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Preliminary studies on the growth, tolerance and phytoremediation ability of sugarbeet (*Beta vulgaris* L.) grown on heavy metal contaminated soil

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ABSTRACT

Sugarbeet (*Beta vulgaris* L.) was tested in a greenhouse pot experiment to evaluate its growth, tolerance ability and remediation potential to soils contaminated with cadmium (Cd) and nickel (Ni). Sugarbeet seeds were pre-germinated and four weeks after sowing the seedlings were transplanted to 36 pots. They were left to grow for 12 weeks and then aqueous solutions of nitrate salts of Cd and Ni were added to the soil. The applied treatments were: only $Cd(NO_3)_2.4H_2O$ addition in quantities (g) of Cd_0:0, $Cd_{0.5}$:0.5, Cd_5:5, and Cd_{10}:10; only $Ni(NO_3)_2.4H_2O$ addition in quantities (g) of Ni_0:0, Ni_1:1, Ni_{10}:10, and Ni_{20}:20; a combination of both salts in quantities (g) of $Cd_0 + Ni_0$: 0 + 0, $Cd_{0.25} + Ni_{0.5}$: 0.25 + 0.5, $Cd_{2.5} + Ni_5$: 2.5 + 5, $Cd_5 + Ni_{10}$: 5 + 10. At the end of the experiment, the determined DTPA-extractable metal concentrations in soil were up to 225.8 mg Cd kg^{-1} and 75.4 mg Ni kg^{-1}. Under the Cd treatment, plant growth remained unaffected, with the exception of the leaves' number, which differed significantly among control (Cd_0) and highly (Cd_{10}) treated plants on the last measurement date. Nickel affected sugarbeets growth and Ni_{20} was lethal to the plants. Under the combined Cd and Ni treatment, the toxicity symptoms were milder than in Ni treatment and only the $Cd_5 + Ni_{10}$ treated plants were affected. The higher accumulation rate of Cd and Ni was recorded in the above-ground biomass, with beets also experiencing cadmium and nickel accumulation. Under Cd_{10} treatment, metal content reached 92.1 mg kg^{-1} in the aboveground biomass and 14.3 mg kg^{-1} in beets, while Ni concentrations under Ni_{10} treatment were up to 283.3 mg kg^{-1} and 45.9 mg kg^{-1} respectively. Under the combined Cd and Ni treatments, Cd concentrations were up to 172.5 and 76.7 mg kg^{-1} in shoots and beets, respectively, while Ni was up to 467.8 and 289.7 mg kg^{-1}, respectively. Metal accumulation (mg per pot) in the single experiments followed the pattern Cd > Ni, mostly due to the higher biomass production in the Cd pots and the higher Cd mobilized content in the soil. In the combined Cd and Ni contamination, Ni accumulation was higher than the Cd accumulation, reflecting a preference by sugarbeet to absorb an ion with lower radius and higher electronegativity. Higher modified translocation factors (mTFs) were obtained for Ni, in the single experiments. In the combined experiment, the mTFs was reduced for both metals due to the combined accumulation of Ni and Cd. Sugarbeet can be considered an interesting candidate for Cd phytoextraction, favored by the biomass produced and accumulation observed but further studies should be conducted in order to evaluate the effect of the soil contamination on the bioethanol yield and quality.

1. Introduction

The increasing population, urbanization, and industrialization during the last two centuries have led to severe anthropogenic impacts on the environment (Steffen et al., 2011; Selinus et al., 2005). Every year, considerable amounts of heavy metals are released into the environment on a global scale, *e.g.* 22,000 t of cadmium (Cd), 93,900 t of copper (Cu), 7,83,000 t of lead (Pb) and 13, 50,000 t of zinc (Zn) in 2002 (Carrillo-Chávez et al., 2014; Turgut and Pepe, 2005). Anthropogenic activity (use of municipal waste, pesticides, fertilizers, emissions from waste incinerators, car emissions, the activity of metallurgical and petrochemical industries, mining, and construction) is responsible for the increment of heavy metals in soils (Fernando et al., 2016). However, although heavy metals contaminated soils represent an environmental burden, there are some crops that are able to tolerate, accumulate and/or stabilize the contamination, contributing to alleviate the risk to humans and ecosystems (Baker, 1981; Chaney et al., 1997). The use of plants (and microbes associated) to remediate heavy

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Table 1
Physical and chemical properties of the soil used in the experiments, before artificial contamination.

Physical	
Size fractions (%)	
Clay	12.1
Silt	18.6
Sand	69.3
Texture	Sandy Loam
Chemical	
pH	7.8
Organic matter (%)	2.1
$CaCO_3$ (%)	21.6

Table 2
Cadmium nitrate and nickel nitrate quantities applied to the soil of each pot per treatment.

Treatments	Treatmen I Cd $(NO_3)_2.4H_2O$ (g)	Treatment II Ni $(NO_3)_2.4H_2O$ (g)	Treatment III Cd $(NO_3)_2.4H_2O$ + Ni $(NO_3)_2.4H_2O$ (g)
Control	Cd_0: 0	Ni_0: 0	$Cd_0 + Ni_0$: 0 + 0
Low	$Cd_{0.5}$: 0.5	Ni_1: 1	$Cd_{0.25} + Ni_{0.5}$: 0.25 + 0.5
Medium	Cd_5: 5	Ni_{10}: 10	$Cd_{2.5} + Ni_5$: 2.5 + 5
High	Cd_{10}: 10	Ni_{20}: 20	$Cd_5 + Ni_{10}$: 5 + 10

metal contaminated soils is a cost-effective and economically attractive phytotechnology that combines soil remediation with minimum disturbance (Cunningham and Ow, 1996; Mulligan et al., 2001). Moreover, some crops, such as energy crops, are able to combine tolerance to contamination with the production of a high yielded biomass that can be further valorized economically (Fernando et al., 2010; Pandey et al., 2016). In addition, cultivating energy crops in contaminated soils reduces the clash with land use by food crops (Dauber et al., 2012).

Sugarbeet (*Beta vulgaris* L.) is a commercially important crop, since it accounts for 30% of the world's sugar production. In the EU-28 (2013), sugarbeet was cultivated on 11 million hectares. In 2015, the EU-28 produced 102 million t of sugarbeet, and more than half of the production came from France (32.9%) and Germany (22.2%) (EUROSTAT, 2017). Besides, sugarbeet is used as a fodder, and in industries, and there is a growing interest in its potential as a source of biofuel once this crop have proven to be a good feedstock for bioethanol production (Maung and Gustafson, 2011; Salazar-Ordóñez et al., 2013).

Production of energy crops in heavy metals contaminated soils have been suggested for perennial grasses, such as miscanthus, arundo and switchgrass, short rotation coppices, such as poplar and willow trees, and annual crops, such as hemp, flax, and kenaf among others (Arora et al., 2016; Ding et al., 2016; Evangelou et al., 2015; Fernando et al., 2014; Papazoglou, 2007). But studies on the tolerance and phytoremediation potential of sugarbeet to soils contaminated with heavy metals are scarce and not relevant. Therefore, the aim of the present study is twofold: (i) to investigate whether sugarbeet accumulates heavy metals, resulting in potential entry into the food chain, and (ii) to combine possible phytoremediation with an energy crop, in order to achieve a low-cost soil decontamination agent via the biofuel production. The tested heavy metals were Cd and Ni. Cadmium is one of the most dangerous heavy metals for plants, since it can be toxic even in low soil concentrations (Alloway, 2005; Kabata-Pendias and Pendias, 2001). Exposure to Cd can have inhibiting effects on the metabolic activities of plants (Keunen et al., 2016; Zouari et al., 2016), disrupt their cellular homeostasis (Howladar, 2014), negatively affect their photosynthesis (Liu et al., 2017), nutrient uptake, transport, and relocation (Zouari et al., 2016), and inhibit plant growth (Gill et al., 2012; Ma et al., 2017). Nickel is a natural component of soil (parent material) and comprises approximately 0.008% of the content of the earth's crust (Ahmad and Ashraf, 2011; Alloway, 2005; Kabata-Pendias and Pendias, 2001). It is an essential mineral nutrient for plants playing an important role in plant metabolism, but the amounts required for normal growth are very low (Ahmad and Ashraf, 2011; Chen et al., 2009). High Ni concentrations may turn toxic to plants inhibiting photosynthesis, seed germination, plant growth and productivity (Ahmed and Häder, 2010; Boominathan and Doran, 2002; Parlak, 2016; Velikova et al., 2011).

2. Materials and methods

2.1. Experimental setup

Surface soil (0–20 cm) taken from the area of Markopoulo, in Attica prefecture of Greece, that has not been exposed to any kind of industrial or municipal contamination was used. The soil was air dried at room temperature, followed by screening with a 2-mm sieve and characterized, obtaining the properties listed in Table 1. Soil texture was determined by the hydrometer method, pH was measured in 1:1 soil/water suspension by glass electrode, the organic matter by the Walkley–Black method (Nelson and Sommers, 1982) and the $CaCO_3$ content by the acid neutralization method (Rowell, 1994).

Pots filled with 13 kg of the air-dried soil were placed in a greenhouse and this implies that the findings derived from this study require field validation. Sugarbeet seeds were pre-germinated and four weeks later one seedling was transplanted in each pot and was regularly watered. During transplantation, efforts were made to select uniform seedlings. The plants were grown for 12 weeks until they reached a height of approximately 20 cm. Then the soil was contaminated with nitrate salts of Cd and Ni as shown in Table 2. Each amount of the metal salts was diluted in 500 mL distilled water and was spiked on the soil of each pot. In the control pots 500 mL of clean distilled water was added. Each treatment (Ni, Cd or both) and each concentration tested was replicated three times. The experiment consisted of three groups (Ni, Cd and Ni + Cd) of 12 pots each, in a total of 36 pots.

2.2. Plant measurements and analysis

During the experiment, the plant height (i.e. the distance of the largest leaf tip from the soil surface) and the number of leaves were recorded every 7 days.

Six weeks after the metal addition, or 22 weeks after sowing, the plants were cut just above the soil surface. The taproot was divided into crown and beet by separating the crown from the beet below the oldest leaf insertion. The crown is the uppermost part of the taproot where leaves emerge, morphologically being part of the shoot. The aboveground biomass was washed thoroughly with tap water, and fresh and dry (at 60 °C for three days) weights were measured. Beets were generously rinsed with tap water and then − in order to remove any metal left in the beet surface- they were immersed in 0.01 M HCl for approximately 5 s (Gardea-Torresday et al., 2005). Their fresh and dry (at 70 °C to constant weight) matter weights were determined.

The dried aerial biomass and beets were ground into powder using a micro hammer mill. Subsamples (1 g) were then digested with concentrated HNO_3 on a hot plate with the addition of H_2O_2 (30%) until clear solutions were obtained. The resulting solutions were diluted to 10 mL and filtered with a Whatman No. 42 filter paper. Cadmium and nickel concentrations were quantified by an atomic absorption spectrometer (AAS: 908 AA, GBC Scientific Equipment Pty Ltd, Australia). All chemicals were of analytical reagent grade. Quality control of the analyses was performed by running standard reference materials of metals (E-Merck, Germany) after every 6 samples.