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Assessment of the Accumulation Ability of *Festuca rubra* L. and *Alyssum saxatile* L. Tested on Soils Contaminated with Zn, Cd, Ni, Pb, Cr, and Cu

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Citation: Pusz, A.; Wiśniewska, M.; Rogalski, D. Assessment of the Accumulation Ability of *Festuca rubra* L. and *Alyssum saxatile* L. Tested on Soils Contaminated with Zn, Cd, Ni, Pb, Cr, and Cu. *Resources* **2021**, *10*, 46. https://doi.org/10.3390/resources10050046

Academic Editor: Eveliina Repo

Received: 26 March 2021
Accepted: 4 May 2021
Published: 9 May 2021

Abstract: The contamination of soils with metals applies, in particular, to areas related to industry, the mining of raw materials and ores, transport, and agriculture. Unlike organic materials, metals cannot degrade over time and need to be reduced, removed, or immobilized in soil. One of the remediation methods for soils contaminated with metals is phytoextraction, which uses plants' ability to accumulate metals in their own tissues. Metals enter the plant organism through the roots and are transported to the aboveground parts, where they are accumulated. In this study, we evaluated the phytoaccumulative abilities of two plant species tested on soils from industrial areas contaminated with metals to different extents (Zn, Cd, Ni, Pb, Cr, and Cu). The research was conducted for three years under the conditions of a pot experiment. In order to obtain four soils with varying degrees of metal contamination, two soils from industrial areas, G1 (contaminated) and G2 (uncontaminated), were mixed in the following ratios: 1:1, 1:3, 1:7, and 1:9. In the phytoremediation process, *Festuca rubra* L. and *Alyssum saxatile* L. were tested. After analyzing the results of the bioconcentration factor (BCF) for the tested plants, it was noted that both of the tested plants accumulated Cd and Zn more easily, followed by Cu, Ni, and Cr, and then Pb to a lesser extent. The values of factors for Cd and Zn were correlated with the high mobility of these elements compared to other metals and their relatively easy uptake by plants. *Alyssum saxatile* L. has an ability to accumulate Cd compared to *Festuca rubra* L., which is confirmed by the BCF (0.764) and transfer factor (TF) (3.5) (for 1:7 combination) values. The calculated results for the BCFs for Alyssum saxatile L. are less than one for all tested metals, which allows us to state that Alyssum saxatile L. is not an accumulator.

Keywords: metals; accumulation; phytoremediation; bioconcentration factor (BCF); transfer factor (TF)

1. Introduction

Metals occurring in soils can cause different reactions in plants at certain concentrations. There are three groups of plants that differ in the relation between plant and soil: accumulating plants, which accumulate metals mainly in shoots equally for both low and high levels of metals in the soil; indicator plants, where uptake and transport to the shoots are regulated so that the internal concentration reflects the level outside; excluding plants, in which the concentration of metal in the shoot is kept at a low level, despite its high concentration in the environment (up to a certain critical value). One of the very popular biological methods of removing pollutants from soils is phytoremediation. Phytoremediation is based on the use of plants capable of removing, carrying, stabilizing, and/or degrading pollutants in the soil [1]. Plants used in the phytoremediation process are characterized by tolerance to high concentrations of compounds in the soil, a high degree of accumulation or biodegradation of pollutants, the ability to accumulate several pollutants at the same time, rapid growth, high biomass production, and resistance to diseases, pests, and difficult environmental conditions [1].

Phytoremediation techniques result from different mechanisms of plant uptake, transport, accumulation, and release of harmful substances. There are several phytoremediation techniques: phytodegradation, rhizodegradation, phytoextraction, phytostabilization, phytovolatilization, rhizofiltration, and phytodesalination [2–10]. These techniques can also be divided according to the processes of various so-called levels of detoxification of pollutants accumulated in the environment, which means that pollutants are located or transformed, or that as a result of these changes, the pollutants infiltrate other environmental matrices [11–23].

The phytoremediation method is widely accepted by society [24–26], and its costs are much lower than traditional remediation methods. The created plant cover protects the soil against erosion and migration of pollutants in the soil profile [27]. This method has minimal impact on the environment because it does not require any changes in the soil structure [25]. After phytoremediation is completed, the area can be reused for agriculture or as farmland. Phytoremediation is applied to groundwater and shallow soil layers, which is related to the construction of the root systems of the used plants. The limitations of this technique also apply to the plant growth rate and to the length of the growing season [27].

Phytoextraction uses plants' ability to accumulate metals in their own tissues. Metals enter the plant organism through the roots and are transported to the aboveground parts, where they are accumulated [28,29]. This technique can be applied in situ. The remediation process may take from several weeks to several months, depending on the growing season of the applied plants. After this time, the plants are harvested and the metals accumulated in the plant aboveground are permanently removed from the site, together with the collected biomass [30].

Phytoextraction is the removal of metals from contaminated soil through their intensive uptake by the plant and accumulation in the shoots [1,7,9,24–26,31]. This method consists of three stages: mobilizing metals in the soil, accumulating mobilized pollutants in plant roots, and transporting them to the shoots [8], where they accumulate [27]. The pollutants posing a threat are stored in vacuoles or react with compounds present in the cytoplasm [27]. The effectiveness of the technique depends on the selection of the right plant. The plants used must be tolerant to high concentrations of metals in the soil and should also be able to intensively absorb pollutants in shoots with a simultaneous large increase in plants' biomass [26,31,32]. Metals or other pollutants can bind directly with protein functional groups, resulting in changes in their conformation and functioning [33]. Metals can interact with the uptake of other elements from the soil, and these interactions can be of a synergistic or antagonistic nature [33–35]. Antagonistic interactions lead to decreased uptake of certain minerals and to nutrient deficiencies. For instance, *Aeluropus littoralis* L. plants exposed to Cd exhibit a hampered accumulation of Fe, Cu, Mn, and Zn in shoots [33,36].

Exposure to metals can influence the contents of chlorophyll by decreasing it [33,37]. All of the described processes result in hampered growth and reduced fitness of metal-stressed plants. Although the direct effect of metals is relatively well studied, there is limited information on plants' post-metal stress performance [33].

Hyperaccumulators can be categorized according to the consistency of their metal accumulation behavior. We distinguish between 'obligate' and 'facultative' hyperaccumulators. The obligate hyperaccumulator species are endemic to some type of metalliferous soil and always exhibit metal uptake at the level defined for hyperaccumulation. Facultative hyperaccumulators, on the other hand, are species with populations in which (some) individuals are hyperaccumulators and other individuals of the same species are not [38]. There is evidence that hyperaccumulators preferentially grow roots in areas of high metal concentration, have elevated levels of uptake into root cell symplasm, and have reduced root vacuolar transport [39]. Nominal threshold criteria are part of an operational framework, complemented with a suite of characteristics that include a bioconcentration factor greater than one (but often >50), a shoot-to-root metal concentration quotient greater than one, and extreme metal tolerance ('hypertolerance') due to effective biochemical detoxification [40]. The facultative hyperaccumulator category covers a variety of situations. It includes particularly those species that occur on both metalliferous and non-metalliferous soils, showing hyperaccumulation from only the metalliferous ones. On normal soils, such plants do not hyperaccumulate, either because they cannot do so because of a genetic difference or because of the low availability of the metal in question [41].

Due to the high content of metals, the biomass collected after the phytoextraction process may pose a threat to humans, animals, and the environment. Therefore, it is subjected to neutralization procedures, which consist of storing the contaminated biomass material in landfills as hazardous waste or incineration. Another way of neutralizing contaminated biomass is subjecting it to the pyrolysis process. Plants can be pyrolyzed in installations for waste processing [27].

The aim of this study was to assess the accumulation abilities of two plant species tested on soils from industrial areas contaminated with metals to different degrees. In the phytoremediation process, *Festuca rubra* L. and *Alyssum saxatile* L. were tested; these were selected for the different mechanisms of plant reaction to metals.

2. Materials and Methods

2.1. Characteristic of Soils

Two soils marked as G1 and G2 were used for the pot experiment. Soil G1 (Figure 1a) was taken from an industrial waste landfill, where it was stored in the form of a heap. This soil was used to clean metallurgical carts/wagons and therefore was contaminated with metals. This soil was characterized by a brown color, specific smell, alkaline pH (8.0), high content of carbonates (4.23 ± 0.12%), organic carbon level of 5.28% (±0.10), total nitrogen level of 1.22% (±0.09), and a high degree of saturation of the sorption complex with exchangeable alkaline cations: Ca^{2+}, 25.2 (±2.3); Mg^{2+}, 6.64 (±0.91); K^{+}, 0.15 (±0.01); Na^{+}, 1.02 (±0.03) (cmol(+)/kg soil).

(**a**)

(**b**)

Figure 1. Soils used in pot experiment: (**a**) G1, soil from slag heap at metallurgical waste site in Krzemionki Opatowskie; (**b**) G2, soil from slag heap located on the west side of Ostrowiec Świętokrzyski Steelworks.

Soil G2 was taken from a heap formed during the construction of the Ostrowiec Świętokrzyski Steelworks (Figure 1b). The examined soil was characterized by a yellow color, earthy smell, and a small content of skeletal fraction (approx. 5.0%, which consisted of fine pebbles and limestone). It had an alkaline pH (7.6), low content of carbonates (0.85% ± 0.05), organic carbon level of 0.90% (±0.05), total nitrogen level of 0.21% (±0.07), and a high degree of saturation of the sorption complex with exchangeable alkaline cations: Ca^{2+}, 12.2 (±1.1); Mg^{2+}, 0.65 (±0.43); K^{+}, 0.12 (±0.01); Na^{+}, 0.04 (±0.02) (cmol(+)/kg soil).

The soils used in this research had different granulometric compositions: loamy sand (G1) and light clay (G2). These soils contained 88% (G1) and 59% (G2) of the sand fraction, 7% (G1) and 21% (G2) of the dust fraction, and 5% (G1) and 20% (G2) of the loam. The total concentrations of metals in soils G1 and G2 are presented in Table 1.

Table 1. Total metal concentration in soils G1 and G2.

Soil	Zn	Cd	Ni	Cr	Pb	Cu
	(mg/kg d.m. soil)					
G1	11,032 ± 348	35.5 ± 2.5	291.3 ± 15.3	784.9 ± 35.9	9885 ± 316	1748 ± 71
G2	66.1 ± 4.3	0.40 ± 0.05	13.2 ± 1.1	9.30 ± 0.79	15.9 ± 1.3	14.2 ± 1.1

In accordance with the Ordinance by the Polish Minister of the Environment on 1 September 2016 on the method of assessing soil contamination [42], metals are one of the main environmental hazards. The above-mentioned Ordinance defines, among other factors, the permissible contents of pollutants in soil, depending on particular soil characteristics and the depths and groups of land. The examined soil samples were classified as the fourth group of land, i.e., industrial areas, and the assessed layer of soil was 0–0.25 m below ground level. None of the examined soil combinations exceeded permissible values of cadmium (15 mg/kg), nickel (500 mg/kg), and chromium (1000 mg/kg) according to the Ordinance. The permissible values were exceeded for lead (600 mg/kg) in all soil combinations. In the case of zinc (2000 mg/kg), the permissible limits were exceeded for all soil samples in the 1:1 and 1:3 combinations, whereas exceedance for copper (600 mg/kg) was observed only in the 1:1 combinations.

2.2. Characteristics of the Pot Experiment

The pot experiment was conducted for three years in the vegetation hall of the Plant Physiology Department of Warsaw University of Life Sciences—SGGW—in Warsaw, Poland. In the pot experiment, Wagner-type pots were used, each filled with 10 kg of soil. In order to obtain soils of varying degrees of metal contamination, soils G1 and G2 were mixed in the following ratios: 1:1, 1:3, 1:7, and 1:9. In total, eight soil combinations were obtained (four soil combinations for each tested plant) in triplicate in a completely random system. The pot experiment was conducted under controlled conditions of soil moisture (60% of field capacity).

Uniform mineral NPK fertilization was applied to all the pots. Fertilizers were introduced into the soil before sowing the seeds. The basic fertilization was: 0.39 g of N, 0.13 g of P_2O_5, and 0.26 g of K_2O per pot, which is equivalent to doses of 102 kg of N, 34 kg of P_2O_5, and 68 kg of K_2O per hectare. In the experiment, the following substances were used: 0.29 g of phosphorus and potassium fertilizer, 0.98 g of ammonium nitrate, and 0.23 g of potassium nitrate per pot. Phosphorus and potassium were used once, and nitrogen was administered in two doses during plant vegetation. During the vegetation period of the plants, their development phases were observed. Two plant species were tested simultaneously: *Festuca rubra* L. (P1) and *Alyssum saxatile* L. (P2).

In order to determine the size of the yield and the content of the collected metals, the tested plants were collected one or three times during the growing season, depending on their species. The first cut of *Festuca rubra* L. was harvested during the tillering phase, the second cut was harvested in the stage of earing, and the third cut was harvested in the flowering stage. The biomass of *Alyssum saxatile* L. was harvested once a year, in the first year in the flowering phase, and in another two years in the fruit's ripening phase. For analysis, the shoots and roots of the tested plants were used.

2.3. Methods

The following physical and chemical properties were determined in soils G1 and G2: granulometric composition with the Casagrande method in Prószyński's modification, according to the Polish Society of Soil Science, pH in 1 M KCl [43], organic carbon content [44], total nitrogen [45], carbonate content [46], and exchangeable alkaline cations (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}) [47].

The total metal content of the soil and plants was established after mineralization of the samples in a 3:1 mixture of concentrated perchloric acid ($HClO_4$) and nitric acid (HNO_3) [48,49]. The content of metals (Zn, Cd, Ni, Cr, Pb, and Cu) in the ammonium nitrate (1 M NH_4NO_3) extract, according to DIN ISO 19730 [50], and in the diethylenetriaminepentaacetic acid (0.2M DTPA) extract, according to PN-ISO 14870:2007P [51], were determined to assess metal forms solubility in the soil solution. In addition, hydrochloric acid (1 M HCl) was used for the assessment of the phytoavailable metal forms in soils (according to the procedure accepted in agricultural and chemical stations in Poland) [47].

The metal content in the studied samples was determined by using atomic spectrometry, with the technique of inductively coupled plasma optical emission spectrometry (ICP-OES), using Thermo Scientific's iCAP 6500 or Varian Axial Vista 720-ES spectrometer. For each series of measurements, blank samples were prepared in parallel, and their values were included when calculating the results for the samples. Dry mass was also included in the calculations. The accuracy of the methodology was verified using the method of standard additions.

2.4. Statistical Analysis

The mean values of the results were calculated based on the samples taken from the pot experiment over the three-year duration of the experiment and are shown in Tables 2–6 and Figures 2–5. Analysis of variance [52] was used for statistical analysis of particular soil variables. If the variables did not meet the criteria, their transformations were used instead, mostly logarithmic functions or inverse values. The least significant difference (LSD) for the Student's *t*-test was used as the basis for comparison [53–56]. Statistical analyses were performed in R (R-project).

Table 2. Total content of metals in soil, dependent on combination.

G1:G2 /Plant	Total Content of Metals in Soil * (mg/kg d.m.)											
	Zn		Cd		Ni		Cr		Pb		Cu	
	Mean	σ	Mean	σ	Mean	σ	Mean	σ	Mean	σ	Mean	σ
1:1/P_1	5095	139	12.80	0.84	140.8	6.0	328.7	18.3	5225	183	748.4	31.5
1:1/P_2	5101	143	12.95	0.67	139.0	6.9	298.2	14.7	4804	133	745.2	37.3
LSD_0.95	118.5		0.72		10.4		26.6		231.7		36.1	
1:3/P_1	2522	86	6.74	0.60	57.7	4.5	180.9	7.6	1892	61	383.9	23.3
1:3/P_2	2154	90	7.22	0.61	63.7	5.7	190.3	9.4	1872	60	412.7	13.3
LSD_0.95	113		0.77		6.7		11.0		78		24.4	
1:7/P_1	1157	49	3.92	0.48	53.0	3.3	77.5	5.2	1087	52	258.2	15.7
1:7/P_2	1201	37	3.78	0.41	41.9	3.4	74.2	7.0	1013	41	265.4	21.8
LSD_0.95	56		0.57		4.3		8.0		60		24.4	
1:9/P_1	1017	41	3.52	0.63	42.6	3.3	57.2	4.3	848.0	25.6	143.7	7.5
1:9/P_2	972.5	44.2	3.05	0.28	25.9	2.5	53.3	4.5	810.2	37.7	129.7	11.5
LSD_0.95	55		0.63		3.8		5.7		41.5		12.5	

* Mean value of the studied variable for individual pot combinations over the years; σ—standard deviation.

Table 3. Metal content after 1 M HCl extraction, dependent on combination.

G1:G2 /Plant	Metal Content after 1 M HCl Extraction * (mg/kg d.m.)											
	Zn		Cd		Ni		Cr		Pb		Cu	
	Mean	σ	Mean	σ	Mean	σ	Mean	σ	Mean	σ	Mean	σ
1:1/P_1	4595	156	11.50	0.82	64.0	4.3	145.0	6.0	4370	204	618.2	31.2
1:1/P_2	4578	138	10.88	2.02	56.3	3.3	124.2	7.1	4051	109	638.4	33.9
LSD_0.95	128.7		1.14		3.9		9.7		152.5		26.5	

Table 3. *Cont.*

G1:G2 /Plant	Metal Content after 1 M HCl Extraction * (mg/kg d.m.)											
	Zn		Cd		Ni		Cr		Pb		Cu	
	Mean	σ	Mean	σ	Mean	σ	Mean	σ	Mean	σ	Mean	σ
1:3/P_1	2252	81	5.18	0.44	39.5	2.8	75.2	5.4	1690	80	347.3	15.4
1:3/P_2	1926	59	5.13	0.43	36.7	3.8	68.5	8.6	1670	54	304.4	18.5
LSD_0.95	92		0.56		4.3		9.2		88		21.9	
1:7/P_1	1045	40	3.26	0.33	23.5	1.6	48.7	6.6	967.0	45.6	227.7	8.6
1:7/P_2	1018	36	2.95	0.54	22.1	1.9	50.5	8.9	902.4	29.4	214.9	13.4
LSD_0.95	49		0.57		2.3		10.1		49.3		14.5	
1:9/P_1	923.7	47.0	2.56	0.28	13.0	1.3	44.6	2.7	688.0	23.2	126.7	6.1
1:9/P_2	856.5	31.5	2.47	0.20	12.9	1.2	39.2	4.2	721.8	23.5	105.1	6.4
LSD_0.95	51.5		0.32		1.6		4.6		30.1		8.1	

* Mean value of the studied variable for individual pot combinations over the years; σ—standard deviation.

Table 4. Metal content after DTPA extraction, dependent on combination.

G1:G2 /Plant	Metal Content after DTPA Extraction * (mg/kg d.m.)											
	Zn		Cd		Ni		Cr		Pb		Cu	
	Mean	σ	Mean	σ	Mean	σ	Mean	σ	Mean	σ	Mean	σ
1:1/P_1	266.9	12.8	1.95	0.18	1.72	0.14	3.68	0.37	660.1	39.1	110.2	6.1
1:1/P_2	245.7	19.0	1.92	0.17	1.57	0.12	3.70	0.25	611.9	24.7	96.9	4.9
LSD_0.95	12.9		0.14		0.11		0.29		31.9		5.23	
1:3/P_1	151.7	10.0	1.27	0.11	<det.	-	3.40	0.33	461.4	18.1	63.2	4.6
1:3/P_2	140.4	8.5	1.19	0.15	<det.	-	3.30	0.28	459.1	23.5	53.7	4.7
LSD_0.95	11.9		0.17		-		0.39		26.9		6.0	
1:7/P_1	101.8	5.6	0.83	0.09	<det.	-	3.10	0.37	295.1	17.4	36.7	2.5
1:7/P_2	98.2	4.9	1.05	0.17	<det.	-	2.93	0.30	298.3	13.3	33.3	2.7
LSD_0.95	6.8		0.17		-		0.43		19.9		3.3	
1:9/P_1	75.4	4.5	0.71	0.09	<det.	-	2.70	0.22	252.9	18.3	29.0	1.9
1:9/P_2	79.8	3.3	0.77	0.10	<det.	-	2.72	0.19	270.5	17.1	26.8	2.7
LSD_0.95	5.1		0.12		-		0.27		22.8		3.0	

* Mean value of the studied variable for individual pot combinations over the years; σ—standard deviation; - no possibility of calculating the value.

Table 5. Metal content after 1 M NH_4NO_3 extraction, dependent on combination.

G1:G2 /Plant	Metal Content after 1 M NH_4NO_3 Extraction * (mg/kg d.m.)											
	Zn		Cd		Ni		Cr		Pb		Cu	
	Mean	σ	Mean	σ	Mean	σ	Mean	σ	Mean	σ	Mean	σ
1:1/P_1	146.9	8.5	1.10	0.07	2.20	0.21	2.08	0.17	6.68	0.59	77.4	5.9
1:1/P_2	137.0	6.9	0.93	0.08	2.30	0.24	<det.	-	6.23	0.55	70.2	4.2
LSD_0.95	8.6		0.02		0.18		-		0.49		3.9	
1:3/P_1	68.5	2.7	0.51	0.08	1.30	0.19	<det.	-	2.52	0.30	37.2	2.9
1:3/P_2	64.6	4.0	0.47	0.04	1.20	0.14	<det.	-	2.98	0.28	34.3	3.1
LSD_0.95	4.4		0.08		0.22		-		0.37		3.8	
1:7/P_1	31.0	1.8	0.29	0.04	<det.	-	<det.	-	2.50	0.20	9.4	0.8
1:7/P_2	34.5	2.5	0.31	0.04	<det.	-	<det.	-	2.70	0.24	9.5	0.5
LSD_0.95	2.8		0.05		-		-		0.29		0.8	
1:9/P_1	22.7	1.2	<det.	-	<det.	-	<det.	-	1.88	0.18	4.4	0.4
1:9/P_2	15.4	1.3	<det.	-	<det.	-	<det.	-	1.87	0.16	3.9	0.3
LSD_0.95	1.6		-		-		-		0.22		0.5	

* Mean value of the studied variable for individual pot combinations over the years; σ—standard deviation; - no possibility of calculating the value.

Table 6. Yield of plant dry mass tested on soils with different levels of metal contamination, dependent on combination.

G1:G2	Plant	Yield of Plant (g d.m.)	
		Mean *	**σ**
1:1	P1—*Festuca rubra* L.	11.82	2.10
	P2—*Alyssum saxatile* L.	0.67	0.60
	LSD_0.95	11.43	
1:3	P1—*Festuca rubra* L.	17.46	1.01
	P2—*Alyssum saxatile* L.	23.25	9.83
	LSD_0.95	8.98	
1:7	P1—*Festuca rubra* L.	19.72	1.37
	P2—*Alyssum saxatile* L.	35.96	16.48
	LSD_0.95	15.04	
1:9	P1—*Festuca rubra* L.	23.64	1.08
	P2—*Alyssum saxatile* L.	37.99	18.22
	LSD_0.95	16.61	

* Mean value of the studied variable for individual pot combinations over the years; σ—standard deviation.

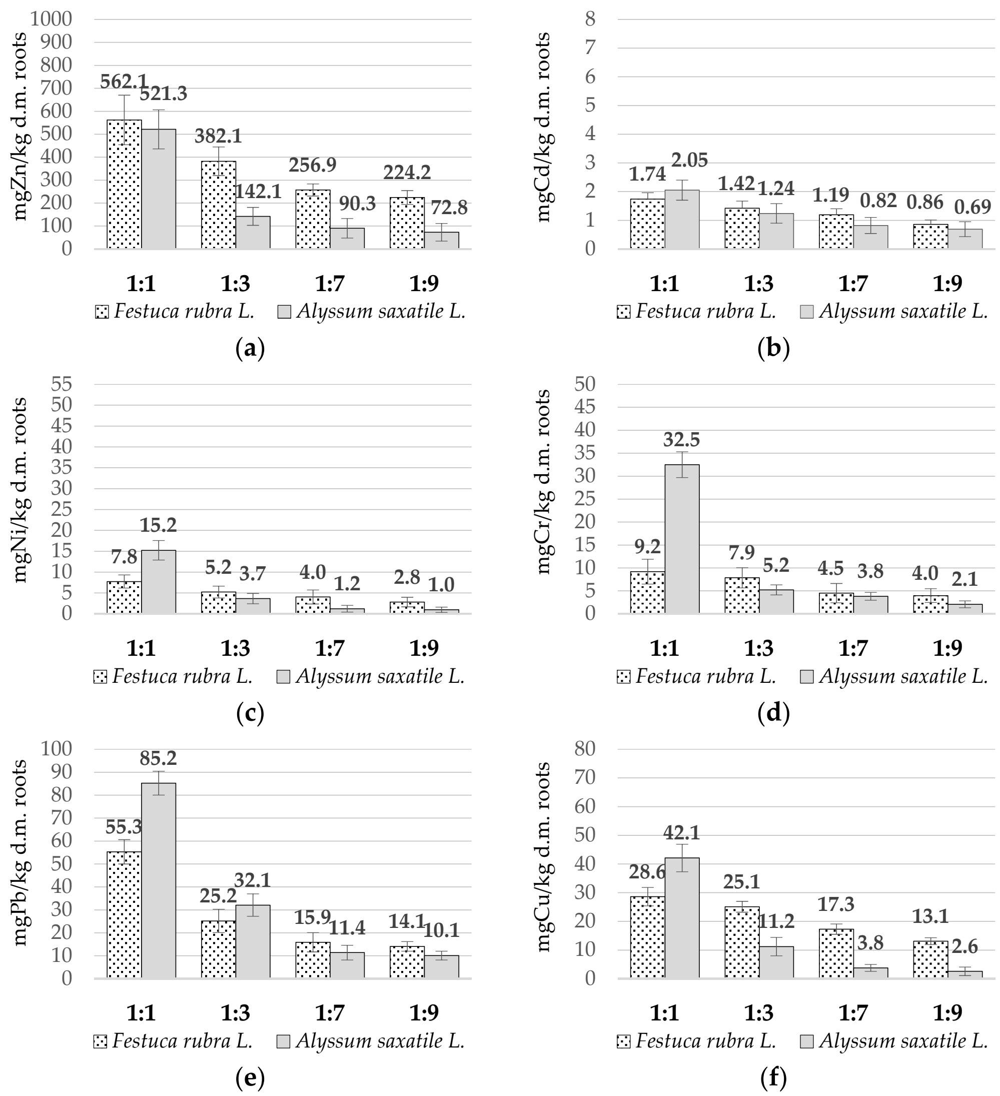

Figure 2. Metal concentration in dry mass roots of plants tested in soils with different contamination levels: (**a**) zinc; (**b**); cadmium; (**c**) nickel; (**d**) chromium; (**e**) lead; (**f**) copper.

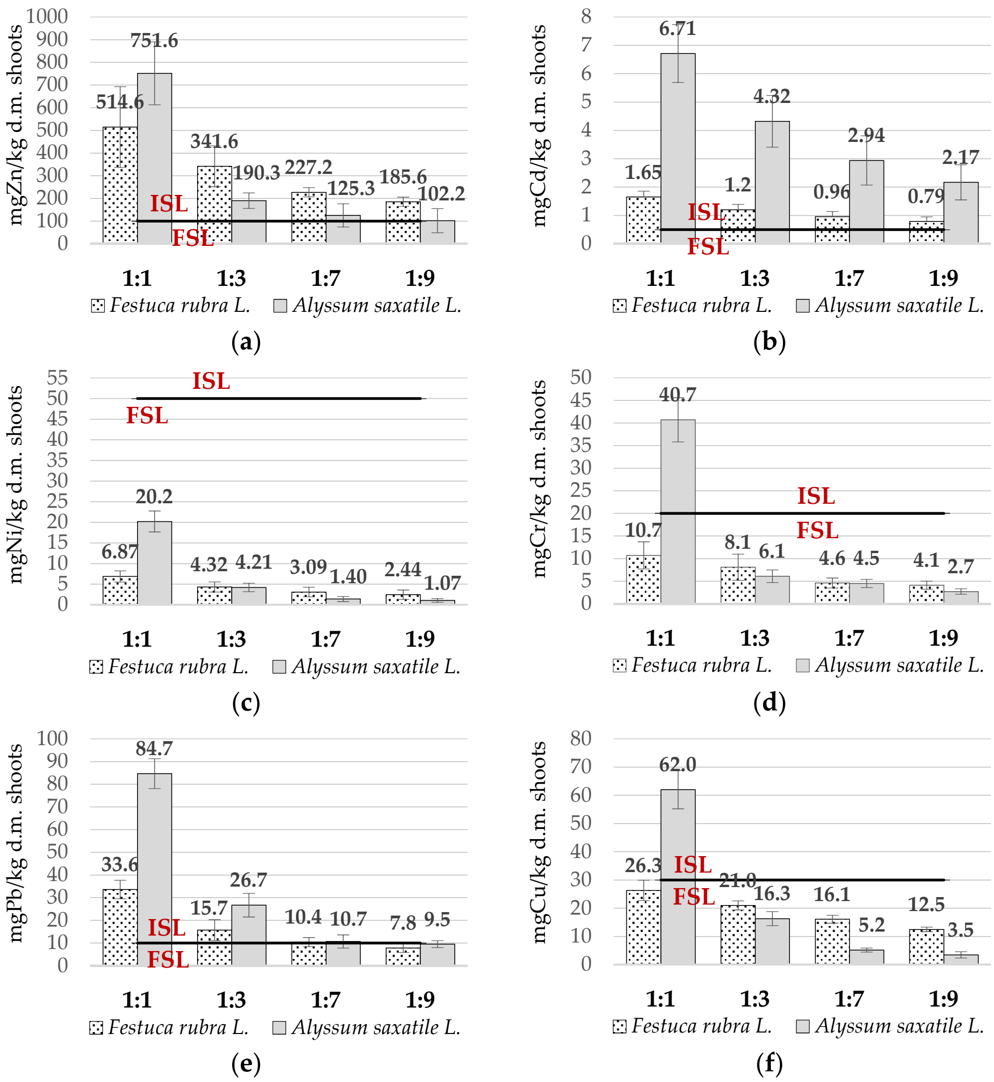

Figure 3. Metal concentration in dry mass (shoots) of plants tested on soils with different contamination levels: (**a**) zinc; (**b**); cadmium; (**c**) nickel; (**d**) chromium; (**e**) lead; (**f**) copper.

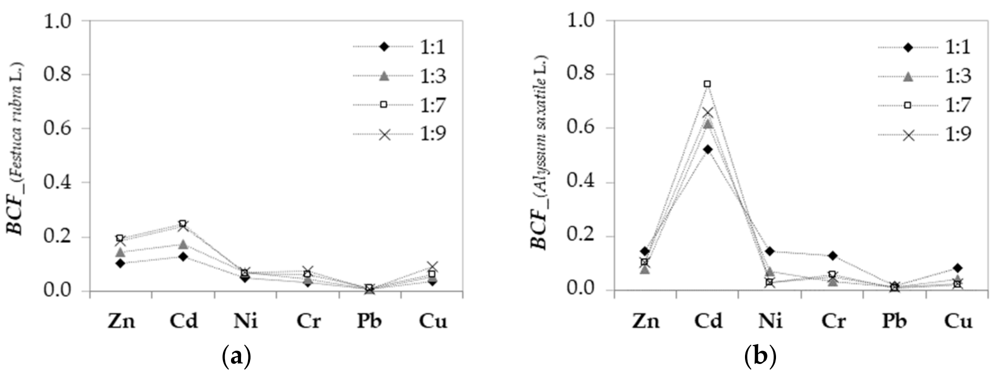

Figure 4. Bioconcentration factors (BCFs) of individual metals in (**a**) *Festuca rubra* L. and (**b**) *Alyssum saxatile* L.

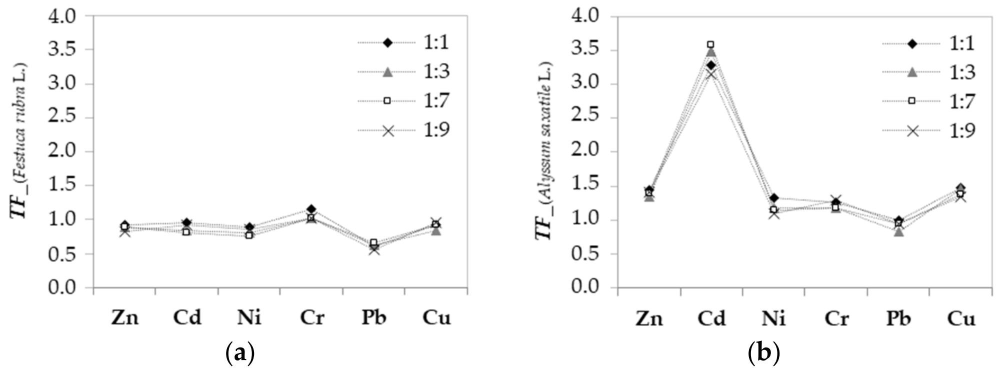

Figure 5. Transfer factors (TFs) of individual metals in (**a**) *Festuca rubra* L. and (**b**) *Alyssum saxatile* L.

3. Results

3.1. Total Content of Metals in Soil

Table 2 presents the results of the determination of the total content of zinc, cadmium, nickel, chromium, lead, and copper in the soil for individual combinations for which both plants were tested. The performed statistical analysis allowed assessment of the occurrence of significant differences between the total metal contents for both tested plants.

As a result of the analysis of zinc, statistically insignificant differences were found between the soils when the different plants were tested using soil combinations 1:1, 1:7, and 1:9. Only in the 1:3 combination of soils showed a significantly lower zinc content when *Alyssum saxatile* L. was tested in comparison to *Festuca rubra* L. There were no significant differences in the cadmium and chromium contents in the tested soils in any combination, taking into account the different plants tested. For the 1:1 combination of soils, significant differences were observed for the nickel and lead contents in the tested soils due to the tested plant species. A significant influence of the tested plant was also noted in soil combinations 1:7 and 1:9, where lower nickel contents were found when *Alyssum saxatile* L. was tested. In particular, the lead content in the soils of *Festuca rubra* L. was significantly higher. The effect of this plant on the lead content was also noted in the soil combination 1:7. As a result of the analysis of variance for copper, the differences between the 1:1 and 1:7 soil combinations were found to be statistically insignificant for both plants.

Of the remaining soil combinations 1:3 and 1:9, the reduction of the copper content was significantly influenced by the species of the tested plants; for the 1:3 combination, lower copper content was noted for *Festuca rubra* L., while for the soil combination 1:9, lower copper content was noted for *Alyssum saxatile* L.

3.2. Metal Forms Extracted with 1 M HCl

In studies using 1 M HCl extraction, very high contents of metal forms were obtained for all of the metals tested. Table 3 presents the results of the determinations of the forms of zinc, cadmium, nickel, chromium, lead, and copper in soil after 1 M HCl extraction for the individual combinations for both plants.

As a result of the analysis of variance for zinc, statistically insignificant differences were found between the soils where both plants were tested using soil combinations 1:1 and 1:7. Only on soil combinations 1:3 and 1:9 did *Alyssum saxatile* L. significantly reduce the forms of zinc after 1 M HCl extraction compared to *Festuca rubra* L. None of the tested plant species significantly decreased the cadmium content in the tested soils. For nickel, a statistically significant difference was found between the individual species of the tested plants only in the case of the 1:1 combination of soils, where *Alyssum saxatile* L. significantly influenced the reduction of nickel forms in the tested soils. Comparing the influence of the tested plant species, a significant decrease in the chromium content was found in soil combinations 1:1 and 1:9 for *Alyssum saxatile* L. in relation to *Festuca rubra* L. When analyzing the influence of the tested plant species, for lead, no significant differences were found in the 1:3 soil combination. Regarding the 1:1 and 1:7 combinations of soils, the highest lead content was noted for *Festuca rubra* L. and the lowest for *Alyssum saxatile* L., which means that *Alyssum saxatile* L. significantly reduced the content of lead forms compared to *Festuca rubra* L. For the 1:9 combination of soils, this relationship was reversed. For copper, a statistically significant difference was found between individual species of the tested plants only for the 1:3 and 1:9 soil combinations.

When comparing the phytoremediative usefulness of individual plant species, in most cases, the differences were not found to be significant. The cases where such significance was demonstrated was for *Alyssum saxatile* L., which had a greater effect than *Festuca rubra* L.

3.3. Metal Forms Extracted with DTPA and 1 M NH_4NO_3

The results obtained after the extraction with the DTPA and 1 M NH_4NO_3 solution are summarized in Tables 4 and 5.

Single extractions were carried out, among others, using solutions of complexing compounds: EDTA, DTPA, and EDTA-AcOH/NH_4OAc [57–60]. In order to determine phytoassimilable forms, i.e., those currently available for plants, and potentially soluble forms, i.e., those that can be absorbed by plants due to changes taking place in the soil, single extractions were carried out with the use of two reagents: DTPA and 1 M NH_4NO_3. Numerous authors [61–63] believe that, for the assessment of the actual solubility of metals in soil solutions, which largely determines the current absorption of metals for plants— especially in contaminated soils—neutral salt solutions are the best extraction solutions.

Table 4 shows that only for the 1:1 combination did the zinc content in the soil significantly decrease (compared to the soil where *Festuca rubra* L. was tested). *Alyssum saxatile* L. did not significantly affect the nickel content in the soil in comparison to *Festuca rubra* L. For the plants tested, no significant effect on the content of cadmium, chromium, or lead was found. Comparing the influence of the tested plants, it was found that for the combinations of 1:3 and 1:7, *Alyssum saxatile* L. had a significantly greater effect on the reduction of copper content in the tested soils than *Festuca rubra* L.

A solution of ammonium nitrate (1 M NH_4NO_3) was used for the extraction of metals from the soils in the experiment due to the specificity of the described soils and the widespread use of this method (e.g., standardization in Germany). The results obtained are shown in Table 5. *Alyssum saxatile* L., only for the combinations of 1:1 and 1:3, did not significantly reduce the zinc content in the soil (compared to the soil where the *Festuca rubra* L. was tested). For Cd, Ni, Pb, and Cu, it is not possible to unequivocally state a higher influence of *Alyssum saxatile* L. than *Festuca rubra* L. on the content of tested metals in the tested soils. On the contrary, a significant difference can be noticed between the action of *Alyssum saxatile* L. and *Festuca rubra* L. for the selected combinations.

The use of a DTPA solution turned out to be the most effective for Pb and the least effective for Cd. The ammonium nitrate solution showed the highest extraction capacity in relation to Zn, and compared to DTPA, it showed a better ability to extract Ni.

3.4. Yield and Metal Content in the Plants

The study determined the yield of the plants of *Festuca rubra* L. and *Alyssum saxatile* L. and showed various tolerances to the content of metals in particular soil combinations. Symptoms of the phytotoxic effect of metals were observed, especially in plants tested using the most contaminated soils (combination 1:1). The metal toxicity in the plants was confirmed by the results of dry matter yields, which are summarized in Table 6. The biomass of the aboveground parts of *Alyssum saxatile* L. increased with a decrease in the metal content in individual soil combinations and was higher than in *Festuca rubra* L.

An important parameter determining the phytotoxicity of a given metal is the plant species as well as its variety [64]. The different sensitivities of plants to individual metals enables the selection of plants capable of growing in concentrations that are toxic [65,66]. The increased content of metals necessary for plants for the proper functioning of plant cells (including Cu, Zn, and Ni) and redundant metals that do not fulfill metabolic functions (e.g., Cd, Pb, and Cr) is unfavorable for their yield [61]. This adversely affects the growth and development of most plant species and consequently leads to phytotoxicity, which is the result of the disruption of physiological processes due to disturbances in the uptake and transport of macro- and microelements. Unfortunately, there is no ideal plant that would meet all the conditions in the phytoremediation process, especially when the soil is contaminated with various metals. Plants may stop growing at high concentrations of metals [67]. The intended efficiency with the use of phytoextraction may be significantly limited due to the high content of metals, bordering on the toxicity threshold [27].

Figure 2a–f show the results for Zn (a), Cd (b), Ni (c), Cr (d), Pb (e), and Cu (f) content in the roots of the two plants tested in each of the soils used in the experiment. For the roots, the ISL and FSL values were not marked because the plants did not have edible roots.

The appearance of plants in each combination confirms the results of other researchers, who stated that germination is an indicator that is slightly influenced by the presence of metals [68]. Many metal-sensitive plants germinate in contaminated soils but then stop growing due to, among other reasons, severely shortened root systems, which was reported for *Alyssum saxatile* L. in the most polluted soil. Various studies have shown that the phytotoxicity of most metals consists primarily of the incomplete and delayed germination of sown seeds, delayed germination, and various types of deformation of the root system as well as inhibition of its growth [69]. Most often, disturbances within the root system caused growth limitation and limited the development of the aboveground parts of the studied plants.

For *Festuca rubra* L., the mean values were higher by 12% for Zn, 12% for Cd, 16.5% for Ni, 39.1% for Pb, and 9% for Cu in the roots than in the shoots. The opposite correlation was found for Cr, as *Festuca rubra* L. accumulated more Cr in its shoots by an average of 5.4%. In *Alyssum saxatile* L., unlike in the case of Festuca rubra L., it was found that most of the tested metals were accumulated in the shoots instead of the roots of the plant, where the average content was higher by 28.2% for Zn, 70.3% for Cd, 14.5% for Ni, 18.1% for Cr, and 29% for Cu. For the Pb content of *Alyssum saxatile* L., the opposite correlation was found, as there was 7.4% more Pb in the roots of this plant than the shoots.

The results of the metal content in the dry matter and shoots of the *Festuca rubra* L. and *Alyssum saxatile* L. are presented in Figure 3.

To evaluate the usefulness of the biomass of the tested plants, determination of the content of metals Zn, Cd, Ni, Cr, Pb, and Cu in the dry matter of *Festuca rubra* L. and *Alyssum saxatile* L. was carried out, along with comparison of the limit levels for the forage suitability and industrial suitability of biomass. Among the tested metals, both plants contained the highest concentrations of zinc, cadmium, and lead. The content of the individual metals in the tested plants increased with a higher degree of soil contamination.

The critical content of metals was adapted for the assessment of the plants for the forage suitability level (FSL) or industrial suitability level (ISL) [70]. In the case of zinc and cadmium (Figure 3a,b), only industrial suitability was found for both tested plants due to exceeding the 100 mgZn/kg d.m. and 0.5 mgCd/kg d.m. thresholds. The content of lead (Figure 3e) was suitable for forage only in the plants tested in the soil combination of 1:9. For nickel (Figure 3c), both plants were found to be useful for forage due to a content of nickel not exceeding the 50 mgNi/kg d.m. threshold. The content of chromium and copper (Figure 3d,f) for both plants was found to be useful for forage, with the exception of the 1:1 soil combination for *Alyssum saxatile* L., where the concentration of Ni exceeded the forage suitability index.

The factors that most severely limit the effectiveness of soil remediation include slight penetration by shortened plant root systems and incomplete coverage of the soil surface by plants [27], which were confirmed by the observations for the tested species of *Alyssum saxatile* L. In the 1:1 combination of soils, significantly higher concentrations of the tested metals were observed in *Alyssum saxatile* L. than in the other tested plants. This proves the high phytoremediation usefulness of the *Alyssum saxatile* L. in heavily contaminated soils. In the other combinations, the contents of zinc, nickel, and copper in *Alyssum saxatile* L.

were lower than in *Festuca rubra* L., the cadmium content was higher, and in the case of chromium and lead, no significant differences were found.

3.5. Bioconcentration Factors (BCFs)

The value of the bioconcentration factor (BCF) determines the relationship between the concentration of metal taken up by the aboveground parts of the plant (shoots) and its concentration in the soil [71,72]. This indicator is the ratio of the metal content in the plant to the total metal content in the soil. The content of metals in plants can vary depending on their ability to move from soil to aboveground parts [10,73]. Figure 4 presents the calculated values of the BCF for individual metals in *Festuca rubra* L. and *Alyssum saxatile* L.

Analyzing the obtained BCF values for Zn, Cd, Ni, Cr, Pb, and Cu, it should be stated that, under conditions of soil contamination with metals, plants accumulate Cd and Zn more easily than Cu, Ni, Cr, and to a lesser extent, Pb. This was confirmed by the bioconcentration factor of 0.249 (for cadmium) noted for the soil combination of 1:7 for *Festuca rubra* L. (Figure 4a). High values for zinc and cadmium testify to the high mobility of these elements compared to other metals and their relatively easy uptake by plants. In contrast, for the same combination as for cadmium, the BCF for lead was 0.01. The lowest values of the BCF found for lead are due to the lowest mobility of lead from soil to plant tissues.

Figure 4b shows the calculated values of the BCF for individual metals in *Alyssum saxatile* L. The values of this factor for cadmium are clearly higher than for the other elements; the bioconcentration factor reached a value of 0.764, meaning that *Alyssum saxatile* L. is not an accumulator plant but instead has selective ability toward cadmium accumulation in its shoots.

3.6. Transfer Factors (TFs)

The transfer factors of individual metals in *Alyssum saxatile* L. and *Festuca rubra* L. are presented in Figure 5.

Metal transfer from roots to shoots is determined by the transfer factor (TF). The transfer factor describes the proportion of the metal in soil or in soil solution that is taken up into the plant [56]. This is defined as the ratio of the concentration of metals in shoots to their concentration in roots [74,75].

In the case of *Festuca rubra* L., all of the tested metals have values below one. In the case of Cr, however, its value slightly exceeds one for all tested combinations (Figure 5a). For *Alyssum saxatile* L., for all metals except Pb, the transfer factor values exceeded one. For Pb, the TF values were below one for each of the tested combinations (Figure 5b).

4. Discussion

Plants are increasingly used to restore the biological balance in industrial and highly urbanized areas. However, the phytoremediation method faces a number of limitations. One of the basic problems of phytoremediation is the relatively long remediation time, which, in the case of metals, may even be over 30 years due to the degree of soil contamination. The rate of removal of pollutants is influenced by factors such as the length of the growing season, soil type, pH value, the appropriate amount of nutrients, water, and plant resistance to diseases and pests [30,76–79]. The process of accumulation of metals in plants is relatively simple. It consists of three phases: ion mobilization, uptake, and transport to plant organs. The intensity of this process is proportional to the concentration of metals. Most of the taken-up ions are retained in the plant's root system by structural elements of the cell wall, e.g., pectin compounds and proteins. An obstacle in the use of plants in phytoremediation processes may also be their insufficient resistance to high concentrations of toxic compounds contaminating the environment [30].

The results of this study showed that the contents of Zn, Cd, Ni, Cr, Pb, and Cu were different in individual plants, depending on the species. In *Festuca rubra* L., among the examined metals, the highest content was found in the case of zinc, followed by lead and copper. The content of individual metals in *Alyssum saxatile* L. increased with the increase in the degree of soil contamination with metals. Regarding the combination of soils heavily contaminated with metals (1:1), significantly higher concentrations of the tested metals were observed in *Alyssum saxatile* L. than in *Festuca rubra* L. In the other combinations, the contents of zinc, nickel, and copper in *Alyssum saxatile* L. were lower than in *Festuca rubra* L., while the cadmium content was higher. For chromium and lead, no significant differences were found. The factors that most severely limit the effectiveness of soil remediation include differences in plant root systems and incomplete coverage of the soil surface by plants [80], which was confirmed by the observations for the tested species of *Alyssum saxatile* L. Plants can accumulate metals from soil only to the depth of penetration through the root system; contaminants lying below the reach of the root system are not accessible to plants and do not undergo remediation [30].

Metals in the soil, after exceeding the toxicity threshold, are responsible for the delay or even end of plant life processes, which was found in this study in the case of *Alyssum saxatile* L. Analyzing the obtained BCF values for Zn, Cd, Ni, Cr, Pb, and Cu, it can be stated that, from soil contaminated with metals, plants accumulate Cd and Zn more easily than Cu, Ni, Cr, and to a lesser extent, Pb. Similar results were obtained by other authors [61], arranging metals according to their decreasing mobility: Cd > Zn > Ni > Cu > Pb. High factors for zinc and cadmium testify to the high mobility of these elements compared to other metals and their relatively easy uptake by plants. The change of metal locations in the series may be related to the high metal content in the soil, which can be observed in the case of Cu–Ni. Cd is the metal most susceptible to accumulation from the soil by plants [81].

Various authors have pointed out the difference in the availability of metals and their various forms for plants. Other researchers [82–84] point out that the uptake of metals from the soil by plants is greatly influenced by bound and bioavailable forms (sometimes divided into bioavailable and bioavailable for plants). The use of various chelating reagents allows the determination of these forms in soil. The author of [82] suggested the use of a 1 M solution of ammonium nitrate to identify readily available forms. For the determination of all forms of metals in soil, other researchers [84] suggested the use of DTPA or EDTA as the solution with the highest efficiency for extracting bound forms potentially available to plants. As a result of metal extraction using a DTPA solution and a 1 M solution of ammonium nitrate, higher metal contents were extracted with DTPA for Zn, Cd, Cu, Pb, and Cr. For ammonium nitrate, only for Ni were higher values obtained in comparison to the DTPA solution. In this research, the applied DTPA solution was the most effective for Pb and the least effective for Ni and Cd. For the ammonium nitrate solution, the highest efficiency was found toward Zn compared to the other metals, and the lowest, toward Cr.

The mechanism of heavy metal ion uptake by plant roots from the soil solution is the result of several processes, such as cation exchange through the cell membranes, intracellular transport, and rhizosphere processes [81]. The root zone of plants plays a major role in the degradation of pollutants, releasing compounds such as sugars, organic acids, and amino acids, which increase the bioavailability of metals and thus cause their greater accumulation in the plant. On the other hand, by releasing organic acids, alcohols, sugars, and enzymes, plants support the development of the soil microflora [85].

The participation of microorganisms in phytoremediation technology consists mainly of the production of proteins, enzymes, and biocatalysts [86]. According to the literature, metals collected from the soil solution accumulate more in the roots than in the shoots, proportional to the increase in their content in the substrate. In the roots, metals are mainly bound to the cell wall. Moreover, fewer metals were found in the protoplast region.

Depending on the properties of the plant, various phytoremediation methods can be applied. When the bioconcentration factor is above one in the roots and the transfer factor is below one, it can be concluded that the phytostabilization of metals can occur within the plant [87–89]. When the bioconcentration factors in the shoots and the transfer factors are above one, it can be concluded that the phytoextraction process can take place in the tested plant, as confirmed by various authors [89–91]. The criterion sometimes proposed for defining hyperaccumulation is the shoot-to-root quotient of metal concentrations, which typically is greater than one in hyperaccumulators [39]. While a useful property in supporting other evidence of hyperaccumulation, this ratio cannot be used alone to define hyperaccumulation for several reasons: the difficulty of sampling roots from many plants, especially trees; the difficulty in analysis of ensuring that roots are free of all soil contamination and externally sorbed metal ions; the fact that the relative concentrations on a dry weight basis of a metal in various plant organs may depend as much on the proportion of structural material in those tissues as on the detailed solution transport processes; a plant with, for example, 10 μg/g metal in the root and 20 μg/g in the leaves, while having a translocation ratio greater than one, is of no special importance in the context of hyperaccumulation; metal partitioning into shoots relative to the roots may depend on external metal supply [41,92], and the higher root-to-shoot biomass ratio of some hyperaccumulators can also contribute to relatively high shoot-to-root metal quotients [41,93].

The plant with the highest accumulation factor was *Alyssum saxatile* L., which, in the case of Cd in the 1:7 soil combination, reached a value (0.764) below one, indicating a low accumulation level of a given metal. At the same time, it was observed that in the case of Ni and Cr, the values of the accumulation factor were higher for a high content of pollutants (1:1 soil combination) for *Alyssum saxatile* L. In the case of *Festuca rubra* L., no increase in the BCF was noted for any of the tested metals, which means that it cannot be used as an accumulator for any of them, regardless of their content.

Phytoextraction is a method that removes metal contamination from soil. Of the plants tested, only *Alyssum saxatile* L. turned out to have a selective ability of accumulation of Cd without being qualified as an accumulating plant. It should be taken into account that the efficiency of phytoextraction by a given plant species may be significantly reduced due to the high metal content in soil, bordering on the toxicity threshold.

5. Conclusions

1. The results of the content of individual metals in the tested plants indicate that *Alyssum saxatile* L. has the selective ability to accumulate cadmium in its shoots without being defined as an accumulating plant. The contents of Cd in its biomass were significantly higher compared to *Festuca rubra* L. For the remaining metals, the differences were less significant. This confirms the opinion that each plant has different characteristic mechanisms reacting to metals: exclusion, indication, and accumulation. However, their reactions differ depending on the content of a given metal in the soil.
2. The wide range of plant tolerances to environmental factors is a very important aspect for the phytoremediation process; however, in heavily contaminated soils, a high metal content may be a problem even for plants accumulating given metals. This was observed in the studies with *Alyssum saxatile* L., where the high concentration of metals in the soil exceeded the toxicity threshold, contributing to the disappearance of the plant's life processes.
3. The calculated results for *Alyssum saxatile* L. for the BCF are below one for all tested metals, which allow us to state that *Alyssum saxatile* L. is not an accumulator. The obtained values for the TF are above one for all tested metals except Pb, which means that this plant does not stabilize metals either.

Author Contributions: Conceptualization, A.P. and M.W.; methodology, A.P. and M.W.; validation, A.P. and D.R.; formal analysis, A.P. and D.R.; investigation, A.P.; resources, A.P.; writing—original draft preparation, A.P., M.W. and D.R.; writing—review and editing, A.P., M.W. and D.R.; visualization, A.P.; supervision, A.P.; funding acquisition, A.P. and M.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the following projects: "An attempt to determine the effectiveness of methods of remediation/detoxification of soils contaminated with metals for the reclamation of degraded industrial and post-mining areas" (504M/1110/0001) and "Assessment of effectiveness of stabilization methods soils contaminated with metals" (504/02097), financed by the Faculty of Building Services, Hydro and Environmental Engineering, Warsaw University of Technology.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in this article.

Conflicts of Interest: The authors declare no conflict of interest.

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