

Original Paper

**Researches upon the Heavy Metals Content of
Silene alba (Caryophyllaceae) Species**ȚÎRCOMNICU VIORELA, BUBULICĂ MARIA-VIORICA, CHIRIGIU L.,
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ABSTRACT The analysis of heavy metals in the leaves and stems of *Silene alba* species has been performed. The mean levels of heavy metals in vegetal dried samples were in normal limits. Differences depend on the presence of organic compounds with ligand character and on the environmental conditions. The results indicate that there is not any real danger by the possible utilization of some pharmaceutical preparations of *Silene albae herba*.

KEY WORDS: *Silene alba*, heavy metals, content.

Introduction

Silene alba (Miller) E.H.L. Krause sin. *Melandrium album* (Miller) Garcke, White Champion, *Caryophyllaceae* family, native to most of Europe, Western Asia and North Africa, is an herbaceous annual, biennial or short-lived perennial plant, growing in most open habitats, particularly wasteland and fields, preferring sunny areas that have rich and well-drained soils [1].

In the specialty papers, there are incomplete data on the chemical composition of *S. alba* species, as follows: phytoecdysteroids (ecdysone, 2-deoxy-20-hydroxyecdysone), flavonosides (apigenin- and luteolin-O,C-glycosides), triterpenoid saponins (gypsogenin and quillaic acid saponins), and polyphenolic acids [2–9].

For the qualitative evaluation of the vegetal products with medicinal and/or aromatic properties, the control of the heavy metals concentrations is mandatory [10].

Because the medicinal plants sources are often selected from different growing regions, significant differences in the amounts of heavy metals uptake and storage in vegetal tissues can be found [10].

It is known that in mountain areas the heavy metals absorption in plants achieve high values because of some environmental conditions related to soil acidity. In addition, the mobility of metal cations at the plant tissue level is stimulated by the presence of minerals rich in heavy metals in the growing field [10].

The purpose of this paper is to study the heavy metals composition of leaves and stems of *Silene alba* species.

Material and Methods**Plant material**

The amount of the harvested vegetal material depends on the analytical purposes, in the first

instance, and of course on the specificity of plant tissues, soil, water and air conditions, on the other hand. Good quality samples are necessary to be collected in sufficient quantities, on sunny weather, in a specific time of day [10].

From the *S. alba* species, twenty vegetal samples were collected at the flowering, in May 2010, from the Botanical Garden of The University of Craiova, Dolj County.

Preparation of plant tissue for analysis

Sample preparation is critical in obtaining accurate data and reliable interpretation of plant analysis results.

The vegetal products should be processed during decontamination, drying, particle-size reduction, storage and organic matter destruction.

Plant material must be cleaned and free of extraneous substances, including soil and dust particles that may influence analytical results.

The decontamination process must be thorough while still preserving sample integrity. Decontamination procedures involving washing and rinsing with deionized water and 0.2% detergent solution (non-phosphate), should only be used for fresh, fully turgid plant samples.

After decontamination, water is removed from the plant tissue, at temperatures under 60°C, to stop the enzymatic reactions and to stabilize the samples.

Plant tissue samples are reduced to 0.5 to 1.0 mm particle size to ensure homogeneity and to facilitate organic matter destruction [10].

Gravimetric determination of ash

The ash represents the residue obtained through the dry ashing of a matter, being made by inorganic compounds.

Dry-ashing is conducted in a muffle furnace at temperature 500 to 550°C for four to eight hours.

For tissues high in carbohydrates and oils, ashing aids may be required to achieve complete decomposition of organic matter. At the end of the ashing period, the vessel is removed from the muffle furnace, cooled, and the ash is dissolved in nitric acid. The final solution is diluted as needed to meet the range requirements of the analytical procedure or instrument utilized [10].

Weigh 0.5 to 1.0 g dried plant material that has been ground and homogenized into a high-form, 30 mL porcelain crucible.

Samples were placed in a cool muffle furnace. Temperature control of the furnace was set to allow gradual increase (two hours) in the ashing temperature and maintain for four to eight hours. After that, the furnace was turned off to allow samples to cool (in one hour). Then, the ash is weighing on analytical balance nearest 0.1 mg.

High temperature oxidation. Heavy metals detection

This method prepares plant tissue for the quantitative determination of the content of Ca^{2+} , Zn^{2+} , $\text{Fe}^{2+/3+}$, Mn^{2+} , Ni^{2+} , Pb^{2+} , Cr^{3+} , by atomic absorption spectrometry (AAS), utilizing high-temperature dry oxidation of the organic matter and dissolution of the ash with 4% nitric acid.

The method detection limit is approximately 0.04%. The method is generally reproducible within $\pm 7\%$ [10].

The tissue samples (leaves, stems) were prepared in the above-mentioned manner.

Heavy metals content was determined using analytical balance, porcelain crucibles, muffle furnace, volumetric labware, deionized water, standard calibration solutions, and an AAS-30 Carl Zeiss Jena (Germany) spectrometer with Photron & Narva cathode (Table 1).

Five standard calibration dilutions (0.001 mg/L to 2 mg/L) were prepared starting from 5 mg/L reference solutions diluted with 4% nitric acid.

Table 1 – Experimental data of AAS-30 Carl Zeiss Jena (Germany) spectrometer with Photron & Narva cathode

Parameters	Mo	Pb	Zn	Ni	Ca	Cr	Fe
Wavelength [nm]	313.3	283.3	213.9	232.0	422.7	357.9	248.3
Lamp current [mA]	15	9	13	12	9	10	11
Air flow [L/hr]	–	590	620	710	630	680	690
Acetylene flow [L/hr]	150	65	65	69	65	65	69
N ₂ O flow [L/hr]	620	–	–	–	–	–	–
Flame type	Red.	Ox.	Ox.	Ox.	Ox.	Ox.	Ox.

Red. – reducing; Ox. – oxidizing.

Results and Discussion

The results of AAS analysis are given as mean and standard deviation (Table 2, Figure 1).

Table 2 – Heavy metals content of *S. alba* samples (leaves and stems)

Heavy metals	<i>Silene albae folium</i>	<i>Silene albae cauli</i>
	[mg%]	[mg%]
Ca^{2+}	1.0632 \pm 0.01	0.0451 \pm 0.01
Zn^{2+}	6.5722 \pm 0.2	3.5662 \pm 0.2
Mn^{2+}	0.2005 \pm 0.002	0.0934 \pm 0.02
$\text{Fe}^{2+/3+}$	0.6364 \pm 0.01	0.2258 \pm 0.01
Ni^{2+}	0.1551 \pm 0.01	0.3191 \pm 0.01
Pb^{2+}	1.2315 \pm 0.2	5.7242 \pm 0.2
Cr^{3+}	0.1269 \pm 0.02	0.0624 \pm 0.002
Sample [g]	1.1328 \pm 0.0002	1.1245 \pm 0.0002
Dried sample [g]	0.9910 \pm 0.0002	0.9815 \pm 0.0002
Ash [g]	0.0948 \pm 0.0002	0.0911 \pm 0.0002
% Dry ashing residue	9.4536 \pm 0.5	10.7739 \pm 0.5

The maximum limits of heavy metals allowed in plants for adequate growing and development are the followings: 300 mg% Ca^{2+} , 10 mg% Zn^{2+} , 5 mg% Mn^{2+} , 100 mg% $\text{Fe}^{2+/3+}$, 0.80 mg% Ni^{2+} , 50 mg% Pb^{2+} and Cr^{3+} [10].

In the vegetal products obtained from *Silene alba* species, the content of the heavy metals can be considered as normal.

All samples contain Pb^{2+} and Cr^{3+} , markers for soil and air pollution.

The levels of Pb^{2+} (normal limits) in vegetal tissues samples appear because the plants collected were relatively closely to roads (cars circulation).

Generally, for most plants the concentrations of some heavy metals are higher in the roots than in the aboveground parts.

This is an important finding, because only the aerial parts of *S. alba* species are usually used as medicinal products.

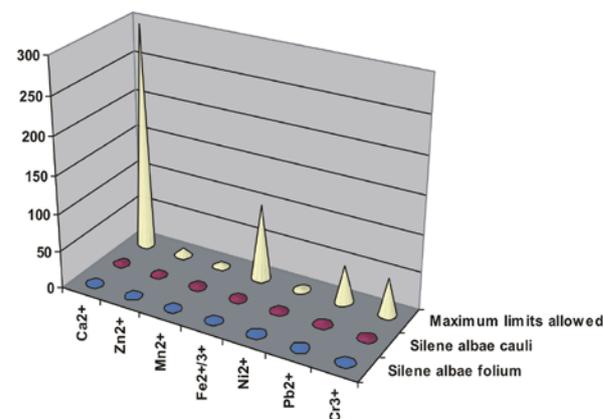


Figure 1 – Maximum limits allowed and heavy metals content of *S. alba* samples (leaves and stems).

High levels of Ca^{2+} , Zn^{2+} , Mn^{2+} , $\text{Fe}^{2+/3+}$ and Cr^{3+} , but in normal limits, have been determined in the stems, because their intake from soil or aerial parts.

The leaves contain high levels of Ni^{2+} and Pb^{2+} , probably as consequence of the bioinorganic mechanisms during the flowering period and of the soil acidity.

AAS analysis confirmed indirectly that the samples were collected from mature *S. alba* species, during the flowering period, when appear complex combinations with divalent (Ca^{2+} , Zn^{2+} , Mn^{2+} , Fe^{2+} , Pb^{2+}) or trivalent (Fe^{3+} , Cr^{3+}) heavy metals.

Collecting samples from polluted zones is not acceptable. In addition, for vegetal products with internal uses (powders, extracts), the soil analysis is recommended.

The quality control of the raw materials (*Silene albae herba*, in our case), before their utilization in pharmaceutical preparations, is the only safe way to prevent high intake of heavy metals.

Soils with high levels of acidity (low pH) and/or heavy metals are not appropriate for good quality of vegetal medicinal products. In these cases, the entire aerials parts or roots may be contaminated with toxic concentrations of heavy metals and the products become unsuitable for pharmaceutical uses.

According to the special writing edited by Yash P. Kalra, "Handbook of reference methods for plant analysis" (1998), under the auspices of Soil and Plant Analysis Council (Washington): "nutrient concentrations that produce the maximum or optimum yields have been established as the critical or optimum levels" [10].

The mineral content is an entity variable in both time and depending on the different parts of the plant itself [10].

A certain part of a plant (or a plant organ) and, in some special cases, the entire plant collected in a particular physiologic period, are the most important sampling parameters. In fact, they are the so-called "startup parameters" [10].

Our results acknowledge that physiologically young vegetal tissues undergo rapid changes in the mineral content, compared to full mature and/or old plants. The harvesting of a vegetal product must be correlated to the accumulation of highest amounts of secondary metabolites, corresponding to the best mineral content, yield and phenophase (morphological characteristics) [10].

In order to solve some agronomic difficulties, for research purposes and for diagnosis of possible mineral nutrition problems, the plant analysis is the most useful tool. The interpretation of plant analysis results must always take into account dissimilar

interactive factors that influence vegetal growth, development and maturation, such as: stable or changeable climate, genetic information, cultural and geographic area, etc. [10].

The analysis of mineral content is a very useful method especially for the regulation of quality and production of grown medicinal plants. Also, through this method, the mineral nutrition of vegetal tissue and the biosynthesis of secondary metabolites can be improved using some peculiar agro-technical processes [10].

Conclusions

1. The content of some heavy metals (Ca^{2+} , Zn^{2+} , Mn^{2+} , $\text{Fe}^{2+/3+}$, Ni^{2+} , Pb^{2+} , Cr^{3+}) of the leaves and stems of *Silene alba* species has been established using atomic absorption spectrometry analysis.

2. The content of the heavy metals can be considered in normal limits, even for Pb^{2+} and Cr^{3+} markers for soil and air pollution.

3. High levels of Ca^{2+} , Zn^{2+} , Mn^{2+} , $\text{Fe}^{2+/3+}$ and Cr^{3+} , but in normal limits, have been determined in the stems then in the leaves.

4. The samples have been collected during the flowering period, when appearing complex combinations with divalent (Ca^{2+} , Zn^{2+} , Mn^{2+} , Fe^{2+} , Pb^{2+}) or trivalent (Fe^{3+} , Cr^{3+}) heavy metals.

5. The results indicate that there is not any real danger by the possible utilization of some pharmaceutical preparations of *Silene albae herba*.

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