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ORIGINAL ARTICLE

METALLOME OF *ORIGANUM VULGARE*: THE UNKNOWN SIDE OF A MEDICINAL AND AROMATIC PLANT USED WORLDWIDE

DIANA S. ANTAL[1#], COSMIN CITU[2#], FLORINA ARDELEAN[1], CRISTINA DEHELEAN[1]*, LAVINIA VLAIA[1], CODRUTA SOICA[1], VICENTIU VLAIA[1] MARIUS BIRIS[2], IOAN SAS[2]

1"Victor Babes" University of Medicine and Pharmacy, Faculty of Pharmacy, 2 Eftimie Murgu Square, Timisoara, Romania
2"Victor Babes" University of Medicine and Pharmacy, Faculty of Medicine, 2 Eftimie Murgu Square, Timisoara, Romania

**corresponding author: cadehelean@umft.ro*
[#]Authors had equal contribution to this research.

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Abstract

Potential health benefits of aromatic and medicinal plants connected to their content in minerals are usually not evoked by the relevant literature. However, they may be significant for persons using regularly phytotherapy, as well as for those adopting diets which are rich in herbal spices. In this context, we performed an extensive investigation of metal and metalloid elements present in different populations of wild-growing *Origanum vulgare* samples from Romania. Forty-nine elements were identified and quantified through inductively coupled plasma - mass spectrometry (ICP-MS). Results were discussed as to their significance for human health. The present study provides, for the first time, a complete set of reference data on the mineral content of *Origanum vulgare* from South-Western Romania.

Rezumat

Aportul elementelor minerale din plantele medicinale şi aromatice la menţinerea stării de sănătate este rareori abordată în literatura de specialitate. Totuși, această contribuţie ar putea fi semnificativă la persoanele care recurg în mod regulat la fitoterapie, precum şi la cele care adoptă o alimentaţie bogată în condimente vegetale. În acest context a fost realizată o analiză extensivă a elementelor metalice şi metaloizilor prezenţi în diferite populaţii de şovârf (*Origanum vulgare)* din flora spontană a României. Patruzeci şi nouă de elemente au fost identificate şi cuantificate prin *inductively coupled plasma - mass spectrometry* (ICP-MS). Rezultatele au fost analizate sub aspectul semnificaţiei lor pentru sănătatea umană. Prezentul studiu oferă, pentru prima dată, un set complet de date de referinţă pentru conţinutul în elemente minerale ale speciei *Origanum vulgare* din sud-vestul României.

Keywords: mineral elements, inductively coupled plasma - mass spectrometry (ICP-MS), *Origanum vulgare*

Introduction

The metallome is represented by metal- and metalloid species in biological systems, defined as to their identity and/or quantity. The study of the metallome, interactions, and functional connections of metal species with biomolecules in biological systems is termed metallomics [20]. This research area is rapidly growing, driven by the increasing interests of scientists in the metals and metalloids within biological systems. Plants contain, to the present knowledge, all natural elements included in the Periodic Table. Some minerals are essential (K, Ca, Mg, B, Cl, Cu, Fe, Mn, Mo, Ni, Zn), others stimulate the plant growth without being essential (Co, Na, Se, Si, Al), while another category, termed bulky elements, has apparently no function in the plant body [31]. Medicinal species and food plants transmit their charge of metallic/metalloid elements to humans, hence they provide a part of the required essential elements and contribute to a balanced natural supplementation of human nutrition [6]. As well, plants may take up toxic elements causing adverse health effects in humans, and for this reason medicinal plants are analysed with regard to their content in mercury, lead, cadmium and arsenic [32].

Oregano (*Origanum vulgare*) is an important culinary and medicinal plant. Six subspecies of *Origanum vulgare* are known (*vulgare, hirtum, gracile, glandulosum, virens* and *viridulum*). Most of the commercial oregano comes from wild plant populations growing in Turkey and Greece [2]. Traditionally, oregano was employed to treat disorders of the upper respiratory tract, coughs, fever, digestive disorders, dysmenorrhea, rheumatic pains, and urinary disorders [1, 17, 18]. More recently, anti-hyperglycaemic and cytotoxic activities were reported [12, 19]. In the food industry, oregano is not only used as a flavouring agent, but also as a preservative, due to significant antimicrobial effects and good antioxidative activity [21]. The U.S. Food and Drug Administration (FDA) recognize *Origanum vulgare* as generally safe for human consumption without limitations on intake, granting the plant a GRAS (generally recognised as safe) status [10].

The secondary metabolite profile of *Origanum vulgare* has been widely investigated. The main constituents are volatile oils, flavonoids, phenolic acids, anthocyans, tannins, and triterpenic acids [13, 23]. The sensory qualities and many of the medicinal properties of oregano are attributed to the volatile oil, highly variable in composition as a function of genotype, developmental stage, environment and even agricultural practice [8, 23]. Carvacrol (associated with a sharp, pungent flavour) and thymol (potent antibacterial) are important chemicals in Greek oregano (ssp. *hirtum*), a typical East Mediterranean taxon [13]. On the contrary, the volatile oil of wild oregano (ssp. *vulgare*) growing in Eastern European countries like Romania mainly contains sabinen (giving a sweet flavour), β-caryophyllen, D-germacrene and trans-β-ocymene, while carvacrol and thymol are absent [27]. Flavonoids (quercetin, apigenin, kaempferol derivatives) and phenolic acids (rosmarinic, ferulic, p-coumaric, caffeic) confer a potent antioxidant capacity to oregano [16, 26, 34].

While the organic constituents of the plant were intensely studied, including the geographical variability, there is scant knowledge about the minerals contained by oregano. Some references on the calcium and magnesium content [1], the composition of a new variety [9] and studies on the growth response of oregano to metal and salt induced stress are available [3, 25]. Until present, no extensive research on the metallome of this widely used plant has been performed. The aim of this study was to identify and quantify most of the metal and metalloid elements (forty-nine) present in wild-growing *Origanum* samples and to discuss the significance of results to human health.

Materials and Methods

Plant material. The identification of the species was performed with the help of "The Illustrated Flora of Romania" [5]. Flowering aerial parts were gathered in the second half of July 2013 from three different sites located in the South Western part of Romania (Aninei Mountains): site 1 - Livada Mare (45.125 latitude, 21.807 longitude), site 2 - Marghitas (45,126 latitude, 21.894 longitude), site 3 - Scocu meadow (44.971 latitude, 21.894 longitude). For each sample, one voucher specimen was deposited in the Herbarium of the Faculty of Pharmacy Timişoara. During the pulverization of the vegetal products, the contact to metallic instruments was avoided in order to prevent adulteration of results.

Analysis of mineral elements For the measurement of the content in Al, B, Ca, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb and Zn (elements present in amounts of milligrams – tens of grams per kg dried plant mass), *Inductively coupled plasma – atomic emission spectroscopy (ICP-AES)* was employed. Samples of 2-3 g of plant material were heated to 105ºC, in order to establish dry mass. Matrix destruction was performed after heating at a temperature of 550ºC for 6 hours, until the plant material was transformed into white ash. The ash was dissolved after boiling in hydrochloric acid 10%, followed by the quantitative transfer of the content in a test-tube. The acid solution was made up with bidistiled water to 25 mL, filtered and measured by ICP-AES without further dilution (apparatus IRIS Interprid II ICP-AES, Thermo Electron, Dreieich, Germany).

Inductively coupled plasma – mass spectrometry (ICP-MS) was used to determine the content in trace and ultra-trace elements present in concentration ranges of micrograms – hundreds of micrograms per kg dry vegetal product: Li, Be, V, Co, As, Se, Rb, Ag, Cd, In, Sn, Sb, Te, I, Cs, Tl, U, lanthanides and thorium. The employed apparatus was X Series ICP-MS (Thermo Electron, Dreiech, Germany, 2004), equipped with a collision cell in order to allow the reduction of polyatomic interferences. Standardization was performed with multi-elemental standard solution (ICP Multi Element Standard Solution XXI Certi PUR Merck); internal standard during measurements was rhodium.

Weighed samples of 0.3-0.4 g dried plant material were placed in Teflon crucibles and 4 mL of nitric acid (sub-boiled), 0.25 mL hydrochloric acid (Merck, ultrapure) and 1 mL hydrogen peroxide (Merck, ultrapure) were added. Mineralization was performed in a closed system with the use of microwave energy (180ºC, 11 bars, MARS 5 microwave oven, CEM GmbH, Kamp-Lintfort Germany). The digestion solutions were transferred into volumetric flasks and made-up to 15 mL with water (nano-pure); 1 mL of each solution was diluted 1:10 and analysed through ICP-MS. For the analysis of mercury, the digestion solutions were examined through atomic fluorescence (apparatus Mercur, Analytik Jena AG, Germany). Iodine was analysed separately, after the pre-treatment of the plant material with tetramethylammonium hydroxide (Merck). Accuracy of experimental data has been verified by a parallel analysis of three certified reference materials: Peach Leaves 1547, Oriental Tobacco Leaves CTA-OTL-1 and Apple Leaves 1515.

Results and Discussion

Following the metalome analysis of the flowering aerial parts of oregano, all researched elements were identified, in very different amounts. Potassium, calcium and magnesium (mineral macro elements) were present in concentrations of some grams per kg dry product (Table I).

Table I

Content of ***s*** and ***p*** block elements (alkaline, alkaline earths, lanthanides and actinides) in *Origanum vulgare* aerial parts from South-Western Romania. Values are expressed per dry weight.

Element		Site 1	Site 2	Site 3	Average	Standard deviation
s Elements (alkaline and alkaline earths)	**Li (μg/kg)**	49	86	4	46.3	41.1
	Na (mg/kg)	59	21	24	34.7	21.1
	K (mg/kg)	19786	13838	13048	15557.3	3683.4
	Rb (mg/kg)	10.3	17.8	28.9	19.0	9.4
	Cs (μg/kg)	9	35	45.2	29.7	18.7
	Be (μg/kg)	5	6	23	11.3	10.1
	Mg (mg/kg)	2362	2537	2889	2596.0	268.4
	Ca (mg/kg)	24610	11668	16513	17597.0	6538.7
	Sr (mg/kg)	113.2	21.3	12.7	49.1	55.7
	Ba (mg/kg)	85.1	25.1	13.3	41.2	38.5
Lanthanides	**Ce (μg/kg)**	151	113	111	125.0	22.5
	Pr (μg/kg)	21	26	15	20.7	5.5
	Nd (μg/kg)	74	178	62	104.7	63.8
	Sm (μg/kg)	14	22	12	16.0	5.3
	Eu (μg/kg)	7.1	5	1.2	4.4	3.0
	Gd (μg/kg)	15.2	23.1	15.9	18.1	4.4
	Tb (μg/kg)	2.2	3	1.8	2.3	0.6
	Dy (μg/kg)	9.5	13.5	7.5	10.2	3.1
	Ho (μg/kg)	2.2	2.5	1.8	2.2	0.4
	Er (μg/kg)	4.3	7.4	3.6	5.1	2.0
	Tm (μg/kg)	1.2	1.2	0.7	1.0	0.3
	Yb (μg/kg)	4.1	7.1	3.7	5.0	1.9
	Lu (μg/kg)	1.2	1	0.3	0.8	0.5
Actinides	**Th (μg/kg)**	19.3	11	7	12.4	6.3
	U (μg/kg)	6.1	6.5	4.5	5.7	1.1

Table II

The content in mineral elements of the ***p*** and ***d*** blocks in *Origanum vulgare* aerial parts from South-Western Romania. Values are expressed per dry weight.

Element		Site 1	Site 2	Site 3	Average	Standard deviation
Transitional (d) elements	**V (μg/kg)**	880	1015	165	686.7	456.8
	Cr (μg/kg)	119	253	127	166.3	75.2
	Mn (mg/kg)	53	28	45	42.0	12.8
	Fe (mg/kg)	36	40	56	44.0	10.6
	Co (μg/kg)	70	265	134	156.3	99.4
	Ni (mg/kg)	3.3	1.7	2.4	2.5	0.8
	Cu (mg/kg)	11.6	6.9	7.7	8.7	2.5
	Zn (mg/kg)	46	32	58	45.3	13.0
	Mo (μg/kg)	351	278	150	259.7	101.7
	Ag (μg/kg)	12	7.6	17.1	12.2	4.8
	W (μg/kg)	22.2	35.3	19.9	25.8	8.3
	Cd (μg/kg)	91	120	149	120.0	29.0
	Hg (μg/kg)	12.44	6.69	11.45	10.2	3.1
Elements of the p block	**Pb (mg/kg)**	0.59	0.37	0.93	0.6	0.3
	Al (mg/kg)	203	107	55	121.7	75.1
	In (μg/kg)	2.2	0	0.6	0.9	1.1
	Tl (μg/kg)	19.2	3	3.9	8.7	9.1
	Sn (μg/kg)	413	122	120	218.3	168.6
	Sb (μg/kg)	14.2	20.4	120.5	51.7	59.7
	B (mg/kg)	60.9	40.9	55.1	52.3	10.3
	As (μg/kg)	759	48	67	291.3	405.1
	Se (μg/kg)	1	31	96	42.7	48.6
	Te (μg/kg)	1.2	3.2	2.7	2.4	1.0
	I (mg/kg)	0.54	0.8	1.4	0.9	0.4

Sodium, manganese, zinc, boron, iron, copper, aluminium, nickel, molybdenum (trace elements in the plant organism) occur in quantities of the order of mg/kg – hundreds of μg/kg. Cobalt, selenium, chromium, lead, cadmium, mercury, the lanthanides and the natural actinides, termed ultratrace elements, were detected in trace amounts, of only some µg/kg (Table II). The high values of the standard deviations indicate the strong relationship between the local conditions and the concentration of minerals in the plants. The most prominent factors associated with the mineral content in plants are genetic (species-specific) and environmental (nature of the weathering rock, pH of the soil, presence of minerals with antagonistic effect) [15]. More recently, phylogenetic analysis highlighted that evolutionary factors may account for over 25% of the total variation in case of at least 21 elements including nonessential ones [30].

Although the contribution of essential minerals in oregano herb to the overall status of essential minerals is modest, some elements do have a more important participation: 2 g dried herb contain 4.2% of the Recommended Dietary Allowance (RDA) for Mn, and 4.4% of the RDA for Ca (EU RDA values taken from [8]). The current analysis presents the way in which a single plant may participate within a balanced diet to the general intake of essential micronutrients. It underlines the usefulness of herbal preparations (spices, herbal teas, encapsulated powders and various dietary herbal supplements) for the provision of micronutrients and the correction of marginal mineral deficiencies [14]. Mediterranean diets, considered to be a model of healthy nutrition, are not only characterized by their composition (in low red meat and animal fat, rich in olive oil, fruits and vegetables) [33], but also by the generous use of herbal spices like rosemary, thyme, oregano, sage, marjoram, garlic and others. While many of the favourable health effects of herbs can be attributed to the content in polyphenols and volatile oils (conferring antioxidative, chemo-preventive, antibacterial, antispasmodic, digestive, and hepato-protective properties [28]), their daily usage has a contribution to the overall mineral status of humans, and may be of relevance to the auxiliary treatment of diseases, as pointed out by other authors as well [6].

Regarding the presence of toxic elements in oregano, their content poses no threats to human health, being low for both the ones subjected to legal limitations (Cd, Pb, Hg, As) [32] as well as for contaminants which are not regularly monitored in medicinal plants (U, Cs, Be, Tl, Sn). It can be stated that *Origanum vulgare* is not prone to the accumulation of toxic elements, unlike medicinal species like *Hypericum perforatum* or *Viola tricolor* [11]. Flowered oregano contains as well a series of elements which have been very little investigated up to the present in foodstuffs or medicinal plants: rare earth elements (lanthanides). Their exact function in plants is unknown [29]. Lanthanide mixtures are used in China as fertilizers for at least 20 years [24]. To date, yield increases for over fifty plant species including cereals, industrial crops, fruits and vegetables, trees and pasture grasses following lanthanide application have been reported. Common yield increases are in the range of 5 to 15 %; improvements in product quality have also been noted [4]. The content of rare earths in oregano is low, and the present knowledge suggests their lack of toxicity in the amounts measured during the present research.

Conclusions

Beyond health benefits imprinted by the organic phytocomplex, the regular utilization of oregano supplies essential minerals. On the other hand, oregano is not prone to the accumulation of toxic metals. In our opinion, the additive contribution of various herbal preparations to the mineral status of humans should not be neglected and constitutes an incentive to a more sustained use of culinary and medicinal herbs. The paper provides a large data set on the content of 49 mineral elements in wild-growing oregano from SW Romania.

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