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Phytoaccumulation and effect of lead on yield and chemical composition of *Mentha crispa* essential oil

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ABSTRACT

Heavy metals such as lead (Pb) accumulated in soil may become a problem for plant growth and human health. An alternative is to grow nonfood crops in these contaminated areas. A pot experiment was carried out in order to investigate the effect of increasing doses (900, 1,800, 3,600, 7,200, and 9,000 mg kg^{-1}) of Pb on the yield and chemical composition of essential oil and phytoaccumulation of garden mint (*Mentha crispa* L., Lamiaceae). It was observed that the length of the root and aerial parts of this species was not significantly affected in different assayed experiments when compared to the control. However, the leaf number, budding, and green mass were very influenced by the presence of Pb in the soil, showing a significant resistance. Regarding Pb phytoaccumulation, it was verified an accumulation of this metal in roots and aerial parts. Although *M. crispa* is a species that tolerates high concentrations of Pb, it is not considered a Pb hyperaccumulator species. Considering the production of essential oil, a high amount of Pb affected significantly its yield, and it was 10 times higher under extreme contamination conditions than the amount of oil produced by the control. The chemical composition of *M. crispa* essential oil was also affected by high Pb doses, and its major component (carvone) concentration varied from 39.3% for cultivation in noncontaminated soil (control) to 90% for all cultivations in Pb-contaminated soils.

Keywords: Mentha crispa; Lead; Soil pollution; Phytoaccumulation; Essential oil; Carvone

1. Introduction

Industrialization and urbanization activities may cause great environmental problems, and one of the most challenging problems is the contamination by heavy metals. A great occurrence of these contaminants may influence the quality of the atmosphere and hydric resources, and may also threaten the health of animals and humans by being part of their food chain [1]. Heavy metals are not degraded biologically and

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remain in the environment for undetermined time, and, therefore, they need to be physically removed or immobilized. Phytoremediation is an emerging technology used to correct contaminated areas due to its cost-benefit, aesthetical advantages, and long-term applicability [2]. This technique is based on the capacity of some plants to accumulate high concentrations of heavy metal ions. One of the main distinguishing characteristics of metal hyperaccumulator plants is the extremely efficient translocation of metals from the roots to the aerial parts.

Within the heavy metals, lead (Pb) is considered an important environmental pollutant which is broadly used in batteries, paints, glass, tubes, and fertilizers [3]. Soils contaminated by Pb may cause severe reductions in yield of many cultivations [4,5]. An increase of Pb amount in the soil may affect plant growth and metabolism, but accumulation depends on the species, cultivar, plant organ, development stage, metal concentration, and the presence of other ions [6,7].

The Pb is absorbed by plants, mainly through the root system, where it accumulates, and sometimes it is translocated to the aerial parts. This translocation barrier happens due to the root endoderm barrier function. In lethal concentrations, this barrier is broken and there is a flow of Pb to vascular tissues and it is deposited in the intercellular spaces, cell wall, vacuoles, and in some cases in the endoplasmatic reticule, decreasing the photosynthetic capacity of the plant [4,6]. After it gets into the cell, Pb inhibits the activity of a lot of enzymes, disturbs mineral nutrition and hydric balance, alters the hormonal state, and affects the structure of the membrane and its permeability [4].

Considering the competition for fertile lands to produce food, the rational use of areas contaminated by heavy metals to plant alternative cultivation like the production of essential oils could increase the profitability levels of farmer who live close to degraded areas, besides providing an ecological olution to the problem [7].

Mint (*Mentha* spp.) is an important aromatic plant. It has received considerable economic importance due to the large demand for its essential oil by the food, pharmaceutical, cosmetic, and hygiene industries [8]. Studies on some species of mint (*Mentha arvensis*, *Mentha citrata*, and *Mentha piperita*) cultivated in soils contaminated by chromium (Cr) and Pb showed that they were somehow influenced by these metals [7]. Although they are species of the same genus, all of them responded differently to contamination. *M. arvensis* leaves were not significantly increased by the presence of Cr or Pb, however the essential oil yield decreased for this species. For *M. citrata*, the essential oil yield and leaf yield decreased, whereas all the production levels (essential oil, leaves, and roots) of *M. piperita* increased. The rising application of Cr and Pb in the soil also changed the chemical composition of essential oil and increased the accumulation of these metals in the roots and aerial parts of all three species when compared to the control.

A recent study, evaluating the accumulation factor for a series of heavy metals in several plants, classified *M. piperita* as a metal hyperaccumulator plant [9]. Conversely, *Mentha aquatica* showed little Pb translocation from roots to leaves [10].

Considering such distinct metal translocation behaviors for some *Mentha* species, this study aimed to assess the effect of Pb on the growth of *Mentha crispa*, popularly known as garden mint, a very common species of this genus in Brazil.

M. crispa is a hybrid species obtained from the crossing between *Mentha spicata* and *Mentha suaveolens* [11]. Its leaves are utilized as tea, and have digestive, calming, tonic, antiseptic, and antiasthma properties. They are also used against biliary disorders, jaundice, constipation, and catarrh [12]. Regarding its essential oil composition, the main component is carvone [13–15]. This compound has economic potential and has been utilized as flavoring, fragrance, budding inhibitor, antimicrobial, environmental indicator, and in medicine [16].

Because of the controversial behavior of the different *Mentha* species in contaminated soils previously cited, and the economic importance of these species in different countries of the world, the objective of the study was to evaluate the effect of Pb on *M. crispa* development, growth, biomass production, and essential oil composition, assessing the impact that this metal may have on the plant and how it behaves to this stress. To develop this experiment, simulated situations of *M. crispa* cultivation in extremely contaminated soils were utilized.

2. Materials and methods

2.1. Experimental setup

The experimental setup was entirely random with six increasing doses of Pb and five replicates displayed in 30 pots. The experiment was carried out under greenhouse (temperature at 23°C ± 2) natural conditions of light at the Campus of Universidade Paranaense—UNIPAR in the municipality of Umuarama, Paraná State, Brazil, from March to August 2010.

2.2. Soil preparation

The soil with sandy texture (Cauiá sandstone formation) was collected from Medicinal Herbarium of

UNIPAR, at the depth of 0–20 cm, dried in a forced air oven at 60˚C for two days. Later, the soil was ground and sieved using porcelain mortar and 2 mm mesh sieve, in order to provide particle sizes homogeneous eliminating possible sorption differences. After that, four kg of soil was weighed and put into the polyethylene pot with 10 dm^3, with cylindrical shape. The soil granulometry found for this kind of soil was 848.81, 122.68, and 28.50 g kg^{-1} for sand, silt, and clay, respectively. The chemical characteristic of the studied soil is presented in Table 1.

2.3. Pb addition standardize the units (mg kg^{-1}) or g kg^{-1}

According preliminary experiments (data not shown) values of maximum adsorption capacity, the Pb in studied soil was 1,800 mg kg^{-1}, the soil was treated with increasing doses of Pb using a stock standard solution ($Pb(NO_3)_2$) containing 1.0 g L^{-1} of the Pb. The following Pb concentrations were added in the soil: 0, 900, 1,800, 3,600, 7,200, and 9,000 mg kg^{-1}. For soil with 0 mg kg^{-1} of Pb concentration, it was named T_0, as a control. For the other Pb additions, the soils were named T_1, T_2, T_3, T_4, and T_5 as being the half (900 mg kg^{-1}), the maximum (1,800 mg kg^{-1}), the double (3,600 mg kg^{-1}), four times (7,200 mg kg^{-1}), and five times (9,000 mg kg^{-1}) the adsorption capacity, respectively. The nitrogen (N) concentration added was kept constant at 2 g kg^{-1} of soil in all treatments. This amount has been selected by the largest amount of added lead nitrate (9,000 mg kg^{-1}) corresponds to 1.22 g kg^{-1} of N in the soil. The amount of lead nitrate to achieve the required Pb concentration was dissolved in 1.0 L of distillated water and stirred until complete dissolution. The soil was split into 30 equal parts (4 kg) for a total of six treatments in quintuplicate, on each significant portion of Pb was added to the solution for each treatment. This solution was added slowly into the soil and mixed constantly, so that the dispersion was homogeneous throughout the soil.

2.4. Garden mint cultivation

After the addition of metal, the soils were incubated for a 30-day period and kept at 60% field capacity of the total pore volume using methodology by Fabian and Ottoni Filho [17] in which daily watering was performed in the late afternoon until harvest time. After the incubation, the garden mint seedlings were planted. The seedlings were collected from aerial stems of matrix plants at the Medicinal Herbarium of UNIPAR, with approximately 10 cm and they were planted one seedling per pot. Periodical visits were done in order to visualize the metal toxicity, which was not observed at any time. After 145 cultivation days, all the plants were collected with aerial part and roots from the pots. The plants were evaluated regarding budding, leaf number (manual counting), and length (aerial part and tap root) using a ruler and caliper.

2.5. Garden mint preparation for analysis

The plants were washed with running tap water and then distilled water. After washing, the material was dried at room temperature and separated in aerial part and roots manually. Half aerial part was used for the extraction of essential oil. The roots and the other half aerial part were used for Pb analysis.

2.6. Oil essential extraction and analysis

The fresh aerial parts of garden mint were submitted to hydrodistillation in a modified Clevenger apparatus [18] and extracted with 500 mL of water for 4 h. The essential oil was collected, dried, and stored in sealed glass vials at 0˚C prior to determination of chemical components.

The essential oil was analyzed by GC–MS (gas chromatography coupled to mass spectrometry) (Agilent 5973 Network Mass Selective Detector) using a fused silica capillary column with an apolar stationary phase (30 m × 0.25 mm × 0.25 μm film

Table 1
Reference values and chemical characteristic of the studied soil for cation exchange capacity (CEC), potential acidity (H^++Al^{3+}), calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), phosphorus (P), carbon (C), and pH in $CaCl_2$ (pH)

Parameters	CEC cmolc dm^{-3}	$H^+ + Al^{3+}$ cmolc dm^{-3}	Ca^{2+} cmolc dm^{-3}	Mg^{2+} cmolc dm^{-3}	K^+ cmolc dm^{-3}	P mg dm^{-3}	C g dm^{-3}	pH
Reference values[a]	2.2–12.5	0.6–5.0	0.3–7.2	0.3–3.3	0.1–0.5	16.1–24.0	0.8–15.9	3.8–6.6
Sampled soil[b]	4.85	2.95	1.0	0.88	0.1	3.54	1.98	4.98

[a]Source: [39]. [b]Laboratory of soil analysis "Solo Fértil" Umuarama, Paraná, Brazil (2012).

thickness). The GC–MS data were obtained using the following conditions: carrier gas He; flow rate 1.0 mL min^{-1}; injection volume 0.1 μL; oven temperature program from 60 to 260°C at 4°C min^{-1} and holding at 260°C for 40 min; injector and detector temperatures were 260 and 280°C, respectively; the ionization mode used was having electronic impact at 70 eV. Identification of the components was achieved from their linear retention indices on DB5 column, determined with reference to a homologous series of C8-C22 *n*-alkanes, and by a comparison of their mass spectral fragmentation patterns with those stored in the data bank (Wiley/NBS library) and the literature [19].

It was not possible to obtain the essential oil to a concentration of 9,000 mg kg^{-1} because of the low amount of plant obtained.

2.7. Pb analysis

The roots and the half aerial part were dried in a forced air oven at 60°C and then weighed until reaching constant mass. Later, the material was ground and passed through a polyester sieve for particle size ≤50 μm. A sample amount of 0.5 g was weighed using five replicates. For acid digestion, it was added to the sample 6 mL of nitric acid (HNO_3, 65% v/v) plus 3 mL of hydrogen peroxide (H_2O_2, 30% v/v). The mixture was poured in a heating plate at 100°C for 90 min and after that filtered in a 50 mL volumetric flask; then, its volume was filled out with deionized water. All glassware were cleaned in nitric acid for three days and washed thrice with deionized water before using. Distilled and deionized water with a resistivity of 18 MΩ from a Millipore water purification system (Millipore, Bedford, MA, USA) was used for the preparation of the samples and standards.

Pb determination was done using a flame atomic absorption spectrophotometer (GBC 932 plus) equipped with deuterium background correction, air-acetylene flame, slit width of 0.7 nm, and analytical line at 283.3 nm. The Pb hallow cathode lamp was used as radiation source with a current of 5.0 mA.

Was used in all analyses certified standards National Institute of Standards and Technology.

2.8. Statistical analysis

One-way analysis of variance—ANOVA followed by *t*-test least significant difference (LSD) post-hoc test ($p \leq 0.05$) was performed for all means using SPSS v.16.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

When analyzing the chemical characteristics of the experimental soil used in this study, it was observed that the amount of all elements was very low compared with reference values (Table 1) to the soils in the region of studied (Caiuá soil formation, Paraná State).

The evaluation of plant growth cultivated in soil treated with increasing doses of Pb is showed in Table 2, like length of the root and aerial part, leaf number, budding, fresh biomass, and percentage of dry mass for five treatments with Pb.

In general, there was no difference for aerial part length due to increasing doses of Pb for all treatments when compared to the control T_0, except for T_3 (Table 2). In the same way, it was also observed that Pb did not affect the length for roots, except for treatment T_4. In this case, the root length difference was about 9 cm lower than the control T_0 (Table 2). This observation was also found by Bekiaroglou and Karataglis [6] and Eun et al. [20] where the growth of the *M. spicata* radicular system may be inhibited due to the restriction caused by Pb excess during the cell division.

About the leaf number, a decrease was found from 150 leaves in T_0 (control) to 34 leaves in T_4. This reduction can also be observed for the amount of buddings in the plant (Table 2). A similar result was observed for Kosobrukhov et al. [21] with *Plantago major* in which increasing doses of Pb forced a reduction of leaf number due to the damage to a lot of physiological and biochemical processes.

From Table 2, the values referring to plant biomass were calculated in relation to the total fresh biomass produced by the plant and the percentage of dry mass. For dry mass, calculated by the percentage in relation to 100 g of plant, it was not verified significant variation due to increasing Pb doses. A similar effect was also observed by Almeida et al. [22] where the dry mass for Jack-bean (*Canavalia ensiformis*) was little affected by the amount of Pb at concentration of 400 mg kg^{-1} in the soil. In another study in a pot experiment done by Scora and Chang [23], they observed that the biomass production of *M. piperita* was not significant affected by the presence of Pb up to 173 mg kg^{-1} in the soil.

Concerning the total fresh biomass, it was observed a significant reduction with increasing doses of Pb when compared to the control T_0, showing that Pb affected the production of garden mint fresh biomass. Zheljazkov and Nielsen [24] found the same behavior for two mint species.

In order to verify the Pb absorption through the plant, atomic absorption analysis for Pb was done in

Table 2
Length (aerial part and root), leaf number, budding, fresh biomass, percentage of dry mass, and essential oil yield (%) of *M. crispa*, cultivated in soil treated with different doses of Pb

Treatment	Length (cm)		Leaf number	Budding	Total fresh biomass (g)	% Dry mass	Essential oil yield %
	Root	Aerial part					
T_0	26.3 ± 3.0^{a}	28.2 ± 1.8^{a}	150 ± 22^{a}	44 ± 1^{a}	44.15 ± 0.15^{a}	22.06 ± 1.79^{a}	0.046 ± 0.002^{d}
T_1	27.7 ± 2.2^{a}	24.7 ± 2.3^{a,b}	59 ± 15^{b,c}	28 ± 2^{b}	28.00 ± 2.00^{b}	19.28 ± 4.36^{a,b}	0.297 ± 0.012^{c}
T_2	23.5 ± 3.8^{a,b}	27.0 ± 4.1^{a}	39 ± 14^{b,c}	18 ± 1^{c}	17.70 ± 0.40^{c}	16.76 ± 0.85^{a,b}	0.321 ± 0.006^{b}
T_3	25.8 ± 3.6^{a,b}	17.2 ± 2.0^{b,c,d}	76 ± 11^{b}	10 ± 1^{d}	9.95 ± 0.87^{d}	21.30 ± 1.14^{a}	0.533 ± 0.012^{a}
T_4	17.2 ± 3.9^{b}	24.3 ± 3.5^{a,c}	34 ± 10^{b,c}	4 ± 1^{e}	3.70 ± 1.40^{e}	23.07 ± 2.25^{a}	0.510 ± 0.006^{a}
T_5	6.07 ± 0.7^{c}	12.32 ± 2.0^{d}	22 ± 4^{c}	2 ± 1^{e}	2.10 ± 0.31^{e}	12.97 ± 3.01^{b}	ND*

Note: Average values and standard deviation (n = 5). Averages followed by the same letters in the column do not differ among themselves by T-LSD test, $p \leq 0.05$.
*ND = Not had sufficient sample.

the roots and aerial parts, and the results are presented in Table 3. In a general way, it can be observed that the higher Pb content in the soil, the higher Pb content in the root and aerial part. However, regarding the T_3 and T_4 treatments for aerial part, there was no significant increase of Pb amount, indicating that there was a saturation of this element.

From Table 3, a higher accumulation of Pb can also be observed in the root when compared to the aerial part, mainly at the T_4 treatment. This behavior was also observed in other studies [5–7,25] and the reason for that can be the root ability to accumulate amounts of heavy metals, and simultaneously restrict their translocation to the aerial part [26]. In plants, Pb moves in the root via apoplast and through the cortex it accumulates near the endoderm, which acts as a partial barrier for Pb translocation from roots to the aerial part [27].

Table 3
Pb concentration in the root and aerial part for *M. crispa* in soil treated with different doses of Pb

Treatment	Pb concentration (mg kg^{-1})	
	Root	Aerial part
T_0	0.20 ± 0.06^{c}	0.27 ± 0.03^{c}
T_1	1.90 ± 0.38^{c}	2.49 ± 0.58^{c}
T_2	54.32 ± 1.99^{b}	18.04 ± 2.81^{b}
T_3	53.61 ± 1.88^{b}	43.13 ± 2.55^{a}
T_4	83.45 ± 2.13^{b}	41.60 ± 6.91^{a}
T_5	610.83 ± 35.77^{a}	39.26 ± 7.61^{a}

Note: Average values and standard deviation (n = 5). Averages followed by the same letters in the column do not differ among themselves by T-LSD test, $p \leq 0.05$.

Another point evaluated in this study is related to the production of garden mint essential oil under increasing doses of Pb and the values are presented in Table 2. Initially, it can be observed that there was difference in the production of essential oil between the control (T_0) and the other treatments. For T_1 and T_2 treatments, the production of essential oil increased over six times. For T_3 and T_4 treatment, the essential oil yield was 10 times higher than the control (T_0).

Regarding the essential oil yield, the amount extracted from *M. crispa* was superior in the treatments with higher Pb doses than smaller doses for this metal, even with leaf number and dry biomass reduced (Table 2). This observation is according to what has been reported in other studies with different species of *Mentha* [7,24,25].

Although the effect of soil contaminants in the production of essential oil still has not been extensively studied [7,25,28], it is known that its production is highly integrated to the plant physiology, depending on its metabolic state [29]. However, there is evidence that Pb can interfere in *Mentha* essential oil yield [25] and this can be confirmed by the results obtained in this study on *M. crispa*.

According to David and Boaro [30], although secondary metabolites are not essential to the producing organisms, they are a way to ensure the species survival and perpetuation, i.e. the plant can induce the production of essential oil in a stress environment in order to guarantee the survival of its species. In this experiment, the production and quality of garden mint essential oil was significantly affected by stress caused by Pb addition in the soil.

Some studies have reported that increasing the amount of N in the soil affects the vegetative growth and the production of essential oil of the mint.

Table 4
Chemical composition and area (%) of *M. crispa* essential oil obtained from five treatments with Pb

Peak	Compounds	T_0	T_1	T_2	T_3	T_4
	Hydrocarbonate monoterpenes					
1	Limonene	t	0.81	1.43	1.39	1.86
	Oxygenated monoterpenes					
2	1,8-Cineole	t	t	t	t	t
3	Linalool	2.44	t	t	1.11	1.99
4	n.i	0.86	t	t	t	t
5	n.i	0.25	t	t	t	t
6	Borneol	0.86	0.94	t	t	t
7	Dihydro carveol	4.38	2.30	3.53	5.16	3.23
8	(+)-*Trans*-carveol	1.39	1.34	t	1.17	t
9	Carvone	**39.31**	**90.85**	**95.04**	**85.32**	**91.53**
10	n.i	0.55	t	t	t	t
11	Bornyl-acetate	0.93	t	t	t	t
12	Dihydro carveol acetate	2.21	t	t	1.88	1.39
13	n.i	0.40	t	t	t	t
14	Dihydro carveol acetate iso	1.51	0.66	t	t	t
15	n.i	0.32	t	t	t	t
	Hydrocarbonate sesquiterpenes					
16	*Alpha*-copaene	0.71	t	t	1.19	t
17	*Beta*-bourbonene	2.24	1.13	t	1.69	t
18	*Alpha*-gurjunene	0.28	t	t	t	t
19	*Beta*-cariophyllene	1.66	1.06	t	1.08	t
20	*Trans-beta*-farnesene	1.47	t	t	t	t
21	*Alpha*-humulene	0.91	t	t	t	t
22	Muurola-4(14),5-diene	0.66	t	t	t	t
23	n.i	0.61	t	t	t	t
24	Germacrene D	2.85	0.92	t	t	t
25	*Beta*-selinene	0.91	t	t	t	t
26	*Alpha*-farnesene	0.80	t	t	t	t
27	*Delta*-cadinene	2.58	t	t	t	t
28	n.i	0.47	t	t	t	t
29	Epi-bicyclosesquiphellandrene	2.45	t	t	t	t
30	Cadina-1,4-diene	1.47	t	t	t	t
31	*Alpha*-cadinene	2.50	t	t	t	t
	Oxygenated sesquiterpenes					
32	Nerolidol	1.81	t	t	t	t
33	Ledol	1.27	t	t	t	t
34	Spathulenol	0.84	t	t	t	t
35	n.i	0.39	t	t	t	t
36	Cariophyllene oxide	0.88	t	t	t	t
37	n.i	0.42	t	t	t	t
38	Viridiflorol	0.88	t	t	t	t
39	Caryophylla 3,8(13) dien-5, α-ol	1.22	t	t	t	t
40	*Beta*-oplopenone	2.20	t	t	t	t
41	*Allo*-Aromadendrene epoxide	1.23	t	t	t	t
42	n.i	0.39	t	t	t	t
43	n.i	0.91	t	t	t	t
44	n.i	0.86	t	t	t	t
45	*t*-muurolol	0.95	t	t	t	t
46	n.i	0.93	t	t	t	t
47	n.i	0.42	t	t	t	t
48	n.i	0.59	t	t	t	t

(*Continued*)

Table 4
(*Continued*)

Peak	Compounds	T_0	T_1	T_2	T_3	T_4
49	n.i	0.46	t	t	t	t
50	Phytol	0.79	t	t	t	t
	Hydrocarbonate diterpenes					
51	Sandaracopimara-8(14), 15-diene	1.28	t	t	t	t
52	n.i	0.62	t	t	t	t
53	Calyculone	0.99	t	t	t	t
54	n.i	0.18	t	t	t	t
55	n.i	0.21	t	t	t	t
56	n.i	0.51	t	t	t	t
57	n.i	0.44	t	t	t	t
58	n.i	0.20	t	t	t	t
59	n.i	0.14	t	t	t	t
	Total identified compounds	99.99	100.0	100.0	99.99	98.61

Note: The values of the areas (%), obtained by CG, were calculated from the relation between the total area and the area of each compound in the sample. The identification was based on the comparison of mass spectra of Wiley 275 spectra libraries. n.i—Nonidentified; t—Traces. The substances are listed from the elution order by DB-5 column (phenylmethylsiloxane 5%). T_0 (Treatment 0) Control, T_1 (Treatment 1) half of the adsorption capacity of soil (900 mg kg^{-1}), T_2 (Treatment 2) maximum adsorption capacity (1,800 mg kg^{-1}), T_3 (Treatment 3) double of the soil capacity (3,600 mg kg^{-1}), T_4 (Treatment 4) four times the maximum adsorption capacity (7,200 mg kg^{-1}).

However, the essential oil quality was not affected; meaning the chemical composition of the essential oil remained virtually the same [31–33]. To avoid any effect of N on the production and quality of garden mint essential oil in this study, ammonium nitrate was added to all treatment, and all treatment with Pb had the same amount of N in the soil.

The influence of high doses of Pb was also evaluated on the chemical composition of *M. crispa* essential oil in order to observe the possible changes in the chemical composition of essential oils extracted in the four treatments. Chromatographic analyses were done by GC–MS of garden mint essential oil cultivated in noncontaminated soil (T_0) and with

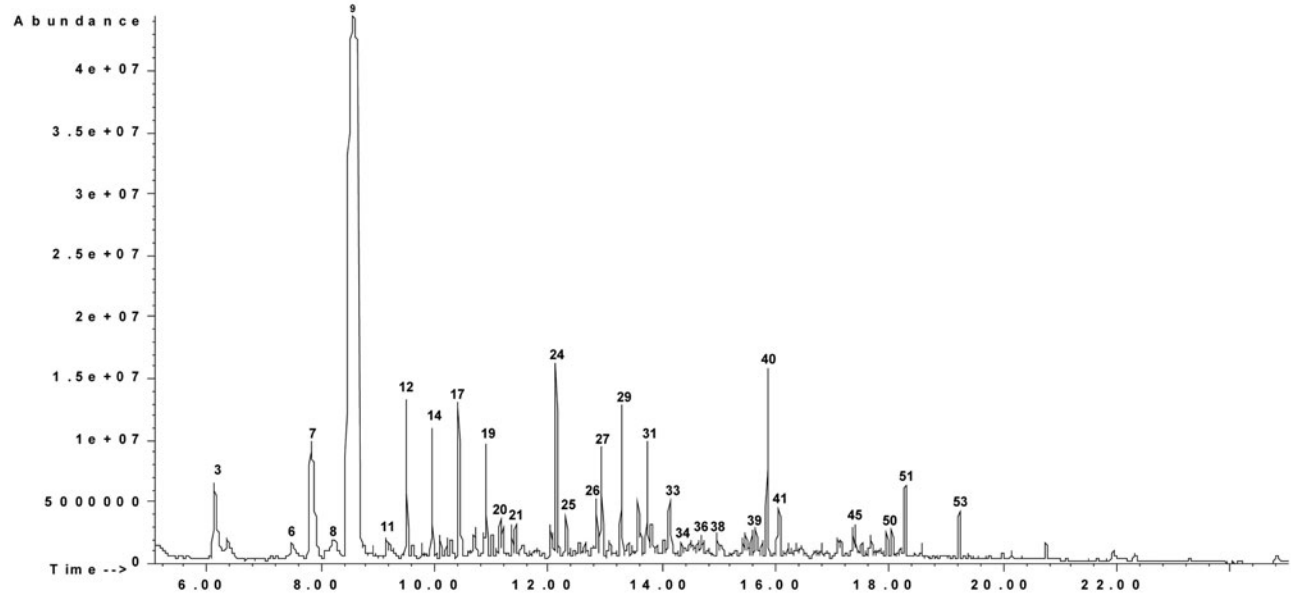

Fig. 1. Chromatogram of volatile fraction obtained for *M. crispa* essential oil obtained without Pb added in the soil (T_0 control). The numbers on the peaks correspond to the identification of the chemical compounds of the oils represented in Table 4.

Table 5
Percentage of classes of chemical compounds present in *M. crispa* essential oil obtained from four treatments with Pb

Classes		T_0	T_1	T_2	T_3	T_4
Monoterpenes	Hydrocarbonate	0.00	0.81	1.43	1.39	1.86
	Oxygenated	55.41	96.09	98.57	94.64	98.14
Sesquiterpenes	Hydrocarbonate	22.57	3.11	0	3.96	0.00
	Oxygenated	17.44	0.00	0.00	0.00	0.00
Diterpenes	Hydrocarbonate	4.57	0.00	0.00	0.00	0.00
Total		99.99	100	100.0	99.99	100.0

Note: T_0 Control, T_1 (Treatment 1), T_2 (Treatment 2), T_3 (Treatment 3), T_4 (Treatment 4).

increasing doses of Pb, identifying the compounds presented in Table 4.

As shown in Table 4, the qualitative and quantitative composition of essential oils for *M. crispa* cultivated in noncontaminated soil (T_0) and in highly contaminated soils with Pb ($T_1 - T_4$) showed significant variations. The individual analysis of essential oil of garden mint cultivated in noncontaminated soil (Fig. 1) shows the detection of approximately 59 compounds (making up 99.99% of the total oil), and 36 of them were identified (Table 4). From Table 5, oxygenated monoterpenes (ca. 55%) with carvone (39.31%) and dihydro carveol (4.38%) were predominantly found in this essential oil, following hydrocarbon sesquiterpenes (~22.6%) and oxygenated sesquiterpenes (~17.4%).

When *M. crispa* was cultivated with high doses of Pb, beyond essential oil yield increased, the high

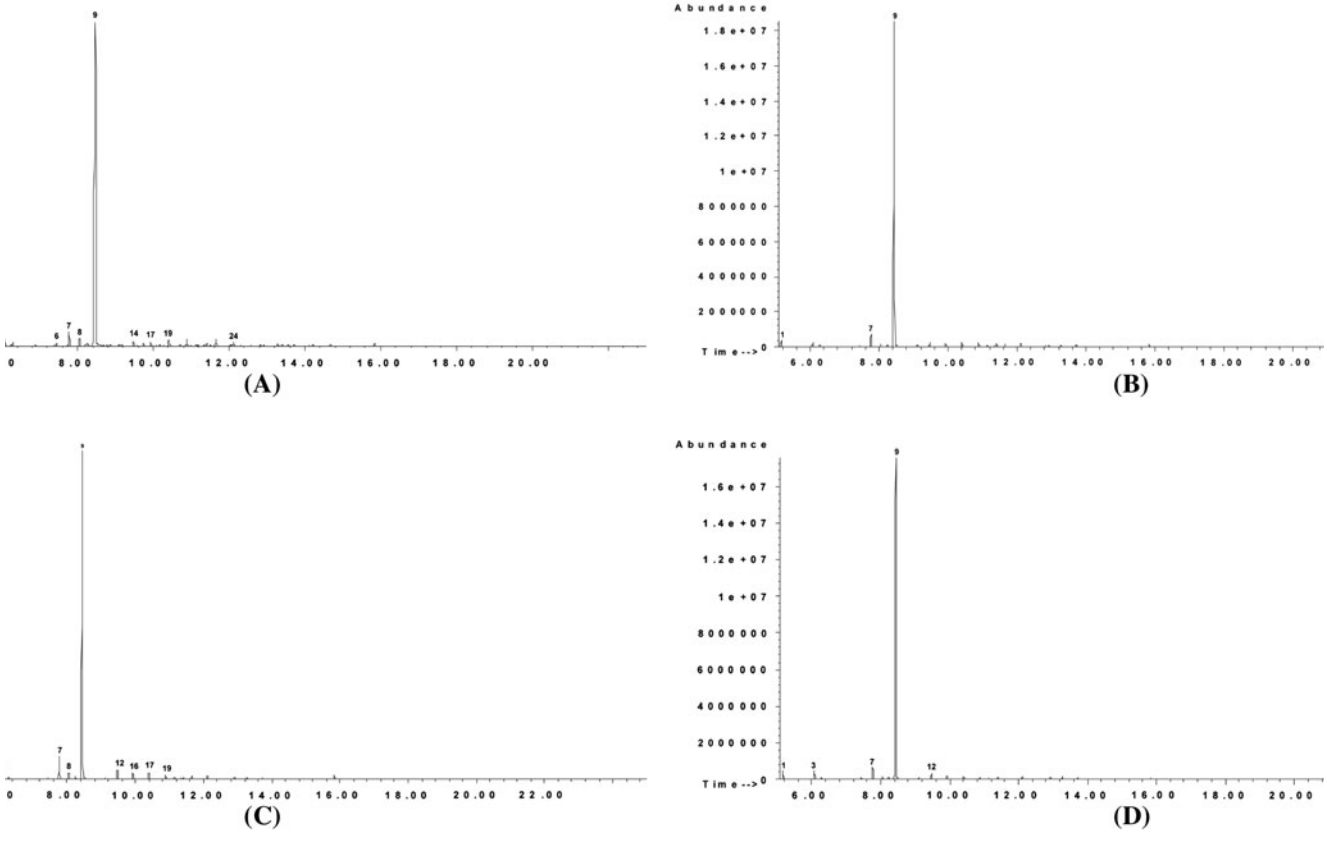

Fig. 2. Chromatograms of volatile fraction obtained for *M. crispa* essential oil with increasing doses of Pb in the soil. T_1—A (900 mg kg^{-1}); T_2—B (1,800 mg kg^{-1}); T_3—C (3,600 mg kg^{-1}) and T_4—D (7,200 mg kg^{-1}). The numbers on the peaks correspond to the identification of the chemical compounds of the oils represented in Table 4.

doses of Pb also influenced its chemical composition (Fig. 2), with a predominance of oxygenated monoterpenes (> 95%), mostly carvone (85–95%). Although carvone is the main compound found for this essential oil of garden mint cultivated in noncontaminated soil (39.2%), Pb translocation from roots to the aerial parts of the plant influenced the production of this compound drastically, indicating a possible alteration of the secondary metabolism of the plant. However, the study done by Scora and Chang [23] in a pot experiment with *M. piperita* showed that the chemical composition of essential oil was not affected by the presence of heavy metals such as Pb.

Biosynthetically, carvone is formed by cyclization of geranyl pyrophosphate to limonene, hydroxylation to *trans*-carveol, and dehydrogenation to carvone [34]. According to Carter et al. [35], this transformation requires four enzymatic steps, including geranyl diphosphate synthase (prenyltransfer), limonene synthase (cyclization), cytochrome P450 limonene hydroxylase (oxygenation), and carveol dehydrogenase (redox transformation).

According to Shewry and Lucas [36], plants constantly undergo several stress situations and can modulate defense responses to overcome these stresses and return to the normal metabolism. There are a lot of stresses undergone by plants like drastic temperature changes, moisture, solar radiation, pest infestation or pathogens, soil composition, etc. Plants can modify the molecular compounds constitution as a response mechanism and a lot of these alterations can be directly related to defense and protection. During their evolution, in order to survive, response mechanisms are developed by plants against damages and diseases, and when activated, they recognize the aggression [36].

Several studies verified an alteration in the expression standard of plant proteins produced by biotic and abiotic stresses, resulting in either inhibition or induction of biosynthesis of specific elements [37]. Thus, carvone superproduction observed in the present study could be interpreted as a defense mechanism of *M. crispa* in response to chemical stress. This mechanism could be explained by the loss or inactivation of specific enzymes and damages in biosynthetic production processes of most of the other secondary metabolites, mainly because of the mobility of this metal inside the plant, favoring the production of the major compound.

Other authors verified that several species of *Mentha* are tolerant to low concentrations of Pb and other metals present in the soil [7,9,10], and that this specific metal may influence the chemical composition of essential oils for this genus species. However, in our contribution about the behavior of *Mentha* under an extreme contamination by Pb in the soil, the composition of the essential oil was drastically influenced, and the production of the major metabolite was predominant. This information raises some questions on the utilization of areas with highly contaminated areas. Would other species of *Mentha* also be so tolerant to Pb as *M. crispa*? Could high concentrations of other metals also drastically influence the chemical composition of *M. crispa* essential oil or other species of *Mentha*? Considering the high contamination conditions by metals in the soil and other genera and families of plants with commercial interest, could essential oil also result in a greater production of major metabolite?

4. Conclusions

It was verified that *M. crispa* was tolerant to Pb and can be cultivated in areas considered degraded with high amounts of this element. Leaf number, budding, fresh biomass, essential oil yield, and chemical composition of the oil were affected by increasing Pb doses. Moreover, a significant increase of carvone fraction was observed after the Pb treatment.

According to Raskin et al. [38], Pb hyperaccumulator plants are able to extract and accumulate in their tissues higher values than 1,000 mg kg^{-1} of Pb in dry matter. In our study, we verified that in the greatest contamination condition (7,200 mg kg^{-1} of Pb, T_4), only 125.05 mg kg^{-1} of this contaminant was found in the roots plus aerial part of this species. With this information, it can be concluded that *M. crispa* is only tolerant and not hyperaccumulator for this heavy metal.

Finally, it would be very interesting to use chemically degraded areas for the cultivation of regional species of *Mentha* (or species of other genera and families) to produce essential oils in order to obtain high concentrations of metabolites that are interesting for the pharmaceutical, cosmetic, or food industries.

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