



Ministério da
Agricultura, Pecuária
e Abastecimento



CIBAMA-UFRO
Centro de Excelencia en Investigación Biotecnológica Aplicada al Medio Ambiente



III Workshop Latino Americano sobre Biobed

ADVANCES IN BIOPURIFICATION SYSTEM FOR PESTICIDE DEGRADATION - CHILE

M.C. Diez

Chemical Engineering Department
CIBAMA-BIOREN

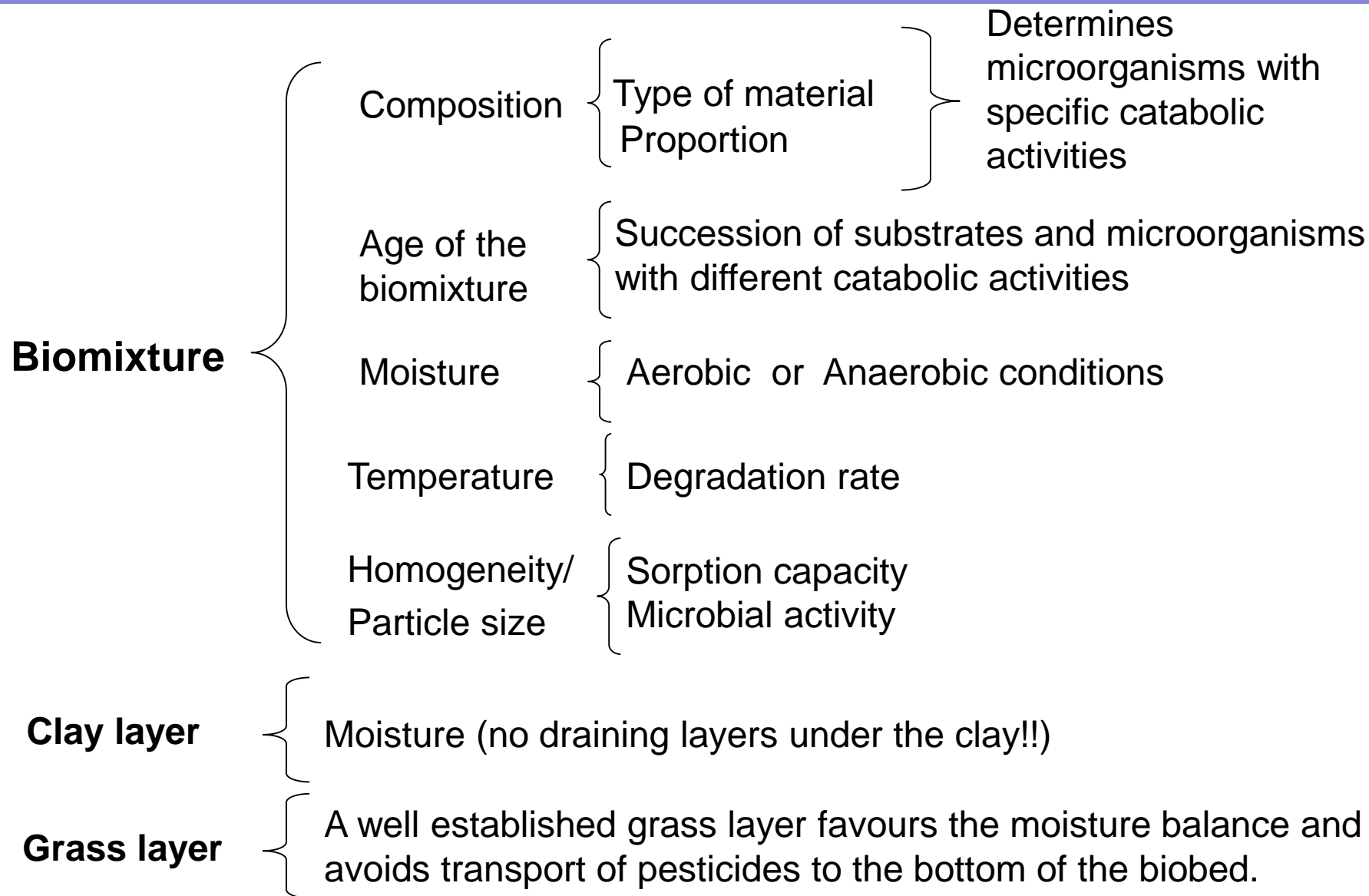
Universidad de La Frontera, Temuco, Chile.

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<https://lechosbiologicos.wordpress.com/>



Factors Affecting the Efficiency of Biobed





Some topics that we have addressed

1

Effect of the type of soil. Incubation Temperature. Alternative materials: oat husk, barley, sawdust, biochar

2

Supplementation of NPK nutrients and activating compounds, in the biomix

3

Development of supports for the immobilization of ligninolytic fungi and their inoculation in the biomix

4

Isolation, selection and development of bacterial consortia degrading organochlorine, organophosphates and other pesticides

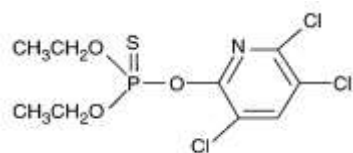
5

Effect of the rhizosphere (columns and ponds) and hydraulic load in the biopurification system (ponds). Single and repeated applications

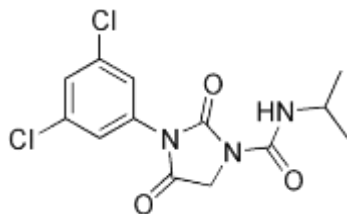


Pesticides Characteristics

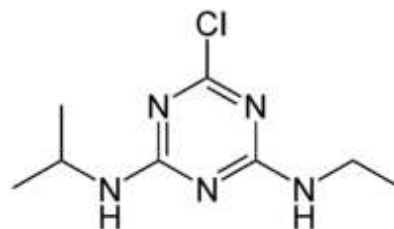
Pesticides	Koc	T1/2 Soils (days)	Water solubility (mg L ⁻¹)	Mobility class
Chlorpyrifos (I)	6000	7-15	1.4	Non mobile
Iprodione (F)	700	42-80	13	Moderately mobile
Atrazine (H)	40-155	35-50	33	Moderately mobile
Isoproturon (H)	36-240	6-28	65	Moderately mobile



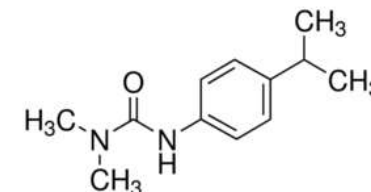
Chlorpyrifos



Iprodione



Atrazine



Isoproturon

Soils and Materials Characterization

Parameter	Soils		
	Clay	Sandy	Trumao
N (mg/kg)	13.0 ± 0.4	9.0 ± 0.1	18.6 ± 0.7
P (mg/kg)	4.0 ± 0.3	20.0 ± 0.9	17.1 ± 0.5
K (mg/kg)	43.0 ± 2.4	149.0 ± 8.4	69.0 ± 1.4
pH _w	5.2 ± 0.4	6.0 ± 0.2	5.6 ± 0.1
Organic Matter (%)	3.0 ± 0.2	2.0 ± 0.5	12.7 ± 0.5
Silt (%)	20.1 ± 0.7	32.6 ± 0.9	34.2 ± 0.4
Clay (%)	68.5 ± 0.8	9.1 ± 0.5	28.7 ± 0.8
Sand (%)	11.3 ± 0.5	58.3 ± 0.4	37.1 ± 0.2

Parameter	Wheat Straw	Sawdust	Barley Husk
Dry weight (%)	91.1 ± 1.8	87.8 ± 1.2	88.8 ± 1.0
Fiber (%)	54.3 ± 1.2	76.6 ± 1.3	12.9 ± 0.5
Lignin (%)	9.9 ± 0.4	20.6 ± 0.6	2.4 ± 0.2
Celullose (%)	41.8 ± 0.7	54.3 ± 0.4	9.6 ± 0.1
Organic Matter (%)	66.1 ± 1.2	61.75 ± 0.6	59.8 ± 0.9
pH	5.9 ± 0.1	4.82 ± 0.1	5.8 ± 0.1
N (mg/kg)	5.6 ± 0.3	2.8 ± 0.1	6.8 ± 0.6



Biomix	Atrazine	Ioproturon	Iprodione	Chlorpyrifos
CS1	89.8 ± 2.4 a	79.1 ± 0.7 a	24.8 ± 1.7 a	76.1 ± 0.9 ab
CS2	66.2 ± 4.2 c	63.6 ± 0.2 b	25.6 ± 1.2 a	86.8 ± 0.2 a
CS3	77.0 ± 0.2 b	53.2 ± 0.2 c	34.3 ± 0.2 a	57.8 ± 0.2 b
SS1	76.5 ± 0.2 b	77.4 ± 2.2 a	52.9 ± 5.2 b	72.9 ± 0.7 ab
SS2	75.0 ± 4.2 b	63.3 ± 1.4 ab	38.1 ± 0.3 c	77.2 ± 1.1 a
SS3	92.7 ± 0.2 a	52.4 ± 1.7 b	68.4 ± 1.2 a	55.9 ± 1.7 c
TS1	73.4 ± 0.4 b	74.4 ± 0.4 a	32.3 ± 1.2 a	76.1 ± 1.2 a
TS2	71.6 ± 0.3 c	44.3 ± 1.2 b	25.9 ± 1.5 a	55.7 ± 1.1 c
TS3	92.7 ± 0.5 a	65.0 ± 0.2 a	23.6 ± 1.1 a	63.7 ± 0.4 b

Soils

CS: Clay Soil

SS: Sandy Soil

TS: Trumao Soil

Materials

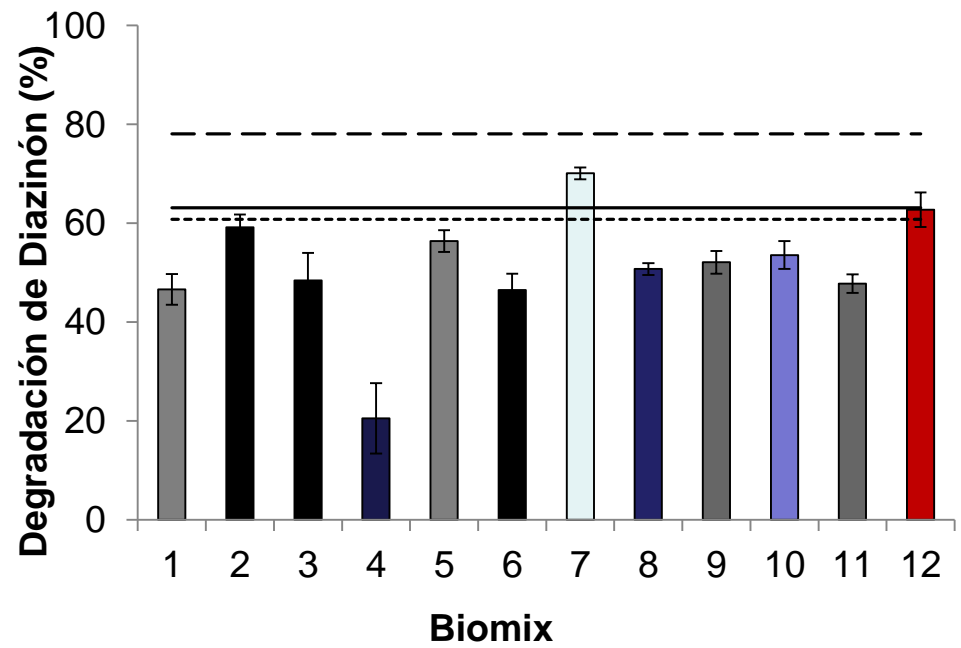
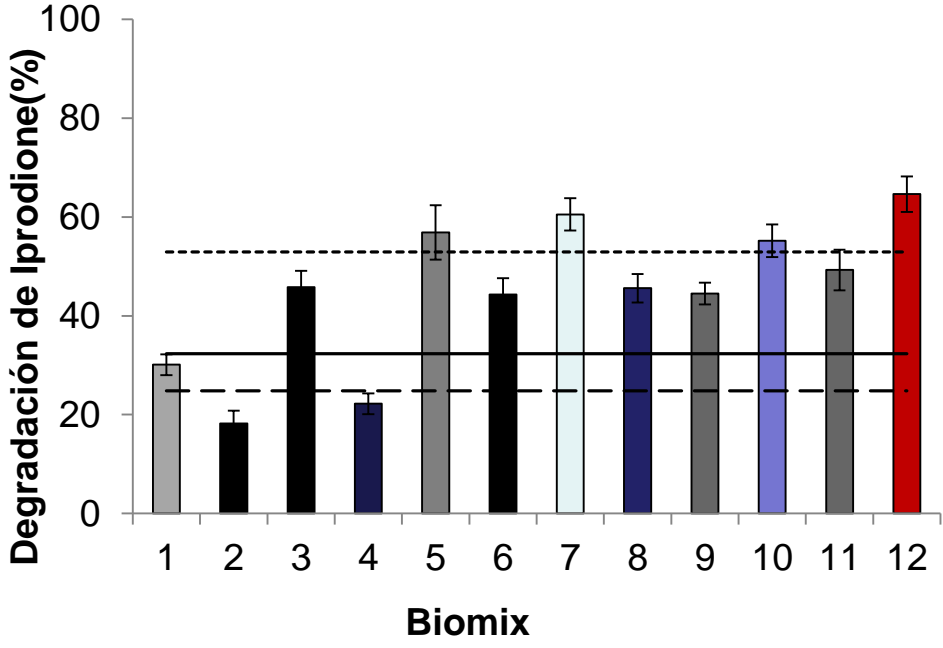
1 soil:peat:straw (1:1:2)

2 soil:peat:straw:sawdust (1:1:1:1)

3 soil:peat:straw:barley (1:1:1:1)

Iprodione and Diazinon Degradation (100 Mg Kg⁻¹)

	Biomix											
	Clay Soil (25%) Straw (50%)				Trumao Soil (25%) Straw (50%)				Sandy Soil (25%) Straw (50%)			
	1	2	3	4	5	6	7	8	9	10	11	12
Peat (%)	20	15	10	5	20	15	10	5	20	15	10	5
Biochar (%)	5	10	15	20	5	10	15	20	5	10	15	20



Influence of novel lignocellulosic residues in a biobed biopurification system on the degradation of pesticides applied in repeatedly high doses

M. Cristina Diez¹ ✉ · Gonzalo R. Tortella¹ · Gabriela Briceño¹ · Maria del Pilar Castillo² · Jorge Díaz³ · Graciela Palma¹ · Carolina Altamirano¹ · Carolina Calderón¹ · Olga Rubilar¹

Chemosphere 92 (2013) 1361–1366



Contents lists available at SciVerse ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere



Technical Note

Degradation of pesticide mixture on modified matrix of a biopurification system with alternatives lignocellulosic wastes



C. Urrutia^a, O. Rubilar^b, G.R. Tortella^b, M.C. Diez^{b,*}

*Journal of
Biobased Materials and Bioenergy*
Vol. 7, 741–747, 2013

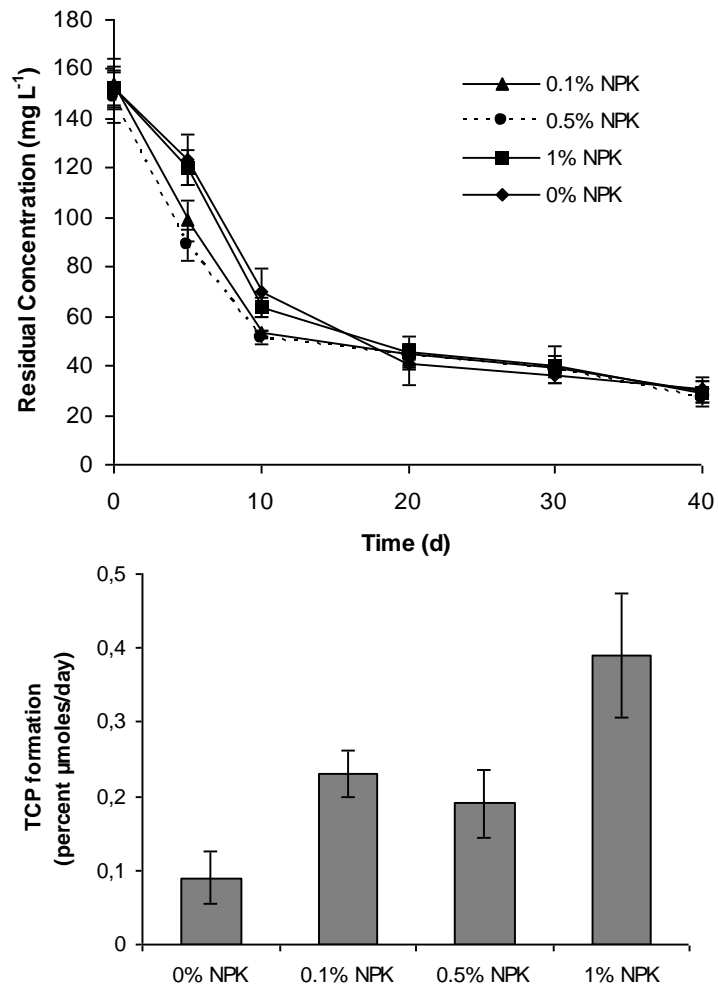
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Biochar as a Partial Replacement of Peat in Pesticide-Degrading Biomixtures Formulated with Different Soil Types

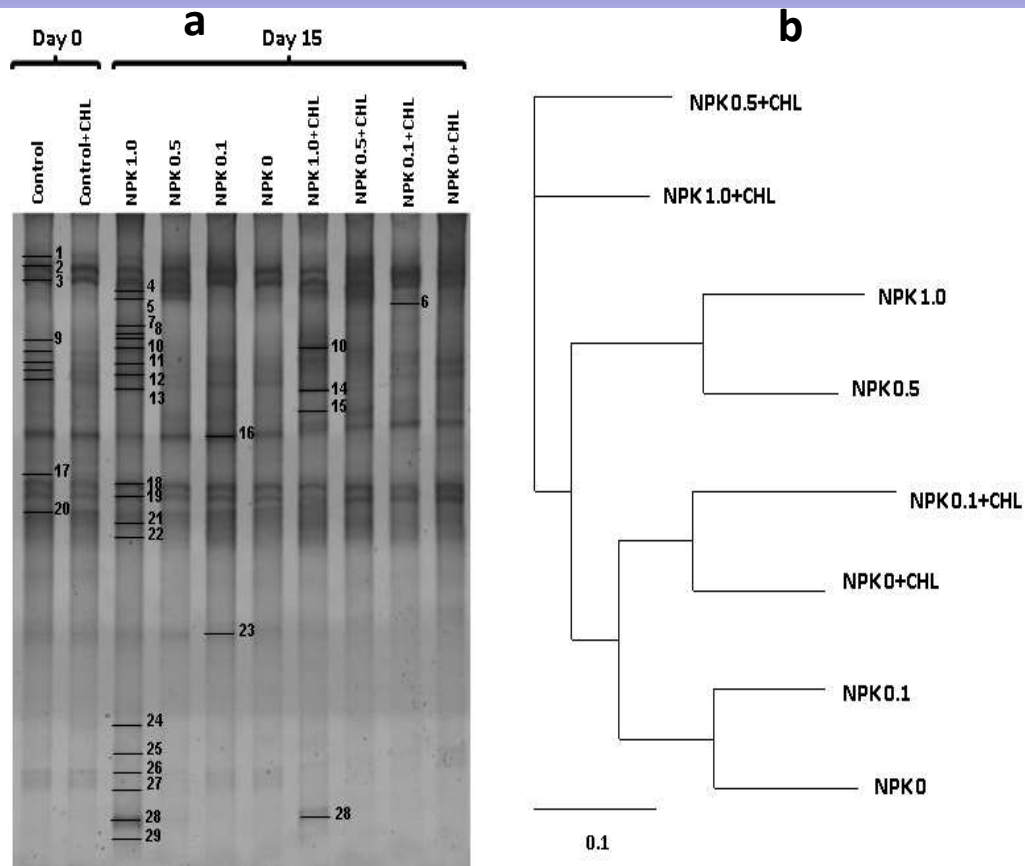
M. Cristina Diez^{1,3,*}, Marcela Levio³, Gabriela Briceño^{2,3}, Olga Rubilar^{1,3}, Gonzalo Tortella^{1,3}, and Felipe Gallardo^{2,3}



NPK Addition - Microbial Diversity Evaluation



Chlorpyrifos degradation and TCP accumulation in a biobed biostimulated with NPK fertilizer after 40 days of incubation. (Initial chlorpyrifos conc. 160 mg kg⁻¹)



DGGE banding pattern (a) and clustering representation (b) of the bacterial 16S rRNA genes amplified with the primers sets EUBf933- GC/ EUBr1387

NPK of 0.5 and 1.0%, modified the bacterial community and affected TCP degradation (Tortella et al., 2010)

Contents lists available at SciVerse ScienceDirect

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journal homepage: www.elsevier.com/locate/chemosphere



Technical Note

Chlorpyrifos degradation in a biomixture of biobed at different maturity stages

G.R. Tortella^{a,*}, O. Rubilar^a, M.d.P. Castillo^b, M. Cea^a, R. Mella-Herrera^a, M.C. Diez^a

J. Soil Sci. Plant Nutr. 10 (4): 464 - 475 (2010)

BIOSTIMULATION OF AGRICULTURAL BIOBEDS WITH NPK FERTILIZER ON CHLORPYRIFOS DEGRADATION TO AVOID SOIL AND WATER CONTAMINATION

G. Tortella^{1,*}, O. Rubilar¹, M. Cea¹, C. Wulff¹, O. Martínez¹, and M.C. Diez^{1,2}

Contents lists available at SciVerse ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod



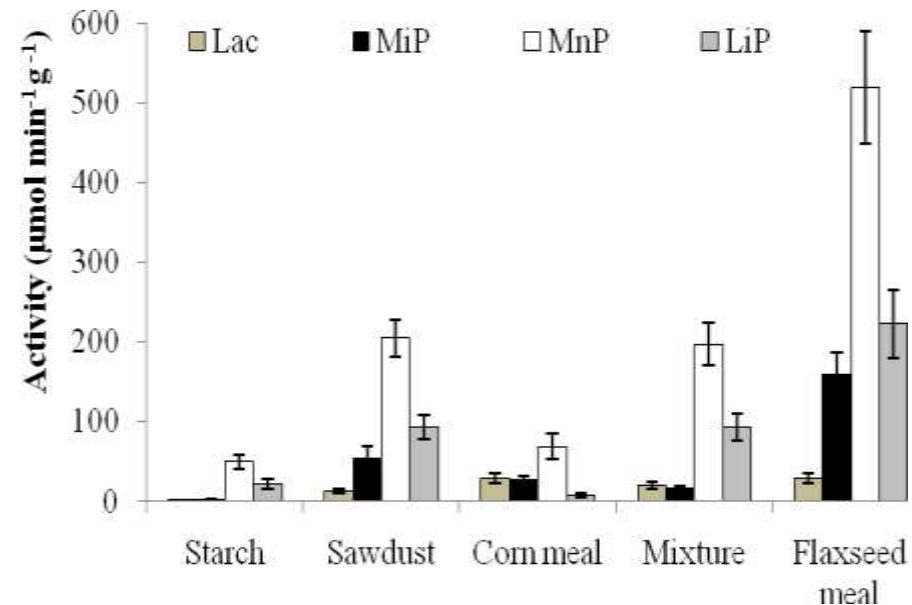
Natural wastes rich in terpenes and their relevance in the matrix of an on-farm biopurification system for the biodegradation of atrazine

G.R. Tortella^{a,b,*}, O. Rubilar^{a,b}, M. Cea^{a,b}, G. Briceño^{a,b}, A. Quiroz^{b,c}, M.C. Diez^{a,b}, L. Parra^{b,c}

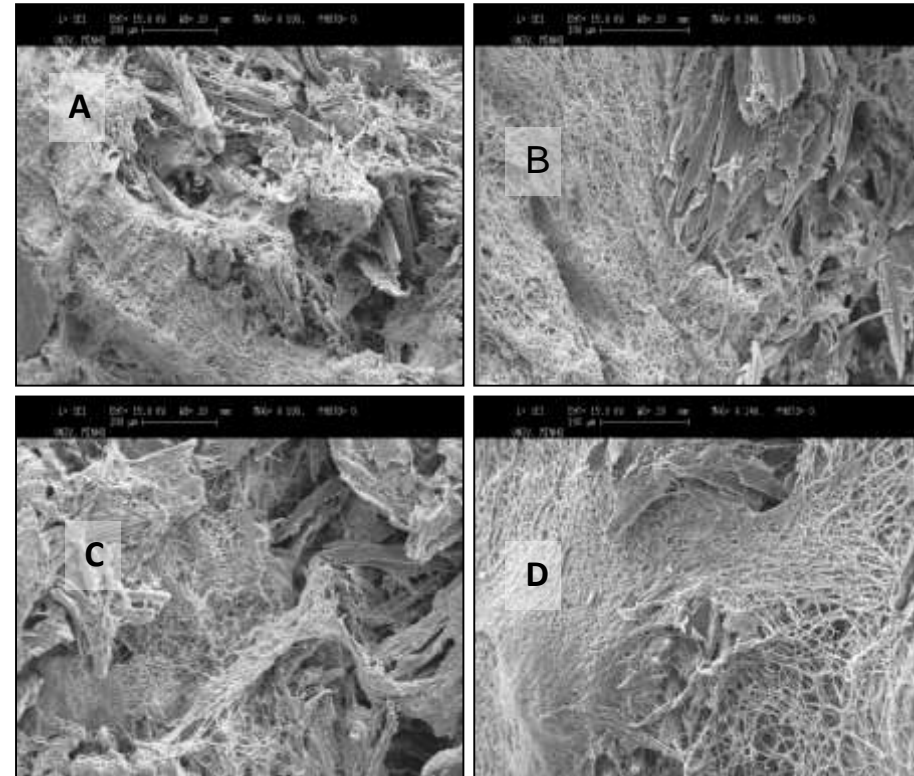
Pellet Formulation for WRF Immobilization



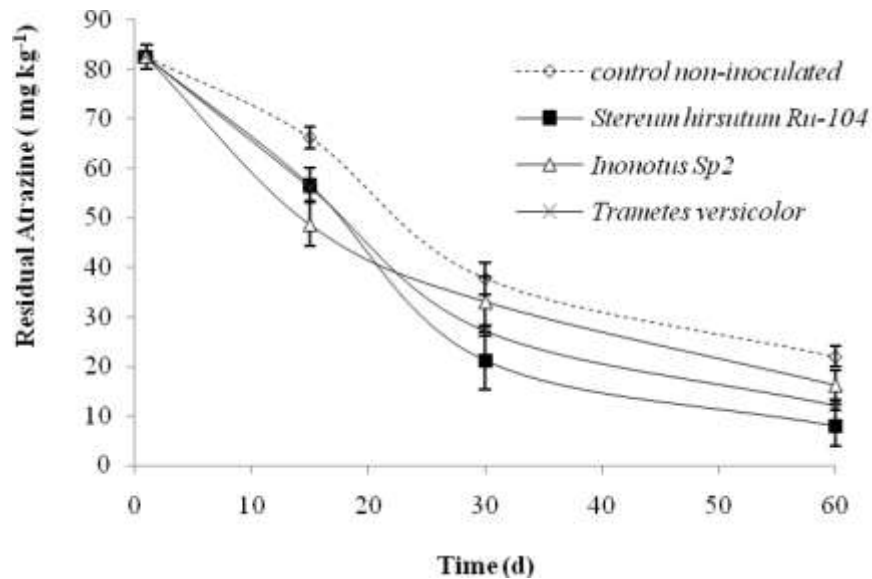
Patent 01395-2013, 16/05/2013.



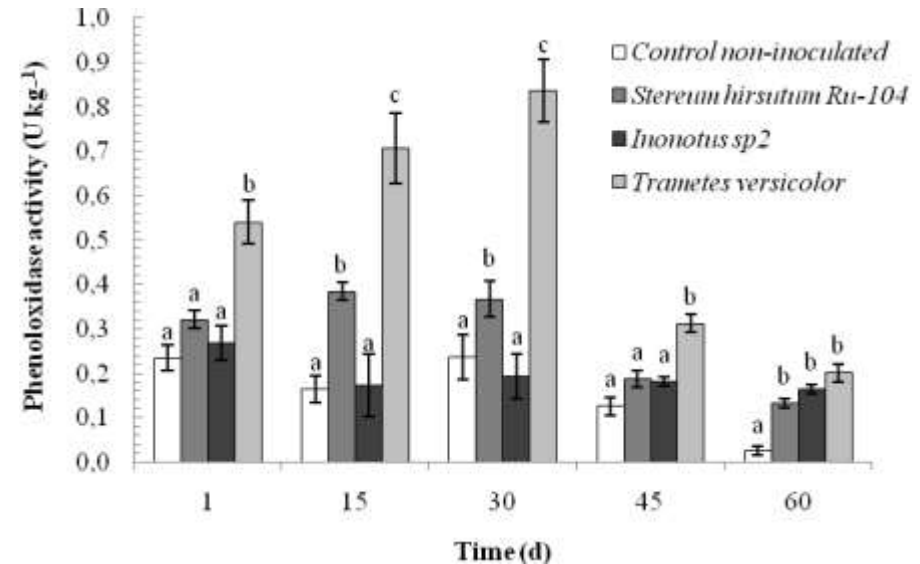
Cumulated ligninolytic activities of *A. discolor* on different materials



Scanning electron microscopy of *A. discolor* overgrown in the formulation F1, 30 days of storage. Core of F1-CPS at 4 °C (A), surface F1-CPS at 25 °C (B), core F1-UPS at 4 °C (C), surface F1-UPS at 25 °C (D).



Residual atrazine (mg kg⁻¹) after 60 days at 20 °C, in a biomixture inoculated with pelletized support of white-rot fungi.



Phenoloxidase activity (U kg⁻¹) after 60 days at 20 °C, in a biomixture inoculated with pelletized support of white-rot fungi.

ORIGINAL ARTICLE

Open Access



Immobilization of the white-rot fungus *Anthracophyllum discolor* to degrade the herbicide atrazine

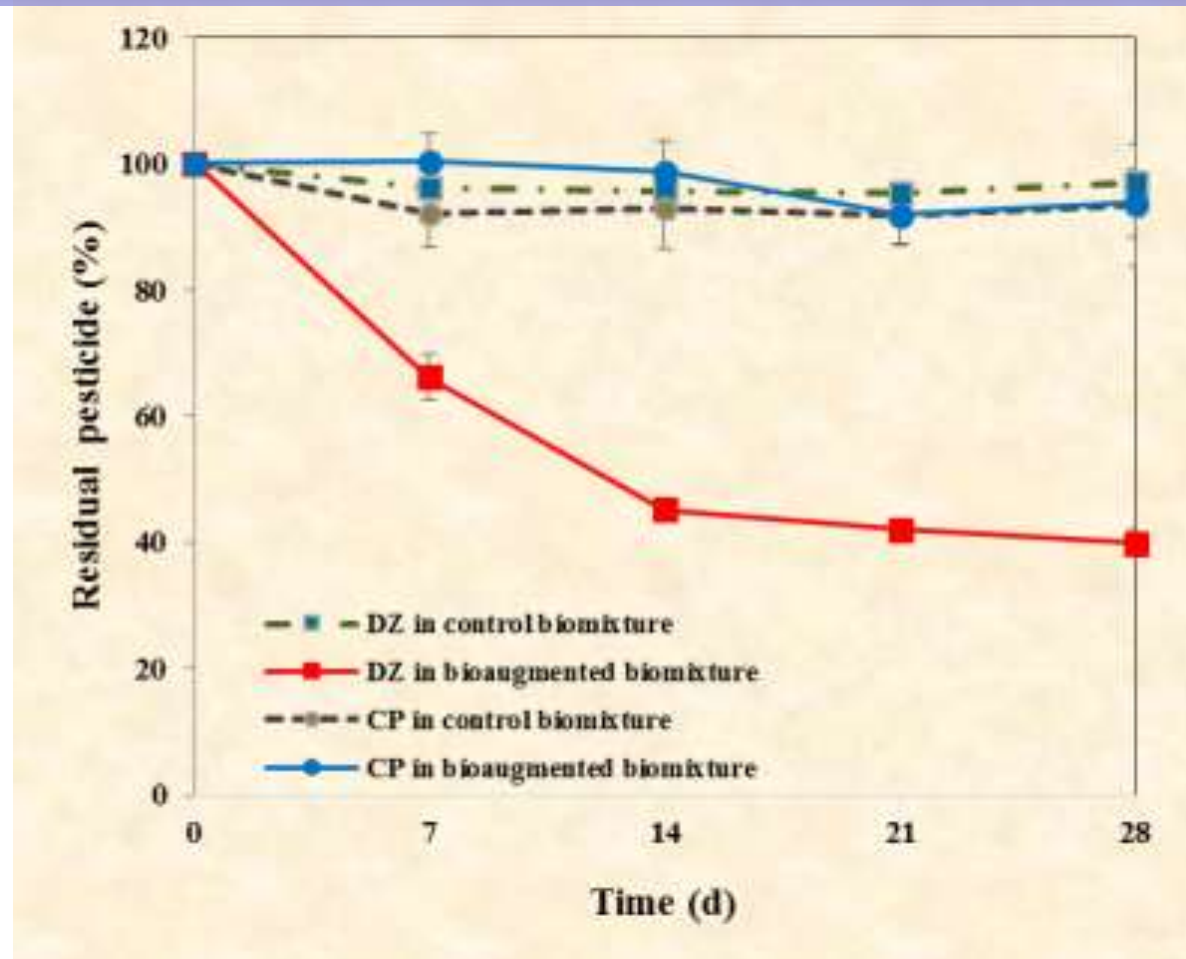
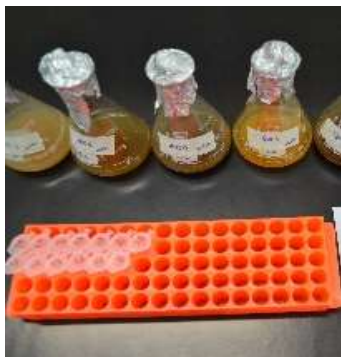
S. Elgueta^{1,2*}, C. Santos², N. Lima³ and M. C. Diez⁴

JOURNAL OF ENVIRONMENTAL SCIENCE AND HEALTH, PART B
2017, VOL. 0, NO. 0, 1–7
<https://doi.org/10.1080/03601234.2017.1330070>



Atrazine, chlorpyrifos, and iprodione effect on the biodiversity of bacteria, actinomycetes, and fungi in a pilot biopurification system with a green cover

Sebastian Elgueta^{a,b}, Arturo Correa^b, Marco Campo^a, Felipe Gallardo^a, Dimitrios Karpouzias^d, and Maria Cristina Diez^{a,c}



Diazinon and Chlorpyrifos removal from sterilized (control) and inoculated biomixture by the *Streptomyces* mixed culture during 4 weeks.

Organophosphorus pesticide mixture removal from environmental matrices by a soil *Streptomyces* mixed culture

Gabriela Briceño^{1,2} · Karen Vergara¹ · Heidi Schalchli^{1,3} · Graciela Palma⁴ · Gonzalo Tortella^{1,2} · María Soledad Fuentes⁵ · María Cristina Diez^{1,3}

International Biodeterioration & Biodegradation 73 (2012) 1–7



ELSEVIER

Contents lists available at SciVerse ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod



Chlorpyrifos biodegradation and 3,5,6-trichloro-2-pyridinol production by actinobacteria isolated from soil

G. Briceño^{a,b,c,*}, M.S. Fuentes^d, G. Palma^{b,c}, M.A. Jorquera^{b,c}, M.J. Amoroso^{d,e}, M.C. Diez^{a,c}

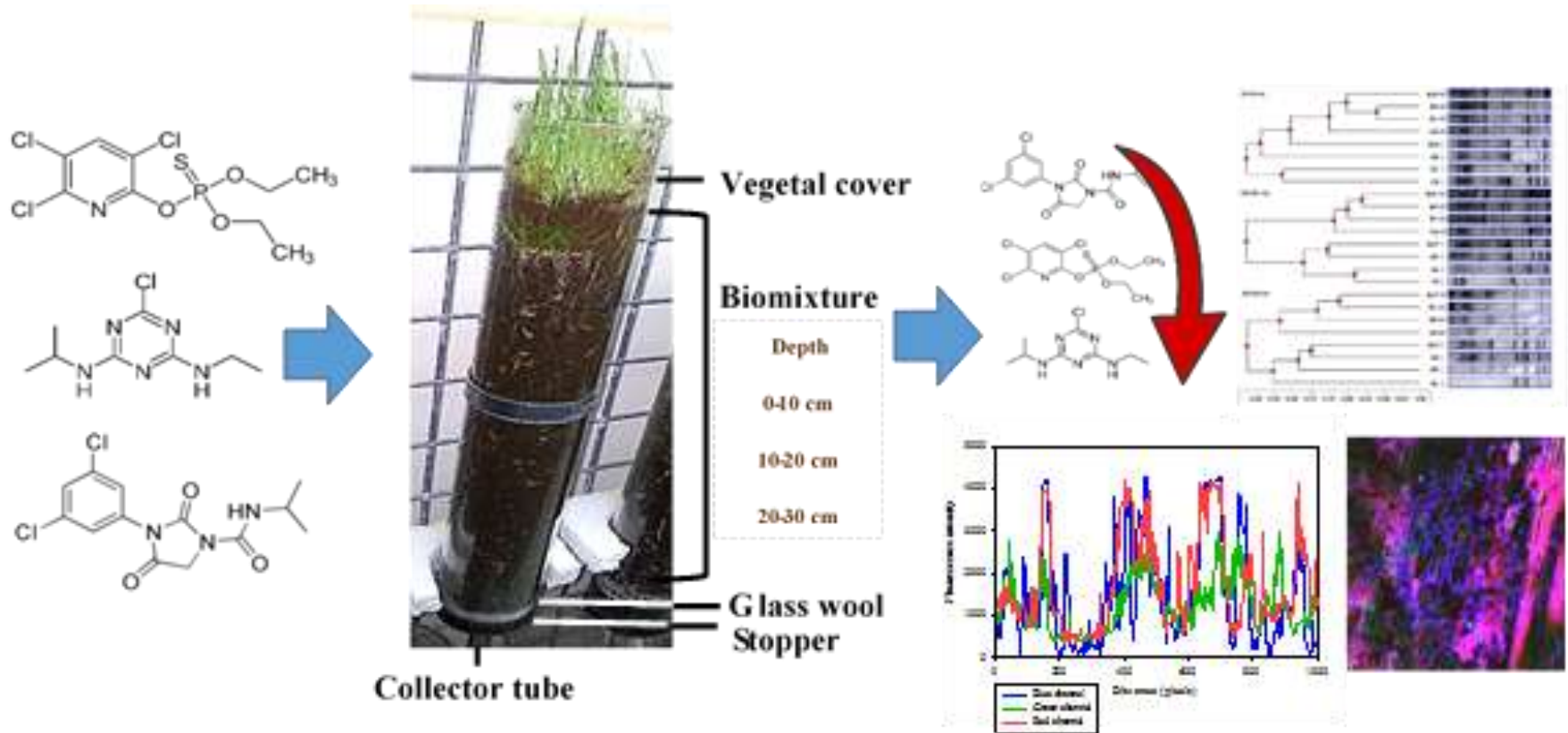
^a Department of Chemical Engineering, Universidad de La Frontera, P.O. Box 54-D, Temuco, Chile

^b Department of Chemical Science, Universidad de La Frontera, Temuco, Chile

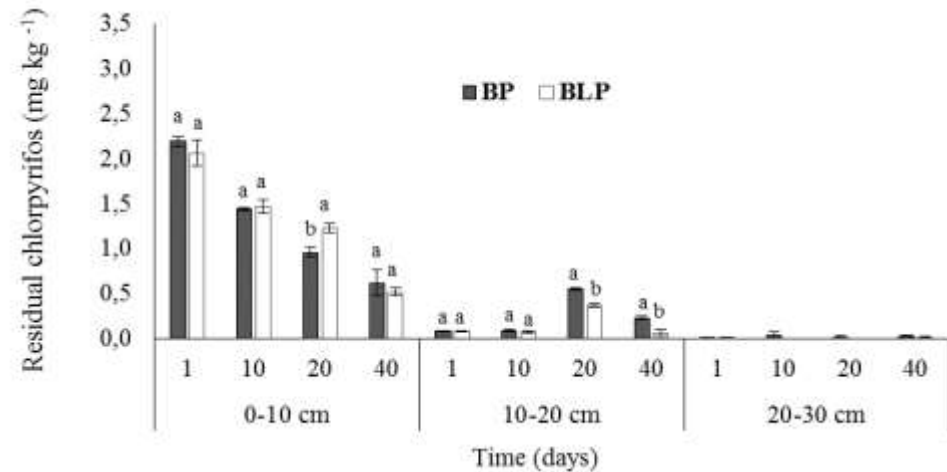
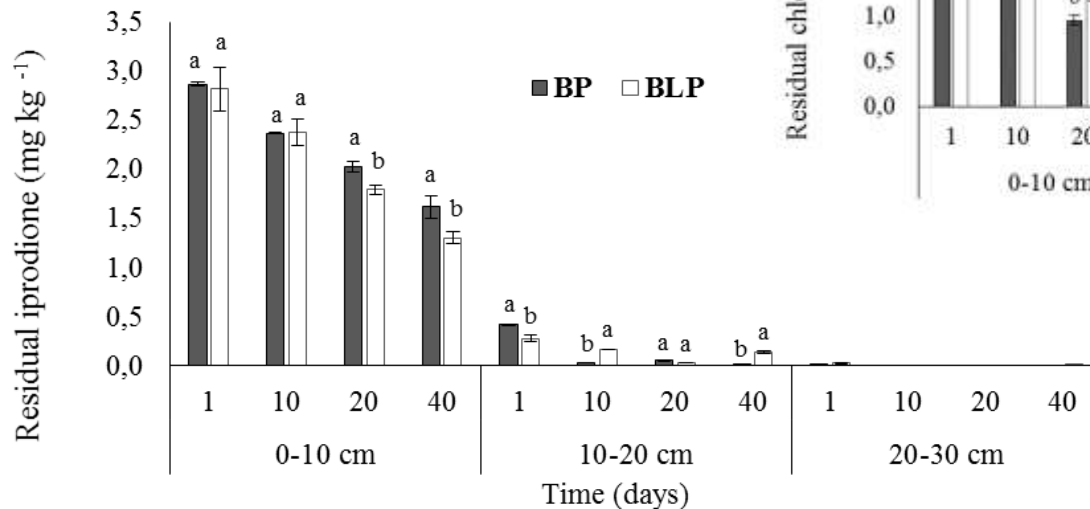
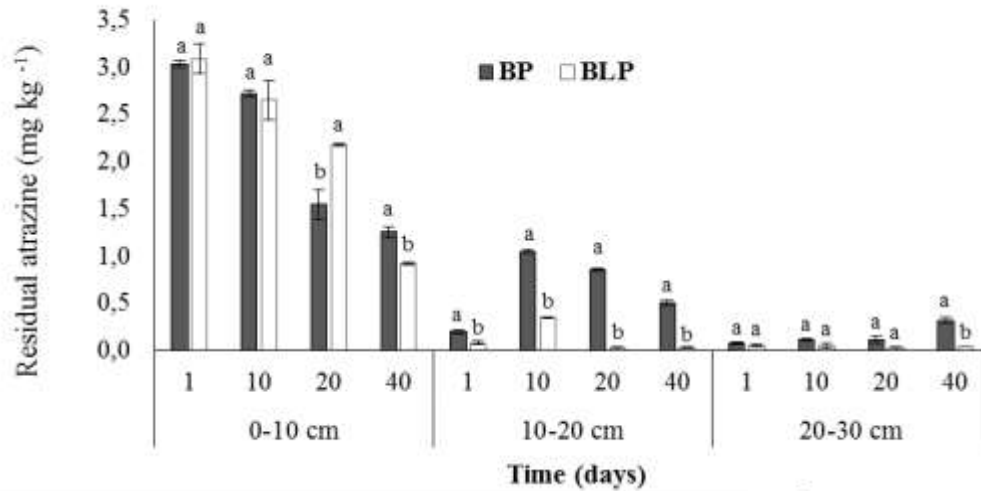
^c Scientific and Technological Biosource Nucleus, Universidad de La Frontera, Temuco, Chile

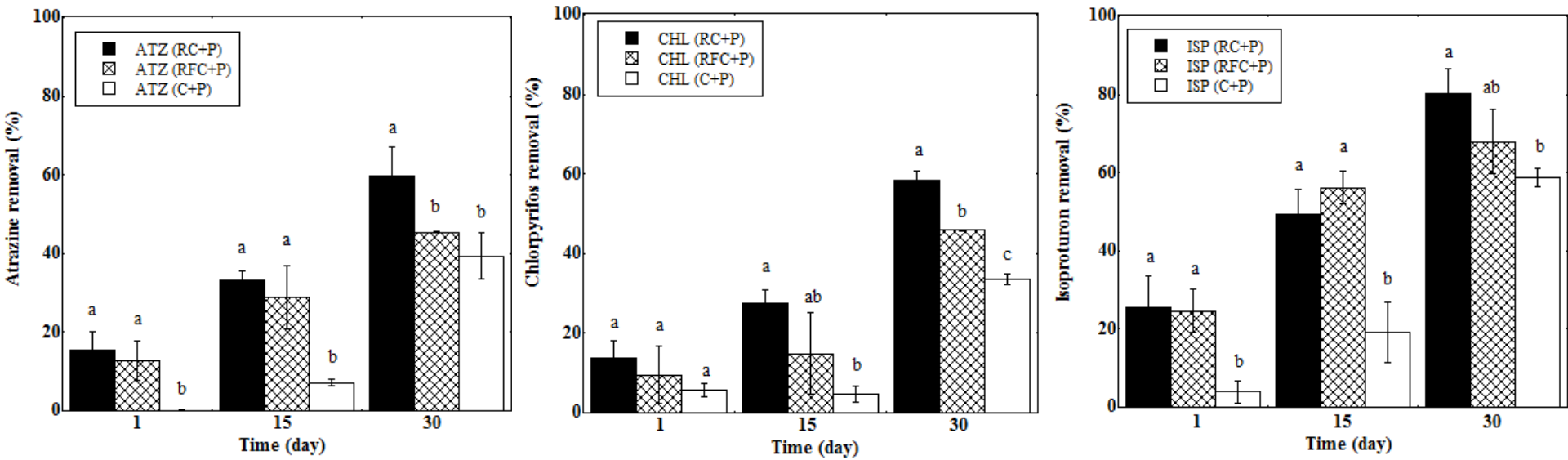
^d Planta Piloto de Procesos Industriales Microbiológicos (Ppimi-Center), Avenida Belgrano y Puaje Caseros, 4000 Tucumán, Argentina

^e Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, 4000 Tucumán, Argentina



Columns Assays Single application





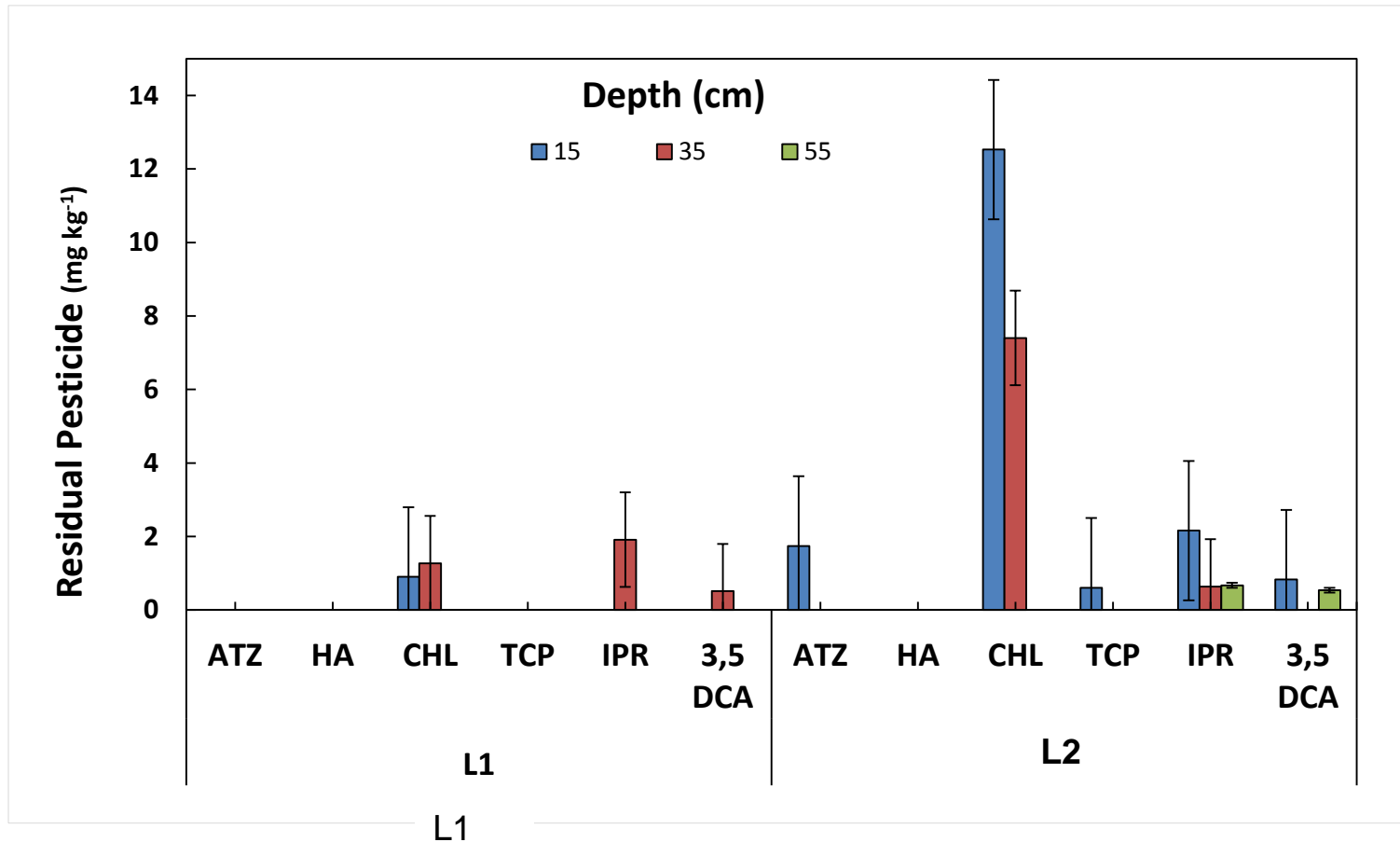
Pesticides removal ATZ, CHL and ISP (5 mg kg⁻¹) in the biomixture with roots (RC+P), without roots (RFC+P) and control(C+P).

Biomixture= soil, peat and oat husk (1:1:2)

Effect of the Rhizosphere on Pesticides Degradation



Units of 1m³ with and without vegetal cover + control without pesticides



Pesticides and metabolites concentrations in the biomix after 60 days at three depth of biobed. With pesticides and vegetal cover (L1), With pesticides without vegetal cover. (L2) Single application . Pond Assay



Contents lists available at [ScienceDirect](#)

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman



Research article

Bioaugmentation and rhizosphere-assisted biodegradation as strategies for optimization of the dissipation capacity of biobeds



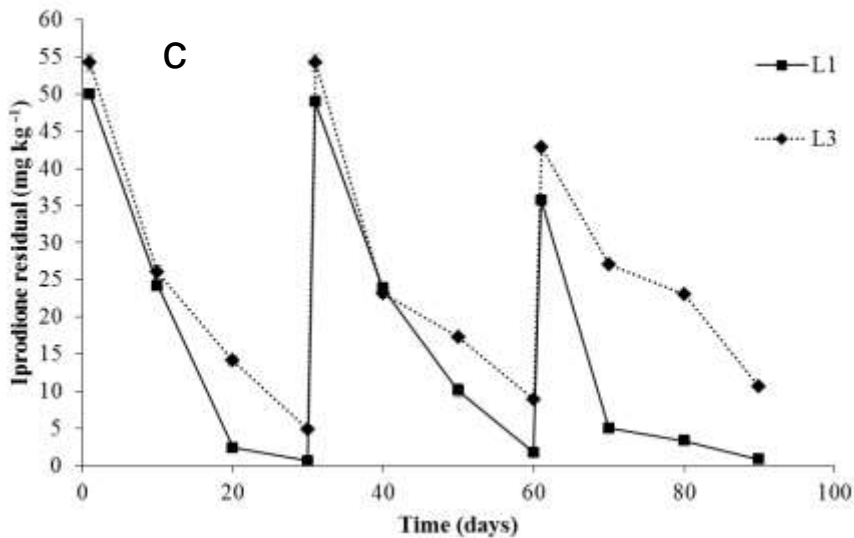
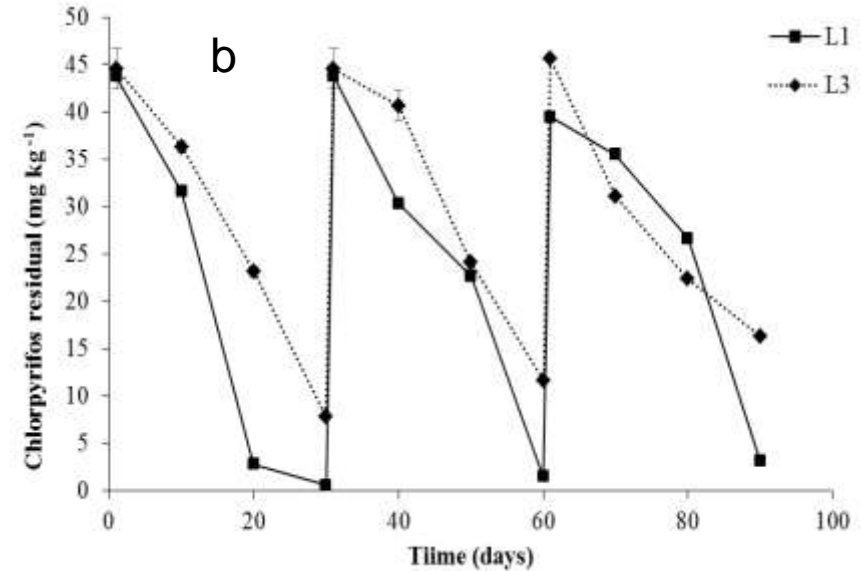
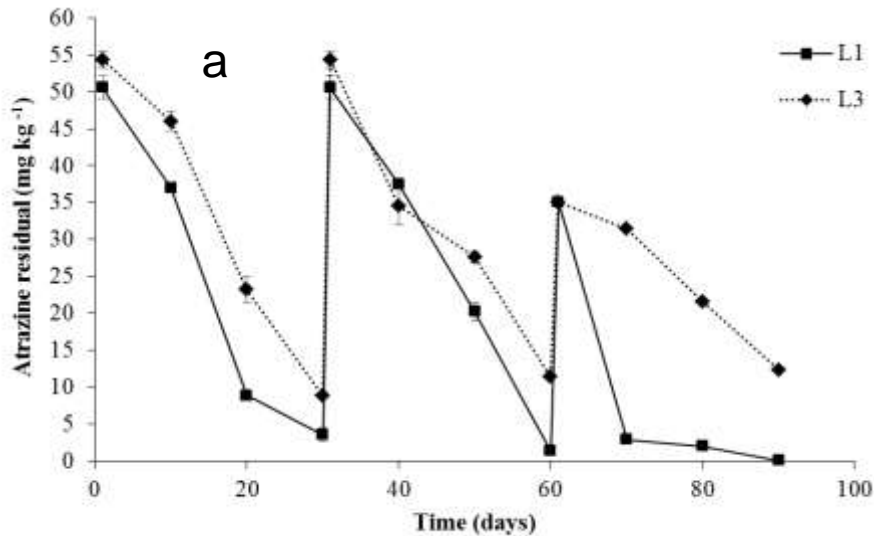
M. Campos ^a, C. Perruchon ^b, P.A. Karas ^b, D. Karavasilis ^b, M.C. Diez ^c, D.G. Karpouzas ^{b,*}

Journal of Soil Science and Plant Nutrition, 2015, 15 (2), 410-421

RESEARCH ARTICLE

Rhizosphere effect on pesticide degradation in biobeds under different hydraulic loads

M.C. Diez^{1,3*}, H. Schalchli^{1,3}, S. Elgueta³, E. Salgado³, N. Millahueque³, O. Rubilar^{1,3}, G.R. Tortella³, G. Briceño³



Concentración residual de Atrazina (a), Clorpirifos (b) e Iprodiona (c) en la biomezcla (15 cm) con 3 replicaciones L1 (con plaguicida con ballica), L3 (con plaguicida sin ballica).

Plaguicida	Periodo (días)	Disipación (%) Sin Cubierta Vegetal	Disipación (%) Con Cubierta Vegetal
ATZ	1-30	83,7	92,8
	31-60	79,0	97,2
	61-90	64,8	99,6
CHL	1-30	82,4	98,7
	31-60	73,8	96,6
	61-90	64,2	91,9
IPR	1-30	91,2	98,8
	31-60	83,7	96,5
	61-90	75,1	97,8

Disipación (%) de los plaguicidas. Promedio de los tratamientos con y sin cubierta vegetal.

Plaguicida	Periodo (d)	t 1/2 (d)	t 1/2 (d)
		Sin Cub Vegetal	Con Cub. Vegetal
ATZ	1-30	10,9	7,2
	31-60	13,7	5,9
	61-90	19	4
CHL	1-30	11,8	4,3
	31-60	14,7	6,4
	61-90	19,8	8,5
IPR	1-30	8,5	4,3
	31-60	11,7	6,2
	61-90	15,5	5,7
Promedio		14,0	5,83

Tiempo de vida media (t 1/2) por plaguicida . Promedio de los tratamientos con y sin cubierta vegetal

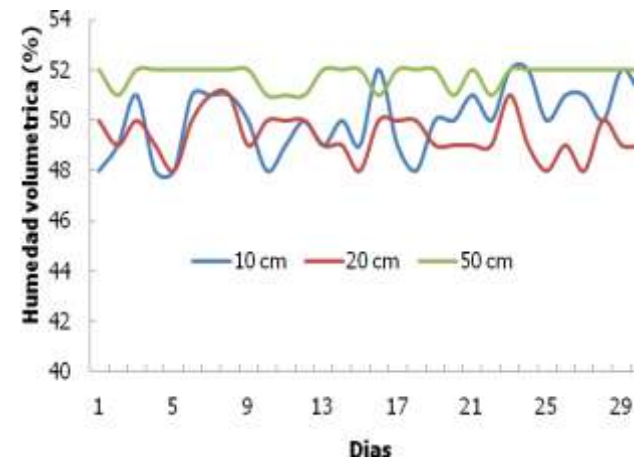
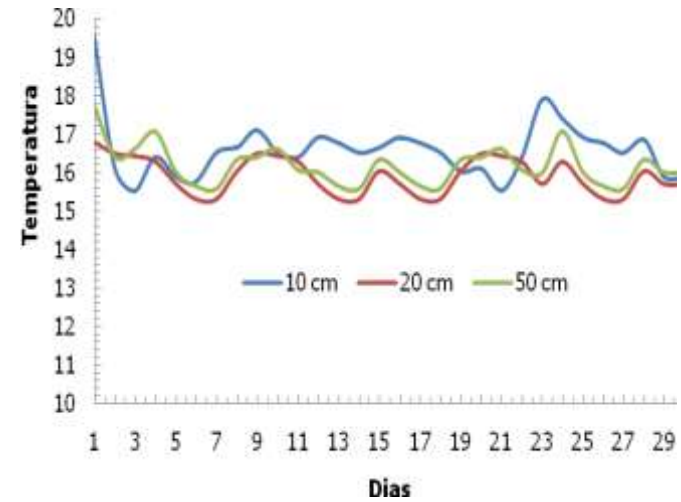
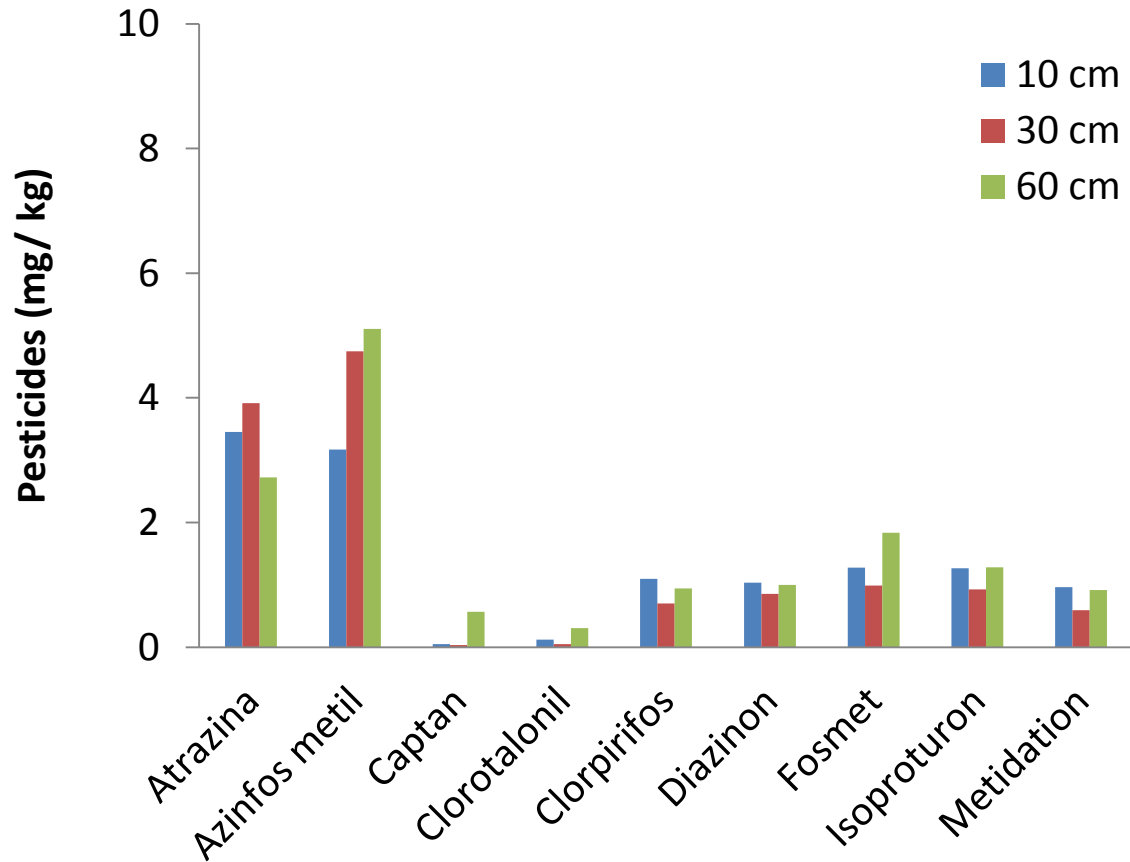
PESTICIDES EVALUATED



Commercial name	Active ingredient	Type	Concentration a.i.
Pyrinex 48 EC	Clorpirifos	Insecticida	480
Captan 80 WP	Captan	Funguicida	800
Clorotalonil 72 SC	Clorotalonil	Funguicida	720
Imidan 70 WP	Fosmet	Insecticida	700
Supracid 40 WP	Metidation	Insecticida	400
Guzation 35 WP	Azinfosmetil	Insecticida	350
Atrazina 500 SC	Atrazina	Herbicida	500
Diazinon	Diazinon	Insecticida	400
Fuego	Isoproturon	Herbicida	500

*P. C= Producto Comercial

DEGRADATION OF PESTICIDES IN BIOBEDS-FARM SCALE



Residual pesticides in the biobed after 120 days of application (inicial concentration 45 mg i.a./kg each)

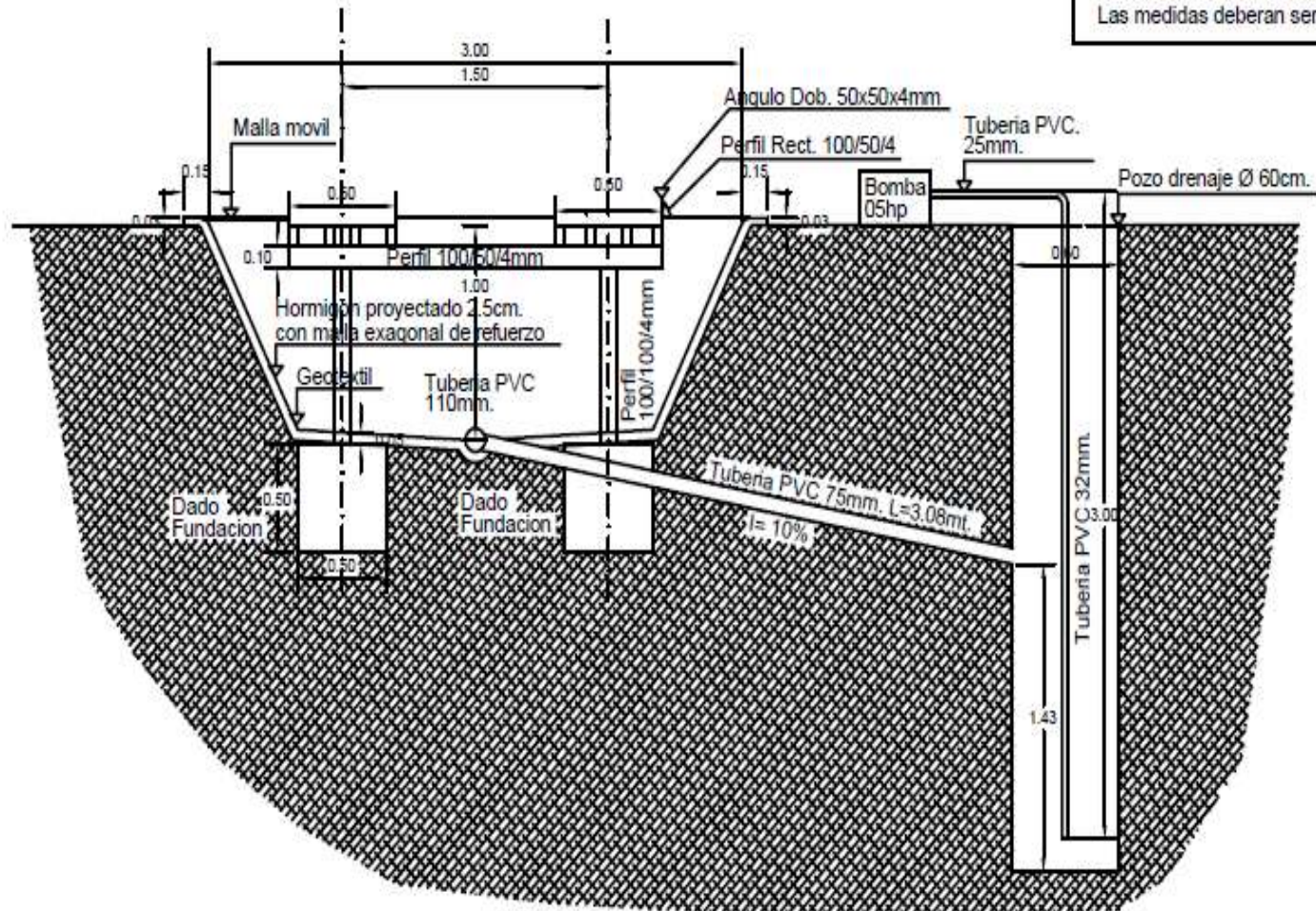
BIOBED CONSTRUCTION



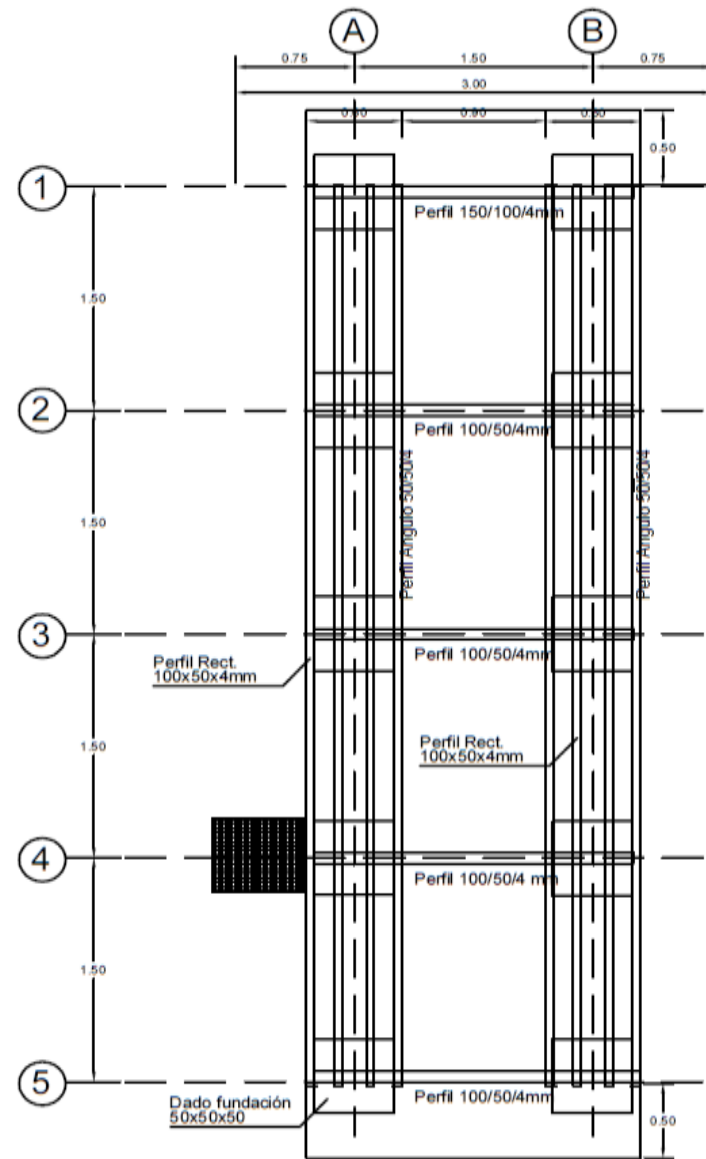
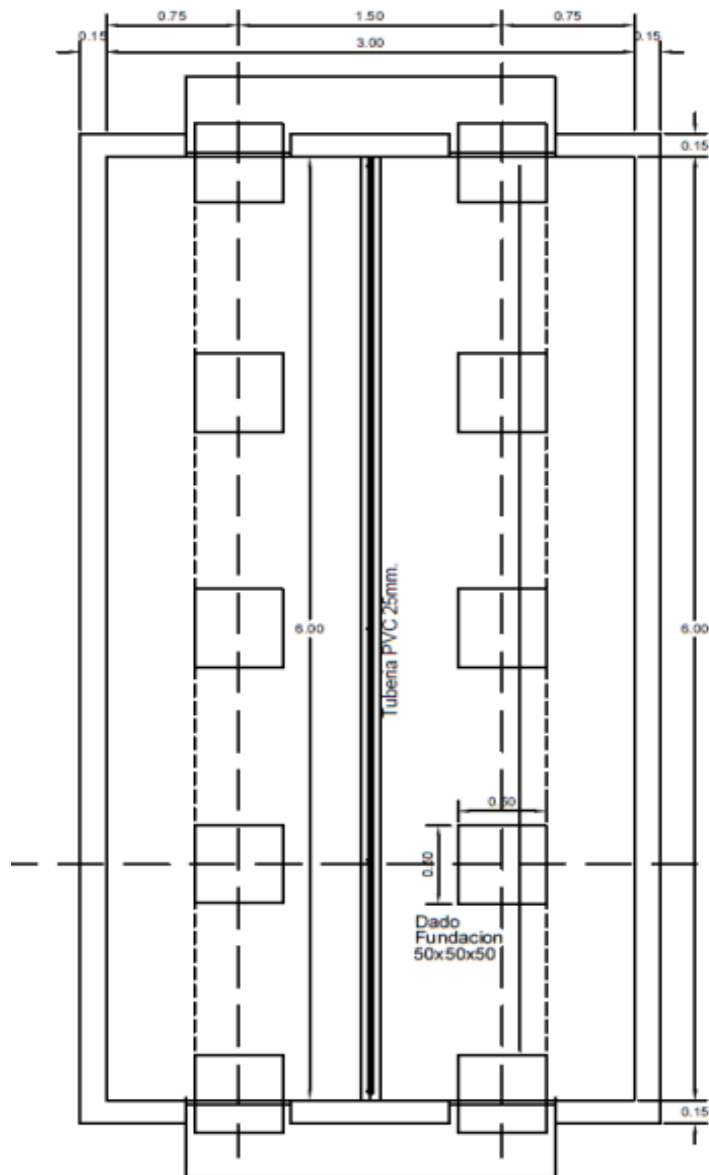
NOTA:

Los materiales a utilizar para la estructura metalica deben ser resistente segun calculo para 5.000 Kg. (Tractor)

Las medidas deberan ser verificadas en obras



BIOBED DESIGN





Excavación



Instalación de fundaciones



Instalación de malla



Revestimiento con hormigón



Instalación de travesaños



Instalación de rampa



Instalación de geotextil



Bomba y sistema de recirculación

CONSTRUCTION



Incorporación de arena y gravilla



Incorporación de biomezcla



Capa de suelo



Instalación de césped



Lecho biológico



Lecho biológico con techumbre

DIFUSION





MANUAL DE CONSTRUCCIÓN Y OPERACIÓN DE LECHOS BIOLÓGICOS

PROYECTO D09R1006

Manejo Adecuado de Residuos de Plaguicidas en la Producción Frutícola de la Región de La Araucanía a través de la Implementación y Difusión de Lechos Biológicos



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III Workshop Latino Americano sobre Biobed

ADVANCES IN BIOPURIFICATION SYSTEM FOR PESTICIDE DEGRADATION - CHILE

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