

## CONTENT OF TOXIC AND ESSENTIAL METALS IN MEDICINAL HERBS GROWING IN POLLUTED AND UNPOLLUTED AREAS OF MACEDONIA

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The aim of this study was to determine and compare Ba, Cr, Cd, Fe, Sr, Pb, and Zn content in medicinal herbs *Urtica dioica* L., *Taraxacum officinale*, and *Matricaria recutita* growing in polluted and unpolluted areas of the Republic of Macedonia. The metal content was determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES). In the unpolluted area of Mt. Plačkovica the metal content in *Taraxacum officinale* was in the descending order: Fe>Sr>Zn>Ba>Cr, while Pb and Cd were below the limit of detection. In the polluted area of Veles, the order was as follows: Fe>Zn>Sr>Pb>Ba>Cd>Cr. Our results suggest that quality assurance and monitoring of toxic metals is needed for plants intended for human use and consumption. Medicinal plants should be picked in areas free of any contamination sources.

**KEY WORDS:** *Ba, Cd, Cr, Fe, Matricaria recutita, Pb, Sr, Taraxacum officinale, Urtica dioica, Zn*

A World Health Organization (WHO) report (1) suggests that in primary healthcare about 70 % to 80 % of the world population rely on unconventional medicine, mainly of herbal origin. Phytotherapy has a very long tradition and in recent years, we have witnessed increasing popularity of over-the-counter (OTC) drugs, nutraceuticals, and medicinal products of plants or other natural resources. Even though medicinal herbs are often advertised as natural and therefore harmless they can produce adverse effects too, including toxic effects, allergic reactions, and drug interactions (2). Herbs such as *Aloe vera*, *Valeriana officinalis*, and *Symphytum officinale* could also be hepatotoxic (3).

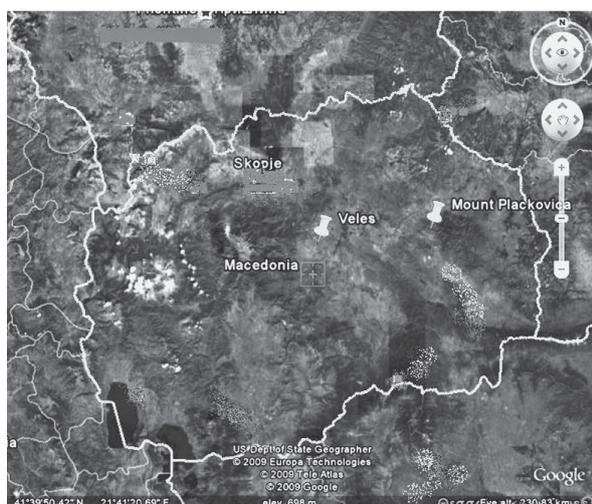
Moreover, they can be contaminated with pesticides, microbes, heavy metals, and toxic chemicals. Pesticides, microbial, and heavy metal

contamination may be related to the environment in which herbs are grown or stored (2). Minerals essential for growth accumulate in different plant parts, but so do metals such as Cd, Co, and Ag, which are of no known use for the plant (4).

Atmosphere, soil, harvesting, and handling play an important role in metal contamination of medicinal plants. Plants are an important link in the transfer of trace elements from soil to man (5). It is therefore important to establish the levels of some metals in common medicinal plants because they can be dangerous if elevated (6). The concentration of heavy metals in plants is one of the criteria for production of drugs. WHO has set maximum permissible levels in raw plant materials only for arsenic, cadmium, and lead (1.0 mg kg<sup>-1</sup>, 0.3 mg kg<sup>-1</sup>, and 10 mg kg<sup>-1</sup>, respectively) (7). Provisional Tolerable Weekly Intake

(PTWI) for mercury is  $5 \mu\text{g kg}^{-1}$  body mass (b.m.), arsenic  $15 \mu\text{g kg}^{-1}$  b.m., lead  $25 \mu\text{g kg}^{-1}$  b.m., and cadmium  $7 \mu\text{g kg}^{-1}$  b.m., as the major toxic elements (8, 9). Furthermore, the European Commission has established lead, cadmium, and mercury limits in food supplements (10) that have been in force since March 2001.

Because of the importance of mineral and trace elements in medicinal herbs, several studies have been carried out to determine their levels by using different methods (11-14). The objective of this study was to quantify and compare the content of Ba, Cr, Cd, Fe, Sr, Pb, and Zn in medicinal herbs *Urtica dioica* L. (*Urticaceae*), *Taraxacum officinale* (*Asteraceae*), and *Matricaria recutita* (*Asteraceae*) growing in a polluted and unpolluted area of the Republic of Macedonia (Figure 1). These herbs are commonly used by local people for infusions and phytotherapeutical purposes. The selected elements are important essential or toxic metals to humans.



**Figure 1:** Sampling locations town of Veles (polluted) and Mt. Plačkovica (unpolluted) in Macedonia

## MATERIALS AND METHODS

### Sampling areas

All samples were collected in May 2009. Samples from the polluted area were taken 10 m to 100 m around the lead and zinc smelting plant “Zletovo” in the town of Veles (Figure 1). We selected this smelting plant because it is an important source of lead pollution in Macedonia, with estimated emission of 83 tonnes per year, according to the 1996 National Environmental Action Plan (NEAP). Several studies of heavy metal

content in soil, vegetables and fruits of this area have confirmed pollution (15-17). Control samples were taken an unpolluted area on Mt. Plačkovica, about 60 km from Veles. The plants were identified at the Department of Pharmacognosy, Faculty of Pharmacy, Skopje, Macedonia. Plants sampled and their locations are described in Table 1.

### Sample collection and preparation

We collected three plants (about 200 g fresh mass) of each species from each location for analysis. For *T. officinale* we took well developed rosette leaves of similar size; for *U. dioica* we used leaves of similar size cut with a non-metal knife and for *M. recutita* we used the flowers. All plant samples were air dried unrinsed, milled in a micro-hammer (without metal parts in it), and stored in clean paper bags.

### Analytical techniques

For all standard and sample preparations we used demineralised water and high purity reagents (Tracepur<sup>®</sup>, Merck, Germany). Standards of selected elements were set by dilution of stock standards, which were prepared using analytical grade salts of metals (Merck, Germany) with  $\text{HNO}_3$  and results were corrected for reagent blanks.

We weighed 0.5 g of each finely crushed item and placed them in polytetrafluoroethylene vessels with 5 mL of 69 %  $\text{HNO}_3$  (Tracepur<sup>®</sup>, Merck, Germany) and 2 mL of 30 %  $\text{H}_2\text{O}_2$  (m/V; Merck, Germany). The mixture was left at room temperature for 1 h and then mineralised in a microwave (MARS CEM XP 1500) in a two-step procedure at 180 °C (Table 2). The digests were filtered on filter paper (Munktell, Sweden), quantitatively transferred to 25 mL calibrated flasks, diluted with demineralised water and analysed for selected metals with an inductively coupled plasma atomic emission spectrometer (ICP-AES) (Varian 715-ES, Varian, USA). Instrument settings are given in Table 3. All results were calculated on a dry mass basis ( $\text{mg kg}^{-1}$  d.m.). Samples were made in triplicate. Each data represents the mean  $\pm$  standard deviation of three samples.

## RESULTS AND DISCUSSION

Figure 2 shows mean values of the investigated metals in the three plant species. In the *Taraxacum* leaves collected in the unpolluted area of Mt.

**Table 1** Plant organ analysed and sampling location

Plant, plant organ investigated	Location (unpolluted area)		
	Plackovica Mountain		
	coordinates		Altitude above sea-level
N	E		
<i>Urtica dioica</i> leaves	41°47'11.58"	22°20'06.49"	740 m
<i>Taraxacum officinale</i> leaves	41°47'11.85"	22°20'08.01"	745 m
<i>Matricaria recutita</i> flowers	41°48'13.42"	22°17'10.80"	382 m
Plant, plant organ investigated	Location (polluted area)		
	Veles area		
	coordinates		Altitude above sea-level
N	E		
<i>Urtica dioica</i> leaves	41°43'30.14"	21°45'51.87"	176 m
<i>Taraxacum officinale</i> leaves	41°43'41.20"	21°46'04.23"	169 m
<i>Matricaria recutita</i> flowers	41°43'46.25"	21°46'00.75"	168 m

**Table 2** Procedure used for the digestion of plant samples

Step	Temperature / °C	Time / min	Power / W
1	180	10 (ramp time)	800
2	180	15 (hold time)	800

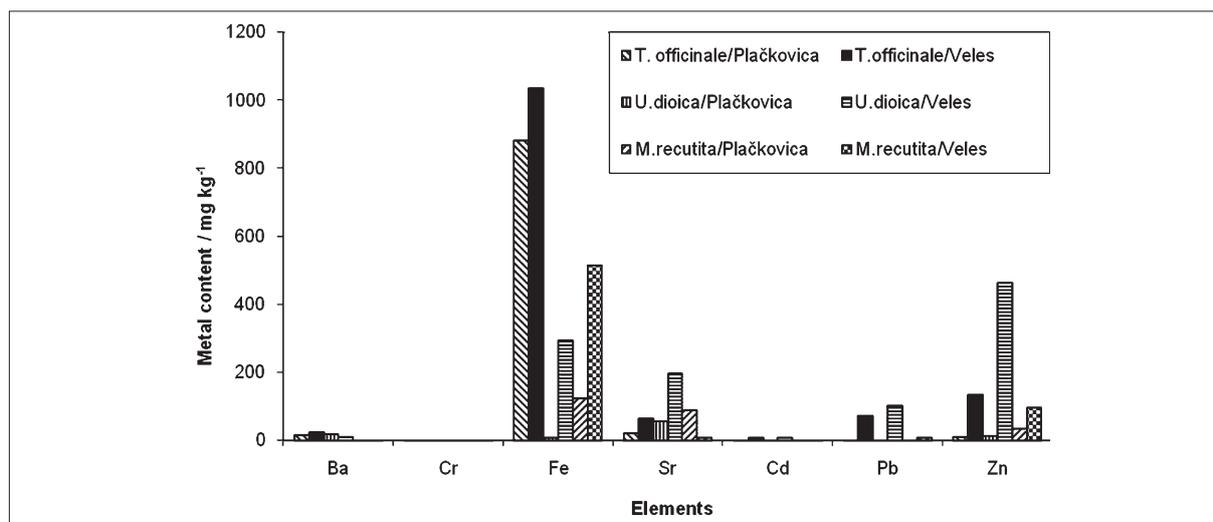
**Table 3** Operating conditions for ICP-AES (Varian, 715ES)

RF Generator					
Operating frequency	40.68 MHz free-running, air-cooled RF generator				
Power output of RF generator	700 W to 1700 W in 50 W increments				
Power output stability	Better than 0.1 %				
Introduction Area					
Sample nebuliser	V- groove				
Spray chamber	Double-pass cyclone				
Peristaltic pump	0 rpm to 50 rpm				
Plasma configuration	Radially viewed				
Spectrometer					
Optical arrangement	Echelle optical design				
Polychromator	400 mm focal length				
Echelle grating	94.74 lines per mm				
Polychromator purge	0.5 L min <sup>-1</sup>				
Megapixel CCD detector	1.12 million pixels				
Wavelength coverage	177 nm to 785 nm				
Conditions for program					
RFG Power	1.0 kW	Pump speed	25 rpm		
Plasma Ar flow rate	15 L min <sup>-1</sup>	Stabilisation time	30 s		
Auxiliary Ar flow rate	1.5 L min <sup>-1</sup>	Rinse time	30 s		
Nebuliser Ar flow rate	0.75 L min <sup>-1</sup>	Sample delay	30 s		
Background correction	Fitted	Number of replicates	3		
Element	Wavelength	Element	Wavelength	Element	Wavelength
Cd	226.50 nm	Cr	267.716 nm	Pb	220.353 nm
Ba	455.403 nm	Fe	238.204 nm	Zn	213.857 nm
Sr	407.771 nm				

Plačkovica, the highest metal content was found for Fe, followed by Sr, Zn, Ba, and Cr, while Pb and Cd were below the detection limit. In samples collected from the polluted area of Veles, the highest content

was again that of Fe, followed by Zn, Sr, Pb, Ba, Cd, and Cr.

Iron is an essential metal for plants and animals. The levels of iron were very high in *T. officinale*



**Figure 2:** Content of selected metals ( $\text{mg kg}^{-1}$ ) in three medicinal plants sampled from the polluted (Veles) and unpolluted (Mt. Plačkovica) area in Macedonia

leaves ( $881.46 \pm 0.4 \text{ mg kg}^{-1}$  for Mt. Plačkovica and  $1036 \pm 4.35 \text{ mg kg}^{-1}$  for Veles) and the lowest in *U. dioica* leaves ( $6.72 \pm 0.2 \text{ mg kg}^{-1}$  for Mt. Plačkovica and  $515 \pm 0.5 \text{ mg kg}^{-1}$  for Veles). The highest Fe content was found in herbs growing in the polluted area. The obtained values are close to those found by Maiga et al. (18) where the highest Fe plant level was  $1500 \text{ mg kg}^{-1}$  or by Kalny et al. (19) ( $522 \pm 225 \text{ mg kg}^{-1}$ ) or by Arceusz et al. (20) ( $307 \text{ mg kg}^{-1}$ ).

Strontium content in our study ranged from  $8.94 \pm 0.13 \text{ mg kg}^{-1}$  in *M. recutita* from Veles to ( $195.8 \pm 0.4 \text{ mg kg}^{-1}$ ) in *U. dioica*, also from Veles. Strontium is a relatively common trace element in the Earth's crust, it is very mobile and therefore readily taken up by plants. Its usual content in different food and feed plants ranges from  $10 \text{ mg kg}^{-1}$  to  $1,500 \text{ mg kg}^{-1}$  d.w. (21). Our results are at the low end of this range.

Zinc is an essential element in all organisms and plays an important role in the biosynthesis of enzymes, auxins, and proteins. It is not highly phytotoxic and the toxicity limit ( $300 \text{ mg kg}^{-1}$  to  $400 \text{ mg kg}^{-1}$ ) depends on plant species and growth stage (21). Several authors (22, 23), find the leaf concentration of  $100 \text{ mg kg}^{-1}$  indicative of environmental pollution with Zn.

All of the herbs growing near the lead and zinc smelting plant in Veles had higher Zn content: ( $465 \pm 2.3 \text{ mg kg}^{-1}$ ), ( $134.1 \pm 0.3 \text{ mg kg}^{-1}$ ), and ( $96.58 \pm 0.3 \text{ mg kg}^{-1}$ ) for *U. dioica*, *T. officinale*, and *M. recutita*, respectively, than control herbs growing on Mt. Plačkovica, where the highest Zn content was found in *M. recutita* flower ( $35.34 \pm 0.2 \text{ mg kg}^{-1}$ ), which

is well below the WHO permissible level of  $60 \text{ mg kg}^{-1}$  (24). Our results are in agreement with earlier findings of high Zn soil contamination in Veles (17). Kalny et al. (19) found the highest Zn level ( $221 \pm 42 \text{ mg kg}^{-1}$ ) in *Crataegus* species.

A large number of plants contain small quantities of Ba ( $4 \text{ mg kg}^{-1}$  to  $50 \text{ mg kg}^{-1}$ ) (25). Larger quantities can be toxic for plants and humans, and inhibit plant growth as well. In our study, Ba content ranged from ( $3.78 \pm 0.1 \text{ mg kg}^{-1}$ ) (*M. recutita*, polluted area) to ( $25.28 \pm 0.2 \text{ mg kg}^{-1}$ ) (*T. officinale*, polluted area). Kalny et al. (19) reported Ba values from ( $25.0 \pm 12.3 \text{ mg kg}^{-1}$ ) to ( $72.6 \pm 9.4 \text{ mg kg}^{-1}$ ).

Chromium is a nonessential element to plants. At  $100 \mu\text{mol L}^{-1} \text{ kg}^{-1}$  d.m., it is toxic to most of the taller plants (26), and according to Allen (22), at levels above  $0.5 \text{ mg kg}^{-1}$  Cr is toxic to all plants. The highest Cr content in our study was ( $1.88 \pm 0.05 \text{ mg kg}^{-1}$ ) in the unpolluted area and ( $2.59 \pm 0.2 \text{ mg kg}^{-1}$ ) in the polluted area. These findings are comparable to results published earlier (18, 27).

According to Kabata-Pendias and Pendias (21), Pb in plants ranges from  $0.1 \text{ mg kg}^{-1}$  to  $10 \text{ mg kg}^{-1}$  d.m. and according to Allen (22) the average content is  $3 \text{ mg kg}^{-1}$ . Klokke et al. (28) propose a Pb toxic range of  $30 \text{ mg kg}^{-1}$  to  $300 \text{ mg kg}^{-1}$ . The degree of heavy metal concentration in leaves seems to be proportional to urbanisation, industrial activity, and traffic density (29).

Some authors have also suggested that normal Cd levels in plants range between  $0.2 \text{ mg kg}^{-1}$  to  $0.8 \text{ mg kg}^{-1}$  and that toxic levels range between

5 mg kg<sup>-1</sup> and 30 mg kg<sup>-1</sup> (21, 28). Our results (Figure 2) show a high Cd and Pb content in all medicinal herbs from the polluted area of Veles. According to the above ranges, Pb and Cd in *U. dioica* and *T. officinale* fall within the toxic range for plants. The highest level of Pb in *U. dioica* leaves was (102.03±0.14) mg kg<sup>-1</sup> and of Cd (7.37±0.04) mg kg<sup>-1</sup>. Similar are the levels of Pb and Cd in *T. officinale* (73.92±0.5) mg kg<sup>-1</sup> and (7.24±0.2) mg kg<sup>-1</sup>, respectively. In contrast, Pb and Cd levels in *M. recutita* were significantly lower [(7.94±0.1) mg kg<sup>-1</sup> and (1.57±0.1) mg kg<sup>-1</sup>, respectively]. This finding is similar to earlier Cd measurements in chamomile plants (30).

Pb and Cd levels in herbs growing in the unpolluted area were below the limit of detection for the method (LD<1 mg kg<sup>-1</sup>) for *T. officinale*. In *U. dioica* leaves the Pb level was (3.86±0.1) mg kg<sup>-1</sup> while Cd remained below the detection limit and in *M. recutita* flowers the Pb level was (1.48±0.05) mg kg<sup>-1</sup> and Cd level (0.13±0.02) mg kg<sup>-1</sup>. Even the highest Pb level is below the WHO permissible limit of 10 mg kg<sup>-1</sup> for Pb in food (24).

The use of medicinal plants is perhaps the oldest way to treat illness (31). Due to widespread and prolonged use of medicinal herbs, their metal constituents could cause a variety of adverse effects (32). Furthermore, metals are sometimes intentionally added to Asian herbal preparations, because the traditional Indian (Ayurvedic) and Chinese medicine believes in their therapeutic properties (33). Therefore, it is not uncommon to find excessive quantities of toxic elements in such formulations. Herbal products from all over the world, such as those from Africa (18, 27, 32), Europe (5, 14, 19), and South America (34) have also been reported to contain high concentrations of toxic elements and pose a serious health risk.

## CONCLUSIONS

Our study has demonstrated that the content of investigated metals in Macedonian medicinal plants from the unpolluted area is below or close to the WHO limits, and that their content in plants growing in the polluted area exceeds these limits. Quality assurance and monitoring of toxic metals is therefore a must for plants intended for human use and consumption. Medicinal plants should be picked in areas free of any contamination sources.

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### **Sažetak**

#### SADRŽAJ TOKSIČNIH I ESENCIJALNIH METALA U LJEKOVITOM BILJU KOJE RASTE U ONEČIŠĆENIM I NEONEČIŠĆENIM PODRUČJIMA U MAKEDONIJI

Cilj je ovoga istraživanja bio utvrditi i usporediti sadržaj Ba, Cr, Cd, Fe, Sr, Pb i Zn u ljekovitom bilju *Urtica dioica* L., *Taraxacum officinale* i *Matricaria recutita* koje raste u onečišćenome odnosno neonečišćenome području u Republici Makedoniji. Sadržaj metala utvrdili smo s pomoću atomske emisijske spektroskopije induktivno spregnutom plazmom (engl. *inductively coupled plasma atomic emission spectroscopy*, krat. ICP-AES). U neonečišćenome području planine Plačkovice, sadržaj metala u *Taraxacum officinale* kretao se kako slijedi: Fe>Sr>Zn>Ba>Cr, dok su Pb i Cd bili ispod granice detekcije. U onečišćenome području blizu talionice olova i cinka u Velesu redoslijed je bio ovakav: Fe>Zn>Sr>Pb>Ba>Cd>Cr. Naši rezultati upućuju na potrebu za osiguranjem kakvoće i praćenjem razina toksičnih metala u biljaka namijenjenih ljudskoj uporabi. Ljekovito bilje valja brati u područjima bez izvora onečišćenja.

**KLJUČNE RIJEČI:** *Ba, Cd, Cr, Fe, Matricaria recutita, Pb, Sr, Taraxacum officinale, Urtica dioica, Zn*

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