Influence of Plant Growth Promoting Bacteria in *Helianthus annuus* (sunflower) grown in Zn and Cd contaminated soils – effects on biomass production, growth parameters and metal accumulation

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Introduction

Pollution of the environment has increased dramatically since the birth of the industrial revolution. The off-site migration of contaminants, when not controlled, can cause serious damage on ecosystems and affect public health. These and other reasons bring up the need for new solutions of remediation, to stop the contaminants dissemination in the environmental compartments.

Phytoremediation - the use of plants to remove or immobilise contaminants - may offer a low cost method for the remediation of contaminated soil. *Helianthus annuus*, one of the most important crops worldwide, is a plant not only with food and energy value, but also with phytoremediation potential - sunflower is a documented metal accumulator and its growth on contaminated land is thus possible. Recent studies also show that sunflower seeds of plants grown in contaminated soils do not have significant levels of metals (when compared to control specimens) and their application in biodiesel production is a possibility.

The obtainment of further economic value through the valorisation of this plant species should be taken into account. Therefore the need to maximize biomass production, allowing not only to revert degraded soil, but also the further use of the biomass produced for other activities from which economical gains can be achieved, such as energy production. The use of plant growth promoting rhizobacteria (PGPR) may constitute a biological alternative to increase crop yield. The enhancement of plant growth using PGPR is documented and more recently these organisms have been used to reduce plant stress associated with phytoremediation of contaminated soils.

Methods

A greenhouse experiment was carried out to assess the influence of the inoculation with selected PGPR on the growth, biomass production and metal accumulation by *Helianthus annuus* in Zn (0, 100 and 500 mg Zn kg^{-1} spoil) and Cd (0, 10, 20, 30 mg Cd kg^{-1} soil) contaminated soils – as described in Figure 1. Bacterial isolates used in this work have previously been characterized as having plant growth promotion abilities and identified as B1 and B2, corresponding to strains within the genera *Ralstonia* (B1) and *Chryseobacterium humi* (B2).

At harvest dry weight of plant sections was determined; plant tissues were then ground and digested at high temperatures with an acid mixture, and metal contents were determined via atomic absorption spectroscopy with flame atomisation (FA-AAS).

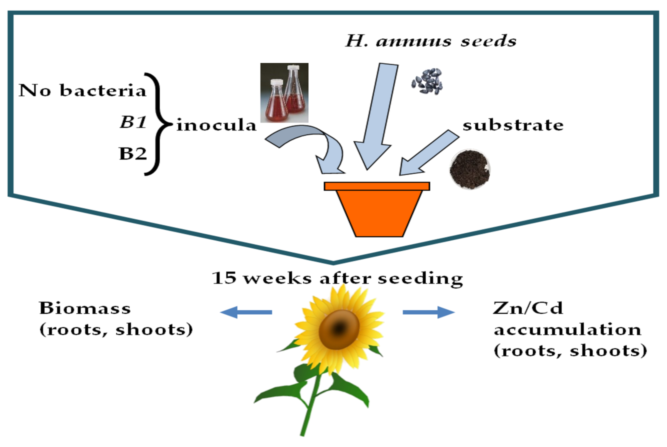
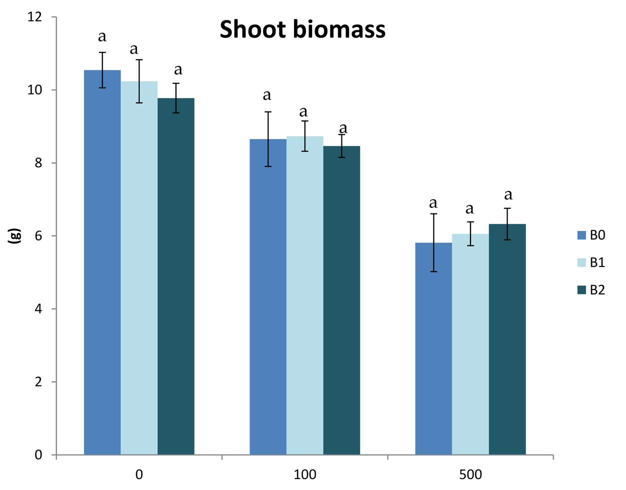


Figure 1: Scheme of the experimental work

Results and Discussion

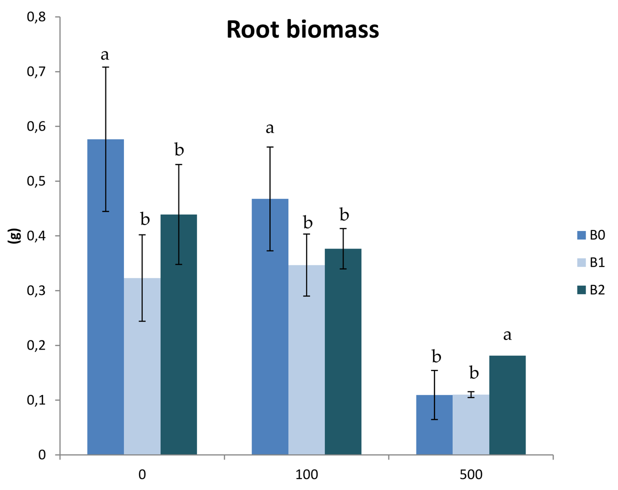
Zn and Cd accumulation in plant tissues – shown in tables 1 and 2 - and dry biomass - shown in figures 2 to 5 - were determined in order to conclude on the influence of the bacterial inoculation and degree of metal exposition on plant development parameters and remediation capacities.

Figure 2: Shoot biomass in plants exposed to Zn



Results are shown as means ± S.D (n=4). Means with different letters in concentration are significantly different from each other ($P < 0.05$) according to the Duncan test.

Figure 3: Root biomass in plants exposed to Zn



Results are shown as means ± S.D (n=4). Means with different letters in concentration are significantly different from each other ($P < 0.05$) according to the Duncan test.

Table 1: Zn accumulation in roots and shoots of *H. annuus*

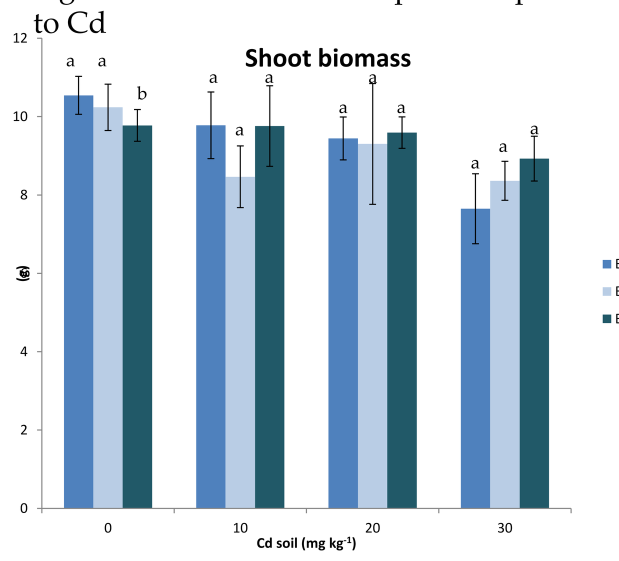
treatment	Zn (mg kg^{-1})					
	roots			Shoots		
	0	100	500	0	100	500
no bacteria	141± 21 a	213± 30 a	443± 80 a	132± 40 a	198± 27 a	468± 33 a
B1	90± 35 b	161± 35 b	430± 43 a	160± 11 a	195± 27 a	326± 39 b
B2	51± 22 b	134± 12 b	312± 20 b	45± 37 b	174± 8 a	374± 31 b

Results are shown as means ± S.D (n=4). Means with different letters in each plant column are significantly different from each other ($P < 0.05$) according to the Duncan test.

Zn

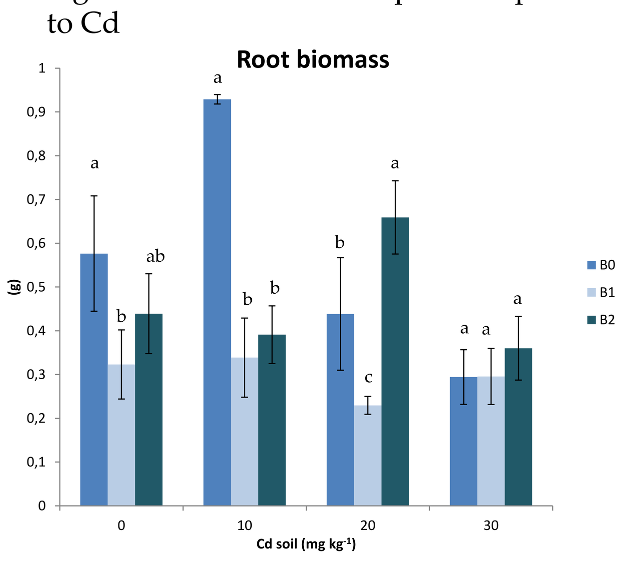
- At low soil concentrations (0 and 100 mg kg^{-1}) inoculation with bacteria decreased root biomass;
- Generally root accumulation was significantly decreased by bacterial inoculation;
- Shoot accumulation was decreased by bacteria inoculation but that was not always significant.

Figure 4: Shoot biomass in plants exposed to Cd



Results are shown as means ± S.D (n=4). Means with different letters in concentration are significantly different from each other ($P < 0.05$) according to the Duncan test.

Figure 5: Root biomass in plants exposed to Cd



Results are shown as means ± S.D (n=4). Means with different letters in concentration are significantly different from each other ($P < 0.05$) according to the Duncan test.

Table 2: Cd accumulation in roots and shoots of *H. annuus*

treatment	Cd (mg kg^{-1})					
	roots			shoots		
	10	20	30	10	20	30
no bacteria	261± 54 ab	529± 20 ab	667± 57 a	196± 11 a	266± 19 a	310± 17 a
B1	324± 70 a	653± 90 a	683± 1 a	195± 8 a	272± 13 a	302± 12 a
B2	196± 25 b	385± 90 b	554± 42 b	193± 15 a	273± 7 a	305± 14 a

Cd was not detected in plants grown in control soil, therefore treatment was omited from the table. Results are shown as means ± S.D (n=4). Means with different letters in each plant column are significantly different from each other ($P < 0.05$) according to the Duncan test.

Cd

- At low soil concentrations (0 and 10 mg kg^{-1}) inoculation with bacteria decreased root biomass
- Inoculation with B2 decreased root accumulation (however not always significantly when compared to the control) while inoculation with B1 had no significant influence
- Bacterial inoculation had no significant effect on shoot accumulation

Conclusions

Inoculation of sunflower with the tested PGPR seems to prevent excessive accumulation of Zn and Cd in the plant tissues, and can present itself as a valuable tool in soil recovery via phytoremediation. This work will surely contribute to the research concerning the importance of the synergy between plant species and soil microorganisms as a valuable biotechnological resource of great use on the re-vegetation and achievement of economical value from under-exploited soils, namely those contaminated with heavy metals.

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