High Cadmium Content in Wild - Growing Medicinal Plants from South- Western Romania

Unexpected Results of a Survey on 29 Species

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The toxicological importance of cadmium, and the fact that Romania is one of the main suppliers of wild-growing medicinal plants, determined us to perform a large-scale investigation of wild-growing species in what their Cd content is concerned, and to evaluate the extraction ratio of this element onto herbal teas. The analysis of 96 samples, carried out through Inductively Coupled Plasma - Mass Spectrometry (ICP-MS), revealed an average Cd content of 0.501 mg/kg in the medicinal plants collected from the Aninei Mountains (South-Western Romania). This value exceeds the limit of 0.30 mg Cd/kg set by the World Health Organization. Wild pansy (Viola tricolor) has a particular capacity to accumulate this element (mean value of 5.2 mg/kg); it should not be wild-collected from the investigated area. Furthermore, products from this species intended for pediatric use should not be commercialized without systematic analysis of Cd content. The average extraction ratio of Cd through decoction is of 19%. Herbal teas may contribute to up to 9% of the Provisional Tolerable Weekly Intake for Cd. The present research presents the first large-scale survey of wild-growing Romanian plants with regard to their Cd content.

Keywords: cadmium (Cd), medicinal plants, ICP-MS, herbal teas, extraction yield

Although Cd has a very low abundance in the outer Earth crust (of 0.2 ppm) [1], its presence in the environment has become significant due to human activities. It is estimated that the anthropogenic enrichment factor is of about 89% [2]. This heavy metal is a well known contaminant of soils and water. Due to its long persistance, Cd represents a major ecotoxicologic concern in environment protection and conservation.

In humans, Cd causes bone demineralisation, renal dysfunction, reduces the absorption of essential minerals (Cu, Fe), and has mutagenic/carcinogenic effects[3]. The very long biological half-life (10-30 years) is a strong contributing factor to this elements toxicity. For the general population, the main sources of Cd exposure are foodstuffs and tobacco smoking. The average intake through food across European countries was evaluated at 2.3μg/kg body weight per week (range from 1.9 to 3.0μg/kg b.w. per week). The Joint FAO/WHO Expert Committee on Food Additives established a Provisional Tolerable Weekly Intake (PTWI) of 7μg/kg body weight (b.w.) per week [4].

Medicinal and aromatic plants are of particular interest for human health. They are employed in the prophylaxis and auxiliary treatment of various diseases due to beneficial effects granted by a complex of secondary metabolites. However, they contain beside the organic actives all known natural elements, some of them beneficial (K, Mg, Ca, Fe, Cu, Mn, I...), other toxic to humans (Pb, Cd, Hg, As, Tl, U....) [5]. Most protocols impose threshold levels on Pb, Cd and Hg in plant prime matters intended for medicinal use. The upper limit of Cd content in medicinal plants was set by the WHO at 300 μg/kg dry weight [6]. An even more restrictive threshold (200μg Cd/kg) was proposed by the German Ministry of Health [7].

Romania is, on European level, one of the major suppliers of medicinal plants collected from the wild [8]. As such it is mandatory to know if the content of toxic elements complies with WHO limitations. There are two implications of this matter: i) from the viewpoint of healthcare, it is essential to employ only a safe prime matter, and ii) from an environmental conservation viewpoint, it is useless to wild-collect plants with a high content of toxic elements. So far there has not been performed an extensive survey of the Cd content of Romanian medicinal plants from the wild; this study aims at performing such a survey on species growing in the Aninei Mountains situated in the South-West of the country and completes previously published data on the content of other toxic elements: As and U [9,10]. The second objective of the current research consists in the evaluation of the Cd extraction rate from plants onto aqueous extracts, as herbal teas are the most widespread form of use of medicinal plants. This procedure is essential in order to understand the relevance of plants' Cd content for human health.

Experimental part

Materials equipment and methods

Plant material. Ninety-six samples representing plant organs of 29 medicinal species employed in phytotherapy were collected from the wild flora of the Aninei Mountains (South-West of Romania). The plants were gathered at altitudes of 400-900 m, from unpolluted sites. After collection, the samples were dried at ambient temperature and deposited in cotton sacks. Previous to the determination of Cd content, plants were brought to powder consistency, and dry mass at 105°C was determined for each vegetal product through heating during two hours in an oven.

Apparatus and analysis parameters. The assessment of the Cd content was performed using Inductively Coupled Plasma - Mass Spectrometry (ICP-MS). The employed apparatus was X Series ICP-MS (Thermo Electron, Dreieich,

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Germany). The parameters of the measurement were as follows: excitation power of plasma: 1360 W; flow rate for plasma gas: 13 L/min; for nebulization gas: 0,9 l/min; for auxiliary gas 0.9 L/min; extraction voltage: -173 V; pole bias: -2 V; hexapole bias: -2V; lens 1: 0.3 V; lens 2: -20.4 V; lens 3: -127.1 V; torch position: horizontal - 54 V, vertical - 577 V; amount of doubly charged ions CeO/Ce < 2% and Ba2+/Ba < 4%; measurement time 10 s in threefold repetitions; sampling depth 130.

Sample preparation. Weighed samples of 0.3-0.4 g dried plant material were placed in Teflon crucibles and 4 mL of nitric acid (Merck, additionally purified by subboiling), 0.25 mL hydrochloric acid (Merck, ultrapur) and 1 mL hydrogen peroxide (Merck, ultrapur) were added. Mineralization was performed in a closed system with the use of microwave energy (oven MARS 5, CEM GmbH, Kamp-Lintfort Germany), at 180°C and 11 bar pressure, for 20 min. The digestion solutions were transferred into volumetric flasks and made-up to 15 mL with water (nanopure); 1 mL of each solution was diluted 1:10 and analyzed by ICP-MS.

Preparation of the aqueous extract. In order to evaluate the proportion in which Cd passes into solution, we prepared decoctions out of 18 plant products, obtained as follows: 50 mL bidistilled water were added to 2.000 g dried herb, and heated to boiling; the temperature of 100°C was maintained for 15 min. After cooling and filtration, 5 mL extract were introduced in a Teflon crucible, and treated with 3 mL nitric acid subboiled and 0.250 mL hydrochloric acid ultrapur. The solution was microwave-digested, brought to 10 mL, and analyzed by ICP-MS.

Method of analysis. As a first step, a semi-quantitative analysis was performed, allowing the estimation of the Cd concentration ranges in the digestion solutions of the plant materials. Quantitative determinations were carried out with the aid of a calibration curve using ICP Multi Element Standard Solution XXI CertiPUR Merck, diluted to obtain optimal measurement range (between 0.05 - 50 μg/L). Internal standard was rhodium. The limit of detection for Cd was 0.06 μg/L.

Accuracy of data has been verified by a parallel analysis of two certified reference materials: Peach Leaves 1547 and Oriental Tobacco Leaves CTA-OTL-1. The agreement between the concentration indicated by the producer and the one obtained experimentally within the present study certifies the fact that mineralization and determination procedures were carried out quantitatively and correctly (table 1).

Results and discussions

Cadmium is a calcophile element, associated geochemically with Zn in sulphidic minerals. The oxidation processes during weathering liberate Cd as soluble Cd^{2+}. This ion has a high mobility and is weakly adsorbed by organic matter, clays and oxides [11]. Although Cd is not essential to plant metabolism, it is readily taken up by roots and leaves [12]. A linear correlation between the Cd content of plants and the environment has been pointed out by several researches [13]. Cd accumulation capacity differs between species, and some plants like spinach or fungi have a known ability to store high Cd amounts. Such species act as Cd reservoirs in the food chain, and may represent potential health risks. In fact, vegetarians have a higher Cd dietary exposure of up to 5.4 μg/kg b.w. per week [4]. On the other hand, Cd accumulators may be useful in the phytoremediation of polluted soils.

Due to the high toxicity of Cd, its content in medicinal plants is subject to reglementations. In relation to the limit set by the WHO (300 μg Cd/kg dry plant), only 51% of the samples analyzed in the curent research comply (fig. 1). The high Cd content may be explained based on the high geogenous occurence of the element in the rocks and soils of the investigated area (Aninei Mountains), as the weathering rocks here are mostly represented by limestone. All analyzed plants were gathered far from known pollution sites, as such the unexpectedly high Cd content in the samples is not likely to be anthropogenic. In fact, the high occurence of Cd in the soils of Banat region has been pointed out previously [14], with values of Cd content ranging from 1 to 1.8 ppm being measured (compared to uncontamined soils from other regions of Europe, where Cd varies between 0,1 and 1.0 ppm [11], a situation motivated by the calcophile nature of Cd.

Within the analyzed plants, Cd content displays an important interspecies difference. Cd accumulating medicinal plants are: wild pansy (*Viola tricolor),* Saint John's wort *(Hypericum perforatum)*, agrimony (*Agrimonia eupatoria),* chickory *(Cichorium intybus),* wild thyme *(Thymus pulegioides),* birch *(Betula pendula),* stinging nettle *(Urtica dioica),* ribwort *(Plantago lanceolata),* yarrow *(Achillea millefolium),* (table 2). Among the above-mentioned species, wild pansy, Saint John's wort, birch and yarrow are known for their ability to store Cd [15,16]. However, wild pansy from the investigated area exceeds by far the Cd content plants collected elswhere and contains amounts comparable to those measured for food

Table 1
RESULTS OF CADMIUM DETERMINATION THROUGH ICP-MS IN CERTIFIED REFERENCE MATERIALS

Peach Leaves 1547		Oriental Tobacco Leaves CTA-OTL-1	
Certified value (μg Cd/g)	Measured value (μg Cd/g)	Certified value (μg Cd/g)	Measured value (μg Cd/g)
0.026 ± 0.003	0.024	1.12 ± 0.12	1.15

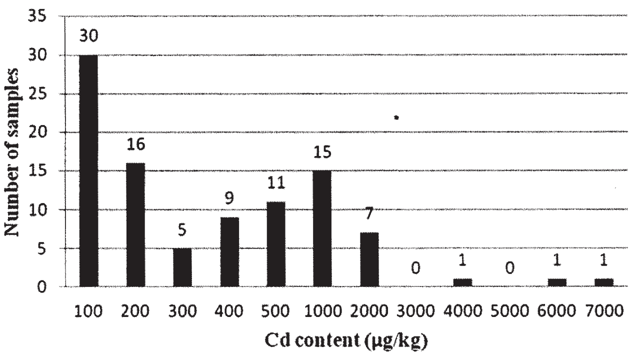

Fig. 1. Frequency distribution of cadmium content in 96 samples of wild-growing medicinal plants

Species	$\bar{x}$	n	Min	Max	Species	$\bar{x}$	n	Min	Max
Geum urbanum	421	3	69	630	*Valeriana officinalis*	105	3	15	206
Primula officinalis	400	2	361	439					
Average Cd content in subterranean parts: 309 ± 177 µg/kg									
Agrimonia eupatoria	557	2	207	906	*Leonurus cardiaca*	169	2	2	335
Artemisia absinthium	515	2	358	671	*Lythrum salicaria*	23	2	17	28
Chelidonium majus	139	2	125	152	*Origanum vulgare*	168	5	78	402
Cichorium intybus	766	2	197	1334	*Taraxacum officinale*	278	3	29	490
Epilobium parviflorum	242	3	31	567	*Thymus pulegioides*	619	7	178	1402
Equisetum arvense	173	4	11	486	*Viola tricolor*	5200	3	3048	6839
Hypericum perforatum	765	7	139	1639					
Average Cd content in aerial parts (*herba*): 740 ± 1363 µg/kg									
Allium ursinum	256	5	6	593	*Plantago lanceolata*	477	3	461	603
Betula pendula	565	4	52	1640	*Rubus idaeus*	282	2	143	420
Corylus avellana	58	2	55	61	*Urtica dioica*	250	5	30	1034
Fragaria vesca	116	3	17	134	*Crataegus monogyna*	202	5	88	419
Fraxinus excelsior	49	2	12	86					
Average Cd content in leaves: 251 ± 177 µg/kg									
Achillea millefolium	616	6	12	1472	*Tilia cordata*	88	2	33	143
Sambucus nigra	61	3	3	139	*Tilia tomentosa*	403	2	252	554
Average Cd content in flowers: 292 ± 266 µg/kg									

$\bar{x}$: average content; n: the number of analyzed samples within each plant organ; Min: minimal Cd content; Max: maximal Cd content;

Table 2
CADMIUM CONTENT OF SOME MEDICINAL PLANTS (µg / kg DRY PLANT)

Species – plant product	Cd (µg/kg) in plant product	Cd (µg/kg) extracted through decoction*	extraction yield (%)
Viola tricolor (wild pansy) – herb sample 1	**6839**	1061	15.5
Viola tricolor (wild pansy) – herb sample 2	**5714**	1057	18.5
Viola tricolor (wild pansy) – herb sample 3	**3048**	695	22.8
Cichorium intybus (chicory) - roots	**1261**	147	11.6
Filipendula ulmaria (meadowsweet) - flowers	**794**	29	3.6
Achillea millefolium (yarrow) - flowers	**547**	106	19.4
Primula officinalis (cowslip) - rhizomes	**439**	50	11.4
Crataegus monogyna (hawthorn) - leaves+flowers	**419**	61	14.6
Althaea officinalis (marshmallow) - leaves	**357**	19	5.3
Leonurus cardiaca (motherwort) - herb	**335**	157	46.9
Hypericum perforatum (St John's wort) - herb	**310**	107	34.5
Cichorium intybus (chicory) - herb	**197**	68	34.5
Thymus pulegioides (wild thyme) - herb	**178**	20	11.2
Ononis spinosa (restharrow) - roots	**140**	13	9.3
Sambucus nigra (elder) - flowers	**139**	16	11.5
Valeriana officinalis (valerian) - rhizomes	**94**	26	27.7
Taraxacum officinale (dandelion) - herb	**29**	9	31
Equisetum arvense (horsetail) - herb	**11**	3	27.3

* Values represent the extractible Cd amount through decoction (15 minutes) from the indicated plant product; preparations were made of 4% plant material in bidistilled water.

Table 3
CADMIUM CONTENT OF SOME AQUEOUS EXTRACTS OBTAINED FROM MEDICINAL PLANTS

plants grown in Cd-contaminated areas [17], although in the present case this cause is excluded. An important remark is the ability of most plants to take up and tolerate high Cd amounts (table 2), this means that for most wild-collected plants it is not possible to predict the Cd content based on the name of species. This agrees with the recently published viewpoint that Cd composition of plants lacks evolutionary control [18], while the concentration of elements like V, Ca, Al and Zn correlate to phylogenetic factors.

Taking into account the popularity that aqueous extracts enjoy as pharmaceutical form in phytotherapy, we evaluated the proportion in which medicinal plants transfer their Cd charge to herbal teas. Results show a rather stable extraction yield, with most of the values (15 out of 18) being below 30% (table 3). The easiest passage into water is specific to Cd from motherwort, Saint John's wort and chicory herbs (>30%), while Cd from meadowsweet can be extracted in the lowest proportion (3.6%). These variations can be explained by each plant's different chemical composition, where saponins, tannins, mucilages, flavonoids etc. create specific *p*H values and redox potentials, or involve Cd in complex combinations with low solubility.

With view to the Cd Provisional Tolerable Weekly Intake (PTWI) recommended by the WHO (70 µg/day for a subject weighing 70 kg) [4], the aqueous extracts of wild pansy herb contribute to this amount in the highest degree. Herbal teas prepared from 6 g of this plant (the equivalent of 3 cups per day prepared from 2 g each) may supply up to 6.37 µg Cd under a highly bioavailable form, representing 9.1% of the (PTWI). This may raise concerns in children, as wild pansy is often recommended in pediatrics, including for the reconstitution of bottle milk from powder.

Conclusions

The analysis of the Cd content from 96 samples of various medicinal plants collected from the wild flora of South-Western Romania shows that only 51% of the samples comply with the upper limit set by the World Health Organization (300 μg Cd/kg dry plant). These unexpectedly high Cd contents may be explained by the high geogenous occurence of the element in the area, which mainly contains limestone weathering rocks. Particularily high Cd contents were measured in wild pansy, Saint John's wort, agrimony, chickory, wild thyme and birch. The wild collection of these species from the area is not recommended as most of the samples surpass WHO limitations. Wild pansy intended for pediatric use should undergo particular control due to the marked Cd accumulation capacity of this plant.

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