8th International Conference on Engineering for Waste and Biomass Valorisation

WasteEng20

May 31-June 4, 2021
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Biofuel Production from Phytoremediation Derived Sunflower Biomass

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Biofuel Production from Phytoremediation Derived Sunflower Biomass

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February, 2021

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Lepabe Laboratory for Process Engineering, Environment, Biotechnology and Energy

100 M ha of degraded/contaminated soils in the world (USEPA)

Why a special concern with heavy metal contamination?

Non degradable → we can only change their bioavailability

Only a few can be considered as nutrients for plants (ex. Zn)

Hazardous to human health and the ecosytems in general

International laws (Netherlands, Germany, Canada, USA, European legislation) **impose limits to the contamination in soil**

Recovery of contaminated soils

Physical and Chemical Treatments: expensive; may hinder soils for further applications; harmful for the existing animals and plants

Pedogenic processes on parent material

Weathering

Dust particles from rocks and volcanic ash

Natural occurrence of HM in soils (usually at low concentrations)

- Burning of fossil fuels
- Mining and smelting of ores
- Metallurgical industries
- Municipal waste and sewage
- Fertilisers and pesticides

Persistent pollution of the soil with HM

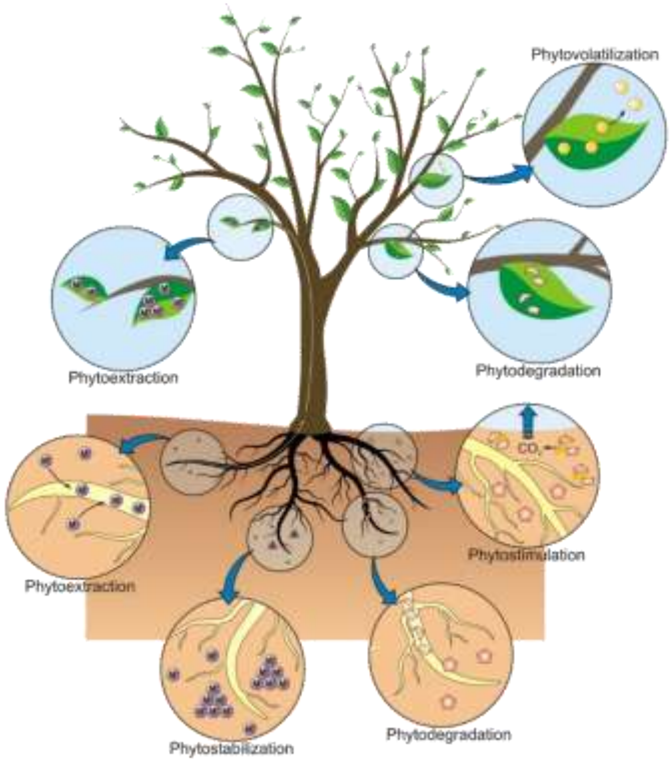
Main technologies for the remediation of HM contaminated soils

Soil washing	200 €/m³
Soil vapour extraction	120 €/m³
Soil flushing	145 €/m³
Solidification	330 €/m³
Stabilisation / Immobilisation	330 €/m³
Vitrification	205 €/m³
Electrokinetics	300 €/m³
Thermal desorption	330 €/m³
Encapsulation	
Biological treatments	90 to 200 €/m³
Phytoremediation	**30 €/m³**

Use of plants and associated microorganisms to treat the contaminated soil

Phytoremediation advantages:

- Economically viable
- Solar energy driven
- Improvement of soil quality
- Soil functions maintenance
- Reduction of soil erosion and contaminant dispersion and leaching via groundwater
- Mitigation of the heavy metal contamination of soils



Phytoremediation inconvenients:

- Time consuming
- Limited to non phytotoxic concentrations
- Limited to root depth
- **Fate of the produced biomass**

Metal extraction (only applicable to metals with value, ex. Ni)

Biofortified crops production (only applicable to metals that are nutrients, ex. Se)

Incineration (high temperatures may vaporize some metals, ex. Hg)

Application of crops suitable for energetic valorisation

Application of energy crops with remediation potential for further valorisation:

- Utilization of degraded soils for valuable applications
- Gradual decontamination of the degraded soils for further agricultural applications
- Biomass production with added value

Bioenergies:

- Demand increases at an annual pace of ca. 20%
- Biomass production has doubled with an increase of only 10% of the agricultural exploitation area (FAO)

The use of biomas produced in degraded soils ensures the release of more agricultural soil area for food production

Contribution to the design of a solution for the Food vs. Fuel dilemma

Plants and Microorganisms

Plant growth under heavy-metal stress conditions

- Hormonal and nutrition imbalance
- Susceptibility to diseases
- Detrimental effect on plant growth and development

Plant Growth Promoting Rhizobacteria (PGPR)

Arbuscular mycorrhizal fungi (AMF)

- Improvement of plant nutrition
- Production of growth regulators
- Improvement of plant tolerance to toxics
- Enhancement of metal translocation or reduction of mobility/availability of metals

Objetives

- Growing plants with energetic value (sunflower)
- Analysis of the phytoremediation potential of the tested combinations
- Oil extraction from produced plants seeds
- Bioetanol production from produced plants stove
- Biodiesel production via transesterification using the generated oil and bioethanol

Sunflower propagation in selected soils

Soils

Private Property - Porto (Agricultural Soil - Control)

Chemical Complex - Estarreja (Industrial Soil)

Panasqueira Mine– Barroca Grande (Mine Soil)

Geoderma

Selection of metal resistant plant growth promoting rhizobacteria for the growth and metal accumulation of energy maize in a mine soil — Effect of the inoculum size

Highlights

Mine land valorization through energy maize production enhanced by the application of plant growth-promoting rhizobacteria and arbuscular mycorrhizal fungi

Journal of Hazardous Materials

Arsenic, lead and nickel accumulation in *Rubus ulmifolius* growing in contaminated soil in Portugal

Heavy Metal Accumulation in Plant Species Indigenous to a Contaminated Portuguese Site: Prospects for Phytoremediation

Chemosphere

Inoculating *Helianthus annuus* (sunflower) grown in zinc and cadmium contaminated soils with plant growth promoting bacteria – Effects on phytoremediation strategies

Soils

Control: Agricultural soil (S. Gens Farm)

- Oporto, Portugal

Parameter	Value
Cd (mg kg^{-1})	<1.8 (L.O.D)
Zn (mg kg-1)	37± 3

Industrial: Soil from the banks of Estarreja stream

- Aveiro, Portugal
- Adjacent to the industrial chemical complex present in the area
- Place of discharges of solid residues and liquid effluents from the facilities nearby in the past, with subsequent infiltration of contaminants in the soils of the area

Parameter	Value
Cd (mg kg^{-1})	1.6 ± 0.5
Zn (mg kg-1)	599± 12

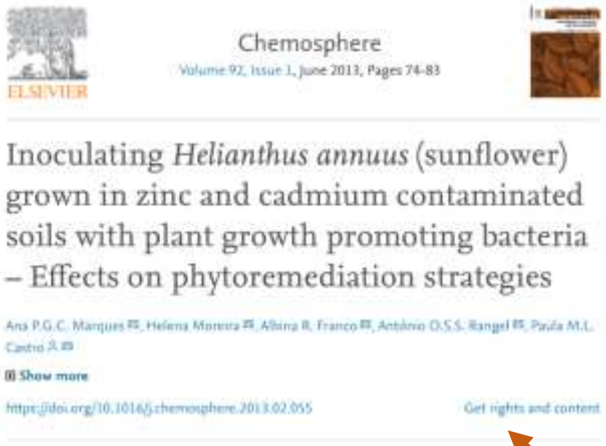
Considered as concerning according to the Ontario norms (>200 mg Zn/kg and >1.4 mg Cd/kg)

Mining: Heap of Barroca Grande Mine, Panasqueira

- Castelo Branco, Portugal
- Active mine
- Heaps and lagoons in the open
- Leaching to Casinhas stream, running to Zêzere river

Parameter	Value
Cd (mg kg^{-1})	9.7± 0.78
Zn (mg kg-1)	486± 15

Microrganisms and plants



Chemosphere
Volume 92, Issue 1, June 2013, Pages 74-83

Inoculating *Helianthus annuus* (sunflower) grown in zinc and cadmium contaminated soils with plant growth promoting bacteria – Effects on phytoremediation strategies

Ana P.G.C. Marques, Helena Moreira, Albina R. Franco, António O.S.S. Rangel, Paula M.L. Castro

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https://doi.org/10.1016/j.chemosphere.2013.02.055 Get rights and content

Applied Soil Ecology
Volume 105, September 2016, Pages 36-47

Promotion of sunflower growth under saline water irrigation by the inoculation of beneficial microorganisms

Sofia I.A. Pereira, Helena Moreira, Konstantinos Argyras, Paula M.L. Castro, Ana P.G.C. Marques

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https://doi.org/10.1016/j.apsoil.2016.03.015 Get rights and content

Highlights

- Watering sunflower with saline water decreased plant biomass production.
- Inoculation with microbial inoculants induced sunflower biomass rates.
- Inoculation reduced nutrient imbalance and improved K^+/Na^+ ratios in plant tissues.
- Microbial inoculation improved soil enzymes activities.

Growth promoting rhizobacteria (*Ralstonia eutropha* 1C2)

- Isolated from a metal contaminated area
- Capable of producing in vitro plant growth promoting substances
- Capable of increasing sunflower biomass in vivo

Arbuscular mychorrizal fungi (*Rhizophagus irregularis*)

Known for promoting plant growth and increasing the resistance of plants in stress conditions

Sunflower

Sunflower propagation in selected soils

Greenhouse preparation

Containers with 1 m^3 lined with plastic and perforated to allow water draining (soil capacity of 1 ton)



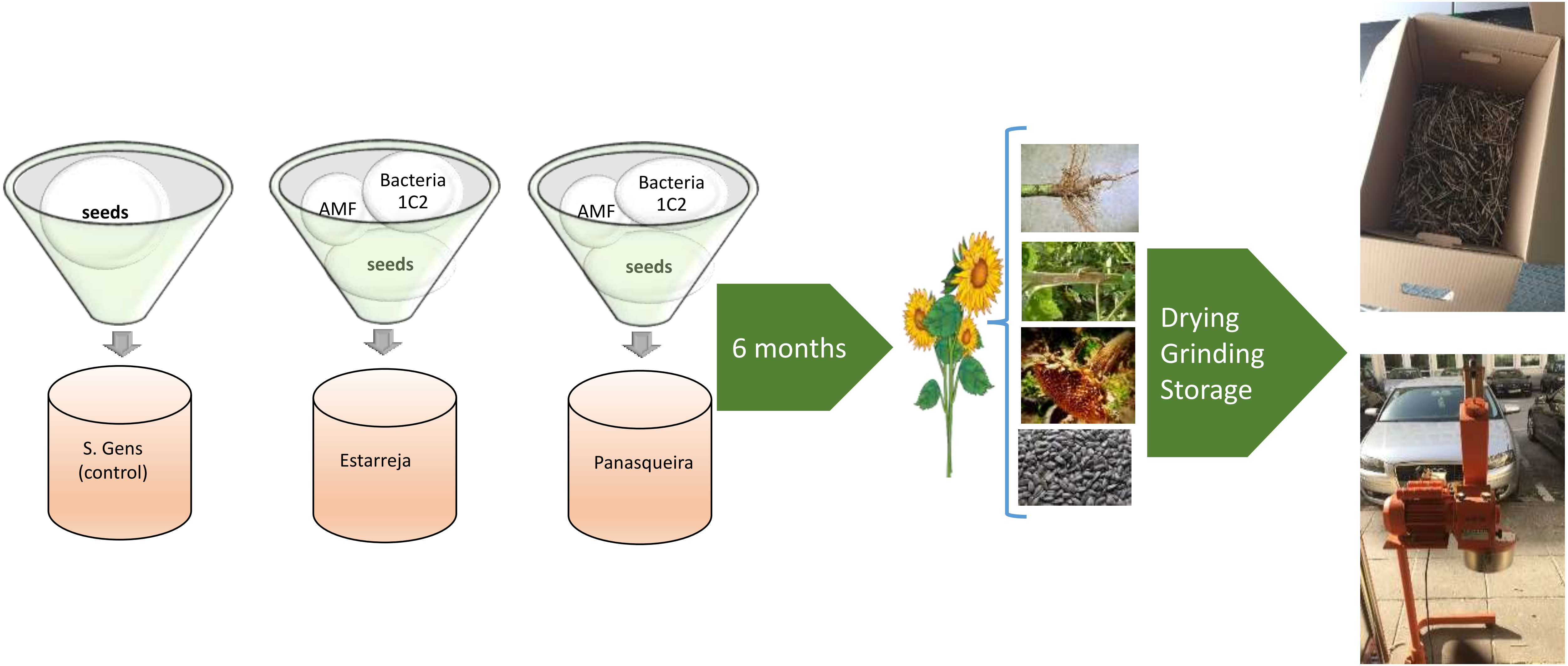
Soil collection


MINING


INDUSTRIAL

CONTROL (AGRICULTURAL)

Experimental design



*all plants growing in the Panasqueira mine soil died before harvesting

Plant growth and soil phytoremediation: Results

Treatment	Zn (mg/kg dry weight)				Cd (mg/kg dry weight)			
	root	stem	flower	seeds	root	stem	flower	seeds
Control	67 ± 3	56 ± 5	36 ± 11	2 ± 1	1.6 ± 0.2	1.0 ± 0.1	n.d	n.d
Estarreja	434 ± 6	343 ± 9	129 ± 7	4 ± 2	24 ± 2	15 ± 2	5.3 ± 0.6	0.5 ± 0.2

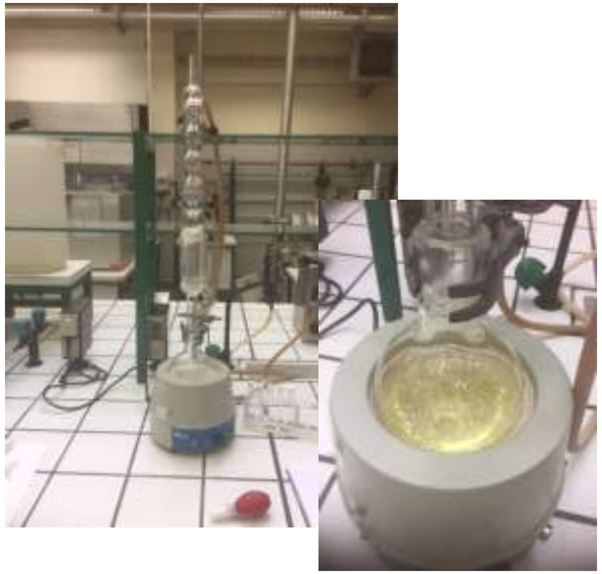
Treatment	Biomass (g)			
	root	stem	flower	seeds
Control	33.66	750.12	223.77	62.57
Estarreja	19.35	620.21	199.36	51.92

ACCUMULATION => Control (bellow phytotoxicity levels) < Estarreja

BIOMASS => Control > Estarreja

Estarreja => Zn_{root}, Zn_{stem} e Zn_{flower} > phytotoxic levels (100 mg/kg)
Cd_{root}, Cd_{stem} e Cd_{flower} > phytotoxic levels (5mg/kg)

Oil extraction



- Several solvents were tested namely hexane, heptane, octane, etanol, isopropyl and propyl alchool (contact time determined by the stabilisation of the refraction index of the extracted liquid)

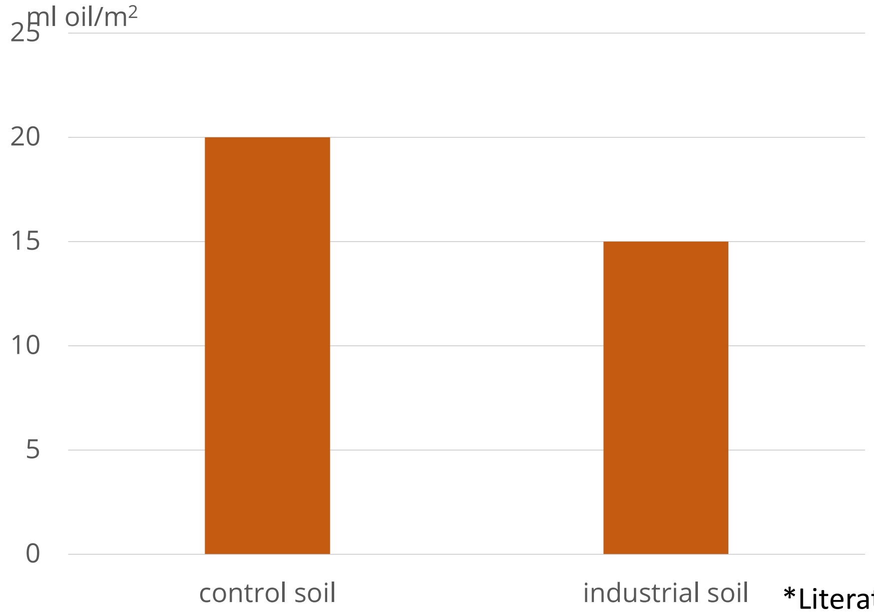
- Optimisation of oil production resulted in the following protocol:

Soxlet extraction with n-hexane (lower contact time)

↓

Vacuum evaporation at constant temperature with a rotary evaporator

Oil extraction



*Literature =80 ml/m^2

VOLUME => Control > Estarreja
Similar extraction yields ca. 32%

	Zn (mg/l)	Cd (mg/l)
Control	n.d.	n.d.
Estarreja	1.8 ± 0.3	n.d.

Zn => The oil produced in Estarreja soil presents low metal levels; no Zn was detected for the oil derived from plants growing in the control soil

Cd => Not detected in any of the tested oils

Bioethanol fermentation

- Optimisation of ethanol production resulted in the following protocol:

Acid pretreatment (85 ºC, 6h, 50 rpm)

pH adjustement to 5 (optimum for enzyme functioning)

Enzymatic hydrolysis with *Viscozyme* L. (50 ºC, 2h, 50 rpm)

Fermentation with *Saccharomyces cerevisiae* (72h, 30 ºC)

Vacuum evaporation at constant temperature with a rotary evaporator

Bioethanol fermentation

	Production yield % (m/v)	Total yield (ml/m^2)
Control	27.8	280
Estarreja	19.4	162

PRODUCTION YIELD

Controlo > Estarreja

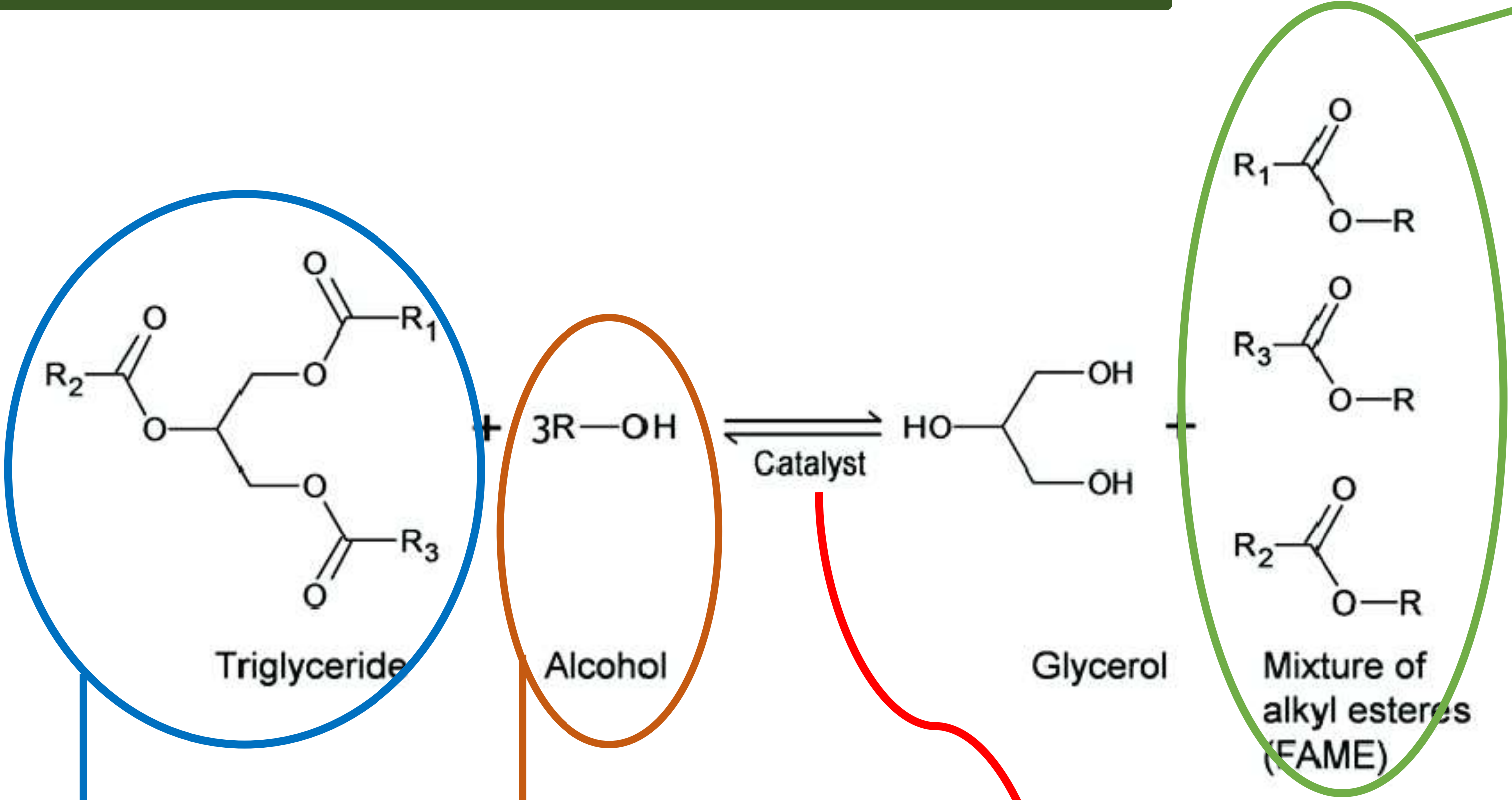
	Zn (mg/l)	Cd (mg/l)
Control	n.d.	n.d.
Estarreja	1.1 ± 0.1	n.d.

Zn and Cd

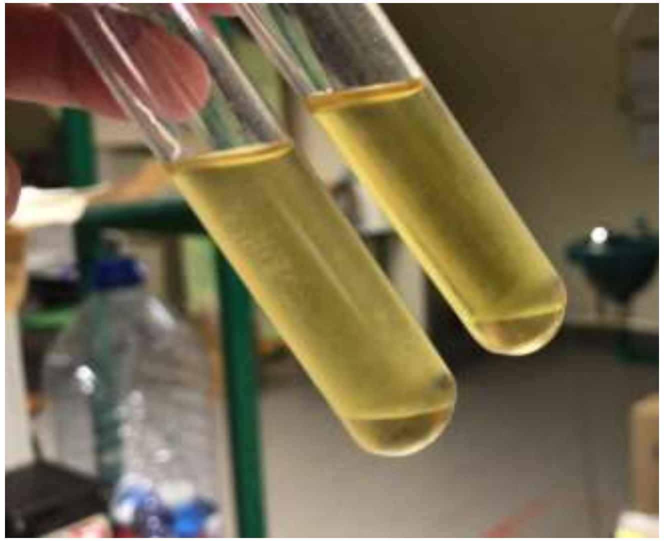
=> Low levels found for the ethanol produced from Estarreja grown plants;

=>no metals were detected for the ethanol produced from control plants

Biodiesel production



BIODIESEL



Extracted oil

Produced bioethanol

Enzymatic catalyis ✗
Alcaline catalysis ✗
Acid catalysis ✓

NO METALS WERE DETECTED IN ANY OF THE TREATMENTS

Thank you for the attention!

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