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Feasibility Study on Reducing Lead and Cadmium Absorption in Sweet Basil (*Ocimum basilicum* L.) With Using Active Carbon

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ABSTRACT

In order to reducing the risk of heavy metals concentration in plant tissues which are among the edible plants by human, find the active carbon ability in heavy metals removal from Lead (Pb) and Cadmium (Cd) contaminated soils and its effect on growth and nutrient absorption of Sweet basil, this greenhouse experiment was conducted in the faculty of agriculture, Islamic Azad University, Karaj branch, Iran. The experiment was conducted in factorial in the form of a completely randomized design with four replications and three treatments: different levels of active carbon (0. 5, 10, 15 and 20 $g.kg^{-1}$ soil), Lead (0 and 4 $g.kg^{-1}$ soil) and Cadmium (0 and 0.08 $g.kg^{-1}$ soil). Results showed that active carbon significantly affected the sweet basil trials. It was observed that the Pb and Cd absorption was reduced in soil contaminated with heavy metal when active carbon was applied. The optimum results were obtained through the application of active carbon in 20 $g.kg^{-1}$ soil treatment. Totally, our results showed that active carbon could improve the basil growth in heavy metal contaminated soil by inhibition of Pb and Cd translocation into the plant tissues. Moreover, it was found that Sweet basil has a high ability in heavy metals removals in polluted regions.

Keywords*:* *Heavy metals, Root dry weight, Soil pollution.*

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INTRODUCTION

Heavy metals are available in various levels in soil and many of them are necessary for biological cells. However, all of the heavy metals are toxic in high concentrations. A heavy metal with undesirable concentration could be considered as a pollutant (McIntyre, 2003). Heavy metals are a major group of inorganic pollutants which contaminated the large scales of lands worldwide. Recently, researchers have focused on the use of cheaper and more efficient technology such as active carbon in order to remediate and purify the contaminated soils. Heavy metal accumulation can reduce the soil quality and consequently the crop safety and also has a lot of risk for human, animal and ecosystems health (Garbisu and Alkorta, 2003; Halim *et al*., 2003). Lead (Pb) is one of the more permanent elements which its half-life in soil is estimated from 740 to 5900 year (Kabata-Pendias, 2010). Natural background concentrations of Pb in soil range from 10 to 30 Pb mg.kg^{-1} soil (Alloway, 1995). The Pb originating from sludge could remain in soil for 150 years after its application (Kumar *et al*., 1995) and also can cumulate in biologic tissues for high durations, as its half-life in blood is estimated to be one month and in the skeleton 20 to 30 years (Järup, 2003). Accordingly, the use of Pb-polluted vegetables could be a main source of diseases in humans. Cadmium (Cd) is another heavy metal that is found in the agricultural fields. Cd contamination is a non-reversible accumulative process, with the estimated half-life in soil varying between 15 to 1100 years (Kabata-Pendias, 2010). The half-life of Cd in human body is estimated to be 10 years and a life-long intake can therefore lead to a Cd accumulation in kiddies, consequently resulting in tubules cell necrosis (Godt *et al*., 2006). The high plant-soil mobility of Cd helps it to be easily accumulated in plant tissues, while high accumulation of Cd in plants not only adversely affects crop yield and quality, but gives rise to a threat to human health via the food chain. Active carbon, as an absorbent, has several applications. This compound is mainly produced from pyrolyzation of plant materials which is then activated. Active carbon has small and low-volume pores that increase the surface area available for adsorption or chemical reactions (Bansal *et al*., 1988; Bansal and Goyal, 2005; Rodríguez-Reinoso, 1997; Van Hulle *et al*., 2008). Active carbon is widely used in agricultural sector and many of its useful effects were reviewed by Dias et al. 2007. Although active carbon can help to increase the soil quality and then food safety, some of the plants also are able to improve the soil quality through heavy metal absorption; the process is known as phytoremediation. Briefly, phytoremediation is the use of plants to clean up environmental pollution (Pilon-Smits and Pilon, 2002; Pivetz, 2001; Van Aken, 2008). Plant species used for phytoremediation should be characterized with high ecological adaptability for growth in different soils and climates, fast growth rate, appropriate root morphology and heavy metal tolerance. Sweet basil (*Ocimum basilicum* L., Lamiaceae) is a commonly used for culinary and medicinal purposes (Putievsky and Galambosi, 1999). The genus is native to the tropical and sub-tropical regions (Lewinsohn *et al*., 2000). Aromatic leaves of Sweet basil are used fresh or dried as a flavoring agent for food, confectionery products, and possess antimicrobial activity (Grayer *et al*., 1996; Hornok, 1992). The use of aromatic plants is suggested as alternative crops for Cd and Pb polluted soils and it has been demonstrated that basil can be grown in soils enriched with Cd and Pb, without risk of heavy metals transfer into the essential oil, and without significant alteration of the essential oil composition that may impair marketability (Zheljazkov *et al*., 2006). Regarding the risks of heavy metals accumulation in soil, plant tissue and human body, and the role of active carbon in reducing their concentration in soil, the present research was conducted to find the active carbon ability in heavy metal removal in the contaminated soils under basil cultivation.

MATERIALS AND METHODS

Field and Treatments Information

The research was carried out as a greenhouse experiment in the faculty of agriculture, Islamic Azad University, Karaj branch, Iran. The experiment was conducted in factorial in the form of a completely randomized design with four replications and three factors, including Active Carbon (AC_1: 0, AC_2: 5, AC_3: 10, AC_4: 15 and AC_5: 20 $g.kg^{-1}$ soil), Lead (Pb: 0 and 4 $g.kg^{-1}$ soil) and Cadmium (Cd: 0 and 8 $mg.kg^{-1}$ soil). Pots were filled with a sandy-loam soil (EC, 2.14; pH, 7.6; Pb concentration, 22 ppm). The soil was treated with active carbon originating from Lemon wood. The active carbon type (Micro, Meso and Macro) and its distribution were determined by electron microscope. In order to induce Pb and Cd pollution, $Pb(NO_3)_2$ (CHEM. LAB Belgium, 99%) and $CdCl_2$ 2 ½ H_2O= 228.35 (CHEM. LAB Belgium) were used respectively. The given salts were dissolved in distilled water and then equally added into the soil in the pots. The polluted soil was kept for one month in order to fix the salt in soil structure. After one month from pollutant application, the powdered active carbon was mixed with soil. Pots were irrigated from bottom. The Sweet basil seeds (10 seed) were planted in the pots. After 90 days from seeding, plants were harvested and were kept in 60°C oven for 48 h. Plant fresh and dry weights were recorded. The plant root was washed and dried in 60°C oven for 48 h.

Heavy metals concentration assay in plant tissue

The powdered plant sample was weighted in 0.1 g and transferred to test tube. Then, 0.5 g of selenium was added to the mixture. Thereafter, 10 cc sulfuric acid was added to the samples. In the distillation stage, the digested sample was poured into the distillation flask accompanied by distilled water and 10 drops of phenolphthalein. In the titration stage, 1 N sulfuric acid was prepared and was added to the solution drop by drop. In order to determine the Pb concentration in plant tissue, 0.1 g of plant sample was first weighted and kept in the hot stove to full burning. Then, 2 N was prepared and the sample washed with chloridric acid and poured into the volumetric flask and diluted in 25 cc with warm water. Thereafter, the extracts were filtered and their Pb concentration was determined by atomic absorption. A same method was used to determine Cd concentration in tissue.

Statistical analysis

Data were analyzed by SAS software (Ver.8) through analysis of variance procedure. The means of treatments were compared by Duncan multiple test at 5% probability level.

RESULTS AND DISCUSSION

Results showed that leaf dry weight was significantly affected by active carbon treatment and also the interaction of Pb and Cd (Table 1). The highest amount of leaf dry weight was obtained by AC_5 which showed 2.89 $g.pot^{-1}$ increase in comparison with control treatment (AC_1, Fig. 1).

Table 1. Analysis of variance for Basil traits affected by heavy metal contaminants.

S.O.V	df	Leaf dry weight	Stem dry weight	Root dry weight	Leaf fresh weight	Stem fresh weight
Active carbon	4	9.595**	4.736**	1.073**	89.83**	133.48**
Pb	1	5.278**	1.012**	0.153**	44.67**	79.42**
Cd	1	8.714**	6.806**	1.143**	78.63**	106.43**
Active carbon × Pb	4	0.236	0.140	0.036**	5.53	3.92
Active carbon × Cd	4	0.352	0.201	0.015	35.91**	4.09
Pb × Cd	1	1.541*	2.665*	0.043*	9.12	9.49*
Active carbon × Pb × Cd	4	0.355	0.376	0.012	8.44	3.34
Error	40	0.23	0.087	0.0061	3.71	1.59
CV (%)	-	18.51	19.79	9.14	15.13	14.02

ns, * and **: non-significant, significant at 5% and 1% probability levels, respectively.

Leaf dry weight was reduced by Pb and Cd in 0.98 and 0.87 g.pot^{-1} values, respectively, in comparison with control treatment (without application of Pb and Cd, Fig. 2). The interaction effect showed that both Pb and Cd significantly reduced the leaf dry weight in sweet basil (Fig 2). Similar to our results, it has been reported that Cd causes a destructive effect on the chlorophyll structure and can reduce the photosynthesis area in plant (Kabata-Pendias, 2010). On the other hand, in a research carried out on *Jatropha curcas* L., it has been reported that leaf growth was inhibited by Pb application (Shu *et al.*, 2012) which a similar occurrence has also been observed in our experiment. It was observed that stem dry weight was significantly affected by active carbon treatment and also the interaction of Pb and Cd (Table 1). Among the active carbon treatments, the control treatment (AC_1) showed the lowest stem dry weight while the highest stem dry weight was seen in high active carbon treatment (AC_5) which showed 2.03 g.pot^{-1} increase in comparison with control treatment (AC_1, Fig. 3). The increase of stem dry weight in active carbon treatment can be attributed to the heavy metal absorption by active carbon. Studying the interaction effect showed that stem dry weight was significantly reduced by pollutants, as the control treatment (Pb_0Cd_0) had the highest value of the stem dry weight while the lower stem dry weight was observed in $Pb_{400}Cd_{80}$ treatment (Fig 4). The main effect of Cd on the plant can be occurred through inhibition in the energy production chains in the mitochondria and chloroplast (Das *et al.*, 1997). Results showed that root dry weight was significantly affected by the treatments and their interactions (Table 2). Although the root dry weight showed an increasing trend with increasing level of the active carbon, adding Pb in the medium caused a slight fall in this increasing trend (Fig. 5). However, it was observed that active carbon increased root dry weight in both application and non-application of Pb (Fig. 5). The highest value in root dry weight was observed with highest amount of active carbon (AC_5) and with the non- application of Pb (Fig. 6). It was observed that both Pb and Cd reduced the root dry weight and this decrease was intensified by Pb and Cd interactions, as the lowest value was obtained in $Pb_{400}Cd_{80}$ treatment. Previously, the root growth reduction by Cd has been reported in the *Trifolium subterraneum* (Joner and Leyval, 1997). There are some other reports (Kopittke *et al.*, 2007; Lagriffoul *et al.*, 1998; Liu *et al.*, 2000; Sandalio *et al.*, 2001) representing that plant roots are seriously damaged by heavy metals (especially Pb and Cd).

Table 2. Analysis of variance for element content variation in Sweet basil.

S.O.V	df	Cd content of leaf	Cd content of stem	Cd content of root	Pb content of leaf	Pb content of stem	Pb content of root
Active carbon	4	24.03**	3.69**	39.11**	56.73**	153.34**	1143.55**
Pb	1	13.54**	1.78	15.91*	64.56**	148.50**	2832.09**
Cd	1	38.01**	2.24*	19.58*	34.94**	176.99**	995.57 **
AC × Pb	4	3.52	1.15	6.78	4.45	13.46	278.51*
AC × Cd	4	3.16	1.49*	7.45	7.09	10.12	109.08
Pb × Cd	1	5.97 *	1.79	13.38*	31.08**	35.19*	44.88
AC × Pb × Cd	4	2.94	1.11	5.94	5.42	11.66	109.96
Error	40	1.41	0.46	3.21	3.53	6.68	64.12
CV (%)	-	22.36	16.42	22.18	17.36	19.31	18.15

ns, * and **: non-significant, significant at 5% and 1% probability levels, respectively.

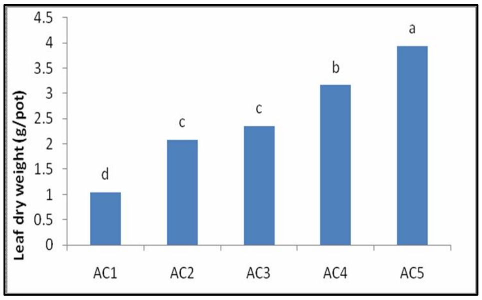


Fig. 1. Mean comparison of leaf dry weight of basil affected by different levels of active carbon via Duncan test at 5% probability level.

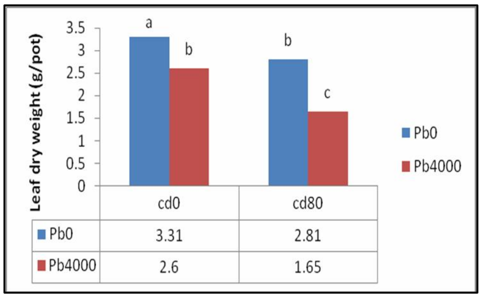


Fig. 2. Mean comparison of interaction effects of Pb and Cd on sweet basil leaf dry weight via Duncan test at 5% probability level.

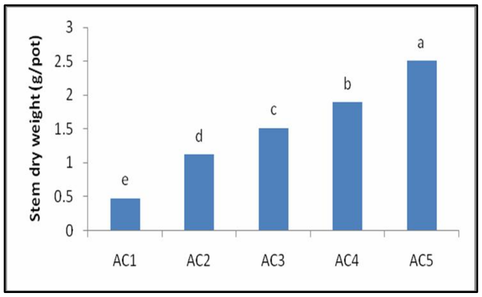


Fig. 3. Mean comparison of stem dry weight of basil affected by different levels of active carbon via Duncan test at 5% probability level.

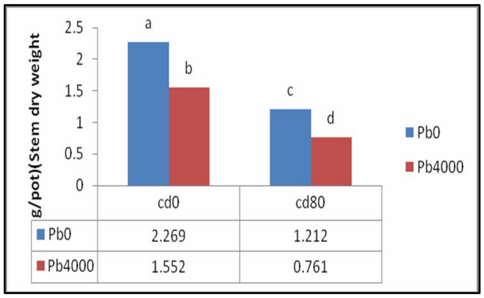


Fig. 4. Mean comparison of interaction effects of Pb and Cd on Sweet Basil stem dry weight via Duncan test at 5% probability level.

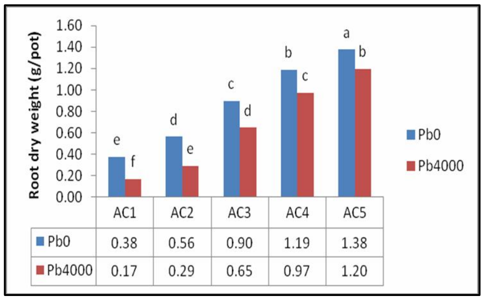


Fig. 5. Mean comparison of interaction effects of active carbon and Pb on sweet basil root dry weight via Duncan test at 5% probability level.

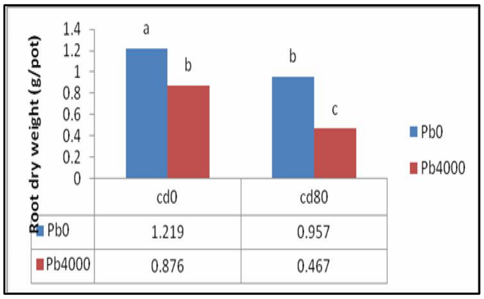


Fig. 6. Mean comparison of interaction effects of Pb and Cd on sweet basil root dry weight via Duncan test at 5% probability level.

Since root is the first part of the plant to sense the heavy metals, its behavior is determinative for plant survival in soils contaminated with heavy metals. Analysis of variance showed that leaf fresh weight was significantly affected by Pb application and also the interaction effect of active carbon and Cd (Table 1). Leaf fresh weight was almost reduced in 4 g.pot^{-1} value by applying the Pb in the medium (Fig. 7). The highest value of leaf fresh weight was obtained by applying 15 and 20 g.kg^{-1} of active carbon (Fig. 8). This increase was alleviated by Cd adding in the sweet basil growing medium (Fig. 8). Reducing the stomata conductance (Nilsen and Orcutt, 1996) may be one of the main reasons for leaf fresh weight reduction under Cd pollution. It has been reported that the Calvin cycle is the most sensitive to Cd toxicity in plant and according to this it can be outlined that photosynthesis and consequently leaf growth is the main target for Cd toxicity (Krupa and Moniak, 1998). According to leaf growth reduction in the sweet basil due to heavy metals, it can be outlined that the heavy metal doses was reached to a toxic threshold, because the optimum growth was inhibited. The stem fresh weight was also significantly affected by active carbon levels and also interaction of Pb and Cd (Table 1). Active carbon application caused a significant increase in the stem fresh weight, as an almost five-fold increase was observed with 20 g.kg^{-1} active carbon application, in comparison with control treatment (without active carbon, Fig. 9). The stem fresh weight was decreased by pollutant application, as a three-fold reduction was observed by adding the Pb and Cd in the sweet basil growing medium (Fig. 10). Stems create a communication pathway between plant leaf and roots and according to this, unwanted variation in their growth affects other parts of the plants. Results showed that experimental factors had a significant effect on Cd absorption in sweet basil leaf (Table 2). Increase of the active carbon in sweet basil growing medium caused a significant decrease in Cd absorption in leaves (Fig. 11). The highest Cd absorption was occurred in AC_1 treatment (without active carbon) while the AC_5 (the highest active carbon level) was more effective than other treatments in inhibiting the Cd translocation into the leaves, as reduced Cd absorption about 4.365 mg.kg^{-1}. With this regard, the ability of activated carbon in removal of heavy metals in several crops cultivation have been reported (Boona mnuayvitaya *et al*., 2004; Goel *et al*., 2005; Kadirvelu and Namasivayam, 2003; Rao *et al*., 2006). The Cd content of Cd treated plant was increased in 6.28 mg.kg^{-1}, in comparison with control treatments (Fig. 12). When Pb and Cd were simultaneously applied, the Cd absorption was reduced in comparison with Cd separately applied condition, in 2.4 mg.kg^{-1} values. It means that a competitive process has been occurred between Pb and Cd in their absorption in sweet basil leaves. The Cd absorption in the Sweet basil stem was significantly affected by the interaction of Cd and active carbon (Table 2). The increase of Cd absorption in the plant stem treated by external application of Cd was alleviated in all levels of active carbon treatments (Fig. 13). Under active carbon free condition, Cd absorption was 0.112 mg.kg^{-1} in sweet basil leaf, while under 20 g.kg^{-1} of active carbon Cd absorption was decreased to 0.009 mg.kg^{-1} (Fig. 13). It seems that active carbon prepared adsorbing spaces to fix the Cd cations and banned Cd from moving into the plant tissues. The Cd and Pb trapping by activated carbon obtained from the *Phaseolus aureus* hulls was reported by Rao *et al*., (2009).

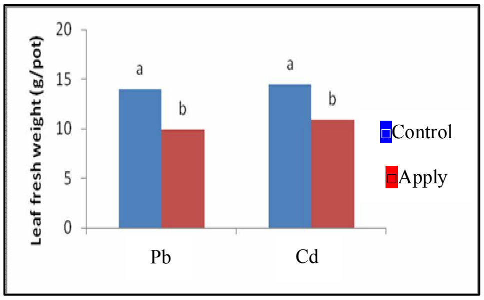
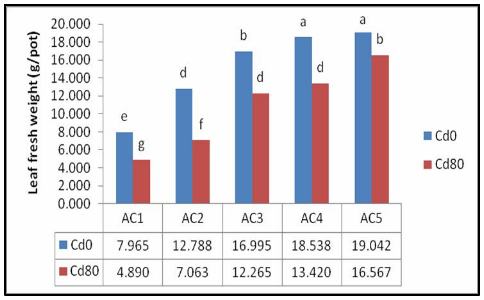


Fig. 7. Mean comparison of the leaf fresh weight in pollutant treatments via Duncan test at 5% probability level.



	AC1	AC2	AC3	AC4	AC5
■Cd0	7.965	12.788	16.995	18.538	19.042
■Cd80	4.890	7.063	12.265	13.420	16.567

Fig. 8. Mean comparison of interaction effects of active carbon and Cd on leaf fresh weight via Duncan test at 5% probability level.

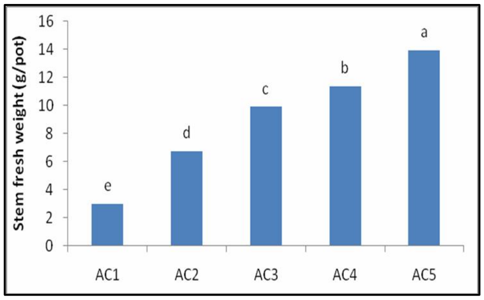
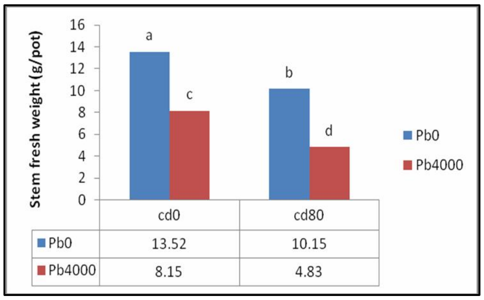


Fig. 9. Mean comparison of stem fresh weight affected by different levels of active carbon via Duncan test at 5% probability level.



	cd0	cd80
■Pb0	13.52	10.15
■Pb4000	8.15	4.83

Fig. 10. Mean comparison of interaction effects of Pb and Cd on sweet basil stem fresh weight via Duncan test at 5% probability level.

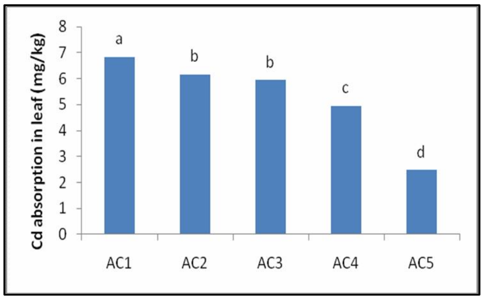
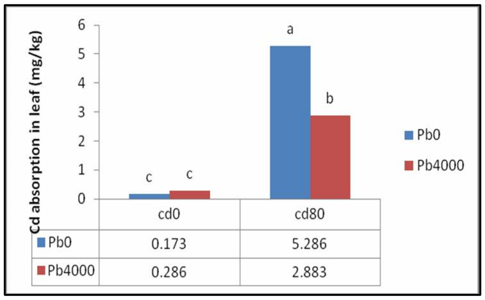
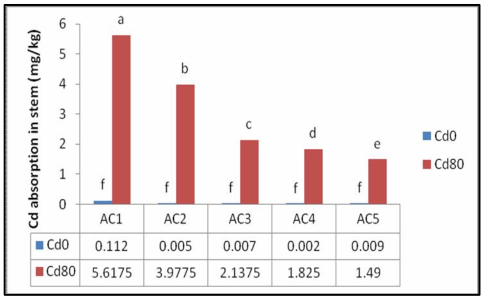


Fig. 11. Mean comparison of the Cd absorption in leaves affected by different levels of active carbon via Duncan test at 5% probability level.



	cd0	cd80
■Pb0	0.173	5.286
■Pb4000	0.286	2.883

Fig. 12. Mean comparison of interaction effects of Pb and Cd on Cd absorption in sweet basil leaves via Duncan test at 5% probability level.



	AC1	AC2	AC3	AC4	AC5
■Cd0	0.112	0.005	0.007	0.002	0.009
■Cd80	5.6175	3.9775	2.1375	1.825	1.49

Fig. 13. Mean comparison of interaction effects of active carbon and Cd on Cd absorption in stem via Duncan test at 5% probability level.

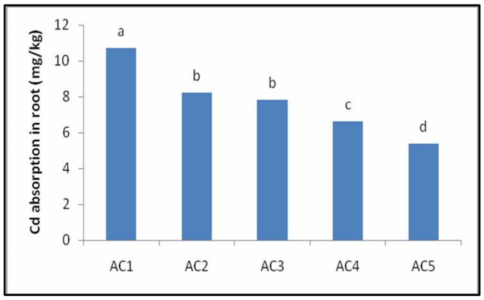


Fig. 14. Mean comparison of the Cd absorption in root affected by different levels of active carbon via Duncan test at 5% probability level.

Results showed that Cd absorption in sweet basil root was significantly affected by active carbon levels and interaction effects of Pb and Cd (Table 2). The maximum Cd was absorbed by sweet basil root under active carbon free condition (AC_1) while the Cd absorption in the root was decreased to a minimum value by applying 20 g.kg^{-1} active carbon, which showed 5.32 mg.kg^{-1} reduction in comparison with control treatment (AC_1, Fig. 14). The Pb application caused a decrease in Cd absorption in the sweet basil root in 3.35 g.kg^{-1} values (Fig, 15); meaning that sweet basil root had an absorbing system which does not allow it to work specifically and absorb heavy metal dependent on their availability in the medium. Previously, a competitive process (antagonism) has been suggested (Kabata-Pendias 2010) to justify the transferring the heavy metal ions into the plant tissues. Same to our results, Cd increase in the basil tissues (leaf, stem and root) under Cd-contaminated soil has been reported (Zheljazkov, et al. 2006); meaning that basil has ability to remove Cd in the polluted soil and can used for phytoremediation purposes. Moreover, it can be noted that basil cultivation in the Cd-contaminated soils could have an optimum yield for farmers and accordingly could be suggested as a compatible crop for this regions. It was observed that Pb absorption in sweet basil leaf was significantly affected by active carbon levels and also interaction effect of Pb and Cd (Table 2). The Pb absorption in the leaf was decreased by the active carbon application and the lowest Pb in the leaves was obtained in the AC_5 treatment which showed 11.6 mg.kg^{-1} reductions in comparison with control treatment (Fig. 16). It was observed that plants treated with Pb had the highest concentration of Pb in their leaves (10.9 mg.kg^{-1} increase, Fig. 17). In addition, it was observed that Cd application reduced the Pb content of the leaf (Fig. 17). Our results are confirming that active carbon trapped Pb and reduced the Pb concentration in the sweet basil growing medium; consequently resulting in the better growth for basil. Previously, the Pb removal by active carbon has been reported by many researchers (Badmus *et al.*, 2007; Goel *et al.*, 2005) and our data also can confirm the previous findings. Results showed that Pb absorption in sweet basil stem was significantly affected by active carbon and interactions of Pb and Cd (Table 2). It was observed that Pb absorption in the sweet basil stem was decreased when the active carbon levels increased (Fig. 18). The lowest absorption of Pb in stem was observed in the plant treated with 20 g.kg^{-1} of active carbon, which showed 20.6 mg.kg^{-1} decrease in comparison with control treatment. It seems that the stem capacity to accept the heavy metal (Pb and Cd) is clearly defined because it was seen that the Pb and Cd content in sweet basil stem was almost equal (15.13 and 15.97 mg.kg^{-1}, respectively). Moreover, Pb absorption in the stem was reduced by adding Cd in the growing medium (Fig. 19). It means that Cd could replace with Pb in the stem tissue. In a same research, it has been shown that active carbon originated from peanut husks remarkably absorb Pb and showed high affinity to Pb^{+2} (Ricordel *et al.*, 2001), which is consistent with the our results. Analysis of variance for Pb absorption of the sweet basil root showed that this trait was significantly affected by Cd availability and also interaction effect of active carbon levels and Pb (Table 2). Also the Pb absorption in the sweet basil root was reduced by use Cd application (Fig. 20).

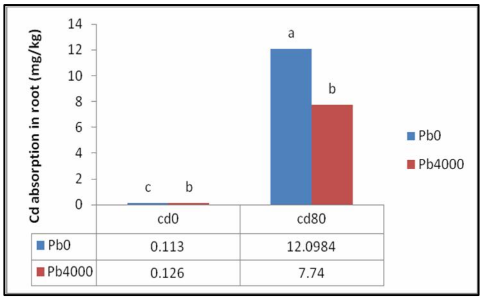


Fig. 15. Mean comparison of interaction effects of Pb and Cd on Cd absorption in Sweet Basil roots via Duncan test at 5% probability level.

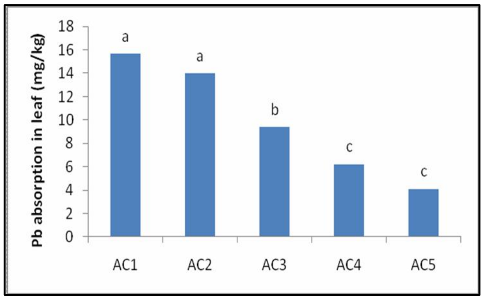


Fig. 16. Mean comparison of the Pb absorption in leaf affected by different levels of active carbon via Duncan test at 5% probability level.

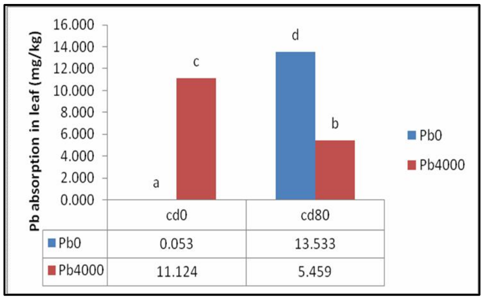


Fig. 17. Mean comparison of interaction effects of Pb and Cd on Pb absorption in sweet basil leaves via Duncan test at 5% probability level.

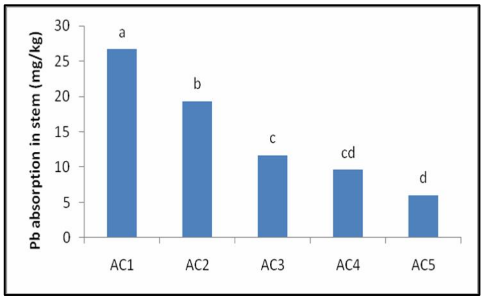


Fig. 18. Mean comparison of the Pb absorption in stem affected by different levels of active carbon via Duncan test at 5% probability level.

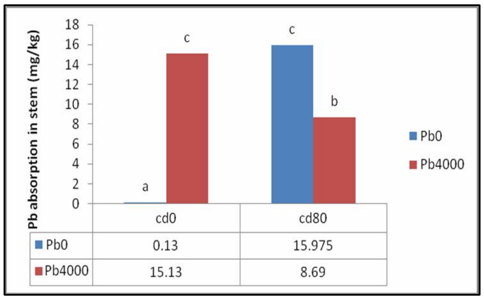


Fig. 19. Mean comparison of interaction effects of Pb and Cd on Pb absorption in sweet basil stem via Duncan test at 5% probability level.

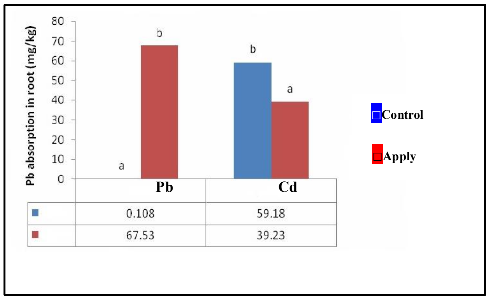


Fig. 20. Mean comparison of the Pb absorption in the sweet basil root under pollutant treatments via Duncan test at 5% probability level.

The same process has been reported by Zheljazkov *et al.* (2006) who observed that Cd availability diminished Pb accumulation in basil. In addition, it was observed that the root Pb content was decreased by increase in the active carbon level (Fig. 21), as the lowest value of Pb in sweet basil root was observed in the plants treated with 25 g.kg^{-1} of active carbon (AC$_5$). The Pb content in sweet basil root in AC$_5$ treatment showed 55.8 mg.g^{-1} decreases in comparison with control treatment (Fig. 21). The root Pb was increased by external application of Pb, increase was diminished by active carbon application (Fig. 21). It means active carbon was able to trap Pb ions. It has been reported endoderm layer could act as an inhibitor factor for Pb absorption in plant (Sharma and Dubey, 2005) which can result in more accumulation of Pb in roots in comparison to other parts of plant.

In our research, it was clearly observed that Pb concentration in root was remarkably more than stem and leaf of sweet basil. Similar to our research, significant accumulation of Pb in basil treated with external Pb has been reported (Zheljazkov *et al*., 2006) demonstrating the basil ability to trap Pb from polluted regions.

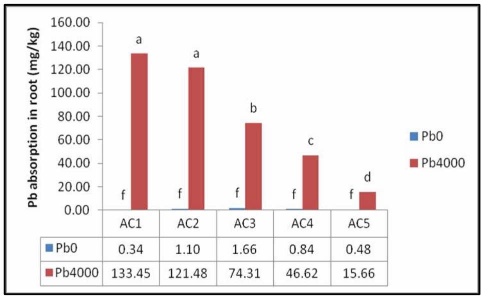


Fig. 21. Mean comparison of the interaction effects of active carbon and Pb on Pb absorption in root via Duncan test at 5% probability level.

CONCLUSION

It was generally concluded that active carbon, due to having nano prosites, is able to trap heavy metal ions in a non-selective manner, as the heavy metals removal by active carbon was observed in our research. It was also seen that Cd and Pb were transferred to the plant tissues in competitive process. Moreover, it was also found that sweet basil has high ability in the heavy metal removal in contaminated regions and can be cultivated for phytoremediation purposes. According to our finding, the use the active carbon is recommended the heavy metal contaminated regions, because of preparing a safe growing condition for food crops is important and increases the food quality.

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