

Investigation of the Use of *Melampyrum Sp.* Extract Samples to Assess Metals Contamination

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Abstract

The aim of this study was to evaluate the metallic contamination of *Melampyrum sp.* extract samples. Chemical characterization of *Melampyrum sp.* extract was performed by measuring metal content in samples from three different sites. The sites were chosen based on information general, for were contaminated by heavy metals. *Melampyrum sp.* Extracts, prepared from spectrophotometric determination, with standard metal solutions obtained in accordance with ISO 11466 with atomic absorption spectrometry method. *Melampyrum sp.* extracts, samples presented high contents of copper (Cu), cadmium (Cd), manganese (Mn), Iron (Fe), Hg, Mo, Pb, Zn, Cr and nickel (Ni). Amounts of heavy metals above the admitted limit by the OMS have been discovered. The content of heavy metals in *Melampyrum sp.* extract was generally within safe limits, and compared well with levels in foods from Food Codex, though some metals contents were high.

Keywords: heavy metals, *Melampyrum sp.* extract, contamination, spectrophotometric determination

1. Introduction

The hemiparasitic genus *Melampyrum* (*Orobanchaceae*) is an important part of the European flora and is most diverse in the Balkan Peninsula followed by Caucasus and temperate Europe [1]. *Melampyrum* originated probably in the Mid Tertiary [2], evolved a number of species a few of which migrated outside Europe and the Caucasus and constituted taxa that do not occur in Europe [3]. Having survived the Quaternary climatic cycles, temperate species of *Melampyrum* maintained a high diversity, unlike most of the European Tertiary flora [4]. Their diversity might have even increased as a result of isolated evolution in glacial refuges. The genus *Melampyrum* is still actively speciating, which has resulted in the evolution of several complexes of closely related microspecies that are hardly distinguishable from each other.

The origin and distribution pattern of individual microspecies are supposed to have been predominantly affected by the migration of populations, their isolation and subsequent coming into contact in the late Pleistocene and Holocene [3, 5-7]. The genus *Melampyrum* includes 30 species spread across much of the temperate and low Arctic areas of the Northern Hemisphere. The centre of its diversity, where two-thirds of the species in the genus can be found, is in south – east Europe and the Caucasus [8]. Species within the genus *Melampyrum* are often highly variable, showing a great deal of ecotype variation, particularly in its southern European distribution [6,9]. *Melampyrum bihariense* Kern and *Melampyrum arvense* belongs to Romania's spontaneous flora being framed, from the systematic point of view, in the *Melampyrum* type, Scrophulariaceae family.

Until now the *Melampyrum* genus, respectively *Melampyrum bihariense* species has been little studied (was not studied) in Romania, but few data that were found show the existence of aucubine in other species of this genus. The *Melampyrum* name results from two Greek words: "melas" – black and "pyros" – wheat, the seeds of this plant having grain wheat aspect, except the fact that they are black colored. International literature is the one that specifies the composition of two species: *Melampyrum arvense* and *Melampyrum cristatum*, but this is far too little to be a term of comparison. The two species contain: aucubin, gardosid metil ester. The iridoides represent a monoterpene pentamethylene – pyrinic group, but their presence in the plant was signaled since 1846. The physiological role of this substance is little studied, but the bitter taste and the antibiotic properties of some of them lead to the hypothesis of a possible role in the protection of plants [16].

In *Melampyrum bihariense* Kern. and *Melampyrum cristatum* L. it was determinate the presence of some polyphenolcarboxylic acids of the iridoide, mucilage, saponins, carotenoids, phytosterols [17].

The problem of environmental pollution due to toxic metals has begun to cause concern now in most major metropolitan cities. The toxic heavy metals entering the ecosystem may lead to geoaccumulation, bioaccumulation and biomagnification. Heavy metals like Fe, Cu, Zn, Ni and other trace elements are important for proper functioning of biological systems and their deficiency or excess could lead to a number of disorders [10,11].

Heavy metal contamination has adverse effects on the agroecosystems, such as loss of high quality farmland and pollution of soil and groundwater, enhanced demand for clean water, contamination of urban areas and increased public health problems [12,13].

Recent studies by several investigators have shown that atmospheric factors can contribute significantly to contamination with heavy metals. Metal deposition patterns, though depending to a considerable extent on climatic conditions (i.e. wind and rainfall distribution), generally decline exponentially with distance from source of pollution [14,15].

The present study was made to estimate the concentration of metals in these special types of plants.

2. Materials and Method

The two *Melampyrum* species, *Melampyrum bihariense* and *Melampyrum arvense* were harvested in the 30th of May, 2009, from Mures by the Faragau Lake. The tested part was dried leafs. The sample were collected from the areas where were some heavy metals present in the soil. It was present on this type of non-fertilized soil.

Samples mineralization. In porcelain capsules, previously brought at constant weight by drying at 105°C, was weighing a 1,000g sample. The capsules with samples were introduced into drying stove at 50 – 60°C for almost 8 hours. Then the temperature was increased at 105°C for 5 – 6 hours. After this time, the sample capsules were took out from drying stove and introduced into calcinations oven, at cold. It was increased progressively the temperature at 200 – 250°C and it was maintained to complete calcinations of the samples.

Then the temperature was increased to 500°C and the samples were calcined for 6 – 8 hours to a white ash was obtained. The samples that were incompletely calcined were treated with 1ml concentrated HNO₃, drying on the sand bath and then calcined at 500°C for others 2 hours.

After cooling, the ash was treated with 0,5 ml bidistilled water and 1 ml hydrochloric acid 6N and was evaporated to dry, on the sand bath; the operation was repeated for two times. The residue was dissolved in small portions of 5 ml HCl 0,5N, passed quantitatively into a 50 ml glass flask and completed to exactly 50 ml with HCl 0,5N solution.

The glass flask content was finally filtered in a perfectly dry flask. For each sample set was achieved a control sample. Heavy metals concentration was determinate by atomic absorption spectrometry. The heavy metals were measured from the obtained hydrochloric solution by pulverization in the air – acetylene flame and measurement of the absorbance, respectively emission at the characteristic wavelength for each analyzed element. It was used an atomic absorption spectrophotometer, controlled by PC. For the spectrophotometer calibration were prepared sets of etalons of different concentrations in HCl 0,5N solution for each analyzed element, starting to the concentrated standard solutions.

The concentration (C) for each determined element was calculated with the following formula:

$$C \text{ (mg/kg or ppm)} = a \cdot f / m$$

where: f = dilution factor; a = element content indicated by apparatus (mg/l); m = sample initial weight.

Samples were prepared for quantitative determination by mineralization using the system for rapid mineralization of many organic and mineral samples for further analysis by methodological spectrophotometric, turbidimetric and titrimetric (SR EN-13804:2003, Determination of microelements).

Spectrophotocolorimetric determination is a physical – chemical method whose principle is based on comparing the intensity of staining intensity color sample for analysis of known concentrations and different solutions. Metals were determined by atomic absorption spectrophotometer AAS, Analytik Jena AG, (SR EN ISO 6869:2002, Determination of metals and ISO 78 – 4:1998, standard plans) [18-20].

Table 1. Major and trace elements content (mg/kg) of *Melampyrum sp.* extracts samples

Heavy metal	Content of heavy metals in <i>Melampyrum sp.</i> extracts	
	<i>Melampyrum bihariense</i> (mg/kg)	<i>Melampyrum arvense</i> (mg/kg)
Hg	0.065 ± 0.006	0.065 ± 0.006
Mo	3.0 ± 0.1	3.8 ± 1.7
Fe	0.559 ± 0.05	0.460 ± 0.03
Mn	10.06 ± 0.05	10.07 ± 0.07
Cu	1.090 ± 0.006	2.057 ± 0.004
Zn	22.3 ± 0.5	9.7 ± 0.5
Ni	3.410 ± 0.04	3.410 ± 0.04
Cr	2.81 ± 0.07	1.32 ± 0.09
Cd	0.03 ± 0.01	0.77 ± 0.13
Pb	0.11 ± 0.05	0.13 ± 0.12

Values are means of triplicate samples (± SD)

There is considerable variation of elements content from sample to sample.

The graphs/figures 1 to 10 show in a comparative manner within the concentration found by laboratory analysis in the case of the two species of *Melampyrum* which were the subject of investigation.

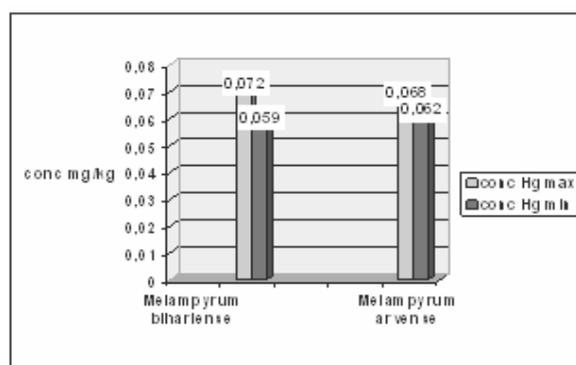


Figure 1. Comparative evaluation for Hg concentration

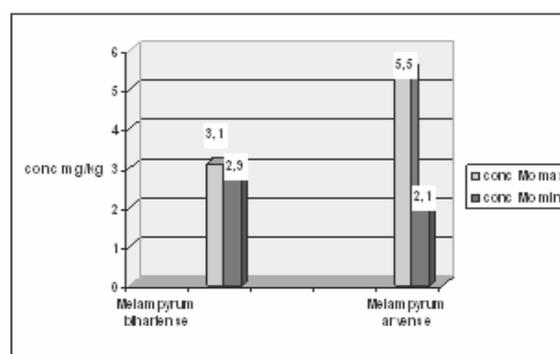


Figure 2. Comparative evaluation for Mo concentration

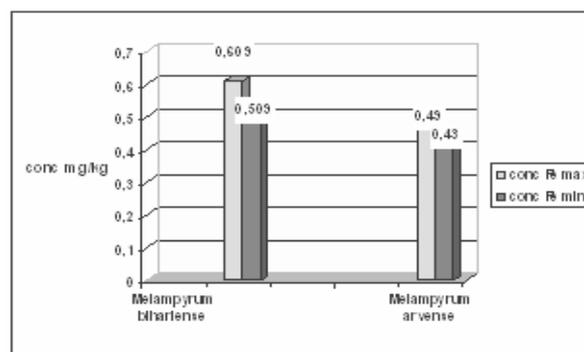


Figure 3. Comparative evaluation for Fe concentration

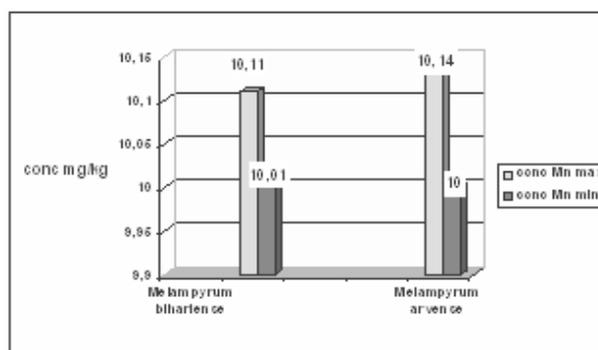


Figure 4. Comparative evaluation for Mn concentration

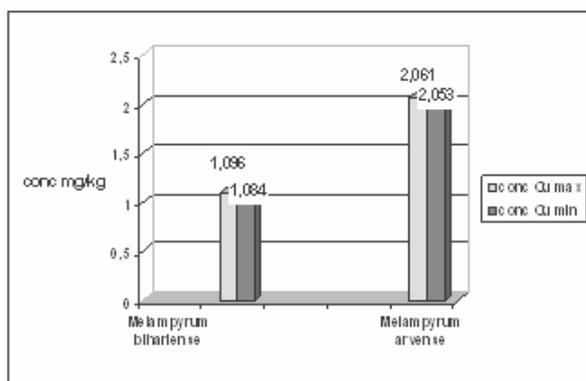


Figure 5. Comparative evaluation for Cu concentration

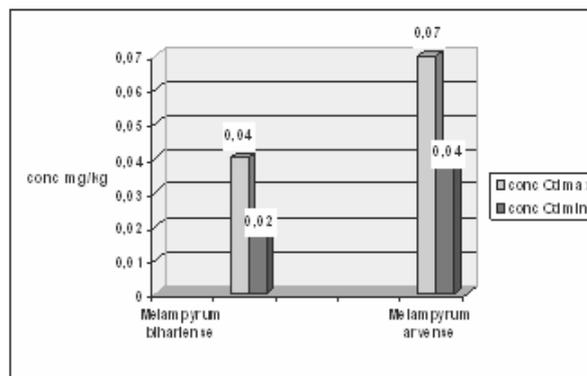


Figure 9. Comparative evaluation for Cd concentration

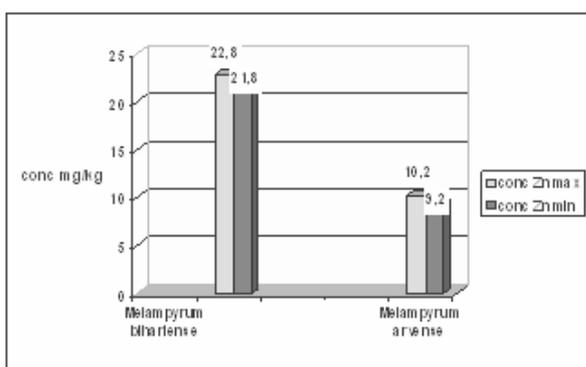


Figure 6. Comparative evaluation for Zn concentration

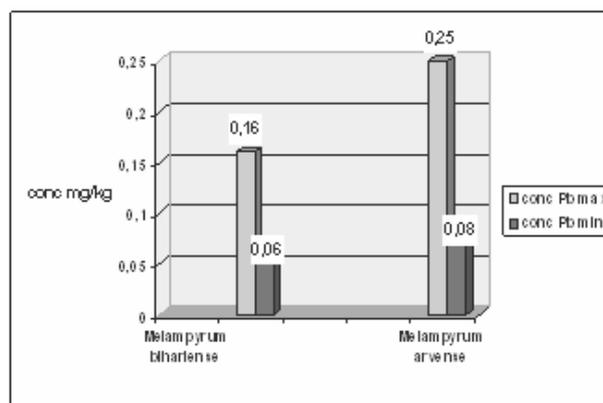


Figure 10. Comparative evaluation for Pb concentration

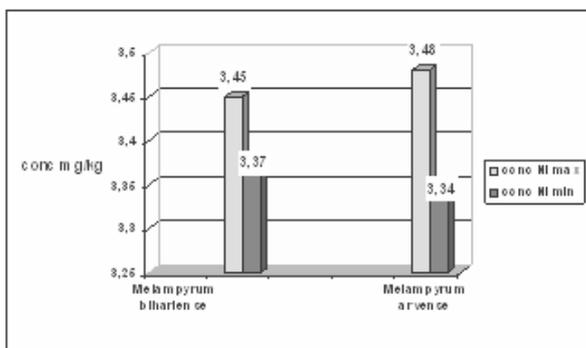


Figure 7. Comparative evaluation for Ni concentration

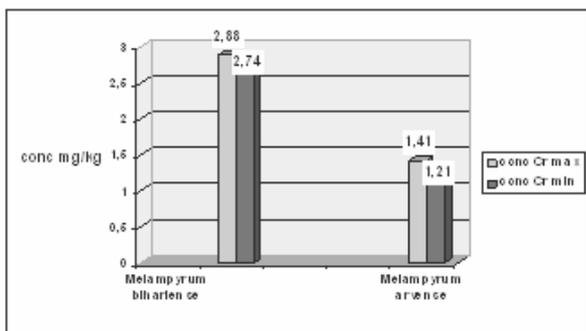


Figure 8. Comparative evaluation for Cr concentration

Melampyrum sp. extracts samples revealed normal concentrations for all samples and all items identified. Two toxic metals, non – essential, Cd and Pb, have high values reported as ppm [21-23].

The species of plants investigated represent atypical forms, which may be included in the characterization of bio-ecosystem of origin, and can respond to external changes bioclimatic. At the same time they can be sources of compounds phytoactive with potential utility in the medical field.

For these reasons metal concentration in the plant is extremely important as a witness to the local environmental changes. The studies on *Melampyrum* species use as source material for potential drugs not found in the literature so the possibility of making comparisons is reduced.

It is recommended that future research in this domain to expand area and align the metallic charge of all ecosystem components plants, water, and soil for an integrative vision.

4. Conclusion

The level of essential trace elements as well as heavy metals in plants depends on geoclimatic conditions of the region, anthropogenic activities, plant species and plant part used.

Our study revealed metal concentrations within acceptable limits after the FDA and WHO standards, noting that research should be extended to specific areas.

The concentration of metals in vegetation will provide baseline data and there is a need for intensive sampling for quantification of the results.

The monitoring of the quality of plant, soil and water, represent a very important action for prevention and sanogenetically environment.

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