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Heavy metal bioaccumulation in *Zea mays* L. i *Medicago sativa* L. in the area of Zenica

Abstract

In the period March-September 2018, research was conducted on the content of heavy metals: iron (Fe), manganese (Mn), zinc (Zn), nickel (Ni), lead (Pb), vanadium (V), molybdenum (Mo), cadmium (Cd), chromium (Cr), copper (Cu) and cobalt (Co) in the soil and plants of corn (Zea mays L.) and alfalfa (Medicago sativa L.) at three locations in the Zenica area (Gradišće, Stranjani and Šerići). Soil analysis shows that the soil's average concentrations of manganese, nickel, vanadium, cadmium, and molybdenum exceed the permitted limits. Based on the measured values, higher concentrations of vanadium (4.93 mg/kg), cadmium (0.251 mg/kg), chromium (5.03 mg/kg), and copper (10.05 mg/kg) are present in the roots of corn. More manganese (170.7 mg/kg) is concentrated in the corn's leaf, and zinc (19.95 mg/kg) in the corn's grain. Most iron (1301.5 mg/kg) is concentrated in alfalfa roots, while nickel (2.43 mg/kg) and lead (4.92 mg/kg) dominate in its above-ground part. The results show that the bioaccumulation of heavy metals is more pronounced in the above-ground part of alfalfa and the roots of corn.

Keywords: heavy metals, corn, alfalfa, soil

INTRODUCTION

Heavy metals are increasingly present in the environment, especially in industrialized areas. The most common sources of soil contamination with heavy metals in the Zenica area are metallurgical and thermal power plants that have been active for many years. The increased content of heavy metals in the surface part of the soil, plants, and atmosphere in mining and metallurgical areas has been documented in numerous studies such as [1], [2], [3] etc. Combustion of fossil fuels and incineration of waste contributes significantly to the emission of As, Se, Hg, Cd, Cr, Ni, and Zn [4].

The content of heavy metals in the soil has increased compared to the natural state and the limit values prescribed by the Rulebook on the determination of permitted amounts of harmful and dangerous substances in the soil and methods of their examination [5], which is a consequence of their anthropogenic redistribution during the integral operation of the Ironworks Zenica. Research conducted by the Federal Institute for Agropedology in Sarajevo [6] and the Metallurgical Institute "Kemal Kapetanović" in Zenica [7] prove that the soil around Ironworks Zenica is heavily contaminated with heavy metals (manganese, nickel, cadmium, and zinc). A study conducted in 2010 [8] based on analyses of plant crops (leaves of fruit crops, lettuce, chard, nettles, alfalfa) in the area of Zenica concluded that the plants were contaminated with lead, cadmium, zinc, copper, nickel, cobalt and chromium. Heavy metals from the soil accumulated in plants enter the food chain and their excessive amount can have a toxic effect on humans and animals.

This paper presents the results of researching heavy metals concentration and their distribution in different parts of corn and alfalfa collected in polluted sites in the Zenica area, to determine their ability to bioaccumulate heavy metals, and their potential use for soil decontamination.

Deficiencies or excesses of certain metals can be reliably determined on a leaf, stem, or root [9], considering their necessary presence in plant tissue. The behavior of heavy metals in the soil and their accumulation in plants depends on many factors, such as the composition and pH value of the soil, the content of organic matter, the adsorption complex, and other factors [10].
The absorption and storage of heavy metals from the soil into plants occurs through a series of transport mechanisms that normally serve to absorb micronutrients [9, 11].

MATERIALS AND METHODS

The research was carried out in three localities in the area of Zenica, established according to the system of cyclic circles at different distances, from 2.78 to 16.4 km as the crow flies from the dominant industrial sources of dust and heavy metal emissions (metallurgical and thermal energy plants in Zenica). The distances, longitude, latitude, and altitude are shown in Table 1.

Table 1. Distance longitude, latitude, and altitude of research sites from industrial emission sources

No.	**Location**	**Distance (km)**	**Latitude**	**Longitude**	**Altitude**
1.	Gradišće	2,78	44^0 14’13” N	17^0 52’8” E	528 m
2.	Stranjani	4,88	44^0 13’14” N	17^0 50’54” E	565 m
3.	Šerići	16,4	44^0 21’30” N	17^0 48’23” E	809 m

To obtain a representative sample, from each location, several soil samples were taken from a depth of 25 cm. After drying in the air, a sample was reduced using the quartering method, and then the remaining sample was shredded, sieved, and packed for chemical analysis.

To monitor the content of heavy metals in the soil, soil samples were taken before and after planting corn and alfalfa. Initial soil analysis is given in Table 2 and the soil analysis after removing plants is shown in Tables 3, 4, and 5.

The plant material was sampled as whole plants with roots, and then the fraction samples were made from root, leaf, and grain in the case of corn and root and the above-ground part of the plant in the case of alfalfa. Fraction samples were air-dried for one month and then crushed. The content of heavy metals in corn and alfalfa samples was done using atomic absorption spectrophotometry according to PERKIN ELMER Analytical Methods for Atomic Absorption Spectrometry.

Limit values of heavy metals (HM) depending on soil texture are defined by the Rulebook on the determination of permitted amounts of harmful and dangerous substances in the soil and methods of their examination [5] are shown in Table 2. The textural features of the soil of the investigated locations are powdery-loamy. Limit values for manganese and iron, given in Table 2, are taken from the Federal Institute for Agropedology[6]. These soil limit values refer to soils with an acidic reaction. The specified values can be increased by 25% for alkaline and carbonate soils.

Results and discussion

The initial soil analysis shows that the average concentrations of manganese, nickel, vanadium, and cadmium are higher than the permitted limit value at all locations in the Zenica area. Elevated molybdenum concentrations in the soil were registered at sites, Gradišće and Stranjani (Table 2).

Table 2. Initial soil analysis at research sites Gradišće, Stranjani, and Šerići

Metal (mg/kg)	**Research site**			**Limit value (mg/kg)**
	Gradišće	**Stranjani**	**Šerići**	
Fe	42.000 (4.2%)	34.633 (3.5%)	22. 700 (2.3%)	5%
Mn	**1417**	**1667**	**3063**	1.000.
Zn	29	21	19	150
Ni	**111**	**66**	**46**	40
Pb	59	25	23	80
V	**104**	**67**	**44**	40
Mo	**20**	**24**	5	15
Cd	**1.4**	**1.36**	**3**	1.0
Cr	78	41	16	80
Cu	29	31	49	65
Co	<1	<1	<1	45
pH in H_20	7.7	7.6	6.2	-
pH in KCl	7.0	6.8	5.6	-

Note: Shaded numbers indicate exceeding limit values.

The content of heavy metals in fraction samples of corn and alfalfa as well as the content of heavy metals in the soil after the removal of plants are presented in the following tables.

Tabela 3. Content of heavy metals in plants and soil after removal of plants at research site Gradišće (mg/kg)

Metal	Corn				Alfalfa		
	Root	Leaf	Grain	Soil after removal	Root	The above-ground part	Soil after removal

Fe	1146.3	238.8	57.36	45.890 (4.589 %)	462.5	339.1	43.590 (4.359 %)
Mn	27.12	76.32	9.98	**1400**	18.00	24.49	**1440**
Zn	4.93	7.39	19.95	10	<0.01	4.92	10
Ni	<0.01	<0.01	<0.01	**80**	<0.01	<0.01	**90**
Pb	<0.01	1.97	2.25	**90**	<0.01	4.92	**70**
V	4.93	<0.01	<0.01	**50**	<0.01	<0.01	<1
Mo	<0.01	<0.01	<0.01	<1	<0.01	<0.01	<1
Cd	<0.001	<0.001	<0.001	<0.1	<0.001	<0.001	<0.1
Cr	4.93	2.46	2.49	**90**	<0.01	<0.01	**100**
Cu	7.40	4.92	<0.01	50	<0.01	2.45	60
Co	<0.01	<0.01	<0.01	<1	<0.01	<0.01	<1

1 **Table 4.** Content of heavy metals in plants and soil after removal of plants at research site Stranjani (mg/kg)

Metal	Corn				Alfalfa		
	Root	Leaf	Grain	Soil after removal	Root	The above-ground part	Soil after removal
Fe	997.5	110.4	30.10	35.500 (3.55%)	943.8	549.5	36.100 (3.61 %)
Mn	105.8	52.9	20.1	**1630**	32.4	26.7	**1630**
Zn	2.65	<0.01	<0.01	20	2.49	2.43	20
Ni	<0,01	<0.01	<0.01	**60**	<0.01	2.43	**70**
Pb	<0.01	4.60	<0.01	50	<0.01	<0.01	10
V	<0.01	<0.01	<0.01	**60**	<0.01	2.43	<1
Mo	<0.01	<0.01	<0.01	<1	<0.01	<0.01	<1
Cd	<0.001	<0.001	<0.001	<0.1	<0.001	<0.001	<0,1
Cr	<0.01	2.30	2.51	70	<0.01	2.43	80
Cu	<0.01	4.60	<0.01	40	7.47	7.29	30
Co	<0.01	<0.01	<0.01	<1	<0.01	<0.01	<1

1 **Table 5.** Content of heavy metals in plants and soil after removal of plants at research site Šerići (mg/kg)

Metal	Corn				Alfalfa		
	Root	Leaf	Grain	Soil after removal	Root	The above-ground part	Soil after removal
Fe	1100.7	263.6	19.3	24980 (2.498 %)	1301.5	235.1	26980 (2.698 %)
Mn	105.5	170.7	7.24	**3000**	158.3	67.2	**2730**
Zn	2.51	5.02	2.41	20	2.36	4.80	20
Ni	<0.01	<0.01	<0,01	40	2.36	<0.01	**60**
Pb	<0.01	<0.01	<0.01	20	<0.01	<0.01	70
V	2.51	<0.01	<0.01	<1	<0.01	<0.01	20
Mo	<0.01	<0.01	<0.01	<1	<0.01	<0.01	<1
Cd	0.251	<0.001	<0.001	<0,1	<0.001	<0.001	<0,1
Cr	5.03	2.51	2.41	30	2.36	2.40	40
Cu	10.05	2.51	2.41	**80**	7.09	4.79	**80**
Co	<0.01	<0.01	<0.01	<1	<0.01	<0.01	<1

Results from Tables 3, 4, and 5 show that The iron content in the dry samples of plants is mostly between 19.3 (grain of corn) and 1301.5 (the root of alfalfa) mg/kg, which is fairly low concerning that some ferrophilic plants (spinach, oats) can contain up to 3500 mg/kg of iron [12]. According to
36 the author, the distribution of iron in different parts of corn is as follows: root 44.1%, stem 14.6%,
1 leaves 23%, grain 15.7%, cob 2.7%. Lazarevic et al. find that the concentrations of Fe in the roots and leaves of corn increase with the increase in the concentration of Fe in nutrient solutions [13], which is
30 in agreement with the higher concentrations of iron in the roots of corn due to the high content of Fe in the soil at the Gradišće location (Tables 2 and 3).

Results in above tables show that the concentration of Iron in corn ranges from 19.3 mg/kg in grain to 1146.3 mg/kg in root. The average concentration of iron in corn leaves ranges from 110.4 mg/kg at the Stranjani site to 263.3 mg/kg at Šerići site. The low concentration of Iron in corn leaves on research site Stranjani indicates low mobility of iron in the soil of this research site. Since the critical concentration of iron in leaves is from 50 to 150 mg/kg [14] low mobility of iron could lead to iron deficiency in this research site. The average concentration of Iron in the roots of alfalfa ranges between 460.5 mg/kg and 1143 mg/kg, and from 235.1 to 549.5 mg/kg in the aboveground part of alfalfa (Tables 3, 4, and 5).

The level of manganese in plants in research sites ranges from 7.24 mg/kg in corn grain to 170.7 mg/kg in corn leaves, followed by 158.3 mg/kg in alfalfa roots (Table 3, 4, 5). According to [12], manganese concentration in the above-ground part of alfalfa reaches 86 mg/kg. According to [15], the concentration of manganese in the grain of corn is 5.7 mg/kg, and according to [16], the concentration of manganese in the grain of corn ranges between 2.85 and 4.35 mg/kg. The concentration of manganese in grain of corn on all research sites is higher (Tables 2, 3, and 4) probably due to the significantly high content of manganese in soil (1.43 to 3.06 times higher than the limit value).

The highest concentration of manganese, 158.3 mg/kg, is found in the roots of alfalfa on research site Šerići because the soil on this research site has the highest manganese content.
Manganese is mobile in the plant and it is found in all parts of the investigated plants. The mobility of manganese increases with soil acidity and its reduction to active manganese Mn^{2+}, which is easily absorbed by plants [17], while more oxidized forms such as Mn^{3+} and Mn^{4+} are inactive forms.

The natural zinc content in plants varies from 20 to 100 mg/kg [18]. In cabbage leaves it is 35 mg/kg, wheat grains contain 23 mg/kg, and sugar beet roots 17.5 mg/kg of zinc [12]. The mobility of zinc in the plant is moderate, and it is believed that it is in the xylem in the form of citrate, chelate, or as a free ion [19]. The critical limit of zinc deficiency is 15 – 30 mg/kg in dry matter of leaves [17].
The maximum measured zinc concentration in corn grain is 19.95 mg/kg. According to research performead by [16], the average zinc value in corn grain varies between 13.6 and 25.6 mg/kg depending on the genotype, weather conditions, and soil properties. Varga [20] states that the zinc concentration in corn grain ranges between 19.09 and 22.60 mg/kg and depends on soil cultivation.
The maximum measured zinc concentration in corn leaves is 7.39 mg/kg and in the aboveground part of alfalfa 4.92 mg/kg (Table 3), which indicates a zinc deficiency in the plant that is very important for normal plant development.

The average concentration of nickel in plants ranges from 1 to 10 mg/kg dry matter [19], while in alfalfa ranges from 1.0 to 4.0 mg/kg [12]. Nickel is considered relatively poorly mobile in the soil because it has a high tendency to bind to clay minerals and iron and manganese oxides. The accessibility of nickel increases when the soil pH is less than 6.5 (Table 2, 4, 5) because under these conditions many Ni-components are relatively soluble [21]. This research showed that higher values of Ni are present in alfalfa (in the root 2.36 mg/kg and the aboveground part 2.43 mg/kg Table 4 and 5) in locations where the pH is less than 6.5, while corn fractions (roots, leaf, grain) contain the same amounts of nickel (less than 0.01 mg/kg). According to a study performed by Federal Institute of Agropedology B&H [8] the nickel content in an alfalfa sample at the Gradišće location was 13.90 mg/kg and it can be considered as contaminated. Studies show that the nickel concentration in alfalfa increased proportionally to the increase in the amount of available nickel in the soil [22].

The natural content of lead in plants varies between 0.1 and 10 mg/kg [23, 24]. Plant roots are able to accumulate lead in plants, but their subsequent translocation to aboveground parts is very limited [25]. Scientific research shows the significant accumulation of lead in the leaf [26, 27], and that lead reaches plants primarily through leaves from the air [28, 29]. Wild herbaceous plants such as alfalfa and nettle have significantly higher bioaccumulation of lead in plants than cultivated vegetable and cereal plants, which is a result of the specific genetic conditions of the plants studied and their adaptation to living conditions [8]. The lead content in the alfalfa sample was 12.20 mg/kg at the Gradišće location, which exceeds the limit value for plants.

The greatest biostimulation by lead was in the Gradišće area, due to greater exposure to atmospheric
5 lead deposition because it is closest to metallurgical plants. The least accumulation of lead in plants
was recorded in the Šerić area, which is the furthest from the pollution source. This research showed
that the highest amounts of lead are present in the aboveground part of alfalfa (4.92 mg/kg), and in the
corn leaf (4.60 mg/kg) (Tables 3, 4, 5) and it is consistent with research of Federal Institute of
Agropedology performed in 2010 [8] at Gradišće site.

This study shows that vanadium concentration in corn roots ranged between 2.51 and 4.93 mg/kg,
2 while in alfalfa the maximum vanadium concentration of 2.43 mg/kg was recorded in the aboveground
part of the plant (Tables 3, 4, and 5).

13 Plants contain very little molybdenum, even below 1 mg/kg (0.1-0.5 mg/kg in dry matter), and
relatively higher molybdenum content is in plants from the legume and cruciferous families [19]. The
molybdenum content in alfalfa hay is 0.98 mg/kg [12]. The mobility of molybdenum in plants is
moderate. Plants absorb molybdenum in the form of MoO_4^{2-} and in plants, it exists as an anion, so its
accessibility increases with increasing alkalinity, unlike all other microelements, This study shows that
the molybdenum content in corn and alfalfa is the same and is less than 0.01 mg/kg (Table 3, 4 and 5).

8 The natural content of cadmium in plants varies between 0.05 and 0.20 mg/kg [23]. It has been
determined that 30-60% of cadmium contained in plants comes directly from the atmosphere and 40-
14 60% from the soil. Cadmium ions are mainly retained in the roots, and only small amounts are
3 transported to the aboveground part of the plant [30]. The highest concentrations of cadmium were
found in the roots of plants [31], which was also shown in this study, as 0.251 mg/kg was recorded in
corn roots at the Šerići location (Table 5) because the pH of the soil at the Šerići location is the lowest
and favors cadmium mobility. The cadmium content in other parts of corn and alfalfa is the same and
amounts to less than 0.001 mg/kg, which complies with previous research.

21 According to [24], the amount of chromium in plants usually varies in the range of 0.02 to 0.2 mg/kg
6 of dry matter [24] while according to [18] the amount of chromium in plants varies between 0.2 and
0.4 mg/kg. Plants growing on contaminated soil can accumulate higher amounts of chromium [21].
Chromium is preferentially accumulated in corn roots with a low rate of translocation to leaves [32],
while [29] believes that plants characterized by iron accumulation also accumulate chromium. Higher
values of chromium were recorded in the roots of corn at 4.93 - 5.03 mg/kg and in the aboveground
16 part of alfalfa at 2.45 mg/kg (Table 3,4,5). Given that the critical value of chromium in a plant is 1
mg/kg [8], the chromium content in both sown crops is above the permitted critical value.

The natural content of copper in plants is 2-20 mg/kg [18]. Copper translocation is moderate, in the
form of Cu complexes, usually with amino acids, so plant roots contain it in significant quantities [19].
The amount and availability of copper are significantly affected by the pH reaction of the soil, and its
accessibility increases with soil acidity[8,19], which is in agreement with the higher concentration of
copper in plants at the Šerići location, where the pH is the lowest of the observed locations. (Tables 2
1 and 5). The concentration of copper in corn roots was 10.05 mg/kg and 7.47 mg/kg in alfalfa roots.
16 The copper content in corn grain ranges from <0.01 to 2.41 mg/kg, while [15] found that the copper
43 content in corn grain ranges from 2.2 to 5.8 mg/kg.

3 The amount of cobalt in plants varies from 1 to 40 mg/kg of dry matter [19]. The natural content of
39 cobalt most often varies from 0.02 to 0.4 mg/kg [18]. Alfalfa hay contains 0.38 mg/kg [12]. It is a
component of vitamin B12, and is especially important for legumes because it is necessary for N_2
fixation, so in the event of a cobalt deficiency, organic production decreases [19]. Due to the poor
mobility of cobalt within the plant, only a small amount of cobalt is moved from the stem to the leaves
[33]. This research shows that different parts of corn and alfalfa have the same amount of cobalt and it
38 is less than 0.01 mg/kg (Table 3,4,5) probably due to the low cobalt content in the soil (below 1
mg/kg).

The area of Šerić is further away from the emission center, but the land at this location is more sensitive from the point of view of contamination. Heavy metals in more acidic soils are in an active form fixed to soil particles and they represent a potential danger. The largest amounts of iron, manganese, vanadium, cadmium, chromium, and copper were accumulated by the plants at the location of Šerići, while the highest amount of nickel was accumulated at the location of Stranjani, The largest accumulation of zinc and lead was registered at the Gradišće location where the soil is neutral to weakly alkaline. In such soil, heavy metals turn into hydroxides, and with a further increase in the pH value, they turn into insoluble hydroxides, and if the carbonate content in the soil is high, heavy metals are inactivated, i.e. they become difficult for plants to access [8]. The research results show that the concentration of nickel, chromium, copper, manganese, and lead (Table 2, 3, 4, and 5) in the soil is higher after the removal of plants due to the pollution of the Zenica area with an increased amount of sediment containing heavy metals [7].

Alfalfa (*Medicago sativa* L.) shows a greater ability to bioaccumulate iron in the roots, and higher values of nickel and lead are accumulated in the aboveground part. (Table 3, 4 and 5). The same values of molybdenum, cadmium, and cobalt are present in both fractions of alfalfa (root and aboveground part). Previous research has shown that alfalfa accumulates chromium, vanadium, lead,
20 nickel, molybdenum, and iron better than corn [34]. Plant species that do not prevent the entry of metals into the roots and allow the bioaccumulation of higher concentrations of metals in the tissues of their aboveground organs are most suitable for use in phytoremediation [35].

35 Maize (*Zea mays* L.) has the ability to accumulate in its roots more vanadium, cadmium, chromium, and copper compared to alfalfa. The translocation of iron, manganese, zinc, chromium, and copper
7 from the roots to the aerial parts of the plant is observed. The leaves have higher values of manganese and lead because heavy metals from the roots and the air accumulate in the leaf. Higher values of zinc are present in corn grains. The same values of nickel, molybdenum, and cobalt are present in the aboveground parts of corn. It can be seen that copper and chromium have high mobility in the soil and
6 are easily taken up by plants.

Conclusion

The results of this research conducted in the period March-September 2018 show that the content of iron, chromium, and manganese in corn, as well as iron and chromium in alfalfa, is above the permitted limits for heavy metals in plants growing on polluted land in the area of Zenica. The biggest
33 accumulator of iron is the root of the plant, with values in alfalfa 1301.5 mg/kg and corn 1146.3 mg/kg. Iron and manganese are mobile in plants and are found in both roots and aboveground parts of corn and alfalfa. The highest concentration of manganese was registered in corn leaves (170.7 mg/kg). This research shows higher values of manganese in corn grains at all three locations ranging between 7.24 and 20.1 mg/kg, probably due to increased concentrations of manganese in the soil. Zinc accumulates in the leaf and grain of corn with the maximum measured value of 19.95 mg/kg. Nickel is more abundant in alfalfa (roots 2.36 mg/kg and aboveground parts 2.43 mg/kg) in locations where the pH is less than 6.5, while in all corn fractions content of nickel is the same and is less than 0.01 mg/kg. Lead dominates in the aboveground part of alfalfa (4.92 mg/kg) in the Gradišće site, which is closest to the metallurgical plants, due to the greater burden of this area with atmospheric depositions
5 containing heavy metals. The highest concentration of cadmium is in the roots of corn with 0.251 mg/kg at the Šerići location, which is related to soil acidity, while in other plant parts of corn and alfalfa, low values were recorded and were less than 0.001 mg/kg. Corn roots accumulate higher values of chromium (4.93 – 5.03 mg/kg), while alfalfa has a higher accumulated value in the aboveground part (2.45 mg/kg). Copper mainly accumulates in the roots of the plant. The maximum copper concentration was recorded in corn roots at 10.05 mg/kg, and in alfalfa roots at 7.47 mg/kg. The plant fractions of corn and alfalfa (root, aerial part, and grain) contain the same amount of cobalt
22 and are less than 0.01 mg/kg. Vanadium (4.93 mg/kg), cadmium (0.251 mg/kg), chromium (4.93 mg/kg), and copper (10.05 mg/kg) are more accumulated in corn roots.

The presence of higher concentrations of metals in the aboveground parts of alfalfa makes it more suitable for phytoremediation. Namely, by removing alfalfa cuttings from contaminated locations over a longer period of time, one part of heavy metals whose values exceed permissible concentrations in

7 soil. In order to obtain better and more complete data on this issue, it is necessary to conduct tests with a larger number of samples.

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