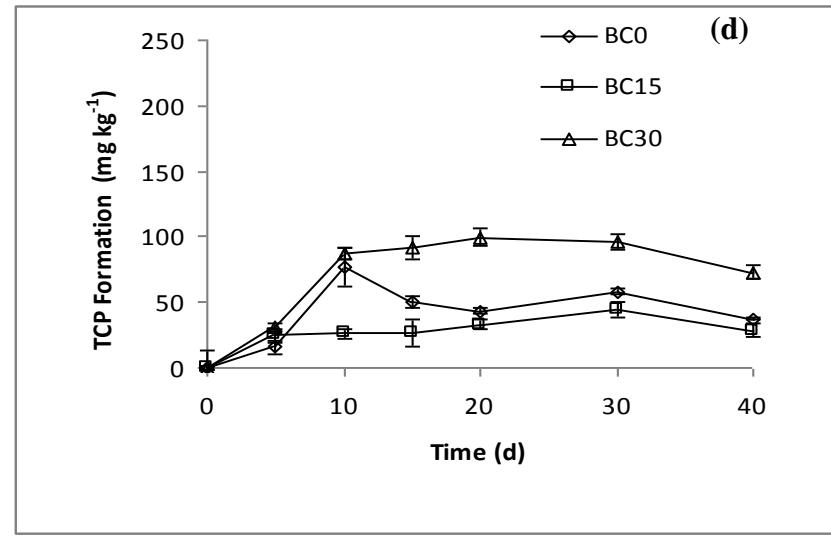
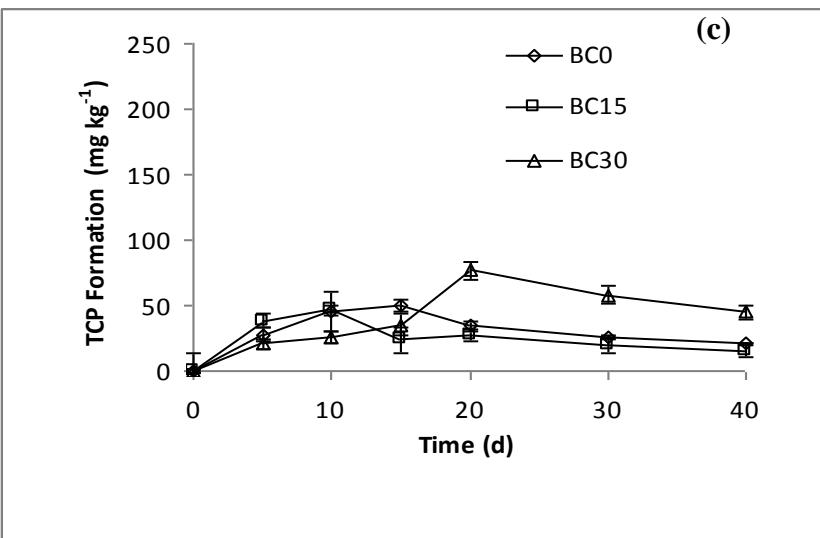
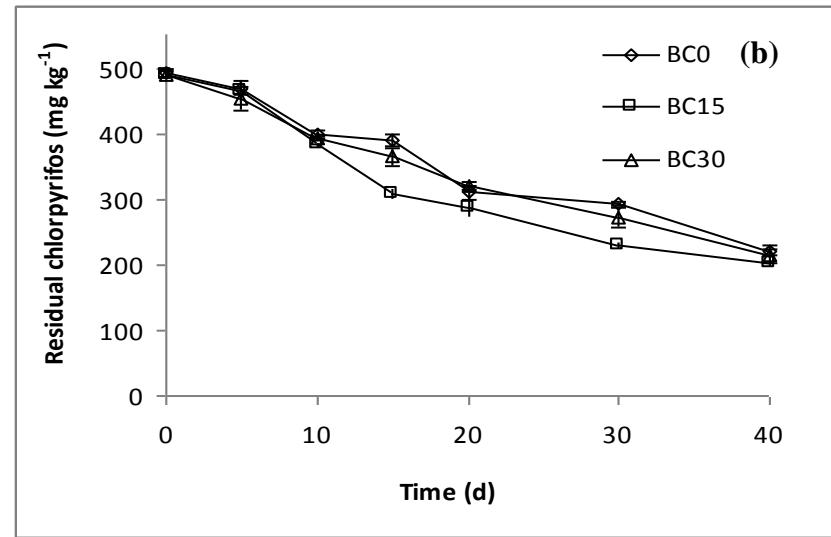
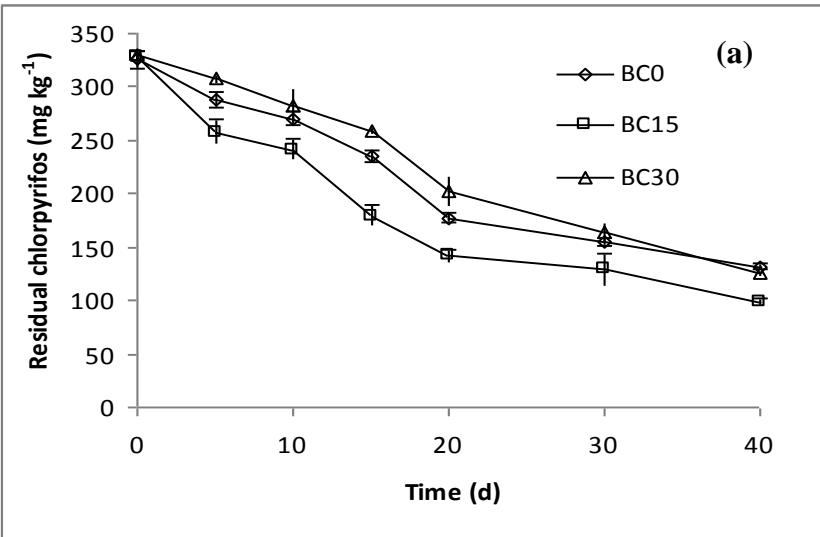


SOME RESULTS- CHLORPYRIFOS



Chlorpyrifos dissipation (a) at **200 mg a.i kg^{-1}** and TCP formation (b) in biomix of biobeds, at different compost times.





Chlorpyrifos dissipation at (a) **320** mg a.i kg^{-1} , (b) **480** mg a.i kg^{-1} and TCP formation in (c) **320** mg a.i kg^{-1} (d) **480** mg a.i kg^{-1} in biomix of biobeds at different compost times.

FDA AND PEROXIDASE ACTIVITY – CHLORPYRIFOS

Biological activity FDA (Fluorescein diacetate) and phenoloxidase activity at different pre-incubation times and Chlorpyrifos concentration (mg kg^{-1}) in the biomix after 40 days of incubation.

Pre-incubation time [days]	FDA [$\text{ug g}^{-1}\text{h}^{-1}$]				Phenoloxidases [U kg^{-1}]			
	Control	200	320	480	Control	200	320	480
0	3.45	8.45	5.65	5.92	0.41	2.10	0.80	0.80
15	4.98	7.23	6.23	6.58	0.48	4.56	0.95	0.90
30	1.23	5.21	3.83	4.96	0.56	0.47	0.71	0.67

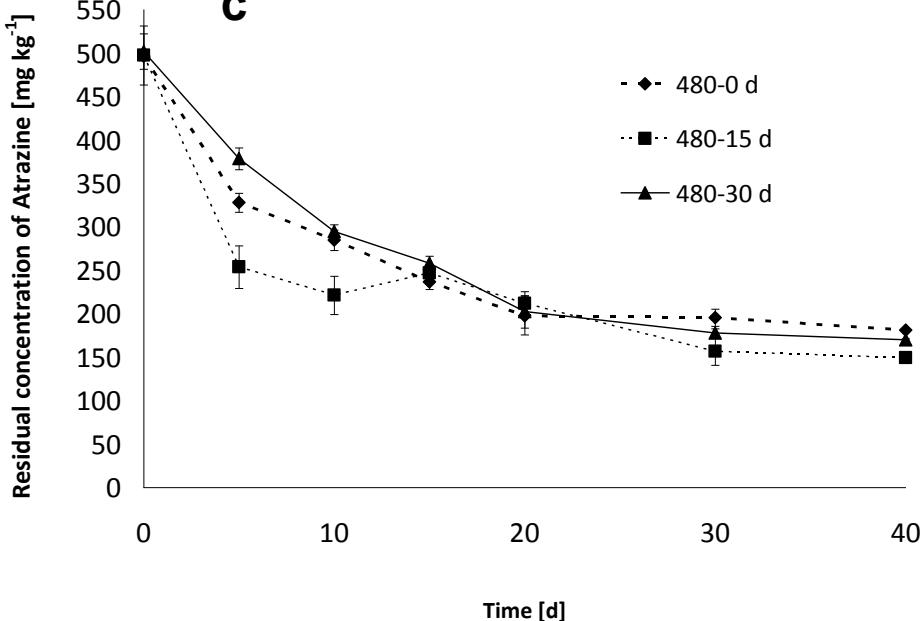
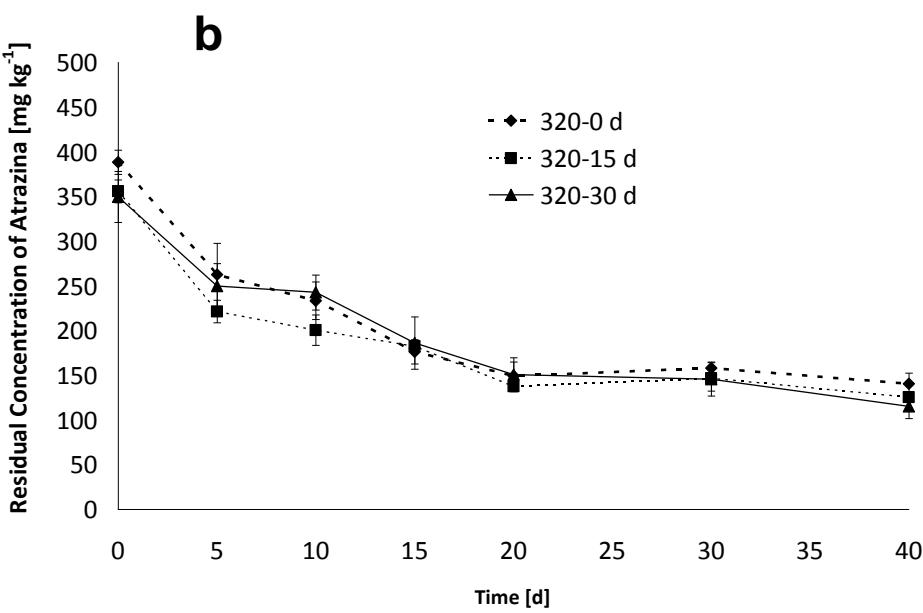
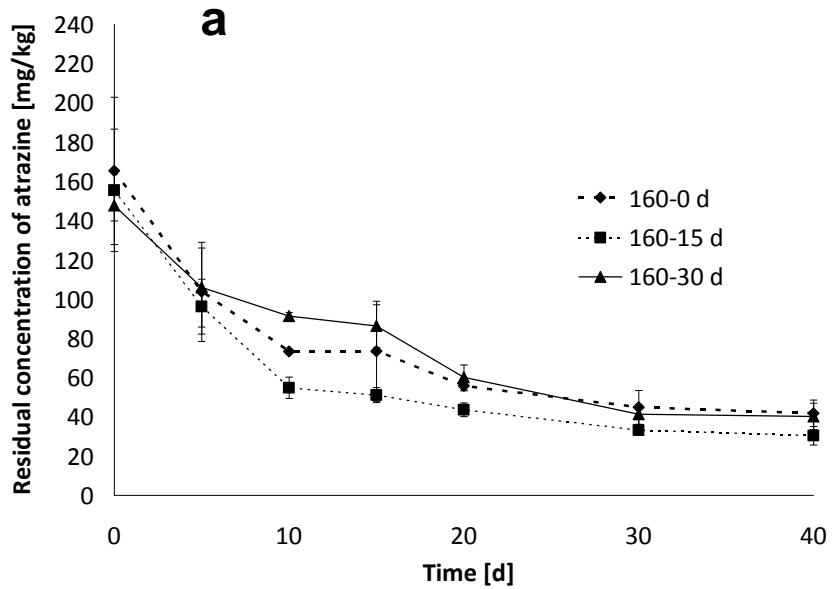
DEGRADATION PARAMETERS OF CHLORPYRIFOS

Values obtained to $t_{1/2}$ and GUS index in biomix of biobeds to different concentration and compost times.

Dose (mg kg ⁻¹)	Sample	K (d ⁻¹)	t _{1/2}	r ²	GUS index
200	BC0	0.03	24.9 b	0.95	0.17
	BC15	0.05	14.3 a	0.91	0.10
	BC30	0.03	23.6 b	0.92	0.16
320	BC0	0.02	29.3 a	0.95	0.20
	BC15	0.03	23.8 b	0.93	0.17
	BC30	0.03	26.1 c	0.98	0.18
480	BC0	0.02	34.4 a	0.96	0.24
	BC15	0.02	30.2 a	0.94	0.21
	BC30	0.02	33.6 a	0.99	0.23

Gus <1.8 Low probability of lixiviation

SOME RESULTS - ATRAZINE



Atrazine dissipation at (a) 160, (b) 320 and (c) 480 mg a.i. kg^{-1} in biomix of biobeds, at different compost times.

ATRAZINE DEGRADATION PRODUCTS

<i>Time of compost</i>	<i>Time</i>	DIA			HA			DEA		
		160	320	480	160	320	480	160	320	480
0	5	48.2	57.7	42.8	43.2	27.8	29.5	8.5	27.8	27.6
	15	42.6	49	42.7	31.6	29.5	24.2	25.7	29.5	32.9
	30	55.5	41.3	44.5	33.8	48.3	31.4	10.5	48.3	24.3
15	5	74.6	73.6	34.4	17.2	16.6	42.9	8.1	16.6	22.6
	15	20.8	22.8	46.6	48.6	34.5	18.8	30.5	34.5	34.4
	30	35.0	32.6	22.1	20.3	19.8	31.7	44.6	19.8	46.2
30	5	68.4	45.9	39.4	15.3	4.85	28.9	16.2	4.85	31.5
	15	82.6	49.7	41.7	17.3	0	29.7	0	0	28.5
	30	70.3	84.8	55.2	29.6	0	37.5	0	6.99	7.1

FDA AND PEROXIDASE ACTIVITY – ATRAZINE

Biological activity FDA (Fluorescein diacetate) and phenoloxidase activity at different pre-incubation times and atrazine concentration (mg kg^{-1}) in the biomix after 40 days of incubation.

Pre-incubation time [days]	FDA [$\text{ug g}^{-1}\text{h}^{-1}$]				Phenoloxidases [U kg^{-1}]			
	Control	160	320	480	Control	160	320	480
0	19.2	7.6	4.52	3.55	1.25	0.21	0.22	0.21
15	23.13	7.8	5.29	3.75	1.64	0.32	0.26	0.70
30	11.36	5.2	3.47	3.17	1.77	0.13	0.21	0.97

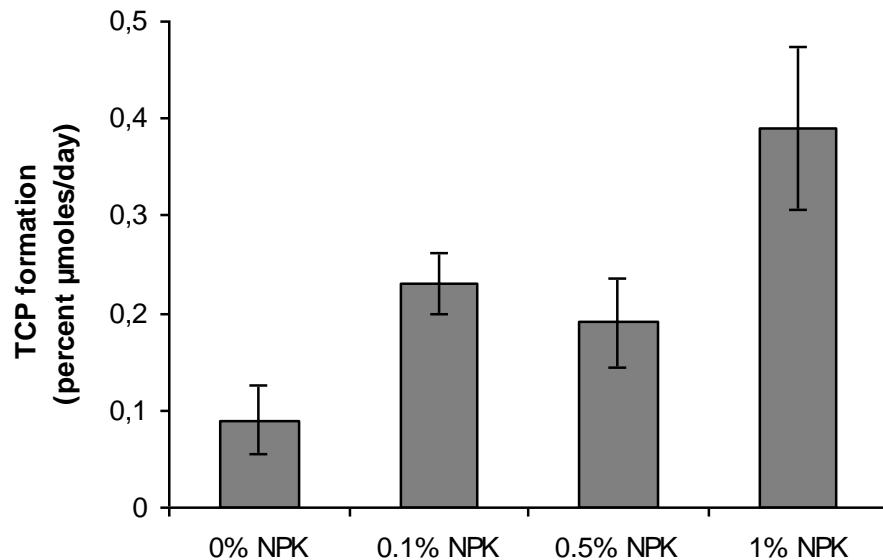
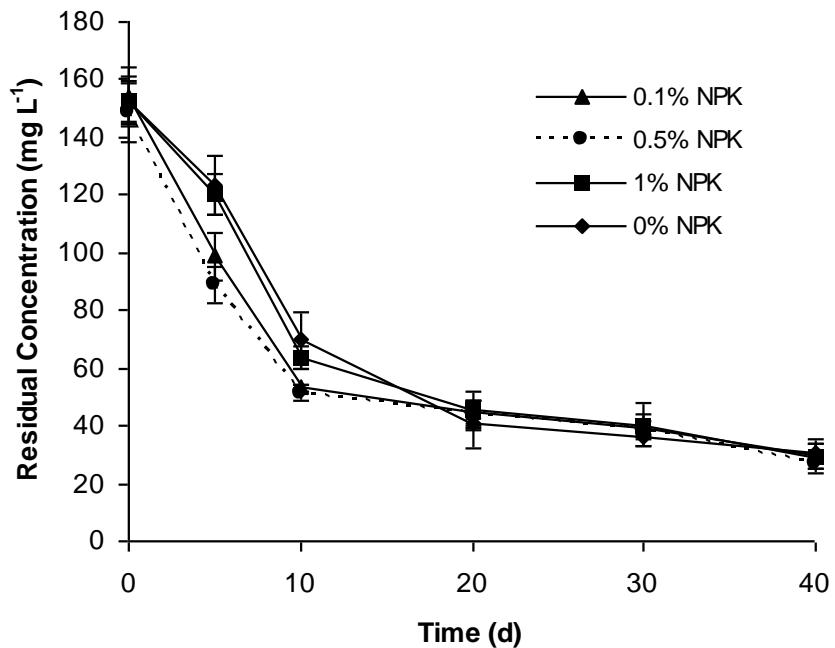
DEGRADATION PARAMETERS OF ATRAZINE

Values obtained to t ½ and GUS index in biomix of biobeds to different concentration and time of compost.

Dose	time of compost	K _d (d ⁻¹)	t _{1/2}	r ²	GUS
160	0	0,031	21,8 ^a	0,90	3,17
	15	0,032	21,1 ^a	0,86	3,15
	30	0,031	22,3 ^a	0,93	3,20
320	0	0,023	30,2 ^a	0,86	3,53
	15	0,022	31,5 ^a	0,88	3,56
	30	0,026	27,9 ^a	0,94	3,44
480	0	0,022	31,8 ^a	0,87	3,58
	15	0,021	32,2 ^a	0,80	3,60
	30	0,022	31,2 ^a	0,83	3,56

Gus > 1.8 high probability of lixiviation

CHLORPYRIFOS – NPK EFFECT

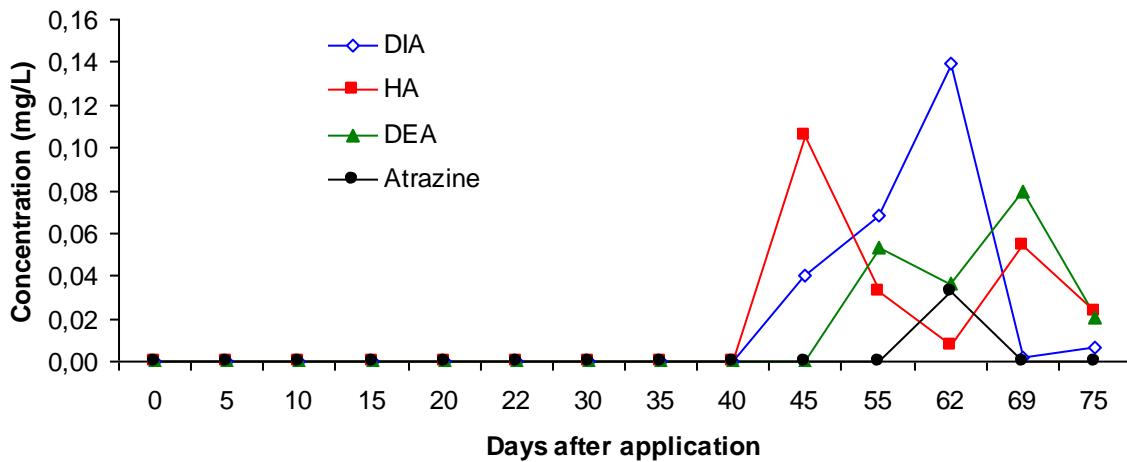


Residual chlorpyrifos and TCP formation in a biomix with different levels of NPK

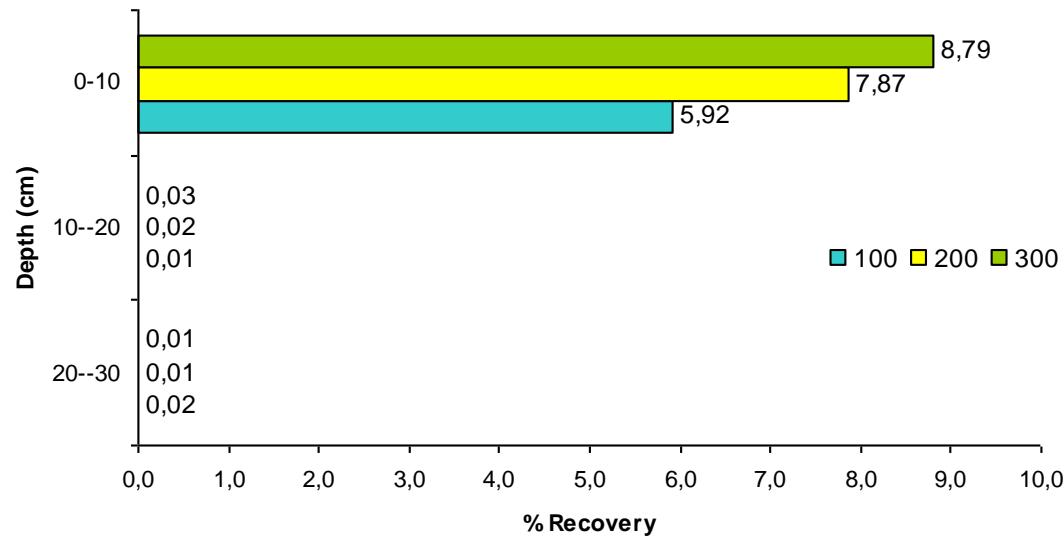
The effect of NPK on chlorpyrifos removal was significant only in the first 10 days

The increment of NPK raised TCP formation probably due to increment on general microbial population instead of TCP degrading bacteria. This aspect will be confirmed by DGGE technique

ATRAZINE – LEACHING ASSAY



Concentration of atrazine and metabolites measured in leachate biomix-filled lysimeters. (2000 mm rainfall simulated and 300 times field dose)

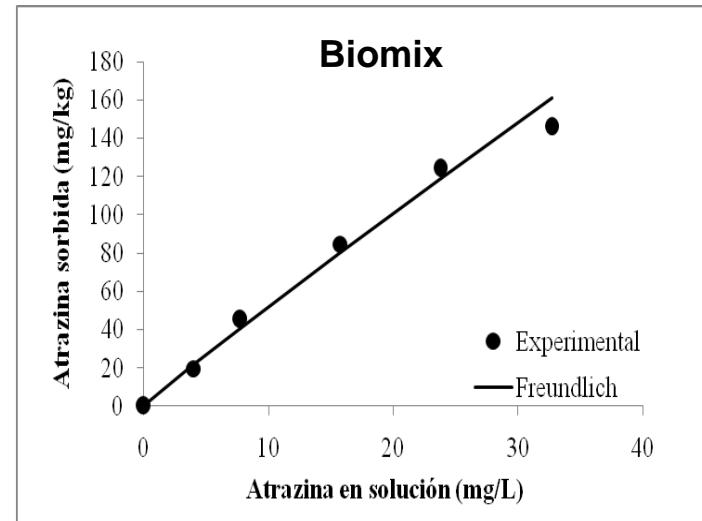
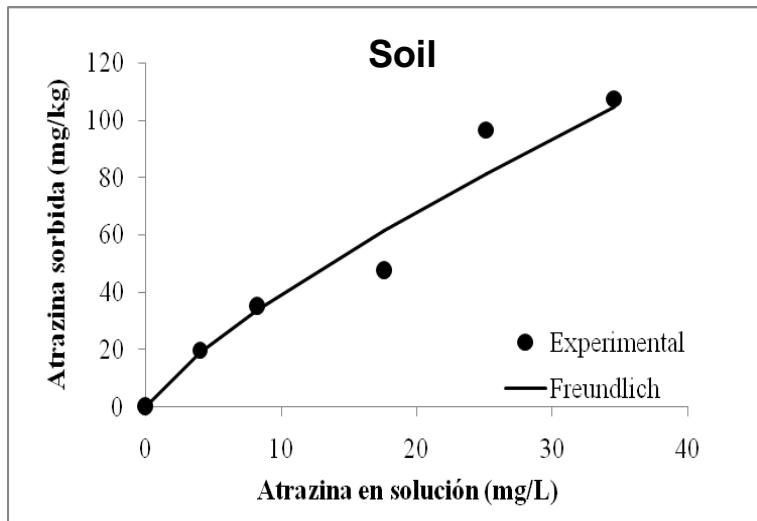


Amounts of atrazine expressed as percentage of the applied dose remaining in biomix-filled lysimeters

RESULT - ABIOTIC EFFECTS

Adsorption coefficients of atrazine and chlorpyrifos. Freundlich parameters (K_f , $1/n$) for soil (a) and biomix (b).

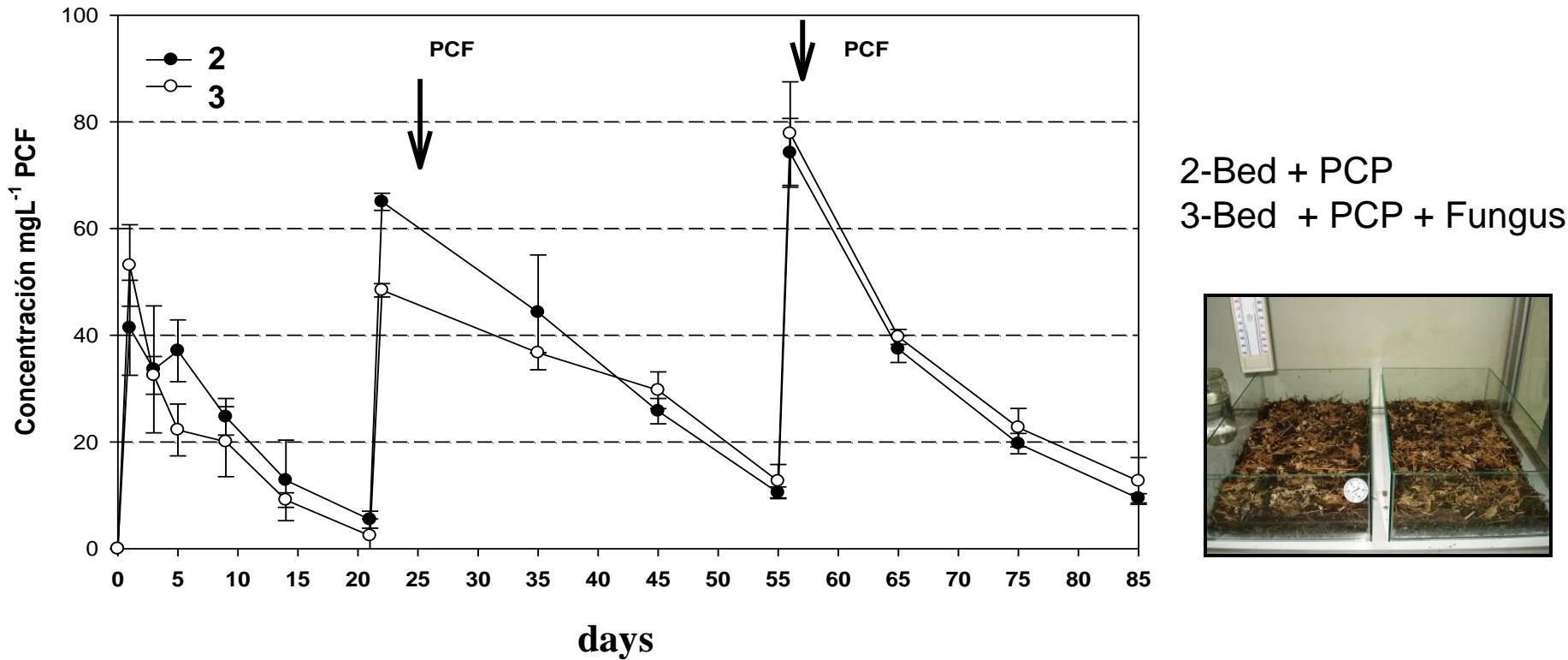
Pesticide	Mobility Class	K_f	$1/n$
Chlorpyrifos	Non mobile	^a 2685 ^b 2798	^a 0.40 ^b 0.47
Atrazine	Moderately mobile	^a 6.47 ^b 5.76	^a 0.78 ^b 0.95



BIOAUGMENTATION OF BIOBEDS

ANTHRACOPHYLLUM DISCOLOR

BIOBED - PCP REMOVAL

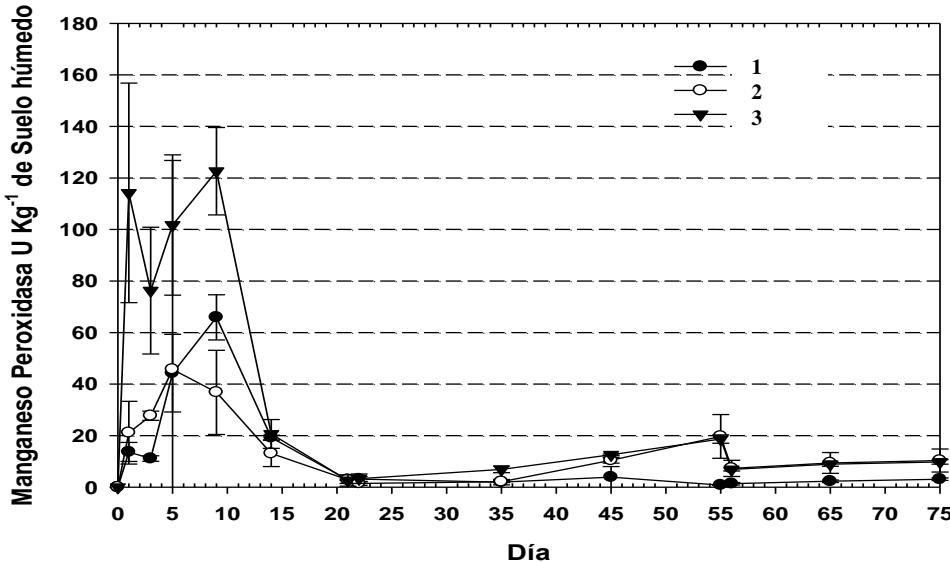


PCP removal in biobeds inoculated and non inoculated.

PCP removal occurred efficiently over the whole incubation period. Over 21 days no differences were found between inoculated and non inoculated biobeds.

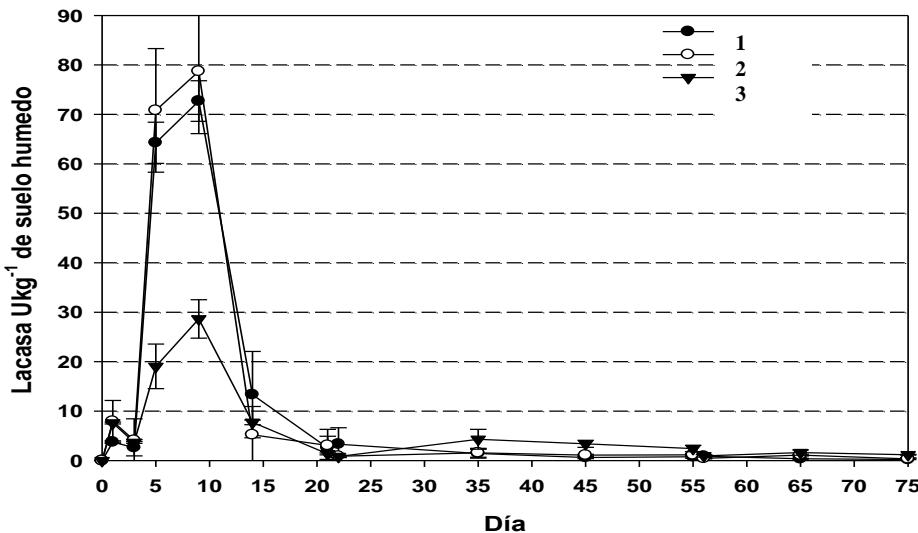
After each application 60, 40 and 20% of PCP was adsorbed

BIOBED OPERATION-ENZYMIC ACTIVITIES



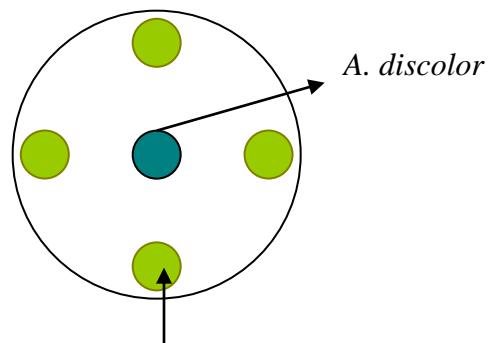
1-Bed
2-Bed + PCP
3-Bed + PCP + Fungus

MnP Activity (U/kg)

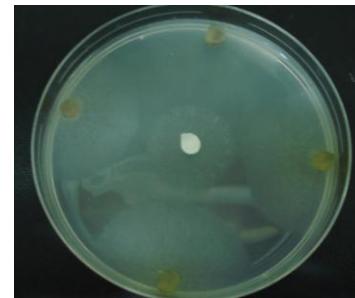


Laccase Activity (U/kg)

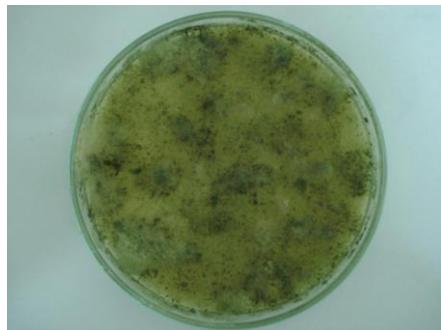
MICROORGANISM COMPETITION



Native Microorganisms



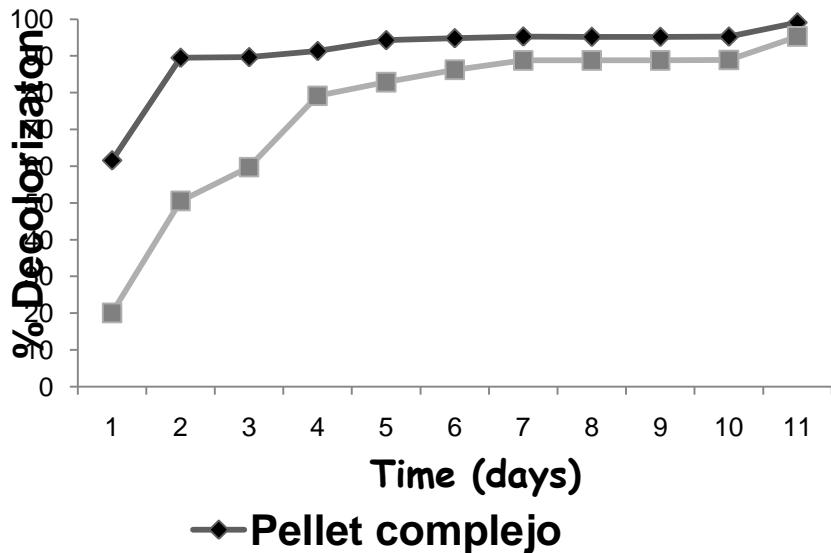
Day 3



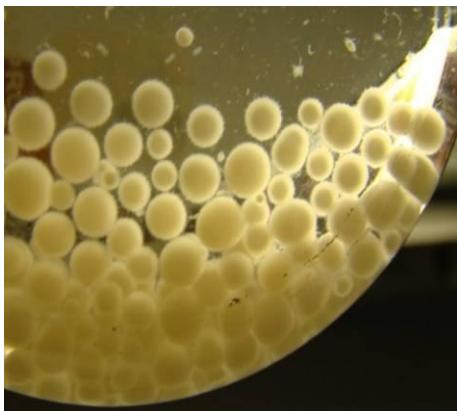
Day 7

Microorganism competition between native and inoculated fungus *A. discolor*.

SIMPLE AND COMPLEX PELLETS OF WHITE-ROT FUNGI



Decolorization of RBBR by complex and simple pellet of *Trametes versicolor*



Simple pellets



Complex pellets

SUPPORT SELECTION

Anthracopyllum discolor growth on different supports after 12 days at 25 °C.

Support	visual growth estimate
Bark	++
Composted Bark	+++
Sawdust	+
Sawdust + apple pomace	+++
Wood chips	+
Wheat straw	++++
Mixture (sawdust+ ground corn + starch)	+++
Ground Corn	++
Corn grains	++++
Starch (from potato)	+
Flaxseed	++++
Chicken feed	++++

++++ (100%) Abundant growth

+++ (75%) medium growth

++ (50%), sparse growth

+ (25%), nil growth

COMERCIAL PELLETS FOR BIOREMEDIACTION PURPOSES

Components as
sawdust,
starch, linseed,
among others



ENVIRONMENTAL BIOTECHNOLOGY CENTER - BIOREN



We wait for you in Chile, Thank you for your attention

