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Heavy Metal Allocation to Pea Plant Organs (*Pisum sativum* L.) from Soil during Different Development Stages and Years

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Abstract: The incorporation of heavy metals contained in soils into the food chain is mediated by plants. Plants show varying abilities to take up and accumulate these elements during vegetative growth. In this study, changes in the content, rate of uptake, accumulation, and translocation of heavy metals during six stages of development of pea plants were determined. In field experiments, two pea cultivars were cultivated in two consecutive growing seasons. The harvested plants were divided into the roots and aerial parts, and at full maturity the seeds were separated additionally. Significant changes in the content of the heavy metals in the separated parts and on average in the entire plant, as well as their bioaccumulation factors (*BAFs*), were most often noted up to the flowering stage of pea plants, after which these values usually did not change significantly. The highest rate of uptake of heavy metals per day of growth was noted between the full flowering stage and the stage when 50% of pods were of typical length. Their translocation factor (*TF*) was most often highest between the three-internode stage and the full flowering stage. The content, uptake, *BAF*, and *TF* of the heavy metals most often varied between years of the study, but did not significantly depend on the pea cultivar. The *BAF* indicates the potential of pea plants to hyperaccumulate lead and zinc and moderate accumulation of other heavy metals in their aerial parts. Excessive concentrations of lead and cadmium disqualified pea's seeds to be used as human food, whereas excessive concentrations of lead prevented their use as fodder. Green mass of pea plants can be used as animal fodder according to the EU directives.

Keywords: bioaccumulation factor; cadmium; chrome; copper; lead; nickel; translocation factor; zinc

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1. Introduction

Heavy metals are a natural component of soils [1–3]. Elevated content of these elements and even pollution of the soil is noted mainly in areas with a high degree of human impact [4–7]. Anthropogenic indirect or direct sources of soil contamination with heavy metals include chemical and electrotechnical industries, coal power plants and the coking industry, oil refineries, metallurgy of ferrous and non-ferrous metals, and transport and agriculture. Plants show sensitivity to both low and high concentrations of heavy metals in the soil. When their content in the soil is at a low/required level, they are a beneficial factor in the growth and development of plants and can improve their physiological and morphological features. Unfortunately, when their concentration is increased to above the threshold of toxicity, they have adverse effects on plants [8,9]. Some heavy metals perform important physiological functions for plants, regulating the course of important vital processes. Copper, nickel, and zinc are essential nutrients for plants and perform important physiological functions. Copper is, among others, a catalyst in the photosynthesis process and a cofactor for many enzymes [10]. Nickel is an activator of urease, which catalyzes the hydrolysis of urea in plant tissues, and a component of other metalloenzymes [11]. Zinc is an important component of carbonic anhydrase and an aldolase stimulator, which are involved in carbon metabolism [12]. Zn is also an integral component of biomolecules such as lipids, proteins, and auxin cofactor, and therefore plays an important role in plant nucleic acid metabolism [13]. Others perform no physiological functions, or their functions are only presumed or as yet unknown [14,15]. The studies conducted so far have not shown a significant role of chrome, lead, and cadmium for a plant. In each of these cases, however, plants are the main link responsible for the translocation of heavy metals from the soil up the food chain and consequently to the human body—the top of the food chain [16–18]. Consumed even in small amounts, they can accumulate in tissues and cause dysfunctions in the body [1,19,20]. Their forms of toxicity to humans include neurotoxicity (Cd, Cu, Zn), nephrotoxicity (Cd, Pb), carcinogenicity (Cr, Ni, Pb), hepatotoxicity (Cd, Cr, Cu, Pb), immunological toxicity (Cd, Cr, Pb), cardiovascular toxicity (Cd, Pb), skin toxicity (Cr), reproductive and developmental toxicity (Cd, Pb), and genotoxicity (Cr) [21]. In small amounts, Zn, Cu, Cr, and Ni are necessary for the human body to function properly.

The content of these elements in food products of plant and animal origin is usually small and does not exceed acceptable limits [22,23]. However, given that there are other routes by which heavy metals can enter the human body (e.g., through the skin or lungs), which are difficult to eliminate, their content in food should be as low as possible. All heavy metals in excessive concentrations are toxic for living organisms [1,24,25]. Excess copper causes chlorosis, necrosis, and dwarfism of plants [26]; nickel interferes with plant metabolism, and inhibits photosynthesis and transpiration [27]; and zinc inhibits photosynthesis and chlorophyll biosynthesis [28]. In the case of excessive content of chrome and lead in the soil, the germination of seeds is delayed, the root system is damaged, and photosynthesis and chlorophyll production are inhibited [29,30]. Excessive concentration of cadmium results in metabolism disorders and reduced nutrient and water uptake [31].

Transfer of potentially toxic elements such as heavy metals from the soil to crop plants depends in part on the availability of these elements and the properties of the crop species [32–35]. Some plant species, known as hyperaccumulators, show a tendency to accumulate large amounts of heavy metals [36,37].

Uptake of heavy metals from the soil by plant roots varies at different stages of the plant's growth and development [38,39]. These elements are then transported in the plant and accumulated in its aerial parts [40].

The uptake of heavy metals by pea plants increases with increasing their amount in the substrate [41]. These authors, after fertilizing pea with increasing doses of sewage sludge, obtained low (less than 1) values of the bioaccumulation factor of cadmium, chrome, copper, nickel, and zinc in pea roots. The value of this factor was found to be greater than 1 for lead. The values of the translocation factor of all the above-mentioned heavy metals were lower than 1, which indicates a low level of their translocation from roots to shoots and to seeds. In the case of cadmium, the accumulation in roots can be up to 45 times higher than that in leaves (for copper 15 times) [42]. Heavy metal concentrations in the pod, shoot, and root are highly correlated with soil pH, organic matter, and heavy metal concentrations [43]. According to this previous study, the significant positive correlation between the concentration of a certain heavy metal in the soil and the same element in pea plant tissues suggests the potential use of this plant for the biomonitoring of the heavy metals.

There are numerous scientific studies describing the accumulation of heavy metals in crop plants, including peas, grown in an environment contaminated with heavy metals [32,33,44,45]. However, there are few studies presenting the rate of uptake, bioaccumulation, and translocation of heavy metals in crop plants growing on uncontaminated soil with these elements.

The aim of the study was to determine the content, uptake, bioaccumulation, and translocation of selected metals which, according to current knowledge, have no physiological functions for plants (Cd, Cr, and Pb) and those included among micronutrients (Cu, Ni, and Zn) in two pea plant cultivars (multi-purpose and fodder cultivars) at various stages of growth and development in terms of usefulness of the harvested biomass for fodder and food. Both cultivars with different types of use are popular in cultivation in the research area.

2. Materials and Methods

2.1. Field Experiment

Polish law sets acceptable limits for content of heavy metals in soils used as arable land, orchards, meadows, pastures, and family garden allotments [46]. Light soils with pH above 6.5 should contain no more than (per kg dry weight of soil) 3 mg Cd, 150 mg Cu and Ni, 250 mg Pb, 300 mg Cr, and 500 mg Zn, while the limits for light soils with pH up to 6.5 are 2 mg Cd, 100 mg Cu, Ni, Pb, 150 mg Cr, and 300 mg Zn. In both years, pea plants were grown on soil that met these standards in full.

A field experiment was carried out in 2015 and 2016 in Siedlce, eastern Poland (52°10′ N, 22°17′ E). Pea plants (*Pisum sativum* L.) were grown on Luvisols (LV) according to a traditional system of pea cultivation (ploughing, fertilization, pre-sowing cultivation—tillage set, and sowing). The most important properties of the soil are given in Table 1. The soils differ slightly in their content of carbon, nitrogen, and heavy metals because pea plants were grown in different fields in the following years, located close to each other. Two factors were investigated in the experiment in a randomized block design in three replications. The first factor was two pea cultivars: (a) multi-purpose cultivar 'Batuta', and (b) fodder cultivar 'Milwa'. The second factor was the growth stage of the plant (according to the BBCH scale—the abbreviation derives from the names of the originally participating stakeholders: "Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie"): (1) BBCH 14, 4-leaf stage; (2) BBCH 33, 3-internode stage; (3) BBCH 55, stage when the first single buds are visible outside the leaves—abbrev. "first bud"; (4) BBCH 65, full flowering stage—abbrev. "flowering; (5) BBCH 75, when 50% of pods are of typical length—abbrev. "50% pods"; and (6) BBCH 90, full maturity—abbrev. "maturity". In total there were 36 plots: 2 cultivars, 6 harvesting dates, number of replications N = 3. Each plot with an area of 1 m^2 (1 × 1 m) used in the experiment was located in a field where the test plant was grown. Pea cultivars were grown in separate plots. Pea plants were fertilized with nitrogen before sowing in an amount corresponding to the application of 30 $kg \cdot ha^{-1}$ N to the soil, i.e., 3 $g \cdot m^{-2}$ N, in the form of ammonium sulphate $(NH_4)_2SO_4$. Phosphorus fertilizer was not applied because a very high content of phosphorus in forms available to plants was determined in the soil. Potassium was applied in an amount corresponding to the application of 100 $kg \cdot ha^{-1}$ K to the soil, i.e., 10 $g \cdot m^{-2}$ K, in the form of potassium chloride (KCl). Pea seeds inoculated with Nitragina (an inoculant containing pea symbiotic bacteria *Rhizobium leguminosarum*) were sown in the first 10 days of April in both years of the study (termed "0"), at a density of 110·m^{-2}. No chemical protection was used, and weeds were removed manually. At the relevant stages of growth, entire pea plants were dug up from the soil to a depth of 0.25 m. All plants were harvested separately from each 1 m^2 plot.

Table 1. Selected soil properties, value of parameter and (±SD), N = 3.

Soil Properties	Unit	Year	
		2015	2016
$pH_{1\ mol \cdot dm^{-3}\ KCl}$	-	6.6	6.5
N_{total}	$g \cdot kg^{-1}$	2.10 (±0.2)	1.45 (±0.1)
C_{total}		34.2 (±0.6)	23.5 (±0.4)
Cd_{total}	$mg \cdot kg^{-1}$	0.324 (±0.1)	0.411 (±0.1)
Cr_{total}		3.07 (±0.1)	2.47 (±0.1)
Cu_{total}		16.99 (±0.5)	11.65 (±0.7)
Ni_{total}		3.32 (±0.1)	3.17 (±0.1)
Pb_{total}		16.01 (±0.8)	13.92 (±0.1)
Zn_{total}		60.18 (±1.6)	66.45 (±2.3)

2.2. Laboratory Work

Plant parts contaminated with soil (roots) were washed with distilled water after harvest. Those harvested from the 4-leaf stage to the 50% pods stage of pea plants were separated for the roots and aerial parts from each plot. In full maturity, seeds were additionally separated because in this stage of development they are the main yield. Hereafter, in all stages of pea growth, all aerial parts of the plant except for the seeds separated in full maturity stage are referred to as the aerial part. Then, the separated parts were dried to a constant weight (at 70 °C) [47]. From the entire mass of separated and dried parts, samples weighing up to 30 g were randomly taken and ground. Samples of plant material were subjected to dry mineralization (ashing) at 450 °C [48]. The chemical compounds contained in the ash were dissolved in 6 mol·dm^{-3} HCl, which was subsequently evaporated. The easily soluble chlorides were transferred to volumetric flasks in 1% HCl solution [48]. The total content of heavy metals in this solution was determined using an Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) spectrometer (Optima 3200 RL, Perkin-Elmer, Waltham, MA, USA). The detection limits of the heavy metals determined were: 0.0018 mg·dm^{-3} Cd, 0.0020 mg·dm^{-3} Cr, 0.0009 mg·dm^{-3} Cu, 0.0036 mg·dm^{-3} Ni, 0.0228 mg·dm^{-3} Pb, and 0.0097 mg·dm^{-3} Zn. In the soils before the experiment, the following were determined:

- pH value—potentiometrically [49];
- total nitrogen and carbon—on a CHN autoanalyzer with IDC detector, Series II 2400, Perkin-Elmer, Valencia, CA, USA;
- total content of heavy metals—after wet mineralization in a mixture of concentrated HCl and HNO_3 acids (3:1 ratio) using an ICP-AES spectrometer (Optima 3200 RL, Perkin-Elmer, Waltham, MA, USA).

All analyses were performed in triplicate. STD GEOCHEM CUSTOM 4 standard reference solutions (PE #: N9307113) were used in the analytical process.

2.3. Weather Conditions

The weather conditions during the growing season varied in the years of the study (Table 2).

Table 2. Rainfall and air temperatures in 2015 and 2016, (Institute of Meteorology and Water Management, National Research Institute in Warsaw).

Month	Total Monthly Rainfall, mm			Average Monthly Temperatures, °C		
	Years		Long-Term Mean 1981–2014	Years		Long-Term Mean 1981–2014
	2015	2016		2015	2016	
III	53.1	46.4	29.6	4.8	3.3	2.0
IV	30.0	50.2	33.4	8.2	8.9	8.1
V	100.2	35.5	60.3	12.3	14.6	13.6
VI	43.3	55.6	72.9	16.5	18.1	16.3
VII	62.6	126.8	67.6	18.7	19.0	18.5
Sum/Means IV–VII	236.1	268.1	234.2	13.9	15.1	14.1

2.4. Calculations

The results obtained in the experiment were used to calculate the uptake, and the factors of bioaccumulation and translocation, of Cd, Cr, Cu, Ni, Pb and Zn, according to the following formulas:

(a) Heavy metal uptake by pea plants, g·ha^{-1} [50], HM_{up}:

$$HM_{up} = Y \cdot C_{plant} \quad (1)$$

where:

Y—obtained dry mass of pea plants (part of pea plants, respectively);
C_{plant}—total heavy metal content (concentration) in pea's plants dry mass (in separated parts, respectively).

(b) Heavy metal uptake by pea plants per 1 day [mg·ha^{-1}], HMD_{up}:

$$HMD_{up} = HM_{up}/D\_No \times 1000 \quad (2)$$

where:

HM_{up}—heavy metal uptake by pea plants;
D_No—the number of days between harvests in the researched development stages.

(c) Bioaccumulation factor of heavy metal [51], BAF_E:

$$BAF_E = C_{plant}/C_{soil} \quad (3)$$

where:

C_{plant}—total heavy metal content (concentration) in pea plants (in separated parts and as averages in plant, respectively);
C_{soil}—total heavy metal content (concentration) in soil.

(d) Translocation factor of selected element [51], $TF_{E.}$ This determines the possibility of translocation of these elements from the roots of plants to their aerial part:

$$TF_E = C_{agp}/C_r \quad (4)$$

where:

C_{agp}—total heavy metal content (concentration in pea's plants aerial part);
C_r—total heavy metal content (concentration) in the roots.

(e) The weighted average heavy metal content was calculated by dividing the total heavy metal uptake by the total amount of harvested mass of pea plants, Wa:

$$W_a = Total\ HM_{up}/Y \quad (5)$$

where:

$Total\ HM_{up}$—total heavy metals uptake by entire pea plants;
Y—obtained dry mass of entire pea plants.

(f) Value of bioaccumulation factor of heavy metals meanly in the entire pea plant, $MeanBAF_E$:

$$MeanBAF_E = W_a/C_{soil} \quad (6)$$

where:

W_a—weighted average heavy metal content in pea plants;
C_{soil}—total heavy metal content (concentration) in soil.

2.5. Statistical Analyses

The results obtained in the experiment were analyzed by ANOVA with the Fisher–Snedecor distribution. LSD values at a significance level of $\alpha = 0.05$ were calculated by the Tukey test. The Statistica 13.1 PL statistics package (StatSoft Inc., Tulsa, OK, USA) was used for the calculations.

For roots, aerial parts, and the entire biomass of pea plants, a three-factor analysis of variance was performed, according to the following model:

$$yijlp = m + ai + bj + cl + abij + acil + bcjl + abcijl + eijl,$$

where:

y_{ijlp}—the value of the examined characteristic;
m—population average;
a_i—the effect of pea's growth stage;
b_j—the effect of pea's cultivar;
c_l—the effect of year;
ab_{ij}—the effect of the interaction of growth stage x cultivar;
ac_{il}—the effect of the interaction of growth stage x year;
bc_{jl}—the effect of the interaction of cultivar x year;
abc_{ijl}—the effect of the interaction of growth stage x cultivar x year;
e_{ijl}—the random error (numbers).

Two-factor analysis of variance was performed for the seeds of pea plants, according to the following model:

$$yijp = m + ai + bj + abij + eijp,$$

where:

y_{ijp}—the value of the examined characteristic;
m—population average;
a_i—the effect of pea's cultivar;
b_j—the effect of year;
ab_{ij}—the effect of the interaction of pea's cultivar x year;
e_{ijp}—the random error (numbers).

3. Results

3.1. Heavy Metal Content in Pea Plants

The content of heavy metals varied significantly between developmental stages and the years of pea plants cultivation (W_a in Tables 3 and 4). On average in the entire pea plants (W_a in Table 3), the content of copper and zinc decreased from the four-leaf stage to the first bud; in chrome, it decreased to the flowering stage; but in nickel, it only decreased to the three-internode stage. W_a content of these heavy metals in other developmental stages of pea plants did not change significantly. Cadmium and lead varied little.

The content of chrome, copper, and zinc in the aerial parts of pea plants was higher in the first two development stages (nickel only in the first) than thereafter. Cadmium and lead concentrations were slightly increased during peak growth.

The content of chromium and zinc in the roots of pea plants decreased from the four-leaf stage to the first bud stage, while that of copper, nickel, and lead decreased only until the three-internode stage. In the following stages of development, the content of these heavy metals in the roots was similar. An exception was the final stage of development, in which the content of chrome, copper, and lead was higher than that in the three previous stages.

The content of the analyzed heavy metals in the seeds and other aerial parts of pea plants harvested at full maturity was lower than in the roots. In the earlier stages, the content of the heavy metals in the aerial parts was also often lower than in the roots.

The content of all heavy metals analyzed (cadmium, chrome, copper, nickel, lead, and zinc) in all separated parts and W_a in the entire pea plants was most often higher in the conditions of their cultivation in 2016 than those in 2015 (Table 4). Only W_a of chrome in the entire test plant and the content of nickel in the seeds did not differ significantly between years of the study.

There were almost always no significant differences in the content of any heavy metals depending on the pea plants cultivar (Table 4). Only the content of zinc in the roots of the 'Milwa' cultivar was higher than that in the 'Batuta' cultivar.

Table 3. Heavy metal content in pea plants in successive growth stages, mg·kg^{-1} DW (mean values ± SD of two cultivars and two successive years with three repetitions each).

Heavy Metals	Parts of Pea Plants	Growth Stages						LSD$_{0.05}$
		Four-Leaf	Three-Internode	First Bud	Flowering	50% Pods	Maturity	
Cd	seeds						0.307 (±0.119)	
	aerial part	0.238 a (±0.095)	0.217 a (±0.062)	0.285 a b (±0.194)	0.373 b (±0.233)	0.316 a b (±0.171)	0.253 a (±0.123)	0.102
	roots	0.439 a b (±0.149)	0.335 a (±0.134)	0.492 b (±0.182)	0.538 b (±0.288)	0.555 b (±0.226)	0.552 b (±0.158)	0.123
	W_a	0.325 a b (±0.059)	0.242 a (±0.047)	0.303 a b (±0.187)	0.382 b (±0.236)	0.324 a b (±0.173)	0.280 a (±0.118)	0.085
Cr	seeds						0.320 (±0.108)	
	aerial part	0.751 c (±0.160)	0.649 b c (±0.131)	0.521 a b (±0.117)	0.398 a (±0.048)	0.407 a (±0.193)	0.415 a (±0.177)	0.140
	roots	6.529 d (±1.741)	4.437 c (±1.221)	3.432 a b (±0.763)	3.107 a (±0.558)	3.191 a (±0.577)	3.997 b c (±0.734)	0.748
	W_a	3.248 d (±0.610)	1.498 c (±0363)	0.769 b (±0.135)	0.544 a (±0.080)	0.509 a (±0.203)	0.449 a (±0.128)	0.241
Cu	seeds						9.09 (±1.92)	
	aerial part	10.27 d (±1.30)	8.96 c (±1.34)	7.82 b (±1.23)	7.18 b (±1.29)	7.35 b (±2.25)	5.99 a (±2.75)	1.00
	roots	13.18 b (±4.22)	8.71 a (±3.94)	9.09 a (±2.33)	7.26 a (±2.27)	9.12 a (±1.62)	13.27 b (±2.36)	2.59
	W_a	12.42 c (±1.77)	9.45 b (±1.10)	7.93 a (±1.02)	7.19 a (±1.25)	7.42 a (±2.17)	7.34 a (±2.21)	1.19

Table 3. *Cont.*

Heavy Metals	Parts of Pea Plants	Growth Stages						$LSD_{0.05}$
		Four-Leaf	Three-Internode	First Bud	Flowering	50% Pods	Maturity	
Ni	seeds						1.52 (±0.35)	
	aerial part	3.70 b (±1.36)	2.13 a (±1.04)	1.53 a (±1.22)	1.51 a (±1.11)	1.68 a (±1.16)	2.17 a (±0.52)	1.03
	roots	16.77 b (±9.29)	10.75 a (±5.69)	7.51 a (±2.32)	6.34 a (±2.31)	6.14 a (±2.97)	6.66 a (±1.74)	5.42
	W_a	9.19 c (±3.67)	4.04 b (±1.50)	2.04 a b (±1.27)	1.78 a (±1.19)	1.85 a (±1.19)	2.03 a b (±0.33)	2.14
Pb	seeds						16.6 (±8.0)	
	aerial part	14.2 a (±6.6)	18.5 c (±1.8)	17.1 b c (±3.7)	16.4 a b c (±6.2)	16.6 a b c (±7.4)	15.5 a b (±7.1)	2.5
	roots	25.9 c (±6.7)	20.3 a b (±5.3)	20.2 a b (±4.3)	17.5 a (±5.9)	17.6 a (±6.6)	24.2 b c (±7.8)	4.1
	W_a	19.7 c (±4.7)	18.8 b c (±2.3)	17.4 a b c (±3.6)	16.5 a b (±6.1)	16.6 a b (±7.4)	16.1 a (±7.4)	2.6
Zn	seeds						60.3 (±19.6)	
	aerial part	90.6 c (±23.4)	84.7 c (±21.6)	70.2 b (±21.5)	65.6 a b (±23.6)	64.6 a b (±25.1)	60.6 a (±24.4)	6.7
	roots	171.6 c (±55.1)	142.6 b (±32.7)	125.3 a (±19.2)	114.4 a (±27.2)	116.6 a (±25.6)	121.0 a (±19.7)	12.0
	W_a	124.2 d (±30.8)	97.1 c (±20.1)	74.9 b (±20.9)	68.2 a b (±24.0)	66.5 a (±25.1)	61.7 a (±22.2)	6.9

a, b, c, d—means for investigated factors with different letters in the rows are significantly different.

Table 4. Heavy metal content in different pea plants cultivars and years, mg·kg^{-1} DW ([1] mean values in maturity ± SD of two successive years or two cultivars, respectively, and with three repetitions each; [2] mean values ± SD of six growth stages, two successive years, or two cultivars, respectively, and with three repetitions each).

Heavy Metals	Parts of Pea Plants	Sources of Variation					
		Pea Cultivars			Years		
		'Milwa'	'Batuta'	$LSD_{0.05}$	2015	2016	$LSD_{0.05}$
Cd	seeds	0.291 [1] (±0.128)	0.323 [1] (±0.120)	n.s.	0.198 [1] a (±0.037)	0.416 [1] b (±0.039)	0.050
	aerial part	0.266 [2] (±0.144)	0.294 [2] (±0.177)	n.s.	0.171 [2] a (±0.047)	0.389 [2] b (±0.161)	0.040
	roots	0.510 [2] (±0.198)	0.460 [2] (±0.213)	n.s.	0.349 [2] a (±0.096)	0.622 [2] b (±0.195)	0.052
	W_a	0.301 [2] (±0.139)	0.317 [2] (±0.169)	n.s.	0.201 [2] a (±0.056)	0.417 [2] b (±0.144)	0.033
Cr	seeds	0.334 [1] (±0.122)	0.306 [1] (±0.102)	n.s.	0.233 [1] a (±0.032)	0.407 [1] b (±0.081)	0.087
	aerial part	0.532 [2] (±0.208)	0.515 [2] (±0.184)	n.s.	0.474 [2] a (±0.229)	0.573 [2] b (±0.140)	0.055
	roots	4.161 [2] (±1.913)	4.064 [2] (±1.073)	n.s.	3.943 [2] a (±1.245)	4.281 [2] b (±1.791)	0.293
	W_a	1.220 [2] (±1.140)	1.120 [2] (±0.956)	n.s.	1.149 [2] (±1.133)	1.190 [2] (±0.967)	n.s.
Cu	seeds	8.69 [1] (±2.12)	9.48 [1] (±1.80)	n.s.	7.35 [1] a (±0.82)	10.82 [1] b (±0.47)	0.86
	aerial part	7.80 [2] (±2.22)	8.06 [2] (±2.21)	n.s.	7.00 [2] a (±2.29)	9.16 [2] b (±1.24)	0.39
	roots	10.61 [2] (±4.02)	9.60 [2] (±3.64)	n.s.	11.21 [2] a (±4.39)	9.00 [2] b (±2.96)	1.02
	W_a	8.56 [2] (±2.59)	8.69 [2] (±2.32)	n.s.	7.96 [2] a (±3.01)	9.30 [2] b (±1.36)	0.47
Ni	seeds	1.59 [1] (±0.37)	1.45 [1] (±0.34)	n.s.	1.64 [1] (±0.20)	1.40 [1] (±0.44)	n.s.
	aerial part	1.90 [2] (±1.10)	2.35 [2] (±1.46)	n.s.	1.55 [2] a (±1.41)	2.70 [2] b (±0.89)	0.50
	roots	9.30 [2] (±5.36)	8.76 [2] (±6.72)	n.s.	6.54 [2] a (±3.19)	11.52 [2] b (±7.15)	2.12
	W_a	3.39 [2] (±3.01)	3.59 [2] (±3.45)	n.s.	2.60 [2] a (±2.61)	4.38 [2] b (±3.54)	0.84
Pb	seeds	16.2 [1] (±7.9)	17.1 [1] (±8.8)	n.s.	9.1 [1] a (±0.4)	24.2 [1] b (±1.7)	1.5
	aerial part	16.0 [2] (±5.5)	16.8 [2] (±6.1)	n.s.	11.5 [2] a (±3.6)	21.2 [2] b (±2.5)	1.0
	roots	21.4 [2] (±7.1)	20.5 [2] (±6.5)	n.s.	16.1 [2] a (±4.7)	25.8 [2] b (±4.7)	1.6
	W_a	17.3 [2] (±5.6)	17.7 [2] (±5.6)	n.s.	12.9 [2] a (±3.6)	22.1 [2] b (±2.4)	1.0
Zn	seeds	59.5 [1] (±23.7)	61.0 [1] (±16.7)	n.s.	42.0 [1] a (±4.6)	78.5 [1] b (±4.9)	4.5
	aerial part	73.3 [2] (±27.9)	72.1 [2] (±22.2)	n.s.	51.2 [2] a (±12.9)	94.3 [2] b (±12.4)	2.6 [2]
	roots	141.0 [2] b (±34.2)	122.9 [2] a (±38.1)	4.7	108.3 [2] a (±21.0)	155.6 [2] b (±34.6)	4.7 [2]
	W_a	84.1 [2] (±34.7)	80.1 [2] (±29.6)	n.s.	60.1 [2] a (±21.5)	104.1 [2] b (±25.1)	4.4

a, b—means for investigated factors with different letters in the rows are significantly different; n.s.—not significantly differ at $p \leq 0.05$.

3.2. Heavy Metals Uptake by Pea Plants

HM_{up} varied significantly between developmental stages and years of pea plant cultivation (summarized in Tables 5 and 6). The total HM_{up} of cadmium, copper, nickel, and lead by entire plants increased significantly from the three-internode stage to the flowering stage, zinc from the four-leaf stage to the flowering stage, but chrome from the three-internode stage to the first bud stage (Table 5). Between the flowering stage and the 50% pods stage, the quantity of heavy metals accumulated in pea plants did not vary significantly. In the case of cadmium and chrome, this held until the end of the growing period. HM_{up} of copper, nickel, and zinc significantly increased from the 50% pods stage to maturity.

The HM_{up} values for the roots of pea plants were highly differentiated. The value of this parameter for lead and zinc tended to be the highest values from the three-internode stage to the flowering stage; in chrome and nickel, from the four-leaf stage to the flowering stage; but in cadmium, from the first bud to the 50% pods stage. The amount of copper accumulated in the roots of the test plant was not significantly dependent on its stage of development.

The HM_{up} of cadmium, chrome, nickel, and lead for the aerial parts of pea plants increased from the four-leaf stage to the flowering stage, but in copper and zinc it increased to the 50% pods stage. The amount of accumulated cadmium, chrome, nickel, and lead in pea plants did not vary significantly between the flowering stage and the 50% pods stage. Between the 50% pods stage and the maturity stage, accumulation of heavy metals in the aerial parts (without the seeds) decreased.

Total HM_{up} values of cadmium, nickel, lead, and zinc by the entire pea plants were higher in the conditions of 2016 than those in 2015 (Table 6). The HM_{up} of chrome and copper did not vary significantly between years of the study.

The HM_{up} of these elements in the separated parts of the pea plants, in which significant differences in uptake were noted, most often also indicated this dependency. An exception was the amounts of nickel accumulated in the seeds and copper accumulated in the seeds and roots, which were higher in 2015 than in 2016.

In most cases, there was no significant variation in HM_{up} for different cultivars of pea plants (Table 6). Only the HM_{up} values of copper and zinc by the roots of the 'Milwa' cultivar were greater than those of the 'Batuta' cultivar.

Variation in HMD_{up} (uptake per day) by pea plants is presented in Tables 7 and 8. The lowest values of HMD_{up} for chrome, copper, and zinc were noted in the period from sowing to the four-leaf stage, but for cadmium, nickel, and lead, they were found to the three-internode stage (Table 7). Their rate of HMD_{up} increased up to that of the 50% pods stage. The highest rate was noted between the flowering stage and the 50% pods stage. After this time, the rate of HMD_{up} decreased. The values for HMD_{up} of cadmium, nickel, lead, and zinc obtained between the 50% pod stage and maturity corresponded to the rate noted between the three-internode stage and the first bud; however, for chrome, this corresponded to the rate between the four-leaf stage and the three-internode stage. The mean rates of HMD_{up} for the entire period from pea seed sowing to plant harvest, in declining order, are as follows (mg·ha^{-1} per day): Zn (16,838) > Pb (4175) > Cu (1924) > Ni (497) > Cr (156) > Cd (79).

There was no significant variation in HMD_{up} depending on the pea plant cultivar (Table 8).

The values of HMD_{up} for chrome and copper were also not significantly different between years of the study. A higher rate of HMD_{up} of cadmium, nickel, lead, and zinc was obtained in the conditions of 2016 than those in 2015.

Table 5. HM_{up} by pea plants in successive growth stages, g·ha^{-1} (mean values ± SD of two cultivars and two successive years with three repetitions each).

Heavy Metals	Parts of Pea Plants	Growth Stages						$LSD_{0.05}$
		Four-Leaf	Three-Internode	First Bud	Flowering	50% Pods	Maturity	
Cd	seeds						0.72 (±0.14)	
	aerial part	0.05 a (±0.02)	0.16 a (±0.7)	0.73 b (±0.45)	1.54 c (±0.67)	1.63 c (±0.66)	0.98 b (±0.45)	0.34
	roots	0.06 a (±0.02)	0.07 a (±0.03)	0.12 b (±0.04)	0.13 b (±0.06)	0.11 b (±0.03)	0.07 a (±0.02)	0.03
	sum	0.11 a (±0.02)	0.23 a (±0.07)	0.85 b (±0.47)	1.67 c (±0.71)	1.74 c (±0.68)	1.77 c (±0.51)	0.34
Cr	seeds						0.77 (±0.16)	
	aerial part	0.14 a (±0.03)	0.48 a (±0.13)	1.36 b (±0.22)	1.94 c d (±0.64)	2.20 d (±0.85)	1.58 b c (±0.51)	0.55
	roots	0.94 c (±0.22)	0.93 c (±0.34)	0.85 b c (±0.23)	0.79 b c (±0.16)	0.67 a b (±0.13)	0.54 a (±0.23)	0.22
	sum	1.08 a (±0.23)	1.41 a (±0.37)	2.21 b (±0.33)	2.73 b c (±0.68)	2.87 c (±0.91)	2.89 c (±0.35)	0.63
Cu	seeds						22.33 (±3.43)	
	aerial part	1.94 a (±0.62)	7.10 b (±1.5)	21.04 c (±5.51)	33.80 d (±7.22)	39.50 e (±5.54)	23.14 c (±9.84)	5.15
	roots	2.21 (±0.93)	1.81 (±0.82)	2.33 (±1.01)	1.86 (±0.59)	1.99 (±0.65)	1.83 (±0.86)	n.s.
	sum	4.15 a (±0.76)	8.91 a (±1.20)	23.37 b (±5.78)	36.66 c (±7.27)	41.49 c (±5.42)	47.30 d (±8.42)	5.08

Table 5. *Cont.*

Heavy Metals	Parts of Pea Plants	Growth Stages						$LSD_{0.05}$
		Four-Leaf	Three-Internode	First Bud	Flowering	50% Pods	Maturity	
Ni	seeds						4.06 (±1.82)	
	aerial part	0.68 a (±0.24)	1.66 a b (±1.09)	3.97 b c (±3.52)	6.28 c d (±3.65)	8.53 d (±5.20)	8.70 d (±2.33)	2.88
	roots	2.39 b (±1.22)	2.23 b (±1.24)	1.79 a b (±0.39)	1.59 a b (±0.47)	1.23 a (±0.42)	0.84 a (±0.21)	1.00
	sum	3.07 a (±1.26)	3.89 a (±1.69)	5.76 a b (±3.82)	7.87 b c (±3.98)	9.76 c (±5.32)	13.60 d (±2.72)	3.08
Pb	seeds						37.74 (±9.13)	
	aerial part	2.84 a (±1.73)	13.86 a (±3.26)	44.75 b (±7.43)	73.57 c d (±17.86)	88.24 d (±30.24)	59.70 b c (±24.47)	16.34
	roots	3.77 a b (±1.03)	4.19 b c (±1.13)	4.92 c (±1.03)	4.38 b c (±1.16)	3.58 a b (±0.88)	3.00 a (±0.75)	1.06
	sum	6.61 a (±1.89)	18.05 a (±4.12)	49.67 b (±7.59)	77.95 c (±18.39)	91.82 c d (±30.93)	100.44 d (±32.14)	16.32
Zn	seeds						143.0 (±15.2)	
	aerial part	17.4 a (±7.5)	64.7 b (±25.7)	182.7 c (±47.6)	292.4 e (±52.0)	341.6 f (±78.0)	232.0 d (±72.2)	46.7
	roots	24.1 b (±4.9)	29.4 c (±6.1)	31.0 c (±7.1)	28.9 b c (±5.5)	24.3 b (±3.4)	15.6 a (±4.2)	5.0
	sum	41.5 a (±11.5)	94.1 b (±29.9)	213.7 c (±49.3)	321.3 d (±53.4)	365.9 d (±78.7)	390.6 e (±73.3)	47.3

a, b, c, d, e, f—means for investigated factors with different letters in the rows are significantly different. n.s.—not significantly differ, at $p \leq 0.05$.

Table 6. HM_{up} by pea plants, $g \cdot ha^{-1}$ ([1] mean values in maturity ± SD of two successive years or two cultivars, respectively, and with three repetitions each; [2] mean values ± SD of six growth stages, two successive years or two cultivars, respectively, and with three repetitions each).

Heavy Metals	Parts of Pea Plants	Sources of Variation					
		Pea Cultivars			Years		
		'Milwa'	'Batuta'	$LSD_{0.05}$	2015	2016	$LSD_{0.05}$
Cd	seeds	0.66 [1] (±0.12)	0.78 [1] (0.13)	n.s.	0.65 [1] (±0.14)	0.79 [1] (±0.12)	n.s.
	aerial part	0.80 [2] (±0.68)	0.90 [2] (±0.84)	n.s.	0.57 [2] a (±0.42)	1.12 [2] b (±0.91)	0.13
	roots	0.10 [2] (±0.05)	0.09 [2] (±0.04)	n.s.	0.08 [2] a (±0.03)	0.11 [2] b (±0.05)	0.02
	sum	1.01 [2] (±0.76)	1.12 [2] (±0.95)	n.s.	0.76 [2] a (±0.52)	1.36 [2] b (±1.01)	0.13
Cr	seeds	0.79 [1] (±0.16)	0.75 [1] (±0.16)	n.s.	0.78 [1] (±0.15)	0.76 [1] (±0.18)	n.s.
	aerial part	1.31 [2] (±0.96)	1.26 [2] (±0.82)	n.s.	1.25 [2] (±0.86)	1.32 [2] (±0.93)	n.s.
	roots	0.80 [2] (±0.27)	0.78 [2] (±0.26)	n.s.	0.87 [2] (±0.24)	0.71 [2] (±0.27)	n.s.
	sum	2.24 [2] (±0.93)	2.17 [2] (±0.85)	n.s.	2.24 [2] (±0.84)	2.16 [2] (±0.94)	n.s.
Cu	seeds	20.95 [1] (±3.89)	23.71 [1] (±2.49)	n.s.	24.51 [1] b (±3.14)	20.14 [1] a (±2.13)	3.30
	aerial part	20.64 [2] (±14.76)	21.54 [2] (±14.81)	n.s.	20.05 [2] a (±14.47)	22.13 [2] b (±15.01)	2.02
	roots	2.21 [2] b (±0.95)	1.80 [2] a (±0.62)	0.28	2.48 [2] b (±0.80)	1.53 [2] a (±0.50)	0.28
	sum	26.34 [2] (±16.36)	27.29 [2] (±18.23)	n.s.	26.61 [2] (±)16.13	27.01 [2] (±18.44)	n.s.
Ni	seeds	4.28 [1] (±2.22)	3.85 [1] (±1.50)	n.s.	5.51 [1] b (±1.18)	2.62 [1] a (±0.94)	1.48
	aerial part	4.39 [2] (±3.58)	5.54 [2] (±5.03)	n.s	3.42 [2] a (±2.99)	6.52 [2] b (±4.99)	1.18
	roots	1.72 [2] (±0.71)	1.64 [2] (±1.11)	n.s.	1.42 [2] a (±0.56)	1.93 [2] b (±1.13)	0.39
	sum	6.82 [2] (±4.22)	7.82 [2] (±5.53)	n.s.	5.76 [2] a (±4.48)	8.89 [2] b (±4.88)	1.21
Pb	seeds	36.04 [1] (±7.10)	39.44 [1] (±11.23)	n.s.	30.27 [1] a (±3.09)	45.21 [1] b (±6.34)	5.59
	aerial part	44.72 [2] (±31.87)	49.60 [2] (±38.69)	n.s.	39.22 [2] a (±27.81)	55.10 [2] b (±40.27)	6.39
	roots	4.10 [2] (±1.23)	3.85 [2] (±1.6)	n.s.	3.58 [2] a (±1.00)	4.37 [2] b (±1.17)	0.42
	sum	54.82 [2] (±36.03)	60.02 [2] (±45.45)	n.s.	47.84 [2] a (±30.12)	67.00 [2] b (±47.77)	6.39
Zn	seeds	136.4 [1] (±17.5)	149.7 [1] (±9.8)	n.s.	140.3 [1] (±18.9)	145.8 [1] (±11.6)	n.s.
	aerial part	184.5 [2] (±122.4)	192.4 [2] (±134.2)	n.s.	156.2 [2] a (±106.5)	220.7 [2] b (±139.8)	18.3
	roots	27.9 [2] b (±8.0)	23.2 [2] a (±5.8)	2.0	24.7 [2] (±7.4)	26.3 [2] (±7.3)	n.s.
	sum	235.2 [2] (±136.0)	240.5 [2] (±153.7)	n.s.	204.3 [2] a (±121.7)	271.4 [2] b (±158.2)	18.5

a, b—means for investigated factors with different letters in the rows are significantly different; n.s.—not significantly differ at $p \leq 0.05$.

Table 7. HMD_{up} by entire pea plants in successive growth stages and their averages, mg·ha^{-1} (mean values ± SD of two cultivars and two successive years with three repetitions each).

Heavy Metals	Intervals between Growth Stages						Averges	$LSD_{0.05}$
	Sowing—Four-Leaf	Four-Leaf—Three-Internode	Three-Internode—First Bud	First Bud—Flowering	Flowering—50% Pods	50% Pods—Maturity		
Cd	4 a (±1)	17 a (±5)	67 b (±41)	142 c (±74)	189 d (±56)	57 b (±16)	79	28
Cr	36 a (±7)	101 b (±27)	171 c (±24)	217 c (±35)	320 d(±89)	93 b (±11)	156	57
Cu	136 a (±26)	599 b (±86)	1798 d (±428)	2854 e (±464)	4630 f (±521)	1526 c (±271)	1924	390
Ni	100 a (±40)	278 a b (±121)	457 b c (±331)	665 c (±395)	1044 d (±479)	439 b c (±88)	497	251
Pb	216 a (±59)	1289 a (±294)	3851 b (±731)	6375 c (±1851)	10,077 d (±2609)	3240 b (±1037)	4175	1450
Zn	1357 a (±354)	6717 b (±2139)	13,663 c (±4668)	26,300 d (±6609)	40,389 e (±5459)	12,602 c (±2365)	16,838	4141

a, b, c, d, e, f—means for investigated factors with different letters in the rows are significantly different.

Table 8. HMD_{up} by entire pea plants, mg·ha^{-1}. (mean values ± SD of six growth stages, two successive years or two cultivars, respectively, and with three repetitions each).

Heavy Metals	Sources of Variation					
	Pea Cultivars			Years		
	'Milwa'	'Batuta'	$LSD_{0.05}$	2015	2016	$LSD_{0.05}$
Cd	76 (±73)	82 (±84)	n.s.	55 a (±50)	103 b (±93)	11
Cr	158 (±109)	155 (±97)	n.s.	159 (±103)	154 (±103)	n.s.
Cu	1925 (±1576)	1922 (±1522)	n.s.	1924 (±1605)	1923 (±1492)	n.s.
Ni	457 (±341)	537 (±477)	n.s.	346 a (±218)	648 b (±503)	98
Pb	4030 (±3428)	4319 (±3757)	n.s.	3540 a (±3056)	4809 b (±3969)	568
Zn	17,198 (±13,277)	17,478 (±14,165)	n.s.	14,961 a (±12,520)	19,715 b (±14,443)	1621

a, b—means for investigated factors with different letters in the rows are significantly different; n.s.—not significantly differ at $p \leq 0.05$.

3.3. Bioaccumulation Factor of Heavy Metals

The *MeanBAF* of chrome, copper, nickel, lead, and zinc was the highest in the first three stages of vegetative growth of pea plants (Table 9). The *MeanBAF* of these heavy metals did not differ significantly from the flowering stage to maturity. In the case of cadmium, *MeanBAF* and *BAF* calculated for the aerial parts changed irregularly, reaching high values in the flowering stage. In the other stages of development, the values of those factors for cadmium did not differ significantly. The *BAF* of chrome, copper, and zinc in the aerial parts was higher in the first two stages of pea plant growth than in subsequent stages. In the case of nickel, the highest value of this factor was obtained in the four-leaf stage.

Table 9. *BAF* of heavy metals in pea plants' successive growth stages (mean values ± SD of two cultivars and two successive years with three repetitions each).

Heavy Metals	Parts of Pea Plants	Growth Stages						$LSD_{0.05}$
		Four-Leaf	Three-Internode	First Bud	Flowering	50% Pods	Maturity	
Cd	seeds						0.81 (±0.23)	
	aerial part	0.64 a (±0.23)	0.60 a (±0.17)	0.75 a b (±0.44)	0.96 b (±0.52)	0.82 a b (±0.37)	0.66 a (±0.26)	0.26
	roots	1.19 a b (±0.34)	0.91 a (±0.39)	1.31 b (±0.37)	1.40 b (±0.62)	1.46 b (±0.4)	1.48 b (±0.28)	0.32
	MeanBAF	0.89 a b (±0.13)	0.66 a (±0.12)	0.80 a b (±0.42)	0.98 b (±0.52)	0.84 a b (±0.37)	0.74 a (±0.23)	0.21
Cr	seeds						0.12 (±0.05)	
	aerial part	0.27 b (±0.04)	0.24 b (±0.05)	0.19 a (±0.06)	0.15 a (±0.03)	0.15 a (±0.09)	0.16 a (±0.08)	0.05
	roots	2.42 d (±0.84)	1.63 c (±0.52)	1.24 a b (±0.25)	1.15 a (±0.30)	1.18 a b (±0.32)	1.44 bc (±0.22)	0.28
	MeanBAF	1.18 d (±0.24)	0.54 c (±0.13)	0.28 b (±0.07)	0.20 a b (±0.05)	0.19 a (±0.09)	0.17 a (±0.06)	0.09
Cu	seeds						0.68 (±0.26)	
	aerial part	0.76 d (±0.23)	0.65 c (±0.14)	0.58 b (±0.19)	0.54 b (±0.19)	0.56 b (±0.27)	0.47 a (±0.28)	0.07
	roots	1.02 b (±0.27)	0.62 a (±0.26)	0.63 a (±0.11)	0.53 a (±0.21)	0.66 a (±0.17)	0.93 b (±0.09)	0.21
	MeanBAF	0.89 c (±0.16)	0.64 b (±)0.12	0.58 a b (±0.17)	0.54 a (±0.19)	0.56 a (±0.26)	0.56 a (±0.26)	0.08
Ni	seeds						0.47 (±0.10)	
	aerial part	1.14 b (±0.41)	0.66 a (±0.33)	0.48 a (±0.39)	0.47 a (±0.35)	0.53 a (±0.37)	0.67 a (±0.17)	0.32
	roots	5.21 b (±2.98)	3.33 a (±1.82)	2.33 a (±0.76)	1.97 a (±0.75)	1.91 a (±0.96)	2.07 a (±0.58)	1.71
	MeanBAF	2.85 c (±1.18)	1.25 b (±0.49)	0.64 a b (±0.41)	0.56 a (±0.38)	0.58 a b (±0.38)	0.63 a b (±0.11)	0.68
Pb	seeds						1.15 (±0.62)	
	aerial part	0.99 a (±0.51)	1.25 b (±0.20)	1.16 a b (±0.32)	1.13 a b (±0.49)	1.14 a b (±0.58)	1.07 a (±0.55)	0.18
	roots	1.75 b (±0.51)	1.38 a (±0.45)	1.37 a (±0.38)	1.20 a (±0.48)	1.21 a (±0.53)	1.66 b (±0.64)	0.28
	MeanBAF	1.34 b (±0.40)	1.27 a b (±0.24)	1.18 a b (±0.32)	1.13 a (±0.49)	1.15 a (±0.58)	1.11 a (±0.57)	0.18

Table 9. *Cont.*

Heavy Metals	Parts of Pea Plants	Growth Stages						$LSD_{0.05}$
		Four-Leaf	Three-Internode	First Bud	Flowering	50% Pods	Maturity	
Zn	seeds						0.94 (±0.26)	
	aerial part	1.42 c (±0.30)	1.33 c (±0.27)	1.10 b (±0.28)	1.02 a b (±0.32)	1.01 a b (±0.35)	0.94 a (±0.34)	0.11
	roots	2.68 c (±0.74)	2.25 b (±0.47)	1.98 a (±0.25)	1.80 a (±0.35)	1.83 a (±0.32)	1.90 a (±0.23)	0.19
	MeanBAF	1.95 d (±0.39)	1.53 c (±0.24)	1.17 b (±0.27)	1.06 a (±0.32)	1.04 a (±0.34)	0.96 a (±0.30)	0.11

a, b, c, d—means for investigated factors with different letters in the rows are significantly different.

The *BAF* calculated for roots was highest in the case of zinc and lowest for cadmium in the first two stages of pea plant growth. The highest values of this factor calculated for roots in the case of chrome and nickel were obtained only in the first tested growth phase of pea plants, while in the case of copper and lead they were found for the first and last tested development phases.

The values of *BAF* for cadmium, copper, and lead for the seeds were higher than those for the aerial parts of pea plants, while the reverse was true for chrome and nickel, and the same values was obtained in the case of zinc. The *BAF* values of all heavy metals were lower in the seeds than in the roots.

The *BAF* of all heavy metals calculated for all separated parts, and *MeanBAF* for the entire pea plants, were higher in the conditions of 2016 than those in 2015 (Table 10). The exception was the non-significantly different *BAF* value calculated for seeds collected in two years of research.

Table 10. *BAF* of heavy metals in pea plants ([1] mean values in maturity ± SD of two successive years or two cultivars, respectively, and with three repetitions each; [2] mean values ± SD of six growth stages, two successive years or two cultivars, respectively, and with three repetitions each).

Heavy Metals	Parts of Pea Plants	Sources of Variation					
		Pea Cultivars			Years		
		'Milwa'	'Batuta'	$LSD_{0.05}$	2015	2016	$LSD_{0.05}$
Cd	seeds	0.77 [1] (±0.25)	0.86 [1] (±0.22)	n.s.	0.61 [1] a (±0.11)	1.01 [1] b (±0.09)	0.14
	aerial part	0.70 [2] (±0.32)	0.77 [2] (±0.40)	n.s.	0.53 [2] a (±0.15)	0.95 [2] b (±0.39)	0.10
	roots	1.37 [2] b (±0.43)	1.22 [2] a (±0.46)	0.12	1.08 [2] a (±0.30)	1.51 [2] b (±0.47)	0.12
	MeanBAF	0.80 [2] (±0.30)	0.84 [2] (±0.37)	n.s.	0.62 [2] a (±0.17)	1.02 [2] b (±0.35)	0.09
Cr	seeds	0.13 [1] (±0.06)	0.12[1] (±0.05)	n.s.	0.08[1] a (±0.01)	0.17 [1] b (±0.03)	0.03
	aerial part	0.20 [2] (±0.08)	0.19 [2] (±0.07)	n.s.	0.16 [2] a (±0.07)	0.23 [2] b (±0.06)	0.02
	roots	1.54 [2] (±0.80)	1.48 [2] (±0.39)	n.s.	1.28 [2] a (±0.41)	1.73 [2] b (±0.73)	0.11
	MeanBAF	0.45 [2] (±0.43)	0.41 [2] (±0.33)	n.s.	0.37 [2] a (±0.37)	0.48 [2] b (±0.39)	0.05
Cu	seeds	0.65 [1] a (±0.28)	0.71 [1] b (±0.27)	0.05	0.43 [1] a (±0.05)	0.93 [1] b (±0.04)	0.05
	aerial part	0.58 [2] (±0.23)	0.60 [2] (±0.24)	n.s.	0.39 [2] a (±0.13)	0.79 [2] b (±0.11)	0.03
	roots	0.78 [2] b (±0.28)	0.68 [2] a (±0.24)	0.08	0.67 [2] a (±0.26)	0.79 [2] b (±0.25)	0.08
	MeanBAF	0.63 [2] (±0.24)	0.63 [2] (±0.22)	n.s.	0.46 [2] a (±0.18)	0.80 [2] b (±0.12)	0.03
Ni	seeds	0.49 [1] (±0.11)	0.45 [1] (±0.11)	n.s.	0.49 [1] (±0.06)	0.44 [1] (±0.14)	n.s.
	aerial part	0.59 [2] a (±0.34)	0.73 [2] b (±0.46)	0.13	0.47 [2] a (±0.42)	0.85 [2] b (±0.28)	0.13
	roots	2.89 [2] (±1.70)	2.72 [2] (±2.14)	n.s.	1.97 [2] a (±0.96)	3.64 [2] b (±2.26)	0.67
	MeanBAF	1.05 [2] (±0.94)	1.12 [2] (±1.09)	n.s.	0.78 [2] a (±0.79)	1.38 [2] b (±1.2)	0.26

Table 10. *Cont.*

Heavy Metals	Parts of Pea Plants	Sources of Variation					
		Pea Cultivars			Years		
		'Milwa'	'Batuta'	$LSD_{0.05}$	2015	2016	$LSD_{0.05}$
Pb	seeds	1.12 [1] (±0.61)	1.19 [1] (±0.68)	n.s.	0.57 [1] a (±0.03)	1.74 [1] b (±0.12)	0.11
	aerial part	1.10 [2] (±0.44)	1.15 [2] (±0.47)	n.s.	0.72 [2] a (±0.23)	1.53 [2] b (±0.18)	0.07
	roots	1.46 [2] (±0.55)	1.40 [2] (±0.52)	n.s.	1.01 [2] a (±0.29)	1.86 [2] b (±0.34)	0.11
	MeanBAF	1.19 [2] (±0.44)	1.21 [2] (±0.45)	n.s.	0.81 [2] a (±0.22)	1.59 [2] b (±0.17)	0.07
Zn	seeds	0.93 [1] (±0.32)	0.96 [1] (±0.21)	n.s.	0.70 [1] a (±0.08)	1.18 [1] b (±0.07)	0.07
	aerial part	0.14 [2] (±0.39)	0.13 [2] (±0.30)	n.s.	0.85 [2] a (±0.21)	1.42 [2] b (±0.19)	0.04
	roots	2.22 [2] b (±0.48)	1.93 [2] a (±0.52)	0.07	1.80 [2] a (±0.35)	2.34 [2] b (±0.52)	0.07
	MeanBAF	1.31 [2] b (±0.51)	1.25 [2] a (±0.42)	0.04	1.00 [2] a (±0.36)	1.57 [2] b (±0.38)	0.04

a, b—means for investigated factors with different letters in the rows are significantly different; n.s.—not significantly differ at $p \leq 0.05$.

In most cases, there were no significant variations between cultivars of pea plants in the values of *BAF* and *MeanBAF* calculated for all heavy metals (for the separated parts and for the average for the entire plant, respectively). The exception was the higher *BAF* of cadmium and zinc obtained in the roots and *MeanBAF* of zinc in the case of the 'Milwa' cultivar compared to 'Batuta'. In addition, *BAF* values of copper in the seeds and of nickel in the aerial parts were higher for 'Batuta' than for 'Milwa'.

3.4. Translocation Factor of Heavy Metals

The *TF* values for cadmium, chrome, lead, and zinc calculated for aerial parts and seeds were little differentiated in the successive stages of pea growth (Table 11). Non-significantly different values of this coefficient were obtained: for cadmium from the four-leaf stage to the 50% pods stage and for seeds; for zinc from the four-leaf stage to the 50% pods stage; and for chrome and lead from the three-internode stage to the 50% pods stage. In the case of copper, the TF value tends to be higher during the period of rapid pea growth than at the beginning and the end of its vegetation.

Table 11. *TF* of heavy metals to aerial parts (Ap) and seeds (S) of pea plants (mean values ± SD of two cultivars and two successive years with three repetitions each).

Heavy Metals	Growth Stages							
	Four-Leaf (Ap)	Three-Internode (Ap)	First Bud (Ap)	Flowering (Ap)	50% Pods (Ap)	Maturity (Ap)	Maturity (S)	$LSD_{0.05}$
Cd	0.60 a b (±0.32)	0.79 b (±0.48)	0.58 a b (±0.29)	0.67 a b (±0.20)	0.55 a b (±0.13)	0.44 a (±0.14)	0.55 a b (±0.14)	0.30
Cr	0.12 a b c (±0.04)	0.15 c d (±0.04)	0.16 d (±0.05)	0.13 b c d (±0.03)	0.13 b c d (±0.05)	0.11 a b (±0.07)	0.09 a (±0.04)	0.04
Cu	0.78 b c (±0.29)	1.17 e (±0.41)	0.93 c d (±0.34)	1.06 d e (±0.30)	0.82 c (±0.28)	0.49 a (±0.28)	0.72 a b c (±0.26)	0.24
Ni	0.29 (±0.23)	0.23 (±0.15)	0.19 (±0.10)	0.22 (±0.11)	0.28 (±0.17)	0.33 (±0.08)	0.25 (±0.10)	n.s.
Pb	0.55 a (±0.23)	0.9 6 b (±0.19)	0.86 b (±0.15)	0.94 b (±0.20)	0.92 b (±0.16)	0.62 a (±0.13)	0.66 a (±0.18)	0.18
Zn	0.54 a b (±0.08)	0.6 1 b (±0.14)	0.56 a b (±0.13)	0.57 b (±0.14)	0.54 a b (±0.12)	0.49 a (±0.12)	0.49 a (±0.14)	0.08

a, b, c, d, e—means for investigated factors with different letters in the rows are significantly different.

The *TF* of cadmium, chrome, copper, lead, and zinc calculated for seeds did not differ significantly from the lowest values obtained for the aerial parts. The *TF* of nickel did not change significantly in successive stages of growth of the test plant.

n.s.—not significantly differ at $p \leq 0.05$.

The *TF* of chrome, copper, lead, and zinc was higher in the conditions of 2016 than those in 2015 (Table 12). The *TF* of cadmium and nickel to the aerial parts, and also of cadmium to the seeds, did not vary significantly between years of the study. The exception was the *TF* of nickel to the seeds, which was higher in 2015 than in 2016.

Table 12. *TF* of heavy metals in pea plants ([1] mean values in maturity ± SD of two successive years or two cultivars, respectively, and with three repetitions each; [2] mean values ± SD of six growth stages, two successive years or two cultivars, respectively, and with three repetitions each).

Heavy Metals	Parts of Pea Plants	Sources of Variation					
		Pea Cultivars			Years		
		'Milwa'	'Batuta'	$LSD_{0.05}$	2015	2016	$LSD_{0.05}$
Cd	seeds	0.56 [1] (±0.19)	0.54 [1] (±0.08)	n.s.	0.48 [1] (±0.14)	0.62 [1] (±0.09)	n.s.
	aerial part	0.54 [2] a (±0.23)	0.68 [2] b (±0.34)	0.12	0.56 [2] (±0.33)	0.65 [2] (±0.26)	n.s.
Cr	seeds	0.10 [1] (±0.05)	0.07 [1] (±0.03)	n.s.	0.05 [1] a (±0.01)	0.12 [1] b (±0.04)	0.04
	aerial part	0.14 [2] (±0.05)	0.13 [2] (±0.04)	n.s.	0.12 [2] a (±0.04)	0.15 [2] b (±0.05)	0.02
Cu	seeds	0.74 [1] (±0.32)	0.71 [1] (±0.21)	n.s.	0.49 [1] a (±0.06)	0.96 [1] b (±0.09)	0.07
	aerial part	0.80 [2] a (±0.35)	0.95 [2] b (±0.40)	0.09	0.69 [2] a (±0.37)	1.07 [2] b (±0.28)	0.09
Ni	seeds	0.26 [1] (±0.10)	0.24 [1] (±0.09)	n.s.	0.32 [1] b (±0.05)	0.17 [1] a (±0.07)	0.09
	aerial part	0.22 [2] a (±0.12)	0.29 [2] b (±0.17)	0.06	0.23 [2] (±0.16)	0.28 [2] (±0.13)	n.s.
Pb	seeds	0.67 [1] (±0.19)	0.65 [1] (±0.18)	n.s.	0.53 [1] a (±0.06)	0.80 [1] b (±0.15)	0.17
	aerial part	0.78 [2] (±0.22)	0.84 [2] (±0.25)	n.s.	0.77 [2] a (±0.29)	0.85 [2] b (±0.17)	0.07
Zn	seeds	0.47 [1] (±0.15)	0.51 [1] (±0.04)	n.s.	0.41 [1] a (±0.08)	0.57 [1] b (±0.06)	0.05
	aerial part	0.51 [2] a (±0.14)	0.59 [2] b (±0.09)	0.03	0.48 [2] a (±0.12)	0.62 [2] b (±0.09)	0.03

a, b—means for investigated factors with different letters in the rows are significantly different; n.s.—not significantly differ at $p \leq 0.05$.

The *TF* of all heavy metals to the seeds and of chrome and lead to the aerial parts were not significantly differentiated for the tested cultivars of pea plants. *TF* of cadmium, copper, nickel, and zinc to the aerial part was higher for the 'Batuta' than for 'Milwa' cultivar.

4. Discussion

4.1. Heavy Metal Content and Uptake by Pea Plants

Recent years have seen increasing demand for food and, at the same time, increasing expectations regarding its quality. Numerous studies have been carried out on the migration of various substances and elements toxic to living organisms into the food chain [52,53]. Among these, heavy metals occupy a special position. They do not undergo biodegradation, and in facilitative conditions they are easily taken up by plants and accumulated in their tissues. It is worth comparing the content of heavy metals in plants intended for direct consumption or for animal feed with binding standards, in order to determine whether they have been exceeded. The acceptable limits on the content in legume vegetables of cadmium and lead, metals posing a serious threat to the human body, are 0.1 mg·kg^{-1} fresh weight for both elements [54]. A higher maximum limit of lead in cereals and the edible seeds of legume plants, amounting to 0.2 mg·kg^{-1} fresh weight, is permitted by European Union regulations [55], and the limit for cadmium in legume seeds is 0.04 mg·kg^{-1} fresh weight [56]. An attempt to convert these standards to acceptable limits for the content of lead and cadmium in the dry matter of legume plants, assuming an average water content of 12% in air-dried seeds, resulted in values of 0.22 mg·kg^{-1} for lead and 0.045 mg·kg^{-1} for cadmium. Comparison of the results of the present study with these standards shows that the content of both elements in the pea seeds exceeded the acceptable limit. This state persisted in both years of the study and for both cultivars. Directive 2002/32/EC of the

European Parliament and of the Council of 7 May 2002 [57] on undesirable substances in animal feed specifies acceptable limits for cadmium and lead in animal feed of vegetable origin, amounting to 1 and 10 mg·kg^{-1}, respectively, and additionally a limit for lead content in green fodder of up to 40 mg·kg^{-1} relative to a feedingstuff with a moisture content of 12%. Conversion of these standards to acceptable limits for the content of cadmium and lead in the dry matter of animal feed resulted in values 1.12; 11.2, and 44.8 mg·kg^{-1}, respectively. The cadmium content of both pea cultivar plants harvested in all development stages in both years fully met this standard. Irrespective of the pea plant cultivar, the average content of lead in the seeds was higher than the standard and disqualified it as fodder. The variation in this trait between the years of the study showed that seeds harvested in the first year met the standard for lead content, but seeds from the second year exceeded it substantially. These results were obtained in conditions of pea cultivation on soil with slightly higher lead content in the first year (16.0 mg·kg^{-1}) than in the second year (13.9 mg·kg^{-1}). In both years, however, these were soils which, according to Polish standards for the content of heavy metals [46], can be used as arable land, orchards, meadows, pastures, and family garden allotments, and thus for cultivation of plants for human consumption and for fodder production. The cause of the excessive content of lead in the seeds may have been the greater availability of lead in the conditions of the experiment conducted in the second year (higher content, uptake, and bioaccumulation factor of Pb) and its higher factor of translocation to the seeds. The experiment in the second year was set up on a different soil than in the first year (although located nearby) (Table 1). Moreover, in the second year of the study, during the period of the fastest growth and development of peas (May–June), less rainfall and higher air temperatures were recorded than the long-term average values of these parameters. Thus, it was a warmer period, with less rainfall in the study area than average. The mentioned period in the second year of the research was warmer than in the first year (higher average air temperature) with less precipitation in May and slightly more in June. In July of the second year of the study, very heavy rainfall was recorded, almost twice as much as the long-term average and slightly more than twice as much as in the first year. Literature data show that in conditions of high air humidity, the uptake of heavy metals by leaves increases [58]. In our study, higher content and uptake of heavy metals were more often obtained in the second than in the first year. However, there are no lead or other heavy metal emitters located in the study area and in the vicinity; therefore, contamination should not occur in this way. Under the conditions of the research conducted in the second year, the processes of mobilization of heavy metals to forms available for plants could have occurred in the soil, which led to the increased uptake of these elements by pea plants. The content of lead in the aerial parts of the pea, which can serve as green fodder, did not exceed 44.8 mg·kg^{-1} for any of the analyzed growth stages, pea cultivars, or years of study. The results indicate that in the light of current standards, the green parts of pea can be used as high-quality fodder, while the seeds harvested in the second year of the study should not be used as animal feed.

According to literature data, a slightly elevated accumulation of lead in pea does not negatively affect the plant because it is tolerant to elevated lead concentrations [59,60]. However, pea is highly sensitive to elevated concentrations of cadmium [60]. In the case of soil with elevated cadmium content, to protect against its toxic effects it is recommended to apply substances with the capacity to adsorb it, such as biochar, gravelly sand [61], farmyard manure, or zeolite [62].

Excessive cadmium concentrations in green pea have been noted in studies by other authors [63]. They report cadmium and chrome content in commercial green pea at the same level of 0.5–1.0 mg·kg^{-1}. The authors did not detect lead in that study, and the level of nickel reached 0.5 mg·kg^{-1}. Among the heavy metals tested they obtained the highest content of copper (up to 30 mg·kg^{-1}) and zinc (up to 40 mg·kg^{-1}). Based on these results, the content of heavy metals in green pea can be ranked as follows: Zn > Cu > Cd = Cr > Ni > Pb. Other authors report the following order of concentrations of heavy metals in the seeds of green pea: Pb > Zn > Ni > Cr > Cd > Cu for plants grown on soils not contaminated with heavy metals; and Cd >Pb > Zn > Ni > Cr > Cu for pea plants grown on contaminated soil [45]. From these two reports, peas may have the potential to accumulate large amounts of various heavy metals. Leading the ranking are zinc, lead, and cadmium. In own study, the levels of cadmium, chrome, and nickel were low, while the concentrations of zinc and lead were high in the pea seeds. The average content of heavy metals in the seeds ranks as follows: Zn > Pb > Cu > Ni > Cr > Cd. With the exception of cadmium, the ranking determined in our own research reflects the tendency of peas to accumulate large amounts of zinc and lead compared to other heavy metals. The high content of zinc in pea seeds confirms their great usefulness as a source of this trace element in the diet for all people, not only for vegetarians [64]. These authors report that zinc absorption from diets rich in legumes, especially after their processing, is comparable to that from diets based on animal protein. Low cadmium content compared to other heavy metals results from its relatively low content in soils (Table 1) [45,65,66]. However, it is a highly toxic element for plants. Progressively, with an increasing concentration of cadmium in the ground, the rate of photosynthesis, chlorophyll content, activities of photosystem, and photosynthetic enzymes decrease [67,68]. Heavy metals such as Cd, Pb, Cu, and Zn taken up by roots cause oxidative stress in the treated pea roots. Under these conditions, an increase in the activity of antioxidant enzymes is observed [69]. These researchers proved that among the above-mentioned heavy metals, the least toxic trace element for pea plants is Zn, while Cu and Cd were the most toxic. In addition, they report that Cu is the trace element that moves most rapidly from roots to shoots; in their study, this value was about 50%. However, copper is less toxic to pea plants than lead or zinc [70].

Peas grown on soil not contaminated with heavy metals or on contaminated soil often contain higher concentrations of these elements in the roots than in the shoots [45]. This was confirmed in the present study, in which the content of the heavy metals in the roots was most often higher than that in the aerial parts and seeds. This proves the presence of mechanisms in pea plants that prevent the movement of some of them from the roots to the aerial parts. Most often, structural elements of the cell wall participate in this process, e.g., through pectin compounds and proteins [71].

In the subsequent phases of growth and development of pea plants, a tendency to increase the uptake of heavy metals by the entire plant was noted, with a simultaneous tendency to decrease their concentration. This relationship can be linked to the dilution effect of their concentration in the stages of rapid growth. Significant dilution effects were most often observed up to the first bud phase of tested plants, while a significant increase in their uptake was most often observed up to the flowering phase. Starting from the flowering phase, there was a tendency to decrease the quantity of heavy metals accumulated in the roots. After summing the uptake of heavy metals in the aerial parts and pea seeds, a tendency to increase their uptake in the entire above-ground mass in all subsequent development stages can be observed. Seed separation at full maturity shows that 40.7% of cadmium, 26.6% of chrome, 47.2% of copper, 29.9% of nickel, 37.6% of lead, and 36.6% of zinc were accumulated in/transferred to the seeds.

The quantities of accumulated cadmium, nickel, lead, and zinc in the entire pea plant's mass were higher in the second year than in the first of study. This dependence should be associated with a much higher content of these metals in the slightly smaller pea biomass harvested in the second year than in the first year of cultivation [72].

4.2. Bioaccumulation Factor of Heavy Metals

The efficiency of migration of heavy metals from the soil to plants was assessed by calculating their bioaccumulation factors (*BAFs*) [73,74]. *BAF* values in the range of 1–10 indicate a hyperaccumulator plant, *BAF* values of 0.1–1 indicate a moderate accumulator plant, *BAF* values of 0.01–0.1 indicate a low accumulator plant, and *BAF* values of <0.01 indicate a non-accumulator plant [75]. The *BAF* values for the aerial parts and seeds of pea in the present study were 0.60–0.96 Cd, 0.12–0.27 Cr, 0.47–0.79 Cu, 0.47–0.67 Ni (1.14 only in the four-leaf stage), 0.94–1.42 Zn, and 0.99–1.25 Pb. These indicate the potential of pea for hyperaccumulation of lead and zinc and moderate accumulation of the other heavy metals in the aerial parts. In the second year of the study, in which the values for lead content were exceeded in the seeds, the *BAF* values for all heavy metals were usually higher in all separated parts of the plant and on average for the entire plant than in the first year. Calculation of the average *BAF* in the entire plant (including the roots, with which heavy metals remain in the soil in practice) and in the entire growing period allows heavy metals to be ranked in declining order as follows: Zn (1.29) > Pb (1.20) > Ni (1.09) > Cd (0.8) > Cu (0.63) > Cr (0.43). These calculations revealed that in the case of pea cultivation on soil uncontaminated with heavy metals, the bioaccumulation factors of zinc, lead, and, additionally, nickel were greater than 1, which indicates the possibility of their hyperaccumulation by pea [45].

4.3. Translocation Factor of Heavy Metals

Analysis of the mobility of heavy metals in the soil–pea system was supplemented by determining their capacity for translocation in the plant. The translocation factor (*TF*) of heavy metals was used for this purpose. *TF* values less than one indicate ineffective metal transfer, suggesting that these types of plants accumulate metals in the roots and rhizomes more than in the shoots or leaves [76]. Based on the average *TF* values in the entire growing period, the following pattern was obtained: Cu (0.85) > Pb (0.79) > Cd (0.60) > Zn (0.54) > Ni (0.26) > Cr (0.13). The mean values did not exceed 1. This indicates that pea accumulates all the heavy metals in the roots to a greater extent than in the aerial parts. The values of this factor for copper and lead, but also for cadmium and zinc, indicate that these metals are more easily translocated from the roots to the aerial parts than in the case of chrome and nickel. This is especially concerning in the case of lead and cadmium, as these heavy metals perform no physiological functions, and at high concentrations can have toxic effects. From the four-leaf stage to full maturity of pea plants, the *TF* values were in the following ranges: 0.44–0.79 Cd, 0.09–0.16 Cr, 0.49–1.17 Cu, 0.19–0.33 Ni, 0.55–0.96 Pb, and 0.49–0.61 Zn. These indicate that only the content of copper in the aerial parts in some stages of development was higher than that in the roots. Galal et al. [45] also obtained the highest *TF* values for lead and copper in pea grown on soil that was not contaminated with heavy metals. Their and our research shows that these metals are relatively easily translocated from the roots to the aerial parts of the test plant.

5. Conclusions

The content of the analyzed heavy metals in the separated parts and on average in the entire pea plants, as well as their bioaccumulation factors (*BAFs)*, were usually highest at the start of growth and decreased until the flowering stage, after which the values stabilized. Between the three-internode stage and the full flowering stage, their translocation factor (*TF*) most often was the highest. In the phase of full maturity of pea plants, no significant differences were obtained in the *TF* factor for the aerial parts and to the seeds. Between the flowering stage and the stage when 50% of pods were of typical length, the highest rate of uptake of heavy metals per day (HMD_{up}) of growth was noted. The *BAF* indicates a moderate accumulation of cadmium, chrome, copper, and nickel, as well as a tendency to hyperaccumulate lead and zinc in pea plants. Higher content, HMD_{up}, *BAF*, and *TF* of heavy metals were most often obtained in the second than in the first year of the study. The pea variety usually had no significant effect on the values of the tested parameters. Compared to the applicable standards for the content of heavy metals, the green mass of pea plants was suitable as uncontaminated animal feed, while the seeds contained excessive concentrations of lead, preventing their use as fodder, and excessive concentrations of lead and cadmium, preventing their use as human food.

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