

Original Paper

**Researches upon the Heavy Metals Content of
Rubus caesius L. (*Rosaceae*)**RUXANDRA IONCICĂ⁽¹⁾, MARIA-VIORICA BUBULICĂ⁽¹⁾, L. CHIRIGIU⁽¹⁾,
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ABSTRACT The analysis of heavy metals in the leaves and stems of *Rubus caesius* 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 utilization of pharmaceutical preparations of *Rubii caesii folium*.

KEY WORDS: *Rubus caesius*, heavy metals, content.

Introduction

Rubus caesius L., dewberry, European dewberry (*Rosaceae*), is a species native in Europe, including Britain, from Scandinavia south and east to Spain, Siberia and Western Asia. It is herbaceous, common in Romania's flora, along hedgerows, amongst shrubs and in roughdry meadowland, usually on basic soils [1–4].

In the specialty papers, there are very few data on the chemical composition and pharmacological action of the *R. caesius* species.

From the phytochemical point of view, leaves and fruits of *Rubus* species (*Rubi idaei folium*, *Rubi fruticosi folium*) contain a variety of compounds such as tannins, flavonosides (quercetol- and camferol glycosides), anthocyanins (in fruits), pentacyclic triterpene acids (ursolic acid, oleanolic acid), polyphenolic acids, pectins, organic acids (malic, citric, izocitric, succinic, litospermic, and oxalic acid), volatile oil (traces), sugars, vitamins (ascorbic acid), lipids, mineral salts [4–9].

Rubus species have some pharmacological actions, such as astringent, antidiarrhoeal, mild laxative, depurative, nutritional, antiseptic, tonic, haemostatic, diuretic, cicatrizing, anti-inflammatory, antioxidant [4, 6–8, 11–18].

For medicinal purposes, leaf infusion and decoction, syrup and marmalade from the blue-black fruits with sour and astringent taste are used starting from the *Rubus* species [4, 6–8, 12–18].

The heavy metals concentrations, depending on soil properties and other environmental factors, represent an indication of quality of medicinal herbs [19].

The aim of our paper was to analyze the content of heavy metal cations in the leaves and stems of *R. caesius* species harvested from the South-West of Romania.

Material and Methods**Sampling**

The sampling is a very important phase due to the specificity of soil, climate, plant developmental stages, and collecting time [19].

From the *R. caesius* species, twenty vegetal samples were collected at the flowering, in May 2009, from the surroundings of Bucovăț Forest, 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 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 [19].

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 [19].

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 $\pm 7\%$ [19].

Table 1 – Experimental data

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.

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.

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 *R. caesius* samples (leaves and stems)

Heavy metals	<i>Rubii caesii folium</i>	<i>Rubii caesii cauli</i>
	[mg%]	[mg%]
Ca^{2+}	0.9568 ± 0.01	0.2293 ± 0.01
Zn^{2+}	4.1302 ± 0.2	3.2157 ± 0.2
Mn^{2+}	0.0655 ± 0.002	0.1492 ± 0.02
$\text{Fe}^{2+/3+}$	0.8544 ± 0.01	0.1920 ± 0.01
Ni^{2+}	0.1148 ± 0.01	0.3214 ± 0.01
Pb^{2+}	1.5462 ± 0.2	4.0084 ± 0.2
Cr^{3+}	0.1304 ± 0.02	0.0815 ± 0.002
sample [g]	1.1279 ± 0.0002	0.6557 ± 0.0002
dried sample [g]	0.9710 ± 0.0002	0.6094 ± 0.0002
ash [g]	0.0848 ± 0.0002	0.0211 ± 0.0002
% dry ashing residue	8.733 ± 0.5	3.462 ± 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+} [19].

In the vegetal products obtained from *Rubus caesius* 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).

Among the most abundant plant species of mine tailings, dewberry (*R. caesius*) accumulate significant amounts of Pb^{2+} , Cu^{2+} and Zn^{2+} . Considering the translocation features, dewberry accumulates heavy metals primarily in the underground organs (roots) [20].

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 leaves and fruits of *R. caesius* species are usually used as medicinal products.

High levels of Pb^{2+} , Ni^{2+} and Mn^{2+} , but in normal limits, have been determined in stems, because their intake from soil or aerial parts.

The leaves contain high levels of Ca^{2+} , Zn^{2+} , $\text{Fe}^{2+/3+}$ and Cr^{3+} , 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 *R. caesius* 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+}) metallic cations.

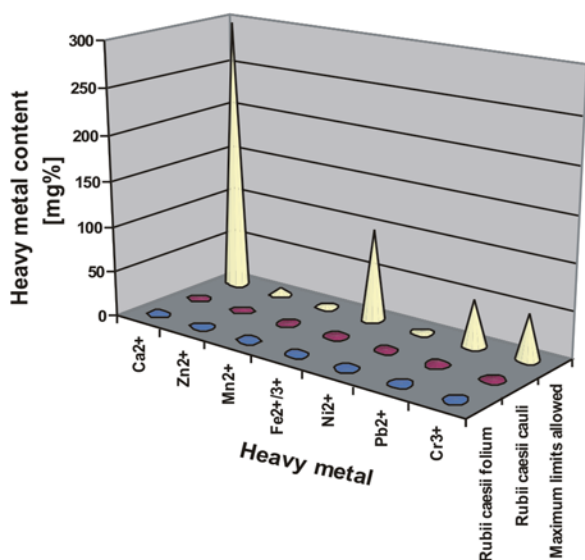


Figure 1 – Maximum limits allowed and heavy metals content of *R. caesius* samples (leaves and stems).

The soil analysis and the avoiding of collecting samples from polluted zones are recommended for vegetal medicinal products with internal uses as powders and extracts. Therefore, the only safe way to prevent high intake of heavy metals through pharmaceutical preparations of *Rubii caesii folium* is quality control of the raw materials before their further utilization. Special attention has to be paid if the medicinal product originates from mineral-carriers of heavy metals and low soil pH, which could induce high content of heavy metals in the raw material (aerial parts).

The content of heavy metals in vegetal products varies depending on numerous factors, among which indicate: soil, climate, water, air, growth stage, genetic specificity, mineral nutrition state, the part selected from plant itself, the sampling time, the rainfall contribution, etc. [19].

It is well known that the amount of metal cations depends on physiological state of vegetal tissues, young individuals showing rapid changes compared to mature or old plants. Therefore, it is assumed that the medicinal products harvested from the plants at the middle of their development period may have a normally and well-balanced content of heavy metal cations, in stable environmental conditions, without pollution [19].

Conclusions

The content of some heavy metals (Ca²⁺, Zn²⁺, Mn²⁺, Fe^{2+/3+}, Ni²⁺, Pb²⁺, Cr³⁺) of the leaves and stems of *R. caesius* species has been established using atomic absorption spectrometry analysis.

The content of the heavy metals can be considered in normal limits, even for Pb²⁺ and Cr³⁺ markers for soil and air pollution.

High levels of Mn²⁺, Ni²⁺ and Pb²⁺ have been determined in the stems then in the leaves.

The samples have been collected during the flowering period, when appearing complex combinations with divalent (Ca²⁺, Zn²⁺, Mn²⁺, Fe²⁺, Pb²⁺) or trivalent (Fe³⁺, Cr³⁺) metallic cations.

The results indicate that there is not any real danger by utilization of pharmaceutical preparations of *Rubii caesii folium*.

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