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ORIGINAL PAPER

Uptake of Cd, Pb, and Ni by *Origanum syriacum* produced in Lebanon

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Abstract Trace metals are found naturally in soil. However, the increase in industrial and agricultural polluting activities has increased trace metal contamination and raised high concerns in the public health sector. The study was conducted on *Origanum syriacum*, one of the most consumed herbs in the Middle East, and was divided into three parts. (1) Pot experiment: to study the effect of Cd, Pb, or Ni levels in soil on their uptake by *O. syriacum*. (2) Field samples: collected from major agricultural regions in Lebanon to analyze Cd, Pb, and Ni concentrations in soil and leaves. (3) Sale outlets samples: to measure the levels of Cd, Pb, and Ni in *O. syriacum* tissues in the market. Results showed that there was a positive correlation between levels of Cd, Pb, and Ni in soil and those in *O. syriacum* tissues. None of the field samples contained Pb or Ni that exceeded the maximum allowable limits (MAL). Three samples collected from heavily poultry-manured soil contained Cd higher than the MAL. Samples collected from sale outlets did not exceed the MAL for Ni but two exceeded the MAL for Cd and one for Pb. Trace metal contamination is not a major concern in *O. syriacum* produced in Lebanon. Only one mixture sample from a sale outlet was higher in Pb than the MAL and three samples from heavily manured fields exceeded the MAL for Cd.

Keywords Zaatar · Thyme · Trace metal · Heavy metal · Environmental pollutants

Abbreviations

AHPA	American Herbal Products Association
ATSDR	Agency for Toxic Substances and Disease Registry
AUB	American University of Beirut
b.w.	Body weight
DTPA	Diethylenetriaminepentaacetic acid
du	Dunums
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
FAO	Food and Agricultural Organization
FDA	Food and Drug Administration
h	Hours
JECFA	Joint Expert Committee for Food Additives
MAL	Maximum allowable limits
MENA	Middle East and North Africa
TE	Trace Elements
TEA	Triethanolamine
Trt	Treatment
WHO	World Health Organization
yr	Year

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Introduction

Trace metals (heavy metals) are elements with densities and atomic weights five times that of water and occur naturally in soils; however, increased industrial, agricultural, medical, and domestic activities could have caused severe contamination in some soils in the world (Tchounwou et al. 2012). The environmental contamination by these metals in some soils has reached high levels that became of concern to the public health sector. Globally, the agricultural sector has highlighted this issue as a major one because high trace metal levels in plant tissue may have detrimental effects on human health with major implications in the food chain as a whole. To this end, consumption of foods with high trace metals levels has been reported to have negative effects on cellular organelles, enzymes, DNA, and nuclear proteins, thereby increasing the incidence of renal, neurological, carcinogenic, and mental disorders in humans (Tchounwou et al. 2012).

Origanum syriacum (thyme) has been traditionally collected from the wild, and recently became mostly cultivated, and is commonly consumed by the population in the MENA region. In addition to being consumed as food, it was reported to have varied therapeutic uses including treatment of gastrointestinal problems, respiratory diseases, hemorrhoids, sexual diseases, and ameliorating the poisonous effects of animals' bites (Lukas et al. 2009). *Origanum syriacum* has also been reported to be effective against infections by *Candida albicans* (Kassaify et al. 2008).

In Lebanon and neighboring countries, *O. syriacum* is mainly used as food. The plant is considered one of the most important staple herbs in the region in view of its low cost, flavorful nature, and diverse culinary applications. It is consumed in Mediterranean countries in an admixture of other ingredients, referred to locally as "*zaatar,*" almost on a daily basis. The basic mixture of "*zaatar*" includes dried ground *O. syriacum* leaves, sesame seeds, sumac (Rhus), and salt. *O. syriacum* is mostly consumed in the form of "*Man'oushe,*" the famous Mediterranean pastry (Mama's Lebanese kitchen 2011). People from different walks of life frequently grab a *Man'oushe* on their way to work/school because it is filling, cheap, tasty and widely available. *Origanum syriacum* is also consumed on its own as green leaves; people have it for breakfast mixed with chopped onions and olive oil with labne (strained yogurt) on the side or as an ingredient in salads.

Would a medicinal herb as *O. syriacum,* which is renowned for its efficacy in treating diseases be a vehicle for trace metal accumulation in the human body? If the answer is in the affirmative, then *O. syriacum* consumption should be reduced to mitigate the development of chronic disorders that might be precipitated by trace metal accumulation in humans.

International organizations and governmental authorities have set regulations or standards for trace metal exposure through inhalation and consumption (air, water and food) to curb their effects on human health. Some of these international organizations and governmental authorities include FAO, WHO, EPA, FDA, CODEX, EFSA, Canadian, and Australian governments among others. In Lebanon, there are no special regulations for trace elements in food and environment; however, the ones of FAO and WHO are applied.

The objectives of this study were to (1) study the effect of applying different levels of Cd, Pb, or Ni (common environmental pollutants) to soil and their accumulation in *O. syriacum* plants, (2) study the concentrations of Cd, Pb, and Ni in several *O. syriacum* plants and soil samples collected from fields in different geographical locations in Lebanon, and (3) measure the concentrations of Cd, Pb, and Ni in *O. syriacum* samples collected from several sale outlets in the country.

Materials and methods

Pot experiment

A completely randomized design pot experiment (CRD) was conducted in the greenhouse at the American University of Beirut (AUB) for each of the three trace elements (Cd, Pb, and Ni), the three mostly common non-nutritive elements usually found in animal manure (Irshad et al. 2013). Each trace element was spiked with five levels (Table 1) and replicated three times, 15 pots/trace element. Each pot was filled with 4 kg of soil. The soil in the pots was mixed with a solution containing the specified amount of trace metal and 0.5 g of 20-20-20 + TE fertilizer. The control pots received only fertilizers and distilled water. The concentration of Cd was much lower than

Table 1 Levels of Cd, Pb, and Ni in the pot experiment

Cd (mg/kg)	Pb (mg/kg)	Ni (mg/kg)
Treatments		
0	0	0
10	100	50
20	200	100
50	500	200
100	1000	500

Pb and Ni because Cd is mobile in the plant and concentrates on the active exchange sites in the roots. Therefore, if applied in high concentrations (as Pb and Ni), it will form toxicity (Kabata-Pendias 2001).

Planting and harvesting

Origanum syriacum seedlings were obtained from a private nursery. Two seedlings were planted in each pot and the plants were harvested after 10 weeks. The shoots (stem and leaves) of the plants were cut, washed, plotted, and dried in an oven for 48 h at 60 °C. The dried shoots were ground and placed in clean plastic containers for analysis.

Tissue analysis

One gram of ground tissue was placed in a ceramic crucible and ashed for 3 h at 600 °C. The ash was dissolved in 10 mL of 65% nitric acid, diluted to 25 mL with deionized water, and placed in a clean falcon tube (Benton Jones 2001). The Cd, Pb, and Ni concentrations in the solutions were measured by atomic absorption spectrophotometer (Thermo 3500 AAS). A standard plant tissue sample (NIST-1547), from the National Institute of Standards and Technology, U.S. Department of Commerce, Gaithersburg, MD 20899, was included in the analysis as an analytical check.

Origanum syriacum: field samples

Ten *O. syriacum* plants (one plant/sample) and the soil around their roots were collected together from cultivated fields in major *O. syriacum* producing regions in Lebanon, where *O. syriacum* is mostly cultivated (Fig. 1), for the measurement of Cd, Pb, and Ni. The soil samples (without the roots) were also analyzed for physical and chemical properties (Bashour and Sayegh 2007).

Origanum syriacum: sale outlet samples

Fourteen *O. syriacum* samples were collected randomly from different sale outlets in Lebanon for measurement of Cd, Pb, and Ni levels. Of these samples, four were dry ground leaves and the rest (10 samples) were dry ground leaves mixed with sesame, sumac, and salt (Fig. 1).

Soil analysis

The physical and chemical properties of the soil used in the pot experiment and those collected from different fields were analyzed according to Bashour and Sayegh (2007). A soil sample, NIST-2709, from the National Institute of Standards and Technology, U.S. Department of Commerce, Gaithersburg, MD 20899, was included as an analytical check.

Statistical analysis

The design of the pot experiment was a Completely Randomized Design (CRD). The study was composed of three independent experiments, where each trace metal (Cd, Pb, and Ni) is the independent variable (fixed) and the concentration for each of the trace metals (Cd, Pb, and Ni) is the dependent variable. The statistical analysis was done using the SPSS statistical analysis program, version 23.0. One-way ANOVA test was performed at a significance level of 0.05 to compare between means of independent variables. The statistical analysis indicated no outliers at a given standard deviation level ± 3 SD.

Results and discussion

Analysis of soil samples

The soil of the pot experiment and the samples collected from different fields (Table 2), around the roots, exhibited wide variations in properties with ranges of 5–49% for sand, 20–63% for silt, and 11–75% for clay. All the samples were neutral to slightly alkaline (pH 7.37–7.80) and calcareous with a

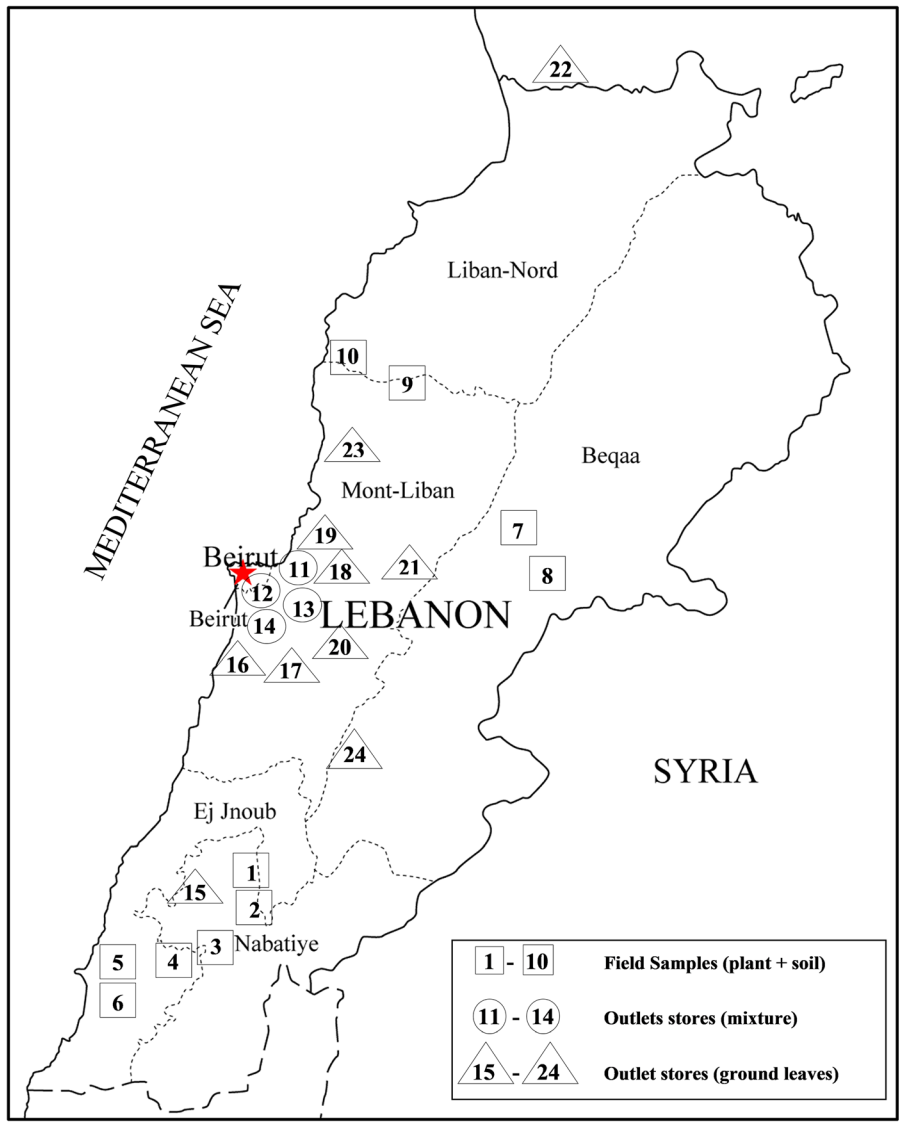


Fig. 1 Map of Lebanon showing the locations of plant and soil sampling sites and outlet stores

range of free $CaCO_3$ contents (3–67%). The EC values were < 1 mS/cm, thereby indicating that the samples were not saline. Available micronutrients were estimated by DTPA/TEA extraction (Lindsay and Norvell 1978), and the results indicated that Fe was very low in all samples except for sample (7) that was sufficient and slightly low in sample (8); Zn was about average except in sample (7) that was high; Cu was high in all samples and very high in sample (7); Mn was low in samples (pot, 1, 2, 3, 4, and 9), slightly high in samples (5, 6, and 10), high in sample (8), and very high in sample (7). According to Su (2014), Cd was medium in samples (1, 2, 3, and 7) and lower than the natural levels in samples (4, 5, 6, 8, 9, and 10). The high micronutrient values in sample (7) were due to excess fertigation with chelated micronutrients.

Analysis of pot *Origanum syriacum* tissue samples

Cd

Origanum syriacum plants grown in potted soil that was contaminated with different levels of Cd (0–100 mg/kg) exhibited a significant increase ($P < 0.05$) of Cd in the tissue with increasing Cd concentration in the soil (Fig. 2). There was a

Table 2 Results of the analyzed soil samples

Parameter	Soil (pot experiment)	Soil samples from different sites in Lebanon									
		1	2	3	4	5	6	7	8	9	10
Sand (%)	23.10	27.42	38.1	49.03	15.78	5	10	20	15	17.87	12.23
Silt (%)	30.80	31.90	50.36	25.60	35.62	20	20	30	25	63.13	40.80
Clay (%)	46.10	40.68	11.54	25.37	48.60	75	70	50	60	19	46.97
Textural class	Clay	Clay	Silt loam	Clay loam	Clay	Clay	Clay	Clay	Clay	Silt clay loam	Silty clay
$CaCO_3$ (%)	26	9	17	3	60	67	43	61	14	37	21
Soil reaction (pH)	7.75	7.62	7.80	7.40	7.49	7.65	7.65	7.59	7.53	7.58	7.37
EC (mS/cm)	0.14	0.09	0.16	0.08	0.82	0.35	0.31	0.42	0.51	0.45	0.54
DTPA—Fe (mg/kg)	0.82	0.71	0.46	0.52	0.50	0.70	0.94	5.60	3.06	0.70	0.55
DTPA—Zn (mg/kg)	0.40	0.81	1.26	1.33	0.79	0.74	1.14	7.18	2.33	0.55	2.16
DTPA—Cu (mg/kg)	0.32	0.80	0.80	0.77	0.56	0.37	0.59	3.03	1.74	0.48	1.68
DTPA—Mn (mg/kg)	0.27	0.19	0.05	0.12	ND	2.81	2.22	46.72	4.58	0.96	2.24
Cd (mg/kg)	0.04	0.13	0.10	0.31	0.03	0.06	0.04	0.32	0.07	0.02	0.02
Pb (mg/kg)	ND	ND	ND	0.10	ND	0.16	0.04	0.13	0.01	0.01	0.07
Ni (mg/kg)	0.21	0.15	0.12	0.20	0.76	0.17	0.19	0.88	0.47	0.21	0.21

ND not detectable

significant difference ($P < 0.05$) between the control and all the other treatments. These results indicate that once Cd is present in the soil, at concentrations $\geq$ 10 mg/kg, *O. syriacum* shoots will accumulate relatively high concentrations of Cd ranging between 1 and 2.7 mg/kg. The concentration of Cd in one of the replicates in Trt 2 (10 mg/kg) was very high which pushed its average concentration to that of Trt 5 (100 mg/kg). Compared to the control treatment, soil contamination with Cd at $\geq$ 10 mg/kg soil led to a fourfold increase in Cd concentrations in the *O. syriacum* tissue. Thus, soil contamination with Cd is of concern because it caused an increase in Cd concentration in the tissue that exceeded the acceptable limits (0.1–0.3 mg/kg tissue) of Australian government, CODEX (2009), and WHO (2007). According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the allowable daily intake for a 70 kg adult is 0.07 mg (AHPA 2009; Smolders 2001). Calculating the allowable intake per adult of *O. syriacum* from the highest contaminated Trt (Trt 5) is 26 g/day. This amount will increase to 280 g/day from the non-contaminated Trt (Trt 1). Thus, it is not a problem for *O. syriacum* produced locally. Ezeabara et al. (2014) proposed safe intake levels for Cd at 0.015–0.050 mg/day for adults and 0.002–0.025 mg/day for children (8–84 g/day from non-contaminated Trt). The results of analysis of the soil and plant samples collected from the fields indicated that any increase in the level of Cd in the soil reflected positively to Cd concentration in the tissue. Cadmium accumulation begins at a young age and accumulates in the body as children grow mainly from ingestion of Cd contaminated foods or inhalation from smoke and dust (Schoeters et al. 2006). Cadmium accumulates mostly in the kidneys but is also found in the muscles, lungs, liver, and blood. Cadmium can stay in the body from 100 days (blood) up to 30 years depending on its component type (fast or slow) and only about 0.01–0.02% are excreted in urine and feces every day. With increased accumulation in the lungs and kidneys, Cd was found to cause lung cancer and kidney failure, two major health disorders (Nordberg et al. 2018).

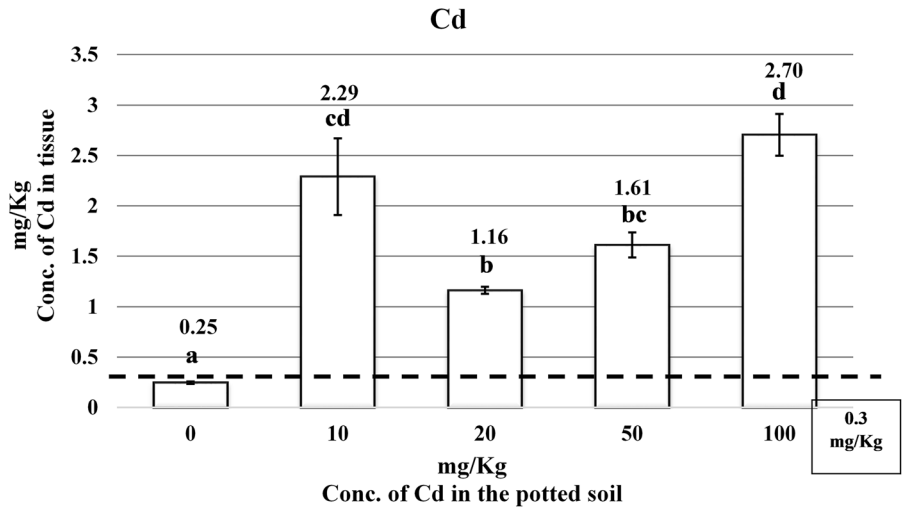


Fig. 2 Concentration of Cd in *Origanum syriacum* tissue

Pb

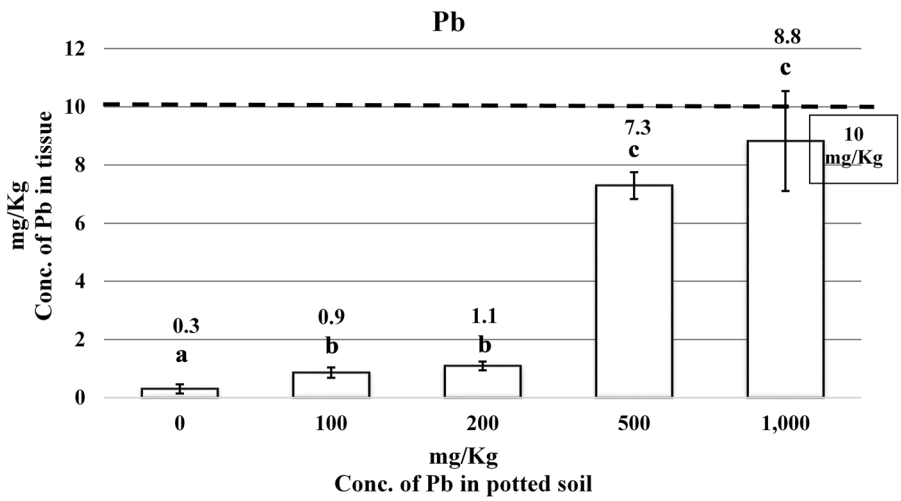
The O. syriacum plants grown in potted soil contaminated with different levels of Pb (0–1000 mg/kg) exhibited a significant increase ($P < 0.05$) of Pb in the tissues with increasing Pb concentration in the soil (Fig. 3). These results agree with those of Lydakis-Simantiris et al. (2016) who reported a significant increase between all treatments of *Thymus vulgaris* and that of the control.

According to the WHO, the maximum limit for Pb in herbal products is 10 mg/kg (WHO 2007). JECFA, on the other hand, has set a daily limit of 0.25 mg for a 70 kg adult (AHPA 2009) while CODEX has set a maximum level of 0.3 mg/kg for leafy vegetables. Ezeabara et al. (2014) proposed safe intake levels of 0.020–0.280 mg/day for adults and 0.010–0.275 mg/day for children. Although contaminating the soil with Pb led to a significant increase in Pb in the tissue, these levels did not reach the maximum limits set by WHO (10.0 mg/kg).

Ni

The results of tissue analysis for *O. syriacum* plants grown in soils contaminated with Ni (0–500 mg/kg soil) showed that the increase in Ni in the soil led to a significant increase ($P < 0.05$) in Ni concentration in *O. syriacum* tissues (Fig. 4). Similar to Cd and Pb, the

Fig. 3 Concentration of Pb in *Origanum syriacum* tissue



higher the Ni concentrations in the soil, the higher the concentrations were in the *O. syriacum* tissues. There was a significant difference ($P < 0.05$) between the control and all the other treatments. These results agree with the findings of Lydakis-Simantiris et al. (2016) where Ni concentration in tissue of *Thymus vulgaris* was highest when the concentration of Ni in the soil was at its highest.

Ingestion of 0.001 mg/kg body weight of Ni has been reported to cause contact dermatitis in sensitive individuals while an intake of 0.0028 mg/kg body weight was needed to affect others (EFSA 2015). Similar to EFSA, ATSDR (2005) has set a daily Ni intake of 0.101–0.162 mg/day for adults.

Analysis of field samples

A total of 10 *O. syriacum* samples were collected from fields in different regions in Lebanon. Six samples were collected from South Lebanon, two from North Lebanon, and two from the central Beqaa region. The concentrations of Cd, Pb, and Ni in *O. syriacum* samples are shown in Figs. 5, 6 and 7.

According to the WHO (2007), the MAL for Cd in herbal tissues is 0.3 mg/kg. A safe intake of Cd for a 70 kg adult is 0.07 mg (0.001 mg/kg b.w). All field samples contained Cd in concentrations lower than 0.3 mg/kg except for three samples that were higher than the MAL (Fig. 5). These samples were brought from the South, from fields that have been receiving high rates of poultry manure (4–6 tons/du/yr) for several years. The reason for their high concentration in Cd is the excessive application of poultry manure for fertilization which contains 1.6 mg Cd/kg dry manure. Therefore, the use of poultry manure in moderation is recommended to limit the increase in Cd level in *O. syriacum* tissue. The maximum allowed daily intake of *O. syriacum* with a concentration of 0.3–0.5 mg Cd/kg for a 70 kg adult ranges between 140 and 233 g. This quantity is considerably high and is not normally consumed by people.

Lead concentrations in *Origanum* tissues of the 10 samples ranged between 1.23 and 8.73 mg/kg (Fig. 6) with the levels being lower than the maximum limit (10 mg/kg) set by the WHO. Moreover, a safe daily intake of lead is 243 µg/adult/day. Therefore, the maximum allowed daily intake of *Origanum* tissue ranges between 40 and 200 g/adult.

Nickel concentrations in the 10 field samples ranged between 0.53 and 2.93 mg/kg (Fig. 7). All samples were lower than the maximum limit set by WHO (10 mg/kg) (Naser et al. 2011) The allowable daily intake of Ni set by the EFSA (2015) for a normal person is 0.0028 mg/kg b.w. However, for people who are sensitive to Ni, a daily intake of 0.001 mg/kg b.w is the limit. Thus, the limit of *Origanum* that can be safely consumed by a 70 kg adult considering a limit of 0.001 mg Ni/kg b.w ranges between 24 and 132 g/adult. Accordingly, an intake of 24 g of *Origanum*/day is safe for Cd, Pb, and Ni, even at the highest concentration levels, which is equivalent to two

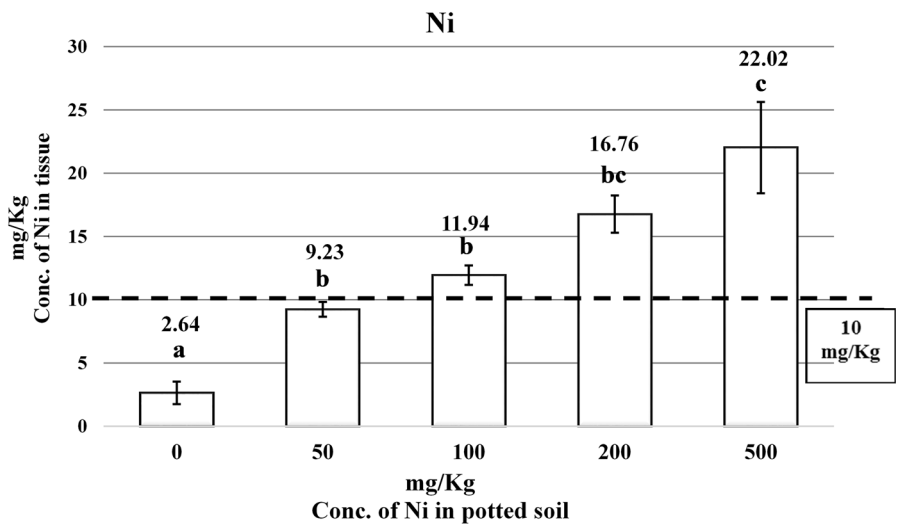


Fig. 4 Concentrations of Ni in *Origanum syriacum* tissue

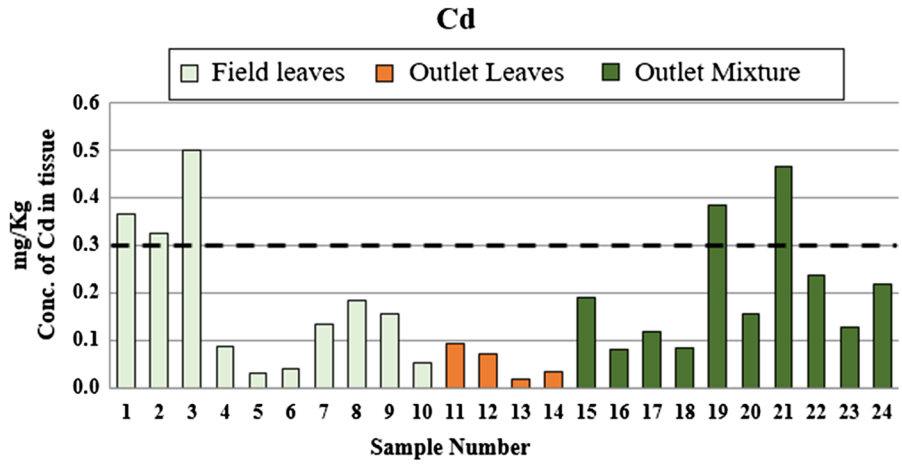


Fig. 5 Concentration of Cd in *Origanum syriacum* collected from different fields and sale outlets in Lebanon and their maximum limits

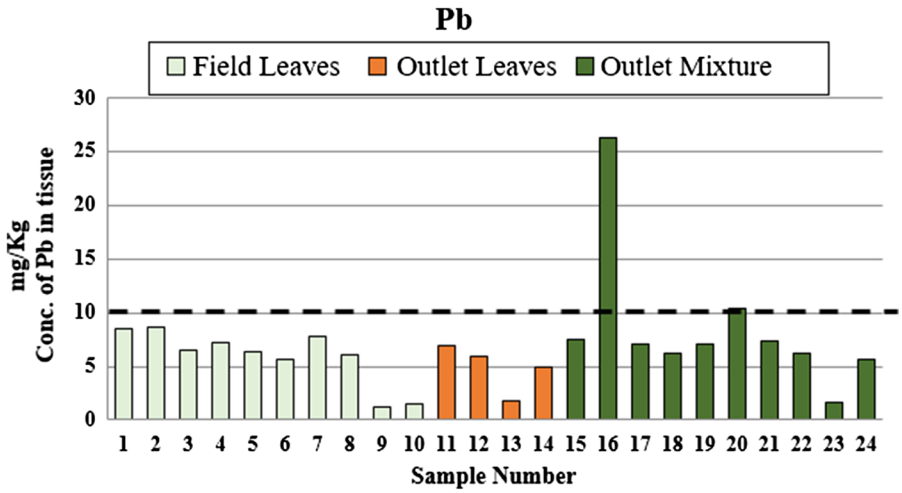


Fig. 6 Concentration of Pb in *Origanum syriacum* collected from different fields and sale outlets in Lebanon and their maximum limits

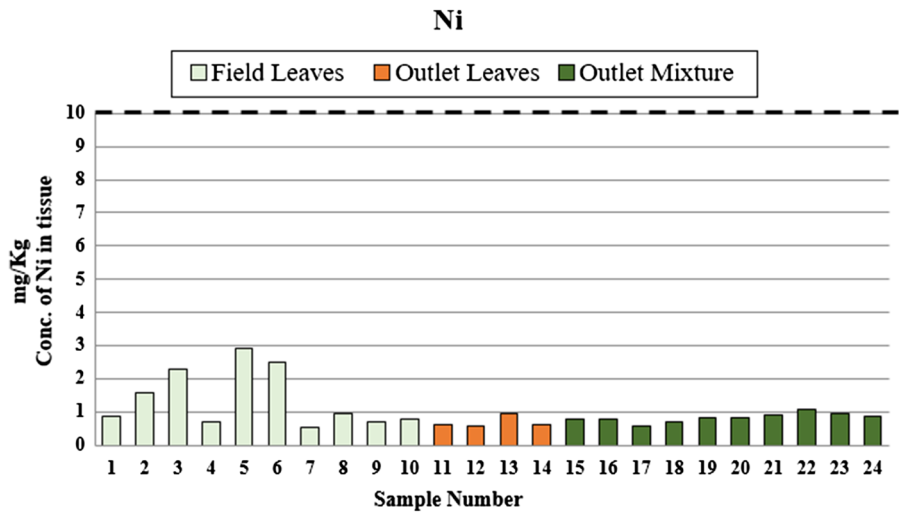


Fig. 7 Concentration of Ni in *Origanum syriacum* collected from different fields and sale outlets in Lebanon and their maximum limits

pastries (*Man'oushe*). To this end, people rarely consume more than 24 g *Origanum*/day.

Analysis of sale outlet samples

The concentrations of Cd, Pb, and Ni in the 14 *Origanum* samples collected from different sale outlets in Lebanon are shown in Table 3 and Figs. 5, 6 and 7. Four samples (11, 12, 13, and 14) were leaf samples, and the other 10 samples (15–24) were mixtures of *Origanum*, sumac, sesame, and salt. Nine samples were collected from stores in Beirut, and the rest were brought from stores in North and South Lebanon.

None of the four dry powder leaf samples was higher in any of the tested trace metals than the MAL set by WHO (2007). Further, out of 14 samples, Cd concentrations in 12 samples were below the MAL set at 0.3 mg/kg by WHO (2007) and two samples were higher than the safe limits. Lead concentrations in the 14 samples ranged between 1.58 and 26.33 mg/kg. The highest Pb concentration was found in the mixture sample that was purchased in Beirut at a concentration of 26.33 mg/kg. This value was more than double the acceptable limit set by the WHO (10 mg/kg). The sample was composed of ground *Origanum* leaves in an admixture with sesame seeds, sumac, and salt. Therefore, the high Pb concentration could have come from impurities in the mixture components. Another

Table 3 *Origanum syriacum* collected from different sale outlets in Lebanon

Sample number	Province	City	Leaves or mixture	Cd (mg/kg)	Pb (mg/kg)	Ni (mg/kg)
1	South	Zawtar	Leaves	0.37	8.55	0.88
2	South	Zawtar	Leaves	0.33	8.73	1.60
3	South	Zawtar	Leaves	0.50	6.48	2.30
4	South	Adsheet	Leaves	0.09	7.28	0.70
5	South	Sour	Leaves	0.03	6.38	2.93
6	South	Sour	Leaves	0.04	5.65	2.50
7	Beqaa	Tal Thnoub	Leaves	0.14	7.79	0.53
8	Beqaa	Terbol	Leaves	0.19	6.12	0.95
9	North	Jbeil	Leaves	0.16	1.23	0.70
10	North	Jbeil	Leaves	0.05	1.51	0.78
11	Beirut	Beirut	Leaves	0.09	6.98	0.63
12	Beirut	Beirut	Leaves	0.07	6.00	0.58
13	Beirut	Beirut	Leaves	0.02	1.72	0.95
14	Beirut	Beirut	Leaves	0.03	4.99	0.60
Average of leaves				0.15	5.67	1.19
15	Beirut	Beirut	Mixture	0.19	7.56	0.78
16	Beirut	Beirut	Mixture	0.08	26.33	0.78
17	Beirut	Beirut	Mixture	0.12	7.02	0.58
18	Beirut	Beirut	Mixture	0.08	6.27	0.70
19	Beirut	Beirut	Mixture	0.39	7.06	0.83
20	South	Zawtar	Mixture	0.16	10.43	0.83
21	Mount Lebanon	Ras el Maten	Mixture	0.47	7.37	0.90
22	North	Arida	Mixture	0.24	6.20	1.08
23	North	Batroun	Mixture	0.13	1.58	0.95
24	South	Chhim	Mixture	0.22	5.64	0.88
Average of mixtures				0.21	8.55	0.83
Maximum limits (WHO)				0.30	10.00	10.00

mixture sample was higher than the Pb MAL by 0.43 mg/kg only. The rest of the samples were within the maximum allowable limit set by the WHO.

Conclusion and remarks

Results show that as Cd, Pb, and Ni concentrations increase in the soil, their levels increase in the *O. syriacum* tissue. There is a positive correlation between the three trace metal concentrations in the soil and those in Origanum tissue. None of the *O. syriacum* samples collected from various fields in Lebanon was high in Cd, Pb, or Ni but two mixture samples purchased from stores in Lebanon were higher than the MAL of WHO. The variation in the concentrations of the trace metals in the mixtures was due to the varied qualities of the mixture (sesame seeds, sumac, etc.). In Lebanon and MENA region, *O. syriacum* is mainly consumed as a mixture of zaatar, sumac, sesame, and salt. From our results, we estimate that consuming 24 g of *O. syriacum* mixture per day by adults and about half the quantity for children is safe and do not lead to the intake of Cd, Pb, and Ni higher than the MAL reported by WHO. However, consuming *O. syriacum* (thyme) grown in Lebanon in salad or as green powder can be at a much higher rate and in the safe levels.

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