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Resilience of caregivers of people with dementia: a systematic review of biological and psychosocial determinants

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ISSN 0103-8478 CROP PRODUCTION

Pb and Cd on growth, leaf ultrastructure and essential oil yield mint (*Mentha arvensis* L.)

Pb e Cd no crescimento, ultraestrutura foliar e produção de óleo essencial de hortelã (*Mentha arvensis* L.)

Caroline Nery Jezler[I*] Pedro Antônio Oliveira Mangabeira[I] Alex-Alan Furtado de Almeida[I] Raildo Mota de Jesus[I] Rosilene Aparecida de Oliveira[I] Delmira da Costa Silva[I] Larissa Correa do Bomfim Costa[I]

ABSTRACT

*Contamination of medicinal plants with heavy metals as Pb and Cd can affect the growth and the essential oil production of the plants and represent a risk to those who consume as medicine. This study aimed to evaluate the effects of absorption and localization of Pb and Cd on growth, ultrastructural aspects of leaves and essential oil yield and composition of **Mentha arvensis**, applied on the soil with increasing concentrations (8, 16, 32, 64 and 128mg kg^{-1}). There was a differential absorption of Pb and Cd by **M. arvensis** mainly concentrated in the roots. Pb was found in small amounts in the leaves while Cd largely exceeded the safety limit without symptoms of toxicity. The ultrastructural analysis revealed the metal accumulation on vesicles surrounding the mitochondria and the presence of electron dense deposits surrounding the mitochondria, nucleus and chloroplasts. Little changes caused by Pb and Cd application were not enough to affect the growth and essential oil yield and composition of **M. arvensis**.*

Key words: *contamination, heavy metals, medicinal plant, menthol.*

RESUMO

*A contaminação de plantas medicinais com Pb e Cd pode afetar o crescimento e a produção de óleo essencial das plantas e representa um risco para quem as consome como medicamento. Este estudo teve como objetivo avaliar os efeitos da absorção e localização de Pb e Cd sobre o crescimento das plantas, aspectos ultraestruturais de folhas e sobre o rendimento e composição química do óleo essencial de **M. arvensis**, os quais foram aplicados ao solo em concentrações crescentes (8, 16, 32, 64 and 128mg kg^{-1}). Houve absorção diferencial de Pb e Cd por **M. arvensis**, que se concentraram principalmente nas raízes. O Pb foi encontrado em baixas concentrações nas folhas, enquanto o Cd excedeu largamente o limite de segurança sem demonstrar sintomas de toxicidade. As análises ultraestruturais revelaram o acúmulo de metais em vesículas ao redor de mitocôndrias e a presença de depósitos eletrodensos ao redor de mitocôndrias, núcleo e cloroplastos. As pequenas mudanças causadas pela aplicação de Pb e Cd não foram suficientes para afetar o crescimento das plantas e o rendimento e composição química do óleo essencial de **M. arvensis**.*

Palavras-chave: *contaminação, metais pesados, planta medicinal, mentol.*

INTRODUCTION

Contamination of soils by heavy metals is the most serious environmental problem and has significant implications for human health. Medicinal herbs may be easily contaminated during cultivation and harvesting. After drying and processing the heavy metals confined in plants enter in the human body and may disturb the normal functions of central nervous system, liver, lungs, heart, kidney and brain, leading to hypertension, abdominal pain, skin eruptions, intestinal ulcer and different types of cancers (JÄRUP, 2003).

Herbal plants are dried parts of plants widely used as raw materials for pharmaceutical preparations and for "self-medication" especially of the poorest population. WHO (1998) recommends that medicinal plants which form the raw materials for the finished products may be checked for the presence of heavy metals, further regulating the maximum permissible limits of toxic metals like cadmium and lead, which amount to 0.3 and 10ppm, respectively (WHO, 1998).

[I]Universidade Estadual de Santa Cruz (UESC), Rodovia Ilhéus/Itabuna, km 16, 45662-900, Ilhéus, BA, Brasil. E-mail: carolinejezler@bol.com.br. *Corresponding author.

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Pb is considered an important environmental pollutant, widely found in batteries, paints, glasses, pipes and chemical fertilizers, mainly the phosphatic fertilizers (CASAS & SORDO, 2006). Soils contaminated with this metal can cause severe reductions in crop productivity (SHARMA & DUBEY, 2005), affecting the essential oils production and composition (ZHELJAZKOV et al., 2006), besides promotes ultrastructural changes as chloroplast dilatation and membranes rupture (HU et al., 2007). Cd is considered a heavy metal found naturally in the soil by mineralization processes of some rocks, but it is widely released into the environment through waste incineration, urban traffic, phosphate fertilizers contamination, among others things. High concentrations of this metal can interfere with plant growth as well as morphological and ultrastructural aspects of its organs (TUNG & TEMPLE, 1996). Medicinal plants exposed to heavy metals can differ in production of secondary metabolites, either suppressing or stimulating production (NASIM & DHIR, 2010).

In Brazil, ***Mentha*** spp. grown in gardens, ensure its popular use as a medicinal alternative for antiemetic, nasal decongestant and flu purposes. The commercial cultivation of ***Mentha arvensis*** L. was enhanced in Brazil mainly for menthol exportation, its majoritary oil compound, which is demanded by pharmaceutical, food, cosmetics and perfumery industries.

Despite the large amount of investigations on heavy metal contents in medicinal plants (ABOU-ARAB et al., 1999; ABOU-ARAB & ABOU DONIA, 2000; CALDAS & MACHADO, 2004), there is little information regarding the effects on the secondary metabolite production and ultrastructural aspects. This study aimed to evaluate the effects of absorption and localization of Pb and Cd, applied with increasing concentrations in soil, on the growth, ultrastructural aspects of leaves and the essential oil production and quality of ***Mentha arvensis***.

MATERIAL AND METHODS

M. arvensis L. propagated by stem cuttings was grown in plastic pots with 5kg of soil homogenized and physico-chemically analyzed before the application of treatments registering the following values: pH 5.3, Al 1.3mmol dm^{-3}, Ca 107mmol dm^{-3}, Mg 45mmol dm^{-3}, K 6mmol dm^{-3}, P 169 mg dm^{-3}, S-SO_4^{-2} 10mg dm^{-3}, Fe 283mg dm^{-3}, Mg 21.1mg dm^{-3}, organic matter 86g dm^{-3}, Pb 14mg kg^{-1}, Cd 0.2mg kg^{-1}, thick sand 392g kg^{-1}, fine sand 212g kg^{-1}, silt 259g kg^{-1}, total clay 137g kg^{-1}, natural clay 33g kg^{-1}.

The experiment was conducted in a completely randomized design with eleven treatments (two metals and five concentrations + control) with five repetitions. The data were submitted to analysis of variance (ANOVA) and regression analyses. The models were selected based on significance ($P \leq 0.05$) of F-test.

The seedlings were grown under greenhouse conditions with the metals Pb $(NO_3)_2$ and $CdCl_2.5/2H_2O$ applied separately, at concentrations of 0, 8, 16, 32, 64 and 128mg kg^{-1} of soil, thirty days after planting. Forty days after the treatments application, the plants were harvested for chemical composition, growth, leaf ultrastructure and essential oil production analyses.

A nitric acid digestion of dried leaves and roots was performed in triplicate, for Pb and Cd determination by optical emission spectrometry with inductively coupled plasma ICP-OES - Varian 710 (Varian, Australia).

The growth variables analyzed were: plant height (PH), stem diameter (SD), leaf area (LA) and number of leaves (NL). The leaf (LDB), stem (SDB), root (RDB) and total dry biomass (TDB) were evaluated and the specific leaf area (SLA = LDB/LA) and leaf area ratio (LAR = LA/TDB) were calculated according to HUNT (1990).

For ultrastructural analysis, the median region of fully expanded leaves were collected from the third node, with four repetitions of each treatment, fixed in glutaraldehyde, post fixed with osmium tetroxide, dehydrated in ethanolic series and embedding in *LR White* resin. Samples were analyzed from the control treatment and the highest concentration of Pb and Cd (128mg kg^{-1}). Semifine and ultrathin cuts obtained in a UC6 Leica ultramicrotome were observed in a Morgagni 268D Transmission Electron Microscope (FEI Company, Soft Imaging System, Germany), operating at 80kV acceleration voltage.

The essential oil content (100g g^{-1} dry biomass) and yield (g $plant^{-1}$), were obtained by hidrodestilation of dried leaves in a Clevenger Apparatus in quadruplicate, for an hour. The quantitative oil analysis was performed in a Varian 3800 Saturn gas chromatograph (Varian, Walnut Creek, CA, USA), equipped with VF5-ms capillary column (30m x 0.25mm x 0.25µm) and flame ionization detector. The temperature started at 70°C, then programmed to reach 200°C at 8°C min^{-1} and up to 260°C at 10°C min^{-1}, being maintained at this

temperature for 5min. Helium was used as carrier gas with a constant flow of 1.2mL min^{-1}. The injection volume was 1µL of 10% oil solution in $CHCl_3$, in reason of *split* 1:10. Qualitative analysis was carried out in mass spectrometer Varian Chromopack 2000 (Varian, Walnut Creek, CA, USA) with the same programming and above-mentioned capillary column. The oil components were identified by comparing their mass spectrum in the literature (ADAMS, 2007) with the spectra database (NIST105) and, also, by comparing retention indices with those of literature. Kovats retention indices (KI) were determined using a homologous series of n-alkanes (C_8-C_{26}) injected under the same chromatographic conditions of samples, using the equation of VAN DEN DOOL & KRATZ (1963).

RESULTS

During the experimental period, the plants showed vigorous and uniform growth, without visible symptoms of phytotoxicity. No significant effects were observed ($P<0.05$) on the growth of ***M. arvensis*** with Pb and Cd application to the soil (Table 1).

The Pb content was not detectable in leaves until the maximum dose of 128mg kg^{-1}, when it was possible to quantify an average of 2mg kg^{-1} Pb in leaf dry biomass. On the other hand, a significant dose dependent response ($P<0.05$) was verified on ***M. arvensis*** roots, reaching a maximum absorption of 50 mg kg^{-1} Pb at 128mg kg^{-1} concentration (Figure 1A).

The Cd content in leaves and roots of ***M. arvensis*** was significantly ($P<0.05$) influenced by the increasing concentration of metal applied to soil, with lower concentrations in leaves than in roots. The largest Cd absorption occurred at 128mg kg^{-1} soil concentration, with maximum absorption of 12 mg kg^{-1} on leaf and 78mg kg^{-1} on root dry biomass (Figure 1B).

The palisade parenchyma cells of control plants exhibited nucleus (nuclear membrane, nucleoli) and organelles with normal aspect (Figure 2A). Chloroplasts containing clear and well-structured grana were observed in these cells, located next to the mitochondria walls and surroundings with intact contours and no evidence of changes (Figure 2D). The presence of starch grains (Figure 2F, G, H) and plastoglobules was also verified in some chloroplasts (Figure 2D, E, F, G, H). On the other hand, in the same cells of plants grown with 128mg kg^{-1} Pb, some changes were observed as invagination of the nucleus (Figure 2B) and cell wall (Figure 2E), disruption of the chloroplast membranes (Figure 2E), in addition to the presence electron dense deposits surrounding the mitochondria (Figure 2H). In plants treated with 128mg kg^{-1} Cd electron dense deposits were also observed around the nucleus (Figure 2C), chloroplasts (Figure 2F), and vesicles with electron dense contents on mitochondrias (Figure 2I).

No significant effects of Pb and Cd application in the soil ($P<0.05$) were observed on the essential oil content (4.26%) and yield (0.36g $planta^{-1}$) of ***M. arvensis***. The chemical composition of the essential oil was not expressive, with only minor variations without any clear trend. The chromatographic analysis of the essential oils enabled the identification of nine volatile components, amounting to about 99% of the chemical composition,

Table 1- Averages of growth variables of ***M. arvensis*** grown with Pb and Cd applied separately to the soil ± standard error ($P<0.05$). Mean values of five replicates.

Variable	Control	Metal	
		Pb	Cd
PH (cm)	37.50 ± 0.47 a	37.58 ± 0.26 a	37.26 ± 0.43 a
SD (cm)	3.90 ± 0.07 a	4.09 ± 0.11 a	4.15 ± 0.07 a
NL (und)	378 ± 11 a	411 ± 7 a	388 ± 8 a
LA (cm^2)	3653 ± 126 a	3700 ± 51 a	3560 ± 56 a
TDB (g)	18.25 ± 0.82 a	20.06 ± 0.50 a	18.84 ± 0.43 a
LDB (g)	8.27 ± 0.35 a	9.03 ± 0.20 a	8.54 ± 0.17 a
SDB (g)	8.01 ± 0.53 a	9.02 ± 0.28 a	8.26 ± 0.26 a
RDB (g)	1.97 ± 0.05 a	2.01 ± 0.06 a	2.03 ± 0.06 a
SLA (g cm^2)	0.00230 a	0.00240 ± 0.00004 a	0.00240 ± 0.00004 a
LAR (cm^2 g^{-1})	200.81 ± 6.08 a	186 ± 3 a	191 ± 4 a

Plant height (PH), stem diameter (SD), number of leaves (NL), leaf area (LA), leaf dry biomass (LDB), stem dry biomass (SDB), root dry biomass (RDB) and total dry biomass (TDB), specific leaf area (SLA) and leaf area ratio (LAR). Means followed by the same lowercase letter between columns do not differ statistically ($P<0.05$).

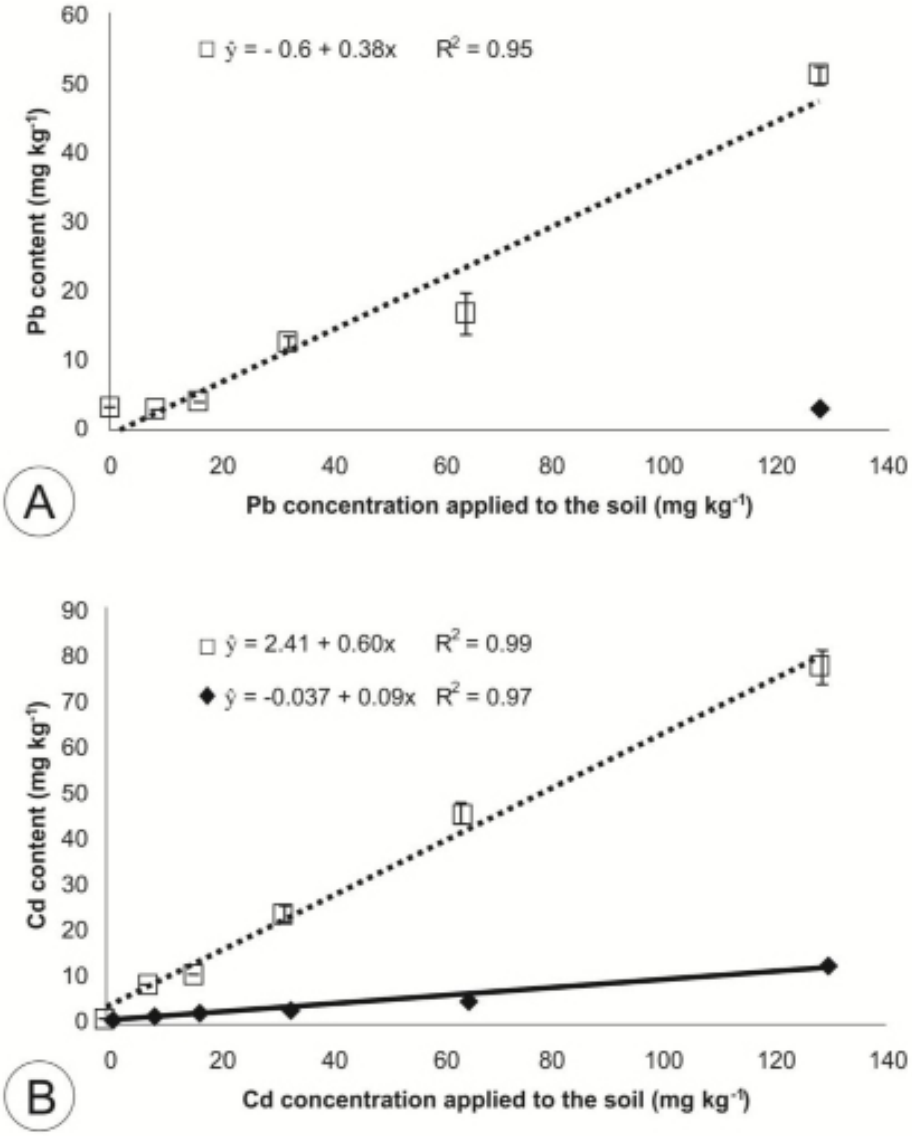

Figure 1- Content variation of Pb (A) and Cd (B) in leaves (—) and roots (·····) of ***M. arvensis*** in relation to the applied doses of Pb and Cd to the soil.

which were divided into aliphatic hydrocarbons (3-octanol), monoterpenes (limonene), oxygenated monoterpenes (neo-isopulegol, isomenthone, menthone, menthol, pulegone and carvotanacetone) and sesquiterpenes (E-caryophyllene) (Table 2). Menthol was the major component of the essential oil of plants in all treatments, responsible for more than 86% of the chemical composition of the essential oil of ***M. arvensis***. Compared to the control treatment, the concentration varied not more than 2% with the application of toxic metals in the soil.

DISCUSSION

The heavy metal concentrations in the soil before application of treatments (14mg kg^{-1} Pb, 0.2 mg kg^{-1} Cd) is in a normal range, since the worldwide average of soil surface, is 15mg kg^{-1} Pb and 0.53mg kg^{-1} Cd (KABATA-PENDIAS & PENDIAS, 2000). The presence of these heavy metals in the soil may result of irrigation with contaminated water, improper exploitation of natural resources and the application of large amounts of phosphate fertilizers and pesticides (CASAS & SORDO, 2006).

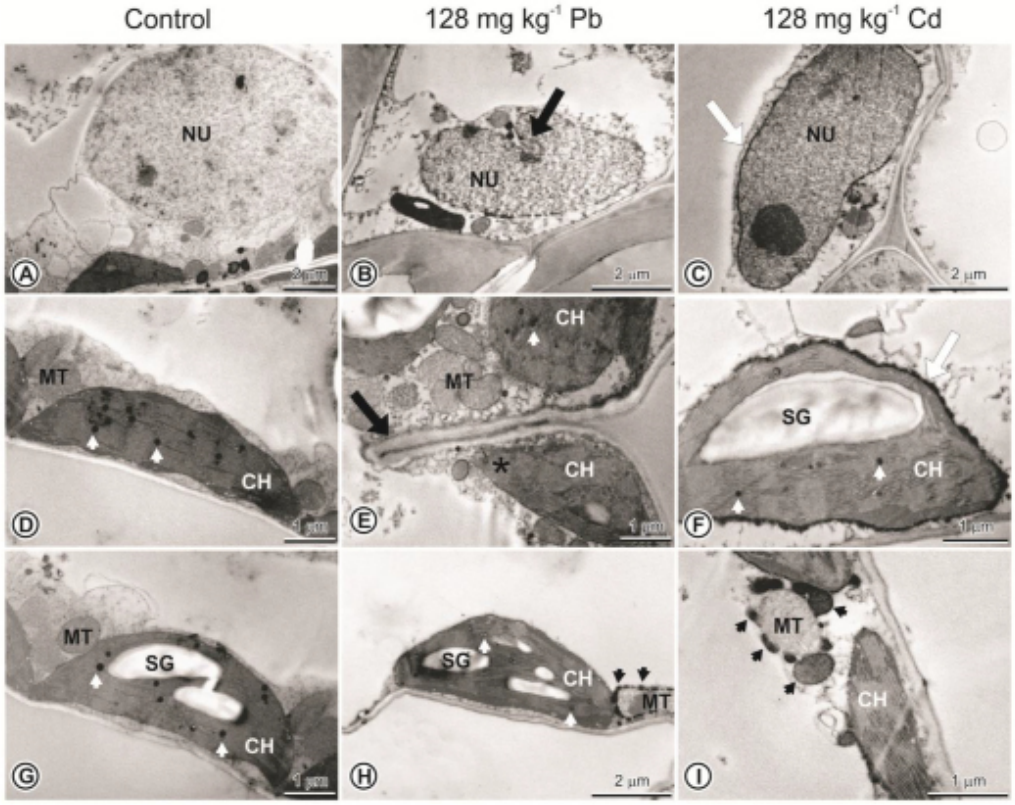

Figure 2- Transmission of electron photomicrographs of palisade parenchyma cells of the mesophyll of ***M. arvensis*** without application of Pb and Cd to the soil (A. D. G) and with application of 128 mg kg^{-1} Pb (B. E. H) and 128 mg kg^{-1} Cd (C. F. I), showing the ultrastructural changes as chloroplast membrane disruption, invagination of cell wall and nucleus membrane, eletrodenses deposits and vesicles with electrodenses deposit around organelles related to the application of toxic metals. NU: nucleus; CH: chloroplast; SG: starch grain; MT: mitochondria; black thick arrow: invagination of the nucleus membrane and cell wall; white thick arrow: electron dense deposits surrounding chloroplasts and nucleus; asterisk(*): disruption of chloroplasts membranes; white arrowhead: plastoglobules; black arrowhead: vesicles with eletrodense contents surrounding mitochondria.

The higher accumulation of Pb in the roots (50mg kg^{-1}) of ***M. arvensis*** in relation to its leaves (2mg kg^{-1}) is sustained by the low mobility of this metal in plants, resulted in less accumulation in shoot organs (KABATA-PENDIAS & PENDIAS, 2000). However, the limited quantity of this metal that penetrates in the root through the cell wall of epidermal cells is retained in the cortex cells, being endodermis the main barrier for limiting Pb translocation to aerial parts (TUNG & TEMPLE, 1996). This Pb retention in the root system can act as a protective mechanism against contamination of the plant shoot (KASTORI et al., 2012), as was checked for ***M. arvensis***. This low translocation of Pb to ***M. arvensis*** leaves is a positive factor, since, normally this is the main commercial part used for essential oil extraction. Furthermore, the amount of Pb in leaves of ***M. arvensis*** did not exceed the limit of 10mg kg^{-1} specified by the WHO (1998).

Similarly, the Cd also showed much higher concentrations in roots (78mg kg^{-1}) than in the leaves (12mg kg^{-1}) of ***M. arvensis***, possibly due the restriction of Cd transport by xylem, which may occur by increased lignin deposition on these cells (LUX et al., 2011). Premature lignification of vessel element adds layers of impermeable cells that reduce transport of both water and metal (LUX et al., 2011). However, the high Cd concentration in ***M. arvensis*** leaves, forbid its popular use as decoction or infusion, once the plant processing in hot water can extract the metals for the tea (ABOU-ARAB & ABOU DONIA, 2000). The Cd concentration found in ***M. arvensis*** leaves exceeded about 40 times the limit of 0.3mg kg^{-1} established by WHO (1998). This emphasizes the

Table 2- Relative percentages of essential oil constituents from leaves of ***M. arvensis*** grown with increasing concentrations of Pb and Cd applied separately to the soil.

Constituents	KI[1]	Metals concentration applied to the soil (mg kg^{-1})										
		Pb						Cd				
		0	8	16	32	64	128	8	16	32	64	128
3-octanol	995	0.83	0.87	0.88	0.79	0.84	0.84	0.86	0.87	0.83	0.88	0.82
limonene	1033	0.59	0.64	0.65	0.86	0.77	0.94	0.57	-	0.67	0.87	0.75
neo-isopulegol	1153	0.56	0.57	0.55	0.55	0.55	0.54	-	0.55	0.54	0.56	0.56
isomenthone	1161	4.40	4.54	4.38	4.77	4.55	5.00	3.57	4.08	4.37	4.50	4.00
menthone	1171	2.11	2.20	2.19	2.19	2.28	2.36	2.21	2.27	2.23	2.25	2.23
menthol	1174	88.4	87.5	88.2	87.2	87.7	86.8	89.5	88.2	87.2	87.8	88.9
pulegone	1244	1.57	2.27	1.56	2.07	1.80	1.86	1.06	1.36	2.54	1.55	1.03
carvotanacetone	1259	0.99	0.89	0.97	0.95	0.96	1.02	1.01	1.11	0.97	1.00	1.03
E-caryophyllene	1425	0.50	0.46	0.55	0.54	0.52	0.58	0.57	0.64	0.58	0.58	0.61
Total		99.9	99.9	99.9	100.0	100.0	99.9	99.9	99.1	100.0	99.9	99.9

KI[1]: *Kovats* Index; - not detected.

danger of metal accumulation in ***M. arvensis*** without phytotoxicity symptoms, representing a potential risk for humans and animals (OLIVER, 1997).

Although some species (***Vetiveria zizanioides***, ***Prosopis juliflora*** and ***Desmanthus virgatus***) register reductions on growth and biomass production in the presence of heavy metals as Pb (ALVES et al., 2008), a possible explanation for the lack of phytotoxicity symptoms and yield of ***M. arvensis*** by Pb and Cd application, would be due to the greater retention of metals in the roots, which act as a barrier to the transport of large quantities of metal to the shoot, thus avoiding the toxic effect of such heavy metals.

Wall invaginations in mesophyll cells of ***M. arvensis*** treated with Pb may be related to an increased strategy surface area as adaptive to provide physiological benefits to increase the ions transport of water under stress conditions (KURKOVA, 2002), since Pb is also related to changes in the water balance of plants (SHARMA & DUBEY, 2005). It can be also associated with a microtubules disruption which may alter the shape and the synthesis of cell wall caused by the action of Pb (LIU et al., 2009). Chloroplast changes observed in cells of ***M. arvensis*** treated with Pb were already described in ***Potamogeton crispus*** treated with Pb, resulting in dilation of chloroplasts and disruption of their membranes (HU et al., 2007), possibly by reducing the concentration of membrane lipids caused by contamination with Pb as noted in the chloroplasts of ***Lycopersicon esculentum*** leaves (DJEBALI et al., 2005).

It is believed that both granules and vesicles with eletrodense contents around the organelles are related to the movement limitation of heavy metals in the cells. Granules with electron dense content around chloroplasts and nucleus of ***M. arvensis*** mesophyll cells treated with Cd, and around ***M. arvensis*** mitochondria of mesophyll cells treated with Pb, were previously observed in ***Aradopsis thaliana*** roots under high concentrations of Cd (BELLENGHEM et al., 2007) and ***Zea mays*** roots treated with Pb (TUNG & TEMPLE, 1996). These structures were described as granular deposits present in the cortex intercellular spaces or in the cell wall. The metal would be transported from the apoplast to the symplast, reducing its movement in the plant (TUNG & TEMPLE, 1996; BELLENGHEM et al., 2007).

Plants exposed to heavy metal stress show changes in production of secondary metabolites, either suppressing or stimulating production (NASIM & DHIR, 2010). Changes in essential oil composition by heavy metals stress are associated with inactivation of specific enzymes of metabolic pathways of secondary metabolites (NASIM & DHIR, 2010). As there were no changes on ***M. arvensis*** essential oil yield and composition, probably Pb and Cd applied in the soil was not sufficient to affect these routes of secondary metabolites production. Similarly, the exposition of ***Anethum graveolens*** and ***Ocimum basilicum*** to Cd, Pb and Cu showed no clear trend of variation (ZHELJAZKOV et al., 2006). Moreover, ***Mentha piperita*** cultivated with Pb and Cd, under maximum concentration of 100 and 1500ppm respectively, showed a significant reduction of essential oil yield, which was mainly associated with the reduction of biomass, number of leaves and total leaf area (AMIRMORADI et al., 2012).

CONCLUSION

Pb and Cd were absorbed by ***M. arvensis*** and concentrated mainly in the roots, but Cd was also translocated to the shoots, exceeding the permissible levels for human consumption. At ultrastrutural level, the metals accumulated on vesicles. The cellular alterations due metals application were not enough to produce phytotoxicity symptoms or affect the growth and essential oil yield of ***M. arvensis***.

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