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Comparative Analysis of the Trace Element Content of the Leaves and Roots of Three *Plantago* Species

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Abstract The primary objective of this study is to perform a comparative analysis of the trace element content of the leaves and roots of three *Plantago* species (*P. maxima* Juss. ex Jacq., *P. major* L., and *P. lanceolata* L.). Trace element levels were assessed by inductively coupled plasma mass spectrometry. The data indicate that the leaves of *P. lanceolata* are characterized by the highest Co, Cr, and Se content, whereas *P. maxima* leaves contained the greatest levels of Si and Zn. In contrast, the highest concentrations of Co, Cr, Fe, I, Mn, Si, and V were detected in the roots of *P. major*. Zn content was also higher in *P. maxima* roots than in the other species analyzed. The toxic trace elements were differentially distributed across the studied species. In particular, *P. lanceolata* leaves contained significantly higher Al, As, Li, Ni, Pb, and Sr levels, whereas the B and Cd content was elevated in *P. major* as compared to the other species. Surprisingly, the leaf Hg level was the lowest in *P. major*, whose levels of Al, As, B, Cd, Ni, Li, and Sr were significantly higher than the other two species. The data indicate that the concentration of most of the essential trace elements was higher in the leaves and roots of *P. major* and *P. lanceolata* than in *P. maxima*, while *P. maxima* had less toxic metals. The obtained data on trace elements content in *Plantago* tissues may be taken into account while using plant preparations in practical medicine.

Keywords *Plantago* · Trace elements · Leaves · Roots · Inductively coupled plasma mass spectrometry

Introduction

Trace elements play an important role in the functioning of the human body. The essential trace elements mainly act as cofactors for various enzymatic systems (generally redox-active metals) or possess regulatory activity [1]. On the other hand, the toxic trace elements—generally heavy metals—impair physiological function via the induction of universal pathogenetic pathways such as oxidative stress, inflammation, and endoplasmic reticulum stress [2]. In particular, the significant role of heavy metals in the development of disease has been demonstrated for endocrine disorders [3], cardiovascular diseases [4], and neurodegenerative states [5].

Phytomedicine is an ancient area of practical medicine that has attracted more attention recently [6]. Plantaginaceae, in general, and Plantago species are characterized by a high content of biologically active compounds such as flavonoids [7], iridoids [8], tannins [9], and other phytochemicals. As a result of rich phytochemical content, the plants are characterized by antioxidant and anti-inflammatory activity [10]. In particular, significant anti-inflammatory effect was demonstrated for preparations from *Plantago major*, *Plantago lanceolata* [11], *Plantago maxima* [12], *Plantago reniformis* [13], *Plantago altissima* [14], *Plantago australis* [15], *Plantago erosa* [16], *Plantago asiatica* [17], *Plantago coronopus* [18], and

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Plantago ovata [19]. Due to a wide geography of *Plantago* species, they have been widely used in traditional medicine in European and Asian countries [20] including Russia [21]. At the same time, *P. maxima*, *P. major*, and *P. lanceolata* are the most widespread in the South Urals with sharply continental climate.

Previous studies have indicated that certain *Plantago* species contain significant levels of trace elements [22]. Essential trace elements may therefore at least partially determine the health benefits of *Plantago* preparations. In particular, it has been shown that zinc [23] and selenium [24] compounds possess significant antioxidant and anti-inflammatory activity. On the other hand, the possibility of heavy metal accumulation from polluted environments [25] should be taken into account due to their proinflammatory and prooxidant activity [2] that may limit health effects of *Plantago* preparations. However, detailed data on the trace element content of *Plantago* species including those growing in the South Urals seem to be lacking. At the same time, such data may be taken into account while using plant preparations in practical medicine.

The primary objective of the current study is thus to perform a comparative analysis of the trace elements content of the leaves and roots of three *Plantago* species (*P. maxima* Juss. ex Jacq., *P. major* L., and *P. lanceolata* L.) growing in the steppe zone of the Southern Urals.

Materials and Methods

Plant Collection and Preanalytic Procedures

The plants were collected at flowering (May 2015) from water meadows near the Ural river in the steppe zone of the Southern Urals, with a sharply continental climate (Orenburg region, selo Kamennoozernoye, 51° 46′ 29.33″ N, 55° 33′ 52.37″ E). The overground leaves and roots of three *Plantago* species—*P. maxima* Juss. ex Jacq., *P. lanceolata* L., and *Plantago major* L.—were collected in five replicates. After collection, the plant organs were dried at room temperature under dark conditions. When drying was complete, the plant organs were ground into a fine powder. Then, 0.05 g of each sample was introduced into Teflon tubes containing concentrated nitric acid for microwave digestion. The degradation was performed for 20 min at 180 °C in a Berghof Speedwave 4 (Berghof Products and Instruments, Germany) system. After cooling of the system, the solutions were brought up to a final volume of 15 ml with distilled deionized water.

The essential (Co, Cr, Cu, Fe, I, Mn, Se, Si, V, Zn) and toxic trace element (Al, As, B, Cd, Hg, Li, Ni, Pb, Sn, Sr) contents of the obtained leaves and roots were assessed using inductively coupled plasma mass spectrometry with a NexION 300D (PerkinElmer Inc., Shelton, CT 06484, USA). The use of dynamic reaction cell technology allowed the removal of the majority of interference without any loss of analyte sensitivity. The system was also equipped with the 7-port FAST valve and ESI SC-2 DX4 autosampler (Elemental Scientific Inc., Omaha, NE 68122, USA). Analysis of every sample was performed in triplicate with the estimation of the mean value that was used in statistical analysis.

The system was calibrated using standard solutions of trace elements (0.5, 5, 10, and 50 μg/l) prepared from a Universal Data Acquisition Standards Kit (PerkinElmer Inc., Shelton, CT 06484, USA) by dilution with distilled deionized water and acidification with 1 % HNO_3. Internal online standardization was also performed using yttrium isotope (^{89}Y). In particular, a standard solution containing 10 μg/l yttrium was prepared from Yttrium (Y) Pure Single-Element Standard (PerkinElmer Inc., Shelton, CT 06484, USA).

Based on trace elements concentration in leaves and plants of the studied *Plantago* species, the translocation factors (TF) were calculated for every element.

Statistical analysis of the data was performed using Statistica 10 software (StatSoft Inc., Tulsa, Oklahoma, USA). The mean and standard deviation (SD) values were calculated. The Mann–Whitney *U* test was used for group comparisons at the $p < 0.05$ significance level.

Results

The data indicate that the leaves of the *Plantago* species contain significant quantities of essential trace elements (Table 1). At the same time, some of the species were characterized by different trace element contents. In particular, the levels of Co, Cr, and V in the leaves of *P. major* and *P. lanceolata* significantly exceeded their values in *P. maxima* by a factor of more than two and three, respectively. The iron and manganese concentrations of the *P. major* and *P. lanceolata* leaves were 67 and 56 % and 60 and 62 % higher than in *P. maxima*. The selenium content of *P. maxima* significantly exceeded the values obtained for *P. major* by a factor greater than three. At the same time, the Se levels of *P. lanceolata* leaves were 73 % higher than in the case of *P. maxima*. No significant difference in the leaves' iodine levels were observed between the studied species. *P. maxima* was characterized by the greatest silicon and zinc content of the leaves. In particular, the silicon level in *P. maxima* exceeded that of *P. major* and *P. lanceolata* by factors greater than 2 and 3, respectively, whereas the zinc levels were 2 and 81 % higher, respectively.

The results of the study demonstrate the heterogeneity of the distribution of toxic trace elements in leaves between the three studied *Plantago* species. The aluminum and strontium contents of *P. major* and *P. lanceolata* were nearly two and three times higher than in *P. maxima*. The level of arsenic in *P. maxima* leaves was also half that of the two other *Plantago* species. *P. lanceolata* was characterized by the highest levels

Table 1 Essential and toxic trace elements content (μg/g) in plant leaves

Element	*P. maxima*	*P. major*	*P. lanceolata*
Co	0.070 ± 0.003	0.182 ± 0.016[a]	0.276 ± 0.040[a, b]
Cr	0.098 ± 0.002	0.251 ± 0.062[a]	0.333 ± 0.029[a, b]
Cu	7.182 ± 0.920	7.356 ± 0.770	4.880 ± 0.453[a, b]
Fe	58.684 ± 3.566	98.224 ± 16.931[a]	91.814 ± 7.007[a]
I	0.209 ± 0.124	0.332 ± 0.086	0.195 ± 0.121
Mn	18.430 ± 0.943	29.490 ± 1.374[a]	29.976 ± 4.931[a]
Se	0.119 ± 0.028	0.033 ± 0.005[a]	0.206 ± 0.039[a, b]
Si	165.400 ± 14.775	60.854 ± 6.720[a]	51.300 ± 18.896[a]
V	0.083 ± 0.006	0.187 ± 0.041[a]	0.271 ± 0.033[a, b]
Zn	35.426 ± 1.463	34.872 ± 1.777[a]	19.572 ± 2.272[a, b]
Al	24.658 ± 0.890	48.352 ± 11.482[a]	90.888 ± 12.210[a, b]
As	0.046 ± 0.011	0.087 ± 0.008[a]	0.111 ± 0.065[a]
B	15.704 ± 1.275	23.334 ± 0.956[a]	19.324 ± 0.468[a, b]
Cd	0.013 ± 0.003	0.056 ± 0.009[a]	0.039 ± 0.007[a, b]
Hg	0.065 ± 0.015	0.012 ± 0.004[a]	0.023 ± 0.008[a, b]
Li	0.091 ± 0.012	0.138 ± 0.008[a]	0.409 ± 0.069[a, b]
Ni	1.346 ± 0.044	1.872 ± 0.058[a]	2.888 ± 0.242[a, b]
Pb	0.127 ± 0.114	0.162 ± 0.014	0.191 ± 0.016[b]
Sn	0.036 ± 0.041	0.021 ± 0.004	0.053 ± 0.033
Sr	34.838 ± 8.473	75.206 ± 7.844[a]	127.400 ± 12.178[a, b]

Data expressed as Mean ± SD

[a] Significant difference in comparison to *P. maxima* ($p < 0.05$)

[b] Significant difference in comparison to *P. major* ($p < 0.05$)

Table 2 Essential and toxic trace elements content (μg/g) in plant roots

Element	*P. maxima*	*P. major*	*P. lanceolata*
Co	0.428 ± 0.134	1.242 ± 0.054[a]	0.429 ± 0.149[b]
Cr	1.708 ± 0.757	3.504 ± 0.253[a]	1.096 ± 0.395[b]
Cu	2.718 ± 0.143	10.140 ± 0.577[a]	10.394 ± 0.127[a]
Fe	507.600 ± 221.634	820.400 ± 72.923[a]	359.000 ± 164.528[b]
I	0.267 ± 0.088	0.728 ± 0.051[a]	0.497 ± 0.162[a, b]
Mn	26.332 ± 5.498	49.834 ± 2.380[a]	21.396 ± 6.059[b]
Se	0.090 ± 0.031	0.062 ± 0.022	0.079 ± 0.012
Si	53.310 ± 28.004	92.836 ± 6.180[a]	70.428 ± 2.987[b]
V	1.712 ± 0.436	9.482 ± 1.012[a]	2.664 ± 0.651[b]
Zn	26.884 ± 1.342	22.886 ± 2.197[a]	15.150 ± 1.827[a, b]
Al	430.400 ± 184.465	710.000 ± 53.521[a]	257.000 ± 118.899
As	0.169 ± 0.054	0.638 ± 0.022[a]	0.207 ± 0.078
B	17.192 ± 1.101	23.148 ± 0.751[a]	20.268 ± 1.818[a]
Cd	0.029 ± 0.005	0.518 ± 0.038[a]	0.082 ± 0.013[a, b]
Hg	0.004 ± 0.000	0.004 ± 0.000	0.004 ± 0.000
Li	0.472 ± 0.170	1.009 ± 0.158[a]	0.428 ± 0.171[b]
Ni	3.646 ± 0.759	9.774 ± 0.298[a]	4.042 ± 1.043[b]
Pb	0.270 ± 0.088	0.731 ± 0.051[a]	0.823 ± 0.428[a, b]
Sn	0.024 ± 0.005	0.022 ± 0.002	0.023 ± 0.007
Sr	37.878 ± 3.144	63.162 ± 2.558[a]	39.512 ± 2.793[b]

Data expressed as Mean ± SD

[a] Significant difference in comparison to *P. maxima* ($p < 0.05$)

[b] Significant difference in comparison to *P. major* ($p < 0.05$)

of lithium and nickel in the leaves. The greatest boron and cadmium levels where seen in *P. major* leaves, exceeding the respective values for *P. maxima* and *P. lanceolata* by 49 and 21 % and by 331 and 44 %. It is notable that the highest mercury concentration was observed in *P. maxima* leaves; this was more than five and two times higher than that in *P. major* and *P. lanceolata*, respectively. The lead content of *P. lanceolata* leaves significantly exceeded the *P. major* values by 18 %, whereas the *P. maxima* values did not differ significantly from the other *Plantago* species examined. At the same time, no significant difference in the tin levels of leaves was observed between the studied species.

Distinct patterns of essential trace element contents were observed in *Plantago* roots (Table 2). In particular, the greatest cobalt content was detected in *P. major* roots; this was more than twice the corresponding values in the other studied *Plantago* species. The chromium level in the roots of *P. major* was more than two and three times higher than in *P. maxima* and *P. lanceolata*, respectively. The iron and manganese content of the roots of *P. major* significantly exceeded the corresponding values for *P. lanceolata* by a factor of more than 2. At the same time, the Fe and Mn contents of *P. maxima* roots were respectively 38 and 47 % lower than that of *P. major*. Root levels of iodine and silicon were also greatest in *P. major*. The *P. maxima* roots were characterized by the least copper concentration, which was more than three times less than that of *P. major* and *P. lanceolata*. It is notable that the highest levels of vanadium were observed in *P. major* roots, and that these values exceeded the respective values of *P. maxima* and *P. lanceolata* by factors of more than 5 and 3. In contrast to the leaves, no significant differences were observed between the selenium contents of the three species' roots. However, the difference in zinc content was greater for the roots than for leaves. In particular, the zinc content of *P. maxima* was 18 and 77 % higher than that of *P. maxima* and *P. lanceolata* roots, respectively. At the same time, no significant differences were observed between the levels of Co, Cr, Fe, Mn, Si, and V in the roots of *P. maxima* and *P. lanceolata*.

The distribution of the toxic trace elements in the plants roots was quite different from that seen in the case of the leaves. Aluminum concentration was greatest in *P. major* leaves, being 65 and 176 % higher than in *P. maxima* and *P. lanceolata*, respectively. At the same time, the arsenic levels in *P. major* exceeded the values in the other studied *Plantago* species by a factor of more than 3. The boron levels in *P. maxima* leaves were 26 and 15 % lower, respectively, than the *P. major* and *P. lanceolata* values. A significant accumulation of cadmium was observed in *P. major* roots. In particular, its levels were more than 16 times and six times higher, respectively, than in *P. maxima* and *P. lanceolata*. The lithium and nickel content of *P. major* leaves was more than twice as high than the values observed for *P. maxima* and *P. lanceolata*. The greatest strontium levels were also seen in *P. major* roots. At the same time, the highest lead content was detected in *P. lanceolata* roots, exceeding that of *P. maxima* and *P. major* by factors of 300 and 13 %, respectively. It is notable that the mercury and tin contents of the roots did not differ significantly between the species studied.

The obtained data (Table 3) indicated that the highest TF for Co, Cr, Fe, Mn, V, Al, Li, Ni, and Sr were observed in *P. lanceolata*. No significant difference in TF values for was detected between *P. maxima* and *P. lanceolata* in translocation factor for Se, Zn, As, and Cd. At the same time, *P. major* was characterized by the lowest translocation of Se and Cd and maximal TF values for Zn. Oppositely, the *P. maxima* was characterized by the highest TF values for Cu, Si, and Hg in comparison to the other species studied. No significant difference in I, B, Pb, and Sn translocation was observed between the studied *Plantago* species.

Table 3 Translocation factors for trace elements in the studied *Plantago* species

Element	*P. maxima*	*P. major*	*P. lanceolata*
Co	0.176 ± 0.051	0.146 ± 0.010	0.692 ± 0.196[a, b]
Cr	0.070 ± 0.037	0.071 ± 0.015	0.348 ± 0.161[a, b]
Cu	2.651 ± 0.391	0.729 ± 0.106[a]	0.469 ± 0.039 [a, b]
Fe	0.133 ± 0.051	0.119 ± 0.011	0.314 ± 0.169[a, b]
I	0.798 ± 0.328	0.455 ± 0.113	0.367 ± 0.138
Mn	0.722 ± 0.139	0.593 ± 0.046	1.459 ± 0.337[a, b]
Se	1.680 ± 1.324	0,596 ± 0.243	2.681 ± 0.743[b]
Si	3.805 ± 1.729	0.654 ± 0.035[a]	0.734 ± 0.293[a]
V	0.051 ± 0.013	0.020 ± 0.004[a]	0.110 ± 0.042[a, b]
Zn	1.322 ± 0.113	1.530 ± 0.088[a]	1.318 ± 0.303
Al	0.069 ± 0.036	0.068 ± 0.012	0.436 ± 0.229[a, b]
As	0.300 ± 0.139	0.137 ± 0.018[a]	0.530 ± 0.155[a]
B	0.917 ± 0.097	1.009 ± 0.050	0.959 ± 0.086
Cd	0.480 ± 0.200	0.108 ± 0.014[a]	0.490 ± 0.142[b]
Hg	17.944 ± 4.123	3.294 ± 0.974[a]	6.500 ± 2.130[a, b]
Li	0.211 ± 0.070	0.140 ± 0.028	1.061 ± 0.379[a, b]
Ni	0.384 ± 0.092	0.192 ± 0.008[a]	0.742 ± 0.138[a, b]
Pb	0.473 ± 0.330	0.222 ± 0.024	0.312 ± 0.203
Sn	1.470 ± 1.476	0.932 ± 0.132	2.638 ± 2.428
Sr	0.914 ± 0.176	1.192 ± 0.130[a]	3.224 ± 0.217[a, b]

Data expressed as Mean ± SD

[a] Significant difference in comparison to *P. maxima* ($p < 0.05$)

[b] Significant difference in comparison to *P. major* ($p < 0.05$)

Discussion

In this study, it was found that three *Plantago* contain significant levels of essential elements and that the concentration of most of these elements was higher in the leaves and roots of *P. major* and *P. lanceolata* than *P. maxima*. On the other hand, *P. maxima* also had rather lower levels of toxic metals.

Plantago species can accumulate trace elements, including toxic metals, from air, soil, and water in their roots and leaves and can act as biomonitors of environmental pollution [25, 26]. The results of Petrova et al. [25] showed that the concentration of essential (Cr, Cu, Zn) and toxic elements (Al, Cd and Pb) in the leaves and roots of *P. lanceolata* was several times higher than in our study. These significant differences may be the result of the fact that Petrova et al. collected plants from polluted urban areas. According to Petrova et al. [25], *P. lanceolata* has the ability to accumulate copper; in this study, we observed that *P. lanceolata* and *P. major* accumulate this element, especially in the roots, while *P. maxima* tends to accumulate it in the leaves. *P. lanceolata* also showed a tendency to bioaccumulate Zn; however, in our study, we observed that the Zn content of *P. major* and *P. maxima* in both the leaves and roots was significantly higher than in *P. lanceolata*. We found higher concentration of zinc in the leaves than in the roots, which may indicate that a large amount of this element is air-derived. A similar distribution between the roots and leaves was observed in the case of selenium in *P. maxima* and *P. lanceolata*.

The *Plantago* species showed a tendency to accumulate aluminum. This element mainly enters the plants through the roots and the usual concentration of Al in vascular plant species is 200 mg/kg.

It is known that the normal cadmium concentration in plants is 0.2–0.8 mg/kg and the results obtained in this work did not exceed that limit. Plants take up this element from the soil through the roots and from the air through the leaves. In the present work, we found that *P. major* in particular accumulated a large amount of Cd in the roots (0.5 mg/kg d.w.).

The normal concentration of lead in plants is 0.1–10 mg/kg. The lead content of the leaves and roots of the *Plantago* species examined here did not exceed the upper value of this range. This element is taken up by plants through the soil and the air [26, 27]. It is worth noting that *P. lanceolata* accumulated the greatest amount of Pb in the leaves and roots.

Similar to our study, Kurteva [28] found that the copper and iron contents of *P. major* were higher than in *P. lanceolata* in an area near a copper mine; the content of zinc in *P. lanceolata* was also more than three times higher than in our study [28]. Rabai et al. found much higher concentrations of essential and toxic elements in the leaves of *P. lanceolata*, and only the zinc level was comparable with our results [29]. *P. lanceolata* was analyzed by Nadgórska-Socha et al. [30] who, in comparison with our results, found higher levels of

Cd, Zn, and Fe and lower levels of Cu, Mn, and Pb in *P. lanceolata* from uncontaminated areas for Poland. In other studies analyzing samples of *Plantago* leaves from Poland, lower levels of Fe and Zn and higher levels of Pb and Cd were found than in the case of the present results. However, in another study from Poland, leaf and root samples of *P. major* contained higher levels of Zn, Cd, and Pb than our samples [31].

It seems that *Plantago* species may have rather high nutritional values, and less toxic properties, on account of their higher levels of Fe, Zn, Cu, and Mn and their lower levels of Pb and Cd than other medicinal plants [31, 32]. However, on the basis of the results of other studies, it can be said that the level of mercury in *Plantago* species was higher than in other herbal plants [33]. Tocainoglu [34] showed that there were higher levels of Pb and other metals in commonly consumed medicinal herbs than in *Plantago*.

In some frequently used herbs—such as mint, chamomile, and sage—the amount of Fe and Cu was higher than in Plantago, but it is worth noting that these elements were poorly extractable into infusions and that the bioavailability of these elements from these herbs was rather low [34–36].

Generally, the toxic metal levels in the leaves of all three *Plantago* species examined in this study did not exceed WHO limits and the other national limits for toxic metals in herbal materials [37]. However, it was observed that the roots accumulated greater amounts of metals being in agreement with the earlier indicated distribution of metals in plant roots and shoots [38]. In particular, the concentration of Cd in the roots of *P. major* exceeds the WHO limits and the national limits of Canada and Thailand [37].

Hypothetically, the difference between *Plantago* species in metal tissue concentration may occur due to regulation of metal absorption from soil. In particular, it has been demonstrated that root excretion of organic substances affects metal absorption [39]. Root exudates may also contain various polyphenols [40, 41] that possess metal-chelating properties [42]. Therefore, it is supposed that polyphenol content in plant tissues may affect metal absorption. This supposition is in agreement with the results of our previous study that demonstrated the highest polyphenol levels in *P. maxima* [43] that is characterized by the lowest heavy metal concentration. Correspondingly, the lowest content of phytochemicals [43] and the maximal metal level were detected in *P. major*.

It appears that, due to their minerals contents and other health properties, *Plantago* species (and especially their leaves) could be favorably used for prevention and symptomatic treatments. *Plantago* species have antioxidant and anti-inflammatory properties, and the World Health Organization has approved *Plantago* compounds for the treatment of high cholesterol and glucose [26]. These health properties may be associated with the level of essential trace elements: in particular, Se, Zn, and Cu may account for antioxidant activity and Zn, Cr, and V for increasing insulin sensitivity [44–48]. The results of some studies have confirmed the connection between essential trace element levels and the pharmacological functions of *Plantago* [49, 50].

In conclusion, Plantago species—especially *P. major* and *P. lanceolata*—contain a significant number of necessary elements. The concentration of toxic elements in the leaves does not exceed the limit, though it does appear that *P. maxima* accumulates smaller amounts of toxic elements than the other two species.

Our results show that the content and distribution of these elements in *Plantago* are not homogenous and depend on physicochemical differences and the ability of minerals to accumulate in tissues. As the leaves and roots of *Plantago* species accumulated toxic metals, including Pb and Cd from the soil and air, appropriate conditions for the growth of these plants should be ensured. The difference in trace elements content in *Plantago* tissues should be taken into account while using plant preparations in practical medicine. However, further studies are required to assess the bioavailability of trace elements from the studied *Plantago* species.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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