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Effect of Cadmium and Lead on Quantitative and Essential Oil Traits of Peppermint (*Mentha piperita* L.)

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Abstract

Cadmium (Cd) and lead (Pb) are particularly noteworthy metals that can pollute the air, soil and water contributing to serious environmental problems. Tests were done on concentrations of Pb and Cd; treatments tested in the experiment were as follows; Cd concentrations (10, 20, 40, 60, 80, 100 ppm) and concentrations of Pb (100, 300, 600, 900, 1200, 1500 ppm) and control. Tests were done on *Mentha piperita* L. in a greenhouse, arranged as a randomized complete block design with three replications. Rhizomes with uniform weight were planted in pots 30×50×35 cm. Plants were irrigated with Cd and Pb chloride after germination of all rhizomes. Results demonstrated that with increasing concentrations of Cd and Pb there was a decrease in fresh and dry weights, main stem height, leaf area per plant, leaf number, number of nodes per main stem and essential oil of peppermint compared to the control. Fresh weights were decreased at 100 ppm of Cd and 1500 ppm of Pb, 18.16% and 24.55%, respectively compared to the control at the first harvest. At the second harvest, these decreases were 15.24% and 32.72%, respectively. At the highest concentrations of Cd and Pb, dry weight of peppermint was dropped 22.92% and 39.01% at the first harvest. For the second harvest, decreased dry weights were 25.88% and 26.77% respectively. It seems that peppermint can tolerate waste water or soil polluted with medium range of Cd and Pb concentrations and the essential oil percentage was not affected by these concentrations.

Keywords: heavy metals, leaf area, medicinal plant

Introduction

In the last decade medicinal plants have become more widely used in medicine (Blumenthal, 1998) and industry (Lorenzi and Matos, 2002). Medicinal plants are currently important plants in ecological agriculture. Cultivation of these plants in polluted soil or soils irrigated with waste-water may contribute to the contamination of end products. Furthermore these polluted products are not safe for human consumption and may create serious health problems (Rai *et al*., 2004). Research has shown that some medicinal plants can be cultivated in soil contaminated with heavy metals without evidence of these hazardous elements in end products (Zhejazkov *et al*., 2008). Peppermint (*Mentha piperita* L.) is an aromatic plant from the family of Lamiaceae, which is a hybrid of *Mentha aquatica* L. and *Mentha spicata* L. This plant has traditionally been used in medicine (Blumenthal, 1998). Peppermint oil is rich in menthol, which is a great component in the manufacturing of perfume and essence (Lorenzi and Matos, 2002). Peppermint cultivate in some Porivnce like Fars, Markazi, South Khorasan, North Khorasan, Kermanshah province. Peppermint is cultivated in some parts of Iran as a medicinal plant for industrial purpose and fresh use. Mostly Peppermint is cultivated by small holder farmers differed from year to year (100 to 1100 ha) and the cultivation area statistics is not clear. Industrialization has been the cause of much environmental pollution and one of the most significant pollutants is that of heavy metals. Heavy metals can cause air, water and soil pollution and may create serious problems in the biosphere (Emese *et al*., 2009; Street *et al*., 2007). Heavy metals exist worldwide as their use is widespread in industry (Chen Wang and Wang, 2005; Singh *et al*., 2004). Irrigation of soil with waste-water polluted with heavy metals not only causes soil pollution, but may also affect food quality and security because of gradual accumulation of heavy metals (Muchuweti *et al*., 2006). Cd and Pb are more noteworthy than other heavy metals because they are more persistent in the environment (Pendey *et al*., 2007). Heavy metals may accumulate in the food chain becoming hazardous for humans and livestock that are more sensitive than plants in terms of absorption of these elements (Liu *et al*., 2006). Toxic effects of heavy metals may cause damage to

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human DNA, particularly in children (Baudouin, 2002). For example toxicity from lead causes damage to the nervous system and consequently causes loss of intelligence and short-term memory, decreased learning ability and carcinogenic diseases (Soghian, 2009). Plants suitable for phytoremediation may be identified through investigation of germination parameters, plant growth and production under heavy metals contaminated media. It was reported that increasing amounts of certain heavy metals causes a decrease of germination and plant growth parameters in many plants (Jun *et al*., 2009; Marques *et al*., 2007; Sengar *et al*., 2008). Loss of plant biomass by increasing levels of Cd toxicity occurred because processes of chlorophyll synthesis and photosynthesis were inhibited (Padmaja, 1990). Scora and Chang (1997) reported that *Mentha peperita* L. planted in polluted soil contaminated with Cd at 0.12 to 6.1 ppm concentrations did not demonstrate altered biomass or essential oil components, but Zheljazkov and Nielsen (1996) indicated that with increasing Cd, Cu, Pb, Mn and Zn concentrations, fresh yield and essential oil yield decreased. Scavroni demonstrated that peppermint can be cultivated as a good phytoremediator in polluted soil as it was capable of developing without accumulation of these metals in its tissue (Scavroni *et al*., 2005). Zheljazkov *et al*. (2006) reported that with increasing concentrations of Cd, Pb and Cu from 10 to 100 ppm, dry matter of peppermint, basil (*Ocimum basilicum* L.) and dill (*Anethum graveolens* L.) were not affected. The main goal of this study was investigation of Cd and Pb impact on fresh and dry weight, stem height, number of nodes in main stem, leaf area, number of leaf per plant and essential oil percent of peppermint.

Materials and methods

The experiment was done in the research greenhouse of the Agricultural Faculty of Ferdowsi University of Mashhad in 2011. Experimental treatments were Cd concentrations (10, 20, 40, 60, 80 and 100 ppm), Pb concentrations (300, 600, 900, 1200 and 1500 ppm) and control (Khorrami Vafa *et al*., 2012 a, b; Zheljazkov *et al*., 2006). The treatments were arranged basis on a randomized block design with three replications. Solutions were prepared with Cd and Pb cholride and distilled water was used as the control. Peppermint was cultivated with uniform weight rhizomes harvested from the research farm of Ferdowsi University of Mashhad, Iran. Every rhizome had two buds and 6 rhizomes were planted in pots of dimensions 30×50×35 cm. The soil of pots was brought from the farm of Ferdowsi University of Mashhad. Irrigation of pots with Cd and Pb solutions was done on the basis of field capacity when all rhizomes were germinated. Distilled water was used for the control. After irrigation with Cd and Pb solutions, all pots were irrigated with distilled water for subsequent applications. Nitrogen, phosphorus and potassium fertilizers were applied according to results of the soil analysis. First and second harvests were carried out at the stage of 10 percent flowering. After every harvest, number of leaves per plant, main stem height, number of nodes per main stem, leaf area per plant and fresh weight of plants were measured. All plants were dried in shading for 72 hours and dry weights of plants were determined. Essential oil percentage was measured by clevenger with distilled water. In this method 30 grams of peppermint leaves were dried and soaked in 400 ml of distilled water and then were boiled in a round-bottom flask. After 120 min, heating was stopped and essential oil percentage was measured. (Croteau *et al*., 2006). Data were analyzed by MSTATC software. All means were compared with Duncan's Multiple Range Test at 5 % level.

Results and discussion

Fresh and dry weight

Results of analysis of variance indicated that various concentrations of Cd and Pb had significant impact ($p \leq 0.01$) at the first harvest (Tab. 1 and 2). Increasing doses of Cd caused a decrease of fresh weight. This decline was 18.16% at 100 ppm Cd and was 24.55% at 1500 ppm of Pb compared to the control. At the first harvest, dry weight declined by 25.88% at 100 ppm of Cd and decreased by 39.01% at the Pb concentration of 1500 ppm compared to the control (Tab. 2). Cd and Pb on fresh and dry weights of peppermint had significant impact at 5 ($p \leq 0.05$) and 1 percent ($p \leq 0.01$), respectively (Tab. 3). At the second harvest the highest doses of Cd and Pb caused decreases of fresh weight by 15.24% and 32.72% respectively compared to the control and decreases of dry weight by 22.93% and 26.77% respectively compared to the control, (Tab. 4). Another research tested the effect of $PbNo_3$ concentrations (0, 0.048, 0.48, 4.8 and 48 mM) on *Vicia faba* L. and reported that increasing Pb decreased fresh weight (Ahmed Kamel, 2008). Increasing doses of Cd (0, 2. 5, 5, 10, 20, 30, 30 mg/l, $cdcl_2$) caused a decline of dry weight of the aerial part of *Matthiola chenopodiifolia* L. and all treatments showed significant difference compared to the control (Ghaderian and Jamali Hajiani, 2010). Increasing Cd doses (0, 3, 6, 9, 12 mg/kg) on *Vigna radiata* L. decreased fresh and dry weights in all varieties, but varieties responded differently (Ghani, 2010). It was indicated that the impact of $Cdcl_2$ (0, 2, 6, 10 ppm) and $Pbcl_2$ (0, 50, 100, 500 ppm) had significant effects on dry weight of *Mentha piperita* L., *Anethum graveolens* L., *Ocimum basilicum* L. (Zheljazkov *et al*., 2006). Incremental Cd doses (0, 0.01, 0.02, 0.05, 0.1 ppm) caused a decrease of root and shoot dry weights of *Gossypium hirsutum* L. (Aycicek *et al*., 2008). Tests were done on *Thlaspi caerulescens* L. treated with Cd (2.5 mg/l) for 14 days. Results indicated that aerial dry weight and root dry weight decreased by 14% and 28%, respectively (Wojcik *et al*., 2005). It was indicated that leaf dry weight of *Arabidopsis halleril* L. treated with 2.5 mg/l of Cd decreased by 26% compared to the control (Cosio *et al*., 2004). *Salsola kali* L. was introduced as a tolerant plant Cd and it was reported that a dose of 5 mg/l of Cd caused a decrease in aerial dry weight of 31% (Rosa *et al*., 2004). The effect of 5 mg/l of Cd on corn and pea affected dry weights of aerial parts by 81% and 41%, respectively (Lozano-Rodriguez *et al*., 1997). It seems reduction in fresh and dry weight of peppermint were due to decrease in photosynthesis and respiration (Moya *et al*., 1993), reducing carbohydrate metabolism (Wierzbicka, 1995) and probably chlorosis (Sanita di Toopi and Gobbrielli, 1999). Disturbance in water availability (Patra *et al*., 2004), inhibition of nutrient uptake (Sanita di Toppi and Gabrielli, 1999) and reduced synthesis of protein or degradation of it (Blaestrasse *et al*., 2003) are some of the other reasons for declining of fresh and dry weight of peppermint. Wierzbicka reported that Pb ions caused water deficit because they disrupted the water balance. (Wierzbicka, 1995). Increasing doses of Pb (0, 10, 20, 30, 40 ppm) had significant negative effects on water availability. High doses of Cd in plants may interfere in plant growth in different methods. Enzyme activity (Van Assche and Clijster, 1990), photosynthesis inhibition (Vassilev and Yordanov, 1997), stomal closure (Barcelo and Poschenrieder, 1990) and inhibition of nutrients uptake are all possible effects on plant growth that may have occurred from Cd. Increasing doses of Cd (0, 10, 20, 50, 100 μm/l) caused decrease of root growth resulting in decreased nutrients and water uptake and reduced respiration (Chen *et al*., 2003). Protein synthesis was also affected by Cd. Protein content of *Cicer arietinum* L. exposed to high doses of Cd, decreased by 38% percent compared to the control (Bavi *et al*., 2011). Reduction in protein content may have been due to reduced synthesis or degradation of protein (Blaestrasse *et al*., 2003). It was reported that a reduction of aerial parts of plants as a result of Cd may be because of reduced chlorophyll contents and photosynthesis type I activity (Shah *et al*., 2008). In some concentrations of Cd (10, 20, 40 ppm) or Pb (100, 300, 600, 900, 1200) fresh and dry weight of peppermint were not significant differences(Tab. 2) in the first and second harvest.

Some researchers reported that in hyper heavy metals accumulator plants, dry weight was increased more than control with increasing of heavy metal concentrations (Tang *et al*., 2009). On the basis of experiment of Tang *et al*. (2009) with increment of Cd concentrations from 0 to 44 ppm dry weight of *Arabis paniculata* Franch increased and after 44 to 267 ppm decreased. In their research the dry weights in medium concentrations were not significant. In our study despite of declining of dry weight with increasing of Cd concentrations in medium doses, the differences between treatments in these concentrations were not significant.

Main stem height

Results demonstrated that tested treatments impacted significantly on main stem height at the first harvest ($p \leq 0.05$) (Tab. 1 and 3). Main stem height of peppermint at the highest doses of Cd and Pb decreased by 17.89% and 3.69%, respectively compared to the control. At the second harvest this reduction was 3.05% and 3.6% from Cd and Pb, respectively. Another research reported effects of $CuSO_4$ and $CdSO_4$ (10^{-6} and 10^{-5}) on *Vicia faba* L. to decrease main stem (Kasim, 2005). Increased Cd in the soil of 25 mg/kg caused plant height to decline in *Solanum nigrum* L. (Sun *et al*., 2008). It was reported that plant height of *Anethum graveolens* L. under concentrations of Cd (2, 6, 10 mg/l) and Pb (50, 100, 500 mg/l) decreased, but the height of *Ocimum basilicum* L. was not affected by Cd and Pb (Zheljazkov *et al*., 2006). A study on Cd doses (0, 1, 5, 10, 100, 500 ppm) on *Lycopersicum sculentum* Mill. indicated that a high level of Cd (100 and 500 ppm) caused a significant decrease in plant height (Singh *et al*., 2011). An increment of Cd concentration caused a reduction of plant height in corn especially at concentrations of 100 and 200 ppm (Mihalescu *et al*., 2010). In the afore-mentioned study, with an increased concentration of Cd, yellowness appeared in the aerial part of plants, especially in leaves, consequently leaves gradually dried. In two studies in hydroponic and pot cultivation on *Vetiveria zizanioides* L. using $PbNo_3$ doses both cultivation methods caused a decrease of root and shoot growth. This reduction was significant in hydroponic culture. *Vetiveria zizanioides* L. was introduced as a green phytoremediator and hyper-accumulator because traits of biomass, plant height and essential oil contents were not significantly affected by Pb (Rotkittikhun *et al*., 2010). A plant can be classed as a hyper accumulator if no reduction in plant biomass and height is recorded from cultivation in heavy metals con-

Tab. 1. Analysis of variance (Mean square) of impact of Cd and Pb on fresh weight, dry matter, main stem height , leaf area per plant, number of leaf per plant, number of node in main stem, leaf essential oil in the first harvest of *Mentha piperita* L.

	Degree of freedom	Fresh weight	Dry weight	Main stem	Leaf area	Number of leaf per plant	Number of node per main stem	Leaf essential oil
Replication	2	170.459	77.98	52.88	25025.076	2046.015	16.157	0.54
Treatment	12	48.84	29.50	16.958	2768.415	148.527	1.448	0.031
Error	24	12.376**	6.425**	5.734**	822.668**	29.65**	0.459**	0.015**
Coefficient variation(%)		5.60	8.59	7.35	8.62	6.25	5.32	5.11

*,**: Significant at 5 and 1% probability levels, respectively

taminated soil, these evaluations are important in terms of defining a plant as tolerant to heavy metals (Yang *et al*., 2004). In these plants biomass did not reduce under the lowest doses of heavy metals to critical amounts but with an increment of heavy metals concentrations from critical and later doses, plant growth was inhibited. Inhibition of plant growth was observed as yellowness, reduction of stem height and plant biomass (Wei and Zhou, 2004). In this study the impact of Cd doses (0, 20, 40, 60, 80, 100 ppm) had no significant effect on peppermint at the first harvest (Tab. 2). Increasing concentrations of Pb from 0 to 1200 had no significant effect on peppermint at the first harvest, but with increasing concentrations of Cd from 1200 to 1500 ppm, plant height significantly decreased. Although, plant height reduced at different doses of Cd and Pb, differences between concentrations was not significant (Tab. 4). It seems increasing Cd and Pb concentrations gradually decreased growth of peppermint and decreased cell length which caused to decrease the plant height, as it was mentioned by other researchers. For instance it was reported that when heavy metals were transferred to the aerial parts of plants causing disturbance in cell metabolism and reducing plant height (Shanker *et al*., 2005). Reduction in growth may be due to loss of cell turgor and reduction in mitosis activity or inhibition of cell elongation. For example Cd in plant cells can influence cell walls specially the middle layer of a cell wall that causes an increasing in cell width instead of cell length (Hassan *et al*., 2006).

Leaf area per plant

Effects of Cd and Pb concentrations on leaf area of peppermint was significant at the first harvest ($p \leq 0.01$), but was not significant at the second harvest (Tab. 1 and 3). At the first harvest, leaf area decreased by 7.37% at 100 ppm of Cd and 22.21% at 1500 ppm Pb compared to the control (Tab. 2). Leaf area of *Phaseolus mungo* L. under Cd treatments 0, 10^{-2}, 10^{-4}, 10^{-5},10^{-8} m/l was reported by Siddhu and Khan (2012). Increasing Cd concentrations to above 10^{-2} m/l caused leaf twisting and leaf abscission that reduced leaf area. High doses of Cd in soil (100 and 500 μg/kg) caused a significant reduction in leaf area in tomato (Singh *et al*., 2011). This reduction in leaf area was due to reduced leaf area expansion and leaf senescence. Other research reported a reduction in leaf area of tomato under $CdNO_3$ concentrations (150, 450, 900 mg/kg soil) compared to the control (Zhao *et al*., 2011). Increasing doses of Pb (0, 4, 6, 8 g/kg soil) and Cd (0, 150, 450, 900 g/kg soil) caused a decrease of leaf area of *Phaseolus vulgaris* L by 16.87%, 56.27%, 68.62% and 79.78% for Pb and 22.96%, 63.47% and 84.91% for Cd compared to the control (Bhardwaj *et al*., 2009). It seems that based on literatures the reduction of leaf area of peppermint in our study was due to impact of Pb on hystological changes in leaves (Elzbieta and Miroslawa, 2005).

It was reported that there was a negative relation between the increment of Cd dose and specific leaf area (Barcelo *et al*., 1988 a). Reduction in turgor pressure of plant cells treated by Cd and reduction of cell wall elasticity caused the formation of small cells and fewer spaces between plant cells. Reduction of turgor pressure occurred from a disturbed water balance. Research reported that Cd impacted in terms of decreased water uptake, water translocation and respiration (Vassilev *et al*., 1997). Reduction of water uptake in plants treated with Cd was due to reduced root growth. Hydraulic conductivity of water from roots to xylems depends on the degree of Cd stress and plant characteristics of bean, soybean and *Acer pseudoplatanus* L. that decreased two to four times (Barcelo *et al*., 1988 b; Lamoreaux and Chaney, 1977; Marchiol *et al*., 1996).

The decline in leaf area of peppermint with increasing of Cd and Pb doses were probably due to reduction in cell

Tab. 2. Means of Cd and Pb effects on fresh weight(g), dry matter(g), main stem height(cm), leaf area per plant(cm^2), number of leaf per plant, number of node in main stem, leaf essential oil(%) in the first harvest of *Mentha piperita* L.

Treatment	Fresh weight (g)	Dry weight (g)	Main stem height (cm)	Leaf area(cm^2)	Number of leaf per plant	Number of node per main stem	Leaf essential oil (%)
Control	73.07 a	37.78 a	37.86 a	371.1 a	98.46 a	14.16 a	2.59 a
Cd-10 ppm	64.11 b	30.49 b	33.84 ab	368.1 a	94.49 ab	13.23 ab	2.46 ab
Cd-20 ppm	63.12 b	29.55 b	32.8 b	332.3 abcde	88.54 abcde	12.82 b	2.45 abc
Cd-40 ppm	62.45 b	29.25 b	32.46 b	338.9 abcde	86.55 bcde	12.69 b	2.43 abc
Cd-60 ppm	61.12 bc	28.62 b	31.76 b	309.6 bcde	82.58 cdef	12.41 bc	2.4 bc
Cd-80 ppm	60.46 bc	28.31 b	31.42 b	292.3 cde	80.59 def	12.28 bc	2.38 bc
Cd-100 ppm	59.80 bc	28.00 b	31.08 bc	286.5 e	78.61 ef	12.15 bc	2.38 bc
Pb-100 ppm	65.11 b	30.49 b	33.84 ab	365.9 a	94.49 ab	13.23 ab	2.35 ab
Pb-300 ppm	64.46 b	30.17 b	33.49 ab	355.0 ab	92.51 abc	13.09 ab	2.3 abc
Pb-600 ppm	63.78 b	29.87 b	33.15 b	346.5 abc	90.52 abcd	12.95 ab	2.3 abc
Pb-900 ppm	63.12 b	29.55 b	32.8 b	343.9 abcd	88.53 abcde	12.82 b	2.28 abc
Pb-1200 ppm	61.12 bc	28.62 b	31.76 b	322.2 abcde	82.58 cdef	12.41 bc	2.25 bc
Pb-1500 ppm	55.13 c	23.04 c	27.05 c	291.0 de	74.64 f	11.21 c	2.2 c

Means in treatments followed by similar letter are not significantly different at 5% probability level, using Duncan's Multiple Range Test (DMRT)

Tab. 3. Analysis of variance (Mean square) of impact of Cd and Pb on fresh weight, dry matter, main stem height, leaf area per plant, number of leaf per plant, number of node in main stem, leaf essential oil in the first harvest of *Mentha piperita* L.

	Degree of freedom	Fresh weight	Dry weight	Main stem height	Leaf area	Number of leaf per plant	Number of node per main stem	Leaf essential oil
Replication	2	121.70	17.55	83.28	5260.67	328.615	5.94	0.063
Treatment	12	28.49	8.72	27.24	640.53	41.07	1.26	0.01
Error	24	12.68*	2.69**	10.75*	366.00ns	18.21*	0.42**	0.004**
Coefficient variation(%)	9.58	9.58	8.79	10.75	10.05	8.81	6.28	5.04

*,**: Significant at 5 and 1% probability levels, respectively

division, cell differentiation of cambium cells. Barcelo *et al*. (1988 b) reported that water movement in bean was due to a reduction of vascular ray and vascular bundles caused by Cd induction and cell division, cell elongation and differentiation of cambium cells (Barcelo *et al*., 1988 b). These researches assumed that these events were due to hormonal disturbance but data was not shown in support of this assumption. Another reason for the reduction of water movement was due to the disturbance of vascular structures because of deposition of non-soluble phenolic compounds (Fuhrer, 1982) and lignin and sequestration of calcium oxalate (Van Balen *et al*., 1980). These events caused a reduction of water potential and consequently relative water content in leaves. Therefore, components of leaf water potential, treated by Cd decreased. Reduction of turgor pressure may be due to inappropriate osmotic adjustment because even this is a proper mechanism for stability of water balance in plants (Yancey *et al*., 1982). It was reported that cell turgor in plant leaves treated by Cd had a lower threshold of relative water content compared to the control. The reason for this loss of water was reduced cell wall elasticity due to the decomposition of phenolic and cellulose compounds in cell walls and the replacement of Cd with calcium in the middle lamella inside cell walls (Barcelo *et al*., 1986). Vazquez (1989) reported that reduction of cell elasticity was due to decreased synthesis of cell wall components because of reduced function of Golgi apparatus.

Number of leaf per plant and number of node per main stem

At the first harvest, effects of Cd and Pb concentrations on number of leaves per plant and number of nodes per main stem ($p \leq 0.01$) were significant (Tab. 1). At the second harvest the impact of treatments on number of leaves per plant ($p \leq 0.05$) and on number of nodes per main stem ($p \leq 0.05$) was significant (Tab. 3). Number of leaves per plant decreased at 100 ppm of Cd and 1500 ppm of Pb 21.16% and 24.19% respectively compared to the control at the first harvest. The number of nodes per main stem declined at the highest doses of Cd and Pb 14.19% and 20.83%, respectively compared to the control at the first harvest (Tab. 3). Other research reported that increasing Pb doses (0, 5, 10, 15, 20, 25 µm/M) caused a decrease of leaf number in *Thespesia populnea* L. (Kabir *et al*., 2010). It was reported that incremental concentrations of Cd after 0.1 µm/M caused a reduction of leaf number in tomato. For example 30 days after planting in a concentration of

Tab. 4. Means of cadmium and lead effects on fresh weight(g), dry matter(g), main stem height(cm), leaf area per plant(cm^2), number of leaf per plant, number of node in main stem, leaf essential oil(%) in the first harvest of *Mentha piperita* L.

Treatment	Fresh weight (g)	Dry weight (g)	Main stem height (cm)	Leaf area (cm^2)	Number of leaf per plant	Number of node per main stem	Leaf essential oil (%)
Control	43.02 a	23.64 a	39.78 a	231.3 a	57.33 a	12.18 a	1.27 ab
Cd-10 ppm	39.1 a	19.93 b	31.76 b	194.1 b	49.75 abc	10.53 b	1.29 a
Cd-20 ppm	37.83 a	19.11 b	30.30 b	190.4 b	48.83 bc	10.29 b	1.20 ab
Cd-40 ppm	37.55 a	18.59 b	29.74 b	199.2 ab	51.01 ab	10.15 b	1.21 ab
Cd-60 ppm	36.37 a	18.18 b	28.88 b	181.7 b	46.63 bc	9.96 b	1.18 ab
Cd-80 ppm	36.3 a	18.01 b	28.60 b	177.4 b	45.6 bc	9.88 b	1.16 b
Cd-100 ppm	36.46 a	18.22 b	28.5 b	183.0 b	46.96 bc	9.81 b	1.18 ab
Pb-100 ppm	38.65 a	19.04 b	31.00 b	199.6 ab	51.23 ab	10.66 b	1.25 ab
Pb-300 ppm	38.62 a	18.72 b	30.67 b	191.8 b	49.06 bc	10.53 b	1.22 ab
Pb-600 ppm	37.68 a	18.00 b	30.78 b	186.7 b	48.10 bc	10.38 b	1.24 ab
Pb-900 ppm	36.12 a	17.24 b	30.10 b	186.6 b	47.82 bc	10.41 b	1.23 ab
Pb-1200 ppm	36.72 a	16.95 b	28.96 b	179.2 b	45.98 bc	9.74 b	1.22 ab
Pb-1500 ppm	28.94 b	17.31 b	27.75 b	174.1 b	41.47 c	9.63 b	1.06 c

Means in treatments followed by similar letter are not significantly different at 5% probability level, using Duncan's Multiple Range Test (DMRT)

10 μm/M leaf number reduced by 31.5% compared to the control (Jing *et al*., 2005). It was reported that with an increment of Cd doses (0, 10^{-10}, 10^{-8}, 10^{-5}, 10^{-2} m) leaf number and leaf area of bean decreased (Siddhu and Khan, 2012). It seems absorption and translocations of Cd and Pb in aerial parts of a plant can disturb the cell metabolism in the stem and that will decrease plant height and number of nodes per plant (Shanker *et al*., 2005). Reductions in plant growth may be due to loss of cell turgor and mitosis activity or cell expansion and elongation. These Cd can inhibit the elongation of plant cells by means of replacement Cd instead of Ca in cell walls that increases cell width instead of cell length (Hassan *et al*., 2006). These events may affect the nodes of a stem. It was reported that an incremental dose of Cd reduced plant height, number of leaves and dry weight of tomato (El-Gamal and Hammad, 2003). The reason for this may be the reduction of water uptake, reduction of synthesis of photosynthetic pigments, carbohydrates and soluble sugars. Increment of Cd doses reduced growth and leaf number in soybean, and the maximum reduction of leaf number was 44.4% at 200 ppm of Cd (Abdo *et al*., 2012). Fouda and Arafa (2002) also reported that high doses of Cd reduced plant height, leaf number and leaf area of soybean. An increment of Pb caused water stress and consequently reduced the leaf area, photosynthesis, dry weight and plant height and number of nodes in soybean (Azmat *et al*., 2009).

Essential oil percentage

Impact of Cd and Pb concentrations on leaf essential oil of peppermint was significant ($p \leq 0.01$) at the first and second harvests (Tab. 1 and 3). At the first harvest, essential oil percentage was decreased by 8.10% and 15.05% at the 100 ppm of Cd and 1500 ppm, respectively compared to the control. This reduction was 7.08% and 16.53% at the second harvest compared to the control (Tab. 2 and 4). It was reported that the impact of Cd concentrations (2, 6, 10 mg/l) and Pb concentrations (50, 100, 500 mg/l) on essential oil of peppermint was not significant but decreased essential oil percentages of dill and basil (Zheljazkov *et al*., 2006). The impact of chromium on hypericin content in *Hypericum perforatum* L. was not significant (Tirillini *et al*., 2006). Using the compost consists of Cu (311 mg/kg), Pb (223 mg/kg), Mo (17 mg/kg) and Zn (767 mg/kg) decreased essential oil content of basil, but the essential oil was free of heavy metals (Zheljazkov and Philip, 2003). It was reported that leaf essential oil content of *Lavandula vera* D. C. was not affected by heavy metals (Zheljazkov and Nielsen, 1993). Essential oil of peppermint extracted from leaves and stems may depend on dry matter, which is produced by leaves and stems. More essential oil yield depends on more dry matter of the aerial parts of plants (Czepak, 1998). Although it was indicated that there was no difference between essential oil yields of peppermint cultivated in polluted soil to heavy metals and the control (Scora and Chang, 1997). Essential oil yield of peppermint decreased in plants cultivated in compost contaminated with heavy metals (Topalov and Zheljazkov, 1991). It was demonstrated that terpenoids produced in epidermal glands in peppermint leaves consumed the carbon provided by photosynthesis (Croteau and Johnson, 1984). Synthesis of peppermint essential oil in epidermal glands depends on providing the photosynthetic carbon and disturbance in carbon nutrition that can cause a reduction of essential oils (Srivastava *et al*., 1994). As it was mentioned in literature, it seems synthesis of essential oil of peppermint affected by high doses of Cd and Pb concentrations due to disturbing on carbon translocations and toxic effects of these heavy metals and consequently essential oil percent was reduced.

Conclusion

Increasing concentrations of Cd and Pb decreased fresh and dry weights of peppermint at the first and second harvest compared to the control. Main stem height reduced at the first harvest more than the second harvest. Impact of treatments on leaf area per plant was significant at the first harvest, but at the second harvest it was not significant. Effect of Cd and Pb concentrations on essential oil percentage was significant and increasing doses of Cd and Pb reduced essential oil percentages. Although fresh and dry weights and essential oil percentages decreased with increasing concentrations of Cd and Pb, means comparison indicated that differences between treatments were not significant. It seems that peppermint can be cultivated in conditions of polluted soil or wastewater polluted with Cd and Pb.

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