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Soil-to-Wheat Transfer of Heavy Metals Depending on the Distance from the Industrial Zone

Violeta Mickovski Stefanović [1], Svetlana Roljević Nikolić [1], Mirela Matković Stojšin [1,*], Helena Majstorović [1], Mladen Petreš [2], Dejan Cvikić [3] and Gordana Racić [4]

[1] Tamiš Research and Development Institute, Novoseljanski Put 33, 26000 Pančevo, Serbia; mickovski.stefanovic@institut-tamis.rs (V.M.S.); roljevic@institut-tamis.rs (S.R.N.); majstorovic@institut-tamis.rs (H.M.)
[2] Faculty of Agriculture, University of Novi Sad, Dositej Obradović Square 8, 21000 Novi Sad, Serbia; mladen.petres@polj.uns.ac.rs
[3] Institute for Vegetable Crops, Karadjordjeva 71, 11420 Smederevska Palanka, Serbia; dcvikic@institut-palanka.rs
[4] Faculty of Ecological Agriculture, Educons University, Vojvode Putnika 87, 21208 Sremska Kamenica, Serbia; gordana.racic@educons.edu.rs
* Correspondence: matkovic.stojsin@institut-tamis.rs

Abstract: The accumulation of heavy metals in the environment is one of the most significant environmental problems due to the potential risk to human and animal health. The aim of this study was to analyze the influence of the distance from the industrial zone on the heavy metal content in the soil and vegetative parts of wheat. A field experiment with four wheat genotypes was conducted in the area of the city of Pancevo, Serbia, at three locations at different distances from the industrial zone. By atomic absorption spectrophotometry (AAS), concentrations of five heavy metals (Zn, Pb, Cr, Cu, and Cd) were determined in the soil and wheat. The highest total content of Zn, Cr, Cu, and Cd in the soil (72.5, 27.3, 26.2, and 0.3 mg kg^{-1}, respectively) was found at the location closest to the industrial zone, while the highest content of Pb (28.9 mg kg^{-1}) was recorded at a location that is in the immediate vicinity of a road. Heatmap correlations and PCA analysis show a significant relationship between the content of heavy metals in the soil and the plant. Genotype Pobeda had the lowest content of Cr, Cu, and Cd in the root and the lowest content of all the analyzed heavy metals in the stem. The highest translocation factor of heavy metals was found in the genotype Apache, which had the highest content of Pb, Cr, and Cu in the stem. The highest heavy metal bioaccumulation and translocation were established for Cd content (0.86 and 1.93). The obtained results indicate a potential ecological risk in the immediate vicinity of the industrial zone, while the difference in the accumulation of heavy metals between the studied genotypes opens new aspects for breeding programs.

Keywords: bioaccumulation factor; translocation factor; heavy metals; industrial zone; wheat

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1. Introduction

The rapid growth of the human population, urbanization, and industrialization significantly reduce agricultural areas, but various harmful agents also adversely affect the lands and areas on which agricultural production is organized [1,2]. As these areas are closer to the main polluters in the ecosystem, the effects on cultivated plants become more pronounced [3,4]. In summary, all living organisms are significantly harmed by environmental pollution [2,5]. Environmental toxins include both organic and inorganic pollutants and represent a significant threat to the ecosystem as a whole, seriously impairing its structure and function [6].

According to many studies performed all over the world, anthropogenic industrial activity is a major cause of heavy metal pollution in the soil and environment [7–12].

Additionally, modern agriculture is a substantial anthropogenic source of heavy metals due to the use of pesticides, inorganic mineral fertilizers, and fuel burning [1,13–16]. Heavy metals and metalloids, including Cr, Mn, Co, Ni, Zn, Cu, Cd, Sn, Hg, and Pb, can result in significant toxic impacts [16,17]. The ability of heavy metals to enter water and food supply systems and their inability to break down could have long-term effects on food safety and human health [18,19]. The harmful effects of heavy metals on humans, animals, and plants are contributed by their persistent nature, high toxicity, bioavailability, and potential risk for greater bioaccumulation [20]. Although some heavy metals, including Cu and Zn, are necessary for a plant's growth and development, excessive amounts of these metals can be toxic to the plant [21,22]. On the other hand, Pb and Cd are only toxic to plants and are not known to have any positive impacts on them [23,24]. Such contaminants can cause acute and chronic diseases, such as lung cancer, renal dysfunction, osteoporosis, and cardiovascular diseases [25,26], as well as the occurrence of various neurological diseases in adults and children [17,27,28]. This is especially important because plants grown on polluted soil can accumulate high concentrations of heavy metals and may serve as the main pathway for transferring metals into the food chain [6,29,30].

Due to the aforementioned considerations, it is important to monitor and control the amount of heavy metals in the soil and their absorption by plants. The accumulation of heavy metals in plants occurs both in the roots and in the aerial parts of the plant [31–34], and the study of the bioavailability, accumulation, and translocation of heavy metals has attracted a lot of attention from researchers. Wheat (*Triticum aestivum* L.) is a staple food that is consumed by around half of the world's population and provides almost 20% of the total calories and protein in the daily diet [35–39]. Wheat consumption is increasing globally as a result of the continuing growth in the human population in both suitable and unfavorable regions for agriculture [37–40]. Regarding the contamination of the human food chain by heavy metals, pollution of cereals, notably wheat, is a major issue [41,42]. Therefore, more consideration should be given to the safety of wheat grain in terms of food security and human health.

Previous research has shown that the content of heavy metals in wheat is mainly conditioned by their content in the soil [43], as well as the influence of other factors, such as the content of organic matter, pH of the soil, capacity of exchangeable cations, the mechanical composition of the soil [44–49], the cultivation system [50], temperature, soil moisture, and plant physiology [45]. Atmospheric deposition and prevailing wind direction can also have a significant influence on the distribution and accumulation of heavy metals in soil and plants [11,51–54].

In earlier studies, the analysis of the impact of the industrial zone on the heavy metal content in soil and wheat was mostly carried out in areas close to industrial plants [55,56], while the majority of studies examined the influence of the distance from the industrial zone on the content of heavy metals in soil [57–61]. In this study, experiments were established at various distances from industrial plants to examine the impacts of the vicinity of the industrial zone on the soil-to-wheat transfer of heavy metals. In addition to the effects of the locality, the genotype and phenophase factors, as well as their interactions, were also studied.

The objectives of this research were: (i) to examine the influence of localities with different distances from the industrial zone on the content of heavy metals in the soil and vegetative parts of wheat through different phenological phases; (ii) to evaluate the relationship between the content of heavy metals in the soil and those in the vegetative parts of plants; (iv) to identify wheat genotypes that exhibit a lower tendency to accumulate heavy metals, with the aim of mitigating potential risks to human health.

2. Materials and Methods

2.1. Experimental Design and Plant Material

The study of the influence of the distance from the industrial zone on the content of heavy metals in the soil and vegetative parts of wheat in different phenophases was carried out in the area of the city of Pancevo, Serbia, which represents the industrial center of the South Banat District. A 3-factor field trial was conducted according to a randomized block design with four replications, with the area of the elementary plot amounting to 15 m^2 *and a total plot area of* 250 m^2 *at each locality*. The examined factors were genotype, locality, and phenophase. The experiment was set up at three locations, each at a different distance from the southern industrial zone in Pančevo City (NIS Oil Refinery, Pančevo, Serbia, 44.831° N 20.690° E): Locality 1–L1 (Vojlovica, 44.837° N 20.694° E, 262.0 m from the industrial zone); Locality 2—L2 (Stari Tamiš, 44.857° N 20.746° E, 5002.0 m from the industrial zone); and Locality 3—L3 (the experimental field of the Tamiš Research and Development Institute, 44.938° N 20.720° E, 11.635.0 m from the industrial zone) (Figure 1). The microclimatic conditions of the study areas were at the level of multi-year estimates throughout the two-year research period, with an average annual air temperature of 11.9 °C and a total precipitation of 684.3 mm. According to the Food and Agricultural Organization (FAO) World Reference Base (WRB) [62], the soil on which the micro-examinations were conducted is of the calcic chernozem (CH-cc-ce.ph) type, and the mechanical composition is crumbly-grained loam.

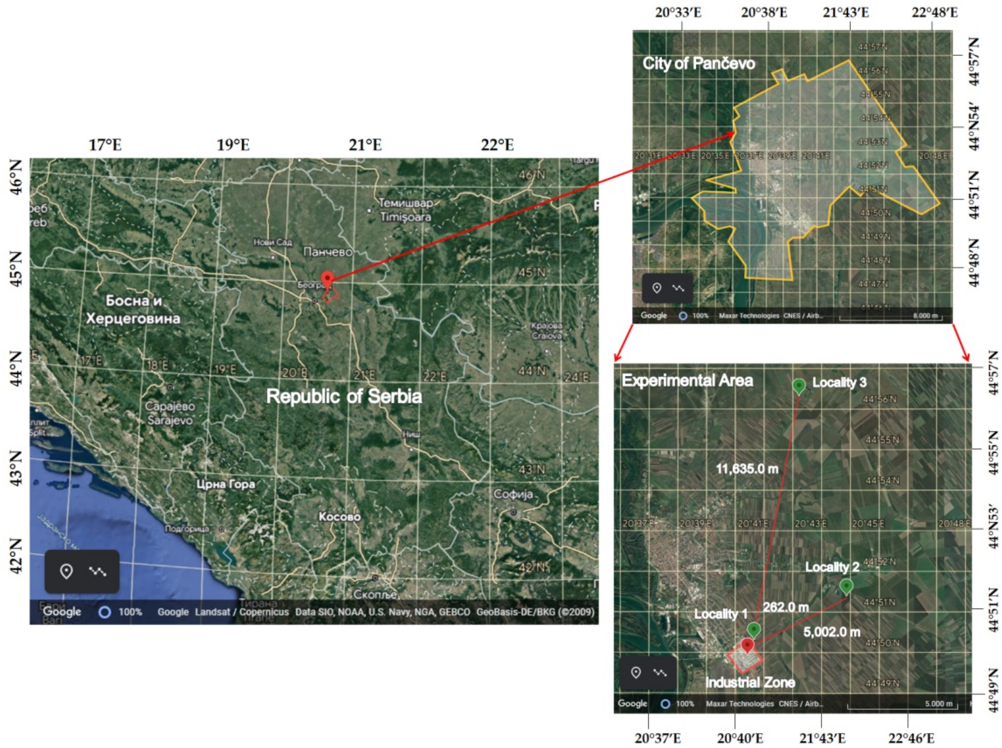


Figure 1. Map of the studied localities (https://earth.google.com/web/ (accessed on15 November 2022) [63].

The experiment was conducted according to a randomized block design with four replications. The elementary plot was 15 m^2. In all three locations, standard agrotechnical measures for wheat production were applied, and the pre-crop was corn. The plowing is carried out using a mouldboard plow at 30 cm, pre-sowing soil preparation using a disc harrow and a harrow, basic fertilization in the autumn with 200 kg ha^{-1} MAP (12:52:0), and top dressing in the spring with 250 kg ha^{-1} AN (27% N). Four common winter wheat (*Triticum aestivum* ssp. *vulgare*) cultivars, Pobeda, Ljiljana, Renesansa, and Apache, were sown in four replications on all three localities in the experiment. Pobeda, Ljiljana, and Renesansa varieties were created at the Institute of Field and Vegetable Crops (Novi Sad, Serbia), while Apache is a modern wheat variety, created in France, which was approved in Serbia in 2007. Sowing was conducted with a pneumatic seeder on the optimal agrotechnical terms for winter wheat, with a seeding rate of 550 seeds per m^2.

Pobeda is a mid-to-late wheat variety. It shows very good tolerance to winter frosts and tolerance to lodging. It is genetically tolerant to powdery mildew (Blumeria graminis DC). Ljiljana is a medium-early variety; the average plant height is about 90 cm; and it is tolerant of lodging and winter frosts. It is genetically tolerant to the pathogens that cause leaf rust (Puccinia triticina) and powdery mildew (Blumeria graminis DC). Renesansa is a medium-early variety. It shows good tolerance for lodging and winter frosts. Apache is an early variety of exceptional quality. It is genetically tolerant to lodging and the pathogenic agent Fusarium sp.

The measurement of the concentration of heavy metals in the root and stem of wheat was carried out in the heading stage (55–59 on the BBCH scale) and in the stage of full maturity (89 on the BBCH scale).

2.2. Soil Sampling and Chemical Analysis

In order to perform agrochemical analysis, soil sampling was carried out before the implementation of agrotechnical measures on the experimental plots. Soil samples were taken from four randomly selected positions of each plot at a depth of 0–60 cm, using a soil auger. The representative sample was made using the random-square method as described by Korunović and Stojanović [64].

The following soil parameters were analyzed:

- Soil pH was measured potentiometrically in a distilled water suspension at the soil-to-water ratio of 1:5 (v/v) [65], according to the SRPS ISO 10390:2007 [66];
- Calcium carbonate content was determined volumetrically in accordance with the methods of Scheibler [67], SRPS ISO 10693-2005 [68];
- Soil organic matter content was determined using the method of Tyurin [69];
- Total nitrogen content was measured using the Kjeldahl method [70], SRPS ISO 11261:2005 [71];
- Available phosphorus and potassium were measured using a solution of ammonium lactate (0.1 M) and acetic acid (0.4 M) (AL-method) according to Egner-Riehm [72], described by Manojlović [73,74].

Total N in soil was measured by Kjeldahl method (Bremner, 1996);

In addition to the chemical properties of the soil, the content of heavy metals: zinc (Zn), lead (Pb), chromium (Cr), copper (Cu), and cadmium (Cd) in the soil and plant samples were determined after wet digestion by concentrated nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) [75,76]. HNO_3 and H_2O_2 were of analytical grade, free of heavy metals and other impurities that could interfere with the analysis, and were purchased from Merck (Darmstadt, Germany). Proficiency testing schemes were used as a quality control measure for analytical determination, which achieves control using certified reference materials (CRMs) with known concentrations of heavy metals. The limit of detection (LOD) was estimated as the mean value of the blank signals plus three times the standard deviation [77]. A series of blank samples (samples that do not contain the analyte of interest) was measured. The LOD values were 0.35, 0.09, 0.04, 0.39, and 0.001 mg kg^{-1} for Zn, Pb, Cr, Cu, and Cd, respectively.

Soil samples were air-dried and crushed into fine-grained particles with a median diameter of less than 2 mm. Then 2 g of the sample was weighed and poured into 20 mL of 60% HNO_3. Moderate boiling was carried out for 2 h. After cooling, 3 mL of 30% H_2O_2 was added, followed by boiling for 15 min. The procedure with H_2O_2 was repeated until no further reaction was observed. After cooling, the samples were quantitatively transferred into borosilicate glass normal vessels of 100 mL and topped up to the final volume with distilled water. The solution was filtered through quantitative filter paper, and the reading was performed on an atomic absorption spectrophotometer, Varian SpectrAA 202 FS (Varian Inc., Palo Alto, CA, USA), in an acetylene/air flame.

2.3. Sampling of Plant Material and Chemical Analysis

In the phenophases of heading and full maturity, plant material samples of four wheat genotypes were collected at all three localities. An individual sample from each elementary plot consisted of 15 wheat plants. The whole plant with the root was sampled, after which the root was physically separated from the wheat stem in laboratory conditions.

Samples of plant material (root and stem, separately) were macerated and dried in an oven at 80 °C. Then 1 g of the prepared sample was weighed and poured into 20 mL of 60% HNO_3. Moderate boiling was carried out for 2 h. After cooling, 3 mL of H_2O_2 was added, followed by boiling for 15 min. The procedure with H_2O_2 was repeated until no further reaction was observed. After cooling, 2 mL of $HClO_4$ was added, and gentle evaporation was performed until dense white vapors of perchloric acid appeared [76]. Then 5 mL of 5 M HCl was added, and then the samples were quantitatively transferred into 50 mL borosilicate glass normal vessels and made up to the final volume with distilled water. The solution was filtered through quantitative filter paper. The reading was performed by atomic absorption spectrophotometry.

Heavy metal content was measured in triplicate in each plant sample.

2.4. Bioaccumulation and Translocation factor

The bioaccumulation potential of the wheat plants was calculated using the following formulas:

Bioaccumulation factor (BAF) [56]:

$$\text{BAF} = \text{heavy metals in the plant (mg kg}^{-1}\text{)/heavy metals in the soil (mg kg}^{-1}\text{)} \quad (1)$$

Translocation factor (TF) [19]:

$$\text{TF} = \text{heavy metals in the stem (mg kg}^{-1}\text{)/heavy metals in the root (mg kg}^{-1}\text{)} \quad (2)$$

2.5. Statistical Analysis

Analysis of the heavy metals' variability was performed using analysis of variance (ANOVA). Multiple comparisons of the mean values of factor variants were performed using the LSD test at two levels of statistical significance (1% and 5%). This analysis was performed using the program IBM SPSS Statistics, Trial Version 22.0 [78]. Statistical data for heavy metal content measured in roots and steam are given in Tables S1 and S2 in the Supplementary Material.

A heatmap analysis of Pearson moment correlation coefficients and correlation matrix analysis by the principal components method (PCA) was performed in order to express the association between heavy metal content in the soil and in different vegetative parts of wheat using the R Project for Statistical Computing, Version 4.2.0, 22 April 2022 ucrt [79].

3. Results

3.1. Heavy Metals Content in Soil

The soil under investigation was highly (L1) to strongly (L2 and L3) carbonated, slightly alkaline, well provided with organic matter, and readily accessible potassium (K_2O), but poor with readily accessible phosphorus (P_2O_5). The lowest $CaCO_3$ content (5.74%) and soil pH (7.38) were observed on L1, with significant differences in relation to L2 and L3. The higher content of $CaCO_3$ at L2 (15.41%) and L3 (15.48%) influenced the increase in pH, with a significant decrease in the content of readily accessible P_2O_5 (2.20 mg 100 g^{-1}) and K_2O (22.50 mg 100 g^{-1}). Although the present differences in the organic matter content between the analyzed localities were insignificant ($p > 0.05$), the total nitrogen content (total N) on L3 (0.20%) was significantly lower compared to the total N content on L1 (2.54%) and L2 (2.69%), which may be a consequence of the different intensity of microbiological processes at the analyzed localities (Table 1).

Table 1. Chemical properties and heavy metal content in the surface layer of soil at the studied localities.

Properties	Locality 1 (L1)	Locality 2 (L2)	Locality 3 (L3)	LSD Test	
				$LSD_{0.05}$	$LSD_{0.01}$
$CaCO_3$ (%)	5.74 ± 0.3 b	15.41 ± 0.02 a	15.48 ± 0.07 a	0.504	0.764
Ph	7.38 ± 0.02 c	8.38 ± 0.01 b	8.60 ± 0.06 a	0.104	0.458
Organic Matter (%)	3.79 ± 0.25 a	4.01 ± 0.32 a	4.03 ± 0.12 a	1.046	0.690
Total N (%)	2.54 ± 0.10 a	2.69 ± 0.17 a	0.20 ± 0.03 b	0.326	0.494
P_2O_5 (mg 100 g^{-1})	9.80 ± 0.9 a	2.20 ± 0.35 b	2.20 ± 0.23 b	1.620	2.454
K_2O (mg 100 g^{-1})	50.00 ± 1.13 a	22.50 ± 1.75 b	22.50 ± 0.64 b	3.547	5.386
Zn (mg kg^{-1})	72.5 ± 1.54 a	67.8 ± 1.02 b	56.0 ± 0.92 c	2.844	4.310
Pb (mg kg^{-1})	15.3 ± 0.43 c	20.8 ± 0.56 b	29.8 ± 0.71 a	1.640	2.486
Cr (mg kg^{-1})	27.3 ± 0.45 a	18.5 ± 0.15 c	20.7 ± 0.23 b	0.860	1.302
Cu (mg kg^{-1})	26.2 ± 0.57 a	21.7 ± 0.93 b	17.8 ± 0.65 c	2.072	3.138
Cd (mg kg^{-1})	0.27 ± 0.001 a	0.13 ± 0.001 b	0.08 ± 0.001 b	0.036	0.054

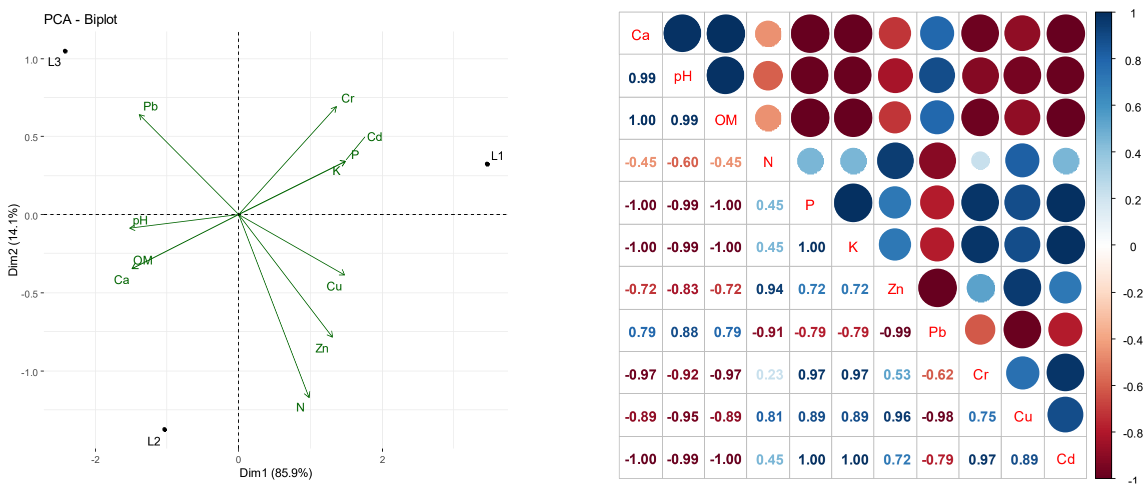
Note: Total content in the soil mean ± SD. Different letters (a, b, and c) indicate significant differences ($p < 0.05$) in the soil's chemical properties between localities.

The results show the statistically significant variability of heavy metal content in the soil among the studied localities. The highest concentrations of Zn (72.5 mg kg^{-1}), Cr (27.3 mg kg^{-1}), Cu (26.2 mg kg^{-1}), and Cd (0.27 mg kg^{-1}) were found in the locality closest to the industrial zone (L1). On the other hand, the content of Pb in the soil at L2 (20.8 mg kg^{-1}) and L3 (29.8 mg kg^{-1}) was significantly higher compared to the Pb content at L1 (15.3 mg kg^{-1}) (Table 1).

The association between the studied soil chemical properties is presented in Figure 2a,b. The total variance is perfectly represented by two principal components (Dim.1 and Dim.2), with eigenvalues > 1, where the first PCA component explains 85.9% and the second one explains 14.1% of the total variance. Analyzed localities are positioned in different quadrants of the PCA biplot, indicating a mutual difference in the soil's chemical properties. The content of the total N had the largest contribution to the total variation. This parameter is in a significant and positive correlation with the content of Zn (0.94) and Cu (0.81) in the soil. Copper (Cu) and zinc (Zn) are both essential micronutrients for plants, and their concentrations in soil are often correlated due to similar sources. The content of P_2O_5 and K_2O is positively associated with the content of Cd and Cr in the soil. These parameters are positioned near the point of the locality closest to the industrial zone (L1). The content of organic matter, $CaCO_3$, and pH value are positioned in the same quadrant of the biplot, with vectors overlapping each other at a sharp angle (<90°). The heatmap correlations also show the interrelationship of these three parameters, which are highly and positively correlated with Pb content and highly significant and negatively correlated with other studied parameters, which may indicate a different source of Pb reaching the soil.

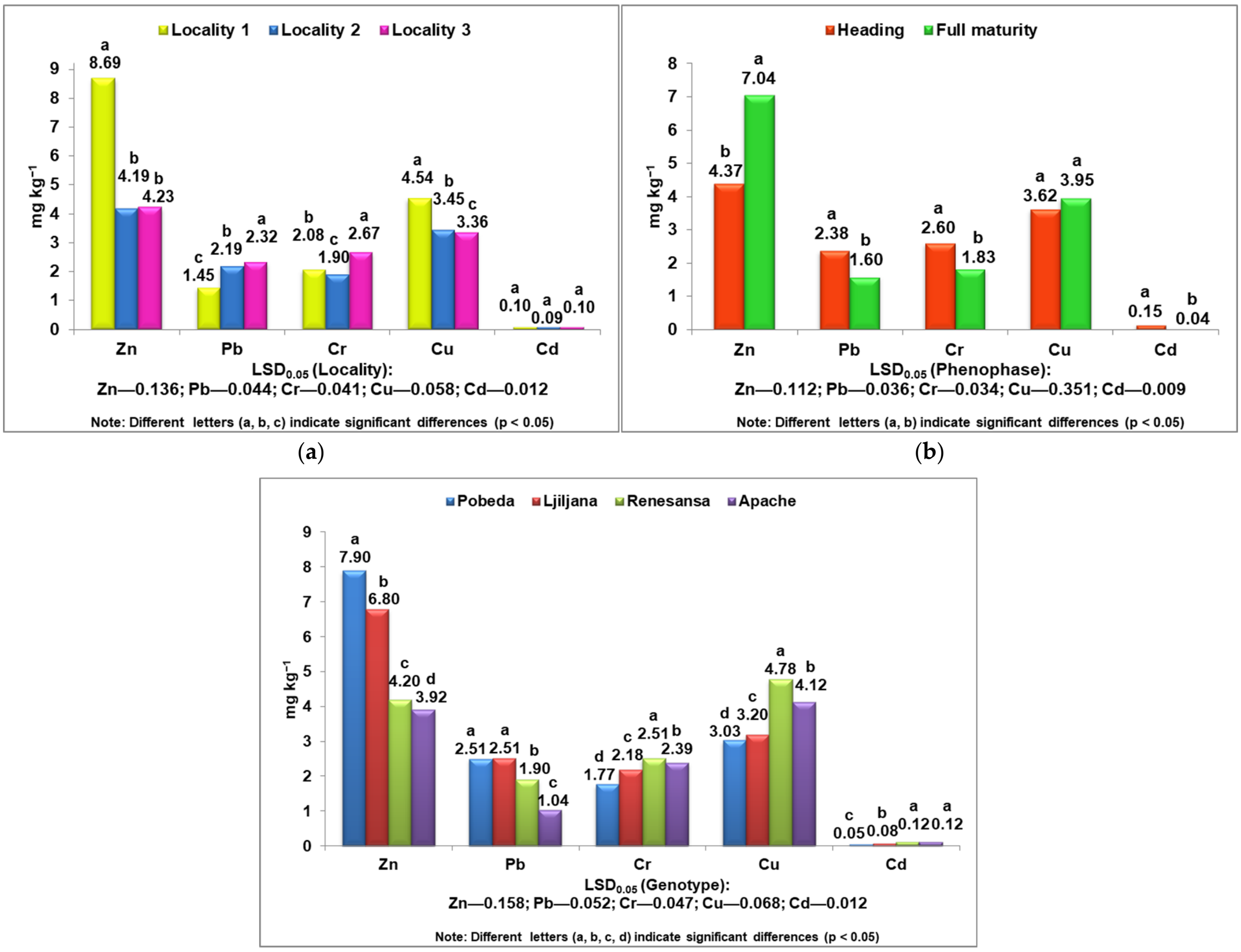
3.2. Heavy Metal Content in Wheat Roots

The analyzed factors (locality, genotype, and phenophase) and their interactions significantly influenced the variability of the heavy metal content in wheat roots (Figure 3 and Table 2). The results indicate that the highest concentrations of Zn (8.69 mg kg^{-1}) and Cu (4.54 mg kg^{-1}) in the plant's roots were found at location L1, which is the closest to the industrial zone, while the highest concentration of Pb (2.32 mg kg^{-1}) and Cr (2.67 mg kg^{-1}) was found in the samples collected at location L3, which is the farthest from the industrial zone (Figure 3a).



(**a**) (**b**)

Figure 2. Principal components analysis (PCA) (**a**) and heatmap of Pearson moment correlation coefficients (**b**) for the soil's chemical properties ($CaCO_3$—Ca, pH, organic matter—OM, total N—N, P_2O_5—P, K_2O—K, Zn, Pb, Cr, Cu, and Cd) at analyzed localities.



(**a**) (**b**)

(**c**)

Figure 3. Heavy metal content (mean values, mg kg^{-1}) in wheat roots: (**a**) averaged per localities (average values of four genotypes in two phenophases); (**b**) averaged per phenophases (average values of four genotypes at three localities); (**c**) averaged per genotypes (average values of each genotype at three localities and two phenophases).

Table 2. Heavy metal content (mean values ± standard deviation of sample means) in roots of four wheat genotypes per locality (average values for both phenophases) and per phenophase (average values for three localities).

Locality (L)	**Genotype (G)**	**Zn**	**Pb**	**Cr**	**Cu**	**Cd**
		Mean Value ± Sd (mg kg^{-1})				
Locality 1	Pobeda	13.38 ± 0.37	1.75 ± 0.04	1.43 ± 0.05	4.42 ± 0.08	0.049 ± 0.007
	Ljiljana	12.55 ± 0.17	2.14 ± 0.05	1.39 ± 0.05	4.82 ± 0.09	0.088 ± 0.007
	Renesansa	4.08 ± 0.09	1.06 ± 0.04	2.64 ± 0.07	4.52 ± 0.09	0.117 ± 0.013
	Apache	4.74 ± 0.12	0.85 ± 0.04	2.85 ± 0.08	4.38 ± 0.07	0.119 ± 0.011
Locality 2	Pobeda	4.25 ± 0.12	2.11 ± 0.07	1.72 ± 0.06	1.65 ± 0.05	0.047 ± 0.007
	Ljiljana	4.39 ± 0.11	2.42 ± 0.07	1.94 ± 0.04	2.68 ± 0.05	0.039 ± 0.006
	Renesansa	4.45 ± 0.10	3.24 ± 0.08	2.38 ± 0.07	5.10 ± 0.07	0.116 ± 0.018
	Apache	3.65 ± 0.09	0.97 ± 0.04	1.53 ± 0.06	4.36 ± 0.09	0.118 ± 0.020
Locality 3	Pobeda	6.06 ± 0.10	3.65 ± 0.06	2.15 ± 0.07	3.02 ± 0.07	0.049 ± 0.008
	Ljiljana	3.45 ± 0.07	2.96 ± 0.06	3.20 ± 0.05	2.08 ± 0.05	0.097 ± 0.008
	Renesansa	4.06 ± 0.08	1.38 ± 0.05	2.51 ± 0.04	4.72 ± 0.07	0.118 ± 0.013
	Apache	3.35 ± 0.07	1.29 ± 0.04	2.78 ± 0.06	3.62 ± 0.07	0.119 ± 0.012
$LSD_{0.05}$ (L × G)		0.273	0.088	0.083	0.117	0.020
Phenophase (P)	Genotype (G)	Zn	Pb	Cr	Cu	Cd
		Mean Value ± Sd (mg kg^{-1})				
Heading	Pobeda	4.76 ± 0.11	3.19 ± 0.06	2.12 ± 0.07	2.75 ± 0.06	0.059 ± 0.006
	Ljiljana	4.80 ± 0.10	3.82 ± 0.07	3.31 ± 0.05	2.73 ± 0.05	0.129 ± 0.009
	Renesansa	4.02 ± 0.09	1.45 ± 0.06	2.64 ± 0.07	4.67 ± 0.07	0.195 ± 0.023
	Apache	3.89 ± 0.09	1.06 ± 0.04	2.33 ± 0.07	4.32 ± 0.07	0.198 ± 0.019
Full maturity	Pobeda	11.04 ± 0.29	1.82 ± 0.05	1.42 ± 0.05	3.31 ± 0.06	0.036 ± 0.007
	Ljiljana	8.80 ± 0.12	1.19 ± 0.05	1.05 ± 0.03	3.66 ± 0.07	0.027 ± 0.005
	Renesansa	4.37 ± 0.08	2.34 ± 0.05	2.39 ± 0.05	4.89 ± 0.08	0.037 ± 0.006
	Apache	3.95 ± 0.09	1.02 ± 0.04	2.45 ± 0.06	3.93 ± 0.08	0.039 ± 0.009
$LSD_{0.05}$ (P × G)		0.223	0.073	0.068	0.096	0.018

The amount of variance explained by the genotype impact was significant for each element (Table S1) since the studied genotypes varied from one another in the amounts of heavy metals (Figure 3c). The greatest effect of genotype was found in the total variation of Cu (29.23%), followed by Pb (22.58%), while the smallest share of this factor was found in the variation of Cr (8.41%) (Table S1). The highest concentrations of Cr (2.51 mg kg^{-1}), Cu (4.78 mg kg^{-1}), and Cd (together with the genotype Apache) were found in the roots of the genotype Renesansa (0.12 mg kg^{-1}). On the other hand, the genotype Pobeda exhibited the greatest amounts of Zn (7.90 mg kg^{-1}) and Pb, along with the genotype Ljiljana (2.51 mg kg^{-1}) in the plant's roots (Figure 3c).

The variability of the concentration of heavy metals in the roots is influenced by the factor of phenophase, whereby 57.66% of the total variation of Cd is explained by the influence of this factor. The share of the phenophase in the total variability of other examined elements is smaller and ranges from 8.04% for Zn to 15.84% for Cr, while this factor did not have a significant impact ($p > 0.05$) on the variability of Cu (Table S1).

A higher concentration of Pb (2.38 mg kg^{-1}), Cr (2.60 mg kg^{-1}), and Cd (0.15 mg kg^{-1}) was detected in the plant's roots during the phenophase of heading in comparison to the concentration of these heavy metals in the phenophase of full maturity (1.60, 1.83, and 3.62 mg kg^{-1}, respectively). The concentration of Zn (7.04 mg kg^{-1}) and Cu (3.95 mg kg^{-1}) was higher in the phenophase of full maturity compared to the phenophase of heading (4.37 and 0.04 mg kg^{-1}) (Figure 3b).

The interaction of the factors had a significant impact on the variability of the content of the examined heavy metals in wheat roots (Table 2). The results of the analysis of variance indicate that the locality and genotype interaction (L × G) had the greatest influence on the variability of the Cu content (24.62%) (Table S1), with the highest concentration of Cu found in the roots of the genotype Renesansa at Locality 2 (5.10 mg kg^{-1}). The content of Zn measured in the genotypes Pobeda (13.38 mg kg^{-1}) and Ljiljana (12.55 mg kg^{-1}) at Locality 1 was significantly higher compared to the content of Zn in the roots of the other genotypes at the investigated locations. On the other hand, the highest Pb concentration was measured in the roots of the genotype Pobeda (3.65 mg kg^{-1}) at Locality 3. Additionally, the highest concentration of Cr was detected at Locality 3, in the genotype Ljiljana (3.20 mg kg^{-1}).

The interaction of localities and phenophases (L × P) had a small share in the variation of analyzed heavy metals. On the other hand, the interaction of phenophase and genotype (P × G) has covered a significant part of the variations of all tested heavy metals. The P × G interaction had the greatest impact on the concentrations of Pb (27.58%) and Cr (21.71%), while it had a small impact on the contents of Cu (3.42%) (Table S1).

In all wheat genotypes, a higher content of Zn was found in the phenophase of full maturity compared to the measured concentration in the phenophase of heading. Contrary to this, in all cultivars, higher Cd concentrations were measured in the heading phenophase compared to the full maturity phenophase. With the exception of the genotype Apache, all cultivars adopted a higher Cr content in the phenophase of heading compared to the phenophase of full maturity. In contrast, the content of Cu was lower in the phenophase of heading than in the full maturity phenophase in all genotypes, except for the genotype Apache (Table 2).

3.3. Heavy Metal Content in Wheat Stem

According to the analysis of variance, the factor of genotype had a greater influence on the concentration of heavy metals in the wheat stem, with the exception of cadmium, than the factors of locality and phenophase (Table S2, Figure 4).

The factor of the locality had almost equal participation in the total variability of the Zn (13.66%) and Cd (12.87%), and the least affected the variability of the Cr (4.43%). On the other hand, the factor of genotype contributed the most to the variation of the Cr content in the wheat stem (47.71%), while twice the share was found in the variation of the Zn (26.99%) and Cu (26.20%), a very small share in the Pb variation (7.16%), and a statistically negligible share in the variation of the Cd (1.66%). The variation in the content of Cd in the wheat stem has been most strongly influenced by the factor phenophase, which had a share in the variation of 44.43%, whereas the variation in the content of other heavy metals was significantly less influenced by this factor (Table S2).

The highest concentration of Zn (9.86 mg kg^{-1}) and Cu (4.24 mg kg^{-1}) was established in the wheat stem on Locality 1, which is closest to the industrial zone. The highest average Pb (2.24 mg kg^{-1}) and Cr (2.44 mg kg^{-1}) in the wheat stem were found at Locality 3, while the highest Cd content (0.18 mg kg^{-1}) was established at Locality 2 (Figure 4a). A significantly higher concentration of all heavy metals, except for Cd, was found in the phenophase of full maturity in relation to the concentration in the phenophase of heading (Figure 4b). For the majority of the examined heavy metals, the differences found across genotypes were mostly significant ($p < 0.01$). The stem of the genotype Apache had the highest average concentrations of Pb (2.26 mg kg^{-1}), Cr (3.86 mg kg^{-1}), and Cu (5.55 mg kg^{-1}), whereas the genotype Ljiljana's stem had the highest average concentration of Zn (12.03 mg kg^{-1}) (Figure 4c).

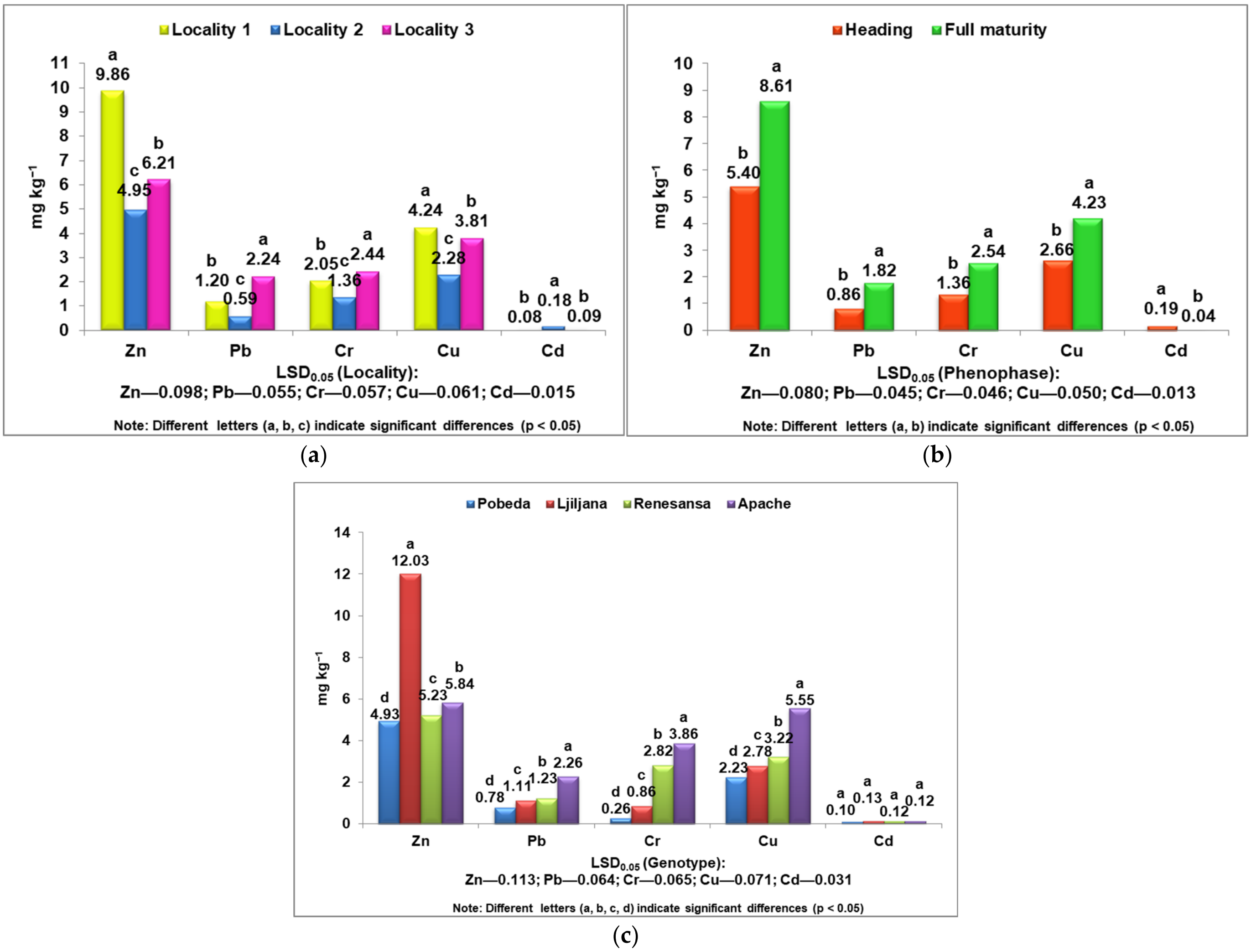


Figure 4. Heavy metal content (mean values, mg kg^{-1}) in wheat stems: (**a**) averaged per localities (average values of four genotypes in two phenophases); (**b**) averaged per phenophases (average values of four genotypes at three localities); (**c**) averaged per genotypes (average values of each genotype at three localities and two phenophases).

The contribution of the locality and genotype interaction (L × G) as well as the locality and phenophase interaction (L × P) was the largest in the total variation of Pb content (30.10 and 17.28%, respectively) (Table S2). The genotype Apache (5.37 mg kg^{-1}) at Locality 3 had the highest concentration of Pb in the wheat stem, followed by the genotypes Ljiljana (2.82 mg kg^{-1}) at Locality 1 and Renesansa (2.28 mg kg^{-1}) at Locality 3. The other genotypes on the analyzed localities had significantly lower Pb concentrations. The highest concentration of Zn was found in the stem of the genotypes Ljiljana (14.94 mg kg^{-1}) and Pobeda (11.74 mg kg^{-1}) at Location 1, while the concentration of Cr and Cu was the highest in the stem of the genotype Apache at Location 3 (5.76 mg kg^{-1} and 7.83 mg kg^{-1}, respectively). The highest concentration of Cd was measured in the genotype Pobeda when grown on Locality 2 (0.24 mg kg^{-1}) (Table 3).

Table 3. Heavy metal content (mean values ± standard deviation of sample means) in the stems of four wheat genotypes per locality (average values for both phenophases) and per phenophase (average values for three localities).

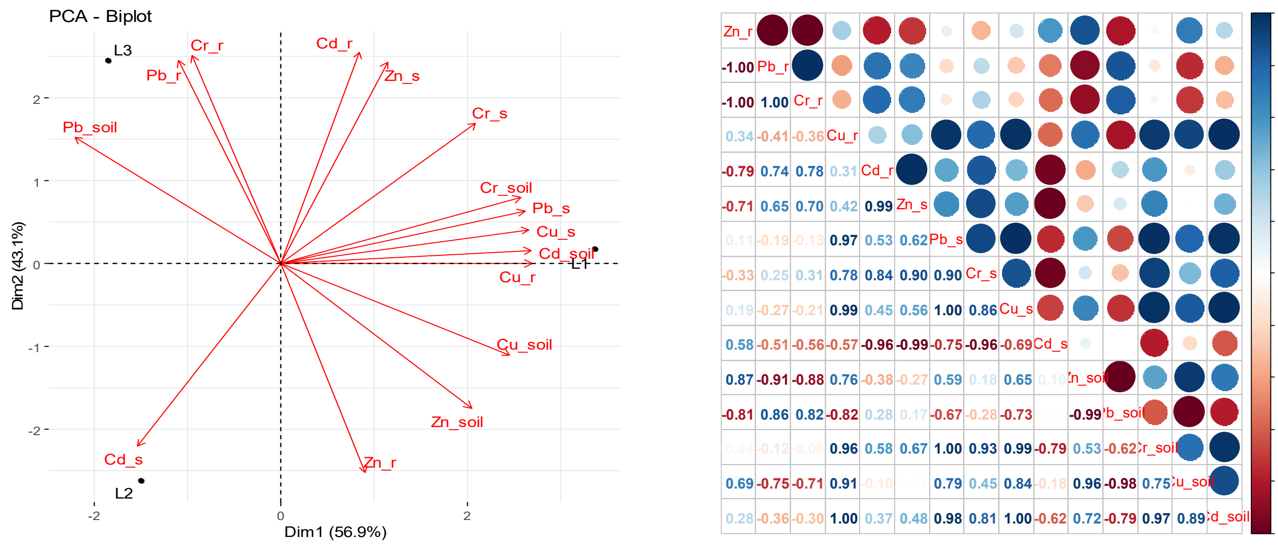
Locality (L)	**Genotype (G)**	**Zn**	**Pb**	**Cr**	**Cu**	**Cd**
		Mean Value ± Sd (mg kg^{-1})				
Locality 1	Pobeda	11.74 ± 0.23	0.25 ± 0.05	0.28 ± 0.05	4.26 ± 0.07	0.037 ± 0.060
	Ljiljana	14.94 ± 0.16	2.82 ± 0.07	1.52 ± 0.05	4.57 ± 0.07	0.046 ± 0.034
	Renesansa	6.33 ± 0.13	0.85 ± 0.07	3.06 ± 0.08	2.70 ± 0.08	0.117 ± 0.019
	Apache	6.56 ± 0.12	0.89 ± 0.07	3.35 ± 0.09	5.44 ± 0.08	0.119 ± 0.017
Locality 2	Pobeda	1.48 ± 0.07	1.01 ± 0.08	0.29 ± 0.05	1.36 ± 0.06	0.237 ± 0.020
	Ljiljana	11.61 ± 0.12	0.26 ± 0.05	0.51 ± 0.06	0.90 ± 0.06	0.216 ± 0.018
	Renesansa	3.97 ± 0.08	0.54 ± 0.06	2.17 ± 0.08	3.31 ± 0.09	0.117 ± 0.020
	Apache	2.76 ± 0.07	0.52 ± 0.04	2.49 ± 0.07	3.55 ± 0.09	0.116 ± 0.017
Locality 3	Pobeda	1.72 ± 0.05	1.07 ± 0.08	0.21 ± 0.05	1.06 ± 0.06	0.018 ± 0.005
	Ljiljana	9.55 ± 0.16	0.23 ± 0.04	0.53 ± 0.04	2.88 ± 0.08	0.099 ± 0.020
	Renesansa	5.37 ± 0.12	2.28 ± 0.08	3.24 ± 0.07	3.64 ± 0.07	0.117 ± 0.018
	Apache	8.21 ± 0.11	5.37 ± 0.09	5.76 ± 0.10	7.83 ± 0.38	0.119 ± 0.018
$LSD_{0.05}$ (L × G)		0.196	0.110	0.112	0.122	0.032
Phenophase (P)	Genotype (G)	Zn	Pb	Cr	Cu	Cd
		Mean value ± Sd (mg kg^{-1})				
Heading	Pobeda	1.55 ± 0.05	0.25 ± 0.05	0.28 ± 0.05	1.72 ± 0.05	0.156 ± 0.02
	Ljiljana	7.37 ± 0.11	1.95 ± 0.06	1.43 ± 0.05	3.16 ± 0.06	0.208 ± 0.02
	Renesansa	6.19 ± 0.13	0.44 ± 0.05	1.55 ± 0.06	2.35 ± 0.07	0.195 ± 0.03
	Apache	6.51 ± 0.11	0.82 ± 0.06	2.20 ± 0.07	3.39 ± 0.07	0.209 ± 0.07
Full maturity	Pobeda	8.30 ± 0.12	1.31 ± 0.08	0.24 ± 0.05	2.73 ± 0.07	0.037 ± 0.05
	Ljiljana	16.70 ± 0.18	0.26 ± 0.05	0.28 ± 0.05	2.41 ± 0.07	0.035 ± 0.07
	Renesansa	4.26 ± 0.09	2.01 ± 0.09	4.10 ± 0.10	4.09 ± 0.08	0.036 ± 0.06
	Apache	5.18 ± 0.08	3.71 ± 0.07	5.53 ± 0.09	7.71 ± 0.10	0.039 ± 0.06
$LSD_{0.05}$ (P × G)		0.159	0.099	0.092	0.100	0.026

The interaction between phenophase and genotype (P × G) had the largest share in the variation of Zn (19.16%) and Cr (18.90%) in the wheat stem (Table S2).

The highest content of Zn in the wheat stem was found in genotypes Ljiljana (16.70 mg kg^{-1}) and Pobeda (8.30 mg kg^{-1}) in the phenophase of full maturity, while the other genotypes had a higher content of Zn in the phenophase of heading. The highest content of Pb, Cr, and Cu in the stem was recorded in the phenophase of full maturity in the genotype Apache (3.71, 5.53, and 7.71 mg kg^{-1}, respectively). With the exception of the genotype Ljiljana, the Pb and Cu content was higher in all genotypes during the phenophase of full maturity. Additionally, the content of Cr was higher in phenophase of the heading in genotypes Ljiljana and Pobeda. In the heading phenophase of all wheat genotypes, the Cd concentration in the stem was 4–5 times higher than it was in the full maturity phenophase (Table 3).

3.4. Association between Heavy Metals Content in Soil and Different Vegetative Parts of Wheat

The association between heavy metal content in soil and different vegetative parts of wheat (root and stem) in the phenophase of heading was estimated through correlation matrix analysis by the principal components method and Pearson moment correlation coefficients (Figure 5a,b). By applying the method of principal components (Figure 5a), the dimensions of the dataset are reduced while maintaining the maximum possible variability that is present in those data. A total of 2 main PCA components with eigenvalues > 1 are extracted from the input data, where the first PCA component explains 56.9% and the second one is 43.1% of the total variance. The vectors of the investigated localities are positioned in different quadrants of the PCA biplot, indicating a mutual difference in heavy metal content in the soil and vegetative parts of wheat. It is established that there is a positive association between the Pb content of the soil and the Pb content of the wheat roots in the phenophase of heading. Furthermore, the vector of Cr content in the soil intersects at a sharp angle (<90°) with the vector of Cr content in the wheat stem, while the significance of the association with Cr content in the root is missing. The Cu content in the soil achieves a positive association with the Cu content in the root and stem of wheat in the phenophase of heading. The vector of Zn content in soil and wheat roots is located in the same quadrant of the biplot, forming a sharp angle. There is no positive association between the Cd content in the soil and the Cd content in the stem and roots. The heatmap analysis of Pearson's correlations shows similar data, where the highest values of correlation coefficients are represented between Cu content in the soil and Cu content in the roots (0.91), then between Cu in the soil and Cu in the stem (0.84). Additionally, the Zn content in the soil has a highly significant and positive correlation with the Zn content in the roots (0.87), as well as the Pb content in the soil with the Pb content in the roots (0.86).

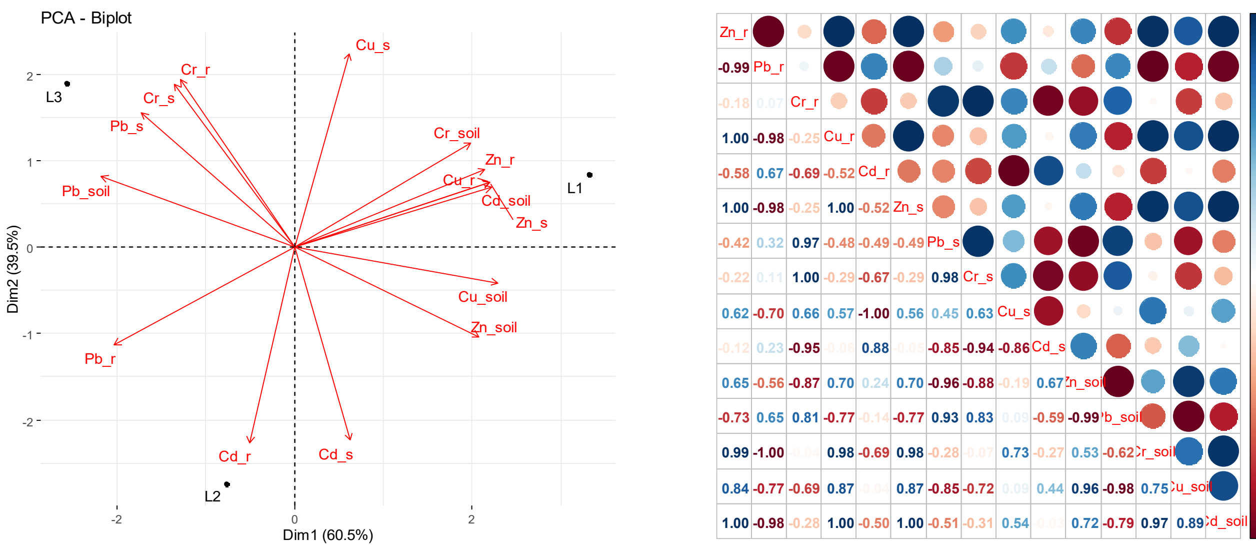


(**a**) (**b**)

Figure 5. Principal components analysis (PCA) (**a**) and a heatmap of Pearson moment correlation coefficients (**b**) for heavy metal (Zn, Pb, Cr, Cu, and Cd) content in the root (r) and stem (s) of wheat in phenophase of heading in different localities (L1—Locality 1; L2—Locality 2; and L3—Locality 3).

Figure 6a,b shows the results of the interrelationships between heavy metal content in soil and heavy metal content in plants examined during the phenophase of full maturity.

The vectors of localities are positioned in different quadrants of the PCA biplot, which indicates the existence of significant differences between localities in the content of heavy metals in soil and plants. The vectors of Pb content in the soil and Pb content in the stem are located in the same quadrant of the biplot, showing a significant mutual connection. Additionally, the soil Pb content vector forms a sharp angle (<90°) with the root Pb content. It was noticed that the vectors of Zn content in the soil, Zn content in roots, and Zn content in stems formed an acute angle with each other (Figure 6a). The content of Cr in the root and stem of wheat is positively correlated, while there is no positive correlation with the total content of Cr in the soil (Figure 6b). The content of Cu in the soil is positively associated with the content of Cu in the roots, while no significant association was achieved with the content of Cu in the stem (Figure 6a). Similar results are shown by heatmap correlations. Specifically, the highest values of correlation coefficients in the phenophase of full maturity are represented between the content of Pb in the root and the content of Pb in the stem (0.95), then between the content of Cu in the soil and the content of Cu in the root (0.87), and between Zn in the soil and Zn in the stem (0.70) (Figure 6b).



(**a**) (**b**)

Figure 6. Principal components analysis (PCA) (**a**) and a heatmap of Pearson moment correlation coefficients (**b**) for heavy metal (Zn, Pb, Cr, Cu, and Cd) content in the root (r) and stem (s) of wheat in phenophase of full maturity in different localities (L1—Locality 1; L2—Locality 2; and L3—Locality 3).

3.5. Bioaccumulation Factor

The calculated values of the bioaccumulation factor (BAF) in the various vegetative organs of the analyzed wheat genotypes at the examined locations are shown in Table 4.

Table 4. The bioaccumulation factor of roots and stems of analyzed wheat genotypes grown at different localities.

Locality	Heavy Metal	Bioaccumulation Factor of Roots					Bioaccumulation Factor of Stems				
		Genotype				Average	Genotype				Average
		G1	G2	G3	G4		G1	G2	G3	G4	
Locality 1	Zn	0.19 a	0.18 b	0.06 c	0.07 c	0.12	0.16 b	0.21 a	0.08 c	0.09 c	0.13
	Pb	0.12 b	0.14 a	0.07 c	0.06 c	0.09	0.02 c	0.19 a	0.06 b	0.06 b	0.08
	Cr	0.05 b	0.05 b	0.10 a	0.11 a	0.08	0.01 c	0.06 b	0.12 a	0.13 a	0.08
	Cu	0.17 a	0.18 a	0.18 a	0.17 a	0.17	0.16 c	0.18 b	0.11 d	0.21 a	0.16
	Cd	0.17 c	0.32 b	0.40 a	0.40 a	0.32	0.14 c	0.17 b	0.41 a	0.40 a	0.28
Locality 2	Zn	0.06 ab	0.07 a	0.07 a	0.05 b	0.06	0.02 d	0.17 a	0.06 b	0.04 c	0.07
	Pb	0.10 c	0.12 b	0.16 a	0.05 d	0.10	0.05 a	0.01 c	0.03 b	0.03 b	0.03
	Cr	0.09 c	0.11 b	0.13 a	0.09 c	0.10	0.02 d	0.03 c	0.12 b	0.14 a	0.08
	Cu	0.08 d	0.13 c	0.24 a	0.20 b	0.16	0.06 b	0.04 c	0.16 a	0.17 a	0.11
	Cd	0.56 c	0.46 d	1.22 a	1.02 b	0.86	2.42 a	2.22 b	1.22 c	1.22 c	1.77
Locality 3	Zn	0.11 a	0.07 b	0.07 b	0.06 b	0.08	0.03 d	0.17 a	0.10 c	0.15 b	0.11
	Pb	0.13 a	0.10 b	0.05 c	0.04 c	0.08	0.04 c	0.01 d	0.08 b	0.18 a	0.08
	Cr	0.11 c	0.16 a	0.12 c	0.14 b	0.13	0.01 d	0.03 c	0.16 b	0.28 a	0.12
	Cu	0.17 c	0.12 d	0.27 a	0.20 b	0.19	0.06 d	0.14 c	0.21 b	0.43 a	0.21
	Cd	0.51 d	1.07 b	1.22 a	1.02 c	1.00	0.24 c	1.07 b	1.22 a	1.21 a	0.93
Average	Zn	0.12 a	0.10 b	0.07 c	0.06 c	0.09	0.07 b	0.18 a	0.08 b	0.09 b	0.11
	Pb	0.11 a	0.12 a	0.09 b	0.05 c	0.09	0.04 c	0.07 b	0.05 c	0.09 a	0.06
	Cr	0.08 c	0.10 b	0.12 a	0.11 ab	0.10	0.01 d	0.04 c	0.13 b	0.18 a	0.09
	Cu	0.14 c	0.14 c	0.23 a	0.19 b	0.17	0.09 d	0.13 c	0.16 b	0.27 a	0.16
	Cd	0.41 c	0.61 b	0.95 a	0.94 a	0.73	0.93 b	1.15 a	0.95 b	0.94 b	0.99

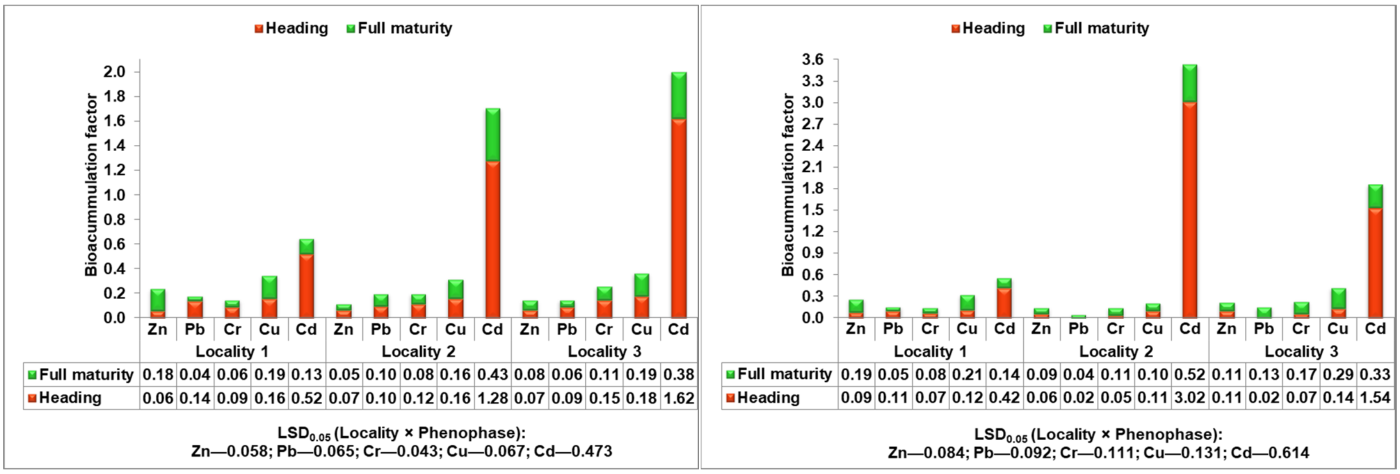
Note: G1—Pobeda; G2—Ljiljana; G3—Renesansa; and G4—Apache. Different letters (a, b, c, and d) indicate significant differences ($p < 0.05$) in the values of the bioaccumulation factor between genotypes.

The BAF values of the examined heavy metals were in the following order: Cd (0.86) > Cu (0.17) > Cr (0.10) > Zn (0.10) > Pb (0.08); therefore, it can be concluded that the bioaccumulation of the studied metals was at the level of weak (Pb) to medium intensity (Cd). When it comes to the average values of root and stem BAF at the analyzed localities, the results showed that the highest BAF value of Zn (0.125) and Pb (0.085) was found at L1, Cd (1.31) at L2, and Cr (0.125) and Cu (0.2) at L3.

The obtained results have shown that the BAF of heavy metals varied among the analyzed wheat genotypes, with some genotypes showing higher levels of heavy metal accumulation than others. On average, for root and stem BAF values, genotype Ljiljana (G2) had the highest BAF values of Zn (0.140) and Pb (0.095); genotype Apache (G4) had the highest BAF values of Cr (0.145) and Cu (0.230); and genotype Renesansa (G3) had the highest BAF value of Cd (0.950). Identifying wheat genotypes with lower levels of BAF for heavy metals can be an effective strategy for reducing the risk of heavy metal contamination in the food chain. The lowest BAF values of the analyzed heavy metals were found in the genotype Pobeda (G1) (Table 4).

There were no significant differences between the average BAF values of roots and stems for Zn, Pb, Cr, and Cu. Wheat stems tend to have a higher BAF value of Cd (0.99) than roots (0.73) (Table 4).

The locality and phenophase interaction (L × P) significantly influenced the variation in the values of the bioaccumulation factor of roots and stems (Figure 7).



(**a**) (**b**)

Figure 7. The bioaccumulation factor in the root (**a**) and stem (**b**) in different phenophases of analyzed wheat genotypes.

At L1 and L3, the values of wheat root BAF of Zn and Cu were higher in the phenophase of full maturity compared to the values of BAF determined in the phenophase of heading, while the values of BAF of other heavy metals (Pb, Cr, and Cd) were higher in the phenophase of heading in relation to the values recorded in the phenophase of full maturity. At L2, the root BAF of Zn, Cr, and Cd was higher in the phenophase of heading, while the values of the BAF for Pb and Cu were equal in both analyzed phenophases (Figure 7a).

The BAF of Zn, Cr, and Cu in the wheat stems was higher in the phenophase of full maturity than in the phenophase of heading in all analyzed localities. The BAF value of Pb was higher in the phenophase of heading in relation to the BAF value in the phenophase of full maturity only at L1. In all the analyzed localities, the BAF value of Cd in the wheat stems was higher in the phenophase of heading compared to the BAF value in the phenophase of full maturity (Figure 7b).

According to the aforementioned, the root BAF of the largest number of analyzed heavy metals was higher in the phenophase of heading, while the stem BAF of most heavy metals was higher in the phenophase of full maturity.

3.6. Translocation Factor

The translocation factor (TF) of the analyzed heavy metals mostly depended on the factor of phenophase. The values of TF for all heavy metals, except for Cd, were higher in the full maturity phenophase compared to the values in the phenophase of heading (Table 5).

Table 5. The translocation factor in different phenophases of analyzed wheat genotypes (G1—Pobeda; G2—Ljiljana; G3—Renesansa; and G4—Apache).

Locality	Heavy Metal	Translocation Factor in Heading					Translocation Factor in Full Maturity				
		Genotype				Average	Genotype				Average
		G1	G2	G3	G4		G1	G2	G3	G4	
Locality 1	Zn	0.36 c	1.74 b	2.13 a	1.71 b	1.49 A	0.75	1.08 a	1.13 a	1.08 a	1.07 B
	Pb	0.08 c	1.34 a	0.51 b	0.52 b	0.61 B	1.00 b	1.00 b	0.99 b	1.61 a	1.15 A
	Cr	0.11 c	1.10 a	0.79 b	1.10 a	0.77 B	1.00 c	1.00 c	1.52 a	1.22 b	1.18 A
	Cu	0.90 b	1.51 a	0.41 d	0.49 c	0.83 B	0.99 b	0.62 d	0.82 c	2.38 a	1.20 A
	Cd	0.59 b	0.41 c	1.02 a	1.00 a	0.74 B	1.33 a	1.04 b	1.02 b	1.04 b	1.08 A
Locality 2	Zn	0.30 d	1.20 a	1.03 b	0.59 c	0.78 B	0.39 d	5.65 a	0.75 c	1.00 b	1.95 A
	Pb	0.11 c	0.08 c	0.22 b	0.50 a	0.29 B	0.91 a	0.17 c	0.14 c	0.59 b	0.45 A
	Cr	0.16 c	0.30 b	0.08 d	0.86 a	0.35 B	0.17 c	0.20 c	1.91 b	3.96 a	1.56 A
	Cu	0.83 b	0.54 c	0.48 d	0.98 a	0.71 A	0.83 a	0.11 c	0.82 a	0.66 b	0.60 B
	Cd	7.87 a	6.69 b	1.00 c	1.00 c	4.17 A	1.34 a	1.37 a	1.02 b	1.06 b	1.16 B
Locality 3	Zn	0.29 d	1.80 b	1.60 c	3.31 a	1.75 B	0.28 d	4.35 a	1.04 c	1.84 b	1.88 B
	Pb	0.06 c	0.05 c	0.32 b	1.17 a	0.40 B	0.58 c	0.15 d	3.14 b	7.07 a	2.73 A
	Cr	0.14 b	0.16 b	0.87 a	0.89 a	0.51 B	0.07 d	0.19 c	1.77 b	3.07 a	1.27 A
	Cu	0.32 d	1.42 a	0.64 c	1.00 b	0.84 B	0.40 d	1.35 b	0.86 c	3.14 a	1.44 A
	Cd	0.66 c	0.94 b	1.01 a	0.99 a	0.90 A	0.18 c	1.35 a	1.00 b	1.01 b	0.88 A
Average	Zn	0.32 c	1.58 b	1.59 b	1.87 a	1.34 B	0.56 d	3.69 a	0.97 c	1.31 b	1.63 A
	Pb	0.08 d	0.49 b	0.35 c	0.73 a	0.41 B	0.83 c	0.44 d	1.42 b	3.09 a	1.45 A
	Cr	0.14 c	0.52 b	0.58 b	0.95 a	0.55 B	0.41 c	0.46 c	1.73 b	2.75 a	1.34 A
	Cu	0.68 c	1.16 a	0.51 d	0.82 b	0.79 B	0.74 c	0.69 c	0.83 b	2.06 a	1.08 A
	Cd	3.04 a	2.68 b	1.01 c	1.00 c	1.93 A	0.95 c	1.25 a	1.01 b	1.04 b	1.06 B

Note: Different lowercase letters (a, b, c, and d) indicate significant differences ($p < 0.05$) in the values of the translocation factor between genotypes among the same phenophase. Different uppercase letters (A and B) indicate significant differences ($p < 0.05$) in the values of the translocation factor between phenophases for each heavy metal.

In the phenophase of heading, the highest TF value of Cd (1.93) was found, followed by a TF value of Zn (1.34), which is in accordance with the significantly higher concentrations of these 2 heavy metals measured in the stems compared to the values in the roots in the given phenophase. The TF values of Pb, Cr, and Cu were below 1, which means that, in the phenophase of heading, the concentrations of these heavy metals in the roots were higher compared to the concentrations measured in the stems (Table 5).

In the phenophase of full maturity, the TF values of all heavy metals were above 1, which indicates that, in the mentioned phenophase, the content of heavy metals in the stems was significantly higher compared to the content of heavy metals in the roots. The highest TF values were found for Zn (1.63), followed by Pb (1.45) and Cr (1.34). This is the result of the pronounced root–stem mobility of the mentioned heavy metals. Additionally, the TF value of Cu (1.08) in the phenophase of full maturity was significantly higher in relation to those in the phenophase of heading (0.79). The lowest value of TF in the phenophase of full maturity was determined for Cd (1.06) and, accordingly, the concentrations of this heavy metal in the roots and stems of wheat were almost equal and very low. Additionally, compared to the values observed during the phenophase of heading, the Cd values in the wheat roots and stems significantly decreased during the phenophase of full maturity (Tables 2 and 3). This decrease in Cd concentration in the wheat stems may be the result of Cd translocation from the vegetative parts to the grain, which is in accordance with high Cd mobility (Table 5).

The analyzed wheat genotypes differed in their capacity for heavy metal translocation from roots to stems. In the phenophase of heading, on average, for all localities, the genotype Apache (G4) had a higher capacity to translocate Zn (1.87), Pb (0.73), and Cr (0.95) and the lowest capacity to translocate Cd (1.00). In contrast, the genotype Pobeda (G1) had the lowest TF values of Zn, Pb, and Cr (0.32, 0.08, and 0.14, respectively) and the highest TF value of Cd (3.04). The genotype Ljiljana (G2) translocated Cu to the greatest extent (TF = 1.16), while the genotype Renesansa (G3) had the lowest ability to translocate Cu (0.51). In the phenophase of full maturity, the genotype Apache (G4) had the highest TF values of Pb, Cr, and Cu (3.09, 2.75, and 2.06, respectively), while the genotype Ljiljana (G2) had the highest TF values of Zn and Cd (3.69 and 1.25, respectively). In this phenophase, the lowest TF values of Zn (0.56), Cr (0.41), and Cd (0.95) were found for the genotype Pobeda (G1), while the lowest TF values of Pb (0.44) and Cu (0.69) were established for the genotype Ljiljana (G2) (Table 5).

4. Discussion

Due to the various sources of heavy metal contamination, their entry into the food chain, and their harmful effects on human health [19,29,30,80], it is important to understand the behavior of heavy metals in soil and their transfer in the soil–plant system as it is a crucial area of research for scientists in the field of environmental protection.

4.1. The Heavy Metal Content in Soil

Industrial activities cause pollution of the atmosphere and the soil near the industrial zone, increasing its acidity and heavy metal content [10,51,52,81]. One of the most important factors that largely determine the capacity of soil to retain heavy metals is its pH value [82–85]. The results of this research showed that the lowest values of pH (7.38) and $CaCO_3$ (5.74%), as well as the highest concentration of Zn, Cr, Cu, and Cd (72.5, 27.3, 26.2, and 0.3 mg kg^{-1}, respectively), was found at Locality (L1), which is closest to the industrial zone. We emphasize that there are other numerous factors that have a significant impact on the content of heavy metals in the soil, such as the type and content of organic matter [86]; the presence and amount of organic and inorganic ligands, including humic and fulvic acids; cation exchange capacity (CEC); redox status (redox potential—Eh); clay mineral content; and nutrients [44,45,47–49,87,88], whose influence was not studied in this research. On the other hand, the highest Pb content was measured in the soil at Locality 3 (L3) (29.8 mg kg^{-1}), which is the furthest from the industrial zone. Recent research has shown that atmospheric deposition from industrial plants contributes to the increase of Pb concentrations in the surface soil layer [89,90]. Keeping in mind that the location with the highest content of Pb in the soil is located in the direction of the flow of the dominant winds, we speculate that the higher content of this metal in the soil at L3 could have originated from the industrial region and that it arrived via atmospheric currents and deposition. Additionally, the comparative study of binding strengths of heavy metals with humic acid points out that the stability constant of the complex formed between Pb ions and humic acid from soil organic matter is higher than the stability constant of the complexes Co, Ni, Cu, and Zn with humic acid [91], as well as that Pb can be highly concentrated in calcium carbonate particles [80]. The highest content of organic matter and calcium carbonate in this study was found exactly at L3, which could additionally affect the status of Pb in the soil at this locality.

Heavy metals characterized by similar values of the main components and positioned close to each other with their vectors are influenced by similar factors, such as similar sources or soil properties [53,57,61,82,92,93]. In our study, the first two principal components explained the largest source of variation, where positive PCA_1 values were found for the heavy metals Cr, Cd, Zn, and Cu, which can indicate a common source of contamination. The same conclusion was obtained by Zhao et al. [60], who also found a positive correlation between the mentioned heavy metals. In accordance with the above, the highest concentration of Cr, Cd, Zn, and Cu was found at L1, which is the closest to the industrial zone. Industrial operations, such as refining, mining, and plastic processing, are the main causes of Cd exposure [94]. In these studies, a two-and-a-half times higher concentration of Cd was found in the soil at L1, compared to the location farthest from the industrial zone (L3). This is in accordance with results reported by other authors [10,11]. On the other hand, the differences in the content of Cr (32%) and Cu (48%) between the nearest and the most distant location were small but significant, which indicates that the increased concentration of Cr and Cu in the soil is caused by industrial activities [61]. The concentration of Zn in the soil also decreases with increasing distance from the industrial area, so the concentration of Zn detected in the soil at L3 was 29% lower compared to the concentration measured in the soil at L1. Ammar et al. [95] also found that in the industrial zone, the concentration of Zn in the soil is 9 times higher than the concentration of Zn in the soil at a distance of 5 km from the industrial zone. It is important to note that the results obtained in different studies depend not only on the type of industrial activity but also on the composition and origin of the substrate on which the research was conducted.

The results of the PCA analysis showed that the content of P, K, and total N in the soil is positively correlated with the content of Cr, Cd, Zn, and Cu in the soil, where it was determined that heavy metal concentrations decrease with increasing distance from the industrial zone.

4.2. Soil-to-Wheat Transfer of Heavy Metals

The bioavailability and uptake of heavy metals by plants are influenced by various factors, including organic matter, granulometric composition, cation exchange capacity, pH value, genetic factors of the plant, root surface, root exudates [87,88,96], and heavy metal content in soil [44,45,47–49]. The concentration of heavy metals in the vegetative parts of wheat varied greatly. Wheat roots and stems have an average content of heavy metals in the following order: Zn > Cu > Cr > Pb > Cd. Chen et al. [97] established the same order of mean heavy metal content values in wheat grain.

The uptake of heavy metals by plants depends on their content in the soil, and their availability increases with a decrease in soil pH [98]. The highest concentration of Zn in the root and stem of wheat was found at L1, which is correlated with the content of this heavy metal in the soil. On the other hand, an increase in soil pH reduces the availability of Zn to plants [99], which is in accordance with the measured lowest concentration of this metal in the roots and stems of wheat at L3.

Plants have different abilities and pathways for the absorption and transport of Pb ions. There are two ways of penetration of Pb into the plant, namely through the leaves and through the roots, i.e., from the air and the soil. The highest concentration of Pb in the roots and stems of wheat was detected at L3, which is positively correlated with the Pb content in the soil. A positive correlation of Pb in the soil with Pb in the wheat plant was established by Yang et al. [43] and Wang et al. [100]. In addition, increasing the Zn content in the soil has an antagonistic effect on Pb uptake, since Pb uses Zn membrane transport proteins for membrane mobility for uptake [101]. In this study, the highest concentration of Pb in the soil and the plant was determined exactly at the location with the lowest Zn content (L3).

Although the amount of Cd in the soil at L1 was the highest, the obtained differences in the concentration of Cd in the root and stem of wheat between the locations were mostly insignificant. In addition, there is no positive association between the Cd content in the soil and the Cd content in the stems and roots. The obtained result indicates that small concentrations of Cd in the soil, which are significantly lower than the maximum allowed value of Cd in soil according to the Regulation on Limit Values of Polluting, Harmful, and Dangerous Substances in Soil (Official Gazette of the Republic of Serbia, 30/2018 and 64/2019) [102], have little influence on its content in the vegetative parts of the plant [103].

The concentration of Cu measured in the roots and stems of wheat was the highest at L1, which is consistent with the concentration of Cu in the soil at L1. Similar results were obtained by Xu et al. [93], stating that Cu in the soil is positively correlated with the Cu content of the wheat plant. However, although the concentration of Cu in the soil at L2 was higher compared to the concentration of Cu at L3, the concentration of Cu in the wheat stem at L2 was lower compared to the concentration of Cu at L3. Conversely, this is probably a consequence of the Cr–Cu interrelationship, because their antagonistic reactions are associated with variable Cr valency. Modern research defines the concept according to which the total concentration of metals in the soil is not a completely reliable indicator of bioavailability due to the different and complex mechanisms of their distribution within the soil–plant system [104]. Similarly, our research also determined that the total content of Cr in the soil is not significantly related to the content of Cr in the vegetative parts of the plant, especially in the phenophase of full maturity. As a detailed analysis of the presence of different forms of metals was not conducted in this study, based on the analyzed parameters of the soil, especially the pH value, we speculate that the hexavalent form of Cr (Cr(VI)), which is more soluble than the reduced trivalent form (Cr(III)) [105,106] is dominant in the soil at L3.

The concentration of heavy metals in roots and stems differed under the influence of the phenophase factor. In the wheat roots, a higher concentration of Pb, Cr, and Cd was found in the phenophase of heading compared to the values measured in the phenophase of full maturity. On the other hand, a higher concentration of all heavy metals except Cd was found in the wheat stems in the phenophase of full maturity compared to the phenophase of heading. According to the aforementioned, in the phenophase of full maturity, there was a sudden decrease in Cd values in both the roots and the stems of wheat compared to the values measured in the phenophase of heading, which is supported by the high mobility of the Cd (TF = 1.93) and its transfer from the vegetative parts to the grain. Additionally, Abbas et al. [107] determined a pronounced translocation of Cd from the root to the stem and grain of wheat. The high translocation of Cd in the wheat plant was established by Chen et al. [97], which may be related to the high solubility and mobility of this heavy metal [25,108,109].

The translocation of heavy metals was mostly dependent on the factor of phenophase, where higher TF values of the largest number of analyzed heavy metals were found in the full-maturity phenophase. In analyzed phenophases, differences in the concentration of heavy metals between the roots and the stems may be related to atmospheric deposition and an above-ground uptake of heavy metals, which is particularly pronounced in the industrial zone. Similar results are reported by other authors [100,102].

The heavy metal content differed among the analyzed wheat genotypes. The lowest concentration of Cr, Cu, and Cd, and the highest concentration of Zn in the roots was found in the genotype Pobeda (G1). The lowest concentration of Zn and Pb in wheat roots was measured in the genotype Apache (G4). These results are in accordance with the obtained values of the bioaccumulation factor (BAF), which point to the conclusion that the genotype Pobeda (G1) has the least ability to uptake the heavy metals, followed by the genotypes Ljiljana (G2) and Renesansa (G3), while the genotype Apache (G4) accumulates heavy metals to the greatest extent. Additionally, the genotype Apache (G4) is characterized by the highest translocation factor (TF) values for heavy metals. Therefore, the genotype Pobeda (G1) stands out as a genotype that uptakes heavy metals with low intensity, while the genotype Apache (G4) showed the greatest ability to accumulate and translocate the examined heavy metals. Hussain et al. [110] found differences in the content of heavy metals between wheat genotypes grown in an organic production system, pointing out that old varieties had a lower ability to bioaccumulate Cd.

5. Conclusions

In this study, the highest content of heavy metals Zn, Cr, Cu, and Cd in the soil was found at the locality closest to the industrial zone, while the highest content of Pb was measured at the locality farthest from the industrial zone, which is located in the direction of the prevailing winds. Through both analyzed phenophases, the content of heavy metals in the soil had a significant positive correlation with the content of heavy metals in the roots and stems of wheat. The bioaccumulation of heavy metals by roots was highest in the phenophase of heading, while the root–stem translocation of heavy metals was dominant in the phenophase of full maturity. On average, for all four wheat genotypes and three locations, a significant increase in the concentration of Zn and Cu in the roots and stems was found in the phenophase of full maturity compared to the phenophase of heading. From the aspect of bioaccumulation and translocation factors and public health risk, the genotype Pobeda stands out as the most suitable genotype. The Apache genotype was characterized by the highest content of heavy metals in the stem and therefore by the highest translocation factor. The obtained results indicate that future breeding programs in the selection of new wheat genotypes should pay more attention to the study of the ability of genotypes to uptake, bioaccumulate, and translocate heavy metals, which could contribute to improving food safety and protecting the health of the population.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13041016/s1, Table S1: Analysis of variance for heavy metal content measured in the roots of wheat in different phenophases and localities; Table S2: Analysis of variance for heavy metal content measured in the stems of wheat in different phenophases and localities.

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