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Phytoremediation Potential and Essential Oil Quality of Peppermint Grown in Contaminated Soils as Affected by Sludge and Time

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ABSTRACT

This study aimed to assess the effect of incubation time and sewage sludge on peppermint biomass, essential oil yield, and Zinc (Zn), lead (Pb), and Cadmium (Cd) concentrations in the plant tissues as well as assessing phytoremediation potential of peppermint grown in contaminated calcareous soils. A greenhouse experiment was conducted by growing peppermint (*Mentha pipertia* L.) in two contaminated soils treated with three levels of sewage sludge (0, 10, and 30 g kg^{-1}) over two incubation times (30 and 120 days). Results revealed an increase in plant biomass and essential oil yield with an increase in incubation time and the sewage sludge amount. The concentrations of Zn, Pb, and Cd in plant tissues increased significantly in all treatments, except Cd concentration in plants growing on 10 g kg^{-1} of sewage sludge and incubated for 120 days. No traces of Zn, Pb, and Cd were found in essential oil of plants treated with 10 g kg^{-1} of sewage sludge. In other treatments, the concentrations of these metals in the essential oil were found within the limits recommended for medicinal plants. Unlike the bioaccumulation factor, the translocation factor of the studied metals decreased with an increase in the sewage sludge amount and incubation time. Although peppermint was not a sustainable plant to lower the pollution load, as the biomass production increased significantly and metals concentrations in essential oil remained within the recommended limits in all treatments, it can be concluded that peppermint can be used for cultivation in contaminated soils treated with sewage sludge.

Keywords: Calcareous soil, Essential oil, Heavy metals, Mint.

INTRODUCTION

Accumulation of heavy metals in soil, especially Cadmium (Cd), lead (Pb), and Zinc (Zn), is of concern in agricultural production systems because of its adverse effect on environmental health, plant growth, and food quality (Khosropour *et al*., 2019). Lead and Cd are recognized as the non-essential elements but could be taken up by plants from soils (Alloway, 2013). Cd is known as the most bioavailable and mobile cationic trace metal, but its biological roles in higher plants are still unknown, therefore, there is no consensus on the tolerance and toxicity of Cd in plant tissues (Alloway, 2013; Sloan *et al*., 2016). Unlike Pb and Cd, Zn is one of the essential elements required in small concentration for plant growth and production of crops, as well as an essential element for animals and humans. It plays a significant role in the structure and catalytic function of plant enzymes, such as alcohol dehydrogenases, superoxide dismutase, and RNA polymerase (Hafeez *et al*., 2013). Overall, the mobility and bioavailability of heavy metals in soils are controlled using a series of soil and plant characteristics such

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as soil texture, pH, and mainly organic matter content. Organic matter is a fundamental component of the soil that affects the chemical, physical, and biological properties (Shaheen *et al*., 2017; Abad-Valle *et al*., 2017). One of the primary valuable sources of organic matter that has been used widely in the farmland for several years is sewage sludge (Eid *et al*., 2019). It induces a great source of macronutrients to soil solution, as well as providing a way of disposing of unwanted waste (Pritchard *et al*., 2010; Mingorance *et al*., 2014). Despite the positive effects on soil properties, sewage sludge contains metal contaminants, and there are some concerns about their environmental fate. Currently, sewage sludge is being disposed in landfills as a means of immobilizing its heavy metal content. According to U.S Environmental Protection Agency (USEPA), 1.3 million tons of sludge is produced per year, requiring proper disposal in landfills for immobilization treatments (Prasad *et al*., 2019). For this reason, its use in agricultural soils should be regulated (Sullivan *et al*., 2006). Several studies have investigated the effects of sewage sludge on field crop growth (Gwenzi *et al*., 2016; Zuo *et al*., 2019); but most of these studies have not investigated the effect of incubation periods on efficacy of sewage sludge and changes in chemistry of metal contaminated soils, especially in calcareous soils.

There is a current opinion that planting of food crops in contaminated soils for phytoremediation involves many environmental risks, including the entry of heavy metals into the food chain of humans and animals through the consumption of these products. Conversely, the existence of aromatic plants growing on contaminated soils has been the focus of several studies because of their potential use in the phytoremediation of heavy metal contaminated soils (Pandey *et al*., 2019). Stanojkovic-Sebic *et al*. (2015) found that the contents of heavy metals in the *Matricaria chamomilla* L., *Mentha* piperita L., *Foeniculum vulgare* Mill., and *Melissa officinalis* L. species grown in contaminated soils were within the levels of recommended limits and these medicinal plants may also be suitable for medicinal extracts. Peppermint (*Mentha piperita* L.) is one of the economically important medicinal plants with species from the family of Lamiaceae. This plant is grown to produce essential oil (Sustrikova and Salamon, 2004). Analytical quantification of the use of medicinal plants as a suitable plant for highly metal-contaminated acidic soils have been done in many studies (Malinowska and Jankowski, 2017; Angelova *et al*., 2016). However, insufficient work has been done to confirm the hypothesis that peppermint could be cultivated as a valuable crop in contaminated calcareous soils treated with sewage sludge without contamination of the consumer product (Prasad *et al*., 2010; Chand *et al*., 2015). Chand *et al*. (2012) observed that peppermint could not be grown as a scavenger in Ni and Pb-polluted soils but could be used in mildly contaminated soils with application of organic matter. Amirmoradi *et al*. (2012) stated that peppermint could tolerate the medium range of Cd (10 and 20 ppm) and Pb (100 and 300 ppm) concentrations in wastewater or polluted soil and these concentrations did not influence the content of essential oil.

Ecofriendly disposal of sewage sludge can be very important as the escalating production of sewage sludge has become a major problem. The current study aimed to provide evidence for the effect of incubation time and suitability of peppermint cultivation in contaminated soil treated with sewage sludge.

MATERIALS AND METHODS

Soils Characteristics

Soil samples were taken from various agricultural lands on the mining site of Zanjan Zinc Industrial Complex, Zanjan, Iran (0-30 cm surface layer) and were tested for heavy metal concentrations. On the basis of critical concentration of Zn, Pb, and Cd, as outlined in previous complimentary work by Kabata-Pendias (1984), soil was divided into two types during this study (Soil 1 and Soil 2). The soils were classified as Typic Calcixerepts in the study area (Soil Survey Staff, 2014). The soil samples were air-dried and passed through a 2 mm stainless steel sieve. Total and available concentrations of Zn, Pb, Cd were determined by acid digestion (HNO_3) (Lindsay and Norvell, 1978), and 0.005 M, DTPA-TEA solutions (Sposito *et al*., 1982), respectively. Cation Exchange Capacity (CEC) was determined by sodium acetate (NaOAC) leaching at pH 7.0 (Chapman, 1965). Available K was analyzed in the solution extracted by 1M, NH_4OAC using flame photometer (FOSS FIAstar 5000 triple) (Haby *et al*., 1990), Soil texture was determined by hydrometer (Gee and Bauder, 1986). Soil pH and Electrical Conductivity (EC) were recorded using pH meter (Mettler Toledo, USA) and EC meter (4010 conductivity meter, Jenway Inc, England), respectively, in saturated paste extract (Thomas, 1996; Rhoades, 1996). Soil organic carbon was determined by the Walkley-Black method (Nelson and Summers, 1996). Calcium carbonate equivalent was estimated by the calcimetric method (Loeppert and Suarez, 1996). Total N and available P were also measured by the Kjeldahl and Olsen method, respectively (Bremner and Mulvaney, 1982; Olsen and Sommers, 1982).

Characterization of Sewage Sludge

Sewage sludge was sourced from the Isfahan Refinery, Iran. The sewage sludge samples were air-dried at 20±2°C and sieved through a 0.5 mm stainless steel sieve. The sewage sludge was characterized for EC and pH using a 1:5 sewage sludge-to-water ratio (Thomas, 1996; Rhoades, 1996). Several other sewage sludge characteristics were determined according to methods previously described for soils.

Set-up of Incubation and Seedling Experiment

A factorial experiment was conducted in a completely randomized design in two contaminated calcareous soil (Soil 1 and Soil 2), using three levels of sewage sludge (0, 10 and 30 g kg^{-1}), and two incubation times (30 and 120 days) with three replications. Samples (500±5 g) of the prepared soils and sewage were put into polyethylene pots (8 cm diameter×7 cm height) and wetted to field capacity. Samples were then incubated at 25±2°C in darkness. At the end of each incubation period, five peppermint rhizomes with the same size [(3.9±0.4) cm length×(0.42±0.03) cm width] were sown into each pot of treated soils. The pots were then placed in controlled light growth cabinets at 20°C (day) and 15°C (night) on a 12-hour light/dark cycle for 85 days. Pots were weighed daily and watered with deionized water as necessary to ensure that soils were maintained at 50% Water Holding Capacity (WHC).

Harvesting of Peppermint and Sampling of Soils

On day 85, plants were harvested and washed thoroughly using deionized water. Peppermint shoots were air-dried at 20±2°C. Peppermint roots were also placed overnight in a drying oven at 45±1°C. The plant tissues were then weighed to record shoot and root dry weight per pot. Dried plant tissues were powdered before being acid digested using 10 mL of 65% HNO_3 per sample and placed on a heating block (Lozano-Rodríguez, 1995). Digested plant samples were then analyzed by ICP-OES (PerkinElmer-Optima 2100 DV, USA) for Zn, Pb, and Cd concentrations. Detection limits of ICP-OES were 0.2, 1 and 0.1 μg L^{-1} for Zn, Pb, and Cd, respectively. The Root Bioaccumulation Factor (RBF) and the Translocation Factor (TF) were calculated according to the following equations (Karami *et al*., 2009):

$$RBF = \frac{C_{root}}{C_{Soil}} \quad (1)$$

$$TF = \frac{C_{Shoot}}{C_{Root}} \quad (2)$$

Where, C_{Shoot} and C_{Root} are the heavy metal Concentrations (mg kg^{-1}) in Shoot and Root, respectively, and C_{Soil} is the total metal Concentration in Soil (mg kg^{-1}).

The essential oil content was then extracted from air-dried plant shoots by the hydro distillation method in a Clevenger type apparatus (Furnis *et al*., 1989). The obtained essential oil was also dried using anhydrous sodium sulfate and then stored in sealed jars at 4°C. Essential oil samples were then analyzed by ICP-OES (PerkinElmer-Optima 2100 DV, USA) for Zn, Pb, and Cd concentrations. Oil yield was calculated by multiplying the oil content by the plant aboveground yield. DTPA-extractable Zn, Pb and Cd concentrations, pH and EC values, as well as the concentration of Dissolved Organic Carbon (DOC) (TOC-VCPH, Shimadzu, Japan), were also done simultaneously on the remaining soil in the pots after plant harvest.

Statistical Analyses

Comparisons of plant metal concentrations and soil characteristics between sewage sludge and non-sewage sludge-treated soils incubated for 30 and 120 days were analyzed using LSD tests in R-3.4.0 software.

RESULTS

Characterization of the Studied Soils and Sewage Sludge

The general physicochemical characteristics of soils and sewage sludge results are shown in Table 1. The results showed that Soil 1 contained a higher total concentration of Zn, Pb, and Cd, as well as amounts of carbonate calcium and organic

Table 1. Physicochemical characteristics of the experimental soils and sewage sludge used in this study.[a]

Characteristics	Soil 1	Soil 2	Sewage sludge
Total Zn (mg kg^{-1})	535.16±4.92	273.44±3.66	555.32±6.79
Total Pb (mg kg^{-1})	227.66±5.31	162.19±2.95	56.74±3.11
Total Cd (mg kg^{-1})	7.50±1.23	3.97±0.87	1.06±0.15
DTPA–extractable Zn (mg kg^{-1})	58.55±2.86	27.81±1.23	49.13±1.54
DTPA–extractable Pb (mg kg^{-1})	23.71±3.11	16.82±1.04	6.25±0.68
DTPA–extractable Cd (mg kg^{-1})	2.87±0.62	0.96±0.33	0.11±0.04
Total N (g kg^{-1})	1.08±0.51	1.21±0.12	38.74±2.11
Available P (g kg^{-1})	0.06±0.01	0.09±0.02	1.17±0.85
Available K (g kg^{-1})	0.11±0.03	0.16±0.02	0.84±0.06
pH	7.61±0.68	7.92±0.54	6.92±0.58
EC (dS m^{-1})	0.80±0.04	0.63±0.07	6.22±0.72
CEC ($cmol_c$ kg^{-1})	17.32±2.54	13.47±1.66	59.46±3.32
Organic carbon (g kg^{-1})	10.80±1.67	8.11±1.74	272.43±4.77
$CaCO_3$-eq (g kg^{-1})	267.01±4.03	463.13±4.88	113.04±3.29
Texture	Clay loam	Sandy clay loam	-

[a] Results are means±standard deviations. Note: The permission for publishing the sewage sludge properties in this manuscript was requested and obtained from the authorized director or representative of the Isfahan Refinery, Iran, prior to the analysis and application of sewage sludge.

Table 2. Chemical characteristics of sewage sludge-treated and untreated soils, sampled 30 and 120 days after treatment application.[a]

Soil	Sewage sludge (g kg^{-1})	Incubation time (Days)	Zn^{B} (mg kg^{-1})	Pb^{B} (mg kg^{-1})	Cd^{B} (mg kg^{-1})	pH	EC (dS m^{-1})	DOC (mg L^{-1})
1	0	30	81.0 ± 3.9^{ij}	39.4 ± 3.7^{h}	3.0 ± 0.02^{e}	7.4 ± 0.01^{fg}	1.2 ± 0.04^{k}	41.1 ± 2.8^{j}
		120	85.6 ± 6.7^{i}	43.7 ± 2.5^{gh}	3.0 ± 0.05^{e}	7.4 ± 0.04^{gh}	1.4 ± 0.10^{ghi}	39.4 ± 1.2^{j}
	10	30	123.2 ± 2.9^{f}	57.7 ± 5.3^{d}	3.2 ± 0.03^{d}	7.2 ± 0.01^{j}	1.8 ± 0.05^{e}	63.9 ± 3.7^{fg}
		120	160.2 ± 1.6^{c}	68.8 ± 5.3^{c}	3.1 ± 0.02^{d}	7.2 ± 0.04^{j}	1.8 ± 0.03^{e}	75.2 ± 3.7^{e}
	30	30	146.9 ± 0.8^{d}	65.8 ± 1.6^{c}	3.3 ± 0.1^{c}	7.0 ± 0.02^{k}	2.3 ± 0.06^{c}	90.3 ± 1.6^{d}
		120	212.3 ± 7.3^{a}	83.6 ± 1.6^{a}	3.7 ± 0.2^{a}	6.9 ± 0.03^{l}	2.9 ± 0.06^{a}	127.9 ± 12.3^{a}
2	0	30	47.1 ± 2.9^{no}	24.4 ± 1.6^{jk}	1.3 ± 0.01^{l}	7.6 ± 0.01^{bc}	0.1 ± 0.07^{lm}	41.0 ± 1.5^{j}
		120	53.7 ± 2.7^{n}	28.1 ± 1.3^{ij}	1.3 ± 0.03^{kl}	7.6 ± 0.01^{cd}	1.3 ± 0.07^{ijk}	40.4 ± 2.0^{j}
	10	30	74.8 ± 1.5^{jk}	46.2 ± 1.9^{fg}	1.4 ± 0.03^{jk}	7.5 ± 0.02^{e}	1.5 ± 0.10^{gh}	62.2 ± 3.3^{g}
		120	104.2 ± 1.3^{g}	51.1 ± 1.9^{ef}	1.3 ± 0.03^{kl}	7.4 ± 0.05^{ef}	1.5 ± 0.04^{e}	75.5 ± 1.0^{e}
	30	30	94.8 ± 0.8^{h}	51.1 ± 3.1^{ef}	1.6 ± 0.04^{i}	7.4 ± 0.01^{fg}	1.9 ± 0.05^{e}	85.8 ± 1.8^{d}
		120	134.3 ± 18^{e}	65.2 ± 3.9^{c}	2.1 ± 0.01^{g}	7.3 ± 0.03^{i}	2.6 ± 0.10^{b}	119.3 ± 5.9^{b}

[a] Results are means±standard deviations. Values with the different lower-case letters within each column are significantly different at $P< 0.05$ according to LSD test. DOC: Dissolved Organic Carbon. [b] DTPA-extractable metal.

carbon than Soil 2. The values of pH and EC were partly similar in both soils and the soil pH value was in the alkaline range. CEC values ranged from 17.32 cmol$_c$ kg^{-1} for the Soil 1 to 13.47 cmol$_c$ kg^{-1} for the Soil 2. Total N, available P, and available K concentrations were higher in Soil 2 than Soil 1. The total concentration of Zn, Pb, and Cd in sewage sludge were also based on current sewage sludge guideline levels, U.S Environmental Protection Agency (USEPA, 2003).

Soil Characteristics after Plant Harvest

Comparisons between sewage sludge-treated and untreated soils showed significant increases in concentrations of DOC, soil EC and significant decreases in soil pH because of the addition of sewage sludge. The increased length of soil incubation time resulted in significant increases in soil EC values and DOC concentrations, and decrease in pH of both soils (Table 2). The concentrations of Zn, Pb, and Cd in the DTPA method significantly increased ($P< 0.001$) as sewage sludge amount and soil incubation time increased. In contrast, after 120 days incubation, concentration of Cd in 10 g kg^{-1} applied sewage sludge was less than 30 days incubation, while there were no significant differences observed between Soil1 and Soil2 in the concentration of this metal (Table 2).

Metals Concentration and Uptake by Plant Tissues

Soil incubation period had a significant effect ($P< 0.01$) on peppermint shoot and root Zn, Pb, and Cd concentrations and shoot metals content (uptake). There were also significant differences ($P< 0.01$) in plant metals concentration and metals uptake between soil types (Table 3).

Comparisons of Zn, Pb, and Cd concentrations and contents in plants grown in soils treated with different levels of sewage sludge revealed significant increases in Zn, Pb, and Cd concentrations and contents as a result of increased sewage sludge levels and incubation time (Table 3).

Table 3. Means of Zn, Pb, and Cd concentrations and shoot uptake by peppermint grown in treated and untreated soils with sewage sludge incubated for 30 and 120 days.[a]

Soil	Sewage sludge (g kg^{-1})	Incubation time (Days)	Zn			Pb			Cd		
			Shoot (mg kg^{-1})	Root (mg kg^{-1})	Uptake[B] (mg pot^{-1})	Shoot (mg kg^{-1})	Root (mg kg^{-1})	Uptake (mg pot^{-1})	Shoot (mg kg^{-1})	Root (mg kg^{-1})	Uptake (µg pot^{-1})
1	0	30	58.32 ± 0.62^{d}	127.31 ± 1.16^{f}	0.30 ± 0.01^{f}	$18.52\pm0.69d^{e}$	55.11 ± 1.23^{f}	0.09 ± 0.01^{ef}	1.23 ± 0.04^{d}	2.59 ± 0.01^{e}	6.43 ± 0.26^{f}
		120	55.73 ± 1.89^{d}	125.03 ± 1.72^{f}	0.28 ± 0.02^{f}	20.18 ± 1.58^{d}	58.74 ± 3.04^{f}	0.10 ± 0.01^{e}	1.18 ± 0.02^{d}	2.54 ± 0.04^{e}	6.07 ± 0.06^{e}
	10	30	67.47 ± 0.50^{c}	153.91 ± 1.37^{d}	0.41 ± 0.01^{e}	22.34 ± 0.55^{c}	77.44 ± 2.60^{d}	0.13 ± 0.002^{d}	1.38 ± 0.02^{c}	3.14 ± 0.01^{c}	8.54 ± 0.18^{de}
		120	104.31 ± 3.40^{b}	263.23 ± 2.95^{b}	0.76 ± 0.05^{b}	27.73 ± 1.65^{b}	101.85 ± 1.97^{b}	0.20 ± 0.01^{b}	1.20 ± 0.01^{d}	2.78 ± 0.04^{d}	8.83 ± 0.30^{cd}
	30	30	101.28 ± 7.55^{b}	239.42 ± 3.00^{c}	0.75 ± 0.06^{b}	27.82 ± 0.65^{b}	101.88 ± 2.51^{b}	0.20 ± 0.004^{b}	1.58 ± 0.01^{b}	3.83 ± 0.05^{b}	11.72 ± 0.12^{b}
		120	112.64 ± 0.84^{a}	298.78 ± 0.47^{a}	0.92 ± 0.01^{a}	33.18 ± 2.53^{a}	124.83 ± 5.39^{a}	0.27 ± 0.02^{a}	1.67 ± 0.02^{a}	4.24 ± 0.03^{a}	13.61 ± 0.17^{a}
2	0	30	25.31 ± 3.03^{g}	58.53 ± 1.85^{i}	0.15 ± 0.02^{g}	6.15 ± 0.15^{g}	21.61 ± 0.96^{h}	0.03 ± 0.01^{g}	0.55 ± 0.04^{g}	1.15 ± 0.03^{j}	3.32 ± 0.24^{h}
		120	25.77 ± 1.07^{g}	59.04 ± 1.55^{i}	0.15 ± 0.01^{g}	6.42 ± 0.58^{g}	23.24 ± 1.20^{h}	0.03 ± 0.01^{g}	0.55 ± 0.02^{g}	1.17 ± 0.04^{j}	3.27 ± 0.14^{h}
	10	30	39.58 ± 0.60^{f}	94.27 ± 1.86^{h}	0.42 ± 0.01^{e}	11.03 ± 0.76^{f}	43.81 ± 1.04^{g}	0.07 ± 0.004^{f}	0.68 ± 0.08^{f}	1.64 ± 0.03^{h}	4.94 ± 0.58^{g}
		120	56.44 ± 1.30^{d}	141.23 ± 1.16^{e}	0.47 ± 0.02^{d}	16.17 ± 1.23^{e}	66.56 ± 5.36^{e}	0.13 ± 0.01^{d}	0.57 ± 0.04^{g}	1.45 ± 0.02^{i}	4.84 ± 0.17^{g}
	30	30	44.73 ± 0.86^{e}	112.62 ± 1.59^{g}	0.42 ± 0.02^{e}	17.43 ± 1.72^{e}	70.54 ± 1.49^{e}	0.16 ± 0.02^{c}	0.84 ± 0.03^{e}	2.16 ± 0.03^{g}	8.13 ± 0.26^{e}
		120	56.02 ± 1.68^{d}	153.49 ± 2.72^{d}	0.59 ± 0.03^{c}	20.72 ± 0.62^{cd}	94.37 ± 1.53^{d}	0.22 ± 0.003^{b}	0.86 ± 0.02^{e}	2.29 ± 0.01^{f}	9.20 ± 0.17^{c}

[a] Results are means±standard deviations. Values with the different lower-case letters within each column are significantly different at $p < 0.05$ according to LSD test. Metals????

[b] Uptake: Shoot metal content.

On the other hand, compared with Zn and Pb, concentrations of Cd in the shoot and root were significantly lower in plants grown in 10 g kg^{-1} sewage sludge-treated soils incubated for 120 days ($P < 0.01$) than plants grown in soils incubated for 30 days.

Plant Biomass and Essential Oil

Shoot and root dry weights significantly increased ($P < 0.01$) as soil incubation time increased from 30 to 120 days (Table 4). Dry weight appeared to decrease with increasing metal concentrations from Soil 1 to Soil 2. The comparison of pooled treatment results for sewage sludge- treated metal contaminated soils gave significant differences in dry weight due to the presence of sewage sludge, such that the highest shoot and root dry matter was obtained in Soil 2 treated with 30 g kg^{-1} sewage sludge after 120-day incubation with 10.6 and 2.87 g pot^{-1}, respectively. There were also significant differences ($P < 0.01$) in essential oil content and yield between treated and untreated soils (Table 4). The essential oil content and yield increased with increasing levels of applied sewage sludge and incubation time in both soils. The highest essential oil content and yield were also recorded in treated Soil 2 with 30 g kg^{-1} sewage sludge after 120-day incubation with 1.31% and 139.72 mg pot^{-1}, respectively.

Metals Concentrations in Essential Oil

A general comparison between Zn, Pb, and Cd concentrations in essential oil of peppermint grown in sewage sludge-treated and untreated soils over 30 and 120 days incubation revealed that the essential oil concentrations of Zn, Pb, and Cd were only detected by ICP-OES in 30 g kg^{-1} sewage sludge-treated soils (Table 5). Except for Cd in Soil 1 treated with 30 g kg^{-1} sewage sludge after 120 days incubation, concentration of these metals in peppermint essential oil were lower than the permissible concentrations in the essential oil of medicinal plant (10, 0.1 and 0.05 mg kg^{-1} for Zn, Pb, and Cd, respectively) (Blagojević *et al*., 2009). However, Zn and Pb concentrations in essential oil showed significant differences between the two incubation times at 30 g kg^{-1} applied sewage sludge in soils (except for Cd).

Table 4. Means of dry weights, essential oil content, and yield of peppermint grown in treated and untreated soils with sewage sludge incubated for 30 and 120 days.[a]

Soil	Sewage sludge (g kg^{-1})	Incubation time (Days)	Shoot dry weights (g pot^{-1})	Root dry weights (g pot^{-1})	Essential oil content (%)	Essential oil yield (mg pot^{-1})
1	0	30	5.23 ± 0.06^{g}	0.93 ± 0.03^{f}	0.79 ± 0.04^{fg}	41.29 ± 1.86^{g}
		120	5.13 ± 0.07^{g}	0.89 ± 0.08^{f}	0.73 ± 0.11^{g}	37.46 ± 6.03^{g}
	10	30	6.16 ± 0.03^{f}	1.36 ± 0.03^{e}	0.84 ± 0.04^{f}	52.10 ± 2.66^{f}
		120	7.34 ± 0.24^{e}	1.74 ± 0.09^{d}	0.98 ± 0.01^{e}	72.01 ± 2.63^{e}
	30	30	7.40 ± 0.02^{e}	1.97 ± 0.01^{c}	1.06 ± 0.03^{d}	78.38 ± 2.18^{d}
		120	8.18 ± 0.04^{d}	2.43 ± 0.31^{b}	1.14 ± 0.01^{c}	93.86 ± 1.06^{c}
2	0	30	6.04 ± 0.05^{f}	1.28 ± 0.08^{e}	0.83 ± 0.01^{f}	50.37 ± 0.91^{f}
		120	5.95 ± 0.06^{f}	1.18 ± 0.04^{e}	0.82 ± 0.01^{f}	49.02 ± 1.12^{f}
	10	30	7.24 ± 0.13^{e}	1.67 ± 0.02^{d}	0.94 ± 0.02^{e}	68.29 ± 0.64^{e}
		120	8.50 ± 0.34^{c}	1.98 ± 0.01^{c}	1.15 ± 0.01^{c}	97.81 ± 3.95^{c}
	30	30	9.61 ± 0.06^{b}	2.47 ± 0.08^{b}	1.22 ± 0.03^{b}	117.46 ± 2.73^{b}
		120	10.60 ± 0.15^{a}	2.87 ± 0.06^{a}	1.31 ± 0.04^{a}	139.72 ± 2.37^{a}

[a] Results are means±standard deviations. Values with the different lower-case letters within each column are significantly different at $P < 0.05$ according to LSD test.

Table 5. Zn, Pb, and Cd concentrations in essential oil of peppermint grown in treated and untreated soils with sewage sludge incubated for 30 and 120 days.^{a}

Soil	Sewage sludge (g kg^{-1})	Incubation time (Days)	Essential oil Zn concentration (mg kg^{-1})	Essential oil Pb concentration (mg kg^{-1})	Essential oil Cd concentration (mg kg^{-1})
1	0	30	nd	nd	nd
		120	nd	nd	nd
	10	30	nd	nd	nd
		120	nd	nd	nd
	30	30	1.06±0.05^{b}	0.35±0.03^{b}	0.05±0.09^{ns}
		120	1.31±0.03^{a}	0.46±0.02^{a}	0.07±0.01^{ns}
2	0	30	nd	nd	nd
		120	nd	nd	nd
	10	30	nd	nd	nd
		120	nd	nd	nd
	30	30	0.60±0.06^{d}	0.21±0.04^{d}	nd
		120	0.72±0.04^{c}	0.29±0.03^{c}	0.04±0.08^{ns}

^{a} Results are means±standard deviations. Values with the different lower-case letters within each column are significantly different at P< 0.05 according to LSD test. nd: Not detectable and ns: Not significant.

Bioaccumulation and Translocation Factors

Root bioaccumulation and translocation factor of Zn, Pb, and Cd showed significant changes over 30 and 120 days incubation time in soils treated with sewage sludge compared to untreated soils (Figures 1 and 2).

Root bioaccumulation factor for Cd in soils treated with 10 g kg^{-1} applied sewage showed a significant decrease after 120 days incubation. Excepting that, comparisons of the root bioaccumulation factor of Zn, Pb, and Cd between treated and untreated soils revealed significant increases as a result of increased amounts of sewage sludge and incubation time in both soils. The maximum values of Zn, Pb, and Cd bioaccumulation factors were, respectively, 0.54, 0.57, and 0.54, observed in plants grown in Soil 1 treated with 30 g kg^{-1} sewage sludge incubated for 120 days (Figure 1).

Conversely, when the incubation time and the applied sewage sludge levels increased, the values of Zn, Pb, and Cd translocation factors showed a downward trend in both soils. The minimum translocation factors of all studied metals were, respectively, 0.36, 0.24, and 0.37, related to Soil 2 treated with 30 g kg^{-1} sewage sludge incubated for 120 days (Figure 2).

DISCUSSION

Generally, sewage sludge degradation is considered one of the main contributing factors to an increase of soil EC, DOC concentrations, and sewage sludge-derived Zn, Pb and Cd as well as a decrease in soil pH values (Roig *et al*., 2012). However, the decomposition of sewage sludge alone did not contribute to the decrease in pH. Thus, a combination of sewage sludge degradation and adsorption to soil surface that results in H^+ desorption are the most likely factors that resulted in the decrease in pH.

The decrease in pH and increase in EC after 120 days of incubation is likely the main factor contributing to the increase in DTPA-extractable Zn, Pb, and Cd concentrations and several studies have linked the solubility of metals to the soil pH (He *et al*., 2017; Xu *et al*., 2019). This

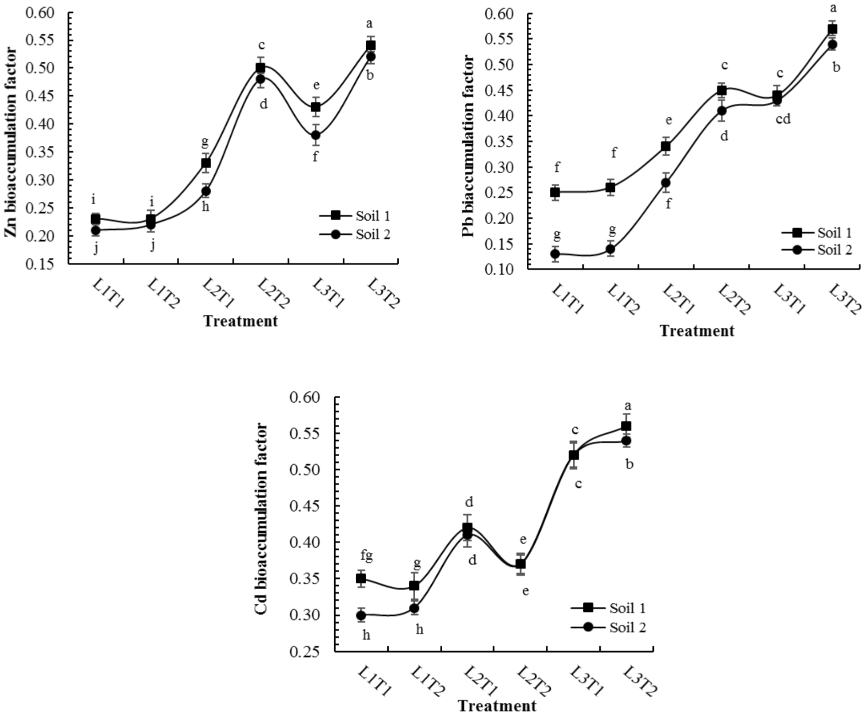

Figure 1. Mean of Zn, Pb, and Cd bioaccumulation factors in root tissue of peppermint grown in sewage sludge-treated and untreated soils over 30 and 120 days incubation times. L1, L2 and L3 are 0, 10 and 30 g kg^{-1} sewage sludge, respectively. T1 and T2 are 30 and 120 days incubation Time, respectively.

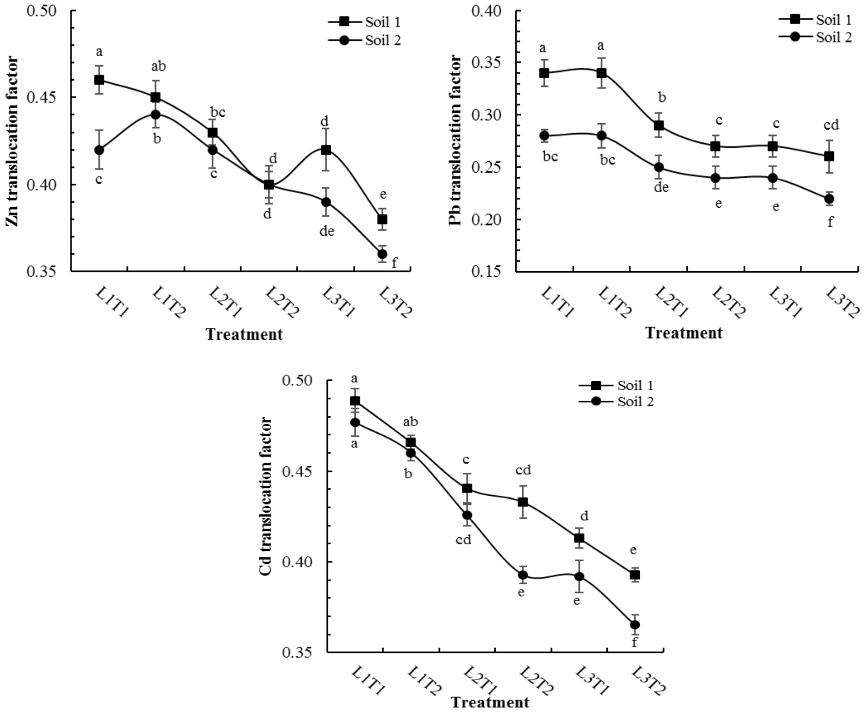

Figure 2. Mean of Zn, Pb, and Cd translocation factors of peppermint grown in sewage sludge-treated and untreated soils over 30 and 120 days incubation time. L1, L2 and L3 are 0, 10 and 30 g kg^{-1} sewage sludge and T1 and T2 are 30 and 120 days incubation Time, respectively.

increase in DTPA-metal extractability by soil EC association is achieved by increasing the formation of soluble chloride-metal complexes (Acosta *et al*., 2011). Another possible reason for the increased concentrations of DTPA-extractable Zn, Pb, and Cd, is the presence of increased amounts of DOC that causes elevated soluble organo-metals complexes. Mohseni *et al*. (2020) found that the desorption rates of Pb and Zn in sewage sludge treated soils were influenced by DOC concentration as a result of increasing soluble organo-metals complexes.

The influences of these parameters present in sewage sludge on DTPA-metal extractability subsequently influenced the increase in Zn, Pb, and Cd concentration in the plant tissues and the plant uptake of these metals. In contrast, the longer soil incubation time of 120 days resulted in significant decreases in the in Cd concentrations in soils treated with 10 g kg^{-1} applied sewage sludge, but not Zn and Pb, compared to an incubation period of 30 days. This is mostly due to a lesser extent dissolved organic carbon in 10 g kg^{-1} compared to 30 g kg^{-1} sewage sludge-treated soils. Therefore, Cd does not compete (lower ionic radius) with Zn and Pb (higher ionic radius) to form organo-metal complexes as a result of a limited amount of DOC (Antoniadis and Tsadilas, 2007; Mohseni *et al*., 2018; Mohseni *et al*., 2020). Consequently, increasing Cd adsorption onto soil surfaces and retaining organo-Zn and Pb complexes in soil solution as well as decreasing Cd concentration in the plant tissues. Saffari *et al*. (2015) to determine the effect of municipal waste compost and incubation time on the mobility of Ni from contaminated calcareous soils found that by increasing the incubation time from 45 days to 90 days, the mobility of Ni decreased as a result of decrease in exchangeable form of Ni. Another possible cause for reduced plant Cd concentration may be linked to the dilution effect of plant biomass on Cd concentration in plant tissues. Azimzadeh *et al*. (2016) found that Ni concentration in plants grown in soils treated with alfalfa green manure decreased due to the dilution effect of plant biomass on this metal concentration in the plant.

Despite the significant increase in the soil metals concentration and salinity as a result of increase in the amount of applied sewage sludge and incubation time, the essential oil content and yield, plus plant shoot and root dry weights, increased. The possible cause for these results is the increase in the amount of organic matter in the solution phase as a result of adding sewage sludge. In particular, organic matter has involved the macronutrients (Kumar and Chopra, 2014). On the other hand, the length of time following sewage sludge application has an important effect on essential oil content, essential oil yield, plant shoot, and root dry weights. Over the incubation period, microbial and chemical decomposition of sewage sludge would most likely contribute to the increase in macronutrients (Mattana *et al*., 2010), as well as the production of significantly higher amounts of essential oil content, yield and plant biomass. Burducea *et al*. (2019) observed that the soils treated with biosolids increased the content of phosphorus by 327% and basil yield by 206%. They stated that the utilization of biosolids to eroded soil improved the concentration of macronutrients, which resulted in significant effects on basil yield and physiology. Additionally, a study by Patel *et al*. (2016) found that the higher essential oil yield and biomass of *Mentha spicata* grown in soil treated with tannery sludge were obtained at 25:75 tannery sludge-to-soil ratio as a result of increased peroxidases, superoxide dismutase, malondialdehyde, catalases, and proline. They stated that the proline and antioxidants play an essential role in the internal detoxification of reactive oxygen species produced due to tannery sludge application.

Unlike Zn, Pb and Cd concentrations in plant tissues, concentrations of these metals in essential oil were not detected by ICP-OES in both 10 g kg^{-1} sewage sludge-treated and untreated soils. To determine the effect of red mud and sewage sludge on essential oil content of lemongrass (*Cymbopogon citratus*), Gautam and Agrawal (2017) found that Cd concentration in the shoot of plants grown in soils treated with 5% red mud + soil: sludge ratio of 2:1 (w/w) was higher than the critical toxicity level. But, it was not transferred from shoot to essential oil, and concentrations of Zn and Pb in essential oil were also less than the critical level determined for food.

Plants have some mechanisms to deal with heavy metals stress. Phytochelatins are generally accepted as the primary peptides responsible for controlling the mobility of metals in plant tissues (Sripriya *et al*., 2016). From these results, formation of phytochelatin-Zn, Pb, and Cd complexes into root cells may be a significant influential mechanism to increase root bioaccumulation and decreased translocation factors of Zn, Pb, and Cd. Besides, increasing the amount of sewage sludge and incubation time could increase nutrients supply, which is used as a precursor to the production of phytochelatins. Chand *et al*. (2012) found higher accumulation of Pb and Ni in the root than shoot of peppermint grown in vermicompost treated soils. They attributed this to the adsorption of these metals in the root cell wall exchange sites and immobilization in root vacuoles. Overall, our findings indicated that peppermint could not be considered as a Zn, Pb, and Cd hyperaccumulator plant. Plants that can accumulate Zn, Pb, and Cd to a concentration higher than, respectively, 10000, 1000, and 100 mg kg^{-1} dry weight are defined as Zn, Pb, and Cd hyperaccumulator plants (Sheoran *et al*., 2009).

CONCLUSIONS

The results obtained from this study showed that the presence of sewage sludge did result in significant increases in soil EC, DOC, and DTPA- extractable Zn, Pb, and Cd, and decrease in soil pH value over the incubation time. Despite the increased concentration of metals as a result of adding sewage sludge, the influence of sewage sludge on the macronutrients supply overtime was probably the main factor that influenced the increased amount of plant biomass and essential oil yield. However, the amount of bioaccumulation factors was significantly lower in plants grown in sewage sludge-treated soils compared with untreated soils over time, thereby demonstrating the use of peppermint as a hyperaccumulator plant as ineffective. Overall, since the concentrations of heavy metals in peppermint essential oil were within the levels of recommended limits, it can be cultivated as an alternative crop in contaminated calcareous soils treated with sewage sludge.

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پتانسیل گیاه پالایی و کیفیت اسانس نعناع فلفلی کشت شده در خاک‌های آلوده متأثر از لجن و زمان

ا. محسنی، ع. ریحانی تبار، ن. نجفی، ش. اوستان و ک. بازرگان

چکیده

هدف از این مطالعه بررسی تأثیر زمان خوابانیدن و لجن فاضلاب بر زیست توده نعناع فلفلی، عملکرد اسانس و غلظت روی (Zn)، سرب (Pb) و کادمیوم (Cd) در بافت‌های گیاه و همچنین ارزیابی پتانسیل گیاه پالایی گیاه نعناع فلفلی کشت شده در خاک‌های آهکی آلوده است. یک آزمایش گلخانه ای با کشت گیاه نعناع فلفلی (*Mentha pipertia* L.) در دو خاک آلوده تیمار شده با سه سطح لجن فاضلاب (۰، ۱۰ و ۳۰ گرم بر کیلوگرم) در طی دو زمان خوابانیدن (۳۰ و ۱۲۰ روز) انجام شد. نتایج نشان داد با افزایش زمان خوابانیدن و مقدار لجن فاضلاب مقادیر زیست توده گیاهی و عملکرد اسانس افزایش یافت. به استثناء غلظت کادمیوم در بافت‌های گیاهان کشت شده در خاک‌های تیمار شده با ۱۰ گرم بر کیلوگرم لجن فاضلاب بعد از ۱۲۰ روز خوابانیدن، غلظت روی ، سرب و کادمیوم در بافت‌های گیاهی افزایش یافت. در اسانس گیاهان کشت شده درخاک‌های تیمار شده با ۱۰ گرم بر کیلوگرم لجن فاضلاب غلظت‌های روی، سرب و کادمیوم قابل مشاهده نبود. در سایر تیمارها غلظت این فلزات در اسانس در حد مجاز برای گیاهان دارویی یافت شد. بر خلاف فاکتور تجمع زیستی ، فاکتور انتقال فلزات مورد مطالعه با افزایش مقدار لجن فاضلاب و زمان خوابانیدن کاهش یافت. اگرچه نعناع گیاهی مناسب برای کاهش بار آلودگی خاک نبود ولی با توجه به افزایش معنادار زیست توده و باقی ماندن غلظت فلزات موجود در اسانس تمامی تیمارها در حد توصیه شده، می‌توان نتیجه گرفت که نعناع می‌تواند برای کشت در خاک‌های آلوده تیمار شده با فاضلاب استفاده شود.