J. Serb. Chem. Soc. 72 (2) 133–138 (2007) UDC 582.936:549.25+549.328.1
JSCS–3543 *Original scientific paper*

Content of heavy metals in *Gentiana lutea* L. roots and galenic forms

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(Received 24 March, revised 10 May 2006)

Abstract: An experimental field for the cultivated production of *Gentiana lutea* L. was established five years ago at the Suvobor Mountain, Serbia. Soil analysis of this area revealed the occurrence of high pseudo-total (Ni – 1270 mg/kg, Cr – 423 mg/kg, Co – 385 mg/kg) and available (especially Ni – 133 mg/kg) heavy metals contents in the soil. Hence, the aim of this research was to evaluate the quality of *Gentiana lutea* L. – roots and galenic forms (liquid extract in 70 % ethanol, spissum and siccum) produced from the roots, because, for most plants, heavy metals accumulate in the root tissue. The amounts of Ni and Cr found in the analyzed roots were very high (54 mg/kg and 14 mg/kg, respectively). The efficiency of ethanol in extracting heavy metals from the roots varied depending on the particular element. The highest efficiency was obtained for Ni (41.3 %), then for Cd (39.5 %), Pb (37.0 %) and Co (30.4 %). According to this, a potential hazard exists for humans, if gentian's galenic forms are produced from the raw material with high heavy metals contents. It is concluded that quality control of the raw material must be carried out before further utilization of gentian.

Keywords: gentian, root, ethanol extract, nickel, chromium.

INTRODUCTION

The control of the heavy metals contents in medicinal and aromatic plants represents one of the factors for the evaluation of their quality.[1] Since these plants originate from different growing areas, great differences in the uptatke and concentrations of heavy metals in the plant tissue can be expected. The high heavy metal content in some medicinal plants arises from their ability to accumulate particular metals, especially cadmium.[1,2] However, high heavy metals uptake can also be found in growing areas located in mountain regions,[3] due to certain properties of these soils, such as acidity and/or the presence of metal-bearing minerals, which favor the mobility of heavy metals in a soil and their high availability to plants.[4]

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doi: 10.2298/JSC0702133R

In Serbia, as well as in Montenegro, there are numerous data on the content of heavy metals in medicinal plants growing in quite different agro-ecological climates.[5–7] However, the data presented in those papers mostly refer to medicinal species, the herb, flowers or semen which are of pharmacological interest (*Hypericum perforatum* L., *Achillea millefolium* L., *Calendula officinalis* L., *Linum usitatissimum* L., *Melissa officinalis* L., *Mentha piperita* L.), while plant roots as a raw material have hitherto not been investigated. Hence, the aim of this research was to evaluate the quality of *Gentiana lutea* L. roots and galenic forms (liquid extract in 70 % ethanol, spissum and siccum) produced from the roots as the raw material, in regard to their content of heavy metals. This type of research is important, since immobilization of ions in roots is one of the possible mechanisms of plants tolerance to metals stress.[8]

EXPERIMENTAL

Sampling

Root samples of *Gentiana lutea* L. were collected from the cultivated field at the Suvobor Mountain (730 m above see level, latitude: 44° 08' 10" N, longitude: 20° 10' 44" E) in November 2002. The cultivated production of gentian was commenced in 1998 using its autochthonous population. Roots samples for chemical analysis and the production of galenic forms of gentian were collected from five locations in the field. Soil samples were also taken from the same locations.

Production of galenic forms

The collected root material was air dried, milled and used for the production of the following pharmacological products: Extractum Gentianae fluidum, Extractum Gentianae spissum and Extractum Gentianae siccum. The extracts were produced in the Institute for Medicinal Plants Research "Dr Josif Pančić" in Belgrade. Liquid extract – Extractum Gentianae fluidum was produced by the method of indivisible percolation,[9] as follows: 150 g of milled root material was overflowed with 370 ml of 70 % ethanol in a percolator and left for 24 h. The total amount of the produced liquid extract was 150 ml, with 19.32 % of dried matter (d.m.). Extractum Gentianae spissum was produced from 50 ml of the liquid extract by evaporation at 50 °C, until a d.m. of 63.41 % was attained (the standard for Extractum Gentianae spissum is 60 % d.m. minimally). Extractum Gentianae siccum was produced from 50 ml of the liquid extract by evaporation at 80 °C until 94.50 % of d.m. was attained (the standard for Extractum Gentianae spissum is 5.50 % of moisture maximally).

Soil analysis

The pseudo-total content of heavy metals (total sorbed Mn, Zn, Cu, Co, Cr, Pb, Ni and Cd) was determined by the AAS method, in an acetylene/air flame after the following decompositions, according to EPA Method 3050:[10] 2 g of air dried and milled samples (to pass through a 2 mm sieve) were digested in a covered Erlenmeyer flask with 20 ml of conc. HNO_3 at 150 °C for 2 h; 3 ml of 33 % H_2O_2 was added after cooling and the content of the Erlenmeyer was brought to boiling for a further 10 min, to ensure complete dissolution (oxidation) of soil organic matter. The procedure with H_2O_2 was repeated once again after cooling. The content was then transferred into a 100 ml volumetric flask with deionized water and filtered through a Whatman No. 40 filter paper into a flask.

The certified reference material, BCR No. 141, was analyzed for pseudo-total heavy metals content (the values obtained in mg/kg were: Mn – 500; Zn – 72; Cu – 32; Co – 9; Cr – 60; Pb – 27; Ni – 29 and Cd – 0.4).

The available content of heavy metals (total extraction of: water soluble, exchangeable and organically bound heavy metals, partial extraction of metals occluded in oxides and secondary clay minerals) was also determined by the AAS method, in an acetylene/air flame, after the following extraction: 20 g of air dried and milled soil (to pass through a 2 mm sieve) was extracted with 0.005 M DTPA for 2 h at room temperature[11] and filtered through a Whatman No. 40 filter paper into a flask.

Galenic forms analysis

Total content of heavy metals (Mn, Zn, Cu, Co, Cr, Pb, Ni and Cd) were determined by the AAS method in an acetylene/air flame, after the following decomposition: 5 ml of Extractum Gentianae fluidum was evaporated at 80 °C and then digested in a covered Erlenmeyer flask with 20 ml of conc. HNO_3 at 150 °C for 2 h; 3 ml of 33 % H_2O_2 was added after cooling and the content of the Erlenmeyer was boiled for a further 10 min. The procedure with H_2O_2 was repeated once again after cooling. Then, 2 ml of $HClO_4$ was added and the content of the Erlenmeyer was evaporated at 100 °C until white fumes of perchloric acid appeared. The content was then transferred into a 50 ml volumetric flask with deionized water and filtered through a Whatman No. 40 filter paper into a flask. The same procedure was performed for the decomposition of Extractum Gentianae siccum (1g) and Extractum Gentianae spissum (1g).

RESULTS AND DISCUSSION

The total concentration of heavy metals in a soil is largely determined by the parent material of the soil. Soils derived from serpentines, a type of ultramafic igneous rock, usually contain high concentrations of Ni, Cr and Co.[12] As the stationary experiment at the Suvobor Mountain, Serbia, for the cultivated production of *Gentiana lutea* L. was established on a soil developed over serpentine layers, a very high pseudo-total content of Ni and high pseudo-total contents of Cr and Co were found in the soil (Table I). The obtained concentrations of these elements exceed their permissible levels for unpolluted soils – 100 mg/kg for Ni, 75 mg/kg for Cr and 65 mg/kg for Co.[8]

TABLE I. Heavy metals content in soil (mg/kg)*

	Mn	Zn	Cu	Co	Cr	Pb	Ni	Cd
Pseudo-total content	1788±134.8 1620–1950	92.2±5.5 85–99	13.2±1.3 12–15	363±57.1 268–385	412±42.5 339–423	57.2±4.35 2–63	1110±161 850–1270	0.26±0.02 0.25–0.03
Available content	18.2±0.8 17–19	5.8±0.4 5.2–6.3	1.3±0.2 1.1–1.5	1.0±0.1 0.9–1.2	4.1±0.4 3.7–4.8	5.0±0.4 4.6–5.6	111.8±15 97–133	0.18±0.04 0.1–0.2

*The results are given as mean, standard deviation and range

As a consequence of the very high pseudo-total Ni in the soil, the acidity of the soil (pH in 1M KCl – 5.37) and the chemistry of Ni in soil,[13] the available content of Ni were also very high and even exceeded the permissible level for its pseudo-total content for unpolluted soils. The available amounts of Cr and Co were not as high as Ni, since these elements are, contrary to Ni, associated with more stable minerals in a soil.[13]

Both the pseudo-total and the available concentrations of the other analyzed heavy metals were within their normal contents for unpolluted soils.

Generally, for most plants, the concentrations of some heavy metals (Ni, Cr, Pb, Cu) are higher in the roots then in the above-ground parts.[14] As it could be expected, in the case where high pseudo-total and available heavy metals contents in a soil are associated with soil acidity, high concentrations of some heavy metals were found in the gentian roots (Table II). The concentrations obtained of both Ni and Cr in gentian root tissue were within their critical concentrations in plants: 10–100 mg/kg and 5–30 mg/kg, respectively.[8] The contents of the other analyzed heavy metals, except Co, can be considered as normal for plants. The content of Co in the gentian root tissue was higher than the normal content in a plant material (0.02–1 mg/kg), but much lower than its critical concentration in plants (15–50 mg/kg). The critical concentration of a heavy metal in plant tissue is the level above which toxicity effects are likely to occur.[13]

TABLE II. Heavy metals content in gentian root tissue (mg/kg)*

Mn	Zn	Cu	Co	Cr	Pb	Ni	Cd
108.8 ± 1.9	32.8 ± 1.3	20,8 ± 1.3	3.4 ± 0.5	14.1 ± 2.1	5.1 ± 0.5	54.0 ± 12.3	0.7 ± 0.1
106 – 111	31 – 34	19 – 22	3.0 – 4.0	11.5 – 16.5	4.5 – 5.5	27.0 – 58.0	0.5 – 0.75

*The results are given as mean, standard deviation and range.

TABLE III. Heavy metals content in gentian galenic forms*

	Liquid extract			Spissum	Siccum
	μg/g	μg/ml	Extraction efficiency/%	μg/g	μg/g
Mn	1.85 ± 0.05	1.76 ± 0.04	4.0	7.46 ± 0.05	9.0 ± 0.7
	1.68 – 2.0	1.6 – 1.9		7.4 – 7.5	8.0 – 10.0
Zn	1.98 ± 0.1	1.88 ± 0.1	14.2	6.82 ± 0.3	9.2 ± 0.8
	1.79 – 2.1	1.7 – 2.0		6.2 – 7.0	8.5 – 10.5
Cu	0.54 ± 0.01	0.51 ± 0.01	6.0	2.0 ± 0.3	3.6 ± 0.7
	0.5 – 0.58	0.48 – 0.55		1.5 – 2.5	2.5 – 4.3
Co	0.44 ± 0.01	0.42 ± 0.01	30.4	0.73 ± 0.08	1.5 ± 0.1
	0.39 – 0.47	0.37 – 0.45		0.6 – 0.8	1.4 – 1.6
Cr	0.26 ± 0.03	0.25 ± 0.02	4.4	1.2 ± 0.4	1.1 ± 0.1
	0.23 – 0.32	0.22 – 0.3		1.0 – 1.9	1.0 – 1.3
Pb	0.80 ± 0.2	0.76 ± 0.2	37.0	1.0 ± 0.1	1.5 ± 0.5
	0.53 – 1.05	0.5 – 1.0		0.8 – 1.2	1.0 – 2.0
Ni	9.5 ± 0.2	9.0 ± 0.3	41.3	40.2 ± 1.5	45.8 ± 1.5
	9.2 – 9.97	8.76 – 9.5		38 – 42	44 – 48
Cd	0.12 ± 0.01	0.11 ± 0.01	39.5	0.25 ± 0.0	0.5 ± 0.1
	0.11 – 0.14	0.1 – 0.13			0.4 – 0.6

*The results are given as mean, standard deviation and range.

Nickel has the highest concentration in the extract of gentian, compared to the other heavy metals (Table III). The extraction efficiency is also the greatest for Ni. On the basis of the obtained extraction efficiency, the analyzed elements can be divided into three groups: ones that are extracted to a very low degree (less than 10 %), Mn, Cr and Cu; elements that are extracted to a medium degree (10–20 %), Zn, and ones extracted to more than 30 % of their total content in the plant tissue, Ni, Cd, Pb and Co. The low and medium degree of extraction indicates strong bonds between the particular metal and organic compounds, the solubility in ethanol of which are low, such as proteins.[15] A high extraction efficiency indicates the presence of the particular metal in inorganic compounds, which are easily soluble in ethanol and/or in organic compounds the solubility in ethanol of which are high, such as lipids.[16]

Although the Ni content of gentian roots is high and a significant amount can easily be extracted with ethanol, the daily Ni intake would not be highly elevated if the produced gentian extracts were correctly employed (20 drops or 1 ml). The amount of Ni input by the investigated extract would, in these cases, be less than 10 μg, which is a small portion of the allowed daily intake. The average dietary nickel intake is approximately 150–200 μg/day. The amount of Ni intake by correct employment of spissum or siccum (20–25 mg/day) produced from raw material rich in nickel would be even smaller.

The amounts of Cr intake *via* gentian extracts, spissum or siccum, are negligible, although its content in the roots is high. This is because of the insolubility of Cr-bearer compounds in ethanol (low extraction efficiency). The intake of Co is also far from levels causing toxic effects, although ethanol has a high efficiency for the extraction of Co from root tissue. This is because the Co concentration in the analyzed roots was found to be close to normal levels for the element in a plant tissue.

The intake of the other investigated heavy metals *via* the analyzed gentian galenic forms is also irrelevant, above all, because of their normal concentrations in the raw material. This is of special importance for Cd, which is one of the most toxic heavy metals.[13]

CONCLUSION

The obtained results indicate that there is not any real danger of elevating the intake of Ni and Cr by consumption of galenic forms of gentian even when produced from raw material with high concentrations of these heavy metals. However, for some elements, in this case Ni, the correct use of the galenic forms precedes such a conclusion. Therefore, the only safe way to prevent high intake of heavy metals through galenic forms of gentian is quality control of the raw materials before their further utilization. Special attention has to be paid if the gentian originates from mountains where mineral-carriers of heavy metals and low soil pH could induce high contents of heavy metals in the raw material (gentian roots).

И З В О Д

САДРЖАЈ ТЕШКИХ МЕТАЛА У КОРЕНУ И ГАЛЕНСКИМ ПРОИЗВОДИМА ГЕНЦИЈАНЕ (*Gentiana lutea* L.)

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Експериментална станица за производњу генцијане (*Gentiana lutea* L.) заснована је на планини Сувобор (Србија) пре пет година. Анализом земљишта са те локације утврђени су повишени псеудо-укупни (Ni – 1270 mg/kg, Cr – 423 mg/kg, Co – 385 mg/kg) и приступачни (посебно за Ni – 133 mg/kg) садржаји тешких метала. Стога је циљ овог рада био евалуација квалитета корена генцијане и галенских производа (течни екстракт у 70 % етанолу, spissum и siccum) добијених из корена, јер се код већине биљака тешки метали акумулирају у ткиву корена. У анализираном корену утврђени су врло високи садржаји Ni (54 mg/kg) и Cr (14 mg/kg). Ефикасност етанола у екстракцији тешких метала из корена варирала је у зависности од елемента. Највећа ефикасност уврђена је за Ni (41,3 %), затим за Cd (39,5 %), Pb (37,0 %) и Co (30,4 %). Према томе, постоји потенцијална опасност за човека при употреби галенских производа добијених из сировине са високим садржајем тешких метала. Закључено је да контрола квалитета сировинског материјала мора да се спроводи пре даље употребе генцијане.

(Примљено 24. марта, ревидирано 10. маја 2006)

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