BIOACCUMULATION OF IRON IN RAPESEED UNDER NATURAL AMENDMENTS INFLUENCE

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Abstract. The present study reveals the results of a phytoremediation process applied to contaminated soil by many heavy metals, located proximity to an energy power plant. Phytoremediation process was studied using the rapeseed cultivation, both in the presence and absence of hemp shives, considered as potential natural soil amendments. The physiological responses of the rapeseed plants, such as variations in length and accumulation of biomass were investigated in other paper. The concentrations of Fe (II) ions have also been determined in soil and plants aiming at locating the metal ions in different organs of the plants. Presence of hemp shives stimulated iron bioaccumulation, promoting the translocation of ions to the aerial part of the plant.

INTRODUCTION

Phytoremediation is a cost effective and eco-friendly “green” remediation technology for environmental cleanup. Among the phytoremediation techniques, the phytoextraction consists in removal of trace elements from soil through their uptake and accumulation by plants (Nascimento and Xing, 2006). By harvesting the resulted plant biomass that contains heavy metals, these are removed from the site. This technique is effective only if the plants accumulate high concentrations of metals/metalloids in shoots (Stingu *et al.* 2010; Stingu *et al.* 2011) and a reasonable amount of biomass is produced (McGrath and Zhao, 2003). Phytoextraction is best suited for the remediation of diffusively polluted areas, where pollutants occur superficially in a relatively low concentration. Phytostabilisation is considered to be another phytoremediation technique, which aims at establishing a vegetation cover and causing in situ inactivation of trace elements by combining the use of metal-tolerant plants with soil amendments. These result in decrease of mobility and toxicity of pollutants concomitant with increase of soil fertility and improvement of plant establishment. The plants recommended for phytostabilisation should retain the metals at the root level restricting their transport to aerial parts thus avoiding the further transfer into the food chain (Wenzel *et al.* 1999).

In this context, the current study examined the possibility to enhance iron tolerance/ bioaccumulation by adding natural products as hemp shives in soil. The relation between natural products and heavy metal tolerance/bioaccumulation was investigated for *Brassica napus* L. plant, cultivated on multi-metal polluted soils which were sampled from the neighbourhood of an energy power plant.

MATERIAL AND METHODS

The soil samples (S1, S2, S3) taken at different distances from the energy power plant (using coal as fuel) (Table 1) and characterized in terms of total dry matter, water content, pH value and content of heavy metal ions especially Cu (II), Pb (II) and Fe (II) (Tanase et al., 2014).

Table 1. Location of soil sampling sites (Tanase et al., 2014)

Soil sample	Location of soil sampling sites
S1	500 m E from the slag and ash storage yard and 1.5 km from the flue gas stack
S2	700 m SE from the slag and ash storage yard and 1.3 km from the flue gas stack
S3	1.8 km NE from the slag and ash storage yard and 300 m from the flue gas stack

The hemp shives used in this study are wastes resulted after beast fibers separation, as a residue in the form of fibers.

Three different samples of contaminated soils were tested in different conditions, both in the absence (S1, S2, S3) and presence (S11, S22, S33) of hemp shives (1g/30cm^2). The plants have been cultivated in greenhouse conditions using those three samples of contaminated soils (100g/pot). *Brassica napus* L. seeds were directly sown into pots. Each sample was replicated in ten pots, and three uniform plants have been spaced evenly in each pot and were allowed to grow.

The cultivated soils were wetted daily with 15 mL tap water for one week, until germination of the plants was observed. From this point, rapeseed plants were wetted every two days with the same amount of water. After 45 days from the beginning of the experiments, the rapeseed plants were separated into roots, stem and leaves which were characterized in order to evidence the different effects of the cultivation conditions on plants growth and development. This result was published in a previewed work (Tanase et al., 2014).

Determination of iron concentration in plants - For iron ions analysis, plant tissues (root, stem and leaves) were digested with a mixture of nitric and hydrochloric acids similar to the case of soil samples (Tanase et al., 2014).

The following parameters have been calculated:

Translocation factor (TF) = metal concentration in shoots (mg/kg)/metal concentration in roots (mg/kg) (Sun *et al.* 2009).

Bioaccumulation coefficient = (metal concentration mg/kg dry plant tissue)/(metal concentration mg/kg soil samples) (Stingu *et al*. 2009).

Recovery rate (%) is the metal ion concentration in the roots, stems, primary leaves reported on concentration of metal ions in the growth medium (Hajiboland, 2005)

Statistical analysis. All the results are expressed as mean ± standard error where n = 3. Comparison of the means was performed by the Fisher least significant difference (LSD) test ($p \leq 0.05$) after ANOVA analysis using program PAST 2.14. Sampling and chemical analyses were examined in triplicate, in order to decrease the experimental errors and to increase the experimental reproducibility.

RESULTS AND DISCUSSION

The characterisation of soil samples showed a multi-metal polluted soil (Tanase et al., 2014).

Table 2. Characteristic of the soil samples (Tanase et al., 2014)

Sample	Water content (%)	pH value	Lead ions concentration (mg/kg)	Copper ions concentration (mg/kg)	Iron ions concentration (mg/kg)	Cadmium ions concentration (mg/kg)	Zinc ions concentration (mg/kg)
S1	19±1.03 [b]	7.53±0.14 [b]	549±13.67 [a]	330±9.93 [b]	36216±24.32 [c]	0.11±0.00 [a]	74±1.43 [a]
S2	15±0.89 [b]	7.43±0.12 [b]	301±8.86 [c]	464±11.42 [a]	51268±43.21 [a]	0.09±0.00 [b]	65±0.92 [b]
S3	25±1.23 [a]	7.72±0.21 [a]	242±5.49 [d]	344±10.78 [b]	24175±21.33 [d]	0.08±0.00 [b]	55±0.59 [c]

Different letters within columns indicate significant differences ($p < 0.05$). Error bars indicate standard error of the mean (n = 3).

Iron ions in the three soils samples tested (S1, S2, S3), have exceeded the alert threshold laid down in regulations (MWFEP 1997). The highest concentration of iron ions was found in the soil sample S2, located at the 700 m SE from the slag and ash storage yard and 1.3 km from the flue gas stack. Also, in the soil samples, other heavy metal ions were found (Cu, Cd, Zn,). The pollution of soil shall be considered to be potentially significant, because runs the risk of migration of these metals deep in soil thus leading to contamination of groundwater (Tanase et al., 2014).

After determining the concentration of iron ions accumulate in various parts of rapeseed plants has shown that supplementing growth media with hemp shives, causes a reduction in the process of accumulation of metal ions for S22 and S33 variants (Fig. 1). In S11 variant, the concentration of iron ions accumulate in plants that have grown in medium with amendments, is higher comparative with S1.

The calculation of translocation, has shown that the presence of hemp shives in contaminated growth medium, leads to a reduction of transport of metal ions to the upper parts of the plants for variations S11 and S22 and an increase in S33variant (Fig. 2). By calculating the value of the recovery rate (%), shows that the amendments represented by the hemp shives, determines improved recovery metal ions in the growth medium in S11 and S22 variants. A special case is recorded in S33 variant, where the recovery rate of metal ions (Fe) is lower compared to the value recorded for plants that have developed in the absence of the hemp shives. Also, the graph there was a significant recovery of metal ions from the root (Fig. 3).

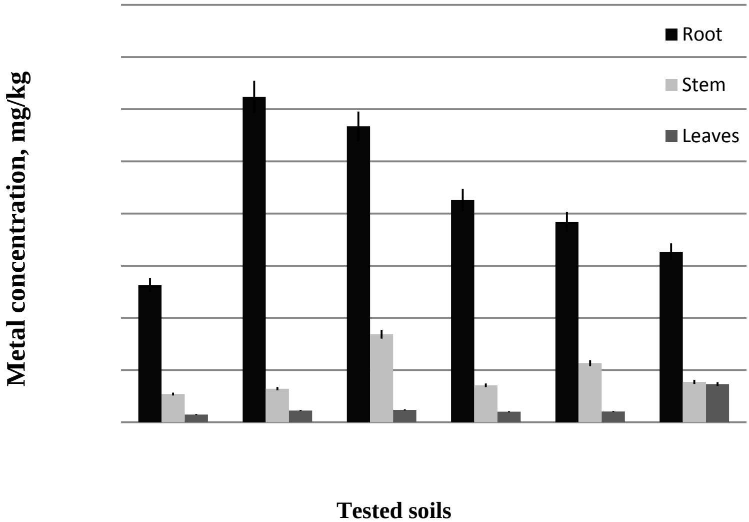

Fig. 1. Bioaccumulation coefficient of Fe (II) in vegetative organs of rapeseed plants. Error bars represent the standard error of means (n = 3)

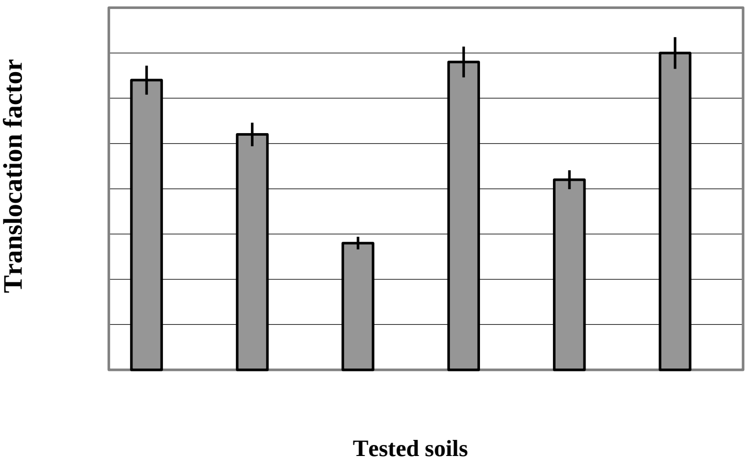

Fig. 2. Translocation factor of rapeseed plants for iron ions. Error bars represent the standard error of means (n = 3)

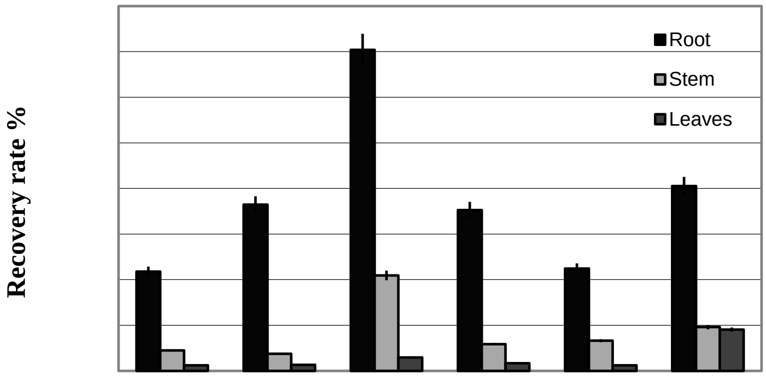

Fig. 3. Recovery rate of iron in vegetative organs of rapeseed plants
Error bars represent the standard error of means (n = 3)

The development of rapeseed plants cultivated is influenced by the presence of heavy metals ions and hemp shives and the following effects were observed in a previous paper (Tanase et al., 2014). An inhibitory effect on biomass accumulation throughout the plants; this consists in decrease in lengths of root and stem or in the amount of biomass accumulated in the different plant organs and a decrease in chlorophyll **a** and **b** contents (Tanase et al., 2014).

CONCLUSIONS

It was determined the concentration of iron ions accumulated in various parts of rapeseed plant. It has been shown that supplementing growth media with hemp shives leads to a reduction of iron ions accumulation process for S22 and S33 soil variants. In the case of S11 variant, the concentration of iron ions accumulated in plants that have in growth medium amendaments is higher than S1.

The calculation of the translocation factor, it is shown that the presence of the hemp shives in growth medium contaminated, leads to a reduction of the transport of metal ions to the upper parts of plants.

By calculating the recovery rate, it appears that the amendaments represented by hemp shives, determines improved recovery of metal ions in the growth medium compared to the value recorded for plants that have developed in the absence of the hemp shives. For all soil variants a significant recovery of iron ions is done in the root.

Bioactive compounds existing in hemp shives soluble in aqueous extracts can modulated the bioaccumulation process of iron ions in *Brassica napus*, depending on heavy metal concentrations.

Presence of hemp shives stimulated iron bioaccumulation, promoting the translocation of ions to the aerial part of the plant.

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