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Effect of trivalent and hexavalent Chromium (Cr) on the total Cr concentration in the vegetative plant parts of spearmint (*Mentha spicata* L.), lemon verbena (*Lippia citriodora* L.) and peppermint (*Mentha piperita* L.)

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Abstract

Three medicinal species, spearmint (*Mentha spicata L.*), lemon verbena (*Lippia citriodora L.*) and peppermint (*Mentha piperita L.*), were grown in pots to study the effect of trivalent (Cr (III) and hexavalent (Cr (VI) Chromium (Cr) on the total Cr concentration in the vegetative plant parts. A completely randomized block design with five concentrations (0, 1, 5, 10, 15 mg kg^{-1}) of Cr (III) and Cr (VI) was laid out. Trivalent Cr was applied as $CrCl_36H_2O$ and hexavalent Cr as $K_2Cr_2O_7$. Total Cr concentration in the plant tissues was measured by two methods: dry ash method (DAM) and microwave-assisted acid digestion method (MADM). Increasing Cr additions to the soil resulted in an increase in the total Cr concentration in the vegetative parts of all the plants measured by both methods and irrespective of the Cr oxidation state (i.e. from 0.72 to 2.55 mg kg^{-1}, from 0.78 to 10.12 mg kg^{-1}, and from 0.43 to 7.41 mg kg^{-1}, in lemon verbena, spearmint and peppermint, respectively, for Cr added as Cr (III) and from 0.82 to 6.02 mg kg^{-1}, from 1.07 to 6.02 mg kg^{-1} and from 1.99 to 11.16 mg kg^{-1}, in lemon verbena, spearmint and peppermint, respectively, for Cr added as Cr (VI)). Uptake of Cr was relatively low in all plants, but the total Cr concentration was significantly lower in plants exposed to Cr (III) than Cr (VI) in both methods of measurement. The total Cr concentration measured by the MADM was significantly higher than the total Cr measured by the DAM.

Keywords: medicinal plants; heavy metals; microwave-assisted acid digestion; dry ash.
Abbreviations: GFAAS_graphite furnace atomic absorption spectrometry; MADM_microwave acid digestion method; DAM_dry ash method; Cr (III)-M_Cr added as $CrCl_36H_2O$ (trivalent) and assayed by MADM; Cr (III)-D_Cr added as $CrCl_36H_2O$ (trivalent) and assayed by the DAM; Cr (VI)-M_Cr added as $K_2Cr_2O_7$ (hexavalent) and assayed by MADM; Cr (VI)-D_Cr added as $K_2Cr_2O_7$ (hexavalent) and assayed by the DAM.

Introduction

In recent years, concern over the contamination of soils by heavy metals from industries has increased. Chromium is a heavy metal that due to its mobility in the plant-soil system and its low affinity for soil colloids can easily enter the food chain, with adverse consequences for human health.

Chromium occurs in soils at concentrations of 10 to 150 mg kg^{-1}, and is the seventh most abundant element on Earth, but 21st in abundance in crystalline rocks (McGrath, 1995). It is a redox active metal and exists in the environment in several oxidation states ranging from Cr(-II) to Cr(+VI). Trivalent and hexavalent Cr are the most stable forms (Kabata-Pendias and Pendias, 2001). These two oxidation states are drastically different in charge, physicochemical properties as well as chemical and biochemical reactivity. Trivalent Cr is the most thermodynamically stable form of Cr in the soil and readily precipitates as chromium hydroxide ($Cr(OH)_3$) and iron–chromium hydroxide ($(Fe,Cr)(OH)_3$) or becomes immobilised after sorption onto soil colloids (Leita et al., 2009). Hexavalent Cr remains thermodynamically metastable in the pore solution and is generally much more mobile in the soil than Cr (III), since Cr (VI) oxyanions (chromate CrO_4, bichromate $HCrO^{4-}$, and dichromate $Cr_2O_7^{2-}$) are not absorbed onto soil colloids under alkaline to sub-neutral conditions. The transport and reduction of Cr (VI) with in contaminated soil ultimately reflects the interdependent effects of chemical, physical and microbial processes (Shanker et al., 2005). Hexavalent Cr can be rapidly reduced to Cr (III) by soil organic matter (Banks et al., 2006).

Nutritionally, Cr (III) is an essential component of a balanced human and animal diet for preventing adverse effects in the metabolism of glucose (Anderson, 1997). Hexavalent Cr is extremely toxic carcinogen and may cause death to animals and humans if ingested in large doses (Syracuse Research Corporation, 1993). Routes of human exposure to Cr compounds include ingestion of contaminated food and water, inhalation of airborne particulates, and contact with numerous manufactured items containing Cr compounds (Syracuse Research Corporation, 1993).

Table 1. Effect of trivalent (Cr (III)) and hexavalent (Cr (VI)) chromium on the total Cr concentration in the vegetative parts of lemon verbena plants (*Lippia citriodora*).

LEMON VERBENA					
Cr (III) supplied plants, mg kg^{-1}	[I] Total Cr mg kg^{-1} (DW)	[II] Total Cr mg kg^{-1} (DW)	Cr (VI) supplied plants, mg kg^{-1}	[I] Total Cr mg kg^{-1} (DW)	[II] Total Cr mg kg^{-1} (DW)
0	0.72 a*	0.25 a*	0	0.82 a*	0.34 a*
5	0.98 ab	0.32 a	5	1.44 a	0.51 a
10	1.24 bc	0.62 b	10	2.93 b	0.97 b
20	1.60 c	0.87 c	20	5.06 c	1.71 c
40	2.55 d	1.56 d	40	7.82 d	2.65 d

[I]mean values of total Cr measured by the MADM; [II] mean values of total Cr measured by the DAM; *Column means followed by the same letter are not significantly different according to Duncan's multiple range test at $p \leq 0.01$

Hexavalent Cr compounds have been estimated to be 10–100 times more toxic than Cr (III) compounds (Katz and Salem, 1994).

The distribution of Cr in environmental systems is controlled by three important reactions: oxidation-reduction, precipitation-dissolution and sorption-desorption (Saleh et al., 1989). All Cr compounds are highly toxic to plants and are detrimental to their growth and development. The toxic effects of Cr are primarily dependent on the oxidation state, which determines its uptake, translocation, reactivity and accumulation (Shanker et al., 2005). Chromium levels in plants growing in 'normal' soils are usually less than 1 mg kg^{-1} DW, rarely exceed 5 mg kg^{-1}, and typically range from 0.02 to 0.2 mg kg^{-1} (DW) (Kabata-Pendias and Pendias, 2001; Pratt, 1966).

Since Cr may enter the human food chain through medicinal plants, it is important to determine their potential toxicity and health risk. Therefore, the objective of this work was to study the effects of different Cr oxidation states (trivalent and hexavalent) on total Cr concentration in the vegetative (herbal) parts of three medicinal plants: spearmint (*Mentha spicata L.*), lemon verbena (*Lippia citriodora L.*) and peppermint (*Mentha piperita L.*), grown in pots under natural environmental conditions.

Results

No visible phytotoxic symptoms were observed due to increasing application of either Cr (III) or Cr (VI) in any of the plant species during the experiment.

Lemon verbena (Lippia citriodora)

The total Cr concentration within the vegetative parts of lemon verbena plants measured by the MADM ranged from 0.72 to 2.55 mg kg^{-1} for Cr added as Cr (III) and from 0.82 to 6.02 mg kg^{-1} for Cr added as Cr (VI), and increased with increasing concentrations of Cr applied to the soil The total chromium concentration measured by the DAM ranged from 0.25 to 1.56 mg kg^{-1} and from 0.34 to 2.65 mg kg^{-1} for Cr added as Cr (III) and Cr (VI), respectively, and increased with increasing concentrations of Cr applied to the soil (Table 1). The total Cr concentration in the vegetative parts of lemon verbena was significantly lower when Cr was applied as Cr (III) compared with Cr (VI) (Fig 1), irrespective of the assay method.

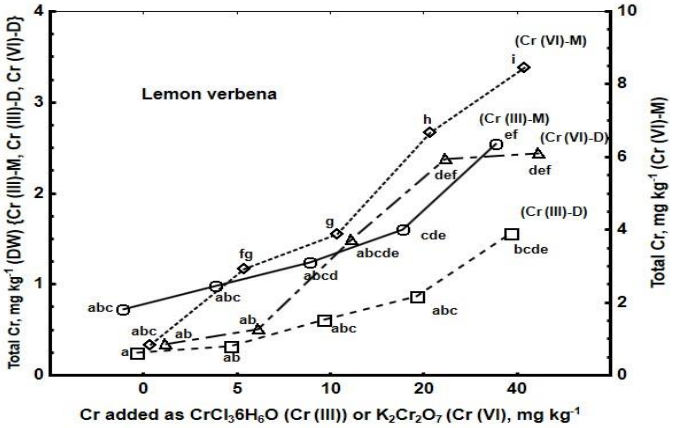

Fig 1. Total Cr concentration in the vegetative plant parts of lemon verbena, in different oxidation states added Cr and in different methods used to measure total Cr (different letters indicate significant differences between the two oxidation states of Cr applied and the two methods used to measure total Cr, according to Duncan's multiple range test at p<0.05).

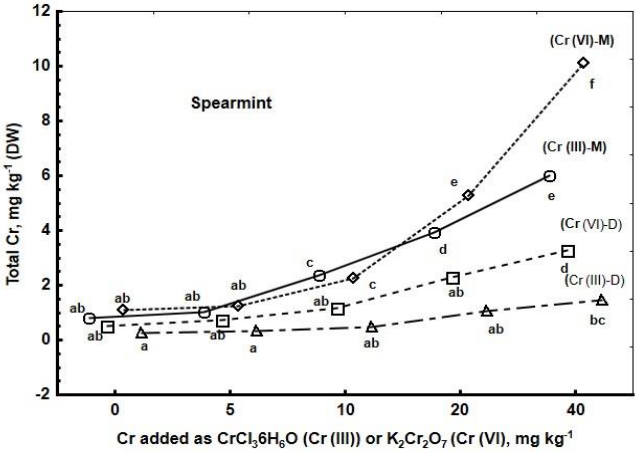

Fig 2. Total Cr concentration in herbal plant parts of spearmint, in different oxidation states added Cr and in different methods used to measure total Cr (different letters indicate significant differences between the two oxidation states of Cr applied and the two methods used to measure total Cr, according to Duncan's multiple range test at p<0.05).

Table 2. Effect of trivalent (Cr (III)) and hexavalent (Cr (VI)) chromium on the total Cr concentration in the vegetative parts of spearmint plants (*Mentha spicata*).

SPEARMINT					
Cr (III) supplied plants, mg kg^{-1}	[I] Total Cr mg kg^{-1} (DW)	[II] Total Cr mg kg^{-1} (DW)	Cr (VI) supplied plants, mg kg^{-1}	[I] Total Cr mg kg^{-1} (DW)	[II] Total Cr mg kg^{-1} (DW)
0	0.78 a*	0.27 a*	0	1.07 a*	0.51 a*
5	1.01 ab	0.34 a	5	1.24 a	0.72 a
10	2.36 b	0.46 a	10	2.25 a	1.15 a
20	5.27 c	1.05 b	20	3.91 b	2.30 b
40	10.12 d	1.47 c	40	6.02 c	3.28 c

[I] mean values of total Cr measured by the MADM; [II] mean values of total Cr measured by the DAM; *Column means followed by the same letter are not significantly different according to Duncan's multiple range test at $p \leq 0.01$

Spearmint (Mentha spicata)

The total Cr concentration within the vegetative parts of spearmint measured by the MADM ranged from 0.78 to 10.12 mg kg^{-1} for Cr added as Cr (III) and from 1.07 to 6.02 mg kg^{-1} for Cr added as Cr (VI), and generally increased with increasing concentrations of Cr applied to the soil. The total Cr concentration in the vegetative parts of spearmint measured by the DAM ranged from 0.27 to 1.47 mg kg^{-1} for Cr added as Cr (III) and from 0.51 to 3.28 mg kg^{-1} for Cr added as Cr (VI), and generally increased with increasing concentrations of Cr applied to the soil. The total Cr concentration in the vegetative parts of spearmint was significantly lower when Cr was added as Cr (III) compared with Cr (VI) (Fig 2), irrespective of the assay method.

Peppermint (Mentha piperita)

The total Cr concentration within the vegetative parts of peppermint measured by the MADM ranged from 0.43 to 7.41 mg kg^{-1} for Cr added as Cr (III) and from 1.99 to 11.16 mg kg^{-1} for Cr added as Cr (VI), and generally increased with increasing concentrations of Cr applied to the soil. The total Cr concentration measured by the DAM ranged from 0.31 to 2.91 mg kg^{-1} for Cr added as Cr (III) and from 0.57 to 4.62 mg kg^{-1} for Cr added as Cr (VI), and generally increased with increasing concentrations of Cr applied to the soil. The total Cr concentration in the vegetative parts of peppermint was significantly lower when Cr was added as Cr (III) compared with Cr (VI) (Fig 3), irrespective of the method.

Discussion

Of the three medicinal plants studied here, the total Cr concentration within the vegetative parts was significantly higher in peppermint than in lemon verbena and spearmint, irrespective of the assay method and the oxidation state of the Cr applied to the soil (Fig. 4, 5, 6, 7).

The higher total Cr concentration in the vegetative parts of all three species when Cr was applied to the soil in the hexavalent form as opposed to the trivalent form, irrespective of the assay method (Fig. 4, 5, 6, 7) may be due to: 1) the better mobilization of Cr (VI) and 2) the independent mechanisms of uptake for the two Cr oxidation states. Hexavalent Cr is more mobile than trivalent Cr (Gardea-Torresday et al., 2005; Zayed et al., 1998; Jean et al., 2008; Han et al., 2004). Gardea-Torresday et al. (2005) reported

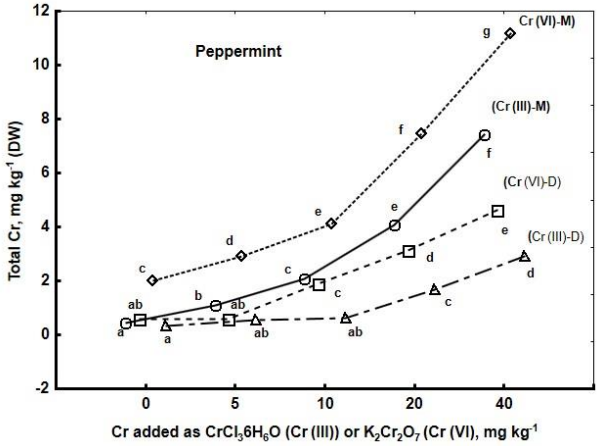

Fig 3. Total Cr concentration in herbal plant parts of peppermint, in different oxidation states added Cr and in different methods used to measure total Cr (different letters indicate significant differences between the two oxidation states of Cr applied and the two methods used to measure total Cr, according to Duncan's multiple range test at p<0.05)

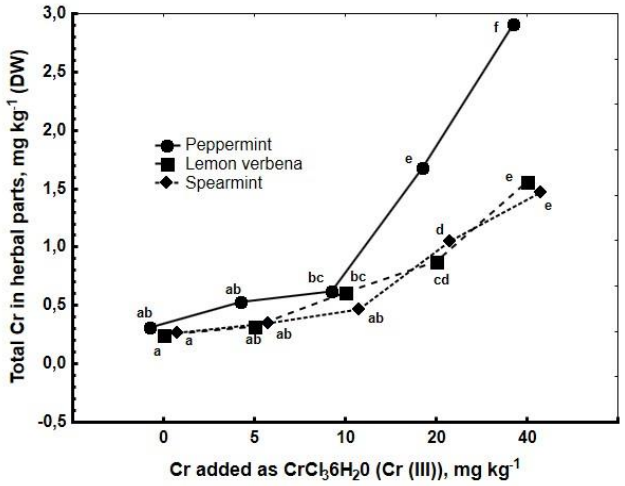

Fig 4. Total Cr concentration in the vegetative parts of peppermint, lemon verbena and spearmint measured by the DAM and with Cr added to the soil in the trivalent form (Different letters indicate significant differences between mean values of plants, according to Duncan's multiple range test at p<0.05).

Table 3. Effect of trivalent (Cr (III)) and hexavalent (Cr (VI)) chromium on the total chromium concentration in the vegetative parts of peppermint plants (*Mentha piperita*).

PEPPERMINT					
Cr (III) supplied plants, mg kg^{-1}	[I] Total Cr mg kg^{-1} (DW)	[II] Total Cr mg kg^{-1} (DW)	Cr (VI) supplied plants, mg kg^{-1}	[I] Total Cr mg kg^{-1} (DW)	[II] Total Cr mg kg^{-1} (DW)
0	0.43 a*	0.31 a*	0	1.99 a*	0.57 a*
5	1.10 a	0.53 a	5	2.90 b	0.57 a
10	2.09 b	0.62 a	10	4.11 c	1.89 b
20	4.0 c	1.68 b	20	7.45 d	3.12 c
40	7.41 d	2.91 c	40	11.16 e	4.62 d

[I] mean values of total Cr measured by the MADM; [II] mean values of total Cr measured by the DAM; *Column means followed by the same letter are not significantly different according to Duncan's multiple range test at $p \leq 0.01$.

that the accumulation of Cr in the upper plant parts is 12 to 18 times higher for hexavalent than for trivalent Cr.

Trivalent and hexavalent Cr have an independent mechanism of uptake (Jean et al., 2008). The uptake of Cr (III) is passive diffusion and this ion interacts with cell walls through cation-exchange sites (Zayed et al., 1998; Jean et al., 2008). Absorption of Cr (VI) requires metabolic energy from the plants. Hexavalent Cr moves more easily from the roots to the upper plants tissues because of absorption, especially of the $Cr_2O_4^{2-}$ form, and as an active process, probably correlates with the sulfate transport system located within the plasma membrane (Pandey and Sharma, 2002; Kim et al., 2006). Since plants lack a specific transport system for Cr, it is taken up by carriers of essential ions such as sulfate or iron; hence chromium can interfere with the uptake of these essential elements (Skeffington et al., 1976).

Irrespective of the form of Cr applied to the soil and the method of assay, the total Cr concentration in the vegetative parts of the three medicinal plants studied here was relatively low (Tables 1, 2, 3) possibly due to the immobilization of Cr by organic matter within the soil (Zayed and Terry, 2003). For example, organic matter has been found to reduce mobile Cr (VI) to the relatively immobile Cr (III) (Banks et al., 2006). However, the fact that in all three species total Cr concentration was higher in plants exposed to Cr (VI) than Cr (III) may be of significance to human health in view of the much higher toxicity of the former.

Finally, the observation that in all cases the total Cr concentration measured by the MADM was significantly higher than that in the dry ash digestion method (Fig. 1, 2, 3) indicates that possibly the use of acid and H_2O_2 in the former increase the the digestion of organic matter. Therefore this method may be more accurate for the measurement of total concentrations of Cr in plant tissues.

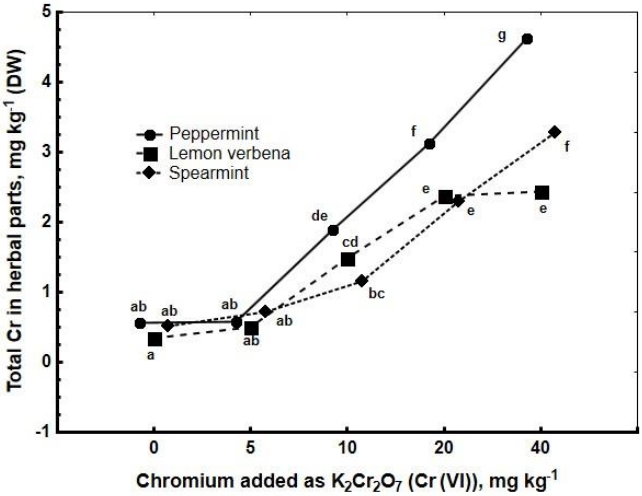

Fig 5. Total Cr concentration in the vegetative parts of peppermint, lemon verbena and spearmint measured by the DAM and with Cr added to the soil in the hexavalent form (Different letters indicate significant differences between mean values of plants, according to Duncan's multiple range test at $p<0.05$).

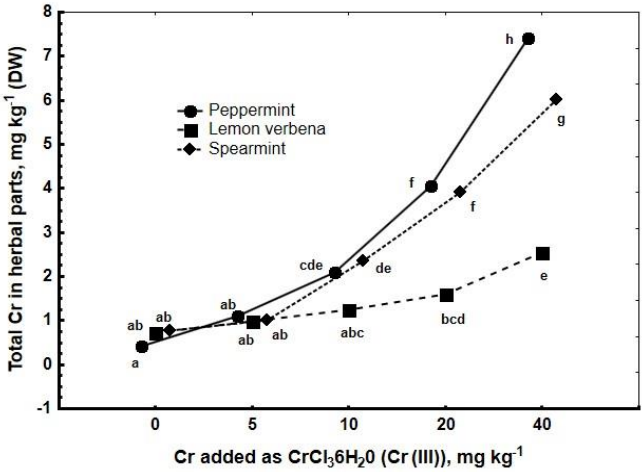

Fig 6. Total Cr concentration in the vegetative parts of peppermint, lemon verbena and spearmint measured by the MADM and with Cr added to the soil in the trivalent form (Different letters indicate significant differences between mean values of plants, according to Duncan's multiple range test at $p<0.05$).

Materials and Methods

Plant material, substrate and pot

Plants of spearmint (*Mentha spicata L.*), peppermint (*Mentha piperita L.*) and lemon verbena (*Lippia citriodora*) derived from rooted cuttings were transplanted to plastic trays containing potting soil (Gramoflor-potting soil, GmbH & Co. Kg., EN 12580) and covered with vermiculite (Agra-Vermiculite) in order to avoid evaporation. The trays were placed in an environmental growth chamber (temperature 20±2°C, relative humidity 90±5%) for 15 days and then transferred to a greenhouse (temperature 18±2°C, relative humidity 70±5%), where plants were individually transplan-

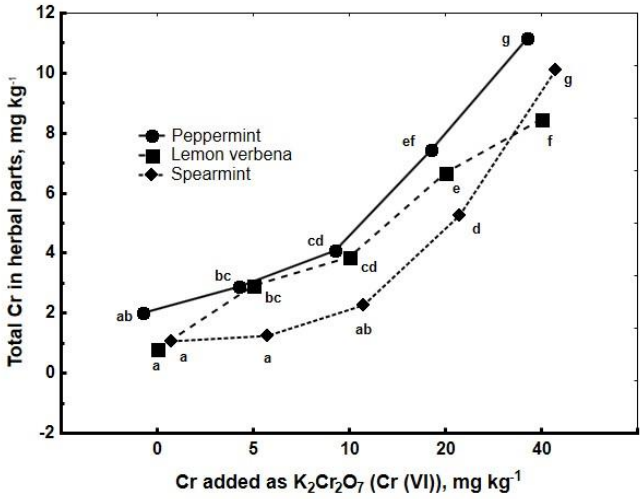

Fig 7. Total Cr concentration in the vegetative parts of peppermint, lemon verbena and spearmint measured by the MADM and with Cr added to the soil in the hexavalent form (Different letters indicate significant differences between mean values of plants, according to Duncan's multiple range test at $p<0.05$).

ted to 2 L pots containing peat (Kekila) and soil (1:1) with pH 7.2 and organic matter content 5.7 mg kg^{-1}.

Experimental design and treatments

After holding in the greenhouse for 15 days, the plants were transferred to the open under ambient environmental conditions and grown for five weeks (total experiment period 65 days). The pots were arranged in a complete randomized block design with five treatments and three replicates per species and Cr source, i.e. a total of 15 pots per plant species and Cr source; in total 90 pots. Trivalent Cr was applied as $CrCl_36H_2O$ and hexavalent Cr as $K_2Cr_2O_7$. Chromium once a week was applied in a volume of 50 ml per pot as Cr (III) and Cr (VI) to the plants at concentrations of 0, 5, 10, 20 and 40 mg Cr kg^{-1}. Soil moisture was maintained at about field capacity.

Plant samples preparation

The aerial plant parts were harvested just prior to anthesis and washed free of all adhering soil in distilled water. The vegetative parts (stems and leaves) were shredded and dried to constant weight at 65^{o}C. The dried samples were finely ground in a centrifuge mill and passed through a 250 mesh sieve. To minimize contamination, acid-washed polyamide pots and agate balls were used.

Laboratory analysis

Total Cr concentration in the vegetative plant parts was measured by two methods, as follows:

The microwave-assisted acid digestion method (MADM) (SW-846 EPA Method 3052, 1996)

Approximately 0.3 g sample was digested with 3 ml HNO_3 and 2 ml H_2O_2 in a microwave digestion system (Berghof speedwave MWS-3^{+}). Each time, 12 samples were placed in the microwave carousel, together with a blank prepared with suprapure acids (Suprapure, Merck). The digested samples were cooled for 30 min, filtered through a Whatman no. 42 filter paper, transferred to a 50 ml flask and brought to volume with distilled water. The clear solutions were analyzed for total chromium by a Thermo Scientific Model iCE 3000 atomic absorption spectrometer equipped with a GF95Z Zeeman Graphite Furnace Atomizer and an FS95 furnace auto-sampler. The source of radiation was a chromium hollow cathode lamp, operating at 100% Lamp Current, which provided a 357.9 nm line, with a spectral bandwidth of 0.5 nm. Data coated Graphite Tubes were used. For all measurements, integrated absorbance with an integration time of 4 s was used. Aliquots of a single stock solution (Cr=1,000±0,002 g/l, Panreac Química S.L.U.**)** were used to prepare calibration solutions by dilution to the desired concentration in 1% HNO_3. The ranges of the calibration curve (5 points) were selected to match the expected concentration for chromium of the sample studied by Graphite Furnace Atomic Absorption Spectrometry (GFAAS). The correlation coefficient r^2 obtained was 0.999. The detection limit (LOD) was calculated as the concentration of an element that gave the standard deviation of a series of ten consecutive measurements of blank solution. The sample volume injected was 20 µl.

The dry ash Method (DAM)

Approximately 0.5 g sample was dry-ashed at 550^{O}C and the residue dissolved in concentrated HNO_3. The Cr concentration of each sample was measured by atomic absorption spectrophotometry using a Thermo Scientific Model iCE 3000 atomic absorption spectrometer equipped with a GF95Z Zeeman Graphite Furnace Atomizer and an FS95 furnace auto-sampler (Baker and Amacher, 1982).

Statistical analysis

Statistical analysis was carried out with the aid of STATISTICA^{TM} Ver. 8.0 (StatSoft 2008) for all the parameters studied. All data were subjected to Duncan's Multiple Range Test to determine the statistical significance of the effects due to treatments with trivalent and hexavalent Cr.

Conclusions

The obtained results showed that: The total Cr concentration in the vegetative plant parts of, peppermint, lemon verbena and spearmint increased with increasing additions of Cr to the soil. The total Cr concentration in plants exposed to Cr (VI) was significantly higher than that of plants exposed to Cr (III), irrespective of the assay method, indicating a higher mobility of hexavalent Cr. However, the uptake of Cr was relatively low in all three plant species. In all three species, the total Cr concentration was found to be significantly higher when measured by microwave acid digestion than by the dry ash method.

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