ORIGINAL PAPER

HEMP SHIVES AS NATURAL AMENDMENTS IN CROPS OF RAPESEED (*BRASSICA NAPUS*) AND FLAX (*LINUM USITATISSIMUM*)

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Abstract The aim of this study is to evaluate the possibility of using hemp shives as natural amendments in a phytoremediation sequence. Thus, plant growth tests were conducted for rapeseed (*Brassica napus*) and flax (*Linum usitatissimum*) in vegetation pots with sandy soil. These were seeded in a cadmium artificial contaminated environment with or without hemp shives. The efficiency of hemp shives as natural amendments was evaluated after 40 days of initiating experimental model, by evaluation of plant growth and development of plants through biometric and gravimetric measurements. Also, the concentration of assimilatory pigments was determined. In order to establish bioaccumulation capacity, degree of recovery and translocation factor, it was determined the concentration of cadmium metal ions absorbed in various parts of rapeseed and flax plants. It has been found that the development of the root system is more pronounced in the presence of hemp shives both in contamination with cadmium ions and in the absence. By determining cadmium concentrations accumulate in various parts of the plant have been shown that supplementing growth media with hemp shives, improves the bioaccumulation process of cadmium ions. According to the results, the hemp shives can be used as amendments in phytoremediation process, enhancing bioaccumulation process.

Keywords: amendments, cadmium, hemp shives

1. Introduction

The concentrations of heavy metals in the soil are currently increasing as a result of industrial activities. These, can cause severe damage to biochemical and physiological process in humans and animals (Jankaite and Vasarevičius 2005; Bech et al., 2012; Choi et al., 2013).

Cadmium (Cd) is a heavy metal that is of great concern in the soil with higher toxicity in living systems. Cadmium toxicity especially affects humans, because of their longevity and the accumulation of Cd in their organs by eating contaminated food (Tudoreanu and Phillips 2004). The pathways by which Cd enters in the environment are through industrial waste from processes such as manufacturing of plastics, mining, electroplating etc. (Adriano 2001; Cordero et al., 2004).

In order to ensure the protection of soil, several researches indicated technologies and methods of phytoremediation to neutralize or block the flow of pollutants and to obtain an efficient protection of soil quality (Stingu et al., 2009, 2011). These include physical remediation, chemical remediation, phytoremediation, and agro-ecological engineering techniques (Chen et al., 1999; Chen 2000). Many of this researches indicated that the uptake of heavy metals by plants can be influenced by soil pH (Eriksson 1989), soil organic matter content and plant species (Bingham et al., 1979).

There has been increasing interest in phytoremediation through the use of plants to reduce concentration of pollutants. Phytoremediation is a cost effective and eco-friendly “green” remediation technology. This technique refers to the use of plants to detoxify a polluted environment (Brooks 1998a). The advantages of phytoremediation are that the procedure is carried out in situ and it is inexpensive compared to other technologies. To remediate metal-contaminated soils, usually the method is phytoextraction. The technology relies on plants that translocate heavy metals from soil through their uptake and accumulate heavy metals (Robinson et al., 2000; do Nascimento et al., 2006). This technique is effective only if the plants accumulate high concentrations of metals in shoots (Stingu et al., 2011) and a reasonable amount of biomass is produced (McGrath and Zhao 2003). The plant biomass is removed from the site and can be burned to reduce its volume.

The use of wastes as amendments to improve soil organic matter level and long-term soil fertility and productivity is gaining importance (Kelechi et al., 2018). Phytostabilization technique is to establish a vegetation cover and causing in situ inactivation of trace elements by combining the use of metal-tolerant plants with soil amendments. The results are decrease of mobility and toxicity of pollutants, increase soil fertility and improvement of plant establishment. The plants recommended for phytostabilization 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, Lou et al., 2018). Another approach is the use of soil amendments: material added to a soil to improve its physical properties as water retention, permeability, water infiltration, drainage, aeration and structure to provide a better environment for roots development (Davis and Wilson 2005). In this context, it is necessary to improve methods for removing, reducing or mitigating toxic substances introduced into soil via human activity.

Novel techniques of phytoremediation involve biostimulation of both plant growth and its remediating potential using extracts and exudates obtained from various biological materials. There is evidence that crude plant extracts or exudates may improve the growth and development of plants potentially exploited in phytoremediation (Hanus-Fajerska et al., 2012; Wiszniewska et al., 2013). Stingu et al., (2012) proposed to exploit natural amendments in phytoremediation of Cd. This study has concluded that polyphenolic extracts from *Picea abies* bark and *Asclepia syriaca* plant stimulated cadmium bioaccumulation, promoting the translocation of heavy metals to the stem and leaves.

In other studies (Tanase et al., 2014) a multi-metal contaminated soil from a site located close to an energy power plant was used to study a phytoremediation process using rapeseed plants, in both the absence and presence of hemp shives as natural amendments. It was found that presence of hemp shives in growth medium, stimulated lead and copper bioaccumulation in rapeseed, and promoted the translocation of heavy metals to the aerial part of the plant. The conclusions were that polyphenolic compounds eluted from hemp shives could properly participate in phytoremediation as natural amendments, increasing the solubilization of heavy metals and improving the phytoextraction process. Another effect could be the reduction of Pb and Cu mobility, decreasing thus translocation, and determining in situ inactivation of heavy metal ions. All these make hemp shives suitable for phytostabilization. The mode of the action and effectiveness of numerous amendments in soil remediation is well known.

The aim of this study is to evaluate the possibility of using hemp shives as natural amendments in a phytoremediation sequence with *Brassica napus* L. and *Linum usitatissimum* L. plants, cultivated on sandy soils.

2. Materials and Methods

2.1 Materials

The hemp shives used in this study are wastes resulted after milling process of hemp stem. After, 70-80% of the material is rejected as shives. Sometimes hemp shives are used as low quality construction materials (tiles that contain fiber, gypsum, cement), as bedding for composting in agriculture, or are converted into fuel briquettes. However, the accessibility of these products led currently to their usage as soil amendment in phytoremediation. For the research, the usage of a sandy soil was proposed in order to eliminate the complicated transformations that could take place in a common soil, and to highlight the influence of the amendment used. Rapeseed and flax seeds were purchased from the “Unisem” seed distribution company, Romania.

2.2 Experimental assessment

Sandy soils contaminated with cadmium chloride $CdCl_2.H_2O$ were tested in different conditions (**Table 1**), both in the absence (RC0, RCd, IC0, ICd) and presence (RCp, RCd+p, ICp, ICd+p) of hemp shives (1g/30cm^2). The plants have been cultivated in greenhouse conditions using three samples of contaminated sand (200g/pot). *Brassica napus* and *Linum usitatissimum* 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 sand used for cultivation was wetted daily with 10 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.

Table 1. Experimental variants

Plants treated	Experimental variants (symbol)	Conditions
Rapeseed ***Brassica napus***	Control (RC0)	200g sand, 3 seeds/pot, water
	Control with hemp shives (RCp)	200g sand, 1g hemp shives, 3 seeds/pot, water
	Cadmium (RCd)	200g sand, 100µg Cd (II)/ g sand, 3 seeds/pot, water
	Cadmium with hemp shives (RCd+p)	200g sand, 100µg Cd (II)/ g sand, 1g hemp shives, 3 seeds/pot, water
Flax ***Linum usitatissimum***	Control (IC0)	200g sand, 3 seeds/pot, water
	Control with hemp shives (ICp)	200g sand, 1g hemp shives/pot, 3 seeds/pot, water
	Cadmium (Icd)	200g sand, 100µg Cd (II)/ g sand, 3 seeds/pot, water
	Cadmium with hemp shives (ICd+p)	200g sand, 100µg Cd (II)/ g sand, 1g hemp shives, 3 seeds/pot, water

2.3 Plantlet analysis

Plant growth and development - To evaluate the influence of natural amendment on plant growth and development in Cd induced stress conditions, after 40 days, the rapeseed and flax plants were separated into roots, stems and primary leaves, followed by biometric measurements of plant elongation and quantitative determinations of biomass. Samples of separated plant have been oven dried at 70°C until constant values of mass were reached and the dry biomass was quantified.

Determination of Cd concentration in plants - For Cd ions analysis, plant tissues (root, stem and leaves) were digested with a mixture of nitric and hydrochloric acids (10.5 mL HCl 12 mol/L and 3.5 mL HNO_3 15.8 mol/L) for 12 hours and, subsequently, they were kept on a hot plate for another 2 hours. The concentrations of cadmium were determined by calibration curves obtained using standards solutions of pure cadmium ions (Fisher Scientific). Calibration curves were generated using three replicates per metal concentration (0.02-5.00 μg/mL) by reading the absorbance at fixed wavelength. Determination of the cadmium ions concentration was carried out by reading a fixed wavelength at 228.80 nm, using the right calibration equation:

$y = 0.128x + 0.0039$, $R^2 = 0.9995$,

obtained for concentrations interval between 0.01-1.8.00 μg/mL.

The concentrations of cadmium ions were determined using a GBC 2008 Avanta Atomic Absorption Spectrophotometer. 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).

Photosynthetic pigments assay - The chlorophyll was extracted in 80% acetone and determined spectrophotometrically by measuring the absorbance at fixed wavelengths of 470, 646, 663 nm. The concentrations of chlorophyll pigments were calculated by using the specific coefficients suggested by Lichtenthaler and Wellburn (1983):

Chlorophyll a (μg/mL) = 12.21 (A 663) – 2.81 (A 646)

Chlorophyll b (μg/mL) = 20.31 (A 646) 5.03 (A 663)

Carotenoids (μg/mL) = (100 x A 470 – 3.27 [Chl a] – 104 [Chl b])/22.

2.4 Statistical analysis

All the results are expressed as mean ± standard error, where n = 3. Comparison of the means was performed by Kruskal-Wallis test and Mann-Whitney U test (post-hoc). Sampling and chemical analyses were examined in triplicate, in order to decrease the experimental errors and to increase the experimental reproducibility.

3. Results and discussions

3.1 Characteristics of hemp shives

In a previous paper was found that the total polyphenols content of hemp shives aqueous extract was 16.41 ± 0.62 g/100g (Tanase et al., 2014). The chromatographic analysis revealed the following components: catechin, caffeic acid, p-coumaric acid and ferulic acid. Thus, the amount of catechins was 5.21 g/100 g plant material and caffeic acid was present in a higher concentration (9.68 g/100g) compared with those of other identified phenolic acids.

3.2 The seed germination of rapeseed and flax

When the growth medium previously contaminated with cadmium ions was supplemented with hemp shives, an increase for rapeseed germination energy and capacity was observed. This shows that the introduction of hemp shives in cadmium ions contaminated environment improves germination rate of rapeseed (**Fig. 1**). In the case of flax, the presence of hemp shives in an environment contaminated with cadmium ions has stimulated the germination energy and capacity. When the medium was contaminated with cadmium ions a decrease of the value for this two studied parameters could be observed (**Fig. 1**). The number of germinated seeds in the environment contaminated with cadmium ions was 12% higher in the presence of hemp shives.

3.3 Growth and development of rapeseed and flax

The development of rapeseed root system was more evident in the presence of hemp shives with or without artificial contamination by cadmium ions (**Fig. 2**). Following biometric measurements for flax, no significant differences were found. When hemp shives were introduced in the soil as amendments, there was an increase in the average length of root and stem.

Concerning the amount of plant biomass accumulated in rapeseed, stimulating effects could be seen in case of plants grown on hemp shives supplemented medium (**Fig. 3** and **4**). The percentages for accumulated biomass in treated plants were over those of the control in all experimental variants (**Fig. 4**). It was found that the soil enriched with hemp shives favored vegetative biomass accumulation in all organs of the plant (rapeseed or flax), especially in the roots (**Fig. 3**) and leaves (**Fig. 4**). When hemp shives were added in Cd contaminated environment, an increase in biomass accumulation in all vegetative organs of the plant was observed.

The concentration of chlorophyll pigments (Chl a + b) synthesized in the leaves was higher in rapeseed and flax grown on hemp shives supplemented environment, compared with the control. The same results were obtained for carotenoids. Analysing the variation of photosynthetic pigments concentration in flax plants, there was a stimulation process for all experimental variants unless Cd ion contaminated soil was mixed with hemp shives. In this case the synthesis of chlorophyll-a and carotenoids was higher compared to the control (with 41.85% and 37%, respectively).

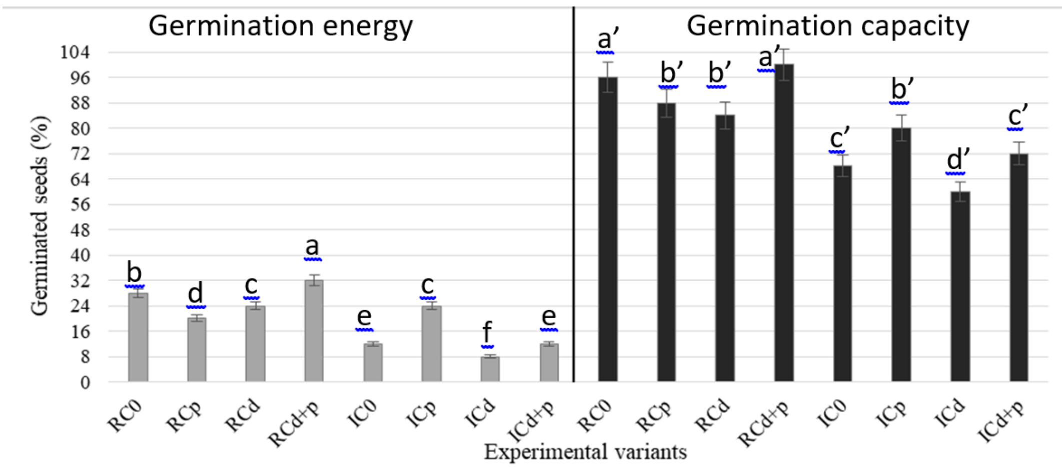

Fig. 1. The influence of hemp shives on germination energy and capacity of rapeseed and flax seeds in cadmium contaminated environment. Different letters means statistical significant differences at $p \leq 0.05$. Error bars represent the standard deviation of means (n = 3). The explanations of experimental variants are in Table 1.

Table 2. Variation of photosynthetic pigments content in leaves of rapeseed and flax plants

Experimental variants	Chl a µg/g	Chl b µg/g	Caroten µg/g	Chl a+b	Chl a/b
RC0	782.75±12.54 g	143.60±2.21 e	187.40±4.7 g	926.35	5.45
RCp	989.57±21.74 e	196.02±3.54 b	209.59±5.44 f	1185.60	5.05
RCd	800.65±10.21 f,g	136.60±5.57 e	183.22±7.41 g	937.25	5.86
RCd+p	900.73±5.22 e,f	170.30±9.71 c	207.24±9.11 f	1071.03	5.29
IC0	1116.91±8.41 c	187.46±8.55 c	317.41±4.54 c	1304.37	5.96
ICp	1269.76±9.55 b	242.18±2.22 a	346.13±3.87 b	1511.94	5.24
ICd	945.29±11.33 e	137.09±5.87 e	284.61±5.21 d	1082.38	6.90
ICd+p	1340.90±22.87 a	201.93±5.11 b	389.93±6.17 a	1542.83	6.64

Different letters means statistical significant differences at $p \leq 0.05$. The explanations of experimental variants are in Table 1.

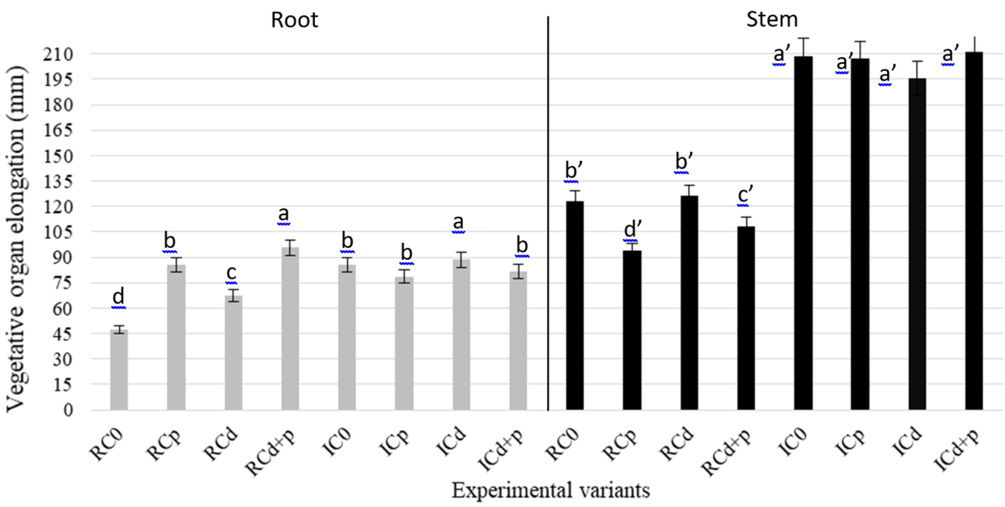

Fig. 2. The influence of hemp shives on root and stem elongation of rapeseed and flax seeds in cadmium contaminated environment. Different letters means statistical significant differences $p \leq 0.05$. Error bars represent the standard deviation of means ($n = 3$). The explanations of experimental variants are in Table 1.

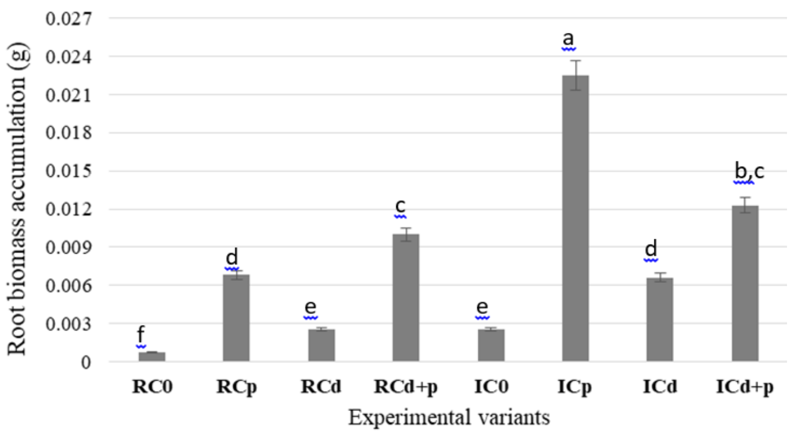

Fig. 3. The influence of hemp shives on rapeseed and flax root biomass accumulation in cadmium contaminated environment. Different letters means statistical significant differences at $p \leq 0.05$. Error bars represent the standard deviation of means ($n = 3$). The explanations of experimental variants are in Table 1.

3.4 Bioaccumulation of cadmium in rapeseed and flax

The determination of cadmium ions accumulated in various parts of the rapeseed and flax plant, showed that soil supplementing with hemp shives improve the bioaccumulation process of metal ions. The presence of the natural amendments (1 g hemp shives) in a contaminated environment with 100µg Cd (II)/g sand stimulated the bioaccumulation process of Cd ions with 6.77% in the case of flax and 42.19% in the case of rapeseed (**Fig. 5**). The same results were obtained for quantification of Cd recovery degree by determining the recovery rate (%).

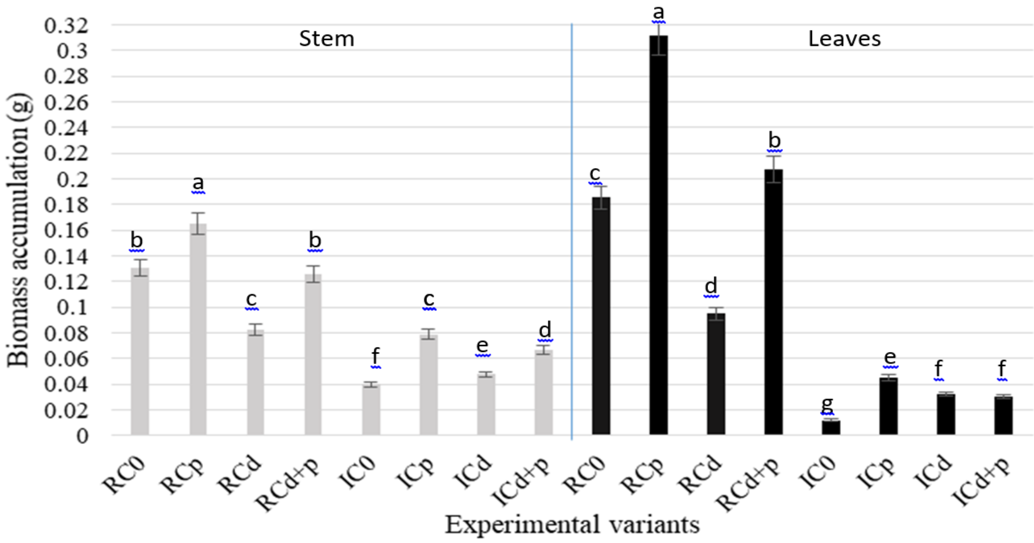

Fig. 4. The influence of hemp shives on rapeseed and flax biomass accumulation in cadmium contaminated environment. Different letters means statistical significant differences at $p \leq 0.05$. Error bars represent the standard deviation of means (n = 3). The explanations of experimental variants are in Table 1.

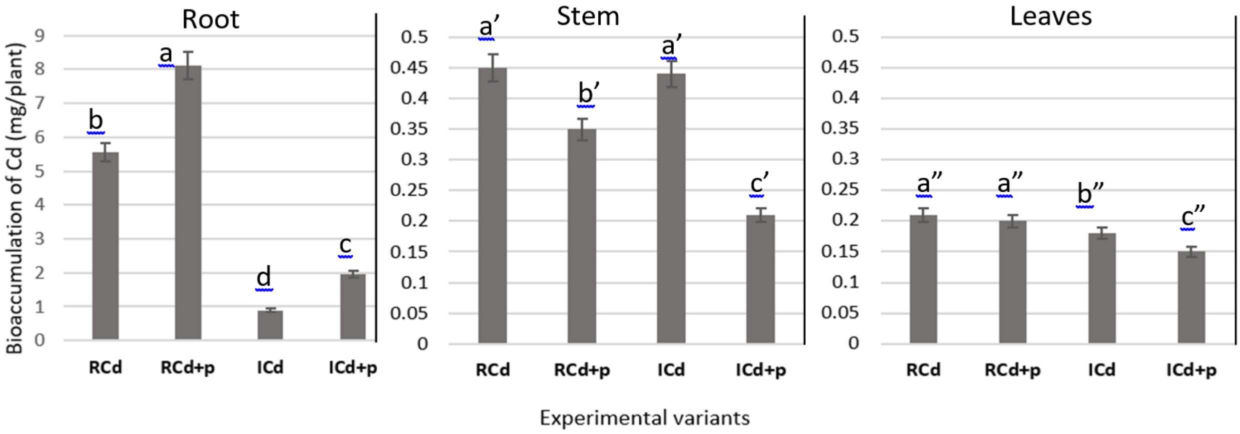

Fig. 5. Cadmium bioaccumulation in rapeseed and flax plants under the influence of hemp shives. Different letters means statistical significant differences at $p \leq 0.05$. Error bars represent the standard deviation of means (n = 3). The explanations of experimental variants are in Table 1.

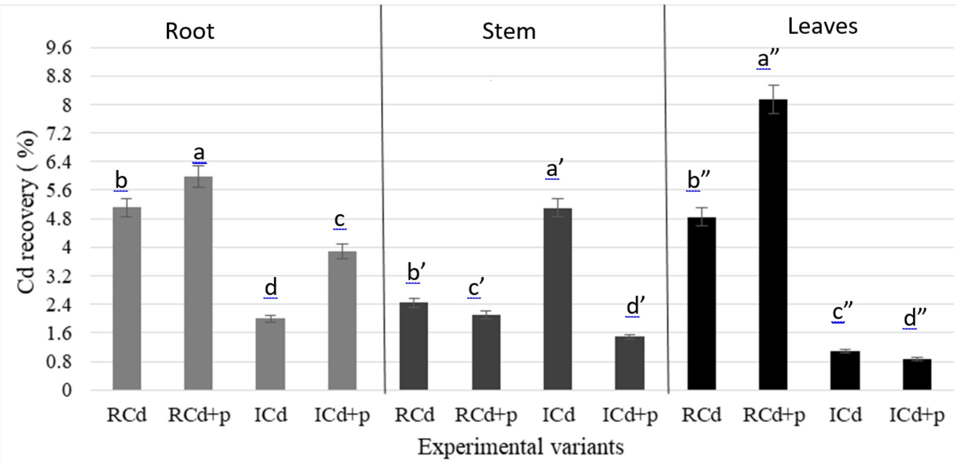

Fig. 6. Cadmium recovery (%) in rapeseed and flax plants under the influence of hemp shives. Different letters means statistical significant differences at $p \leq 0.05$. Error bars represent the standard deviation of means (n = 3). The explanations of experimental variants are in Table 1.

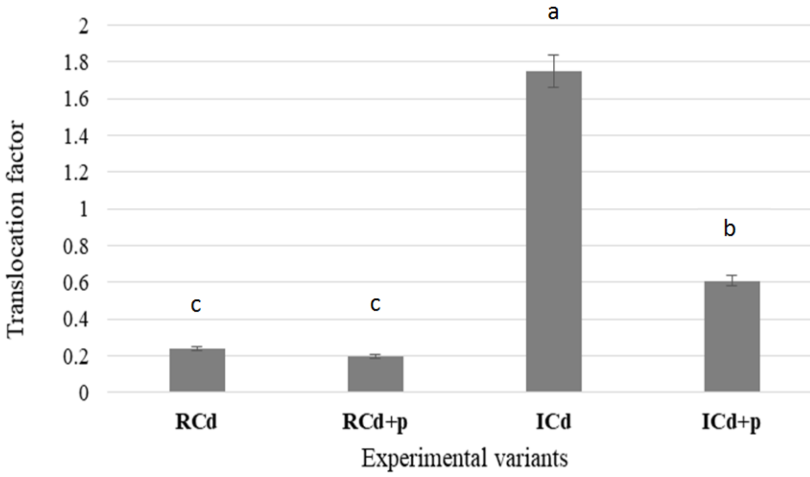

Fig. 7. Translocation factor of cadmium ions in rapeseed and flax plants under the influence of hemp shives. Different letters means statistical significant differences at $p \leq 0.05$. Error bars represent the standard deviation of means (n = 3). The explanations of experimental variants are in Table 1.

In the case of rapeseed there was an improvement with 26% of Cd recovering possibility from environment where hemp shives were added, compared to growth medium without amendments (**Fig. 6**). In the case of flax, growth in the sand with hemp shives, previously contaminated with cadmium ions imparts a positive effect (24.24%) of the recovery degree.

Calculation of a translocation factor - It has been revealed that the presence of hemp shives in sand contaminated with cadmium ions, lead to a reduction in the transport of metal ions to the upper parts of the plants (**Fig. 7**) only in case of flax plants.

It can be concluded that bioavailability of Cd is modulated by plant root exudates and it is attributed to substances from roots, known to contain small organic acids (Nigam et al., 2001). Mobility and bioavailability of Cd and other metals can be controlled by the addition of organic amendment as hemp shives. In soil solution the bioavailability of metals is decreasing with increasing solubilization of soil organic matter, possibly due to the formation of organo-metal complexes (Hernandez-Soriano and Jimenez-Lopez 2012; Wang et al., 2014). In another study, (Stirk and van Staden 2000) cadmium removal by algal biomass was examined.

Hattab et al., (2014a), observed the limited translocation of some metal ions into primary leaves of dwarf bean plants in the result of compost and dolomite limestone addition. Thus, there is evidence that application of organic amendments in the contaminated sites facilitates germination capacity, biomass production, plant survival and productivity for a long-term period and increases plant diversity (Mench et al., 2010).

Sometimes using organic amendments enriches the soil with biochelators. These components can increase mobility and bioavailability of contaminated soil by increasing phytoextraction efficiency.

Doichinova and Velizarova (2013) cultivated seedlings of *Quercus rubra* L. and *Pinus nigra* Arn. in contaminated soil in the presence of paper industry wastes. They observed that amendments improved plant growth conditions in terms of C/N ratio. Despite high concentration of lead and cadmium in the mixture of soil and amendments, alkaline solution limits their uptake by seedlings. For this, agro-industrial wastes could be considered suitable for reducing bioavailability of toxic elements.

Thus, the use of hemp shives as amendments in phytoremediation can lead to enhance the efficiency of the soil cleanup process. However, the quantification and characterization of the natural materials used in phytoremediation are essential. Besides, composition of natural amendments may affect the metabolism of soil microbiota. Studies that include such interactions would be of a significant importance for further evaluation of this approach. It should be highlighted that some waste of both agriculture and industrial origin contain significant amounts of trace metals, and other contaminants detrimental to soils or to the environment (McBride 2003). Therefore, contaminant levels in wastes should be monitored and decision on the use of such products as soil amendments should be preceded by risk assessment.

Conclusions

It has been found that the development of the root system of the studied plants is more pronounced in the presence of hemp shives both in contamination with cadmium ions and in its absence. Soil contaminated with Cd and enriched with hemp shives favors vegetative biomass accumulation in all organs of the rape or flax plant. The concentrations of cadmium ions accumulate in various parts of the plant show that supplementation of growth media with hemp shives, improves the bioaccumulation process of cadmium metal ions. The presence of hemp shives in sand contaminated with cadmium ions, lead to a reduction in the transport of metal ions to the upper parts of the plants. The results obtained allow stating the hypothesis that, hemp shives modulates the bioaccumulation process of cadmium ions in the rapeseed and flax plant. The mechanism is not fully understood but further studies will provide additional information regarding natural amendments involving in bioaccumulation process of metal ions. The hemp shives can be successfully used as amendments in phytoremediation process, enhancing bioaccumulation process.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interst.

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