Analytical control as a tool for the study of biobeds performance

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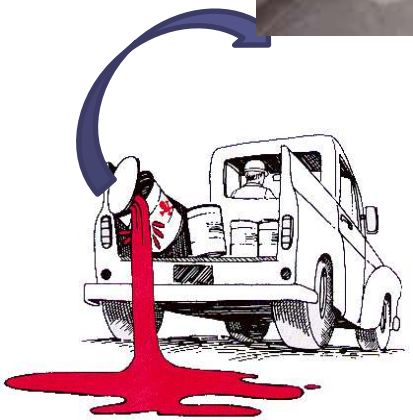
asso



Good Agricultural Practices

- Containers must be triple rinsed. The liquid must be disposed safely. MGAP 19 nov 1996
- Flow and nozzles performance in the application equipment is regulated as well as droplet size.

- Material should be properly organized
- Final application concentration controlled
- Qualified personel

Good Agricultural Practices in Uruguay

6.Management of pesticides

- Selecction of pesticides
- Transport
- Storage.
- Applicators

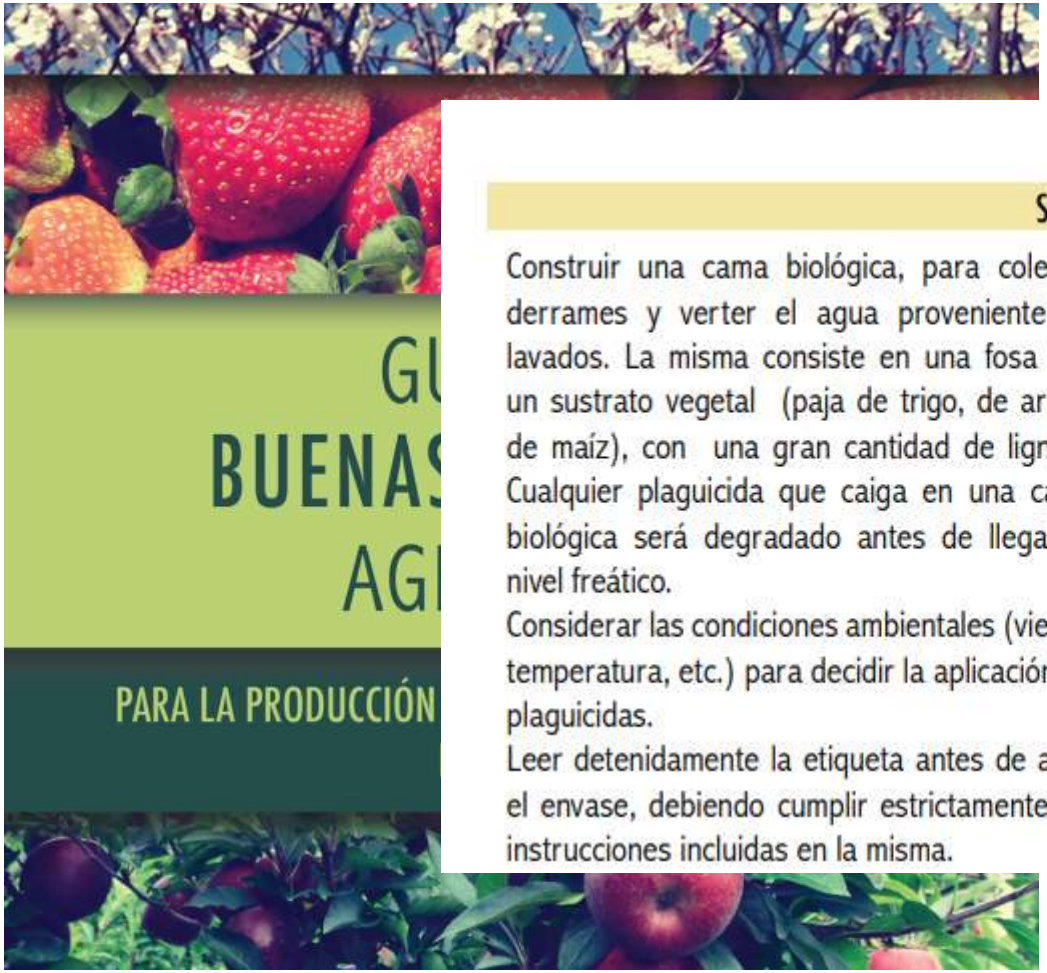
- **Application and handling of pesticides**

- Machinery and application equipment.
- Personal protection equipment.
- Moment of application .Re-entry and entry time . Wait time.
- Surplus management

- **Empty containers**

Uruguay
2014

Good Agricultural Practices in Uruguay



6.Management of pesticides

SE RECOMIENDA

Construir una cama biológica, para colectar derrames y verter el agua proveniente de lavados. La misma consiste en una fosa con un sustrato vegetal (paja de trigo, de arroz, de maíz), con una gran cantidad de lignina. Cualquier plaguicida que caiga en una cama biológica será degradado antes de llegar al nivel freático.

Considerar las condiciones ambientales (viento, temperatura, etc.) para decidir la aplicación de plaguicidas.

Leer detenidamente la etiqueta antes de abrir el envase, debiendo cumplir estrictamente las instrucciones incluidas en la misma.

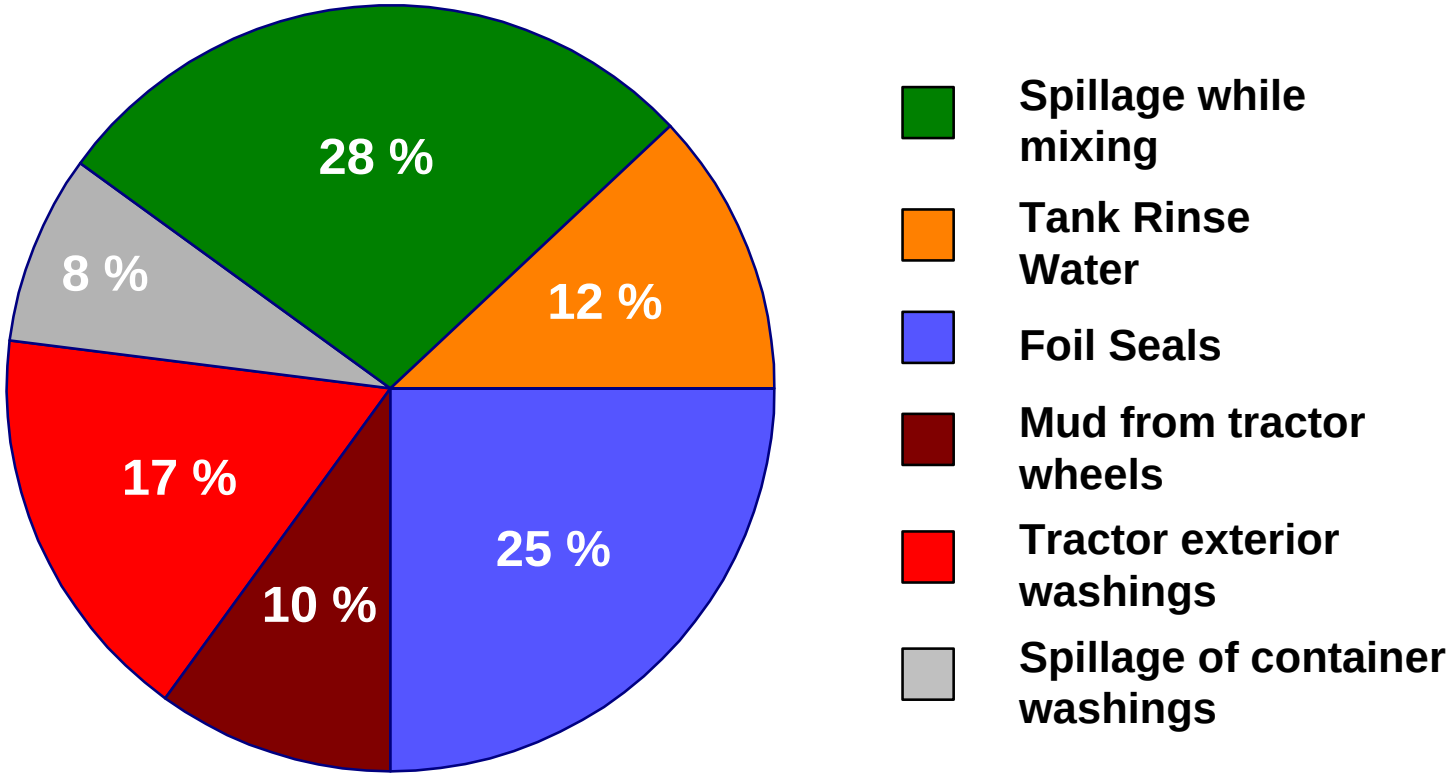
de uso exclusivo para los plaguicidas (balanza, jarra, probeta o jeringa). Lavarlos luego de utilizarlos, y verter el agua resultante en la cama biológica.

Realizar una calibración de los equipos previo a cada aplicación (prueba en blanco) y ajustar la dosis de producto en función del resultado obtenido. Luego de la aplicación lavar la maquinaria y/o equipos utilizados, en la cama biológica o en lugares alejados al menos 40 mts. de fuentes de agua.

No comer, beber, fumar, masticar chicle ni tomar mate durante la aplicación o durante la manipulación de plaguicidas.

Uruguay
2014

Pesticide Pollution sources in farms



Results from Year 1
Bayer CropScience Cherwell Study

Conventional technologies

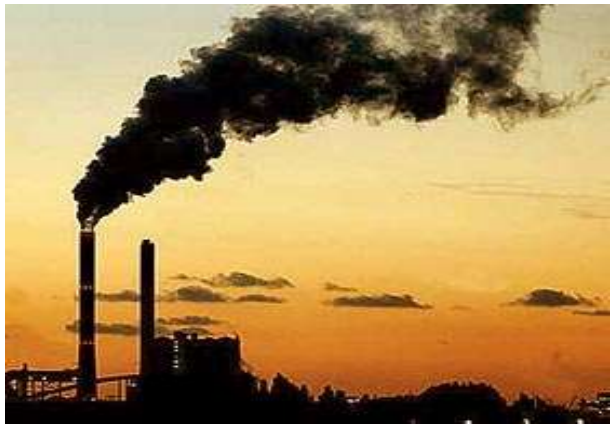
Some conventional technologies for pesticide contamination reduction of impacted places are

Remotion

Icineration

Confinement

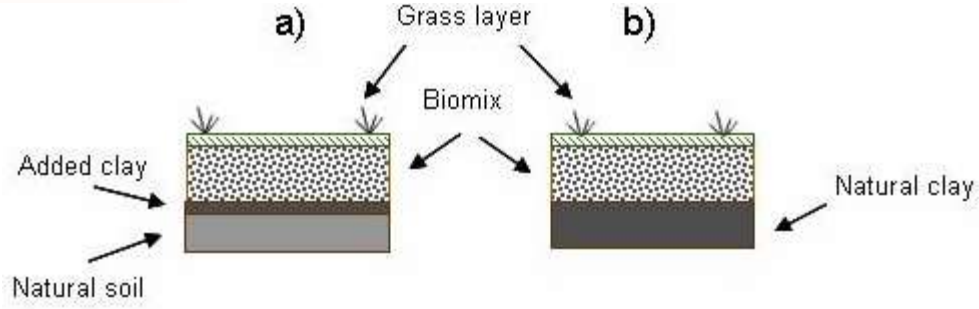
- Expensive technologies
- The contaminant it is not destroyed
- Another environmental compartment is polluted



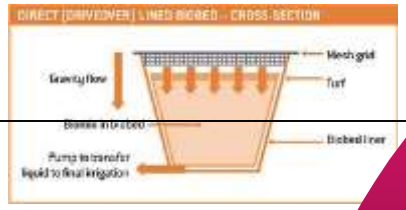
Biobeds

Low cost biotechnological product , that allows the dissipation of agrochemicals from impacted zones.
Biobed composition should contain substances that foster microbial development capable of degrading the contaminant .

Built up form low cost, raw materials, normaly unused by-products from farming activities or biological industrial wastes



Project overview



BIOBED on the field

Design of a BIOBED prototype

Biotransformation "in vitro"

Strain selection

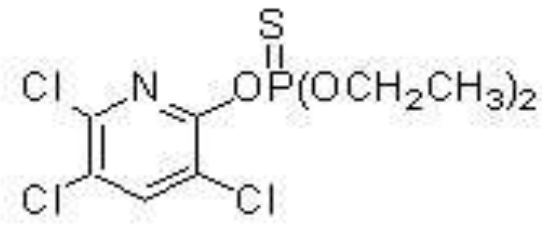
Analytical Methodology

Selected pesticides as model

- Widespread use in agriculture
- Persistent
- High toxicity
- Chlorine containing compound

Chlorpyrifos

Chlorpyrifos organophosphate



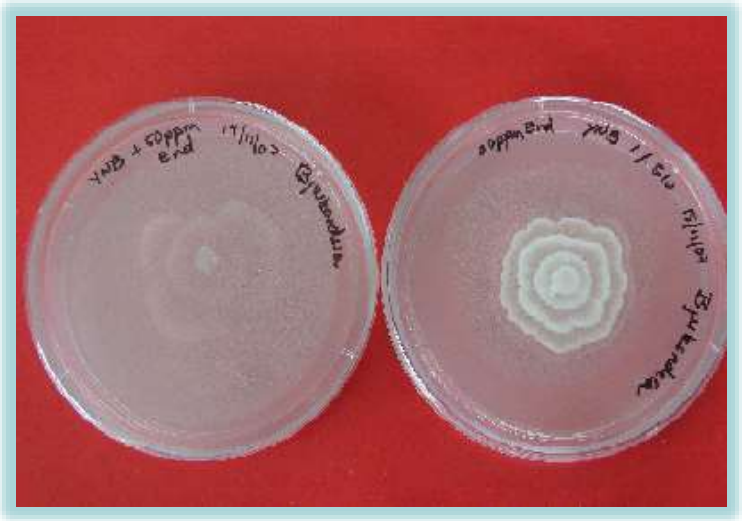
- Highly toxic insecticide
- Slightly persistent
- Endosulfán substitute

Screening of Basidiomycetes

11 native strains of the collection from Cátedra de Microbiología
YNB w/glu and wo/glu+60mg kg^{-1} of chlorpyrifos
(12days at 28±2°C).

- ***P. atropurpuracens(CCM014)***
- ***P. crysosporium(CCM004)***
- ***A. biennis(CCM0106)***
- ***B. adusta(CCM0379)***
- ***T. versicolor(CCM0338)***
- ***P. sanguineus(CCM0374)***
- ***P. cubensis(CCM0417)***
- ***Coriolus sp(CCM0341)***
- ***P. cornucopiae(CCM006)***
- ***G. trabeum(H2130)***
- ***G. recinaceum(CCM7187)***

A. biennis (CCM0106) and *B. adusta (CCM0379)*



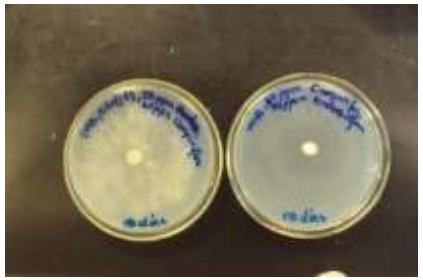
Evaluation of *A. biennis* ability to grow with endosulfan and chlorpyrifos

YNB (1% glu)(wo/glu)

50 mg kg $^{-1}$ endosulfan

→ 8.9 cm±0.15(glu)

→ 7.8 cm±0.15

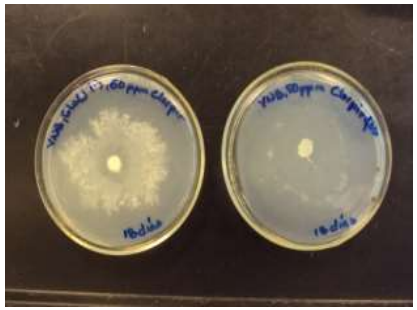


YNB (1% glu)(wo/glu)

60 mg kg $^{-1}$ chlorpyrifos

→ 6.0 cm±0.15(glu)

→ 5.3 cm±0.25



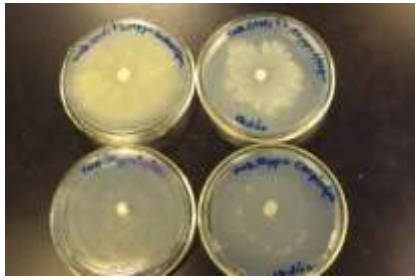
YNB (1% glu)(wo/glu)

50 mg kg $^{-1}$ endosulfan

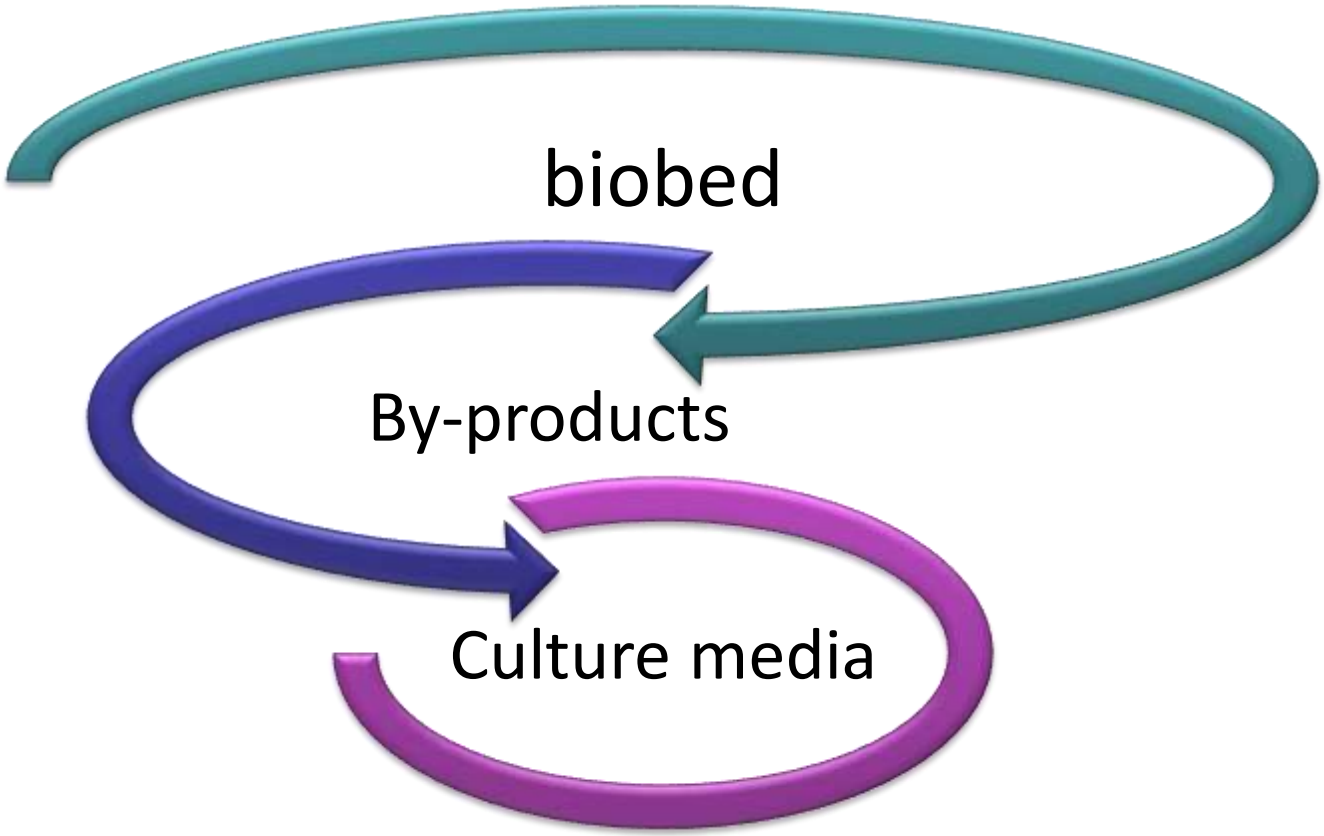
60 mg kg $^{-1}$ chlorpyrifos

→ 7.6 cm±0.20(glu)

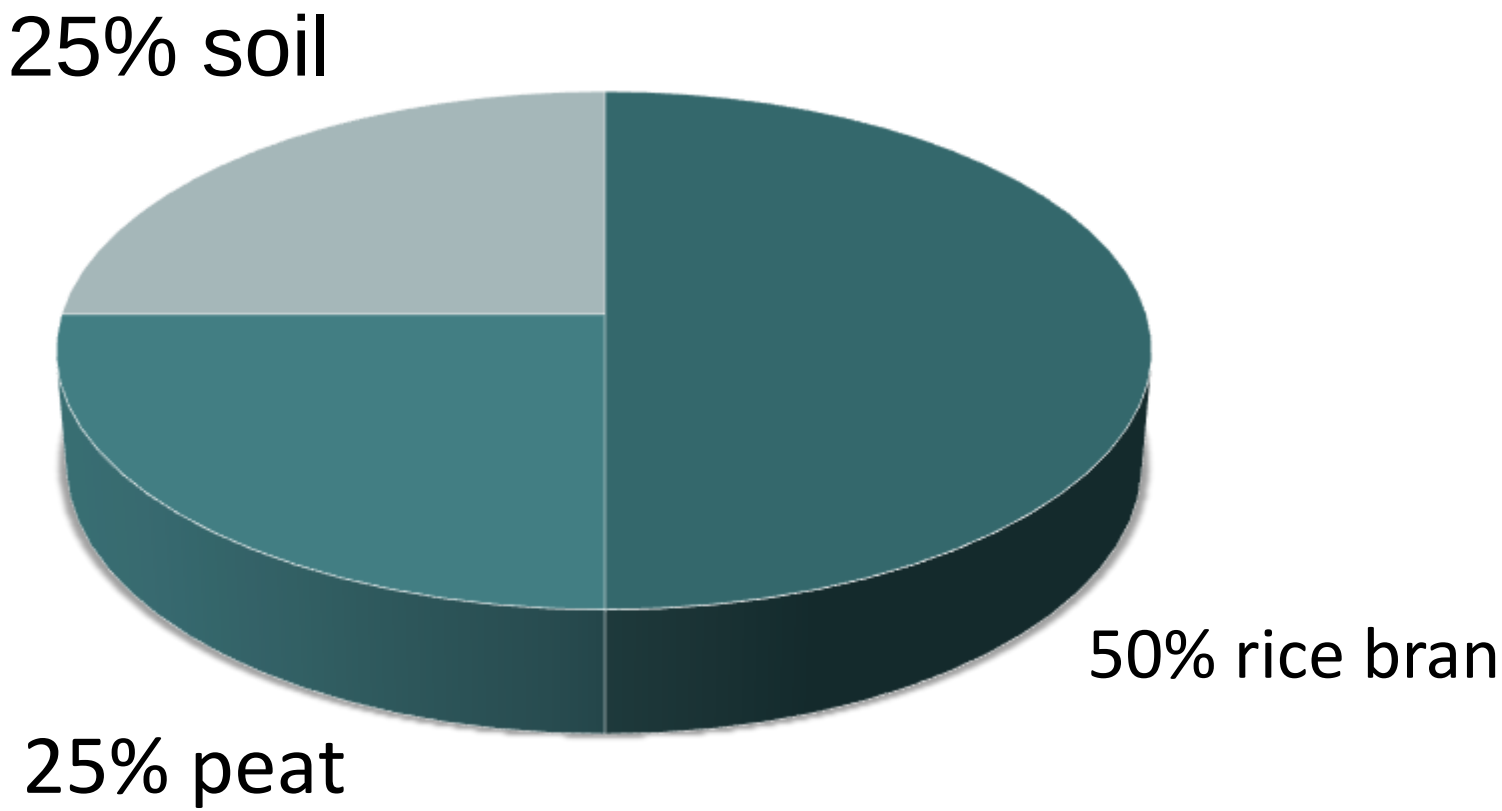
→ 5.2 cm±0.10



Where are analytical methods are needed?



Biobed: typical composition



Castillo M.P,* Torstensson L, and Jonh Stenstrom
J. Agric. Food Chem. 2008, 56, 6206-6219

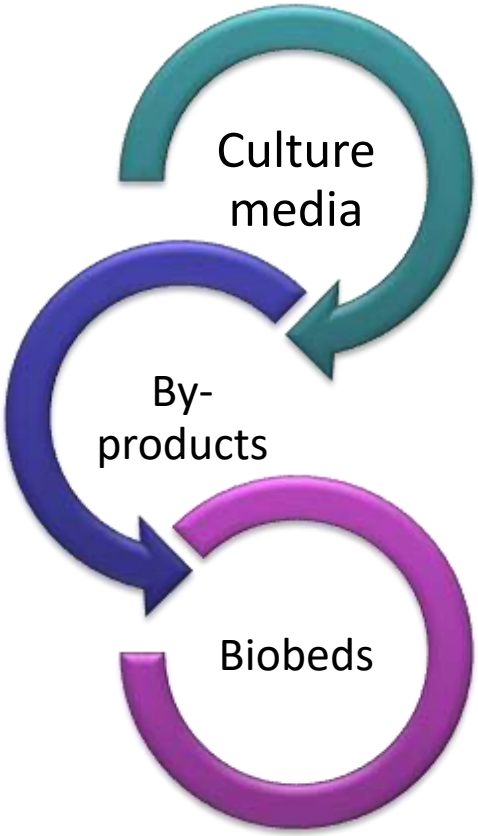
Different matrices to develop the sample preparation

Where analytical methods are needed

- YNB Media+1% de glucose.

- Rice bran + YNB Media + Agar

- Rice bran + Peat + Soil.



Methodologies assayed

- AcOEt Stomacher stirred
- AcOEt Shaker stirred
- **Acetonitrile** shaker stirred :

➢ ACN water miscibility

➢ Single phase extraction of bioreaction compounds

Different methodologies of extraction to evaluate the bioconvertion in the matrix rice bran.

		Steps of extraction and cleanup of the matrix rice bran in culture media							
Methods	Sample (g)	Solvent(mL)/ Agua (mL)	Extraction/ time(h)	Column $NaSO_4$/ Sílica	Volume (mL)	Salting out $MgSO_4$/ NaCl /$NaSO_4$	Extraction/ Time(h)	Centrifuge 3000 rpm(h)	Adjust (mL)/aliquot(mL) /IS/Adjust Vol(mL)
1	15	AcOEt(40)/-	stomacher/5	20g/30g	100	-	-	-	100 /0.1 /Bromophos/1
2	15	AcOEt(40)/-	shaker/24	20g/30g	100	-	-	-	100 /0.1 /Bromophos /1
3	3.5	AcOEt(20)/-	shaker/5	-	-	04/01/2005	manual/1/30	1/6	/3/Bromophos /25
4	15	MeCN(15/15)	shaker/2	-	-	/2/20	shaker/5	1/6	/0.5/Bromophos /25

Method Validation

- Linearity
- % Recovery
- Matrix Effect
- LOD
- LOQ
- Precision (DSR_r)
- Precision (DSR_{wR})

Method validation and quality control procedures for pesticide residues analysis in food and feed. Document No. SANCO/10684/2009

Method Validation

	1 $mgkg^{-1}$		25 $mgkg^{-1}$		60 $mgkg^{-1}$	
	% REC	% RSD	% REC	% RSD	% REC	% RSD
Chlorpyrifos	72	5	109	7	76	10

Lineality

R^2>0.98

LODs:

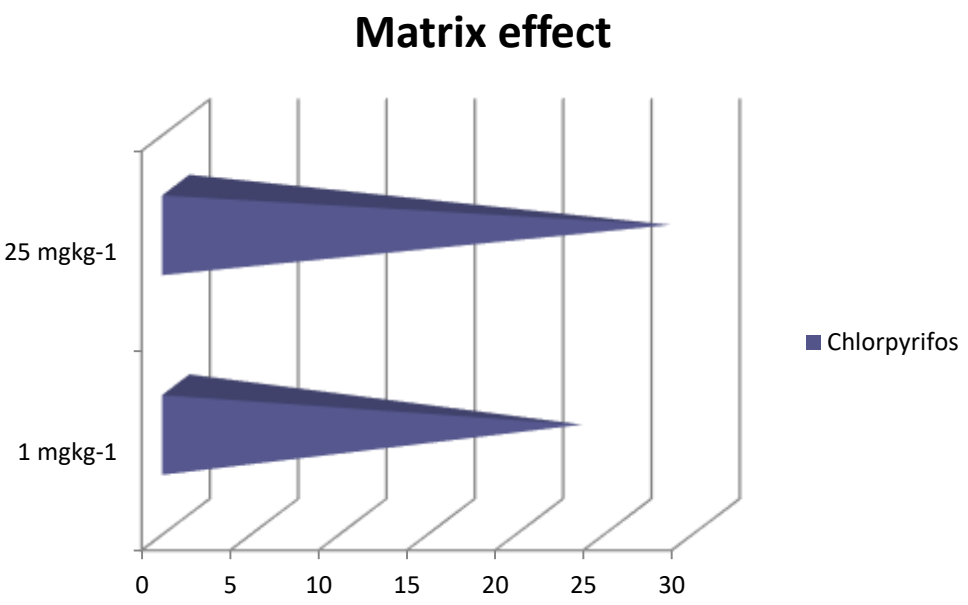
0.3 $mgkg^{-1}$

LOQs:

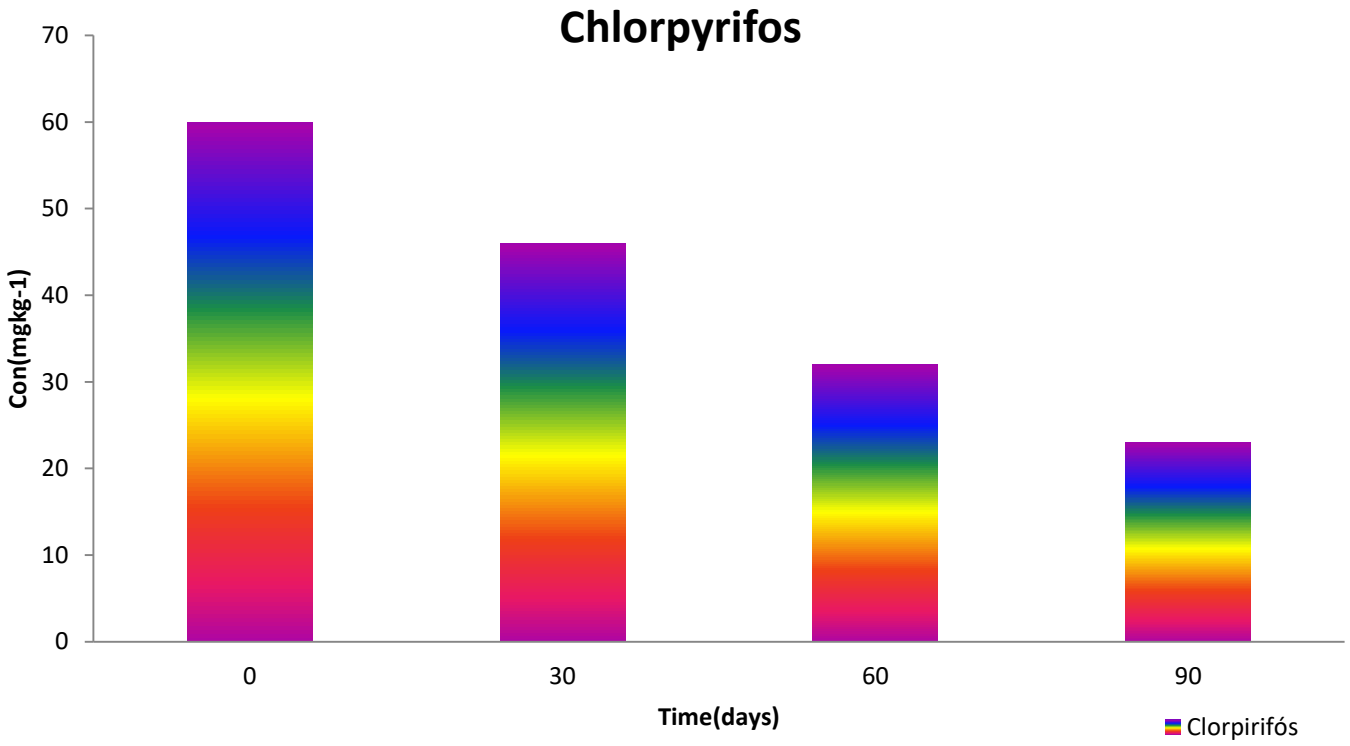
1 $mgkg^{-1}$

Interm REPROD. .3-16%

RSDwR



Chlorpyrifos Bioconversion in rice bran matrix



% Biocon	30 days	60 days	90 days
Chlorpyrifos	74	81	87

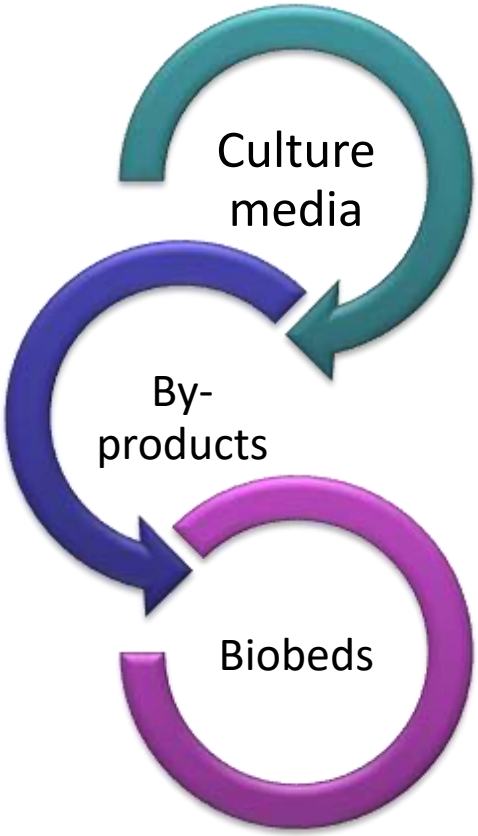
Different matrices to develop the sample preparation

- YNB Media+1% de glucose.

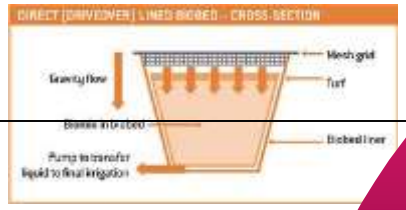
- Rice bran + YNB Media + Agar

- Rice bran + Peat + Soil.

Where analytical methods are needed



Project overview



BIOBED on the field

Design of a BIOBED prototype

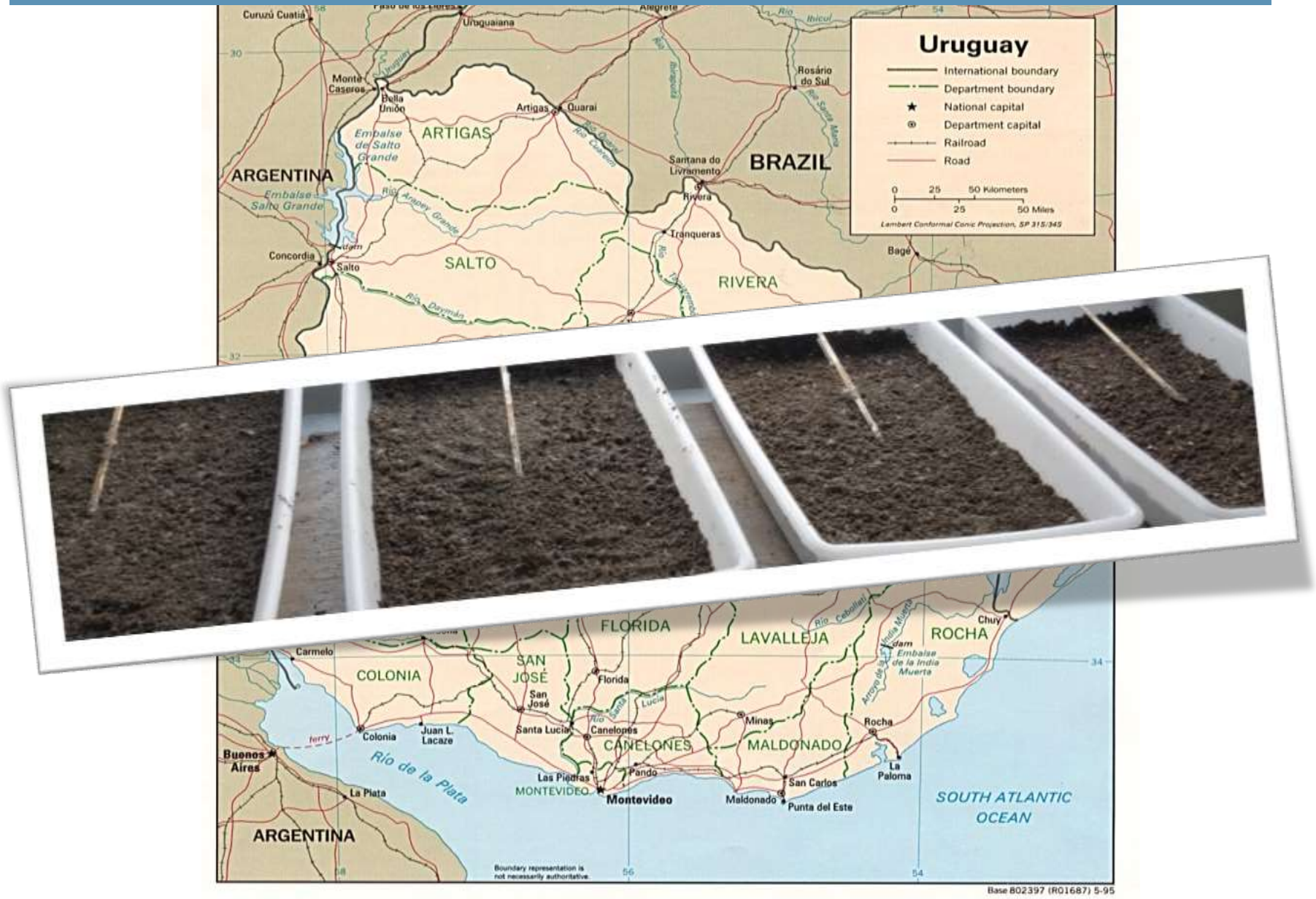
Biotranformation "in vitro"

Strain selection

Analytical Methodology

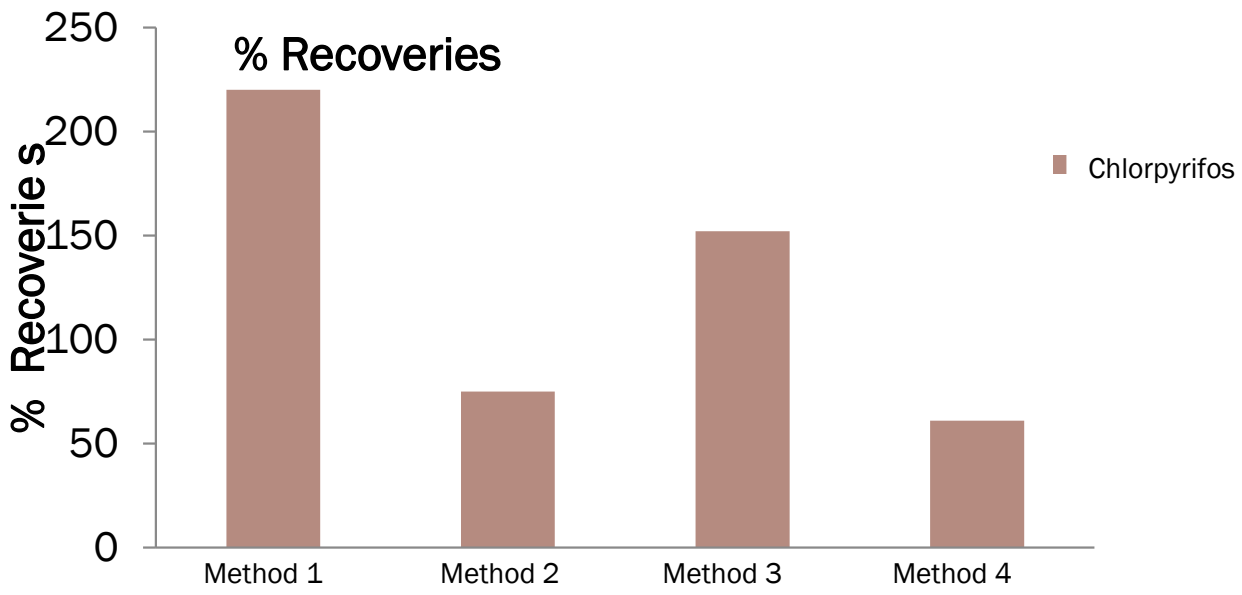
Uruguay
International boundary
Department boundary
National capital
Department capital
Railroad
Road
0 25 50 Kilometers
0 25 50 Miles
Lambert Conformal Conic Projection, SP 31S/34S
ARGENTINA
BRAZIL
ARTIGAS
SALTO
PAYSANDÚ
RIVERA
TACUAREMBÓ
RIO NEGRO
CERRO LARGO
TREINTA Y TRES
DURAZNO
SORIANO
FLORES
FLORIDA
LAVALLEJA
ROCHA
SAN JOSÉ
COLONIA
CANELONES
MALDONADO
MONTEVIDEO
Paso de los Libres
Uruguaiana
Alegrete
Curuzú Cuatiá
Monte Caseros
Bella Unión
Artigas
Quaraí
Rosário do Sul
Santana do Livramento
Rivera
Tranqueras
Bagé
Concordia
Salto
Tacuarembó
Aceguá
Paysandú
Guichón
Melo
Jaguarão
Rio Branco
Concepción del Uruguay
Young
Paso de los Toros
San Gregorio
Kilómetro 329
Blanquillo
Vergara
Gualeguaychú
Fray Bentos
Mercedes
Durazno
Sarandí del Yí
Treinta y Tres
José Pedro Varela
Dolores
Cardona
Carmelo
Florida
San José
Santa Lucía
Canelones
Minas
Rocha
Chuy
Colonia
Juan L. Lacaze
Buenos Aires
La Plata
Las Piedras
Pando
Montevideo
Maldonado
San Carlos
Punta del Este
La Paloma
Río de la Plata
SOUTH ATLANTIC OCEAN
Lago Artificial de Paso del Palmar
Lago Artificial de Rincón del Bonete
Embalse de Salto Grande
Embalse de la India Muerta
Lagoa Mirim
Laguna Merín
Boundary representation is not necessarily authoritative.
Base 802397 (R01687) 5-95

Biobeds in laboratory scale



Selection of the extraction conditions

Methods	Solvent	Extraction	Salting-out	Centrifugation
1	MeCN	shaker	X	X
2	AcOEt	ultrasonido	-	X
3	AcOEt	shaker	X	X
4	MeCN	ultrasonido	-	X

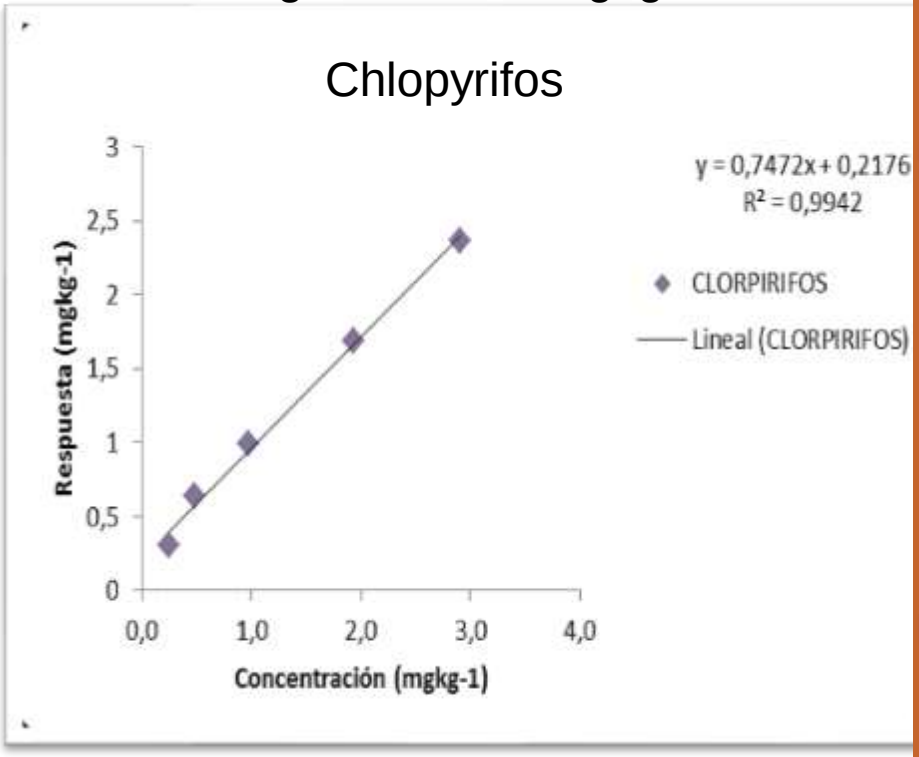


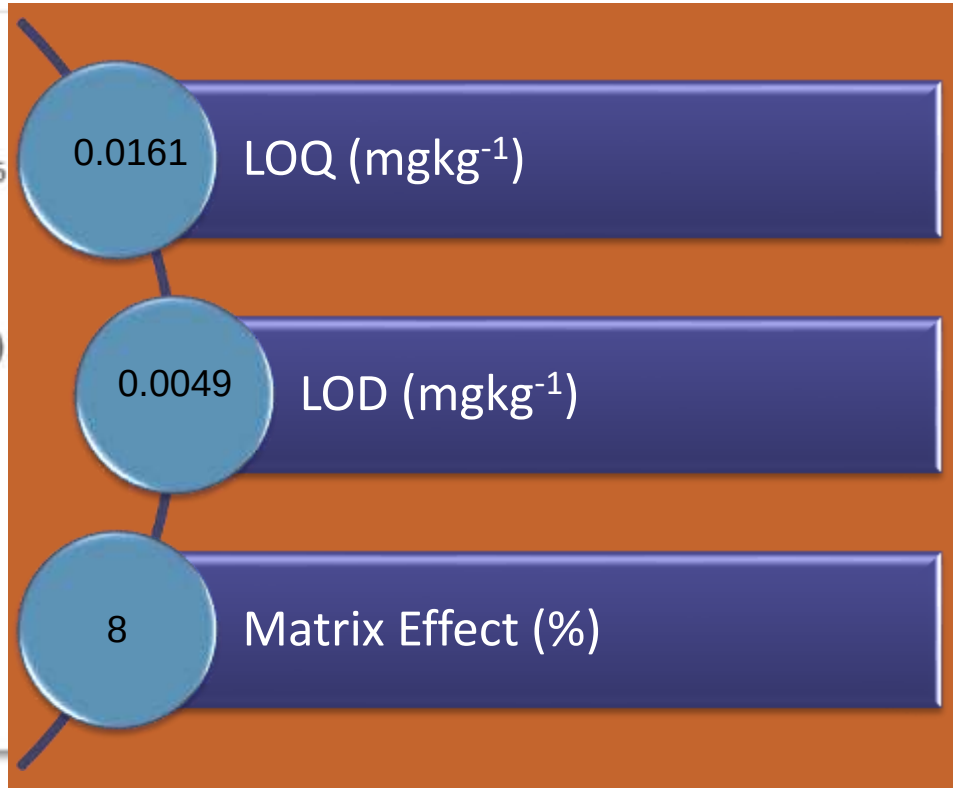
Validation by GC-ECD for soil 1

	Recoveries			
	60 $mgkg^{-1}$		30 $mgkg^{-1}$	
Pesticide	Rec (%)	RSD (%)	Rec (%)	RSD (%)
Clorpyrifos	78	8	72	3

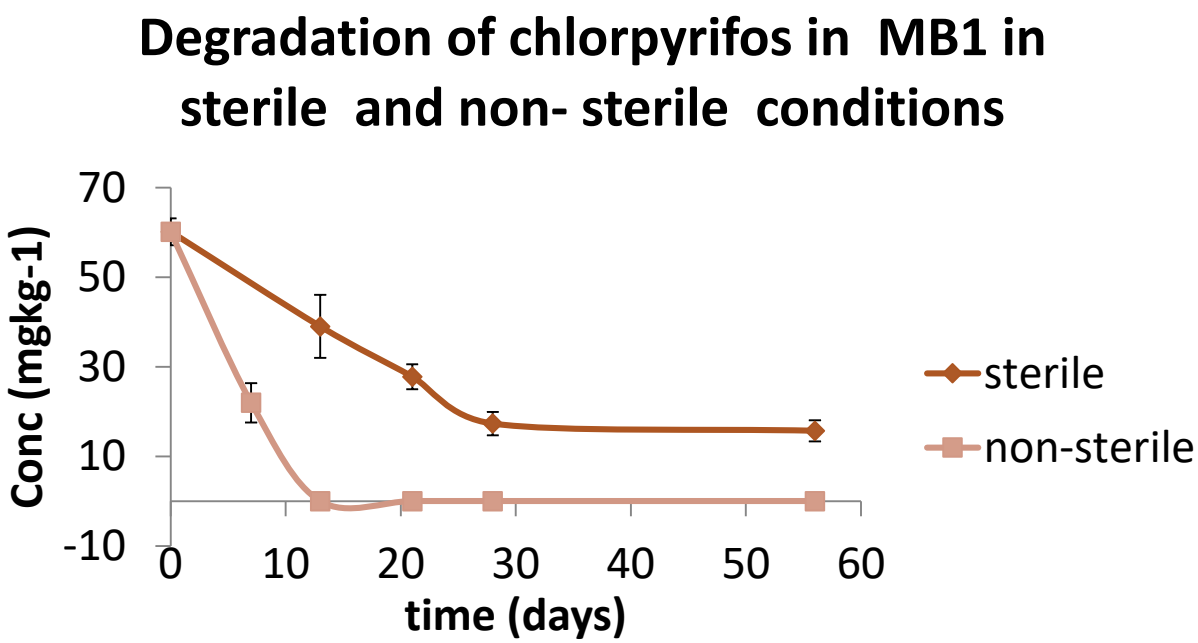
Lineal Range: 0.25-4.0 $mgkg^{-1}$

RSDwR : 8%





Chlorpyrifos bioconversion in the Biobed

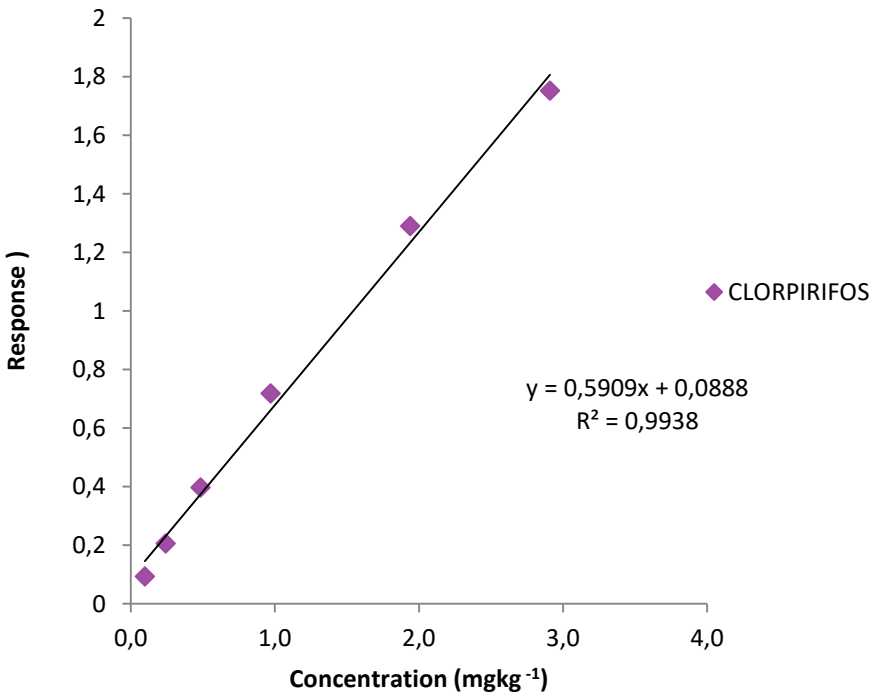


% de Bioconversion	12 days	27 days
Chlorpyrifos- sterile	40	79
Chlorpyrifos-non sterile	64	100

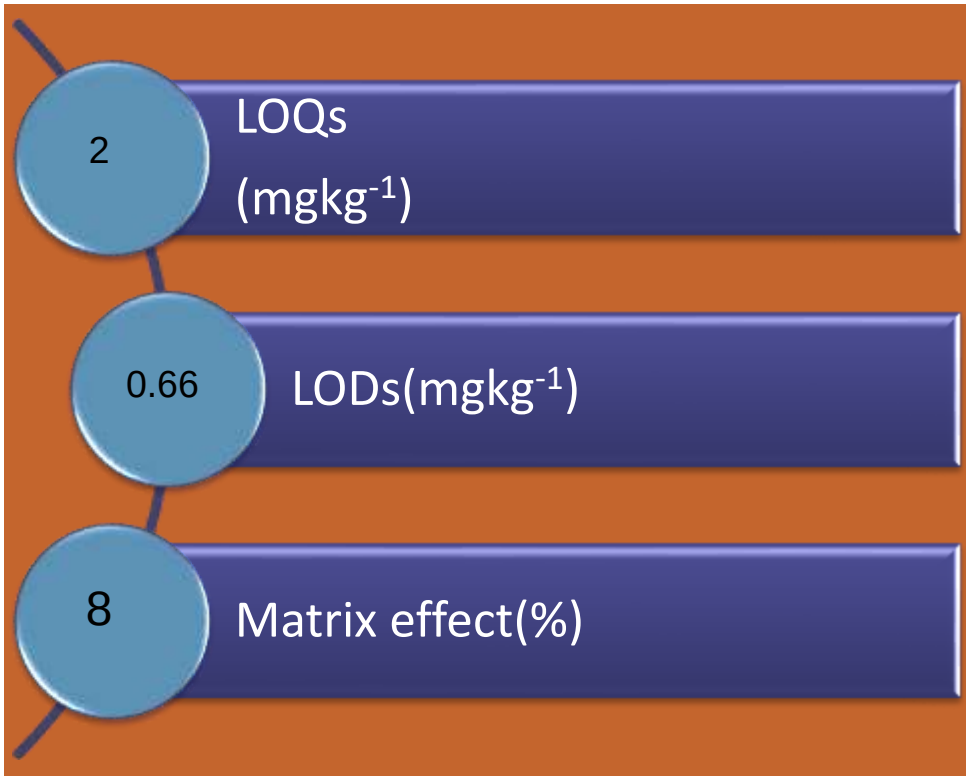
Validation in soil 2 for GC-ECD

Recoveries						
	60 mg/kg		30 mg/kg		2 mg/kg	
Pesticide	Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)
Chlorpyrifos	86	7	75	4	101	16

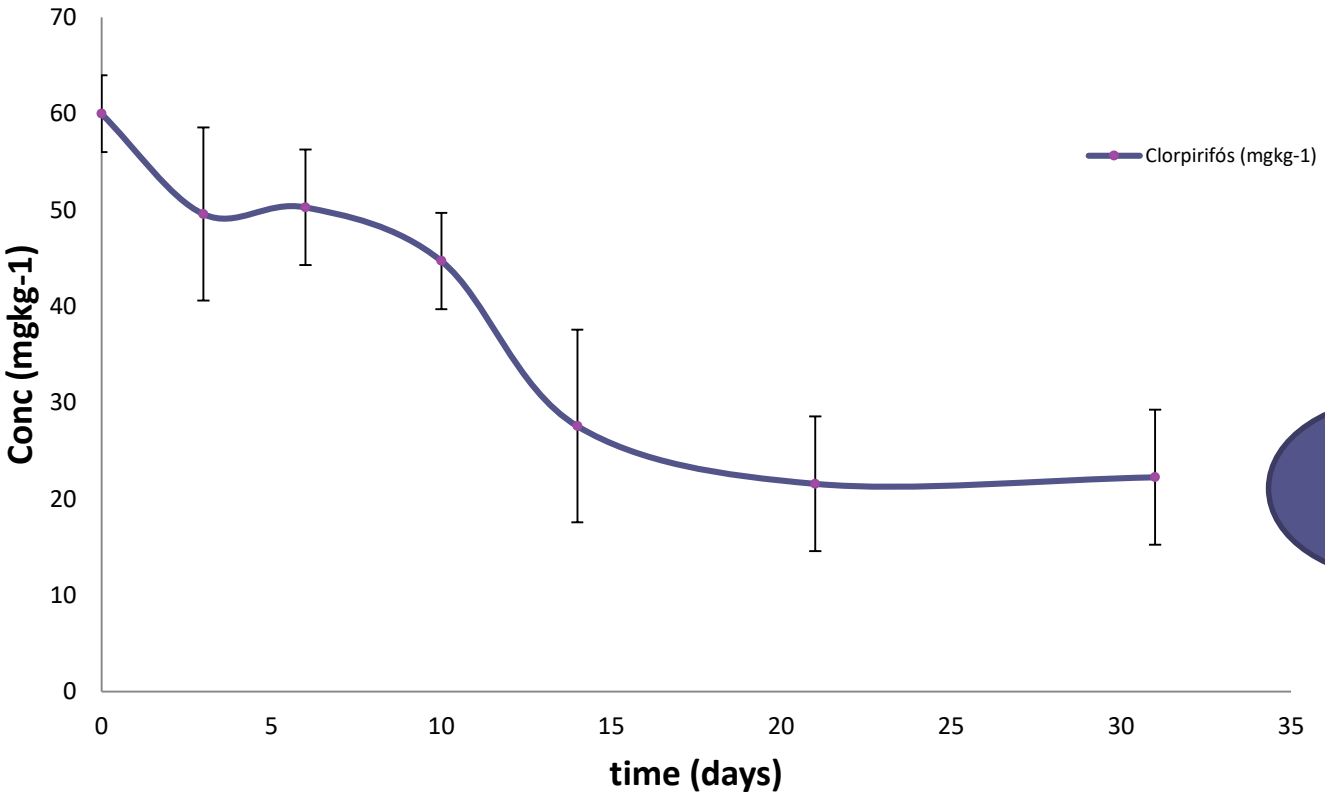
Linearity 0.1-3 $mgkg^{-1}$



RSDwR : 9%

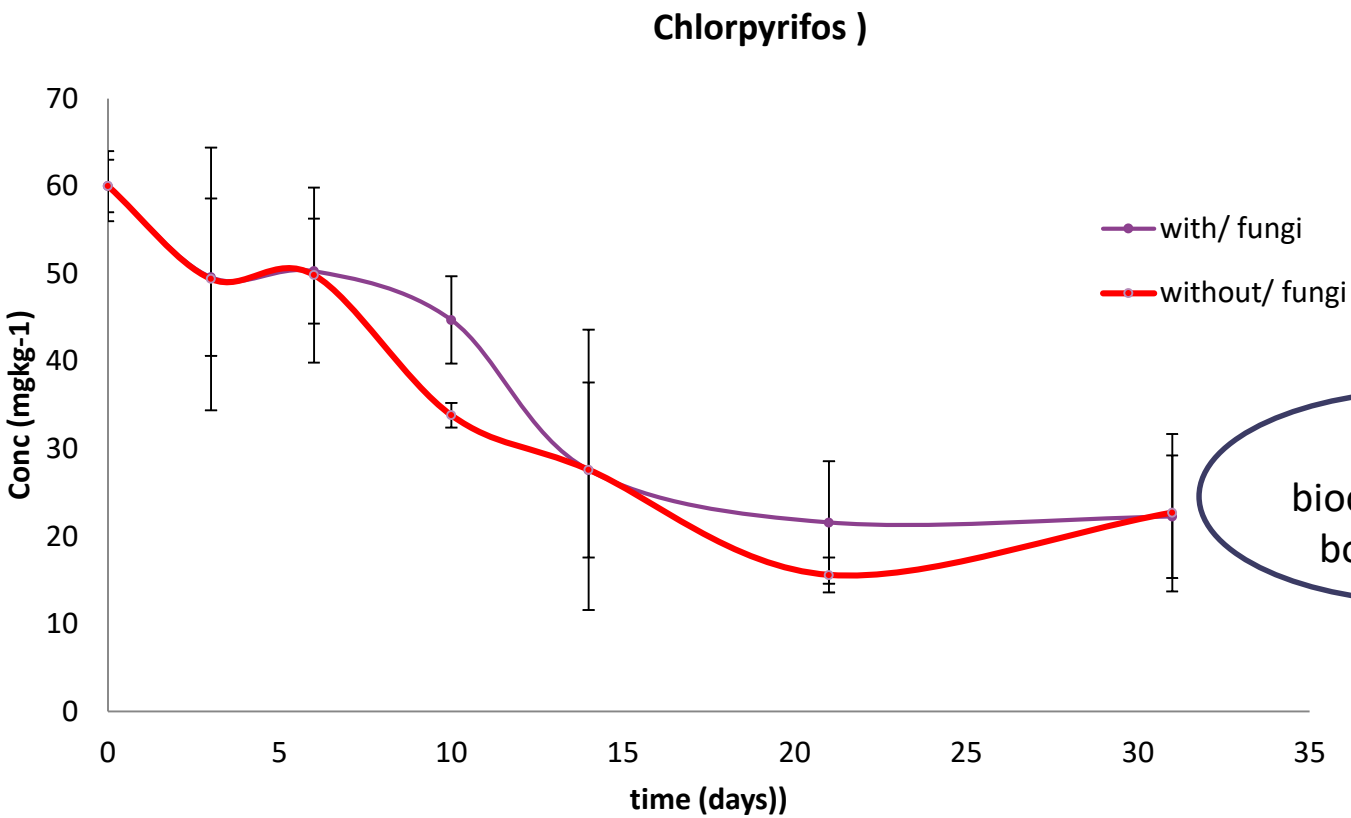


Bioconvertion in the mini-biobeds soil 2



62%

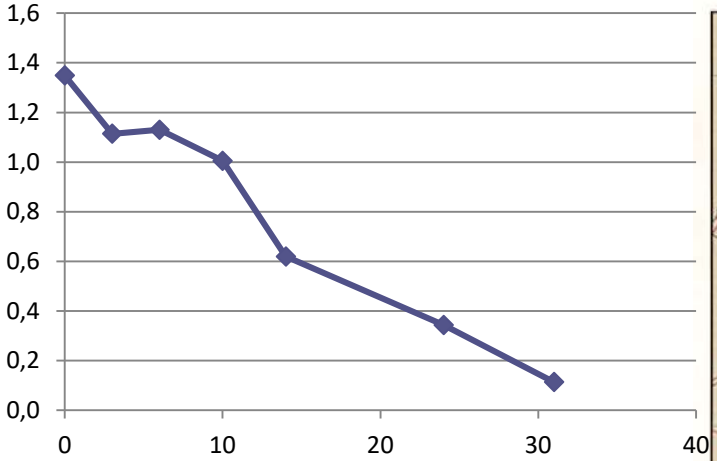
Bioconversion in soil 2



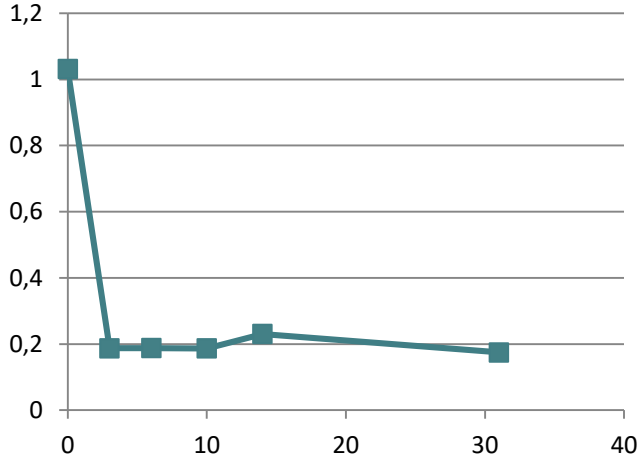
Similar bioconversion in both systems

¿ 3,5,6-trichloro-2-pirydinol (TCP)?

Chlorpyrifos degradation



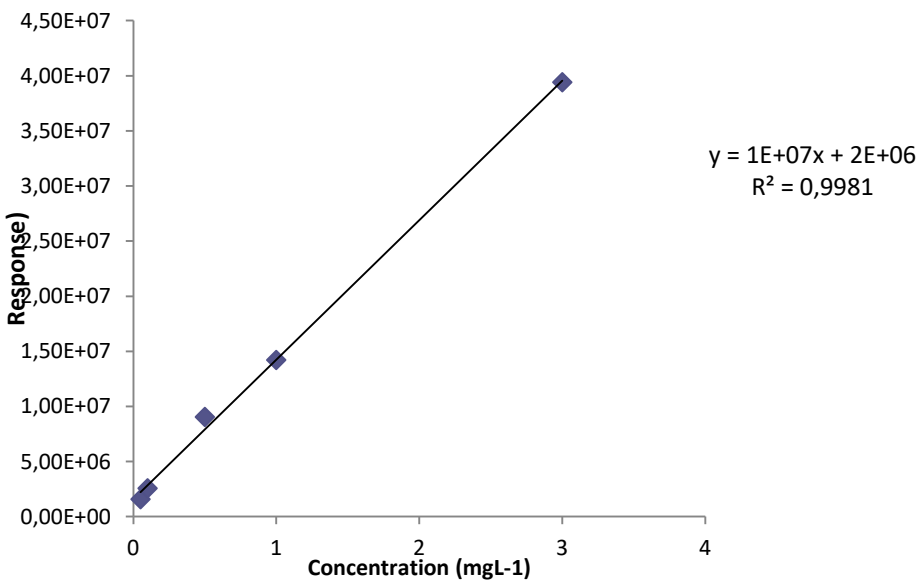
Chlorpyrifos degradation

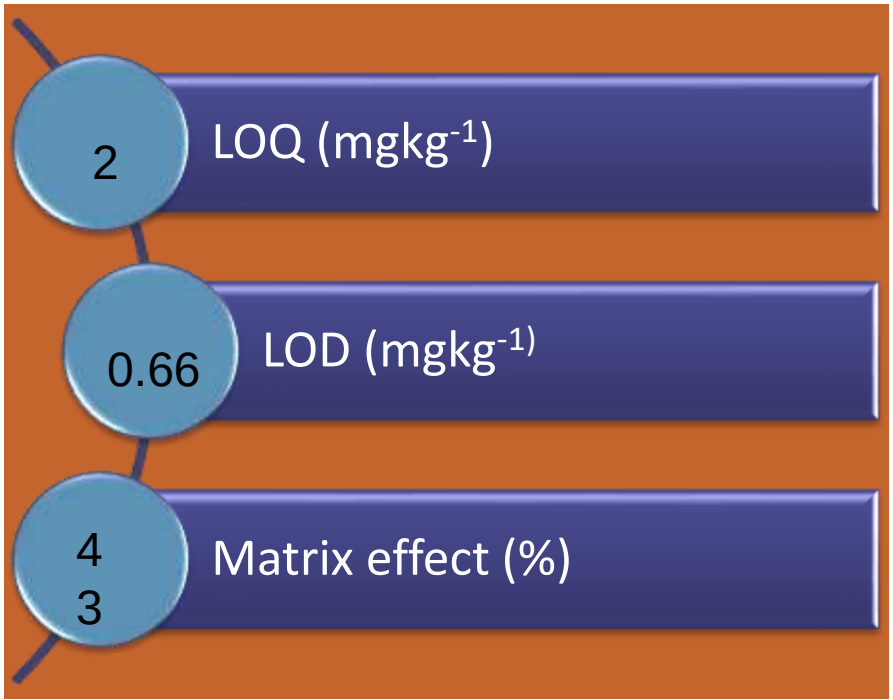


Validation of the methodologies in the biobeds Degradation of chlorpyrifos and TCP (3,5,6-trichloro-2-pirydinol) (solil1) y (soil 2).

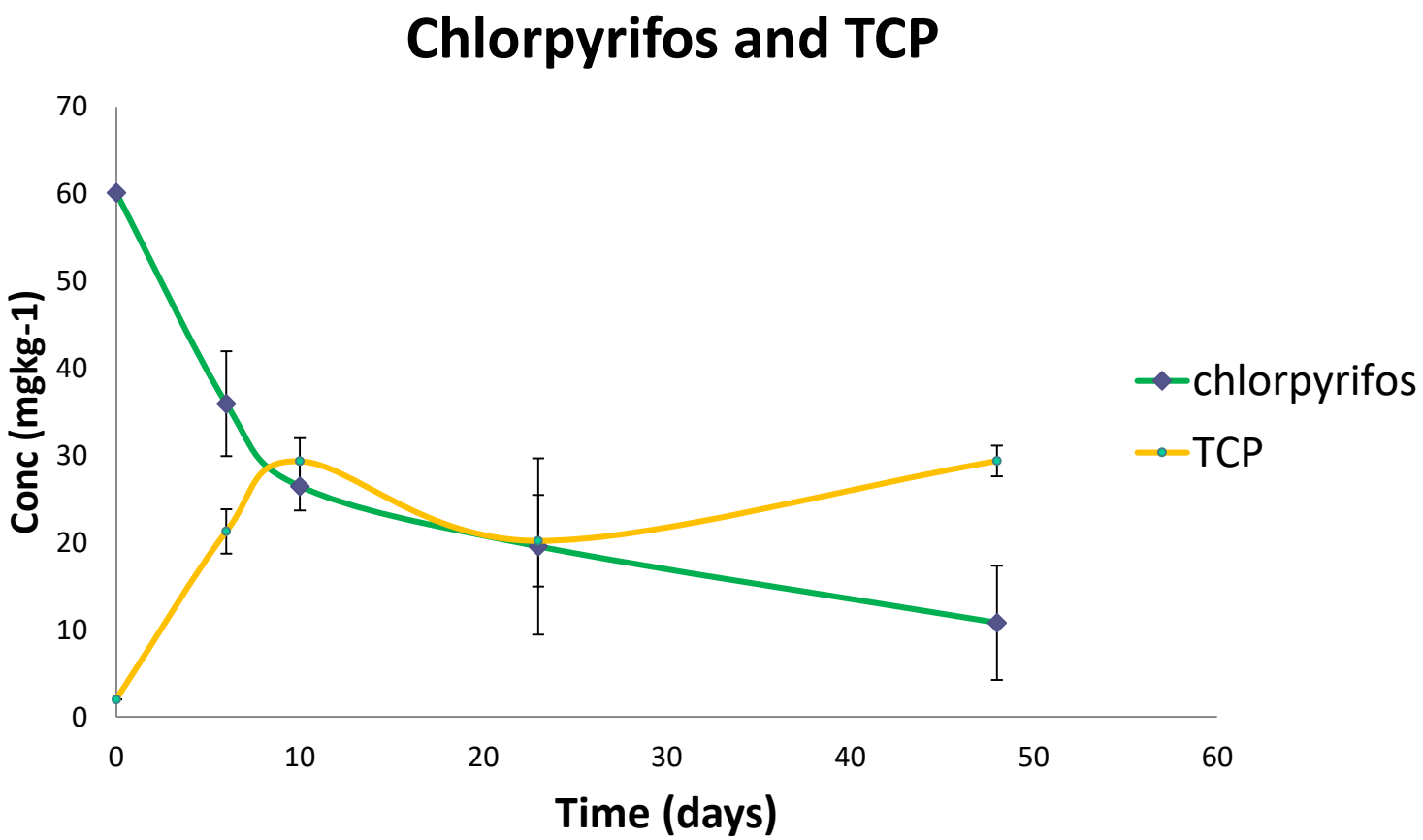
	60 $mgkg^{-1}$		30 $mgkg^{-1}$		2 $mgkg^{-1}$	
	Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)
3,4.5-trichloro-2-piridinol	88	3	113	4	72	10

Linearity:0.025 – 3 $mgkg^{-1}$

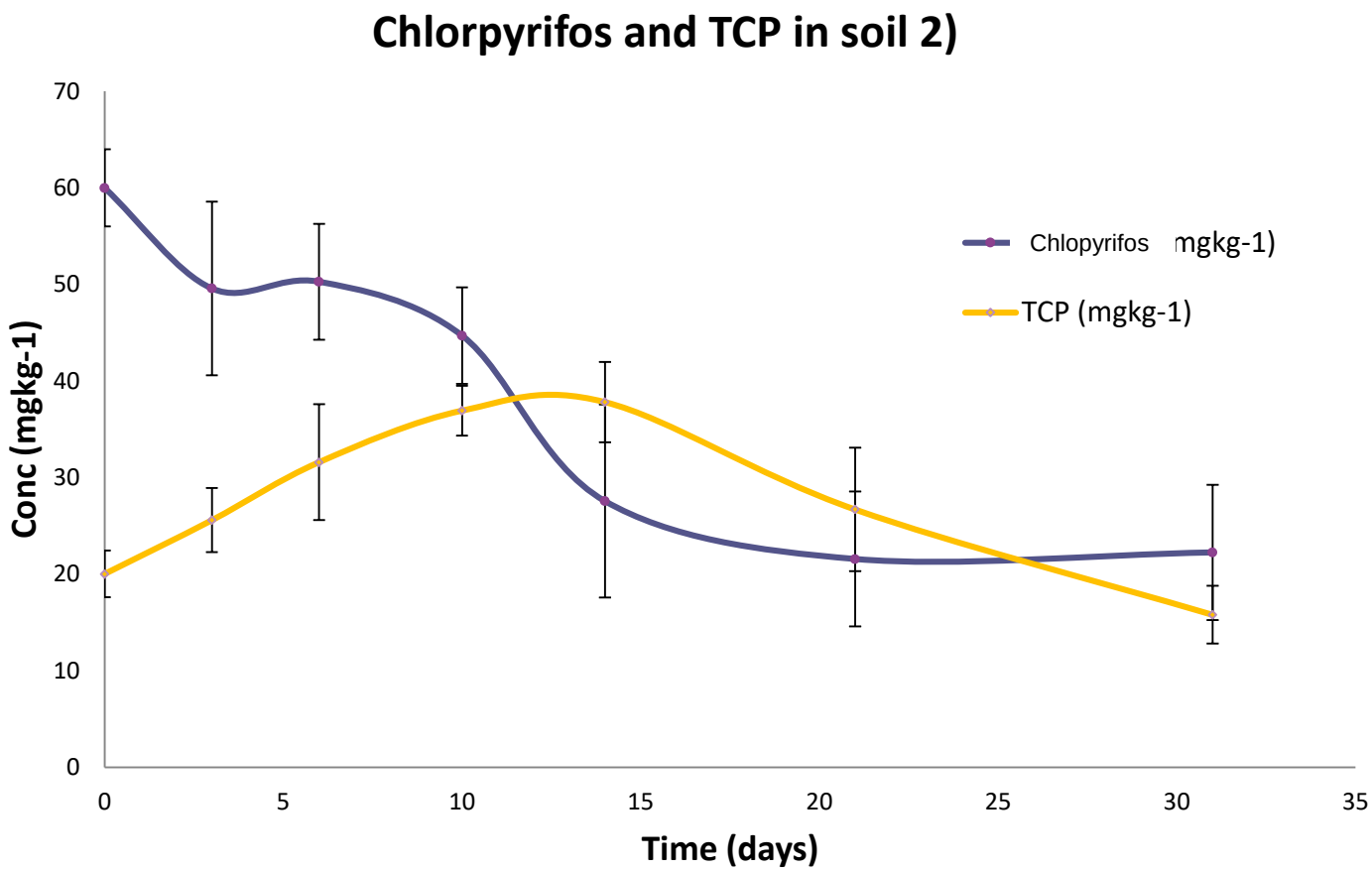




Bioconversion in the matrix soil 1



Bioconversion in the matrix soil 2



Field biobed MB1 o MB2?

Conclusions

- **Three "fit to purpose" methodologies to accurately evaluate chlorpyrifos bioconversion were developed and validated.**

- **The bioconversion in each one of these matrices, for two different White Rot Fungi could be followed and proven**

- ***A. biennis* was able to dissipate 87 % chlorpyrifos in rice bran.**

- **The Biobed developed allowed >90% bioconversion for both pesticides after 30 days**

Thank You!