PLANT SCIENCE CONFERENCE

Microbiota-assisted Phytoremediation of Metal Contaminated Soils by Sunflower

Ana M. Paulo
Paula M.L. Castro
Ana P.G.C. Marques

CATOLICA ESCOLA SUPERIOR DE BIOTECNOLOGIA PORTO

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

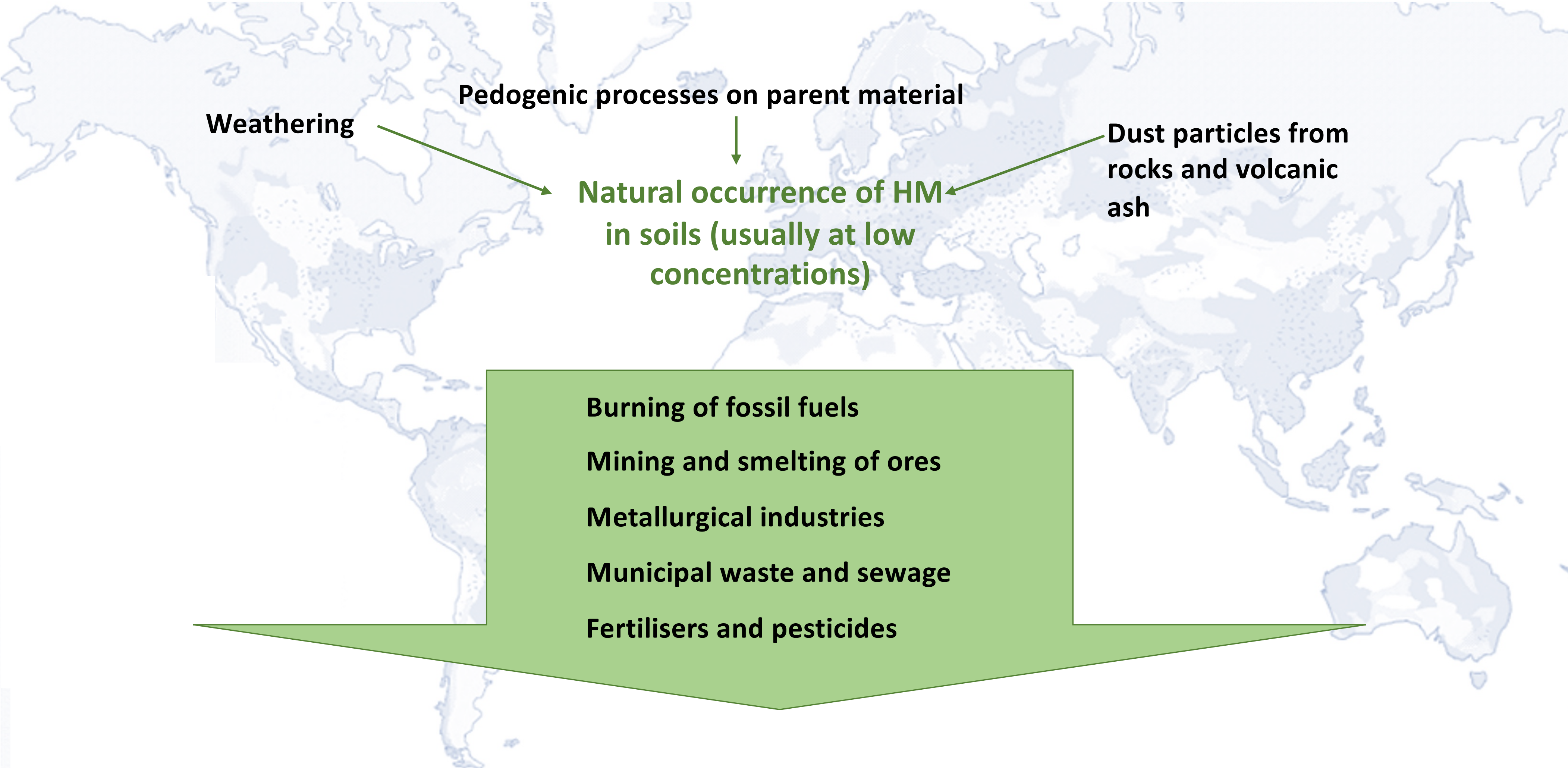
Only a few can be considered as nutrients for plants (ex. Zn)

Hazardous to human health and the ecosystems 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



Persistent pollution of the soil with HM

MAIN TECHNOLOGIES FOR THE REMEDIATION OF HM CONTAMINATED SOILS

Soil washing	200 €/m^3
Soil vapour extraction	120 €/m^3
Soil flushing	145 €/m^3
Solidification	330 €/m^3
Stabilisation / Immobilisation	330 €/m^3
Vitrification	205 €/m^3
Electrokinetics	300 €/m^3
Thermal desorption	330 €/m^3
Encapsulation	
Biological treatments	90 to 200 €/m^3
Phytoremediation	**30 €/m^3**

Use of plants and associated microorganisms to treat the contaminated soil

CHRONOLOGICAL DEVELOPMENTS OF PHYTOREMEDIATION OF HEAVY METAL CONTAMINATED SOILS

Plant communities recognized as **serpentine vegetation** were identified growing on ultramafic soils in the 16th century by Andrea Cesalpino

Margazzi and Vergano (1948)[a] described the accumulation of Ni in *Alyssum bertolonii* from metal contaminated soils

Baker and Brooks (1989)[b] rediscovered *Alyssum bertolonii* and created the term **hyperaccumulator** for plants accumulating 100 times higher levels of metal than the non-accumulating ones

Japanese patent by Utsunomya for the use of plants to extract Cd

First paper describing the use of **transgenics** in phytoremediation, with the use of modified *Brassica napus* L and *Nicotiana tabacum* L. for metal accumulation (1989)

Use of **soil amendments** such as chelates to increase bioavailabilty of metals is first described in papers (Raskin et al., 1997)

First papers questioning the potential of phytoremediation due to it physical limitations (Robson et al., 2006 and Van Nevel et al., 2007)

The term phytoremediation starts to be replaced by the notion of **phytotechnology** (Marmiroli, Marmiroli and Maestri, 2006)

The concepts of **phytomining** and **phytomanagement** are proposed to solve the limitations posed by the lack of revenue of the technology (Lintern et al., 2013; Conesa et al., 2012)

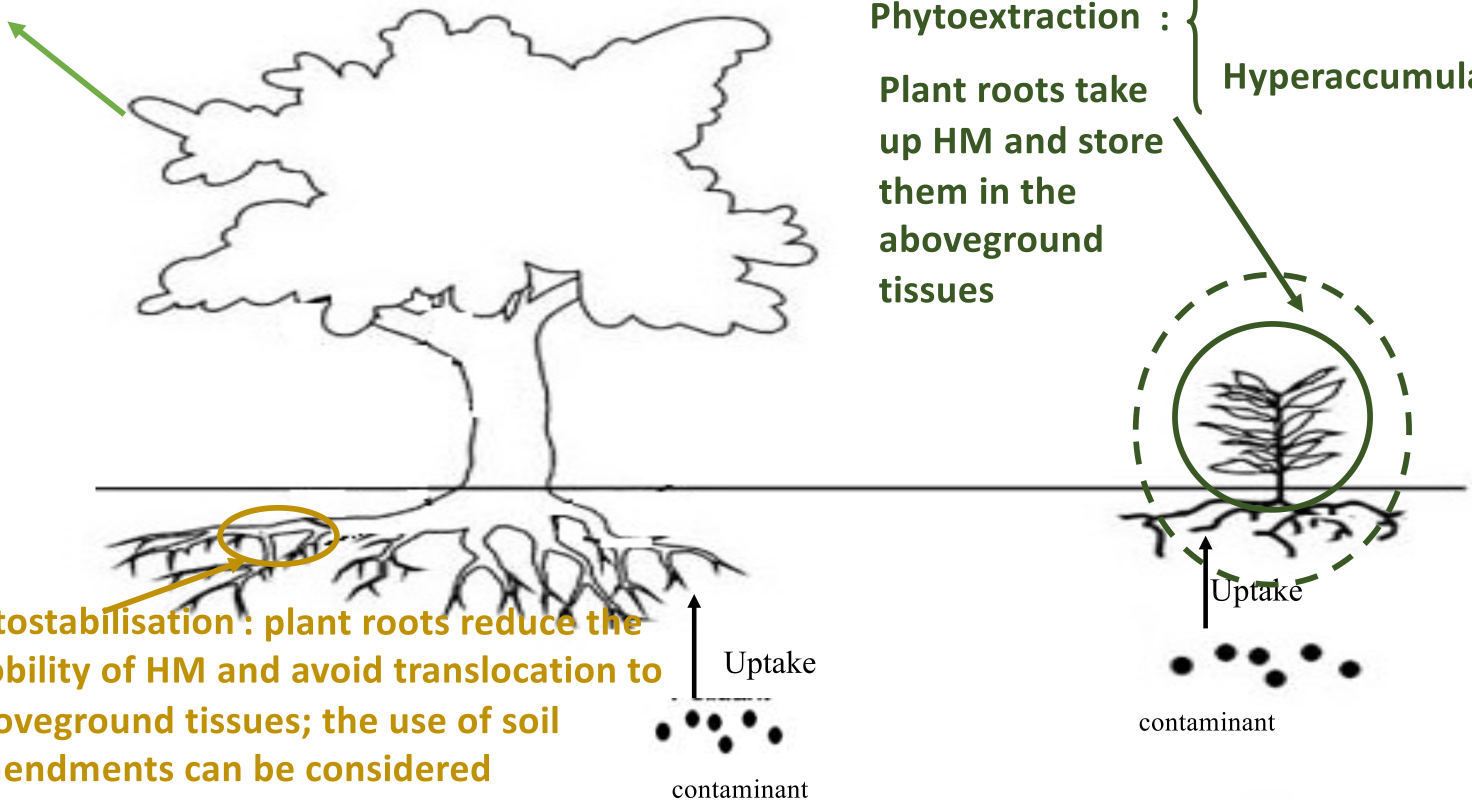
ADAPTED FROM STEPHENSON AND BLACK, 2014

PHYTOREMEDIATION OF METAL CONTAMINATED SOILS

Phytovolatilisation (of volatile metals): HM taken up by the roots pass to the leaves and are volatilised through stomata where gas exchanges occur

Phytoextraction : Chelate- enhanced / Hyperaccumulation

Plant roots take up HM and store them in the aboveground tissues



Phytostabilisation : plant roots reduce the mobility of HM and avoid translocation to aboveground tissues; the use of soil amendments can be considered

PHYTOREMEDIATION OF METAL CONTAMINATED SOILS

Relationship between 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

Objectives

- Use of different soils (contaminated with heavy metals) to grown crops with phytoremediation abilities
- Application of selected bioinoculants
- Determination of biometric parameters for the different plant sections
- Heavy metal accumulation screening for the different plant sections
- Assessment of the microbial dynamics and soil quality variations

Soils

Water Air Soil Pollut (2011) 221:377–389
DOI 10.1007/s11270-011-0797-6

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

Helena Moreira · Ana P. G. C. Marques · António O. S. S. Rangel · Paula M. L. Castro

Received: 13 October 2010 / Accepted: 21 March 2011 / Published online: 6 April 2011

Abstract Phytoremediation is a promising alternative to conventional soil clean-up methods; however, up to date, there is still not enough information on plant species suitable for application in this field of science. Therefore, plant screening on contaminated sites can lead to the identification of further species of interest. In the present study, pedological and botanical characteristics of an industrialised area known for its metal contamination, in special with Zn—Esteiro de Estarreja, in Portugal—were examined in a 1-year screening. Twenty-seven species were found, with a higher occurrence and variability in the summer/spring season. Zinc levels in the tissues of the collected plant samples ranged from 34 mg kg^{-1} in shoots to 2,440 mg kg^{-1} in roots of different species. Species as *Verbascum virgatum*, *Hypochoeris radicata*, *Phalaris arundinacea*, *Conyza bilbaoana*, *Paspalum urvillei* and *Aster squamatus* have shown high Zn shoot accumulation and bioconcentration factors (BCF_{shoots}>1) and high metal translocation factors (TF>1). Others, namely *Spergularia capillacea*, excluded Zn from the shoot tissues and stored the metal at the root zone (BCF_{roots}>1), behaving as tolerant plants. Plants were also screened for arbuscular mycorrhizal fungi colonisation, and only few species showed mycorrhizal presence, namely *C. bilbaoana*, *Hirschfeldia incana*, *Epilobium tetragonum*, *Conyza sumatrensis*, *Pteridium aquilinum*, *P. urvillei* and *A. squamatus*. The present work showed important indigenous species that can cope with installed harsh conditions and with potential for utilisation in phytoremediation strategies, either through metal removal to aerial parts or through its immobilisation in the root zone.

Capsule Twenty seven plant species were found in a one year round survey in a Zn contaminated site and several plants with potential application in phytoremediation strategies, either in metal removal or immobilisation, were found.

H. Moreira · A. P. G. C. Marques · A. O. S. S. Rangel · P. M. L. Castro (✉)
CBQF/Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal
e-mail: plcastro@esb.ucp.pt

H. Moreira
e-mail: helenamoreira@hotmail.com

A. P. G. C. Marques
e-mail: amarques@mail.esb.ucp.pt

A. O. S. S. Rangel
e-mail: aorangel@esb.ucp.pt

Keywords Soil · Phytoremediation · Arbuscular mycorrhizal fungi · Zn · Bioavailability · Survey

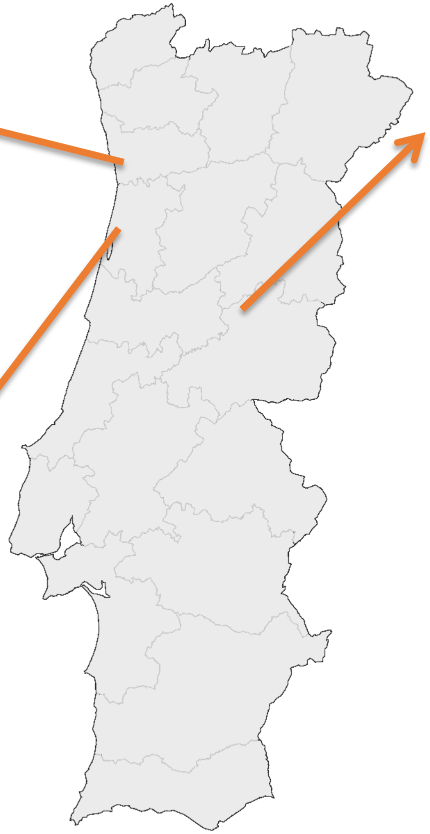
1 Introduction

Pollution of the biosphere with toxic metals has accelerated dramatically since the beginning of the industrial revolution (Nriagu 1979). The primary sources of this pollution are the burning of fossil fuels, mining

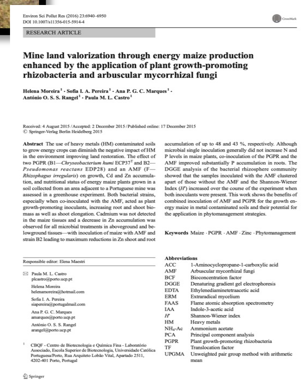
Springer

Private Property - Porto (Agricultural Soil - Control)

Chemical Complex - Estarreja (Industrial Soil)



Panasqueira Mine– Barroca Grande (Mine Soil)



Environ Sci Pollut Res (2016) 23:6940–6950
DOI 10.1007/s11356-015-5914-4

RESEARCH ARTICLE

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

Helena Moreira[1] · Sofia I. A. Pereira[1] · Ana P. G. C. Marques[1] · António O. S. S. Rangel[1] · Paula M. L. Castro[1]

Received: 4 August 2015 / Accepted: 2 December 2015 / Published online: 17 December 2015

Abstract The use of heavy metals (HM) contaminated soils to grow energy crops can diminish the negative impact of HM in the environment improving land restoration. The effect of two PGPR (B1—*Chryseobacterium humi* ECP37^T and B2—*Pseudomonas reactans* EDP28) and an AMF (F—*Rhizophagus irregularis*) on growth, Cd and Zn accumulation, and nutritional status of energy maize plants grown in a soil collected from an area adjacent to a Portuguese mine was assessed in a greenhouse experiment. Both bacterial strains, especially when co-inoculated with the AMF, acted as plant growth-promoting inoculants, increasing root and shoot biomass as well as shoot elongation. Cadmium was not detected in the maize tissues and a decrease in Zn accumulation was observed for all microbial treatments in aboveground and belowground tissues—with inoculation of maize with AMF and strain B2 leading to maximum reductions in Zn shoot and root accumulation of up to 48 and 43 %, respectively. Although microbial single inoculation generally did not increase N and P levels in maize plants, co-inoculation of the PGPR and the AMF improved substantially P accumulation in roots. The DGGE analysis of the bacterial rhizosphere community showed that the samples inoculated with the AMF clustered apart of those without the AMF and the Shannon-Wiener Index (H') increased over the course of the experiment when both inoculants were present. This work shows the benefits of combined inoculation of AMF and PGPR for the growth energy maize in metal contaminated soils and their potential for the application in phytomanagement strategies.

Keywords Maize · PGPR · AMF · Zinc · Phytomanagement

Responsible editor: Elena Maestri

✉ Paula M. L. Castro
plcastro@porto.ucp.pt

Helena Moreira
helenamoreira@hotmail.com

Sofia I. A. Pereira
siapereira@portugalmail.com

Ana P. G. C. Marques
amarques@porto.ucp.pt

António O. S. S. Rangel
arangel@porto.ucp.pt

[1] CBQF - Centro de Biotecnologia e Química Fina - Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Arquiteto Lobão Vital, Apartado 2511, 4202-401 Porto, Portugal

Abbreviations

ACC	1-Aminocyclopropane-1-carboxylic acid
AMF	Arbuscular mycorrhizal fungi
BCF	Bioconcentration factor
DGGE	Denaturing gradient gel electrophoresis
EDTA	Ethylenediaminetetraacetic acid
ERM	Extraradical mycelium
FAAS	Flame atomic absorption spectrometry
IAA	Indole-3-acetic acid
H'	Shannon-Wiener index
HM	Heavy metals
NH_4-Ac	Ammonium acetate
PCA	Principal component analysis
PGPR	Plant growth-promoting rhizobacteria
TF	Translocation factor
UPGMA	Unweighted pair group method with arithmetic mean

Springer

Soils **Agricultural Soil**

- Location: Porto
- Biological agricultural production

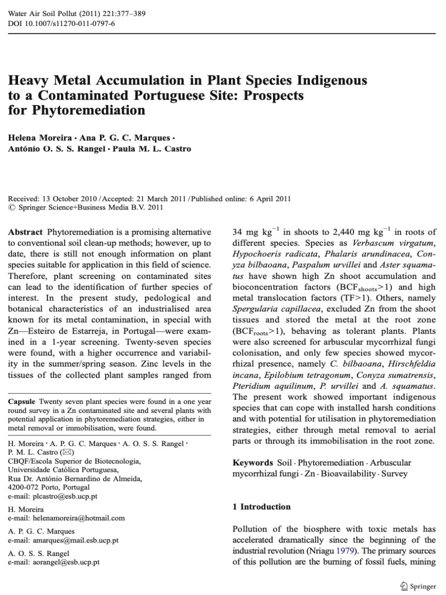
Parameter	Value
Cd (mg kg^{-1})	<1.8 (L.O.D)
Zn (mg kg-1)	37± 3

Private Property

No detectable Cd
Zn at low levels (non phytotoxic)

Soils

Industrial Soil



Water Air Soil Pollut (2011) 221:377–389
DOI 10.1007/s11270-011-0797-6

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

Helena Moreira · Ana P. G. C. Marques · António O. S. S. Rangel · Paula M. L. Castro

Received: 13 October 2010 / Accepted: 21 March 2011 / Published online: 6 April 2011

Abstract Phytoremediation is a promising alternative to conventional soil clean-up methods; however, up to date, there is still not enough information on plant species suitable for application in this field of science. Therefore, plant screening on contaminated sites can lead to the identification of further species of interest. In the present study, pedological and botanical characteristics of an industrialised area known for its metal contamination, in special with Zn—Esteiro de Estarreja, in Portugal—were examined in a 1-year screening. Twenty-seven species were found, with a higher occurrence and variability in the summer/spring season. Zinc levels in the tissues of the collected plant samples ranged from 34 mg kg^{-1} in shoots to 2,440 mg kg^{-1} in roots of different species. Species as *Verbascum virgatum*, *Hypochoeris radicata*, *Phalaris arundinacea*, *Conyza bilbaoana*, *Paspalum urvillei* and *Aster squamatus* have shown high Zn shoot accumulation and bioconcentration factors ($BCF_{shoots}>1$) and high metal translocation factors (TF>1). Others, namely *Spergularia capillacea*, excluded Zn from the shoot tissues and stored the metal at the root zone ($BCF_{roots}>1$), behaving as tolerant plants. Plants were also screened for arbuscular mycorrhizal fungi colonisation, and only few species showed mycorrhizal presence, namely *C. bilbaoana*, *Hirschfeldia incana*, *Epilobium tetragonum*, *Conyza sumatrensis*, *Pteridium aquilinum*, *P. urvillei* and *A. squamatus*. The present work showed important indigenous species that can cope with installed harsh conditions and with potential for utilisation in phytoremediation strategies, either through metal removal to aerial parts or through its immobilisation in the root zone.

Keywords Soil · Phytoremediation · Arbuscular mycorrhizal fungi · Zn · Bioavailability · Survey

Capsule Twenty seven plant species were found in a one year round survey in a Zn contaminated site and several plants with potential application in phytoremediation strategies, either in metal removal or immobilisation, were found.

H. Moreira · A. P. G. C. Marques · A. O. S. S. Rangel · P. M. L. Castro (✉)
CBQF/Escola Superior de Biotecnologia,
Universidade Católica Portuguesa,
Rua Dr. António Bernardino de Almeida,
4200-072 Porto, Portugal
e-mail: plcastro@esb.ucp.pt

H. Moreira
e-mail: helenamoreira@hotmail.com

A. P. G. C. Marques
e-mail: amarques@mail.esb.ucp.pt

A. O. S. S. Rangel
e-mail: aorangel@esb.ucp.pt

1 Introduction

Pollution of the biosphere with toxic metals has accelerated dramatically since the beginning of the industrial revolution (Nriagu 1979). The primary sources of this pollution are the burning of fossil fuels, mining

- Location: Estarreja (Aveiro district);
- Chemical industries (production of sulphuric acid, production of chlorine and caustic soda)

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)

Estarreja Chemical Complex

Discharge of solid residues directly to the soil in the surrounding area	Conducting of the liquid effluents of the factories into the nearby watercourses

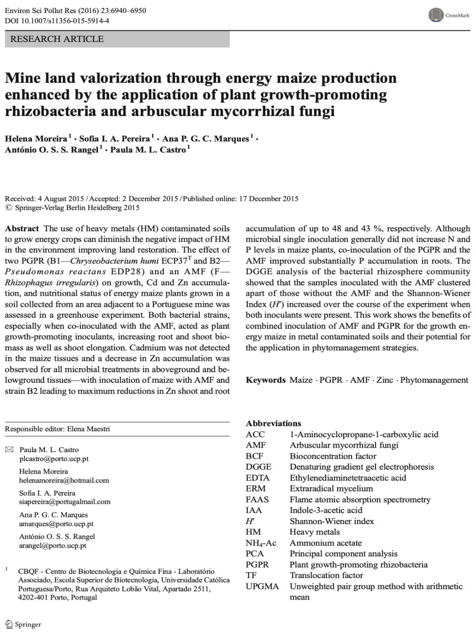
HM reach hazardous levels

Zn and Cd appear as the main contaminants

PLANT SCIENCE CONFERENCE

Soils

Mine Soil


Environ Sci Pollut Res (2016) 23:6940–6950
DOI 10.1007/s11356-015-5914-4

CrossMark

RESEARCH ARTICLE

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

Helena Moreira[1] · Sofia I. A. Pereira[1] · Ana P. G. C. Marques[1] · António O. S. S. Rangel[1] · Paula M. L. Castro[1]

Received: 4 August 2015 / Accepted: 2 December 2015 / Published online: 17 December 2015

Abstract The use of heavy metals (HM) contaminated soils to grow energy crops can diminish the negative impact of HM in the environment improving land restoration. The effect of two PGPR (B1—*Chryseobacterium humi* ECP37[T] and B2—*Pseudomonas reactans* EDP28) and an AMF (F—*Rhizophagus irregularis*) on growth, Cd and Zn accumulation, and nutritional status of energy maize plants grown in a soil collected from an area adjacent to a Portuguese mine was assessed in a greenhouse experiment. Both bacterial strains, especially when co-inoculated with the AMF, acted as plant growth-promoting inoculants, increasing root and shoot biomass as well as shoot elongation. Cadmium was not detected in the maize tissues and a decrease in Zn accumulation was observed for all microbial treatments in aboveground and belowground tissues—with inoculation of maize with AMF and strain B2 leading to maximum reductions in Zn shoot and root accumulation of up to 48 and 43 %, respectively. Although microbial single inoculation generally did not increase N and P levels in maize plants, co-inoculation of the PGPR and the AMF improved substantially P accumulation in roots. The DGGE analysis of the bacterial rhizosphere community showed that the samples inoculated with the AMF clustered apart of those without the AMF and the Shannon-Wiener Index (*H'*) increased over the course of the experiment when both inoculants were present. This work shows the benefits of combined inoculation of AMF and PGPR for the growth energy maize in metal contaminated soils and their potential for the application in phytomanagement strategies.

Keywords Maize · PGPR · AMF · Zinc · Phytomanagement

Responsible editor: Elena Maestri

✉ Paula M. L. Castro
plcastro@porto.ucp.pt

Helena Moreira
helenamoreira@hotmail.com

Sofia I. A. Pereira
siapereira@portugalmail.com

Ana P. G. C. Marques
amarques@porto.ucp.pt

António O. S. S. Rangel
arangel@porto.ucp.pt

[1] CBQF - Centro de Biotecnologia e Química Fina - Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Arquiteto Lobão Vital, Apartado 2511, 4202-401 Porto, Portugal

Abbreviations

ACC	1-Aminocyclopropane-1-carboxylic acid
AMF	Arbuscular mycorrhizal fungi
BCF	Bioconcentration factor
DGGE	Denaturing gradient gel electrophoresis
EDTA	Ethylenediaminetetraacetic acid
ERM	Extraradical mycelium
FAAS	Flame atomic absorption spectrometry
IAA	Indole-3-acetic acid
H'	Shannon-Wiener index
HM	Heavy metals
NH_4-Ac	Ammonium acetate
PCA	Principal component analysis
PGPR	Plant growth-promoting rhizobacteria
TF	Translocation factor
UPGMA	Unweighted pair group method with arithmetic mean

- Location: Beira Baixa (Castelo Branco district);
- Covers an area of more than 2000 ha;
- Most important exploitations: Barroca Grande, Panasqueira, Rebordões and Vale da Ermida;
- Economic exploitation mainly focused on wolframite, cassiterite and chalcopyrite.

Panasqueira Mine – Barroca Grande

Huge tailing piles and two mud dams exposed to atmospheric conditions.

Surface runoff and water percolation leach the tailings to Casinhas stream, which drains to Zêzere river.

HM reach hazardous levels in the surrounding soils

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

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

PLANT SCIENCE CONFERENCE

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)

Microorganisms 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

Show more

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 [a, 1], Helena Moreira [a, 1], Konstantinos Argyras [b], Paula M.L. Castro [a], Ana P.G.C. Marques [a]

Show more

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)

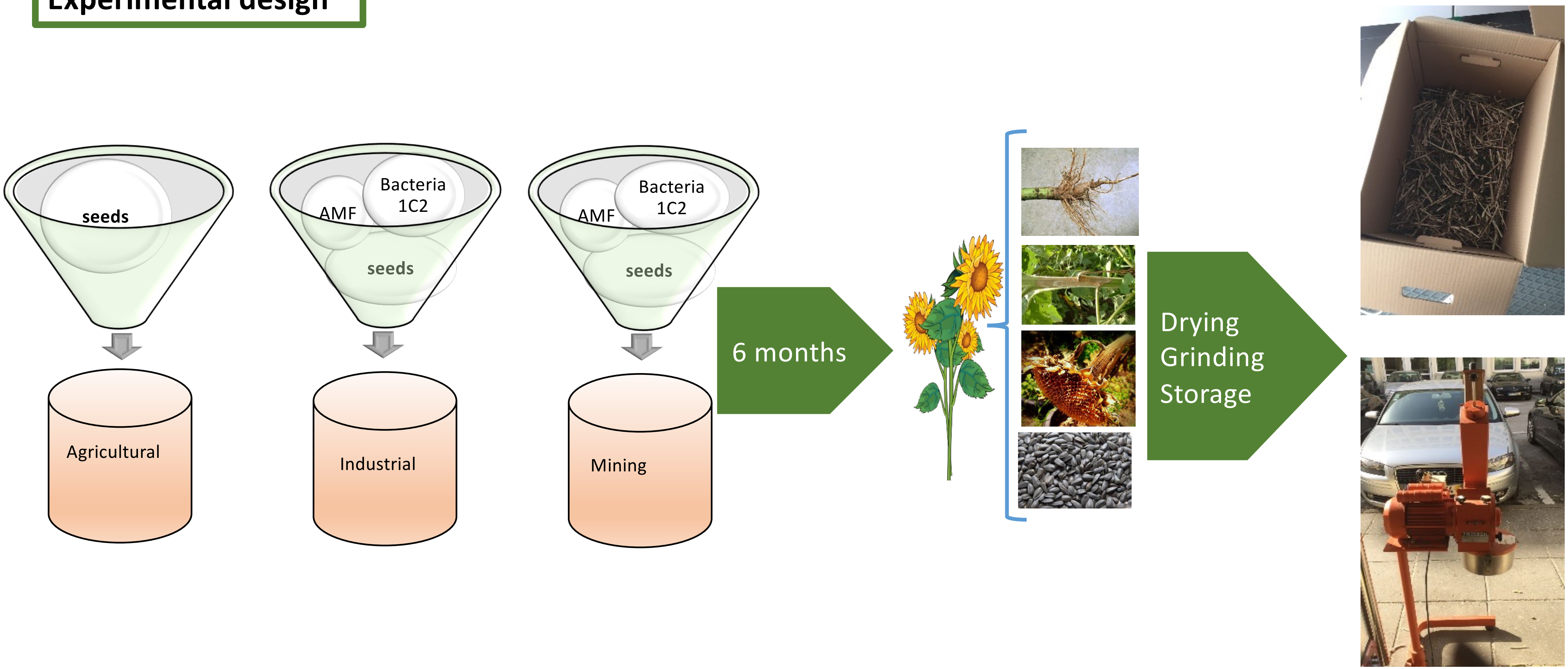
- Isolated from a metal contaminated area
- Capable of producing in vitro plant growth promoting substances
- Capable of increasing sunflower biomass in vivo

Arbuscular mychorrizal fungi (*Rhizophagus irregularis*)

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

Sunflower

Experimental design



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

Results: Plant Biomass and Microbial community

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

BIOMASS => Agricultural > Industrial

-1C2 remained in the contaminated soil until the end of plant growth;

-mychorrizal colonisation in all samples at harvest

- Phytoremediation associated to PGPR induced an improvement of the microbial diversity

Results: Metal levels in soils and plants at harvest

Treatment	Zn (mg/kg dry weight)					Cd (mg/kg dry weight)				
	root	stem	flower	seeds		root	stem	flower	seeds	
Agricultural	67 ± 3 ^{A,c}	56 ± 5 ^{A,c}	36 ± 11 ^{A,b}	2 ± 1 ^{A,a}	$^{***}F_{4,12}$=60,669	1.6 ± 0.2. ^{A,b}	1.0 ± 0.1. ^{A,b}	0 ^{A,a}	0 ^{A,a}	$^{***}F_{4,12}$ =11,193
Industrial	434 ± 6 ^{B,d}	343 ± 9 ^{B,c}	129 ± 7 ^{A,b}	4 ± 2 ^{A,a}	$^{***}F_{4,12}$=2418,335	24 ± 2 ^{B,d}	15 ± 2 ^{B,c}	5.3 ± 0.6 ^{B,b}	0.5 ± 0.2 ^{A,a}	$^{***}F_{4,12}$ =141,505
	t=4,062	t=1,328	t=1,306	t=6,484		t=0,319	t=1,759	t= 14,545	t=16	

ACCUMULATION IN PLANTS => Agricultural (bellow phytotoxicity levels) < Industrial

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

	Zn (mg/kg)		Cd (mg/kg)	
	beginning	harvest	beginning	harvest
Agricultural	18.8 ± 0.70^{a,B}	25.2 ± 2.8^{a,A}	n.d.	n.d.
Industrial	69.4 ± 1.5^{b,B}	82.3 ± 6.3^{b,A}	n.d.	n.d.

SOIL BIOAVAILABILITY
=> Agricultural < Industrial

=> No Cd was detected as bioavailable

PLANT SCIENCE CONFERENCE

Conclusions

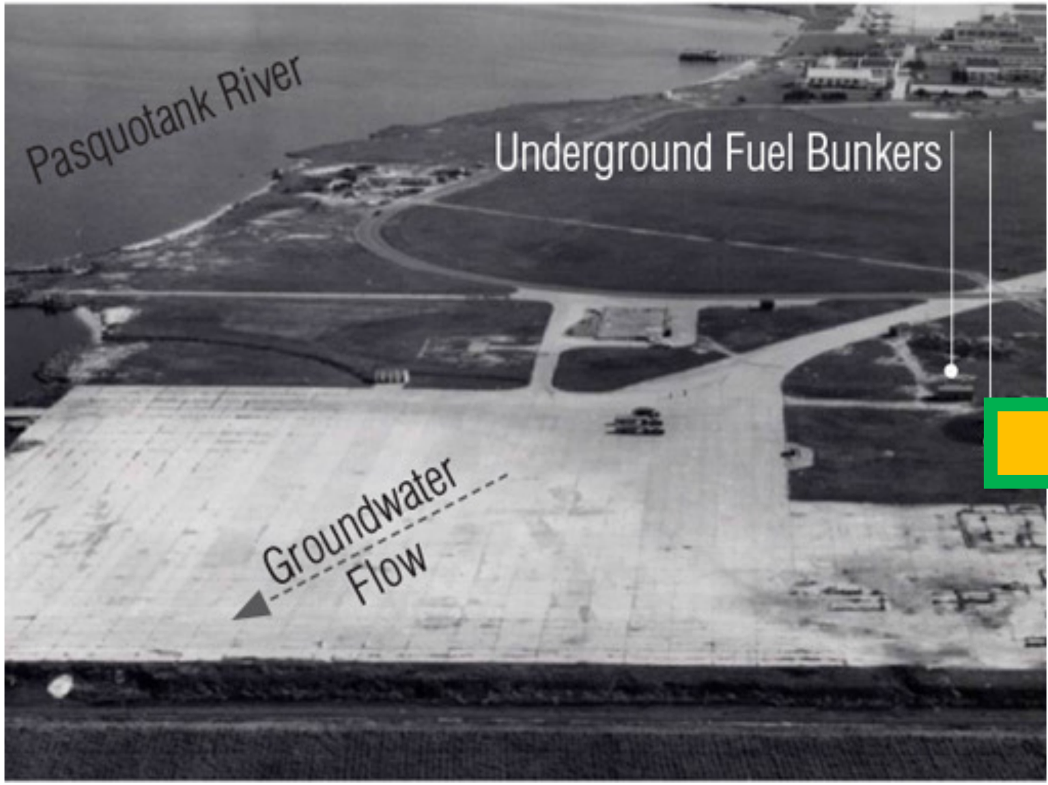
- **Microbiota inoculated sunflower was capable of enduring the hazardous conditions installed and was able to produce significant levels of biomass**

- **Inoculated microorganism were able to persist in a long term experiment and increase soil microbial quality**

- **The metals were mainly accumulated in the root areas independently of the soil in which growth occurred**

Phytoremediation assisted by selected microbiota using energetic crops can be a solution with potential for the production of biomass for energy generation

THANK YOU FOR YOUR ATTENTION!



FCT Fundação para a Ciência e a Tecnologia

UNIÃO EUROPEIA
Fundos Europeus Estruturais e de Investimento

COMPETE 2020
PROGRAMA OPERACIONAL COMPETITIVIDADE E INTERNACIONALIZAÇÃO

Phytoenergy
(PTDC/BTA-BTA/28761/2017)

PLANT SCIENCE CONFERENCE