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THE INFLUENCE OF PHYSICOCHEMICAL CHARACTERISTICS OF THE SOIL ON THE DISTRIBUTION OF HEAVY METALS IN PLANT CULTURES

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Summary

Heavy metals are natural ingredients of many plant cultures, and they are increasingly found in the soil as the main group of inorganic contaminants. The consumption of food products, either fresh or processed, with an increased concentration of heavy metals leads to the deposition of these metals in the body, which further leads to a series of consequences in the form of cardiovascular, respiratory, and neurological problems. The behavior of heavy metals in soil and their accumulation by plants depends on many factors, such as soil structure, soil pH value, organic matter content, adsorption complex and other factors. Therefore, the aim of this work is to present the influence of physical and chemical soil parameters on the distribution of heavy metals (Pb(II) and Cd(II)) in plant cultures and to assess the health risk pollution of the mentioned metals in plant cultures (cucumber, pepper, tomato, eggplant).

Keywords: heavy metals, physicochemical characteristic, soil, plant cultures, health risk

Introduction

Heavy metals, as the main group of inorganic contaminants, are the source of pollution of relatively large areas of the soil, and due to their increased release into the environment, they are receiving increasing attention (Yang et al., 2005). In recent years, pollution by cadmium, one of the most dangerous heavy metals in the environment, is growing rapidly (Jakovljević and et al., 2017). Since many studies increasingly indicate the toxic effect of Cd(II) and Pb(II) on the biological system, soil contamination with these metals has become a major global health and environmental problem.

Cadmium is coming the body through food, drink and inhalation and has a negative effect on the kidneys, liver, bones, and the cardiovascular and respiratory systems (WHO, 1972). Cadmium causes acute and chronic changes in the body and they act cumulatively. When it enters the body, cadmium accumulates in the liver and kidneys, because its biological half-life in the human body is 20 to 30 years. Long-term intake of small concentrations of cadmium can have a major negative effect on human health such as neurotoxicity, respiratory changes. It is important to mention the connection between cadmium and prostate cancer, which IARC classifies as the first cancer group (US.EPA, 1985).

In numerous plant species, the intensity of cadmium transport in the aboveground organs is correlated with its concentration in the nutrient medium. Cadmium adopted from the nutrient medium is mostly retained in the roots, and the share of this element in the stem and leaves of plants is approximately the same or lower than its concentration in the underground part of the plant.

Like cadmium, increasing attention is being paid to lead, which is considered a toxic element for humans and plants. Lead enters the body through food and drink, as well as by inhaling small particles. Ingested lead is mainly deposited in bones, CNS and kidneys. The effects of even a small dose can be seen months and years later. The accumulation of lead in the surface part of the soil is also of great ecological importance since lead has a great influence on the biological activity of the soil. Since lead is generally well bound in all soil types, phytoextraction is limited. When a plant takes up lead, its translocation to higher parts of the plant is very weak. Most of the absorbed lead is concentrated in the roots (Kabata-Pendias, 2001). The intensity of lead contamination of plants decreases with their distance from major roads. Plants poorly absorb lead in inorganic form and transfer it to above-ground organs, except on acidic soils. Organic lead compounds are very quickly taken up and transported to the aerial parts of plants. Lead deposition in most plants is more intense in the roots than in the aerial parts. The great power of the roots in the accumulation of lead could also represent a form of protection of the above-ground part of the plant, since lead in higher concentrations inhibits the elongation of roots and the growth of leaves, inhibits the process of photosynthesis, and affects the morphological and anatomical structure of the plant.

The behavior of heavy metals in the soil is determined by numerous factors that can affect their mobility and accumulation by plants, the most

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important of which are soil structure, soil pH reaction, organic matter content (Pelivanoska et al., 2011). In addition to these, other factors can affect their mobility and harmful effects, such as humidity, calcium carbonate content, hydrated iron and aluminum oxides, cation exchange capacity, redox potential, groundwater level, etc. (Kastori et al., 2003). The characteristic of most toxic elements is that they react with various organic compounds creating stable complexes with ligands containing oxygen, sulfur or nitrogen as electron donors. The toxic effect is based on their irreversible binding to metabolically active groups in amino acids, polypeptides and proteins (Mihaljev et al., 2008).
Therefore, the aim of this work was to examine the physical and chemical characteristics of the soil, determine the concentration of heavy metals (Cd(II) and Pb(II)), and examine the influence of physical and chemical characteristics on the distribution of heavy metals in the most commonly grown vegetable (cucumber, pepper, tomato and eggplant). Correlation analysis determined the degree of soil pollution with heavy metals, determined the connection between the content of heavy metals in plant material and some properties of the soil, as well as the yield of plants, determined the transfer coefficient of the mentioned heavy metals from the soil to the plant, and calculated the risk index in order to assess the impact of heavy metals on human health.

Materials and methods

Materials

The experimental part was carried out at two locations marked as S (Srebrenik) and C (Campus in University of Tuzla) on a total land area of 500 m^2. Soil sampling for analysis was performed according to the instructions of Pernar (Pernar, 2012). In the month of March, an average sample was taken from the mentioned areas at a depth of 0-15 cm. The collected sample was air-dried, and then in an oven at 105 ºC for 3 hours. Along with the soil samples, an analysis of cement dust was performed, which was used for application on the surface of the land, after which plowing was performed in order to also examine the possibility of using cement dust as a substrate for growing vegetable (cucumber, eggplant, pepper, tomato). After 30 days, four plant cultures were planted on both sites with and without added substrate (cement dust), where growth and development were monitored in the March-July period, and after ripening, the fruits were delivered to the laboratory and prepared for analysis on the appropriate parameters.

Methods

The total content of heavy metals (Cd(II) and Pb(II)) in the soil, cement dust and vegetables was determined by the ICP-OES method on the "Perkin Elmer Optima 2100 DV" device. Digestion of samples (soil, dust and plant cultures) weighing 3.0000 g was carried out with 7 ml of concentrated HNO_3 and 4 ml of concentrated H_2O_2 at a temperature of 80 °C, for a total duration of 183 min, in an ultrasonic bath. After digestion, the samples were cooled for 10-15 minutes, and then the flask was topped up to 50 ml (Odobašić et al., 2017). The composition of cement dust was determined by XRD and XRF analysis. The content of humus in the soil and cement dust was determined by the Tjurin method and calculated according to the following equation:

$$\%\ humusa = \frac{(a-b)\cdot 0{,}0005172\cdot 100}{m} \quad (1)$$

where is:
a-volume of Mohr's salt (ml) used for blank x0.1x 10;
b-volume of Mohr's salt (ml) used for the soil sample x0.1x10;
m-mass of the sample.

Calcium carbonate content by volumetric method BAS ISO 10693:2015 and calculated according to the following relationship:

$$\%\ CaCO_3 = \frac{B-S\cdot C_{NaOH}\cdot E.j._{CaCO_3}\cdot 20}{m} x100 \quad (2)$$

where is:
B-volume of NaOH used for the titration of the blank sample (ml);
S-volume of NaOH used for soil sample titration (ml);
E.j.($CaCO_3$)-50.04 (mg/mmol)=50.04 (g/mol);
m-mass of the sample (g).

Results and discussion

Physical and chemical characteristics of the soil and cement dust

Table 1. Physical and chemical characteristics of the soil

Parameters	Soil	
	Location C	**Location S**
pH (H_2O)	6.92	5.19
pH (KCl)	5.71	3.93
Content of organic matter (%)	1.189	0.982
Chloride content (mg/g)	4.88	12.2
Electrical conductivity (μS/cm)	34	14.93
TDS (Total dissolved salt, mg/l)	21.76	9.55

Table 2. Physical and chemical characteristics of the cement dust

Parameters	Cement dust
pH (H_2O)	12.76
pH (KCl)	12.7
Content of organic matter (%)	1.01716
Chloride content (mg/g)	12.203
Carbonate content (%)	76.0608
Electrical conductivity (mS/cm)	5.31
TDS (Total dissolved salt, mg/l)	3398.4

As part of the physical and chemical analysis of soil and cement dust, the pH value (in distilled water and KCl), organic matter content, chloride content, electrical conductivity, carbonate content and the total amount of dissolved salts were determined. The pH analysis in KCl showed that it is a slightly acidic soil at locality C (pH=5.71), and a very acidic soil at locality S (pH=3.93). Using the Tjurin method, the humus content was determined to be 1.189% at location C, and 0.982% at location S, which according to Gračanin's classification indicates that the soil is poor in humus (Table 3.), and that the soil is covered with sediment of different granularity. The electrical conductivity value of a soil sample is proportional to the content of readily soluble salts and exchangeable cations present in the soil. (Pavelić T., 2019) By their dissociation or release in water suspension saturates the soil with nutrients and increases electrical conductivity. Increasing electrical conductivity also increases the concentration of total dissolved salts (TDS). The electrical conductivity value determined by conductometry method was 34 µS/cm for the C location and 14.93 µS/cm for the S location, while the amount of total dissolved salts was 21.76 mg/l for the C location and 9.55 mg/l for location S. Since the XRF and XRD analysis showed that the cement dust is rich in minerals and oxides, the expected result of the high value of electrical conductivity and the content of total soluble salts in the cement dust was also confirmed ie 5.31 mS/cm or 3398.4 mg/l.

Table 3. Limit values for humus content in soil (Method by Gračanin)

Soil supply	Humus %
Very poor in humus soil	<1
Poor in humus soil	1-3
Enough humus soil	3-5
Rich in humus soil	5-10
Extremly rich in humus soil	>10

Based on the obtained results shown in Table 2., it can be concluded that cement dust has an extremely basic reaction (pH=12.70), high conductivity (5.31 µS/cm) and high carbonate content (76.0608%), and that as such it is favorable for application to the soil surface, especially on soil with lower pH values. XRD analysis shows that the cement dust is rich in ferrite (25%) and lime (24%), and to a lesser extent maenite, sylvite, etc. (Fig. 1) XRF analysis shows that cement dust is very rich in CaO (68%), followed by K_2O (9%), SiO_2 (7%), Al_2O3 (4%), Fe_2O_3 and Cl (3%), MgO and Na_2O (1%) (Fig.2).

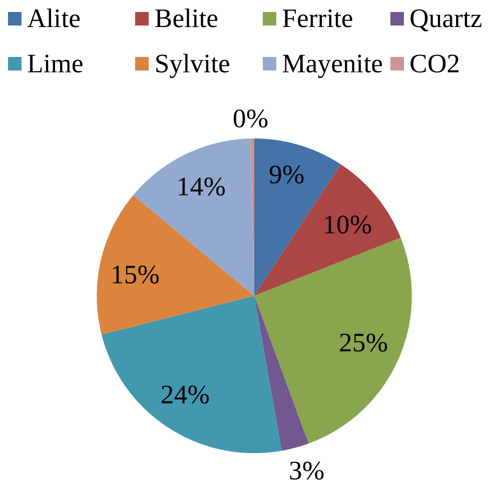

Fig 1. Minerals content in cement dust determined by XRD analysis

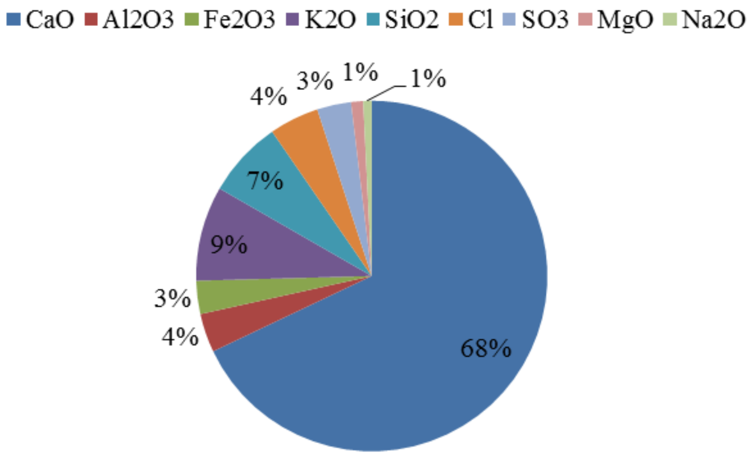

Fig 2. Oxides content in cement dust determined by XRF analysis

The maximum allowed amount (MDK) of dangerous and harmful substances in the soil can damage or change the productive capacity of agricultural land (Jakšić, 2013). Exceeding these values usually come from discharges from factories, spills from landfills, improper use of mineral fertilizers and plant protection products, and as such are defined by the Rulebook on permitted amounts of hazardous and harmful substances in the soil and their testing methods (Official Gazette 52/09). If the land contains a larger amount than the maximum allowed, it is not recommended for agricultural production because it represents a potential danger to human health.

Table 4. MDK Pb and Cd according to the Rulebook on permitted amounts of hazardous and harmful substances in the soil and their testing methods

Heavy metals	MDK for soil (mg/kg)	MDK for cement dust (mg/kg)
Pb	80	500
Cd	1	5

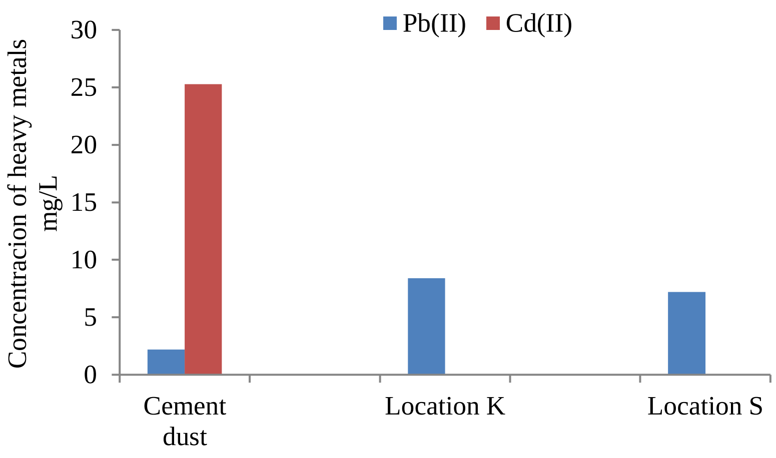

Fig 3. Content of heavy metals in soil and cement dust

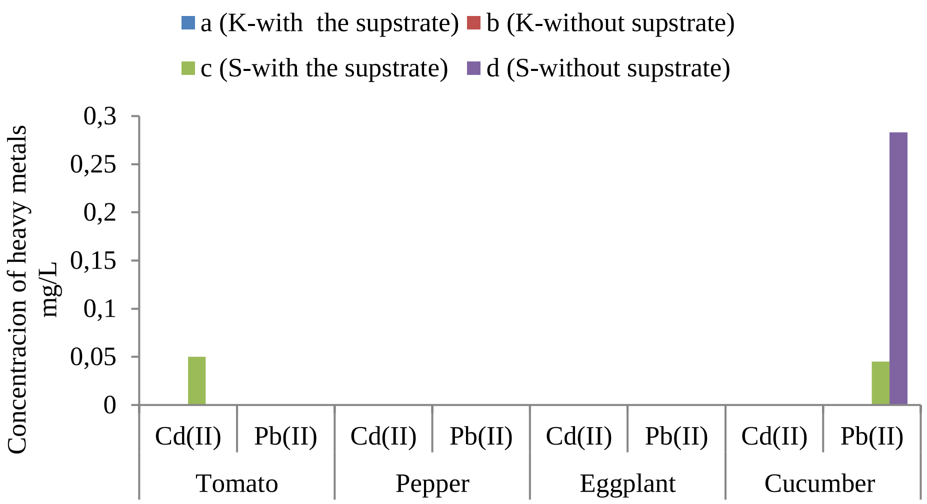

Fig 4. Content of heavy metals in plant cultures (tomato, pepper, eggplant, cucumber)

Determining the content of heavy metals in the soil is a basic indicator for determining the degree of pollution and suitability of the soil for plant production. Based on the presented results, it can be concluded that the soil at location C contains 8.4 mg/kg Pb(II) and 0.05 mg/kg Cd(II), while the analysis of location S showed a Pb(II) content of 7.2 mg/kg, while the Cd(II) content at this location not detected. The stated values do not exceed the permitted values of heavy metals in the soil defined by the Rulebook.

By comparing the obtained values of the content of heavy metals in cement dust with the permitted ones, it can be concluded that the content of the tested heavy metals also does not exceed the permitted values (Regulation on determination of permitted amounts of harmful and waste substances in the soil and methods of their testing, where Article 12 of the above was used of the Rulebook relating to MDK of heavy metals in sludges that are added to the soil in the same way as it was done with cement dust).

The analysis of heavy metals in plant cultures showed that the content of Cd(II) is present in tomatoes at the location S(II) in the sample with the substrate in the amount of 0.05 mg/kg, and Pb(II) in the cucumber in the location S in the sample with the substrate in the amount of 0.045 mg/kg and 0.283 mg/kg in the sample without substrate. Based on the average values of the analyzed elements in the tested samples, their content was in accordance with the Rulebook on maximum allowed concentrations in vegetables (Regulation and maximum allowed amounts for certain contaminants in food Sl.glasnik BIH no. 68). The obtained values of Cd(II) and Pb(II) content show that cement dust is safe for use in agriculture, because research was carried out on several of the most sensitive plant cultures (cucumber, pepper, tomato, eggplant). Also, during the growth and development of the tested cultures on the soil with the addition of cement dust, not a single type of chlorosis or necrosis was observed, and the plant cultures had a normal color and normal growth, which is another indicator of the safe use of cement dust in the soil for the cultivation of the mentioned plants culture.

The degree of soil pollution

Determining the degree of soil pollution is one of the proven ways of determining soil quality, where the necessary remediation measures are provided based on the established actual soil condition (Ahmetović et al., 2020). In many European countries, studies are conducted on the content of toxic metals in the soil, with the aim of managing its flow through the ecosystem and applying protective measures (Lado, 2007). For land used for agricultural purposes, it is necessary to determine the degree of pollution (PD) in order to categorize the land present (Wu, 2010). The degree of pollution is calculated from the following relation:

$$PD\ (\%) = \frac{Cm_{sample}}{C_{lim.value}} x100 \qquad (3)$$

where is:

C_m of the sample - metal concentration in the sample (mg/kg);

$C_{lim.value}$ – limit value of metals in the sample (mg/kg).

Based on the calculated values, the land is classified into one of the categories indicated in Table 5.

Table 5. Classification of soli for agricultural production

Class	Class definition
I	PD<25 %, clean soil, suitable for agricultural production
II	PD=25-50 % soil of increased pollution, cultivation of plants with the necessary protection against the heavy metals imission
III	PD=50-100 % soil of high pollution, cultivation of plants with increased protection measures against heavy metals imission
IV	PD=100-200% polluted soil and unsuitable for cultivation of plants, necessary remedial measures
V	PD>220% extremely polluted soil, ban on the cultivation of plants for human and animal use, necessary to perform comprehensive remediation and recultivation measures.

Table 6. Degree of soil pollution

Heavy metal (mg/kg)	Degree of pollution (PD) %	
	C locality	S locality
Pb	10,5	9
Cd	5	0

Soil pollution is defined as the accumulation of persistent toxic compounds, chemicals, salts, radioactive materials or disease-causing agents in the soil, which have harmful effects on plant growth and animal health (Okrent, 1999). Based on the calculated values, it was determined that the degree of contamination (PD) with lead is 10.5% at location C, and 9% at location S, while the degree of contamination with cadmium at location C is 5%. (Table 6.) Since Cd was not detected in the soil at location S, the level of pollution with this metal is not present either. According to the criteria from Table 5, the land at these locations belongs to Class I, the class of clean land suitable for agricultural production.

In order to determine the phytoremediation potential, the bioaccumulation and translocation factors were determined (Wu et al., 2010). The ability of the plant to accumulate heavy metals is shown as a bioaccumulation factor, which is calculated as the ratio of the metal concentration in the plant to the metal concentration in the soil (Cools, 2010):

$$BF = \frac{C_{plant}}{C_{soil}} \qquad (4)$$

where is:
C_{plant}- metal concentration in the plant;
C_{soil}- metal concentration in the soil.

In order to determine the impact of heavy metals on human health, a risk index and risk assessment on human health were determined. International organizations (FAO/IAEA/WHO, JECFA, US EPA) have defined the maximum permissible concentrations of lead and cadmium based on the weekly metal intake of the average weight of an adult, and they amount to Pb-25 µg/kg b.w./week, Cd 7 µg/kg b.w. /weekly. (JECFA, 1993; FAO/WHO Food Standards Programme, 2002; FAO/WHO Standards Programme, 1998) The risk indices were calculated on the basis of metal concentrations obtained from the analysis of plant cultures and calculated according to the following relationship:

$$Risk\ indices = \frac{weekly\ intake\ of\ metals\ through\ vegetable\ consumption}{PTWI\ metals} x\ 100 \qquad (5)$$

where:
PTWI is the conditionally acceptable weekly metal intake for adults.

Based on the risk index, an assessment of the impact on human health was calculated. Risk assessment is a conceptual framework that provides a mechanism for reviewing information to assess health or environmental outcomes. (National Research Council, 1983). For this purpose, the risk assessment is carried out using the Fine-Kinney method. (Havula, J. et al., 2011) The principle of this method is based on the assessment of metal exposure (E), consequences (C) and event probability (P) and is calculated according to the following relationship:

$$R=E \cdot P \cdot C \qquad (6)$$

where is :
R-risk;
E-exposure;
P-probability of events;
C-consequences.

Table 7. Scoring of exposure and risk assessment to heavy metals

Exposition	**Score**
Permanently	5
Often	4
Random	3
Rare	2
Very rare	1
Possiblity	**Score**
Frequent	5
Probably	4
Random	3
Not significant	2
Without any significance	1
Consequences	**Score**
Catastrophic	5
Very serious	4
Serious	3
Important	2
Not significant	1
Total risk assessment	
Scores	**Situation**
More than od 100	Very high risk
70-100	High risk
40-70	Important risk
20-40	Possible risk
Below 20	Acceptable risk
Risk assessment to heavy metals	**Score**
Pb	
Exposure	4
Possibility	4
Consequences	4
Total risk assessment for Pb	**64**
Cd	
Exposure	4
Possibility	3
Consequences	4
Total risk assessment for Cd	**48**

Exposure assessment (E) aims to determine the extent and nature of contact with substances in different mouths. It is valued from 1 to 5. When it comes to exposure to heavy metals (Cd(II) and Pb(II)) that can enter the human body daily through food and drink as well as by inhalation, risk assessment of exposure to these metals is common (4).

An assessment of the probability of an event (P) with respect to exposure aims to show the likelihood and predicted consequences. It is valued from 1 to 5. The probability of an event related to Cd (II) exposure to human health is estimated as random (3), since cadmium enters the soil through wastewater, phosphate-based fertilizers, sludge, etc. The probability of an event related to human health exposure to Pb (II) is estimated as probable (4) because large amounts of lead are taken up by plants from the air.

Consequences (C) describe possible side effects that may occur. They are rated from 1 to 5. The consequences of exposure to Cd (II) and Pb (II) are assessed as very serious (4) because the intake of any of these metals leads to neurotoxic, respiratory and kidney problems.

Based on the calculated exposure assessment parameters, it can be concluded that there is a important risk of heavy metals (Cd, Pb) to human health. Table 8. shows the value of the risk index for the used plant cultures and the bioaccumulation factor.

Table 8. Values of the bioaccumulation factor and risk index for the used plant cultures

Bioaccumulation factor	**Tomato**				**Papper**			
	1a	1b	1c	1d	2a	2b	2c	2d
Pb	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Cd	N.D.	N.D.	-	N.D.	N.D.	N.D.	N.D	N.D.
	Eggplant				**Cucumber**			
	3a	3b	3c	3d	4a	4b	4c	4d
Pb	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0,00625	0,0393056
Cd	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Risk indices								
	Tomato				**Papper**			
	1a	1b	1c	1d	2a	2b	2c	2d
Pb	-	-	-	-	-	-	-	-
Cd	-	-	0,238	-	-	-	-	-
	Eggplant				**Cucumber**			
	3a	3b	3c	3d	4a	4b	4c	4d
Pb	-	-	-	-	-	-	0,06	0,377
Cd	-	-	-	-	-	-	-	-

*N.D.-not detected

The risk index was determined in tomatoes and cucumbers from location S, while for other plants the risk index was not determined because the concentration of Pb(II) and Cd(II) in peppers, eggplants, tomatoes and cucumbers from location C was not determined. The risk index for tomato at location S with the addition of substrate was 0.238 % for Pb(II); 0.06% for Pb(II) determined in cucumber at location S with addition of substrate, and 0.377% for Pb(II) determined at location S without addition of substrate. As vegetables are often grown near roads, exposure to lead is a common occurrence, so the impact of the risk is also very high. The present lead is incorporated into the plant structure and reaches its edible parts, which is why the consequences of intake of this heavy metal are very high.

Conclusions

The analysis of the soil of localities S and C showed that it is a slightly acidic soil at location C, and a very acidic soil at location S (pH values determined in KCl). The analysis also showed that the mentioned locations are very poor in humus, and that the values of Pb(II) and Cd(II) are in accordance with the limit values determined according to the Rulebook. The analysis of the cement dust that was used as a substrate showed that it is suitable and safe for use in agriculture production.

The analysis of heavy metals in plant cultures showed the content of Cd(II) present in tomatoes at the location S in the sample with the substrate in the amount of 0.05 mg/kg, and Pb(II) in the cucumber in the location S in the sample with the substrate in the amount of 0.045 mg/kg and 0.283 mg/kg in the sample without substrate. The values found are the result of mostly lower soil pH, which can significantly affect the mobility of heavy metals, which is very important from the safety point of view. Based on the calculated values, it was determined that the degree of lead pollution (PD) is 10.5% at location C, and 9% at location S, while the degree of cadmium pollution at location C is 5%, and that the land at both sites corresponds to class I, suitable for agricultural production.

The risk index for tomatoes at location S with the addition of substrate was 0.238 % for Pb(II); 0.06% for Pb(II) determined in cucumber at location S with addition of substrate, and 0.377% for Pb(II) determined at location S without addition of substrate. As vegetables are often grown near roads, exposure to lead is a common occurrence, so the impact of the risk is also very high.

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