



Remediation of a hydrocarbon chronically contaminated soil by combination of persulfate oxidation and bioremediation.

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Polycyclic aromatic hydrocarbons (PAHs) are pollutants of major concern because of their significant toxicity to humans, ecotoxicity, carcinogenicity and mutagenicity

Their principal occurrence in the environment is associated with anthropogenic activities, such as petroleum and gas manufacturing, and coke production

PAHs are strongly hydrophobic and poorly water soluble, resulting in long-term sequestration in various organic domains of the soil matrix

La Plata-Ensenada
petrochemical polo



In general, bioremediation has demonstrated success in elimination of PAHs from contaminated soil.

However, becomes inefficient in lowering long term soil hydrocarbon concentrations, leaving frequently more or less complex residues.

Therefore, a more energetic intervention would be needed,

Chemical oxidation is a promising technology that could bypass the bioremediation limitations.

Persulfate (PS) is being used increasingly for in situ chemical oxidation.



Persulfate generates the highly reactive sulfate free radical ($\text{SO}_4\cdot^-$)

**Oxidation with persulfate followed
by bioremediation**

to evaluate the effect of remediation treatment combining chemical oxidation with ammonium persulfate (PS) followed by bioremediation on the **PAHs elimination** and the **ecological properties of the contaminated soil**

OBJETIVO



Soil from a landfarming unit done long time ago and still now, under natural attenuation ...

So	Properties
Texture	Loam
pH	8,8
EC	634 μ S/cm
OC	2.2%
N _{total}	0.20%
P _(available)	8,33 mg/kg
Fe _(available)	12,6 mg/kg
Fe _{total}	2,3%
PAHs	214 \pm 21 mg/kg
PAHs _(available)	1%
Aliphatic Hydrocarbons	2457 \pm 246 mg/kg

TREATMENTS IN SOIL MICROCOSMS

Microcosms	Treatment	
OxS	Oxidative treatment with amonium persulfate	Persulfate 33 g kg _{ds} ⁻¹ One week
BOxS	Bioremediation followed oxidation	Oxidized soil incubated to 25°C, 25% moisture content, One year
BS	Bioremediation	25°C, 25% moisture content, One year

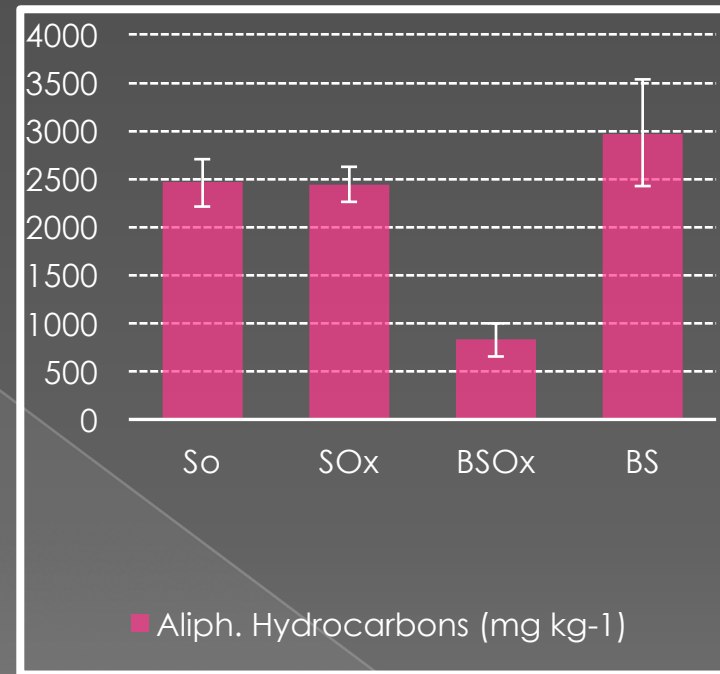
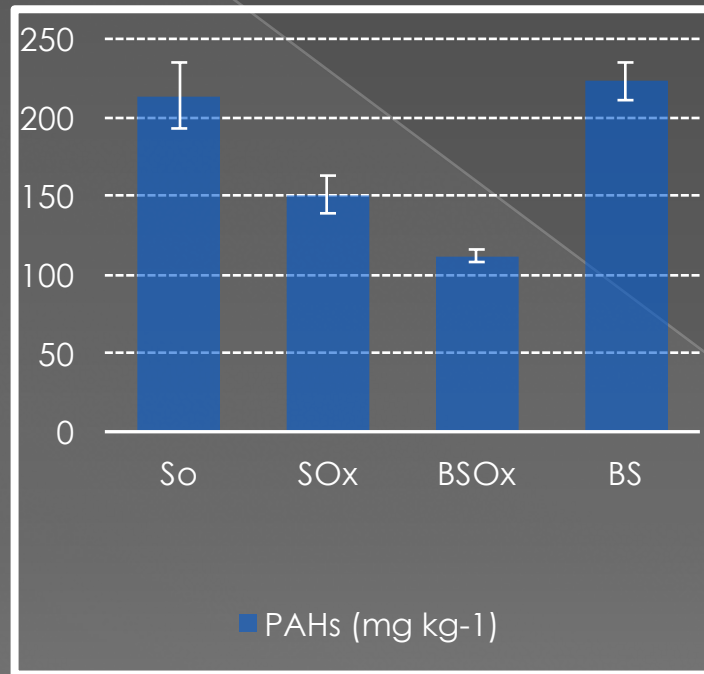


➤ Effect of the **persulfate oxidation** on the physicochemical soil properties ...

Treatments

Microcosms:	S ₀	OxS ₀	BOxS	BS (control)
pH	8.8 ± 0.1 (a)	7.1 ± 0.1 (b)	6.32 ± 0,02 (c)	7.6 ± 0,1 (b)
EC (μS cm ⁻¹)	634 ± 12 (b)	5110 ± 99 (a)	5880 ± 396 (a)	549 ± 42 (c)
OC (%)	2.2 ± 0.8 (a)	2.4 ± 0.1 (a)	2.6 ± 0.1 (a)	2.0 ± 0.9 (a)
N _{total} (%)	0.2 ± 0.1 (b)	0.519 ± 0.003 (a)	0.31 ± 0.04 (b)	0.2 ± 0.1 (b)
P _(available) (mg.kg ⁻¹)	8.3 ± 0.6 (c)	14.5 ± 0.7 (b)	22.0 ± 0.1 (a)	6.0 ± 0.3 (d)
Fe _(available) (mg.kg ⁻¹)	12.6 ± 0.3 (b)	54 ± 2 (a)	<i>nd</i>	<i>nd</i>
Sulphate (mg kg ⁻¹)	97 ± 6 (d)	8407 ± 16 (a)	4894 ± 15 (b)	<i>nd</i>

➤ Effect of the persulfate oxidation and coupled bioremediation treatment on the hydrocarbon elimination (GC-FID)



Microcosms	S ₀	OxS ₀	BOxS	BS
Available PAHs [%]	1 ± 1 (c)	19 ± 4 (b)	30 ± 2 (a)	1 ± 1 (c)

- Effect of the persulfate oxidation and coupled bioremediation treatment on the soil matrix

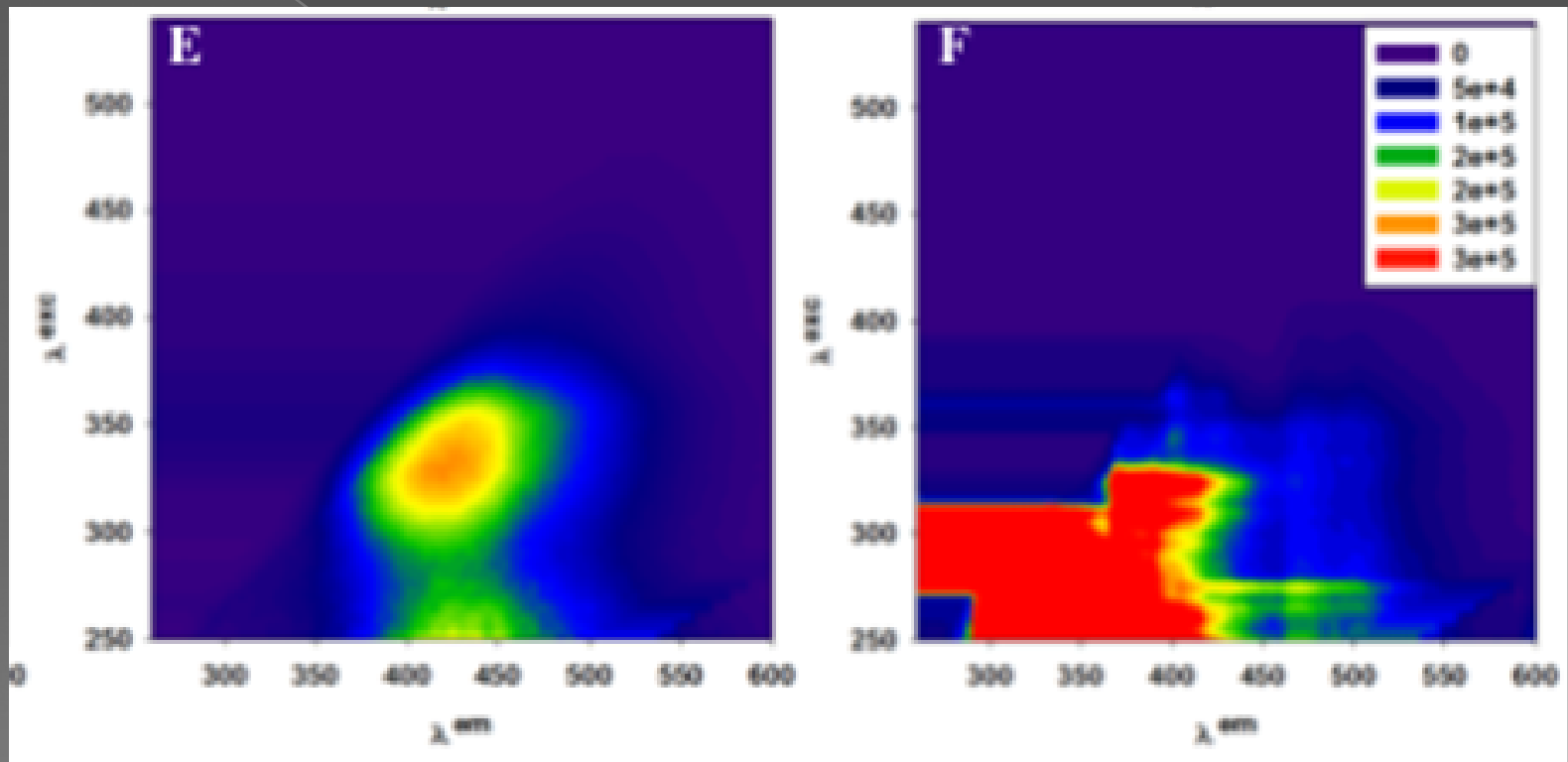
Spectrofluorometric analysis of dissolved organic matter, from the alkaline extract of the soil microcosms.

1- Dissolved total carbon (DTC) and E4/E6 ratio

Microcosms	Treatments			
	S ₀	OxS ₀	BOxS	BS
DTC [mg C L ⁻¹]	96 ± 3 (c)	226 ± 2 (a)	57 ± 2 (d)	46.5 ± 0.8 (e)
E ₄ /E ₆	15.0	17.7	7.1	3.3

Humification index: low value are associated with higher molecular size and condensation degree in the soil organic matter

Spectrofluorometric analysis of dissolved organic matter from the alkaline extract (controls)

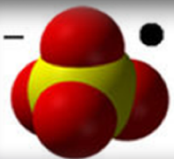


E. Control soil (S).

F. Aqueous solution of 16 PAHs.

➤ Effect of the persulfate oxidation and coupled bioremediation treatment on the soil matrix

2- Excitation-Emission Matrix Spectroscopy (EEMS)

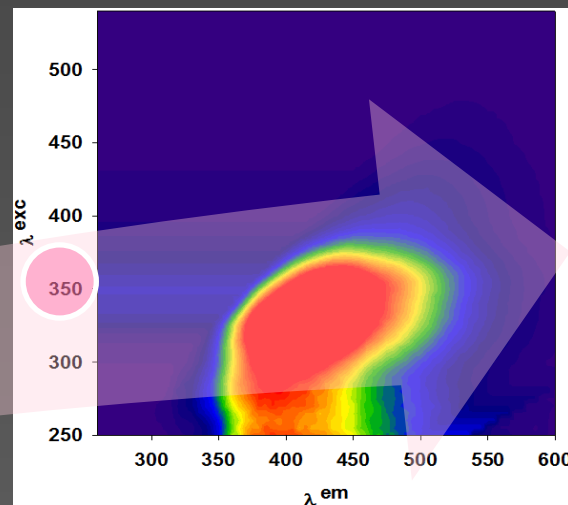
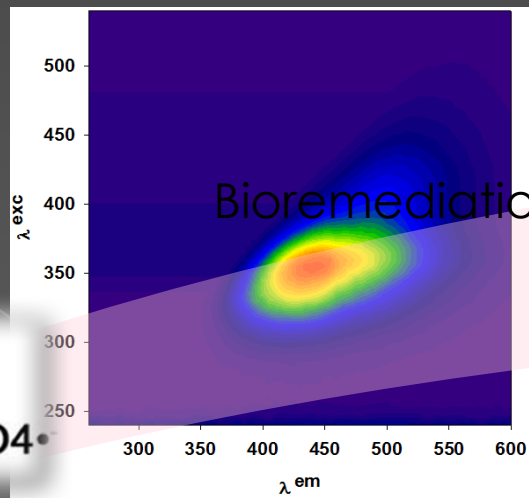


SO₄²⁻

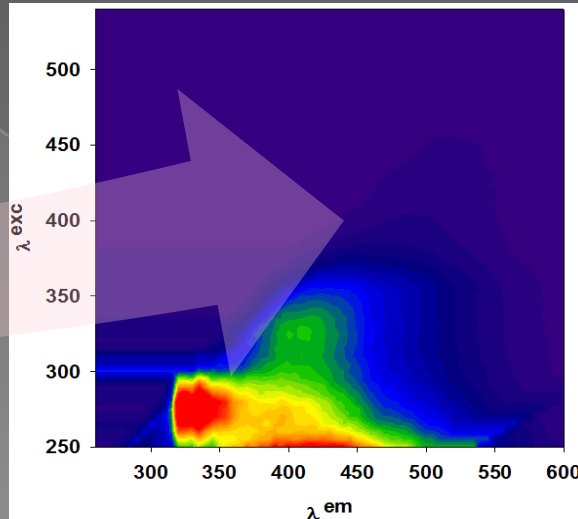
OxS₀

Bioremediation

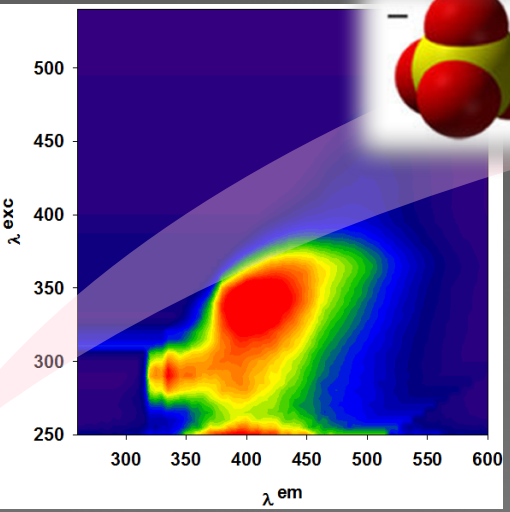
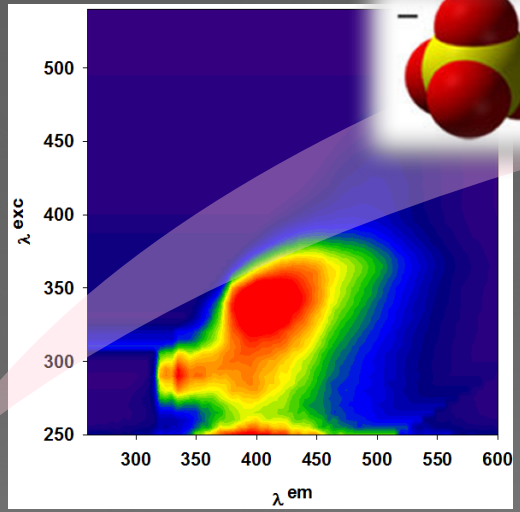
S₀



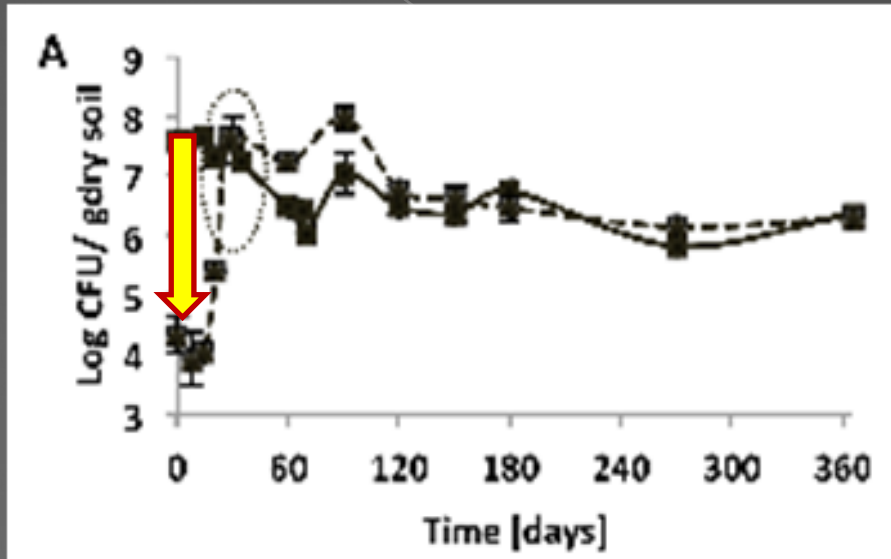
BOxS



BS

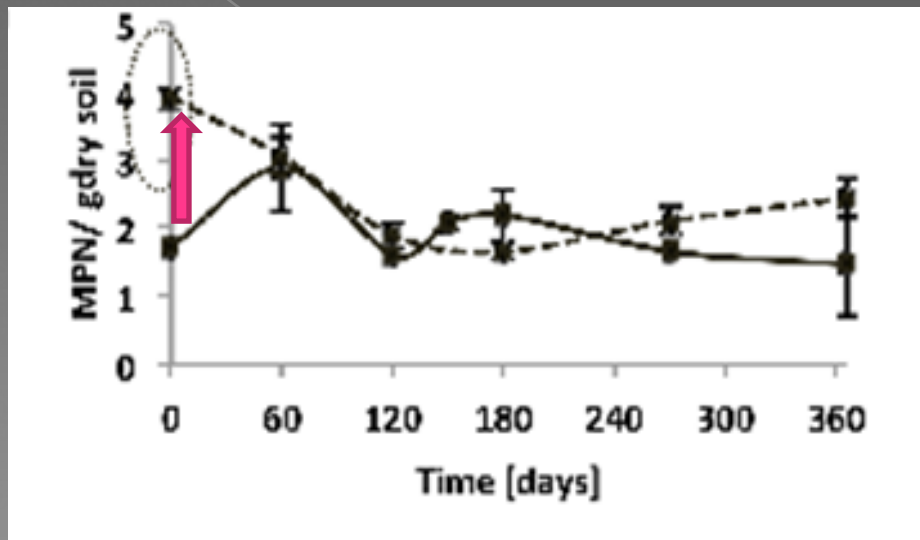


- Effect of the persulfate oxidation and coupled bioremediation treatment on **soil bacterial population density** and enzymatic activities



A. Heterotrophic Bacteria (HB) count.

- D. Most Probable Number of Aliphatic Hydrocarbon degrading bacteria (AHs-DB).



Enzymatic activities determined from each microcosms.

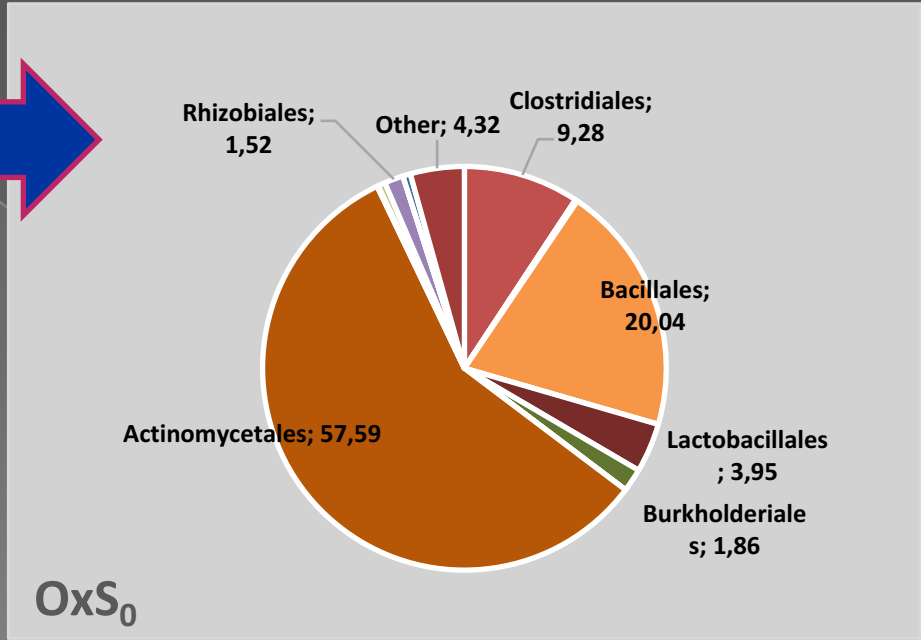
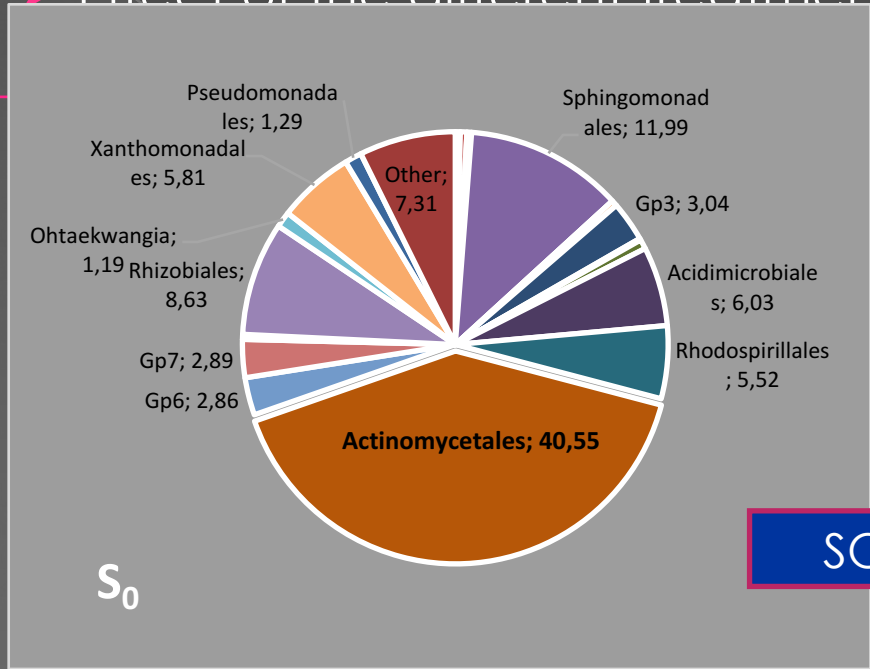
Microcosms	Treatments			
	S ₀	OxS ₀	BOxS	BS
Lipase [μg pNP g _{ds} ⁻¹]	189 ± 4 (b)	267 ± 6 (a)	114 ± 14 (c)	174 ± 3 (b)
Arylsulphatase [μg pNP g _{ds} ⁻¹]	33 ± 1 (a)	35 ± 1 (a)	nd	nd
Alkaline phosphatase [μg pNP g _{ds} ⁻¹]	175 ± 24 (b)	84 ± 3 (c)	157 ± 10 (b)	303 ± 15 (a)
Acid phosphatase [μg pNP g _{ds} ⁻¹]	103 ± 20 (a)	51 ± 6 (b)	nd	42 ± 29 (b)
Urease [μN-NH ₄ ⁺ g _{ds} ⁻¹]	23 ± 6 (b)	nd	204 ± 57 (a)	17 ± 2 (b)

➤ Effect of the persulfate oxidation and coupled bioremediation treatment on the **soil bacterial community**

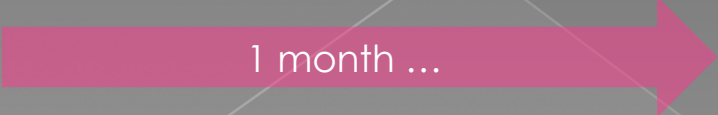
The taxonomic composition and the diversity of the bacterial communities present in OxS₀ and after 1, 5, 9 and 12 months of bioremediation were profiled using pyrosequencing. Samples from S₀ and BS soil microcosms were also analysed.

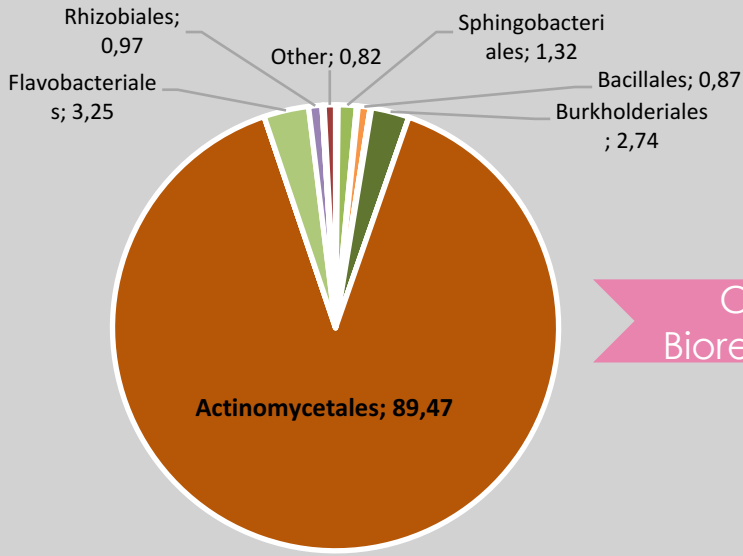
Microcosms	Total sequences	Observed OTUS	Good's Coverage [%]	Hill Numbers		
				⁰ D	¹ D	² D
S ₀	5984	215	84	449	61	44
OxS ₀	3404	92	97	112	28	10
BOxS (1m)	4878	44	97	65	6	3
BOxS (5m)	1094	57	97	71	8	4
BOxS (9m)	959	213	88	286	64	49
BOxS (12m)	767	194	91	234	60	40
BS (12m)	858	160	92	216	47	28

➤ Effect of the different treatments on the relative abundance of
 ...ions

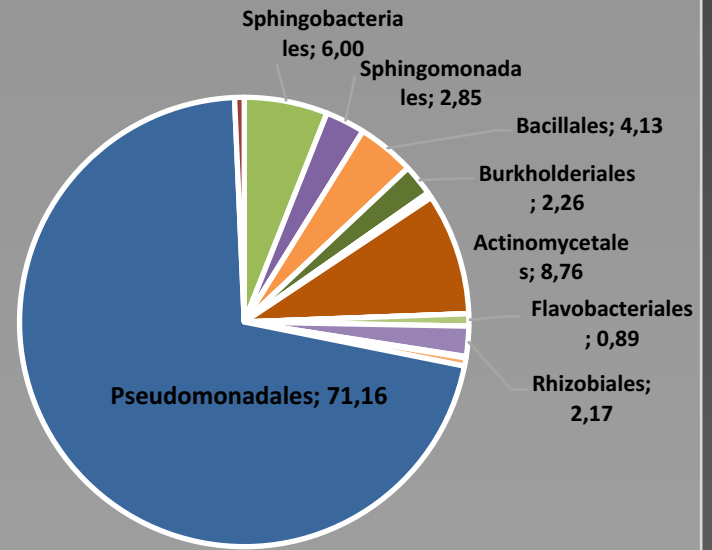


The succession changes was explained by the r-K scheme, which assumes that evolution favors either adaptation to high rates of reproduction (r strategists) or optimal utilization of environmental resources (K strategists) (Atlas & Bartha 1990).



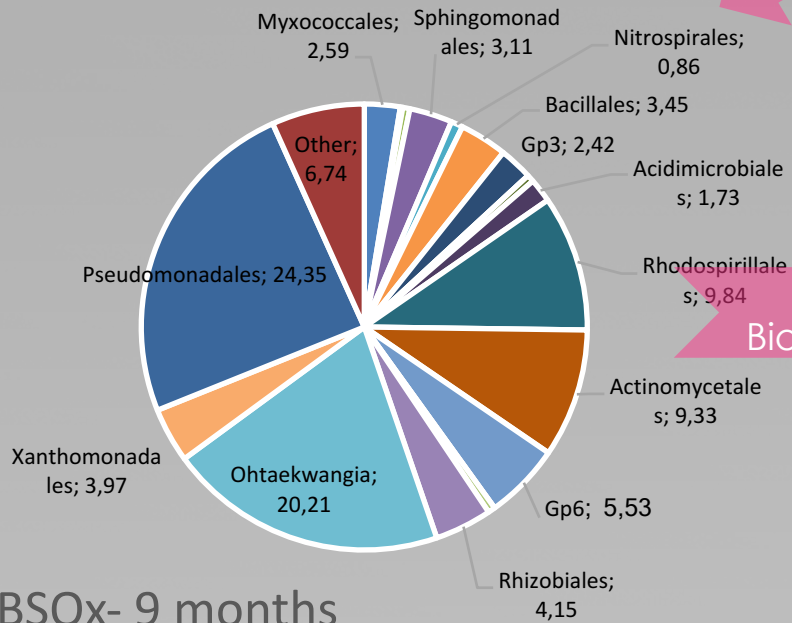


Coupled Bioremediation

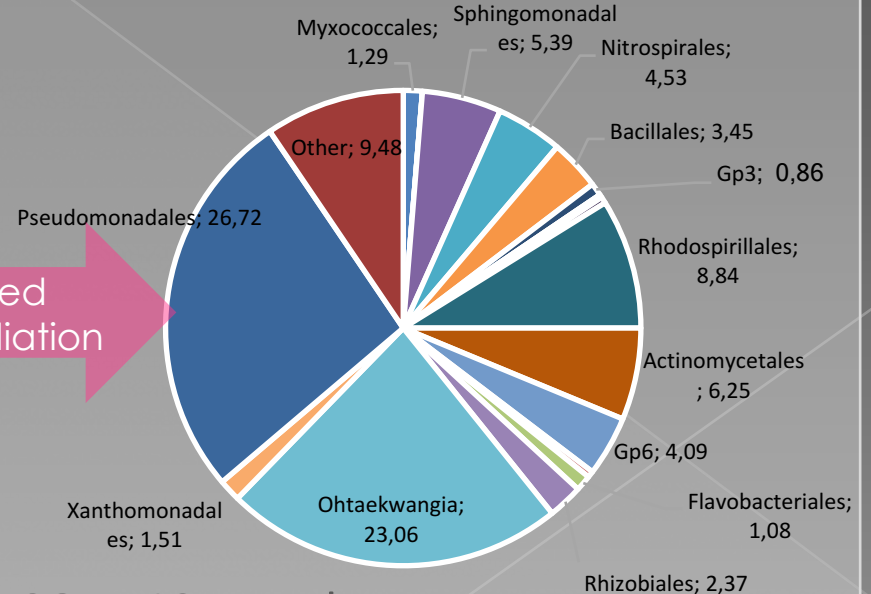


BOxS -1 month

BOxS- 5 months

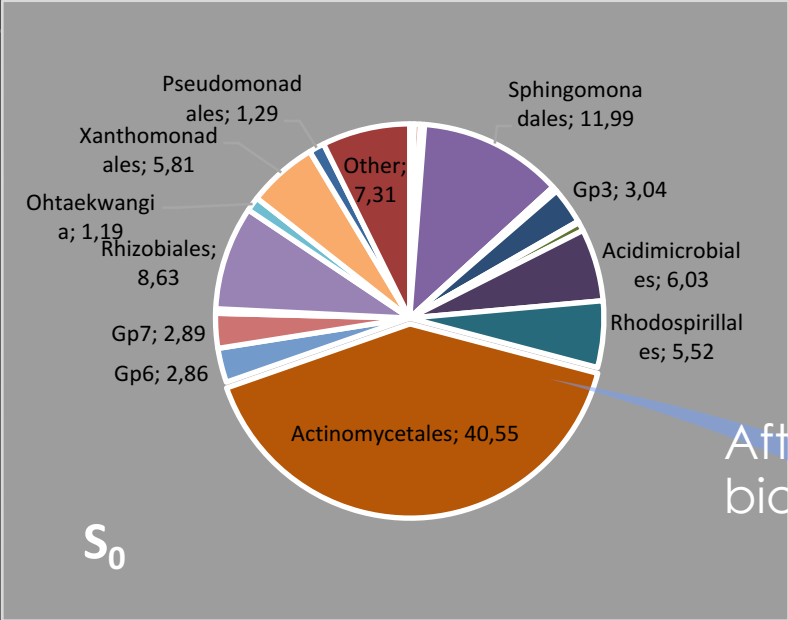


Coupled Bioremediation

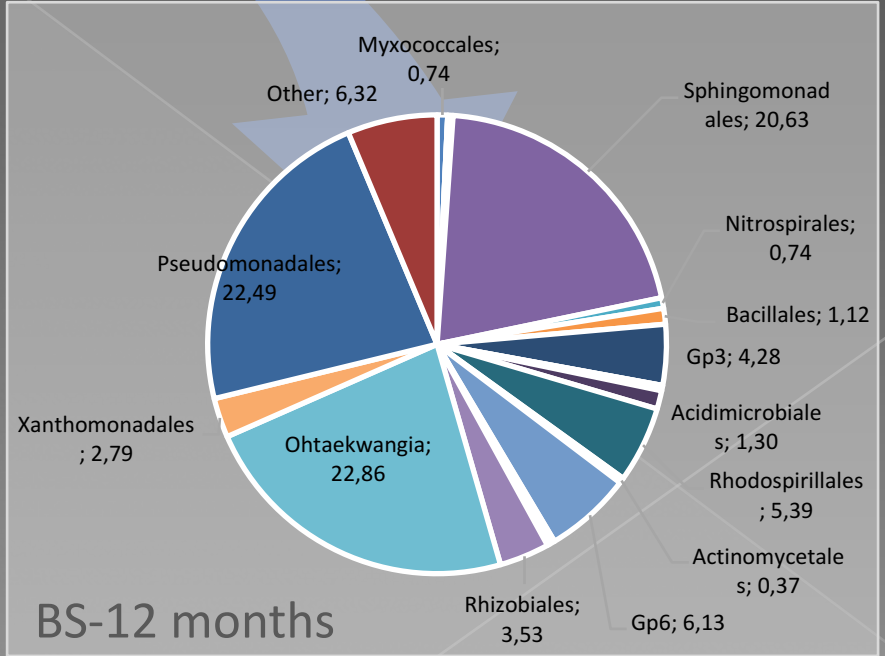
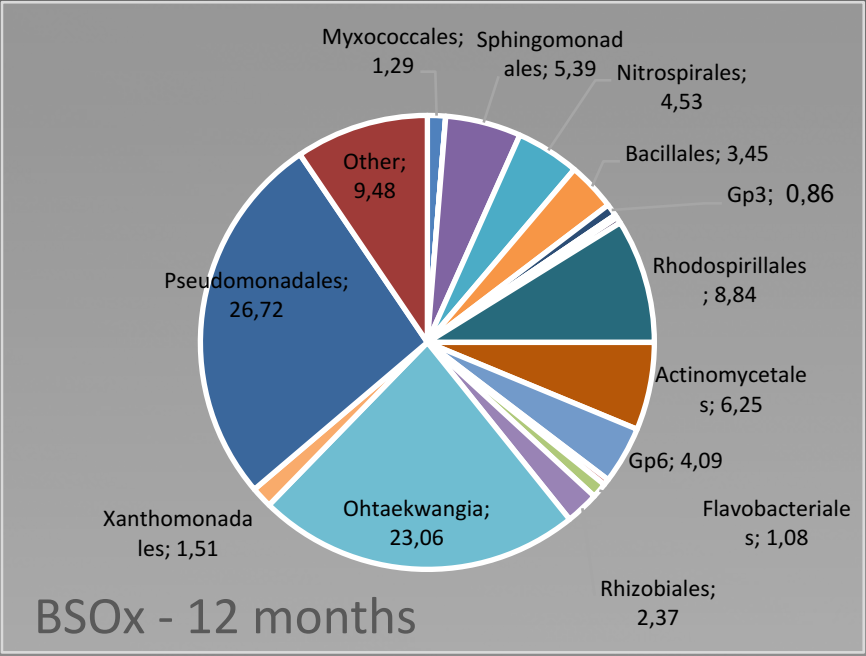


BSOx- 9 months

BSOx - 12 months



After one year of bioremediation



Conclusiones

La oxidación química con PERSULFATO DE AMONIO produjo la eliminación significativa de PAHs en microcosmos de suelo contaminado, de una unidad de *landfarming* ya finalizado.

El tratamiento combinado de OXIDACION QUIMICA + BIORREMEDIACION, permitió la recuperación de la comunidad bacteriana con una eliminación adicional de PAH e HIDROCARBUROS ALIFATICOS.

La OXIDACION produjo la movilización de nutrientes a partir de la matriz del suelo que fueron asimilados por la comunidad bacteriana durante la BIORREMEDIACION, evidenciado a través de la reducción de la relación E_4/E_6 , indicadora del avance de la HUMIFICACION

La comunidad bacteriana del suelo al final del tratamiento combinado (OXID+BIORREM) fue similar a la observada luego del tratamiento control de BIORREMEDIACION, sugiriendo que la alta concentración de SULFATO residual no impidió la recuperación microbiana.

El análisis ESPECTROSCOPICO resulta una herramienta útil en el monitoreo de tratamientos oxidativos que involucren cambios agudos en la matriz de suelos crónicamente contaminados con PAH. Las técnicas de SECUENCIACION MASIVA del ADN, permitió la identificación de las poblaciones bacterianas predominantes que podrían estar asociadas con los cambios observados durante el tratamiento.



Thank you!