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EVALUATION OF SAGE (SALVIA OFFICINALIS L.) AGRONOMIC TRAITS AND ESSENTIAL OIL CONTENT UNDER UTILIZING OF CADMIUM AND LEAD CONTAMINATED WATER

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ABSTRACT

This study was conducted in order to investigation of different cadmium and lead concentrations on agronomic traits and essential oil content at Ferdowsi University of Mashhad, Iran in 2017. The experiment was conducted as factorial based on the randomized complete block design with three replications. The first factor was cadmium concentrations(0, 10, 20 and 40 ppm) and the second factor was lead concentrations(0, 100, 300 and 600 ppm). Sage plantlets were transplanted at 2 leaves stage. The results indicated that effect of cadmium and lead on fresh weight, dry weight, plant height, essential oil content, cadmium and lead absorption by roots and shoots had significant differences. Fresh weight of shoots at 40 ppm of cadmium and 600 ppm of lead concentrations were declined 4.61% and 5.16%, respectively as compared as control. Dry weights of shoots at the highest concentrations of cadmium and lead were decreased 10.83% and 11.08% as compared as the control. The highest absorption of cadmium by shoots was observed at 40 ppm and 600 ppm of cadmium and lead concentrations, respectively. In this research cadmium and lead were not detected in essential oil. Accordingly, it seems the sage could considered for cultivation under using the contaminated waters by cadmium and lead.

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Introduction

Heavy metals exist almost everywhere in the world because they are used in industrial activities[1,2]. Successively accumulation of heavy metals in agricultural soils by contaminated waters not only lead to soil contamination but also cause to decrease of food quality and security[3]. Among poisonous heavy metals, cadmium and lead are marked because of their permanence in the environment [4]. These metals may enter into food chains and damage the human and livestock health because they are more sensitive to heavy metals. Some plants can absorb heavy metals and accumulate in their organs [5,6]. Poisonous effects of heavy metals cause to DNA destruction of human and animals [7]. Lead toxicity in child cause to damage of children nervous systems and consequently lead to get lost of short memory and the intelligence and heart diseases [8]. Selection of the most appropriate plants under utilizing of heavy metals in the first stage need to evaluation of heavy metals doses on plants growth and their reproduction. Increasing of heavy metals concentrations cause to decreasing the growth and seed germination percent of the most plant species [9].

The effect of cadmium on biomass content led to the decline of plant biomass arising from directly inhibition of chlorophyll synthesis and photosynthesis [10]. Scora and chang[11]reported that biomass and essential oil content of peppermint cultivated in cadmium contaminated soil(at 0/12 to 6.1 ppm) were not affected. Although, Zheljazkov and Nielson[12] expressed that increasing of cadmium, lead, copper, manganese and zinc concentrations led to decrease of shoot plant and

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essential oil yield of peppermint. Zheljazkov et al[13] reported that peppermint and basil shoot dry weights and dill(anethum graveolens L.) height under using of cadmium, lead and copper concentrations of 10, 100, 100 ppm, respectively were not affected.
Medicinal properties of lamiaceae family were considered because their healing characteristics from ancient years. One of the most important species of these family is sage(salvia officinal L.). Essential oil of sage has used for the treatment of diseases of the nerves, heart, vessels and blood circulation and respiration[14]. Some medicinal plants are able to accumulate the heavy metals from contaminated soils. Decline of essential oil yields was reported under heavy metal accumulation in their organs in some medicinal plants[13]. The main objective of this study was evaluation of some agronomic traits and essential oil content of sage under using of contaminated waters by cadmium and lead.

Material and Method

This research was done in research greenhouse at Ferdowsi Unversity of Mashhad, Iran in 2017. The soil was analyzed for chemical and physical characteristics(table 1). Seeds were sown into peat moss in the seedling trays. Uniform transplants were cultivated at 2 leaves stage in the soil of boxes(30×50×35 cm). In each box 6 plantlets were cultivated. Plantlet row spacing was 20 cm and the distance on row was 15cm. The experiment was conducted as factorial based on the randomized complete block design with three replications. The first factor was cadmium concentrations(0, 10, 20 and 40 ppm) and the second factor was lead concentrations(0, 100, 300 and 600 ppm). The plants were irrigated by cadmium and lead nitrate.
The irrigation time was determined based on soil field capacity. During the irrigation period, the water did not get out from the boxes as drainage. In each boxes thick plastics were covered up inside the boxes in order to prevent the water drainage. Plants were irrigated by cadmium and lead solutions once a week. Plants were grown for 15 weeks. After this period the plants were irrigated with distilled water until harvesting time. Phosphate and potassium fertilizers were used as based on soil laboratory recommendations. Because the nitrogen levels of different treatments were different, the amount of highest doses of nitrogen for treatments of cadmium(100 ppm) and lead(600 ppm) was considered and the difference amounts of nitrogen for other treatments were calculated. Then the remainder of nitrogen was added to each treatment for creation the nitrogen balance in all treatments. All sage plants were harvested after 180 days at the beginning of flowering stage. After harvesting, fresh shoots and roots were separately weighed. Shoot and roots were spread out on the ground under shadow condition during 4 days. For measuring of the essential oil content, 30 grams of dry leaves were used in Clevenger device[15].
After harvesting soil of each box was dried and was sifted by sieve(2mm diameter). Then the soil was used for determination of cadmium and lead absorption content by Lindsay and Norvell[16] method. Cadmium and lead content were measured by Atomic Absorption Spectroscopy(AAS) device, Model Analyst 700. Dry shoots and roots were milled and were used for measuring of cadmium and lead content by wet digestion method(digestion with nitric acid and perchloric acid). The plant extraction was obtained by wet digestion method was used for Atomic Absorption Spectroscopy(AAS) device, Model Analyst 700[17]. Data were analized by MSTAT-C software and means were compared with Duncan's Multiple Range Test at 5% probability level.

Table 1. Physical and chemical properties of experimental soil

Soil texture			Potassium (mg.kg) Available-K	pH	EC (dS.m)	Sand(%)	Clay(%)	Silt(%)
Organic matter	**Nitrogen (mg.kg)**	**Phosphorous (mg.kg) Olsen-p**						
0.3	1500	1200	1400	7.46	1.2	52	31	17

Result and Discussion

Shoot Fresh/Dry Weight

Different concentrations of cadmium and lead had a statistically significant effect on the shoot fresh/dry weight of Salvia (sage plant) at %1 probability level ($P \leq 0.01$). However, the Cd-Pb interaction was not significant (Table 2). Shoot fresh weight decreased by %4.61 at Cd 40mg/kg in comparison to the control group while it declined by %5.16 at Cd 600mg/kg (Table 3). Shoot dry weight was lowered by %10.83 at maximum Cd concentration while decreased by %11.08 at maximum lead concentration level compared to the control group (Table 3). Ahmad Kamel [18] studied the effect of lead nitrate (0, 0.048, 0.48, 4.8 and 48 mmol/L) on Vicia faba L. . He reported that the fresh weight of Vicia faba L. decreased by increasing lead concentration.
The findings of Ghaderian and Jamali-Hajiani [19] on matthiola chenopodiifolia L. indicated that shoot dry weight had a significant decrease by increasing cadmium in stock solution (0, 2.5, 5, 10, 20, 30 and 40 mg/l of cadmium chloride)specifying that all the treatments had a significant difference with the control treatment group. The effect of different Cd concentrations (9, 6, 3, 0 and 12 mg/kg) on the varieties of vigna radiata L. showed that the fresh/dry weight decreased in all mug bean varieties by increasing cadmium; nevertheless, different mug bean varieties demonstrated distinct reactions to weight decrease [20]. Zheljazko et al. [21] found that cadmium chloride (0, 2.6 and 10 mg/kg) and lead chloride (100, 0, 50 and 500 mg/kg) did not have any significant effect on the dry weight of Mentha Piperita L., anethum graveolens

L. and ocimum basilicum L. Increased cadmium (0, 0.01, 0.02, 0.05 and 0.1 mg/kg) decreased the root and stem dry weight of gossypium hirsutum L. [22]. Decreased plant growth is caused by cadmium toxicity, reduced photosynthesis and respiration, reduced carbohydrate metabolism and chlorosis [23]. Weirzbicka [24] found that lead ions caused water loss [25]. The primary reason for root susceptibility to cadmium is that not only it is more exposed to this element (Cd) than other plant organs but also it accumulates cadmium in itself more than other organs [26]. Second, heavy metals can disrupt the balance of water absorption from soil and its transfer to the root by altering the permeability of the plasma membrane of the root cells [27]. This phenomenon can prevent the growth of cells and subsequently reduce the plant growth.

Plant Height and Number of Sub-branches

The results of ANOVA showed that cadmium and lead had a statistically significant effect on the stem height ($P \leq 0.01$). however, cd-pb interaction was not significant (Table 2). The plant height decreased by %14.17 and %10.83 respectively at cd 40mg/kg and 600 mg/kg in comparison to the control (Table 3). The results of ANOVA indicated that cadmium and lead as well as Cd-Pb interaction did not have any significant effect on the number of sub-branches (Table 2). The effect of copper sulfate and cadmium sulfate (1^{-5} and 10^{-6}mol/l, respectively) on vicia faba L. reduced its plant height [29]. The effect of cadmium (0, 10, 25 and 50 mg/kg of soil) on solanum nigrum L. showed that the plant height did not decrease up to cd 25mg/kg while did at cd 50mg/kg of soil [29]. Zheljazko [21] investigated the effect of cadmium (2, 6 and 10 mg/l) as well as lead (50, 100 and 500 mg/l) on the growth traits of anethum graveolens L.(dill) and ocimum basilicum L. (basil). He found that the aforesaid concentrations did not have any significant effect on the height of basil plant while reduced the height of dill plant. The effect of different concentrations of cadmium chloride (0, 1, 10, 100 and 500 mg/kg) on the growth of lycopersicum sculentum Mill.(tomato plant) indicated that the plant height significantly decreased at high cd concentrations (100 and 500 mg/kg [30]. Increased cadmium reduced the length of maize plant especially at cd 100 and 200 mg/kg [31]. Studies on the maize plant have shown that increased cadmium resulted in chlorosis in the plant aerial parts, particularly the leaves, and the leaves began to dry. In two different studies, researchers have investigated the effect of hydroponic and soilless cultures on vetiveria zizanioides L. They reported that lead nitrate (0, 10, 50 and 100 mg/l) reduced the plant root and shoot in hydroponic and soilless cultivation. There was a significant difference in the reduction of shoot length (plant aerial parts) in hydro culture while there was not any significant difference between the treatments in the soilless cultures. This plant has been introduced as a living green plant and green refiner due to its insignificant reduction in biomass production, height and essential oils during phytoremediation [32]. For a living green plant, the biomass is usually evaluated at the ending point of the maturity stage and one of the most important characteristics of these heavy metal-resistant plants is the insignificant reduction of their plant biomass and plant height [33]. The biomass does not significantly decrease in such plants unless they are exposed to the critical concentrations of heavy metals. Plant growth is disturbed and reduced only when the concentration of heavy metal elements is increased excessively. The symptoms of such growth disorder and reduction are chlorosis, height decrease, and shoot biomass decline [34]. Shanker et al. state that heavy metals transmitted to the plant shoot reduce the plant height due to the disturbance of cellular metabolism in the aerial parts of the plant [35]. In general, reduced growth may be due to the loss of cellular dilatation, decreased mitotic activity and inhibited prolongation of cells. Furthermore, cadmium inhibits cellular proliferation in cells by affecting the cell walls and middle blade as well as increasing the crosslink between cell-wall components [36]

Table2. Analysis of variance of effect of cadmium and lead on shoot and root fresh and dry weight, plant height, number of branches, essential oil, cadmium and lead content in shoot and root (Mean square)

Source of variation	Degree of freedom	Fresh Shoot weight	Dry Shoot weight	Fresh root weight	Dry root weight	Plant height	Number of branches	Essential oil content	Cadmium content in shoot	Cadmium Content in root	Lead Content in shoot	Lead Content in root
(replication)	2	307.476	61.217	146.199	12.06	162.362	39.396	0.039	12.613	559.056	901.35	444.352
(Cadmium)	3	62.225**	36.5**	176.509**	36.952**	20.229**	1.222ns	0.015**	2077.971**	317015.6**	175.003**	4208.285**
(Lead)	3	74.239**	41.6**	155.329**	37.605**	55.888**	0.444ns	0.019*	3.766**	53.464*	118492.03**	526668.655**
(Cadmium vs Lead intraction)	9	0.671ns	1.422ns	1.636**	0.621ns	0.416ns	0.333ns	0.005ns	0.958**	52.623*	48.489*	553.84**
(Error)	30	1.271	1.287	1.776	0.384	1.127	0.529	0.003	0.311	22.359	20.1	43.43
Coefficient variation		5.04	3.14	5.88	6.42	7.32	7.52	8.21	5.21	9.02	9.46	7.62

significant at 5 and 1 % of probability, respectively:**,*

Table 3. Mean comparisons of effect of cadmium and lead on fresh and dry weight(gr) of shoot and root, plant height(cm), number of branches and essential oil content(%)

Traits / concentrations	Shoot fresh weight (per plant)	Shoot dry weight (per plant)	Root fresh weight (gr per plant)	Root dry weight(gr)	Plant heaight(cm)	Number of branches (per plant)	Essential oil(%)
(Cadmium)							
(Control)	110.7 a	38.01a	75.54 a	20.15 a	42.68 a	10.42 a	0.62 a
(10mg/ kg)	109.2 b	36.85b	72.36 b	18.81 b	41.66 b	10.42 a	0.59 ab
(20 mg/ kg)	107 c	35.85c	69.34 c	17.51c	40.77 c	10.08 ab	0.57 bc
(40 mg/kg)	105.6d	33.89 d	66.65 d	16.06 d	36.63 d	9.75 a	0.54 c
(Lead)							
(Control)	110.4 a	38.42 a	74.6 a	20.2 a	43.65 a	10.33 a	0.62 a
(100mg/ kg)	109.3 b	36.8 b	72.88 b	18.79 b	42.27 b	10.33 a	0.60 a
(300mg/kg)	108.1 c	35.32 c	70.02 c	17.48 c	39.92 c	10 a	0.58 a
(600mg/kg)	104.7 d	34.16 d	66.38 d	16.07 d	38.92 d	10 a	0.53 b

Means with the same letters are not significant at 5%(using Duncan's Multiple Range Test)

Percentage of essential oils, the amount of cadmium and lead in salvia essential oils

According to the results, cadmium and lead had a statistically significant effect on the percentage of Salvia essential oil ($P \leq 0.01$). However, Cd-Pb interaction did not show any significant effect on the percentage of essential oils (Table 2). The percentage of essential oils decreased by %12 and %14.51 respectively at maximum Cd-Pb concentrations in comparison to the control group (Table 3). Zheljazko et al. [21] reported that cadmium (10, 6 and 2 mg/l) and lead (100, 50 and 500 mg/l) treatments did not have any significant effect on the percentage of mentha piperita L. (peppermint) essential oils while significantly reduced the percentage of anethum graveolens L. (dill) and ocimum basilicum (basil) essential oils. Trillini et al. [37] found that hypericin levels in the essential oils of hypericum perforatum L. were not affected by the added amount of chrome element to the culture medium. In an experimental study, the use of a compost containing copper (311 mg/kg), lead (223 mg/kg), molybdenum (17 mg/kg) and zinc (767 mg/kg)reduced the percentage of basil essential oils; however, the essential oils of basil plant lacked heavy metals [38]. Zheljazko and Nielsen [39] reported that the amount of essential oils in the fresh forage of lavandula Vera D. C. was not affected by the amount of heavy metals. The peppermint essential oils extracted from the plant shoot and leaves can in part have a role in the production of dry matter by the plant leaves and stems [40]. According Spak [40], the higher the yield of essential oils, the higher the yield of dry matter of the plant. Although Scoura and Chung [11] did not find any significant difference in the essential oil yield of mint plants cultivated in the composts contaminated with heavy metals, Topalov and Zheljazko [41] found that the essential oil yield of peppermint cultivated in composts contaminated with heavy metals decreased significantly. On the other hand, the synthesized terpenoids in the epidermal cysts of the peppermint plant consume the carbons produced through photosynthesis [42]. Consequently, the synthesis of essential oils in the epidermal cysts requires continuous provision of photosynthetic carbon and any disturbance in feeding carbon by heavy metals can decrease the amount of essential oils [43]. In this experimental study, the amount of heavy metals in the essential oils of Salvia was not detected in any experimental treatments. Therefore, no results were found in this regard. This issue indicates that not any cadmium and lead entered the essential oils in the intended concentrations. Stancheva et al. [44] reported that the heavy metals of cadmium, lead, zinc and copper were not detected in sage essential oils at high concentrations. Zheljazko et al. [21] studied the effect of cadmium, lead and copper on peppermint, dill and basil plants. They found that these heavy metals were not transferred from plant tissue to the essential oils during the process of distillation of essential oils. This critical finding reinforced the conclusion that the use of aromatic plants can be an appropriate alternative to crops in the lands contaminated with cadmium, lead and copper, which was in line with the findings of other researchers [12,45].

Absorption of cadmium and lead in plant shoot and root

Different concentrations of cadmium and lead had a statistically significant effect on the absorption of these elements by Sage's shoot ($P \leq 0.01$). The effect of cd-pb interaction was significant on the absorption of cadmium in the plant shoot at %1 probability level while it was significant at %5 probability level on the absorption of lead in the plant shoot. The results of ANOVA indicated the significant effect of cadmium and lead on the amount of cadmium ($P \leq 0.05$) and lead ($P \leq 0.01$) absorption in the plant root (Table 2). The cd-pb interaction had a statistically significant effect on the amount of cadmium ($P \leq 0.05$) and lead ($P \leq 0.01$) absorption (Table 2). The maximum amount of cadmium absorption by the plant shoot was related to the cd 40 mg/kg and Pb 600 mg/kg concentration treatments. The next category belonged to concentration treatments of cd 40 mg/kg along with Pb 300 mg/kg as well as cd 40 mg/kg along with pb 100 mg/kg. Apart from the control treatment, the plant shoot of sage showed the minimum amount of cadmium absorption respectively at cd 10 mg/kg (0 lead), cd 10 mg/kg and pb 100 mg/kg concentration treatments (Table 6). With increased amount of cadmium and lead in irrigation water and soil, the likelihood of their absorption by the plant increased (Table 4).

The amount of cadmium increased by %13.6 in the plant shoot by rising its concentration from 0 to 10 mg/kg at the maximum pb concentration. Moreover, cadmium raised to %21.3 by elevating its concentration from 10 to 20 mg/kg.

However, with an increase of cd 20 to 40 mg/kg in its concentration, its amount rose to only %14.29 in the plant shoot i.e. the amount of cadmium descended by %33 compared to its concentration at cd 20 mg/kg (Table 6). Therefore, it seems that the maximum lead concentrations had antagonistic effects on the absorption of cadmium in the shoot of the sage plant. The percentage of cadmium absorption dropped to %51 in the root of the sage plant by increasing cadmium concentration form Ca 10 to 20 mg/kg at pb 600 ppm; the decrease in the root of the sage was due to the increased cadmium in the shoot of the sage plant. Nonetheless, the increased cadmium from cd 20 to 40 mg/kg reduced its amount by %51.89 in the root of the plant; meanwhile, the amount of cadmium descended in the shoot of the sage plant as well (Table 6). It seems that the antagonistic effects of these heavy metal elements hindered their absorption in both the shoot and root of the sage plant at the maximum concentration of cadmium and lead (40 and 600 mg/kg). With increased cadmium concentration from 0 to 10 mg/kg, the percentage of lead ascended to %0.85 in the shoot of the plant. On the other hand, with increased cadmium dose from 10 to 20 mg/kg and 20 to 40 mg/kg, the percentage of lead rose to %1.29 and %4.07 respectively in the shoot of the sage plant while it ascended to %10.78 and %16.96 respectively in the root of the plant. Thus, the percentage of lead transmitted to the shoot was lower than absorbed cadmium and it seems that lead has less been able to be absorbed by the shoot of the sage plant in the presence of cadmium.

Johna et al. [46] studied the effect of cadmium (0, 150, 300, 600, 450, 700 and 900 μmol/L) and lead (300, 150, 0, 1200, 900, 600 and 1500 μmol/L), as a cd-pb sulfate solution, on the culture medium of brassica juncea L. They found that the plant root hindered the transfer of cadmium and lead to the plant shoot. Increased amount of cd-pb in the culture medium of the plant upraised the absorption of these heavy metals in the shoot of salvia splendens var sello torreador [47]. Factors affecting the absorption of nutrients and heavy metals include pertaining soil and plant factors. Genus, species and genotype are the plant factors that play a critical role [48]. Different plants absorb and transfer various amount of lead to the shoot of the plant [49]. Increased concentrations of cadmium and lead in soil augmented their absorption in the roots of plants while fewer amounts were transmitted to the aerial parts (shoots) [50]. Lead accumulation was higher than cadmium amount in the sage root, which was consistent with the findings of other researchers. Weirzbicka reported that lead stimulates the thickening of the cell-wall of the roots and helps the synthesis of new polysaccharides in the cell walls. The new cell walls will be able create new sites for cation bonding and increase the accumulation of cations[51]. Brennan and Shelley found that accumulation of higher amount of lead may associate with lead confusion as a form of unsorted lead phosphate in the cell wall of the root [52]. Malkowski et al. [50] reported that calcium accumulation in the roots and its transfer to the shoots are less influenced by lead while slowed down by cadmium. Cadmium, which is unable to bond to the root through the apoplast pathway, can compete with the calcium transfer system. Therefore, unlike lead which is in competition with bonding sites in plasma membrane, the effect of cadmium on calcium ions happens across the plasma membrane due to its competition with calcium transfer [50]. Although most studies have highlighted the easy transfer of cadmium from root to stem [53], cadmium transfer from plant root to stem is disturbed at high cadmium concentrations [54]. Lead is mainly absorbed by the plant root and is rarely transferred to the stem [53, 21]. In cases of high levels of lead concentrations in the soil, the percentage of lead transfer from roots to the shoots of basil, dill and peppermint plants increased accordingly [21]. This may contribute to the destruction of plasma membrane due to high levels of lead concentration and reduction of barriers to lead transfer from soil to the plant [55]. According to Mitchell, the absorption of heavy metals is associated with the process of chemiosmosis in the cell membranes of roots [56]. Lead is unevenly distributed in the root cells which hinder lead transfer through symplast or apoplast pathway. Therefore, lead transfer from plant root to plant shoot is constrained. Although tolerance to heavy metal stress and their transfer from plant root to plant stem are negatively correlated and tolerance to heavy metal stress improves by increasing the preservation of heavy metals in the roots, the preservation of heavy metals in the roots per se is not necessarily the factor of resistance [57]. Furthermore, cadmium transfer from plant root to plant shoot is a crucial factor affecting cadmium accumulation in the aerial tissues of the plant.

Table 4. Mean comparisons of effect of cadmium and lead on cadmium and lead contents in shoot and root(mg.kg)

Traits / Concentrations	Cadmium content in shoot(mg/kg)	Cadmium content in root(mg/kg)	Lead content in shoot(mg/kg)	Lead content in root(mg/kg)
(Cadmium)				
(Control)	0 d	0 d	92.11 b	222.8d
10 mg/kg	4.94c	78.26 c	93.82 b	243.1 c
20 mg/kg	8.11 b	172 b	95.88 b	253.4 b
40 mg/kg	29.81 a	376.5 a	100.9 a	267.2 a
(Lead)				
(Control)	10.10 b	158 a	0 d	0 d
100 mg/kg	10.43 b	157.4 ab	41.98 c	153.4 c
300 mg/kg	10.96 a	153.7 b	113.7 b	369.6 b
600 mg/kg	11.37 a	157.1ab	227.1 a	463.5 a

Means with the same letters are not significant at 5%(using Duncan's Multiple Range Test)

Table5. Mean comparisons of interactions of cadmium and lead on fresh and dry weight of shoot and root(gr), plant height(cm), number of branches and essential oil content(%)

Cadmium vs. Lead	**Shoot fresh weight(gr per plant)**	**Shoot dry weight(gr per plant)**	**Root fresh weight(gr per plant)**	**Root dry weight(gr per plant)**	**Plant height(cm)**	**Number of branches**	**Essential oil(%)**
Control	112.9a	39.58 a	78.45 a	21.37 a	44.77 a	10.67	0.66 a
Cd(0)* Pb(100)	111.9ab	38.47 ab	76.79 ab	20.73 ab	43.76 ab	10.67 a	0.64 ab
Cd(0) * Pb(300)	110.3 cd	37.15 bcd	74.84 bcd	19.89	41.67 cd	10 ab	0.61 abc
Cd(0) * Pb(600)	107.8 efgh	36.82 bcd	72.09 ef	18.62 de	40.53 de	10.33 ab	0.57 abcd
Cd(10)* Pb(0)	111.1.abc	38.89 ab	75.76 bc	20.94 ab	43.97 ab	10.67 a	0.63 ab
Cd(10) * Pb(100)	110.3 bcd	37.07 bcd	74.15 cde	19.39 cd	42.98 abc	10.33 ab	0.61abc
Cd(10) * Pb(300)	109.3 cde	36.15 d	71.96 ef	18.11 e	40.76 de	10 ab	0.59 abc
Cd(10) * Pb(600)	106.3 gh	35.28 de	67.57 h	16.79 f	38.94 ef	10.66 a	0.54 bcd
Cd(20)*Pb(0)	109.6 sde	38.35abc	73.3 def	19.85 bc	43.32 abc	10.65 ab	0.61 abc
Cd(20) * Pb(100)	108.5def	36.3 cd	71.25 fg	18.24 e	41.63 cd	10 ab	0.59 abc
Cd(20) * Pb(300)	106.9 fgh	35.12 de	68.31 h	16.88 f	39.23 ef	10 ab	0.57 abcd
Cd(20) * Pb(600)	102.9 i	33.65 ef	64.49 i	15.06 g	38.92 ef	10 ab	0.52 cd
Cd(40) * Pb(0)	108.1 efg	36.85 bcd	70.91 fg	18.64de	42.52 bcd	10 ab	0.57 abcd
Cd(40) * Pb(100)	106.7 fgh	35.37 de	69.35 gh	16.78 f	40.7 de	10 ab	0.55 bcd
Cd(40) * Pb(300)	106 h	32.45 fg	64.98 i	15.03 g	38.01 f	10 ab	0.55 abcd
Cd(40) * Pb(600)	101.7 i	30.89 g	61.37 j	13.78 h	37.29 f	9 b	0.47 d

Means with the same letters are not significant at 5%(using Duncan's Multiple Range Test)

Table6. Mean comparisons of cadmium and lead interactions on cadmium and lead contents in shoot and root(mg/kg)

Cadmium vs. Lead	**Essential oil content(%)**	**Cadmium content in shoot(mg/kg)**	**Cadmium content in root(mg/kg)**	**Lead content in shoot(mg/kg)**	**Lead content in root(mg/kg)**
(Control)	0.66 a	0 g	0 g	0 g	0 i
Cd(0) * Pb(100)	0.64 ab	0 g	0 g	39.52 f	128.5 k
Cd(0) * Pb(300)	0.64 abc	0 g	0 g	105.3 e	335.3 g
Cd(0) * Pb(600)	0.57 abcd	0 g	0 g	223.6 b	427.3 d
Cd(10) * Pb(0)	0.63 ab	4.63 f	80.15 ef	0 g	0 i
Cd(10) * Pb(100)	0.61 ab	4.83 f	85.3 e	41.39 f	150.6 j
Cd(10) * Pb(300)	0.59 abc	5.03 f	15.73 f	108.4 de	368.4 f
Cd(10) * Pb(600)	0.54 bcd	5.26 f	74.42 f	225.5 ab	453.5 c
Cd(20) * Pb(0)	0.61 abc	7.5 e	172.1 cd	0 g	0 i
Cd(20) * Pb(100)	0.59 abc	7.5 e	171.1 cd	48.48 f	161.7 i

Cd(20) * Pb(300)	0.57 abcd	8.33 df	166.3 d	114.6 d	378.3 f
Cd(20) * Pb(600)	0.52 cd	9.1 d	178 c	226.5 ab	473.4 b
Cd(40) * Pb(0)	0.57 abcd	28.27 c	382.2 a	0 g	0 i
Cd(40) * Pb(100)	0.55 bcd	29.40 b	372.7 b	44.55 f	172.9 h
Cd(40) * Pb(300)	0.55 abcd	30.47 a	3.375 ab	126.4 c	396.4 e
Cd(40) * Pb(600)	0.47 d	31.31 a	375.9 ab	232.7 a	499.7 a

Means with the same letters are not significant at 5%(using Duncan's Multiple Range Test)

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