



# OPEN Selenium content and bioaccumulation in *Bidens tripartita* and *Bidens frondosa* under different habitat conditions in Poland and Montenegro

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The subjects of this study were two congeneric species of the genus *Bidens*, the European native *Bidens tripartita* and the invasive *Bidens frondosa*. The aim of this research was to determine: (1) the selenium content of the specimens of these species and the soils in which they grew, (2) the role of habitat parameters in shaping selenium levels in the soil and the plants studied, and (3) the bioaccumulation potential of the plants studied, taking habitat conditions into account. Specimens of both species were collected from riverbanks in Poland and Montenegro, and the Se concentrations in each specimen and in the soil sample from each site were measured. Our studies indicate that the invasive species *B. frondosa* has significantly higher selenium concentrations and greater bioaccumulation abilities (BCF > 1) compared to *B. tripartita*. Despite the average selenium concentration in the soil being higher in Poland than in Montenegro, Se concentration accumulated in *B. frondosa* collected in Montenegro was higher, it was also observed that soil chemical properties affect Se bioaccumulation in this species. The type of land use within each site has also been identified according to CORINE classification. The results of the study indicated a great significance of human impact on the selenium enrichment of plants, as the specimen collected in artificial rivers and areas impacted by anthropogenic pressure contained much higher amounts of Se than plants growing in more natural habitats. The results also illustrated that the invasive species that have the ability to accumulate selenium, like *B. frondosa*, can be an exogenous root of this particular trace element for living organisms.

**Keywords** Selenium, Soil, Bioaccumulation, *Bidens frondosa* L., *Bidens tripartita* L.

Despite the crucial role selenium has in supporting proper growth and well-being of humans and animals, the essentiality of this metalloid for plants is still debatable, although studies show that low concentrations of Se may help plants cope with abiotic stress<sup>1,2</sup> and they play a positive role in the growth and development of plants, yield and fruit quality<sup>3,4</sup>. Selenium exists in nature in both inorganic forms: bioavailable selenate and selenite, and also unavailable to plants selenide and elemental Se, as well as organic: seleno-cystine (SeCys) and seleno-methionine (SeMet)<sup>1,5</sup>. Selenate is more water soluble and easier for plants to absorb, it is the predominant form in alkaline and well-oxidized soils, whereas selenite is most common in anaerobic, acidic soils and wetlands<sup>5,6</sup>.

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The selenium content in soil depends on soil properties, like pH and texture (content of clay particles) and total absorbed bases, and in general it is lowest in acidic and sandy soil<sup>7</sup>. Selenium chemically resembles sulfur and the uptake mechanisms of these two elements in plants are similar; Se is recovered from the soil through sulphate (selenate) or phosphate (selenite) transporters<sup>6</sup>. Some species of plants are considered Se hyperaccumulators and those plants are proven to prefer accumulating available selenium over sulfur, while most plants do not seem to differentiate these two elements. Selenium hyperaccumulators are found among families Brassicaceae, Fabaceae, and Asteraceae, and their presence indicates highly seleniferous soils<sup>8</sup>.

The *Bidens* genus, of the family Asteraceae, includes up to 240 species of annual, or more rarely perennial herbs, most often growing nearby rivers or wetlands worldwide<sup>9–11</sup>. Several species of the genus *Bidens*, of which *B. pilosa* was the most well-studied, are still used in traditional medicine, for they are proven to have antibiotic and antimicrobial effects, and appear to be effective in treating diseases such as malaria<sup>12,13</sup>. *Bidens pilosa* also exhibits anti-diabetic and anti-inflammatory effects as well as it can potentially help regulating allergic response and decrease number of tumor cells<sup>14–16</sup>. The same antibacterial, antifungal and antioxidant properties are observed in *Bidens frondosa* and *Bidens tripartita*<sup>17–19</sup>. Compounds, that determine the usefulness of these species to human health, are polyacetylenes and flavonoids, which can be found in plant extracts<sup>14,19,20</sup>. Another interesting aspect of *Bidens* species is their ability to accumulate some of the heavy metals, such as cadmium, nickel and lead<sup>21–23</sup>, as well as other chemical compounds, like PAH<sup>22,24</sup>. *B. pilosa* is known to be a Cd hyperaccumulator and *B. tripartita* is a Cd accumulator<sup>23,25</sup>. A study conducted on *B. pilosa*<sup>26</sup> showed that this plant tends to accumulate various heavy metals available in the soil, although while most of the absorbed metals (Pb, As, Zn) would mainly be present in the roots, cadmium is accumulated equally in both the roots and the shoots. As metals are mostly absorbed passively, these disparities in their translocation suggest different transport capacity and specific cell membrane permeability, when dealing with certain elements<sup>26,27</sup>. Selenium has been found to play an important role in reducing the uptake of Cd by plants, as well as in mitigating the adverse effects of Cd toxicity in plants, fruits and seeds<sup>2,28–30</sup>. This element in the plant may have a protective function by reducing heavy metal accumulation, if not in the whole plant, then at least in certain parts of it<sup>31–36</sup>.

To the best of our knowledge, species of the *Bidens* genus have not yet been the subject of research regarding selenium content and the possibility of bioaccumulation of this element. In the case of herbs, in addition to their medicinal properties, it is also important to know whether they can be an important source of exogenous antioxidants for humans, including selenium. This prompted the study of two species of herbs from the genus *Bidens*, one of them *B. tripartita* is a European species<sup>37</sup>, while the other *B. frondosa* is an invasive species originating from North America that was first introduced in Europe in the second half of eighteenth century and began spreading across the continent by the end of nineteenth century<sup>38</sup>. *B. tripartita* shows less invasive capacity, being less plastic in terms of regulating the vegetative phase length and more vulnerable to a shortage of water in the soil<sup>37</sup>. The research was conducted in various habitat conditions in remote regions of Europe: Poland and Montenegro. The aim of these studies was to determine: (1) the selenium content in *B. frondosa* and *B. tripartita* and in soils under various habitat conditions, (2) the bioaccumulation potential of the studied plants, taking habitat conditions into account, and (3) the role of habitat parameters in shaping the selenium level in the soil and the studied plants.

## Results

### Chemical composition of soils and selenium content in these soils

In soil samples the selenium content ranged from 0.026 to 1.267 µg/g, whereas in Poland it was in the range of 0.039–1.267 µg/g, and in Montenegro 0.026–0.247 µg/g (Table 1).

In Montenegro *B. frondosa* appeared in extremely diverse alluvial habitats, with a significant limestone skeleton presence. The habitats varied from dry mineral soil, with organic material content with the average of 5.6% to organic soil with the organic mineral content with the average of 32%. These soils were characterized by their composition: amount of general N ranged from 0.17% in the mineral soils to 1.39% in organic soils; available P ranged from an average amount in the mineral soils (266.9 mg/kg) to a very high amount in mineral-organic soils (721.0 mg/kg); available K was a scope from a very low amount in the organic soils (157.4 mg/kg) to a low amount found in the mineral-organic soils (269.5 mg/kg); finally an extremely high amount of available Mg (from 12,515.0 mg/kg to 31,188.0 mg/kg) (Table 2). High amount of calcium carbonate (from 22.0% in organic soil to 41.22% in mineral soil on average) contributed to the basic pH of the soil (pH of KCl > 7.2). Regardless of the rich deposits of carbonates and magnesium salts, these soils did not exhibit significant salinity (on average from 216.5 µS cm<sup>-1</sup> in mineral soils to 1060.0 µS cm<sup>-1</sup> in organic soils).

A positive correlation was found between organic matter, organic carbon, N, C:N, K, salinity, silt, clay, and a negative correlation between sand and selenium content in the soil (Table 3).

In Poland *B. frondosa* was also present in very differentiated habitats of river valleys, however with different properties than in Montenegro. Firts of all soil bedrocks were not composed of alkaline aluwia filled with calcium fragments, but mineral aluwia, which are mostly sandy, with mineral-organic and organic soils and a pH ranging from highly acidic to slightly acidic. These soils were characterised by: available P ranging from an average amount for mineral soils poor in organic matter (265.1 mg/kg on average) to very high amount in mineral-organic soils (1659.4 mg/kg on average); very low amount of available K (on average from 18.3 mg/kg in mineral soils poor in organic matter to 190.0 mg/kg in mineral-organic soils) and finally available Mg ranging from a low amount for mineral soils poor in organic matter (39.0 mg/kg on average) to very high amount in organic soils (902.2 on average) (Table 2). The lack of salinity of soil was determined, however the concentration of salt had a wide range (from 77.8 µS·cm<sup>-1</sup> for mineral soils poor in organic matter to 985.8 µS·cm<sup>-1</sup> in organic soils, on average).

There is a positive, significant impact of organic matter and content of C organic, N on selenium content in the soil (Table 3).

Localities	Type of watercourses	Landscape type	Soil Se (µg/g)	<i>Bidens tripartita</i>		<i>Bidens frondosa</i>	
				Se (µg/g)	BCF	Se (µg/g)	BCF
Poland							
Barycz	S	Pas	0.193	0.032	0.166	–	–
Barycz	S	Pas	0.320	0.030	0.095	–	–
Barycz	S	Agr	0.047	–	–	0.016	0.339
Barycz	S	For	0.096	–	–	0.006	0.060
Barycz	S	For	0.039	–	–	0.004	0.102
Barycz	S	Agr	0.047	0.026	0.553	–	–
Barycz	S	For	0.099	0.021	0.215	–	–
Barycz	S	Pas	–	–	–	0.008	–
Barycz	S	For	0.099	0.028	0.279	–	–
Barycz	S	For	–	–	–	0.069	–
Bóbr	S	Arb	–	–	–	0.014	–
Bóbr	S	Agr	0.076	–	–	0.133	1.764
Brda	S	For	0.117	0.018	0.151	–	–
Brda	S	For	0.122	0.088	0.724	–	–
Brda	S	For	0.364	–	–	0.017	0.046
Brda	S	For	0.150	–	–	0.039	0.261
Brda	S	For	0.219	–	–	0.023	0.105
Brda River	S	For	0.169	0.103	0.610	–	–
Brda	S	For	0.213	0.061	0.285	–	–
Czarna Maleniecka	S	For	–	–	–	0.046	–
Dobrzyca	VS	For	0.222	0.016	0.071	–	–
Ina	S	Pas	0.102	–	–	0.030	0.291
Ina	S	Pas	0.064	–	–	0.000	0.000
Ina	S	Pas	0.066	0.112	1.697	–	–
Korytnica	VS	For	0.279	0.025	0.090	–	–
Luciąża	VS	Agr	0.202	–	–	0.199	0.988
Łupawa	S	For	0.056	0.030	0.532	–	–
Nida	S	Arb	0.605	–	–	0.121	0.201
Nida	S	Pas	–	–	–	0.053	
Nida	S	Pas	0.248	–	–	0.114	0.460
Odra	L	Wet	0.576	–	–	0.151	0.262
Odra	L	Wet	0.221	–	–	0.082	0.370
Odra	L	Wet	0.519	–	–	0.010	0.019
Odra	L	Pas	0.302	–	–	0.037	0.121
Odra	L	Pas	0.487	–	–	0.125	0.258
Odra	L	Pas	0.213	–	–	–	–
Odra River	L	Wet	0.203	–	–	0.085	0.420
Odra	L	Wet	1.267	–	–	0.267	0.211
Odra	L	Pas	0.294	0.156	0.533	–	–
Odra	L	Pas	0.206	–	–	0.098	0.478
Odra	L	Wet	0.308	0.062	0.200	–	–
Parsęta	S	For	0.091	–	–	–	–
Postomia	S	Pas	0.272	0.016	0.058	–	–
Postomia	S	Pas	0.225	0.024	0.106	–	–
Postomia	S	Pas	0.368	0.021	0.057	–	–
Postomia	S	Pas	0.369	0.320	0.868	–	–
Radew	S	Pas	0.181	0.024	0.130	–	–
Radew	S	Pas	0.183	0.036	0.195	–	–
Radew	S	Pas	0.121	0.046	0.381	–	–
Rega	S	For	0.136	–	–	0.008	0.058
Rurzyca	VS	For	0.135	–	–	0.028	0.207
Sąpólna	VS	Pas	0.064	0.016	0.257	–	–
Słupia	S	Pas	0.161	–	–	0.068	0.423
Warta	M	For	0.511	–	–	0.016	0.032
Continued							

Localities	Type of watercourses	Landscape type	Soil Se (µg/g)	<i>Bidens tripartita</i>		<i>Bidens frondosa</i>	
				Se (µg/g)	BCF	Se (µg/g)	BCF
Warta	M	For	0.511	–	–	0.066	0.128
Warta	M	Pas	0.182	0.046	0.250	–	–
Warta	M	Pas	0.173	0.042	0.240	–	–
Warta	M	Pas	0.252	0.133	0.529	–	–
Warta	M	Pas	0.439	0.104	0.236	–	–
Warta	M	Pas	0.149	0.032	0.212	–	–
Warta	M	Pas	–	0.042	–	–	–
Warta	M	Agr	0.121	0.028	0.231	–	–
Warta	M	Agr	0.188	0.057	0.303	–	–
Warta	M	Pas	0.224	0.130	0.580	–	–
Warta	M	Pas	0.253	0.069	0.273	–	–
Warta	M	Pas	0.294	0.046	0.157	–	–
Warta	M	Agr	–	0.032	–	–	–
Warta	M	Agr	0.072	0.033	0.461	–	–
Warta	M	Agr	0.149	0.033	0.221	–	–
Widawka	S	Arb	–	0.014	–	–	–
Widawka	S	Arb	0.458	–	–	0.059	0.129
Widawka	S	Arb	–	–	–	0.042	–
Montenegro							
Bečića	VS	Urb	0.108	0.097	0.901	–	–
Grdevica	VS	Urb	0.177	0.101	0.570	–	–
Grdevica	VS	Urb	0.074	–	–	0.154	2.098
Ditch in the Grdevica valley	AR	Urb	0.065	–	–	0.176	2.710
Koložun	VS	Agr	0.091	0.049	0.537	–	–
Koložun	VS	Agr	0.089	–	–	0.095	1.063
Dich in the Koložun valley	AR	Urb	0.087	0.072	0.822	–	–
Dich in the Koložun valley	AR	Urb	0.051	–	–	0.207	4.042
Morača	S	Wet	0.026	–	–	0.129	4.959
Morača	S	Wet	0.202	–	–	0.040	0.196
Morača	S	Wet	0.100	0.084	0.832	–	–
Morača	S	Wet	0.088	–	–	0.119	1.349
Morača	S	Wet	0.068	–	–	0.075	1.094
Morača	S	Urb	0.129	0.099	0.762	–	–
Morača	S	Wet	0.091	0.050	0.553	–	–
Morača	S	Wet	0.074	0.067	0.910	–	–
Morača	S	Wet	0.078	0.071	0.905	–	–
Morača	S	Urb	0.198	–	–	0.268	1.356
Morača	S	Urb	0.218	–	–	0.348	1.597
Ricavač	VS	Urb	0.038	0.010	0.259	–	–
Veštica	VS	Urb	0.135	0.116	0.855	–	–
Veštica	VS	Urb	0.118	–	–	0.211	1.783
Zeta	S	Agr	0.186	–	–	0.128	0.688
Zeta	S	Urb	0.172	0.093	0.541	0.247	1.433
Zeta	S	Agr	0.193	–	–	0.143	0.738
Zeta	S	Agr	0.147	–	–	0.010	0.067
Zeta	S	Agr	0.217	–	–	0.176	0.811
Zeta	S	Agr	0.190	–	–	0.157	0.830
Zeta	S	Agr	0.247	–	–	0.181	0.734
Zeta	S	Agr	0.072	0.032	0.447	–	–
Zeta	S	Wet	0.099	0.055	0.555	–	–
Zeta	S	Wet	0.071	0.075	1.054	–	–
Zeta	S	Agr	0.058	0.020	0.340	–	–
Zeta	S	Wet	0.060	0.057	0.950	–	–
Zeta	S	Agr	0.078	0.061	0.780	–	–
Zeta	S	Wet	0.056	–	–	0.034	0.596
Continued							

Localities	Type of watercourses	Landscape type	Soil Se (µg/g)	<i>Bidens tripartita</i>		<i>Bidens frondosa</i>	
				Se (µg/g)	BCF	Se (µg/g)	BCF
Zeta	S	Wet	0.049	–	–	0.024	0.496
Zeta	S	Agr	0.238	–	–	0.096	0.403
Zeta	S	Wet	0.051	–	–	0.030	0.585
Ditch in the Željeznica valley	AR	Urb	0.069	0.022	0.311	–	–

**Table 1.** Selenium content (µg/g) in soil and herbs of *B. tripartita* and *B. frondosa* and bioconcentration factors (BCF) of selenium in these herbs in Poland and Montenegro (type of watercourses: *L* large river, *M* medium river, *S* small river, *VS* very small river, *AR* artificial watercourses; landscape types: *Urb* urban fabric, *Arb* arable land, *Pas* pastures, *Agr* heterogeneous agricultural areas, *For* forest, *Wet* inland wetlands).

	Organic matter	SOC	TN			P	K	Mg	Salinity	CaCO <sub>3</sub>
Location	(%)			C:N	pH in KCl	mg/kg			µS·cm <sup>-1</sup>	%
Montenegro	Organic soils (OM > 30%)									
	32,0	15.23	1.39	11	7.2	277.7	157.4	12,515.0	1060.0	22.0
	Organic-mineral carbonate soils (OM—10–30%)									
	12.7	6.50	0.48	14	7.52	721.0	269.5	31,188.0	371.4	34.4
	Mineral carbonate soils (OM 1–10%)									
	5.6	2.84	0.17	17	7.49	266.9	195.2	25,072.5	216.5	41.22
Poland	Organic soils (OM > 30%)									
	42.8	20.81	1.67	12	5.77	1053.8	171.0	902.0	985.8	–
	Organic-mineral soils (OM 10–30%)									
	18.5	9.54	0.74	13	5.15	1659.4	190.0	478.0	825.6	–
	Mineral soils (OM—1–10%)									
	6.3	3.15	0.23	13	5.81	440.8	136.9	289.0	328.9	–
	Mineral soils (OM < 1%)									
	0.6	0.21	0.02	8	5.75	265.1	18.3	39.0	77.8	–

**Table 2.** Chemical composition of soils in sites with *Bidens frondosa* (average values).

Species	Country	Loss on ignition (organic matter)	C org. /caries	N	C:N	pH KCl	P mg/kg	K mg/kg	Mg mg/kg	Salinity	Sand	Silt	Clay
<i>Bidens frondosa</i>	Poland	<b>0.491</b>	<b>0.499</b>	<b>0.427</b>	0.155	−0.084	0.239	0.300	0.238	0.370	−0.307	0.255	0.220
	Montenegro	<b>0.596</b>	<b>0.610</b>	<b>0.599</b>	<b>0.377</b>	0.081	−0.47	<b>0.432</b>	0.338	<b>0.400</b>	− <b>0.646</b>	<b>0.631</b>	<b>0.606</b>
<i>Bidens tripartita</i>	Poland	−0.73	−0.086	−0.097	0.200	0.105	0.103	−0.018	−0.097	−0.213	<b>0.467</b>	−0.403	− <b>0.493</b>
	Montenegro	−0.004	0.011	−0.001	0.079	0.193	0.232	0.031	0.190	−0.013	−0.227	<b>0.339</b>	<b>0.496</b>

**Table 3.** Results of the Spearman’s correlation between soil selenium content and other soil parameters in *B. frondosa* and *B. tripartita* habitats in Poland and Montenegro. Statistically significant parameters have been bolded ( $p < 0.05$ ).

The second analyzed species, *B. tripartita*, was present in a bit less differentiated habitats. In Montenegro *B. tripartita* grew on clay alluvial soils, rich in organic matter (4.4% on average) and mineral-organic soils (12.4% on average). These soils had basic pH (pH of KCl ranging from 7.28 in mineral-organic soils to 7.55 in mineral soils, on average), thanks to the significant percentage calcium carbonate present in the soils (from 25.9% in mineral-organic soils to 37.0% in mineral soils, on average). Furthermore they were characterised by the presence of: total nitrogen from 0.13% in mineral soils to 0.46% in mineral-organic soils, on average; available P ranging from high amount (375.5 mg/kg in mineral soils, on average) to very high amount (1010.4 mg/kg in mineral-organic soils on average); very low amount of available K (from 181.9 mg/kg in mineral soils to 225.6 mg/kg mineral-organic soils, on average) and a very high amount of available Mg (from 14,315.4 mg/kg in mineral soils to 19,858.0 mg/kg in mineral-organic soils, on average). The salicity of the soils was ranging on average from 198.3 µS·cm<sup>−1</sup> in mineral soils to 355.8 µS·cm<sup>−1</sup> in mineral-organic soils on average (Table 4).

It was found that soil selenium content was positively correlated silt and clay (Table 3). In Poland this species was present in the river valleys in diverse habitats—mostly living on sandy soils rich with caries and mineral-organic soils as well as sporadically present in organic soils. These soils had an average

Location	Organic matter	SOC	TN	C:N	pH in KCl	P	K	Mg	Salinity	CaCO <sub>3</sub>
	(%)					mg/kg			μS·cm <sup>-1</sup>	%
Montenegro	Organic-mineral soils (OM 10–30%)									
	12.4	5.17	0.46	11	7.28	1010.4	225.6	19,858.0	355.8	25.9
	Mineral soils (OM—1–10%)									
	4.4	2.00	0.13	15	7.55	375.5	181.9	14,315.4	198.3	37.0
Poland	Organic soils (OM > 30%)									
	37.6	18.00	1.61	11	6.40	2242.0	239.0	666.0	1355.3	–
	Organic-mineral soils (OM 10–30%)									
	16.3	8.23	0.72	12	5.88	1723.5	132.0	463.0	715.2	–
	Mineral soils (OM—1–10%)									
	4.82	2.66	0.21	13	6.06	624.8	61.4	227.0	540.7	–
	Mineral soils (OM < 1%)									
	0.7	0.29	0.03	10	6.00	354.0	8.3	24.0	63.0	–

**Table 4.** Chemical composition of soils at sites with *Bidens tripartita* (average values).

of mildly acidic pH (pH of KCl ranging from 5.88 in mineral-organic soils to 6.40 in organic soils), they had negligible salinity (ranging from 63.0 μS·cm<sup>-1</sup> in mineral soils, lacking in organic matter, to 1355.3 μS·cm<sup>-1</sup> in organic soils, on average). Furthermore they were characterised by the presence of the following elements: the amount of P ranging from high amount in mineral soils, lacking in organic matter (354.0 mg/kg on average), to very high amount in organic soils (2242.0 mg/kg on average); the amount of K ranging from very low amount in mineral soils, lacking in organic matter (8.3 mg/kg on average) to low amount in organic soils (28.6 mg/kg on average); lastly the amount of Mg ranging from low in mineral soils, lacking in organic matter (24.0 mg/kg on average) to a mediocre amount in organic soils (666.0 mg/kg on average) (Table 4).

Selenium content in soils was varied and positively correlated with sand and negatively with clay. (Table 3).

### Variability of selenium concentrations in *B. frondosa* and *B. tripartita* in different habitat conditions

The selenium content in plants ranged from 0.001 to 0.348 μg/g (Table 1), with significant statistical differences depending on the species (U–M Whitney test: U = 1208, Z = 1.95,  $p = 0.049$ ). Clearly higher selenium content was found in *Bidens frondosa* (Fig. 1).

Both in Poland and Montenegro there were reports higher selenium content in *Bidens frondosa* (Fig. 2), but the significant statistical differences was only in Montenegro (U–M Whitney test: U = 588, Z = 0.23,  $p = 0.82$ ; U–M Whitney test: U = 95, Z = 2.97,  $p = 0.003$  respectively).

When analyzing differences in selenium content in specimens of particular species in particular countries, it was observed the higher selenium content in Montenegro than in Poland both for *B. frondosa* (U–M Whitney Test: U = 159.0, Z = -3.389,  $p = 0.0007$ ) and *B. tripartita* (U–M Whitney Test: U = 235.0, Z = -2.124,  $p = 0.034$ ) (Fig. 3).

In case of *B. frondosa* in Poland the amount of Se in the plant was not dependant on the most of the soil parameters in any significant way, with the exception of silt, which showed a significant negative influence (RS = -0.481) (Table 5). On the other case in *B. tripartita*, there is a positive relationship between the Se content in plants and the Se content in the soil (RS = 0.426) and negatively correlation in clay fraction (RS = -0.450).

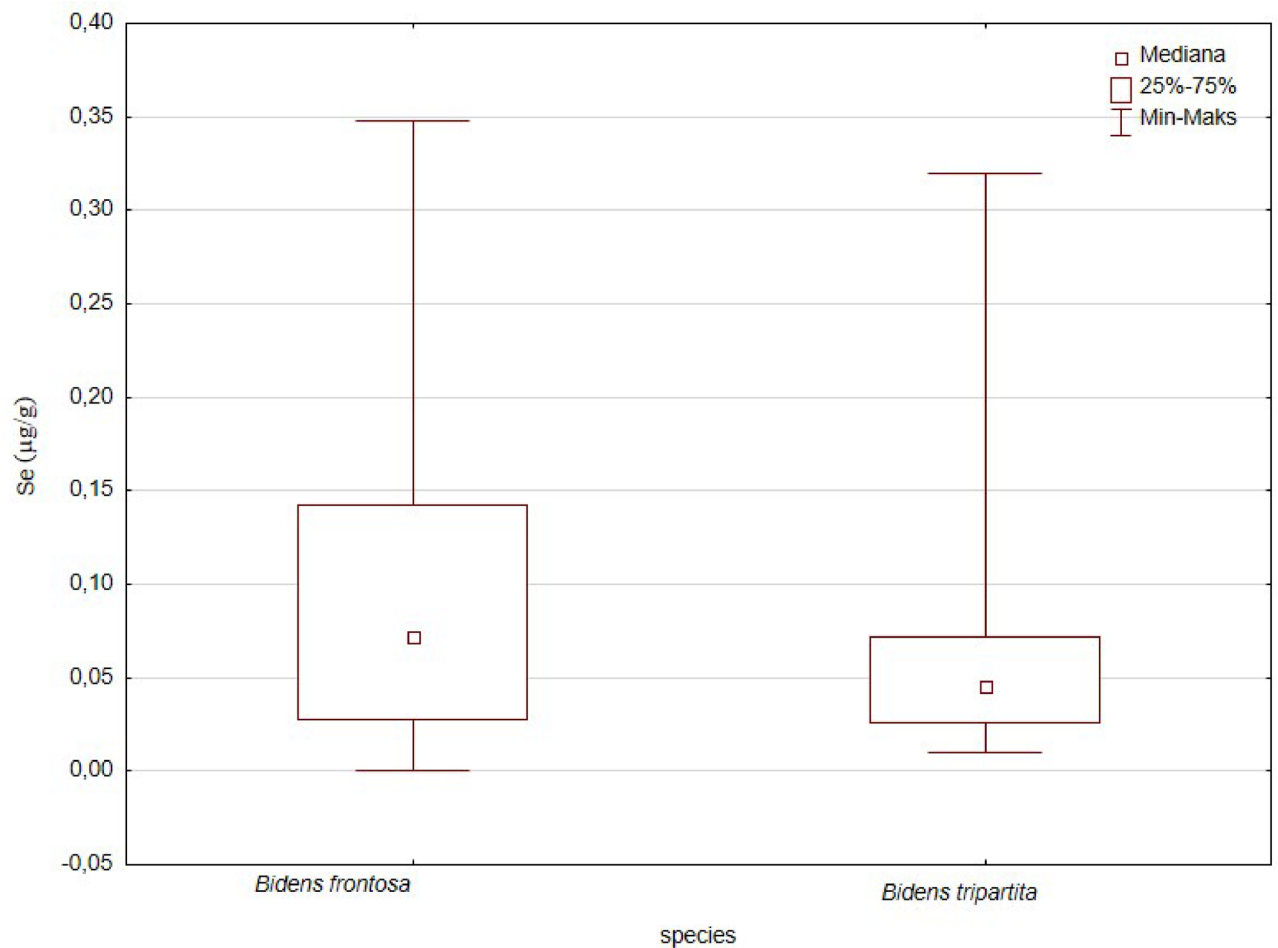
With the *B. frondosa* in Montenegro there was not determined any significant correlation between the amount of Se in the plant and the amount of Se present in the soil ( $R_s = 0.376$ ,  $p = 0.053$ ). It was, however, observed that there was a positive influence of K and Mg and a negative influence of sand (Table 5). For *B. tripartita* from in Montenegro, there is a positive correlation between the Se content in plants and the Se content in the soil ( $R_s = 0.492$ ).

For *B. tripartita* from in Montenegro, there is a positive correlation between the Se content in plants and the Se content in the soil ( $R_s = 0.492$ ). In addition, selenium content in plants was positively correlated with organic matter, organic carbon, N, K, Mg, salinity, silt, clay, but negatively with sand (Table 5).

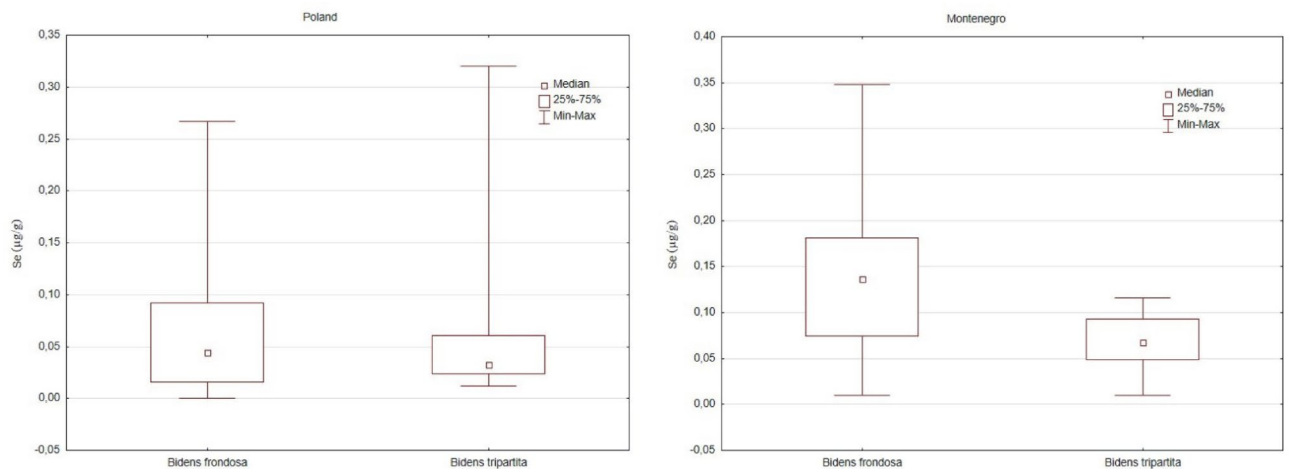
Differences in selenium content, depending on the type of land use in *Bidens frondosa*, were not statistically significant in Poland (K–W test: H (4, N = 32) = 7.028208  $p = 0.1344$ ); but they were in Montenegro (K–W test: H (3, N = 22) = 14.63326  $p = 0.0022$ ). In specimens collected in Poland, the biggest difference was between island wetlands and forest (post hoc test results—multiple comparison of ranks for all samples  $p = 0.031$ ), while for Montenegro it was between urban fabric and pastures (post hoc test results—multiple comparison of ranks for all samples  $p = 0.001$ ) (Supplementary material 1, Fig. 4).

However, in the case of *Bidens tripartita* both for Poland and Montenegro, no statistically significant differences were found, which would prove the relationship between the selenium content in plants and the nature of the environment (K–W test: H (4, N = 38) = 4.492770  $p = 0.3434$ ; K–W test: H (3, N = 19) = 4.948684  $p = 0.1756$  respectively).

The selenium content in the studied plants also differed depending on the type of river they were located on. In Poland the selenium content in *Bidens frondosa* was high for plants located on very small and large, and in *B. tripartita* for plants located on large rivers (Supplementary material 2, Fig. 5). In case of *B. frondosa*, the



**Fig. 1.** Whitney's U–M test results. Differences in selenium content ( $\mu\text{g/g}$ ) in the tested specimens of *Bidens frondosa* and *Bidens tripartita*.



**Fig. 2.** Whitney's U–M test results. Differences in selenium content ( $\mu\text{g/g}$ ) in the tested specimens of *Bidens frondosa* and *Bidens tripartita* in particular countries.



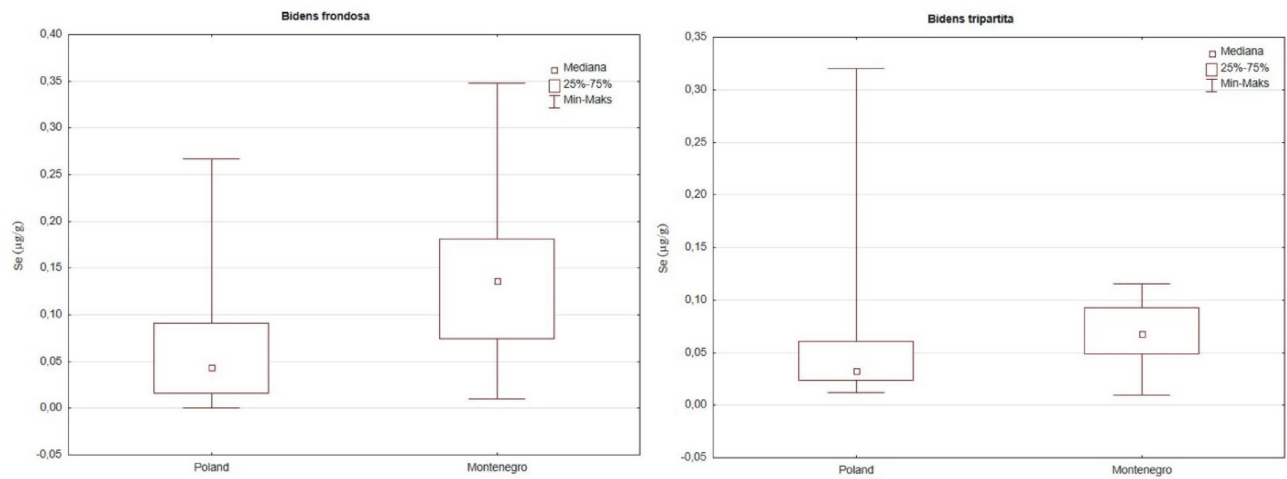


Fig. 3. Regional differences in selenium content (µg/g) in particular species.

Species	Country	Selenium µg/g	Loss on ignition (organic matter)	C org. /caries	N	C:N	pH KCl	P mg/kg	K mg/kg	Mg mg/kg	Salinity	Sand	Silt	Clay
<i>Bidens frondosa</i>	Poland	0.380	−0.180	−0.051	−0.225	0.304	0.250	0.290	0.137	−0.269	−0.310	0.356	−0.481	−0.202
	Montenegro	0.376	0.214	0.184	0.213	0.198	0.169	0.023	<b>0.391</b>	<b>0.411</b>	0.114	−0.414	0.312	0.361
<i>Bidens tripartita</i>	Poland	<b>0.426</b>	−0.018	−0.003	−0.013	0.060	−0.125	0.363	0.012	−0.206	−0.118	0.302	−0.247	−0.450
	Montenegro	<b>0.492</b>	<b>0.434</b>	<b>0.462</b>	<b>0.445</b>	−0.007	0.016	0.109	<b>0.407</b>	<b>0.337</b>	<b>0.430</b>	−0.352	<b>0.553</b>	<b>0.505</b>

Table 5. Results of the Spearman’s correlation between selenium content in specimens of *Bidens tripartita* and *B. frondosa* and soil parameters. Statistically significant parameters have been bolded ( $p < 0.05$ ).

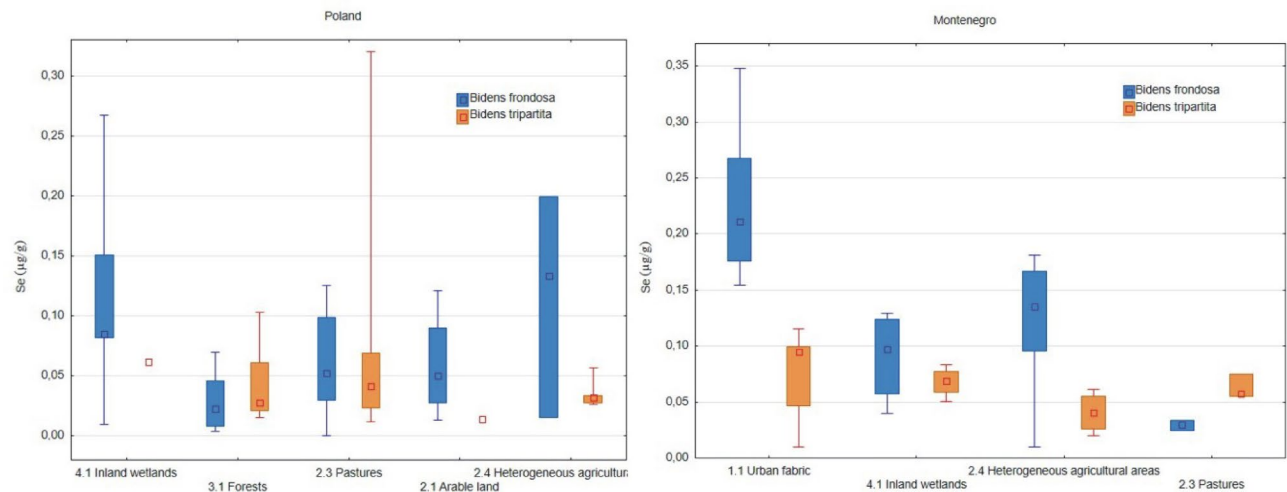
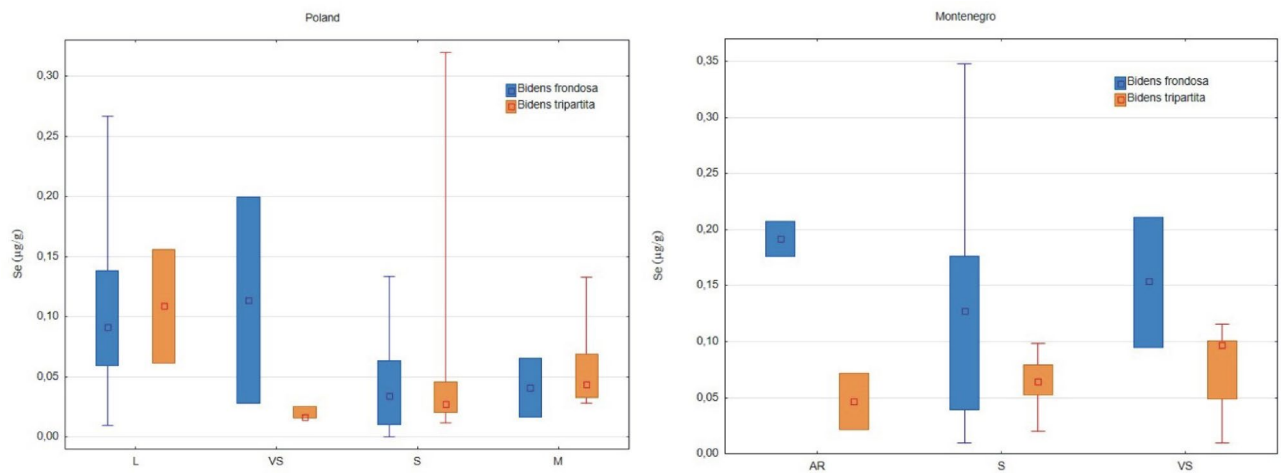


Fig. 4. Differences in selenium content (µg/g) in *Bidens frondosa* and *Bidens tripartita* depending on land: □, Median; □, 25–75%; □, Min–Max.

differences in selenium content depending on the size of the watercourse were statistically insignificant (K–W Test:  $H(3, N = 32) = 5.992045$   $p = 0.1120$ ). However, in case of *B. tripartita*, the differences in selenium content depending on the size of the watercourse were statistically significant (K–W Test:  $H(3, N = 38) = 12.93661$   $p = 0.0048$ ). For *B. tripartita*, post-hoc tests showed significant differences between large and very small river ( $p = 0.044$ ), and between very small and medium river ( $p = 0.047$ ).





**Fig. 5.** Differences in selenium content ( $\mu\text{g/g}$ ) in *Bidens frondosa* and *Bidens tripartita* depending on the type of watercourse:  $\square$ , Median;  $\square$ , 25–75%;  $\text{I}$ , Min–Max.

In Montenegro the selenium content in *Bidens frondosa* was high for plants located on artificial rivers, and in *B. tripartita* for plants located on very small rivers (Fig. 5). In case of both species, the differences in selenium content depending on the size of the watercourse were statistically insignificant (K–W Test: H (2, N = 22) = 1.930016  $p$  = 0.3810; K–W Test: H (2, N = 19) = 0.948421  $p$  = 0.6224).

#### Bioaccumulation of selenium in *Bidens tripartita* and *Bidens frondosa*

The studied herb species differed in selenium accumulation. The bioaccumulation coefficient (BCF) for *B. frondosa* ranged from 0.019 to 4.959, while for *B. tripartita* it ranged from 0.057 to 1.054 (Table 1). Both species also showed differences in selenium accumulation depending on their region of occurrence. In *B. frondosa* specimens from Poland, the lowest BCF value was 0.019, and the highest was 1.764, with an average of 0.311, while for specimens from Montenegro, these values were 0.067, 4.962, and 1.347, respectively. For *B. tripartita* from Poland, the lowest BCF value was 0.057, and the highest was 0.868, with an average of 0.298, while in specimens from Montenegro, it was 0.259, 1.054, and 0.678, respectively.

#### Discussion

Selenium concentrations in soil vary in relation to natural events and human activity. The content of this trace element in soil typically ranges from 0.01 to 2.0 mg/kg, and averages 0.4 mg/kg in the world's soils<sup>38,39</sup>. The selenium content is highly variable and depends on the type of soil and other characteristics of a given location, such as frequency of flooding or method of use. The concentrations of selenium are in general significantly lower in soils which developed in a moderate European climate, in comparison to soils from tropical and subtropical regions<sup>40</sup>. In countries from the balkan region the dominant types of soils are slightly acidic luvisols and brown soils, which show low concentration of selenium<sup>7</sup>, through which the average is equal to 0.27 mg kg<sup>-1</sup><sup>41</sup>. In Poland selenium in sandy soils is equal to on average 0.14 mg/kg (0.06–0.38 mg/kg), in clay soils it is equal to on average 0.3 mg/kg (0.18–0.6 mg/kg), in alluvial soils (in the river valleys) it is equal to on average 0.22 mg/kg (0.12–0.34 mg/kg) and lastly in organic soils it is equal to on average 0.7 mg/kg<sup>42</sup>. The results of our tests confirm this data. Soils in Balkans are reported to be mainly acidic to neutral, and selenium content is very low. Montenegrin soils are more genetically diverse. Typical grey-brown forest soils and podzols with an acidic reaction are found in mountainous areas above plateaus, while alkaline terra rossa soils, products of dolomite and limestone weathering, are found in the coastal zone<sup>43</sup>. Weathering products of dolomite, limestone, and other rocks are also deposited in river valleys. Our studies showed that *B. frondosa* and *B. tripartita* species were found in river valleys with classical carbonate-rich alluvial sediments with an alkaline reaction. It is known that there is a selenium deficiency problem in this region (selenium deficiency in soil occurs below 500  $\mu\text{g/kg}$ )<sup>44</sup>. Jović<sup>45</sup> reported that selenium content in the soils in Serbia and Montenegro ranges from 39 to 440  $\mu\text{g/g}$  (average 230  $\mu\text{g/g}$ ), primarily associated with the silt and clay fraction. The author notes that available forms account for only 1.2–28.2% of the total forms. Our study supports this observation, as the highest recorded concentration of Se in soil samples from Montenegro was 0.246  $\mu\text{g/g}$  (246  $\mu\text{g/kg}$ ), and mean value was 0.143  $\mu\text{g/g}$  (143  $\mu\text{g/kg}$ ). Thanks to silt and clay, clay soils have higher absorptory qualities, which allows more Se absorption. Because of this clay soils have more Se than sandy soils or alluvial soils, however it does not mean that all of the available forms of Se in these clay soils are able to be absorbed by plants<sup>42</sup>.

In soils from both Montenegro and Poland in which *B. frondosa* grew, a positive relationship between organic matter (OM) abundance and soil selenium content was observed in the presented study. Selenium can form complexes with organic matter, and the fraction of Se associated with OM sometimes accounts for up to 40–50% of the total selenium content in the soil<sup>46–48</sup>. In case of *B. frondosa* in Montenegro soils, a positive correlation of the concentration of selenium was noted, not only with OM, but also with some of the other parameters of soil,

including silt and clay, and a significant negative correlation with sand. In this country positive relations between the amount of Se and silt and clay were also present in the subsoil of *Bidens tripartita*. The presented results are compatible with the ones acquired by Matos et al.<sup>49</sup> in regards to the correlation of Se with clay and sand, however there was no correlation there with silt.

Selenium is a metalloid that can compete with sulphur and phosphate when absorbed by the plant root system. There is a relationship between the rate of Se accumulation in plant tissues and its concentration and speciation in soil. The results of our research suggest that there is a relationship between the content of Se in soil and in plants, although the connection between these values differed depending on plant species. In case of *B. tripartita*, the significant positive correlation was in the plants from Poland and Montenegro, while it was non-significant in *B. frondosa*. A high total selenium content in the soil does not always indicate a high content of bioavailable Se<sup>10,22,32,50</sup>. The content of this micronutrient in plants sometimes differed significantly within a species, depending on the region of origin. This was the case with *B. frondosa*, which when collected in Montenegro contained significantly more selenium in its tissues than specimens of the same species growing in Poland, even though the average selenium concentration in the soil is higher in Poland than in Montenegro. It is possible that the higher uptake by *B. frondosa* from Montenegro was due to differences in soil types and other soil properties, as well as the dominance of bioavailable forms of Se. Therefore, the generally predicted relationship, that the total soil Se content is positively correlated with the concentration of this element in the plant, is not straightforward.

The soils where *B. frondosa* grew in Montenegro were alkaline, while in Poland they were mostly acidic, which may have influenced the levels of this trace element in the plants. In alkaline soils, the dominant form of Se is soluble selenates (VI), while in acidic soils, large amounts of Se are immobilized<sup>32</sup>. Organic matter accumulated in the soil also contains significant amounts of Se, which gradually becomes available to plants during its transformation and mineralization<sup>32</sup>. Our studies showed that in *B. frondosa* as well as *B. tripartita* the concentration of Se in the plant was correlated with a higher amount of soil parameters in Montenegrin soils, rather than in Polish soils. With that, the positive correlation of K and Mg could be connected with the structure of the bedrock (big contribution of the limestone skeleton), which affects the pH of the environment. In the neutral and basic environment K, Mg and Se are more available, than in the acidic environment. Moreover in Montenegro, in regards of *B. tripartita*, there was a positive correlation determined between Se in the plant and the salinity, which can have a connection with the salts Ca and Mg, with a basic pH, which come from limestone skeleton. In Poland this correlation is insignificant for both of the species, regardless if it was negative. Because of the acidic pH of the environment the salts of an acidic character can dominate there, which can effect negatively the absorption of Se by the plant. Selenium uptake by plants depends not only on the plant species and the soil parameters discussed, but also on many other factors, including redox potential and environmental factors such as microbial activity and moisture content<sup>6,51</sup>.

In regards to different types of spatial development and land use, the highest concentrations of Se were reported in the material collected on heterogeneous agricultural areas and inland wetlands in Poland. In Montenegro, concentration of Se in plants from the heterogeneous agricultural areas was high as well, but it was the highest in the urban fabric. All of these high values of Se were found in *B. frondosa*. Previous studies suggest that even strong anthropogenic pressure does not significantly impact the natural levels of Se in soil<sup>52,53</sup>. However, agricultural crops are often additionally treated with selenium fertilizers in order to obtain Se-enriched food, especially in regions with a documented selenium deficiency. Edible plants are the main source of selenium in human diet, and lack of this element causes various diseases, such as Keshan disease, hence the need to purposely increase the Se concentration in the consumed crops<sup>1</sup>. This could be the reason that selenium in the examined agricultural areas was of more soluble and bioavailable fraction and explain the enhanced uptake in plants growing there. Agriculture and industrial processes are nowadays the main sources of selenium in the environment (except for natural sources, which are volcanic eruptions and geological processes)<sup>54</sup>, so it is not surprising that the plants from urban and agricultural areas showed the highest Se content among examined specimens.

In personal studies it was determined that only in Poland, and only in case of large and medium sized lakes, does *B. tripartita* show higher concentration of Se in comparison to the invasive *B. frondosa*, which, in both countries, accumulates selenium the most successfully in small rivers, and in Montenegro also in man-made rivers. In the research concerning the content of selenium in macrophytes from running waters<sup>55</sup>, it was stated that the highest Se concentrations were observed in macrophytes collected in streams flowing through villages or near fields, because a significant amount of bioavailable selenium in the environment has anthropogenic source; it can be deposited via plant fertilisers as well as animal feed and excrements<sup>55</sup>. The results of our study also showed an importance of the anthropogenic influence, considering the high Se content in artificial river in examined *Bidens* specimen. Selenium can as well originate from groundwater flowing through rocks rich in certain minerals, and from there it can be deposited into streams<sup>41</sup>; that could also explain the high concentration of this element in *Bidens* tissue from the smallest rivers.

The two species examined in this paper, *Bidens frondosa* and *B. tripartita*, often compete with each other over the habitat across European regions. The invasive *B. frondosa* usually exhibits greater competitive abilities, including reaching higher biomass and height than its native competitor<sup>56</sup>. Our studies indicate that *B. frondosa* has a significantly higher selenium concentrations and greater bioaccumulation abilities (BCF > 1) compared to *B. tripartita*. This may be related to its invasive characteristics, as invasive plant species often utilize various resources and have a greater ability to uptake nutrients and other elements from the environment, including the potentially toxic ones<sup>57</sup>. In case of *B. frondosa* the accelerated growth in the early stages of ontogeny can play a significant role, because it allows it to have an earlier and fuller utilization of the habitats resources<sup>56</sup>. Considering that these plants grow on soils with low selenium content, the accumulation of this element in

the plants does not indicate toxicity of the habitat, but can provide an exogenous source of selenium for living organisms.

## Conclusions

Data obtained as the results of our study showed a positive correlation between the selenium content in the soil and in the tissue of the examined *Bidens* species. Higher Se concentrations were found in *B. frondosa* than in *B. tripartita*, which may be linked to the greater invasive abilities of the former, resulting in more intense uptake and accumulation of soil components. It was also observed, that plants growing in Montenegro reached a higher selenium content than plants of the same species collected in Poland, despite the mean value of Se content in the soil samples in Montenegro being lower than in Poland. The reason could be a different ratio of bioavailable and soluble selenium forms to the total Se content in the examined sites. We also established a great significance of the human impact on the selenium enrichment of plants, as the highest Se concentrations were observed in the plants growing in the areas that were the most influenced by anthropogenic pressure, such as agricultural crops, urban fabrics and artificial rivers.

## Material and methods

### Study area

The research was conducted in the area of two countries—Poland and Montenegro.

Poland is located in the central part of Europe, and extends from the Sudetes and Carpathian Mountains in the south to the Baltic Sea in the north. Poland is mainly located in the Central European Lowlands, from where most of the samples were taken. The others came from the area of the Polish Uplands located to the south<sup>58</sup>. Poland has a moderate transitional climate with both oceanic and continental influences. The geographic location and the predominance of western temperate zone circulation, translates into a greater influence of the Atlantic<sup>59</sup>. According to the Köppen-Geiger climate classification, most of Poland belongs to the oceanic, humid temperate climate (Cfb), generally characterized by cool summers and mild winters<sup>60</sup>.

In Poland, under the influence of climate, vegetation, topography, water, type of bedrock and anthropogenic pressure, different types of soils have formed. On clay formations of glacial or weathering origin Cambisols and Luvisols soils developed; on sandy formations (postglacial, fluvioglacial, aeolian) Podzols, Arenosols and Regosols soils; on highly humid areas Histosols soils; in river valleys Fluvisols (Alluvial soils) of different grain size and moisture content (sands, silts, clays) and Gleysols soils<sup>61,62</sup>.

In Poland, *Bidens frondosa* and *Bidens tripartita* grow mainly in wetlands, such as river banks, marshes and floodplains. Such areas have periodically flooded Alluvial soils, Gleysols and Histosols that are generally strongly moistened. They account for about 8–10% of Poland's soils. These soils are usually fertile, rich in humus and nutrients with a slightly acidic to neutral pH (pH 6–7), less often acidic and alkaline. In addition, these species also occur on clay and sandy soils, and even moist anthropogenic soils in ruderal habitats<sup>63</sup>.

Montenegro is situated on the Balkan Peninsula, and lies within the Dinaric Alps which act as a barrier between Mediterranean and continental air masses. Samples were taken in the southern part, where there is a Mediterranean climate with hot and dry summers and changeable, rainy weather in winter—Csa, and with warm summers—Csb (according to Köppen-Geiger criteria)<sup>60,64</sup>. Due to the topographic conditions of with high relief dynamics, under the combined influences of surface and groundwater, precipitation and temperature, vegetation, and under anthropogenic influence, many types of soils have developed, of which the most dominant in southern Montenegro are the following soil types: Calcomelansol; Eutric Cambisol, Terra rossa, Alluvial soils. District Cambisol with high acidity is most widespread in north-eastern Montenegro, while the coastal zone is dominated by alkaline terra rossa soils<sup>43</sup>.

In Montenegro, the studied *Bidens* species grow on soils of similar origin and moisture conditions as in Poland, but with different chemical characteristics. This is due to the different parent rock—the sediments are often enriched in limestone and dolomite<sup>43,65</sup>.

The surveys were conducted in river valleys of various sizes and artificial watercourses. The following classification of rivers<sup>66</sup> according to the size of the river basin was adopted (very small—lower than 1.000 km<sup>2</sup>, small—1–10.000 km<sup>2</sup>, medium—10.001–100.000 km<sup>2</sup>, large—0.1–1 mln km<sup>2</sup>) (Table 1).

The investigated part of Montenegro belongs to the Adriatic catchment area. The biggest water courses are the Zeta and the Morača that supply Skadar Lake, then the waters are discharged into the Adriatic by the Bojana River. Other smaller rivers and streams like Ricavač, Željeznica, Grdevica, Koložun, Bečića, Veštica, flow directly into the Adriatic sea<sup>67</sup>.

The vast majority of Poland's area belongs to the Baltic Sea drainage basin. The Wisła River basin covers the largest area, within which research was conducted in the valleys of the Nida, Czarna Maleniecka, and Luciąża rivers. The Odra River basin is the second largest, where samples were taken in the valleys of the Odra, Bóbr, Warta, Widawka, Postomia, Ina, Korytnica, Dobrzyca, Rurzyca. Also included in the study were the rivers of the Przymorze, draining directly into the Baltic Sea (Śapólna, Parsęta, Radew, Rega, Łupawa, Śłupia)<sup>68</sup>.

Economic human activity leads to the transformation of the natural landscape, and has a major impact on surface water. It contributes to the pollution of water and soils developed in river valleys<sup>69</sup>. The quality of surface water in Montenegro is threatened by various sources such as insufficiently treated domestic and industrial wastewaters (chemical and pharmaceutical industry), intense mining (lead–zinc, bauxite, barite) and quarry, traffic, intensive tourism, different water activities on rivers (rafting, kayaking, fly-fishing), presence of active agriculture and intensive livestock, meat and food industry in river valleys<sup>70</sup>.

The quality of water in Poland is most strongly influenced by agriculture, especially in terms of eutrophication. The municipal and industrial sectors are now less disruptive, but still contribute to river pollution. The largest municipal and industrial point sources discharged sewage primarily to Poland's main rivers. The impact of

mining is also not insignificant, especially in terms of climate change, leading to increased temperatures and reduced rainfall<sup>71,72</sup>.

### Data collection and analytical procedures

The studies were conducted in 2017–2020 (July–October), in places of river valeys with high coverage of *Bidens* sp. (*Bidentetea tripartiti* communities). The collected material included a total of 113 samples of *Bidens* sp.—58 samples of *B. tripartita* L. (39 from Poland and 19 from Montenegro) and 55 samples of *B. frondosa* L. (33 from Poland and 22 from Montenegro). All samples came from separate locations. One to three plant specimens were randomly harvested at each location. The collected specimens were dried and preserved using standard methods. For each plant sample soil samples were collected using Egner's soil sampler from the plant root zone (0–20 cm). Each sample were air-dried, crushed, and were subjected to selected physicochemical analyses (texture by Bouyoucos method with Casagrande and Prószyński modification), weight percentage of soil skeleton (over 2 mm diameter) [%], acidity (pH in 1N KCl) determined by the potentiometric method, total nitrogen (TN) and total carbon (TC) content was determined using the elemental CHNS analyser (Costech Instruments Elemental Combustion System) [%], calcium carbonate content by Scheibler's method [%], organic matter content by loss on ignition at 550°C [%], the organic carbon (SOC) content was calculated by subtracting the amount of carbon contained in calcium carbonate (CaCO<sub>3</sub>) from the total carbon content (TC), the amount of available forms of soil nutrients (P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O, MgO) extracted in 0,5 M HCl, electrolytic conductivity [uS·cm<sup>-1</sup>] by the potentiometric method.

The selenium (Se) concentration was determined using spectrofluorimetry with a SHIMADZU RF-5001 PC analyzer (Shimadzu, Duisburg, Germany). The process involved wet digestion of samples (whole plants) in concentrated HNO<sub>3</sub> (at 230 °C for 180 min) and HClO<sub>4</sub> (at 310 °C for 20 min). Following this, 9% HCl was introduced to convert selenate VI to selenate IV. Subsequently, selenate IV was complexed with 2,3-diaminonaphthalene (Sigma), and the resulting complex was extracted with cyclohexane (Chempur, Piekary Śląskie, Poland). Fluorescence measurements were taken at an emission wavelength of 518 nm and an excitation wavelength of 378 nm.

To verify the method's accuracy in the analysis of *B. frondosa* and *B. tripartita*, the BCR-402 White Clover Certified Reference Material was utilized. The recovery rate was established at 92% of the reference value, providing assurance of the methodology's reliability.

### Statistical analysis

The bioconcentration factor (BCF) of Se was defined as the ratio of the Se concentration in the plant to that in the soil.

The Spearman correlation was used for statistical analysis of correlations between selenium content in plants and in the soil and between soil selenium content and other soil parameters. Statistical significance of differences: in selenium content, depending on the type of land use; in selenium content, depending on the type of river were tested using non-parametric Kruskal–Wallis test. To indicate significant differences between specific variables, a multiple rank comparison for all samples test post hoc was used. Statistical significance of differences: selenium content depending on particular species; selenium content depending on countries; were tested using non-parametric U-Mann–Whitney test. All statistical analyses were done by Statistica 13.0 software.

### Data availability

The datasets generated during the current study are available in the Figshare repository, <https://doi.org/10.6084/m9.figshare.28603394>.

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## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

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