Aida Yakovlevna Tamakhina et al /J. Pharm. Sci. & Res. Vol. 10(5), 2018, 1263-1266

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Bioaccumulation of Heavy Metals by Medicinal Plants of the *Inula* Genus

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Abstract

The heavy metal pollution is one of the causes for the risk of medical use of medicinal plant raw materials and preparations on their basis. The expansion of preparation areas and uncontrolled gathering of wild medicinal plants contribute to this. The **scope of the study** was to identify the specificity of bioaccumulation and translocation of phytotoxic (Pb, Cd) and essential metals (Cu, Zn, Mo) with the *Inula* genus medicinal plants. **Methods.** To achieve this goal, the content of heavy metals has been identified in soil, underground and aboveground *Inula helenium* L., *I. britannica* L., *I. germanica* L. phytomass under control conditions (slightly polluted soils) and pollution (roadside areas, shore of the sediment pond of Tyrnyauz tungsten-molybdenum combine). The ratios of bioaccumulation and translocation of heavy metals were calculated. The data were statistically processed using Microsoft Excel 2010. **Results.** The existence of a moderate and strong relationship between the Mo, Cd and Pb content in the *Inula* species' phytomass and in the soil has been established. A generic feature of the *Inula* species is the bioaccumulation of Mo, Cu, Cd, regardless of the degree of soil pollution with heavy metals; accumulation of Cd, Cu, Zn in conditions of technogenic pollution. The generic feature of heavy metals' accumulation is associated with the characteristics of plant metabolism and the type of adaptive strategy. The results of the study show the need to control not only the content of toxic metals in medicinal plant raw materials but also the essential trace elements (Mo), which in high concentrations can become dangerous for the human health.

Key words: *Inula, heavy metals, coefficient of bioaccumulation, translocational coefficient, adaptive strategy.*

INTRODUCTION

The heavy metal pollution is one of the causes for the risk of medical use of medicinal plant raw materials and preparations on their basis. The expansion of the preparation areas and uncontrolled gathering of wild medicinal plants significantly contribute to this [1]. The relevance of assessing the heavy metals' content in medicinal plants is due to the possibility of cumulation of certain elements by plants, the selective accumulation of certain metals, potential genotoxicity and carcinogenicity of various metal salts, and the high sensitivity of the individual age groups to the toxic effects of metals [2]. The total content of the element in the soil, the relative amount of the bioavailable form in the soil, the type of the plant, the phase of development and the features of the distribution of elements among the organs, and the adaptation of plants to these geochemical conditions are the main factors determining the content of the elements in plants [3]. From the point of view of pollution of soils and plants with heavy metals, the greatest danger comes from the territories adjacent to large cities, industrial sites and highways [4, 5]. Despite the potential toxicity of many heavy metals accumulated by plants in geochemical anomaly areas, only the maximum content of lead, cadmium and mercury in the medicinal vegetative raw material has been established on the territory of the Russian Federation [6]. In this context, the study of bioaccumulation of metals with different toxicity degrees by medicinal plants and the possibility of prediction of pollution of vegetable raw materials based on the generic feature of accumulation of trace elements are of practical interest.

The **scope of the study** was to identify the specificity of bioaccumulation and translocation of phytotoxic (Pb, Cd) and essential metals (Cu, Zn, Mo) with the *Inula* genus medicinal plants.

MATERIALS AND METHODS

The object of the study were the species of wild perennial grasses of the Inula (*Inula helenium* L., *I. britannica* L., *I. germanica* L.) genus growing in the territory of the Kabardino-Balkarian Republic.

Underground and aboveground phytomass was selected during the period of mass flowering of plants (July 2015-2017) in five replications on the background areas and areas polluted with heavy metals. The plants growing on soils with a low degree of pollution served as the control ones: C1 - forest edge on the outskirts of Nalchik (*I. helenium* L.), C2 - city park of Nalchik (*I. britannica* L); and C3 - steppe phytocenosis in the vicinity of the Altud rural settlement (*I. germanica* L.). The polluted areas included the roadside section of the M-29 (P1) highway, where the *I. helenium* L. plants were selected, and the shore of the sediment pond of Tyrnyauz tungsten-molybdenum combine (P2), where the samples of *I. britannica* L. and *I germanica* L plants were selected. Soil samples in the studied areas were selected at the depth of 10-30 cm in three replications. The regional average level was used as the background content of heavy metals in soils [7]. The gross content of heavy metals (Cu, Zn, Pb, Cd, Mo) in soil samples and samples of underground and overground phytomass were determined by atomic absorption spectrometry with electrothermal atomization on the MGA-915 device. The samples had been previously salinized using the Minotaur-2 microwave mineralizer [6, 8]. To assess the entry of elements into plants, the bioaccumulation ratio (BAR) separately for aboveground and underground phytomass and the translocation ratio (TR) were calculated [9]. The values obtained were compared with the standards for the maximum permissible content of heavy metals [6]. The data were statistically processed using Microsoft Excel 2010.

RESULTS AND DISCUSSION

The gross content of heavy metals in the control samples of the soil was below the background one or was about it (by Zn, Cd and Mo). On the P1 pollution area the excess background on copper, zinc, lead and cadmium 1.4; 1.5; 2.2; 2.3 times, respectively, was observed. In the P2 pollution area, the concentration of Cu, Zn, Pb and Mo exceeded the background 4.5; 10.2; 1.5 and 92.7 times, respectively (Table 1).

In the control plants, heavy metals accumulated mainly in the aboveground phytomass. At the same time, their content, both in the underground and in the aboveground phytomass, exceeded the gross content of metals in the soil (Table 2).

Table 1 - The gross content of heavy metals in the soil of the studied areas, mg/kg

Area	*Cu*	*Zn*	*Pb*	*Cd*	*Mo*
C1	3.56±0.84	22.11±2.75	4.85±1.53	0.44±0.12	0.96±0.05
C2	4.35±1.22	5.72±1.62	4.21±1.24	0.20±0.06	1.10±0.02
C3	4.92±1.31	21.73±2.53	3.07±1.45	0.21±0.04	1.00±0.03
P1	12.40±2.15	33.42±3.48	29.33±4.52	1.16±0.11	1.14±0.02
P2	40.28±3.57	230.16±5.37	19.06±3.26	0.23±0.05	102.00±4.71
Background	9.00	22.60	13.00	0.50	1.10

Table 2 - The content of heavy metals (mg/kg) in the aboveground (Cab) and underground (Cun) phytomass of the elecampane species in control (C) and polluted (P) areas

Species	Area		Cu	Zn	Pb	Cd	Mo
I. helenium	C	Cab	15.40±3.32	20.62±5.87	5.46±1.17	1.53±0.44	6.53±2.04
		Cun	4.21±1.63	16.83±4.24	2.14±0.64	0.45±0.08	4.12±1.23
	P	Cab	29.9±2.54	23.48±5.61	12.51±2.37	2.06±0.19	8.12±3.17
		Cun	8.86±3.91	18.75±3.18	5.03±1.14	0.28±0.04	6.37±2.51
I. britannica	C	Cab	6.03±2.44	22.63±4.39	5.54±1.72	0.97±0.09	7.22±3.11
		Cun	3.21±1.15	4.02±1.21	5.10±1.68	0.88±0.07	3.55±1.05
	P	Cab	4.60±1.28	18.07±2.14	0.58±0.07	0.47±0.06	13.51±4.87
		Cun	5.68±2.17	20.63±3.18	0.71±0.11	0.35±0.09	110.34±21.26
I. germanica	C	Cab	7.12±2.11	25.37±4.25	3.46±1.18	0.28±0.05	5.42±1.23
		Cun	4.81±1.12	6.15±1.13	2.82±1.02	0.22±0.06	4.18±1.08
	P	Cab	16.20±3.58	14.32±2.37	0.93±0.12	0.76±0.10	28.65±4.12
		Cun	5.68±2.71	18.45±3.15	1.43±0.53	0.31±0.09	132.56±28.52
Maximum allowable content, mg/kg			30.00	50.00	6.00	1.00	-

The relation degree between the concentration of metals in the phytomass and the gross content in the soil was strong for Mo ($r = 0.72$), weak for Cd and Pb ($r = 0.35$-0.43), and very weak for Cu and Zn ($r<0.2$). The accumulation of Cd and Pb by the aboveground phytomass of plants was due to the soil and foliar intake of metals. The intake of Cu and Zn into the phytomass depends to a large extent on the biological significance of the ions for plants.

It was stated that the concentration of essential trace elements (Cu, Zn) and toxic metals (Cd, Pb) in plants of the *Inula* genus growing in control and contaminated areas did not exceed the maximum allowable content for medicinal plants and herbal dietary supplements. The exceptions were the *I. helenium* L plants, in the aboveground phytomass of which the content of Pb and Cd exceeded the standards 2 times. In this case, the underground phytomass of *I. helenium* L. was contaminated to a much lesser extent and was quite suitable for medicinal teas. The feature of the *Inula* genus plants is represented by the bioaccumulation of molybdenum by the underground and aboveground phytomass, which sharply increases under conditions of an increased content of this element in the substrate. In the underground phytomass of *I. britannica* L. and *I. germanica* L., growing on the lower terraces of the tailing pit and on the shore of the sediment pond, the molybdenum content in the underground phytomass increased 31.1-31.7 times in comparison with the control. Based on the existing standard for the maximum permissible level of Mo in coarse and succulent fodder (1 mg/kg) [10], it can be argued that the actual concentration of the element exceeding the standard more than 100 times is toxic to animals and humans.

In the conditions of pollution in the aboveground and underground phytomass of all species, the content of heavy metals increases. In the *I. helenium* L. aboveground phytomass the concentration of microelements remained higher than that of the underground one in contrast to *I. britannica* L. and *I. germanica* L. The contents of Zn and Pb in the aboveground phytomass, as well as that of the Cu, Zn, Pb, Cd in the *I. helenium* L. underground phytomass, were below the gross content in the soil.

In the conditions of pollution, higher concentrations of Cu, Zn, Pb and Mo were noted in the *I. britannica* L. underground phytomass. The concentration of Cu, Zn, and Pb in the phytomass is lower than the gross content in the soil. The concentration of Zn, Pb and Mo is increased in *I. germanica* L. underground phytomass. The content of all metals, except for Mo, in the *I. germanica* L. phytomass is below the gross content in the soil.

The results of the analysis of the ratios of bioaccumulation and translocation of metals made it possible to establish the generic specificity of the accumulation of phytotoxic and essential elements (Table 3).

Table 3 - The ratios of bioaccumulation and translocation of heavy metals.

Species	BAR				TR*	
	aboveground phytomass		underground phytomass			
	C	P	C	P	C	P
	Cu					
I. helenium L.	4.40	2.41	1.20	0.71	3.66	3.37
I. britannica L.	1.40	0.11	0.75	0.14	1.88	0.81
I. germanica L.	1.45	0.40	0.98	0.14	1.48	2.85
	Zn					
I. helenium L.	0.93	0.70	0.76	0.56	1.22	1.25
I. britannica L.	3.97	0.18	0.70	0.21	5.63	0.88
I. germanica L.	1.17	0.14	0.28	0.18	4.12	0.78
	Pb					
I. helenium L.	1.14	0.43	0.45	0.17	2.55	2.49
I. britannica L.	1.32	0.03	1.21	0.04	1.05	0.82
I. germanica L.	1.13	0.05	0.92	0.07	3.68	0.65
	Cd					
I. helenium L.	3.48	1.78	1.02	0.24	3.40	7.36
I. britannica L.	4.85	2.04	4.40	1.52	1.44	1.34
I. germanica L.	1.33	3.30	1.05	1.35	1.27	2.45
	Mo					
I. helenium L.	6.80	7.12	4.29	5.59	1.58	1.27
I. britannica L.	6.56	0.13	3.23	1.08	2.03	0.12
I. germanica L.	5.42	0.28	4.18	1.29	1.30	0.22

Note: *TR>1 – bioaccumulation of the element; TR<1 – the physiological barrier that prevents the entry of an element into the plant tissue.

Table 4 – Ranges of bioaccumulation and translocation of heavy metals by phytomass of *Inula species*

Elecampane species	BAR	
	control	pollution
I. helenium L.	*Mo>Cu>Cd>Zn>Pb*	*Mo>Cu>Cd>Zn>Pb*
I. britannica L.	*Mo>Cd>Zn>Pb>Cu*	*Cd>Mo>Zn>Cu>Pb*
I. germanica L.	*Mo>Cu>Cd>Pb>Zn*	*Cd>Mo>Cu>Zn>Pb*
	TR	
I. helenium L.	*Cu>Cd>Pb>Mo>Zn*	*Cd>Cu>Pb>Mo>Zn*
I. britannica L.	*Zn>Mo>Cu>Cd>Pb*	*Cd>Zn>Pb>Cu>Mo*
I. germanica L.	*Zn>Pb>Cu>Mo>Cd*	*Cu>Cd>Zn>Pb>Mo*

Under the conditions of weak or moderate soil pollution, the *Inula* species bioaccumulate the essential elements (Cu, Zn, Mo) and phytotoxic metals (Cd, Pb) in the aerial organs, which is due to the peculiarities of plant metabolism. Copper is a part of the active center of polyphenol oxidase - key enzymes in the biogenesis of phenolic compounds, a number of pigments, some vitamins, auxins, proteins, and saponins. The dehydratase butyryl-CoA (fatty acid oxidation enzyme) and ascorbate oxidase also contain copper ions in the active sites. The copper-containing plastocyanins are involved in photosynthesis [11, 12].

Zinc is a part of the active sites of many enzymes (in particular, enzymes for the synthesis of polyphenols). It increases the resistance of plants to drought and hyperthermia [13]. Molybdenum is an activator of flavoprotein enzymes. It affects the synthesis of carbohydrates, amino acids, chlorophyll, ascorbate, coumarins and cardenolides, and takes part in photosynthesis [11, 14]. Cu, Zn and Mo are activators of enzymes involved in the biogenesis of polyphenols [12]. The ability of *Inula* genus plants to accumulate lead and cadmium is due to the lack of physiological barriers that prevent excessive accumulation of metals in the aerial part.

Under the conditions of heavy contamination of soils with heavy metals, bioaccumulation of essential and toxic metals in *I. helenium* L. plants persists and increases for Cd (TR = 7.6). The decrease in TR values for Cu, Zn, Pb and Mo in *I. britannica* L. and for Zn, Pb and Mo in *I. germanica* L. plants testifies to the barrier role of underground organs that concentrate most of the metals in the conditions of technogenic pollution.

Inula species are characterized by the bioaccumulation of molybdenum, copper and cadmium, regardless of the degree of soil contamination with heavy metals, as well as the accumulation of cadmium, copper and zinc in conditions of technogenic pollution (Table 4).

The generic specificity of accumulation of heavy metals is associated with the peculiarities of plant metabolism and the type of adaptive strategy [15]. It is very important in the light of intercellular communication under stress conditions [16, 17]. *I. helenium* L. shows patience only in the conditions of limited resources; therefore, an increased accumulation of heavy metals is noted in unfavorable places of growth (near highways). In *I. britannica* L. and *I. germanica* L. the species' patience as the predominant element of adaptive strategy causes a high level of adaptation of plants to contamination of the substrate, the ability of the aboveground phytomass to accumulate significant volumes of heavy metals, and the presence of physiological barriers that prevent excessive entry of elements into the organs of the plant.

CONCLUSION

In the conditions of technogenic pollution with heavy metals, the ecological factor is the leading one for the formation of the elemental composition of plants of the *Inula* L. genus. For Mo, Cd and Pb, a reliable relationship has been established between the level of their accumulation in phytomass and the gross content in the soil. The intake of Cu and Zn into the phytomass to a large extent depends on the biological significance of the ions for plants. *Inula* species are characterized by the bioaccumulation of Mo, Cu, Cd, regardless of the degree of soil pollution with heavy metals; accumulation of Cd, Cu, Zn in conditions of technogenic pollution. The nature of the accumulation of heavy metals in the aerial part of plants indicates an unequal level of the physiological barrier for essential and toxic metals. Highly toxic elements are characterized by a more pronounced physiological barrier that prevents their entry to the assimilative and generative organs of plants. The generic feature of heavy metals accumulation is associated with the characteristics of plant metabolism and the type of adaptive strategy. Therefore, the forecast of heavy metals' content in the elecampane phytomass is based on the study of the places of their growth and the determination of the level of patency. The ecologically safe *I. helenium* L., *I. britannica* L. and *I. germanica* L. medicinal raw materials should be prepared in places of their natural growth far from industrial objects and highways. The ability to bioaccumulate molybdenum by the *Inula* species in ecologically safe areas can be used in the development of dietary supplements for the prevention of molybdenum deficiency. The results of the study show the need to control not only the content of heavy metals in raw materials, but also the essential trace elements (Mo), which in high concentrations can become dangerous for the human health. This is especially important for species of technogenic habitats (*I. britannica* L., *I. germanica* L.), which can find practical use for sanitation and phytoremediation of contaminated areas.

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