Chapter

Effect of the Degree of Soil Contamination with Cd, Zn, Cu i Zn on Its Content in the Forder Crops and Mobility in the Soil Profile

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Abstract

In the micro plot experiment, the effect of varying light and medium soil contamination with heavy metals on their content in rye green fodder, serradella forage and barley, and the migration of Cd, Pb, Cu, and Zn in the soil profile was evaluated. Plants accumulated more heavy metals on light soil and soil with low organic matter content. Under the influence of increasing heavy metal contamination of soils, cadmium content increased more in fodder plants than lead content. After 14 years from the introduction of different rates of metals into the top layer (0–30 cm) of the two soils studied, a relatively large movement of heavy metals in the soil profile occurred. The amount of leached metals depends mainly on the rate of a given element. The more contaminated the soil, the heavier the metals that leached to lower genetic levels of soil. An increase in mobility was obtained alongside an increase in soil contamination with the heavy metals studied. By analyzing the mobility coefficients, they can be ranked in the following decreasing sequence: on light soils: Cd > Cu > Zn > Pb and on medium soils: Cd > Zn > Pb > Cu.

Keywords: fodder plants, pollution level, heavy metals, organic carbon, bioaccumulation index

1. Introduction

One of the major problems of our time is heavy metal contamination of soils associated with the progressive development of civilization [1]. At the same time, more and more emphasis is being placed on the quality of crops for consumption, fodder, and processing. Therefore, from the point of view of modern agriculture, the yield of fodder crops must be correlated with their quality. The content of heavy metals in soils is of great interest to ecologists, biologists, agricultural producers, and environmentalists alike [2]. This issue becomes particularly important under conditions of heavy metal contamination of cultivated soils [3]. In the absence of practical ways to permanently remove excess heavy metals from soils, efforts are made to bind them to the soil in such a way that the possibility of uptake by plants is reduced to a minimum [4]. One way of counteracting the negative effects of heavy metal contamination of soils and plants is the application of organic matter in the form of organic fertilizers of various types, for example, manure, peat, and composts [5]. On the other hand, in the case of moderate contamination, it is proposed to introduce agrotechnical measures to reduce the bioavailability of metals from the soil and their content in plants to a level that does not endanger animal and human health [6] (soil liming).

The natural environment (soils, waters, and plants) is usually contaminated with not one, but several heavy metals. Therefore, the Department of Agricultural Chemistry of the Warsaw University of Life Sciences has undertaken research to assess the impact of the degree of soil contamination with presumably contaminating elements in content in the forder crops. An additional objective of these studies is to assess the impact of various factors on the mobility of individual metals in the soil by determining the mobility coefficients in the conditions of crop management techniques, balanced mineral and organic fertilization, and soil liming. For this purpose, in 1987, a unique microplot experiment was established on soils artificially contaminated with copper, zinc, lead, and cadmium oxides (up to the pollution level of classes I, II, and III, according to the regulation of the Minister of the Environment) [7]. The soils were diversified in terms of pH (through liming), organic matter content (through the addition of brown coal), and the grain size composition of the humus level (Ap) (strong clay sand and light silt clay).

A research facility was used for the study, where the soils were contaminated simultaneously with several heavy metals, that is, Cu, Zn, Pb, and Cd, with contamination of each of these metals occurring in classes 0, I, II, and III. The soils were characterized by different pH, humus, and fluffy content.

The paper assesses the influence of artificial contamination of soils with heavy metals of light and medium soils on the movement of heavy metals deep into the soil profile, depending on selected physical and chemical properties and content thereof in fodder plants.

Four metals were selected for the study, that is, Cu, Zn, Pb, and Cd included in the group of metals with a very high risk for the environment [8] and forage. In terms of threat to feed quality, Cd and Pb are the most dangerous, while the toxic ones include Cu and Zn [9].

2. Heavy metal content of soils and plants

2.1 Sources and status of heavy metal contamination of soils in Poland

Heavy metals are generally referred to those metals, which possess a specific density of more than 5 g/cm^3 and adversely affect the environment and living organisms [10]. These metals are quintessential in maintaining various biochemical and physiological functions in living organisms when in very low concentrations; however, they become noxious when they exceed certain threshold concentrations. Zinc and copper are elements essential for life and development of organisms, while cadmium and lead have no specific physiological function and are even harmful to plant development and human health [11]. Under conditions unaltered by man, the natural content of heavy metals in soils depends primarily on the abundance of the bedrock, on weathering and soil-forming processes, and on the granulometric composition [12].

The natural source of heavy metals in soils is the parent rock from which the soils were formed. In Poland, 80% of arable land has a natural and 17.6% elevated heavy metal content. Consequently, only 3% of Poland's arable land is described as contaminated [13, 14]. Weakly and moderately contaminated soils account for 2.7%, while higher and very highly contaminated soils occupy 0.27% and 0.08% of the agricultural area, respectively [15]. Agricultural pollution results from the inappropriate use of soil conditioners produced from sewage sludge. Therefore, the agricultural use of municipal wastewater and industrial sludge for fertilization and liming of soils must be under constant control of the quantity and quality of harmful components introduced. Soil contamination can be localized or diffused [16]. Soil contamination of a local nature affects small areas, and their occurrence is most often associated with intensive industrial activities, inadequate waste storage and treatment, and the extraction and processing of minerals (e.g., metal ores) [11]. Threats from pollution of a point source nature mainly concern industrial and post-industrial, urbanized areas and areas near transport routes and landfills [17].

2.2 Heavy metal toxicity to plants

The toxicity of heavy metals is due to their biochemical role in metabolic processes and the extent to which they are absorbed and excreted by living organisms [18, 19]. The detrimental effect of heavy metals on living organisms is to induce enzyme dysfunction. Heavy metals can block enzyme systems and induce physiological changes that lead to cell and tissue death. In plants, damage to the root system occurs, followed by stunted growth. Depending on the sensitivity of the plants to heavy metals, chlorosis and necrosis appear on the leaves, which are not always characteristic of individual heavy metals. Plants readily take up heavy metals from the soil. An excess of trace elements in the plant causes metabolic disturbances. This usually reduces yield quality, plant germination, and delays emergence. Excessive amount of heavy metals reduces the intensity of photosynthesis, inhibits plant growth, and, above all, causes underdevelopment and distortion of roots [20]. Low concentrations of toxic elements do not adversely affect plant yield. However, at excessive levels, heavy metals have a toxic effect, accumulating in the tissues, retarding plant growth and development, resulting in the plant yielding less or dying completely. Heavy metals damage plants, deform and contribute to weakening the plants' resistance to diseases and pests. The assimilative surface area is reduced due to numerous necrotic spots as a result of the harmful effects of heavy metals, which consequently reduces the efficiency of photosynthesis [21]. When the tolerance limit of heavy metal content in fodder and consumer plants is exceeded, symptoms of poisoning can be expected in animals and humans [22, 23].

The phytotoxicity of heavy metals is largely genetically determined, which is why there are strong species and cultivar variations among plants [24]. This is related, among other things, to the unequal action of resistance mechanisms in individual species and even varieties. One such mechanism in plants tolerant to excess lead is a reduction in the movement of lead from the roots to the aboveground parts. Lead in such plants is stored in the cell walls of the roots as hardly soluble inorganic compounds [25, 26]. Another important mechanism concerning cadmium and copper involves the binding of these elements by a specific plant protein, phytochelatin [27]. It is generally believed that dicotyledonous plants are more susceptible to the harmful effects of heavy metals compared to monocotyledonous plants [28]. Considering the way, the plants are used, it is found that vegetables are less resistant to the toxic effects of heavy metals, compared to agricultural plants [29, 30].

Heavy metals have the ability to move through the trophic chain and, once the tolerance limit is exceeded in the case of fodder or food crops, increased incidence of chronic or acute disease in animals and humans is to be expected. In the environment, heavy metals are present in plants in trace amounts of less than 0.1% d.m. Despite such small amounts, their excessive accumulation can lead to contamination of the entire ecosystem. Kabata-Pendias [28, 31] distinguished four groups of trace elements in terms of their potential threat to the environment. Thus, cadmium, lead, zinc, and copper are categorized as being of very high risk, while nickel belongs to the group of medium environmental risk [32]. In terms of threat to feed quality, Cd and Pb are the most hazardous, while Cu and Zn are among the toxic ones [9].

2.3 Forms and mobility of heavy metals in soil

Heavy metals enter the soil mainly as insoluble compounds [33]. They then undergo different transformations in the soil depending on their physical and chemical properties. The behavior of heavy metals is governed by two opposing groups of phenomena. The first involves processes that reduce the ability of the metals in question to migrate. These obviously include sorption processes, precipitation of insoluble compounds from solution, and biogenic accumulation. This results in the accumulation of heavy metals in the topsoil horizons. The second group is the processes that increase the mobility of heavy metals: desorption, solubility, and decomposition of organic compounds [34, 35].

The solubility of heavy metals in the soil solution, on the one hand, and accumulation in the soil, on the other hand, are mainly influenced by soil properties such as pH, granulometric composition, sorption complex capacity, organic matter content and type, and oxidation-reduction conditions [35].

As the pH value increases, the ability of the soil to bind metal ions into forms that are difficult to dissolve increases. At low pH, all heavy metals, except molybdenum, are available to plants. Therefore, their phytoavailability poses great problems in acid and light soils, which constitute more than 60% of Polish soils [36]. On the other hand, according to Czarnowska and Szymanowska-Sieńczewska [37], the amount of heavy metals present in soluble forms depends primarily on their total content in soils. According to Stępień et al. [38, 39], the cadmium content of soils is significantly influenced by the organic matter content. These authors argue that organic matter currently acts as a buffer in nature for pollutants of anthropogenic origin. The buffering capacity of soil toward these pollutants is mainly based on their immobilization in insoluble compounds and their complete or partial detoxification.

The strong sorption properties of the soil, especially of the organic matter it contains, meaning that heavy metals are largely retained in the topsoil humus layer. The danger that a metal poses to the environment depends not only on the amount in which it is introduced into the soil, but above all, on the chemical form in which it occurs [40].

Heavy metals are found in soils in different forms, which determine their solubility and bioavailability [41] state that, for agricultural purposes, five fractions in which heavy metals occur in soils can be separated:

Exchangeable fraction: These are metals that are weakly bound to the soil solids and readily released into the soil solution.
Carbonate fraction: It includes metals bound to carbonates, readily passing into soil solution at low soil pH.
Iron and manganese oxide fraction: It forms the binder or coating, covering the mineral parts of the soil. Metals in this group are sensitive to changes in redox potential.
Organic fraction: Metals bound to soil organic matter and released gradually during mineralization, which, when spread out over time, results in the periodic retention of heavy metals in forms unavailable to plants.
Another fraction is the *residue fraction*: These are metals associated with silicate minerals and are found in stable compounds.

The authors consider the exchangeable and carbonate fraction to be easily soluble and available to plants. Heavy metals in water-soluble and exchangeable form should be considered as easily mobile in the soil available to plants, while the others are taken up to a lesser extent. Carbonates under suitable conditions (low pH) and oxides (low redox potential) can supplement the soluble and exchangeable form [42]. Under conditions favoring oxidation, metals bound to organic matter can also become available to plants. The active form remains in a state of very rapidly establishing equilibrium with the mobile form and, in most chemical tests, all of the active forms and some parts of the mobile form are determined.

Considering ecological and agronomic aspects, attention should be paid mainly to reactive (readily soluble, highly active, and directly available to plants) or potentially mobile forms of heavy metals (potentially available to plants, active, and leached), which are extracted from the soil by means of weakly inert salts or with chelating solutions [43].

The solubility of cadmium in soil solution, compared to other metals, is high [21, 24]. Its mobility is largely related to the source of soil contamination. Thus, in soils formed from cadmium-rich rocks, the mobility of this element is lower than in soils contaminated with it due to industrial activities [45, 46]. In light soils with elevated cadmium content, the proportion of its exchangeable forms may exceed 50% of the total content [47]. Gorlach and Gambuś [48] emphasize that the solubility of cadmium in soil solution and the limitation of its uptake by plants is strongly influenced by soil organic matter. In contrast, according to Ristovic [49, 50], the activation of soluble forms of cadmium is mainly related to the decrease in soil pH. Under conditions of higher pH, the solubility of chemical combinations of cadmium decreases and the adsorption of this element on soil colloids increases [51]. In soils with a pH of 4.5–5.5, cadmium is highly mobile, and at higher values, it becomes immobilized, forming carbonates [52]. Under the influence of the introduction of municipal waste into soils, cadmium becomes mobilized.

Lead, unlike cadmium, is not very mobile in soil and only a small part of it is soluble in mild solvents [53]. This element is rarely present in soil in the form of Pb cation^{+2}, but it forms complex ions $PbOH^+$ and $Pb(OH)_4^{2-}$, which significantly regulate sorption and desorption processes. It is strongly bound by most soil components, especially by Fe and Mn concretions. It is also sorbed by clay minerals, aluminum and iron hydroxides and by organic matter [28]. Weber [54] emphasizes that, in addition to soil type and organic matter content, the solubility of lead is also influenced by soil pH. Increased calcium content promotes higher soil pH and reduces the uptake of lead by plants. Lead is poorly mobile in contaminated soils, especially light soils, but the proportion of soluble and phyto-available forms is high [55]. The significant amount of this metal in the exchangeable fraction indicates the danger of lead desorption and mobilization from the soil. In acidic soils, on the other hand, lead forms predominate in organic compounds, which can both increase and decrease its migration. Higher solubility of lead occurs in light acidic soil than in less acidic clay soil. The mobile forms of lead in acidic soils occur mainly as Pb cations^{2+}, $PbHCO_3^+$, and organic complexes. In alkaline soils, on the other hand, the predominant forms are: $PbOH^+$ and $Pb(CO_3)_2^{2-}$.

Zinc in the soil environment forms complex ions, for example, $ZnOH^+$, ZnO_2^-. All zinc compounds dissolve under the influence of weathering processes (easily soluble form most accessible to plants), at low soil pH, while the released ions form mineral combinations with high mobility. Organic matter sorbs zinc under certain acidic conditions in the soil; at pH 5.8, zinc is bound by 60% humic acids; at $pH < 5.8$, sorption disappears. The resulting bonds are persistent and, as a result, a fairly significant amount of zinc is observed in the surface horizons of organic and mineral soils. Zinc is one of the most mobile trace elements [56]. This is influenced by both its exchangeable forms and its compounds with organic matter. Organic matter forms fairly stable bonds with zinc, and therefore, there is an accumulation of zinc in the surface horizons of mineral soils and in organic soils. The addition of municipal waste or various zinc compounds to the soil increases the mobile forms of this element. An increase in soil pH decreases its desorption (solubility) in the soil. However, the formation of complex anions and organic-mineral combinations can maintain its high mobility also in alkaline soils. Zinc concentrations in natural soil solutions range from 60 to 2200 g/l, with the highest values in light acidic soils.

Copper in the soil is not very mobile, so its loss by leaching is generally low at approx. 30 g, year/ha. In soils, copper is strongly bound by clay minerals and organic matter, and is also lost as sulfides, sulfates, carbonates, giving poorly mobile forms [57]. Therefore, only a small proportion of copper occurs in soils in mobile forms—in soluble and exchangeable forms. Organic matter plays the main role in binding copper, but microorganisms also play an important role in binding this element. The bioavailability of copper bound to an organic substance depends on the fines weight. Thus, organic compounds with a low weight, which are released during the decomposition of organic matter or are introduced together with municipal waste increase the mobility of copper. In contrast, peat organic matter, as well as humic acids, immobilize copper, more or less permanently.

The content of soluble forms of copper in the topsoil of our soils is 1–5 ppm, but varies from 1–30 ppm, representing a few to several percent of its total content [58]. The bioavailable copper content depends on the amount and type of organic matter. There is less plant-available copper in organic soils because it is strongly bound there. All soil minerals show a high copper binding capacity, which is dependent on the environmental pH.

In an acidic environment there is a high mobility of cationic forms of copper—Cu^{+2}, $CuOH^+$, $Cu_2(OH)_2^{2+}$, and as pH increases, mobile anionic forms prevail—$Cu(OH_3)_2^{2-}$, $Cu(OH)_{3-}$, $Cu(OH)_4^{2-}$ [51]. Copper is subject to constant accumulation in the surface horizons, is strongly bound there and is not subject to displacement deep into the profile. The phytoprecipitation of copper can be reduced with lime and organic matter (peat).

2.4 Factors affecting heavy metal content in plants

Industrial pollution causes the introduction of many heavy metals into soils, which combine with the sorption complex or pass into the soil solution. Dynamic equilibrium states are established in the sorption complex, changing under the influence of external factors, that is, mineral fertilization, precipitation, change in pH [59]. In turn, the uptake of heavy metals by plants from soils is a function of many factors, both biological (plant properties) and environmental (soil properties, climatic factors), [28]. On the other hand, the different biochemical properties of elements determine their susceptibility to bioaccumulation (uptake by plants). Thus, under similar soil conditions, cadmium and zinc are more intensively taken up by plants than manganese, lead or copper. The factors considered to have the most significant influence on the plant uptake of heavy metals are the total content of potentially bioavailable elements in the soil, the concentration of the elements concerned in the soil solution and their relative quantitative proportions, and the flow of elements from the solid to the liquid phase of the soil and then to the roots. As a result of the above-mentioned factors, nickel and lead are considered to have low mobility and therefore low bioavailability, while cadmium and zinc are considered to have high mobility. Through biological selection processes of chemical elements, plants can regulate, to a certain extent, their chemical composition. However, the action of physiological barriers is largely limited with respect to heavy metals and is also subject to breakdown under the influence of their excessive concentration [60]. Therefore, the content of heavy metals in terrestrial plants is generally positively correlated with their occurrence in soils, as well as in lower-lying bedrock, which is used for geochemical prospecting studies.

Under natural conditions, there is a great variation among plant species and varieties in the intensity and selectivity of trace element uptake. Species differences are large even among vegetation occurring in the same habitat. Metal concentrations also vary according to the specific plant part and developmental stage. Plants take up heavy metals, not only through the root system from the soil, but also through the leaves and other above-ground parts, on which compounds of these elements fall in the form of dissolved rainwater or dust. An example of this is lead in cereal plants (in the above-ground parts and even the grain), which is approximately 90% derived from atmospheric pollution fallout. Therefore, determining the source of excessive trace elements in plants as well as in the trophic chain is not easy and generally requires detailed research [28].

Plants are an important link in the movement of metals from the soil to animal and human organisms. For concentrations of metals tolerated by plants but harmful to humans and animals, for example, cadmium, zinc, and lead, the action of the biological barrier of selective uptake of elements by plants is not effective [61]. Trace element mobilization processes in soils are of particular importance in cases of soil contamination by these elements. In general, elements of anthropogenic origin are found in more active forms, and the degree of their immobilization depends on the properties of the soils, especially the reaction and granulometric composition [51]. General regularities are known that determine the degree of harmfulness of heavy metal contamination, depending on soil properties. There is always a significantly higher bioavailability of metals from very light acid soils compared to their bioavailability from heavy soils with neutral pH [62]. Calcium carbonate might act as a strong absorbent for heavy metals and could be complex [63]. The effect of soil type on metal mobility is similarly influenced, and therefore light acidic soils, which have very low buffering and filtering properties, have an increased risk of groundwater and groundwater contamination. Heavy soils, on the other hand, sometimes referred to as “resistant” soils to chemical contamination, can accumulate and immobilize significant amounts of certain elements. Metal fixation in heavy soils is intermittent, as any change in the conditions of the soil environment, for example, increase in acidity and changes in the quality or quantity of organic matter and in the redox potential, can affect their mobilization and therefore easy bioavailability [64].

Various factors influence the bioavailability of heavy metals in plants, but the most important of these is the quantitative content of these elements (hence the establishment of limit numbers to determine the amount that is safe for humans, animals, and the environment) and the physicochemical properties of the soil, which determine the form in which the metals in question are present and therefore their mobility. Research has shown that the three most important soil properties in this respect are the granulometric composition, pH, and humus content of the soil. These properties, together with the metal content of the soil, determine which contamination class the soil belongs to. The granulometric composition of the soil (content of clayey parts) not only determines the content and mobility of trace elements but also shapes the sorption capacity of the soil (restriction of mobility of macro and microelements) [15]. The heavy metal binding capacity of aluminum, iron, and manganese oxides. The heavy metal binding capacity of the above-mentioned oxides is influenced by: their quantitative and qualitative content and the soil pH. An acidic soil pH reduces the sorption capacity of aluminum, iron, and manganese oxides. Lead combines most easily with the oxides in question, while manganese oxides have the highest sorption capacity [47]. Also, the presence of clay minerals in the soil limits the availability of heavy metals to plants. As the humus and clay mineral content increases, the bioavailability and solubility of heavy metals decrease. This relationship is particularly evident in acidic soils. This is particularly true for lead, which is most highly bound to clay minerals. This is followed by copper, zinc, and cadmium [65]. A large amount of plant-available zinc can be immobilized by introducing sufficient montmorillonite into the soil [66]. The same clay mineral can be used for cadmium, lead, and copper. In addition to montmorillonite, soils can be treated for heavy metals using zeolites and bentonites, both synthetic and mineral [67].

An important factor limiting the availability of toxic elements is the soil pH. Heavy metals dissolve more readily in acidic soil and are therefore better available to plants. However, they differ in this respect [62]. The best example is zinc and cadmium, for which a drop in $\text{pH} < 6.0$ results in increased activity, which is not observed to such a great extent for lead and copper. Therefore, liming is an essential treatment, especially for reducing the availability of zinc and cadmium to plants. Czuba et al. [68] report, “the effect of pH on the uptake of heavy metals depends on the plant species and the metal.” This statement was confirmed in an experiment in which the effect of pH on the heavy metal content of aboveground parts of sunflower and maize was studied. The most significant differences were seen when examining the phytoavailability of zinc: in sunflower, (with a decrease in $\text{pH} < 6.5$, the zinc content in the above-ground parts increased significantly: at $\text{pH} = 3.0$, it was about 800–900 mg/kg d.m., at $\text{pH} = 6.5$, it was about 80–90 mg/kg d.m.), in maize, the difference was not so significant, (zinc content in the above-ground parts, at $\text{pH} = 3.0$, was about 150–200 mg/kg d.m.; and at $\text{pH} = 6.5$, about 70–80 mg/kg d.m.). Similar differences were observed for other metals, that is, lead, copper, cadmium, and nickel [68].

The natural occurrence of humus compounds in the soil and organic compounds, that is, humus or compost, also reduce the degree of heavy metal contamination of plants. For this reason, among others, a common cultivation procedure used by farmers is to fertilize the soil with peat, compost, or manure. The better the organic matter is decomposed, the greater the effectiveness of the treatments. This is because humus binds heavy metals into stable complexes [69].

3. Materials and methods

3.1 Study area location

The experiment was carried out on microplots at the experimental station in Skierniewice (Poland) (Lat:51°58′ N; Long:20°10′ E), which belongs to the Faculty of Agriculture and Biology at the Warsaw University of Life Sciences under natural climatic conditions. The climate at the site is temperate with a mean annual rainfall for the years 1921–2020 at 536.1 mm, a mean annual temperature 8.1°C, and insolation at 1714 h. This climate is considered as Dfb, according to the Köppen-Geiger climate classification (warm humid continental climate).

The experimental factors in the experiment were: two types of soil (light soil-loamy sand with 17% of particles <0.02 mm and medium soil-light dusty clay with 25% of particles <0.02 mm), three levels of Corg concentration (6, 9, and 12 g $C{\cdot}kg^{-1}$ of the soil), four levels of heavy metal contamination of soils Zn, Pb, Cd, and Cu (natural content—0, increased content—I, weak contamination—II, medium contamination—III).

The microplots were supplied with vitrified clay pipes with a diameter of 0.4 m and a height of 1.2 m high. They were buried vertically in the soil, which in 1987, were filled with layers of Albic Luvisol soil [70]: (1) ochric (Ap) 0–30 cm (the surface horizon that has been plowed or cultivated)—17% of particles <0.02 mm; (2) luvic (Et) 30–50 cm (the subsurface horizon of eluvial loss of silt and clay)—13% of particles <0.02 mm; (3) agric (Bt) below 50 cm (the subsurface horizon of illuvial accumulation of clay and humic substances)—25% of particles <0.02 mm.

In 2004, the arable soil layer was removed from each pot to a depth of 30 cm. In half of the microplots, a soil material of strong clay sand composition was used, while in the second half of light dusty clay. Appropriate amounts of calcium carbonate, lignite, and heavy metals (Cd, Cu, Zn, and Pb) were added to the 0–30 cm layer. As a result, soils representing four levels of contamination with four abovementioned heavy metals were obtained. Single doses of individual heavy metals per microplot (56 kg of soil) were: zinc—3.2 g in the form of ZnO, lead—28 g in the form of PbO, copper—1.3 g in the form of CuO, and cadmium—35 mg in the form of $CdSO_4$–H_2O. In this way, research objects with different acidification levels (pH 4.2, 4.9, and 5.9) were created, three levels of organic carbon (approx. 6, 9, and 12 $g{\bullet}kg^{-1}$), two levels of floating parts (17% and 25%), and four levels of soil contamination with heavy metals [natural content (0), elevated (I), polluted in the 1st degree (II), polluted in the 2nd degree (III)]. The classification mentioned above enables us to assess the extent of soil contamination with heavy metals taking their bioavailability into account. The experiment consisted of 216 ground microplots. In the experiment, different plant species were cultivated in the following years of the experiment (cereals, maize, rapeseed, mustard seed, and coarse-seeded legumes).

3.2 Collection and processing of soils

Soil samples were taken for testing in 2018 after the harvest of spring barley for grains. Soil samples were collected at the end of the growing season (September–October) from 0 to 25 cm soil layer. Next, the soil was prepared for analysis in accordance with the ISO11465 standard. The soil material was determined for: organic C by direct method PN-EN 15936, pH in the suspension KCl ($mol{\cdot}d.m.^{-3}$) PN-EN 10390, heavy metals were determined in three levels (top layer Ap, Et, Bt) in the extract of 1 M HCl, while in level Ap 3 with methods: total (in royal water acc. to PN-ISO 11466) and in 1 mol·d.m.$^{-3}$ HCl [71]. The content of cations in the solution was determined by the AAS method. In order to validate the method for accuracy and precision, certified reference material was used.

The movement of heavy metals in the soil profile was determined on the basis of the mobility coefficient CM (increase in heavy metal in the Bt and Et layer compared to the control). The obtained results were analyzed statistically by means of variance analysis (using Tukey's test) and single-factor regression. Statistical calculations were performed using the program statgraphics plus). Results are the means from three replications.

3.3 Plant material analyses

- Total heavy metal content of Zn, Pb, Cd, and Cu by atomic absorption spectrometry. The plant samples were combusted in a mixture of acids HNO_3, $HClO_4$, and H_2SO_4 in a volume ratio of 20:5:1 using the microwave technique in a closed system.

- The following indices were calculated for the forage crops studied [72]:

- The tolerance index (Ti), which indicates the degree of growth inhibition of the test plant when grown on contaminated substrate according to the formula:

- Ti = plant d.m. of plants on contaminated substrate/plant d.m. of plants on control substrate.

- A contamination index (C), which indicates the amount of contamination of a plant on a relative scale, that is, the ratio of the toxic ion in a plant growing on contaminated soil to its level in control plants growing under uncontaminated environmental conditions according to the formula:

  C = metal content of contaminated plant/metal content of control plant.

- The metal bioaccumulation index (A), which determines the increase in heavy metal taken up by the plant relative to its increase in the substrate according to the formula:

  A = increase in metal content of plant/increase in metal content of substrate.

4. Results

4.1 Influence of soil contamination levels of heavy metals on their content in forage crops

4.1.1 The content of Zn, Pb, Cd, and Cu in rye forage

On both soils, an increase in the level of heavy metal contamination of the soils resulted in an increase in the heavy metal content of the rye green fodder. The heavy metal content in rye green fodder at pollution level III was many times higher compared to sites with natural content. The degree of effect of soil contamination with heavy metals on their content in rye green fodder varied for individual metals and depended significantly on the soil species. On the light soil, as the contamination increased, the content of Cd and Pb increased the most in rye green fodder and the content of Cu increased the least. A similar relationship occurred on medium soil.

Critical heavy metal contents in feed: Cd—1, Pb—10, Cu—30, and Zn—100.

The series of changes in the increase of heavy metal content in rye forage on both light and medium soil at pollution level III takes the following form: Cd > Pb > Zn > Cu. It is noteworthy that already at level I of soil contamination with heavy metals, that is, at relatively low contamination, a significant accumulation of these metals in rye green fodder was found, especially of Cd and Zn, that is, mobile elements (**Table 1**). In rye green fodder, exceedance of the permissible heavy metal contents occurred for Zn on light soil at pollution levels I, II, and III, on medium soil at pollution levels II and III contamination levels, and for Cd on light soil at level III of soil contamination with heavy metals.

The content of Zn, Pb, Cd, and Cu in serradella.

In both light and medium soil, increasing the amount of heavy metals in the soil resulted in an increase in their content in serradella. The degree to which heavy metal contamination of the soil affected the content of heavy metals in serradella varied for individual metals and depended, as in the case of rye, on the soil species. On light soil, serradella increased the most in Zn, Cd, and Pb content, and on medium soil, Cd and Zn content increased the most. In contrast, Cu content increased least in serradella on light and medium soil. Serradella grown on light soil significantly accumulated more heavy metals compared to plants grown on medium soil (**Table 2**).

Species soils	Level pollution. Soils with metals Heavy	Heavy metal content							
		Zn		Pb		Cd		Cu	
		mg·kg^{-1} d.m.	%	mg·kg^{-1} d.m.	%	mg·kg^{-1} d.m.	%	mg·kg^{-1} d.m.	%
Light	0	45.9	100	0.30	100	0.03	100	5.2	100
	I	**105.6**	230	0.41	137	0.15	500	7.0	135
	II	**202.7**	442	1.60	533	0.50	1667	15.0	441
	III	**276.5**	602	3.20	1067	**1.10**	3667	14.8	435
	On average	157.7	—	1.40	—	0.44	—	10.5	—
Average	0	34.3	100	0.20	100	0.02	100	3.4	—
	I	72.0	210	0.30	150	0.10	500	4.7	138
	II	**112.2**	327	0.80	400	0.21	1050	8.7	256
	III	**184.6**	538	2.10	1050	0.94	4700	12.9	379
	on average	100.8	—	0.83	—	0.32	—	7.4	—

Table 1.
Heavy metal content of life for green fodder in relation to light and medium soil contamination levels.

Species soils	Level pollution and soils with metals Heavy	Heavy metal content							
		Zn		Pb		Cd		Cu	
		mg·kg^{-1} d.m.	%	mg·kg^{-1} d.m.	%	mg·kg^{-1} d.m.	%	mg·kg^{-1} d.m.	%
Light	0	**180.1**	100	3.42	100	**2.80**	100	8.9	100
	I	**199.3**	111	3.71	108	**3.60**	129	11.3	127
	II	**288.0**	160	4.64	136	**4.00**	143	12.9	145
	III	**344.9**	192	5.44	159	**4.42**	158	13.1	147
	On average	253.1	—	4.30	—	3.70	—	11.5	—
Average	0	**140.9**	100	3.00	100	**1.35**	100	7.7	100
	I	**170.5**	121	3.23	108	**2.34**	173	9.7	126
	II	**270.7**	192	3.90	130	**2.80**	207	11.1	144
	III	**317.3**	225	5.20	173	**3.72**	275	12.0	156
	On average	224.9	—	3.82	—	2.55	—	10.1	—

Table 2.
Heavy metal content of serradella in relation to light and medium soil contamination levels.

Critical heavy metal contents in feed: Cd—1, Pb—10, Cu—30, Zn—100

The series of changes in the heavy metal content of serradella on light soil at pollution level III takes the following form: Zn > Cd > Pb > -Cu, and on medium soil: Cd > Zn > Pb > Cu. In addition, already at level I of soil contamination with heavy metals, slight contamination resulted, as in the case of rye forage, also a significant accumulation of these metals in serradella, especially Cd and Cu. At contamination level I, there was no such large increase in the content of toxic elements, as was the case with rye green fodder, indicating that the increase in heavy metal content in plants depends on the plant species. Exceedance of the permissible heavy metal contents in serradella occurred for Zn and Cd on light and medium soil at all contamination levels.

The content of Zn, Pb, Cd, and Cu in barley

The increase in the content of heavy metals in barley grain that takes place under the influence of an increase in soil contamination coincides in terms of the extent of the changes to serradella and rye greens. On light soil, at soil contamination, level III heavy metals in barley grain increased the most in Cd and Pb content and the least in Cu content. In contrast, on medium soil, Cd and Pb content increased the most, while Zn content was the least (**Table 3**).

Critical heavy metal contents in feed: Cd—1, Pb—10, Cu—30, and Zn—100.

The series of changes in the heavy metal content of barley grain on light soil at pollution level III takes the following form: Cd > Pb > Zn > Cu, and on medium soil the following shape takes place: Cd > Pb > Cu > Zn. In barley straw on light soil at pollution level III, Cd and Zn content increased the most

Species soils	Level pollution. Soils with metals Heavy	Heavy metal content							
		Zn		Pb		Cd		Cu	
		mg·kg^{-1} d.m.	%	mg·kg^{-1} d.m.	%	mg·kg^{-1} d.m.	%	mg·kg^{-1} d.m.	%
Light	0	26.2	100	0.32	100	0.12	100	2.7	100
	I	46.1	176	0.44	137	0.25	208	3.6	133
	II	64.6	247	0.74	231	0.60	500	5.5	204
	III	82.2	314	1.50	469	0.90	750	7.2	267
	On average	**54.8**	—	**0.74**	—	**0.50**	—	**4.7**	—
Average	0	22.1	100	0.24	100	0.10	100	1.90	100
	I	37.2	168	0.33	137	0.15	150	2.74	144
	II	49.0	222	0.54	225	0.43	430	4.02	212
	III	70.8	320	0.85	354	0.70	700	6.20	326
	on average	**44.8**	—	**0.50**	—	**0.34**	—	**3.71**	—

Table 3.
Heavy metal content of barley grain in relation to light and medium soil contamination levels.

and Cu the least, while on medium soil, as in grain, Cd and Pb content increased the most and Cu the least. In barley straw on light soil, at pollution level III, the decreasing series of heavy metal contents takes the following form: Cd > Zn > Pb > Cu, and on medium soil the following shape: Cd > Pb > Zn > Cu (**Table 4**).

Species soils	Level pollution. Soils with metals Heavy	Heavy metal content							
		Zn		Pb		Cd		Cu	
		mg·kg^{-1} d.m.	%	mg·kg^{-1} d.m.	%	mg·kg^{-1} d.m.	%	mg·kg^{-1} d.m.	%
Light	0	30.3	100	0.53	100	0.21	100	3.1	100
	I	72.7	240	0.80	151	0.50	238	4.4	142
	II	99.9	330	1.05	198	0.85	405	6.6	213
	III	**220.0**	726	3.20	604	**1.70**	809	10.2	329
	on average	105.7	—	1.40	—	0.81	—	6.1	—
Average	0	24.6	100	0.31	100	0.13	100	2.5	100
	I	55.8	227	0.60	193	0.35	269	3.4	136
	II	80.1	326	0.81	261	0.60	461	4.9	196
	III	**160.4**	652	2.05	661	1.00	769	7.7	308
	on average	80.2	—	0.94	—	0.51	—	4.6	—

Table 4.
Heavy metal content of barley straw in relation to light and medium soil contamination levels.

Critical heavy metal contents in feed: Cd—1, Pb—10, Cu—30, and Zn—100

In barley grain, there were no exceedance of forage heavy metal limits. However, in barley straw, exceedance occurred for Zn at contamination level III, on light and medium soil, and Cd on light soil.

Average bioaccumulation indices (A).

The average value of the bioaccumulation index (5) of Zn in rye green fodder was 1.12, Cd 0.27, Pb 0.007, and Cu 0.23. The high value of the bioaccumulation index of Zn indicates a high propensity of rye to accumulate this element. In the case of serradella, the highest average bioaccumulation index values were obtained for Cd and Zn, 1.30 and 1.11, respectively, indicating a high predisposition of serradella to accumulate these elements under soil contamination conditions and for Pb and Cu, 0.009 and 0.08, respectively. Significantly lower bioaccumulation index values were obtained for rye grain: Zn 0.36, Cd 0.17, Cu 0.21, and Pb 0.004. This indicates that under soil contamination conditions, grain accumulates lower amounts of heavy metals compared to rye and serradella green fodder. Also, barley straw under conditions of soil contamination with elements accumulates lower amounts of these elements compared to rye and serradella green fodder, as evidenced by lower bioaccumulation coefficients (**Table 5**).

Level of soil pollution	Serradella	Rye green fodder	Barley straw	Barley seeds	Mean
		Zn			
I	1.1	2.2	2.3	1.7	**1.8**
II	1.7	3.8	3.2	2.3	**2.7**
b	2.0	5.7	6.9	3.1	**4.4**
		Pb			
I	1.0	1.4	1.6	1.3	**1.3**
II	1.3	4.8	2.3	2.2	**2.6**
III	1.6	10.9	6.3	4.0	**5.7**
		Cd			
I	1.5	4.8	2.5	1.8	**2.6**
II	1.7	12.5	4.1	4.7	**5.7**
III	2.1	38.6	7.7	7.4	**14.0**
		Cu			
I	1.3	1.3	1.3	1.4	**1.3**
II	1.4	2.6	2.0	2.0	**2.0**
III	1.5	3.2	3.1	3.0	**2.7**

Table 5.
Average bioaccumulation index values (A) of Zn, Cd, Pb, and Cu.

Plant	Zn	Cd	Pb	Cu
Serradella	1.11	1.30	0.009	0.08
Rye green fodder	1.12	0.27	0.007	0.23
Barley grain	0.36	0.17	0.004	0.21
Barley straw	0.74	0.42	0.009	0.07
Mean	**0.83**	**0.54**	**0.007**	**0.15**

Table 6.
Average values of the heavy metal contamination rate (C) of plants.

Average contamination rates (C).

The analysis of the degree of heavy metal contamination of plants shows that, in general, the index is higher on light soils than on medium soils under the same dose of heavy metals. At contamination level I (**Table 6**), the highest contamination degree index values were obtained for Zn in rye green fodder and barley straw, 2.2 and 2.3, respectively, and for Cd in rye green fodder 4.8 and in barley straw 2.5. In contrast, at contamination level III, the highest contamination index values for rye green fodder and barley straw were obtained for Pb and Cd.

4.2 Influence of soil species on heavy metal content of forage crops

Soil type, regardless of the level of contamination, significantly influenced the heavy metal content of forage crops. On medium soil, the heavy metal content in rye forage compared to light soil was 13 to 58% lower. The greatest reduction in content was for Cd by 58% at pollution level II and Pb by 50% at pollution level II, respectively (**Table 7**).

The heavy metal content of serradella on medium soil was 5–52% lower compared to that on light soil. The greatest reduction in content occurred, as in the case of rye forage, for Cd and Pb, by 35% at contamination level I and 16% at contamination level II, respectively (**Table 8**).

Level pollution	Species soils	Heavy metal content (mg·kg^{-1} d.m.**)*								
		Zn		Pb		Cd		Cu		Heavy metal content series
		**	%	**	%	**	%	**	%	
0	Light	45.9	100	0.30	100	0.03	100	5.2	100	Cu > Cd = Pb > Zn
	Average	34.3	75	0.20	67	0.02	67	3.4	65	
I	Light	105.6	100	0.41	100	0.15	100	7.0	100	Cu = Cd > Zn > Pb
	Average	72.0	68	0.30	73	0.10	67	4.7	67	
II	Light	202.7	100	1.60	100	0.50	100	15.0	100	Cd > Pb > Zn > Cu
	Average	112.2	55	0.80	50	0.21	42	8.70	58	
III	Light	276.5	100	3.20	100	1.10	100	14.8	100	Pb > Zn > Cd > Cu
	Average	184.6	67	2.10	66	0.94	85	12.9	87	

**For average pH and Corg.*

Table 7.
Heavy metal content of life for green fodder according to soil species at different levels of contamination.

Level Pollution	Species soils	Heavy metal content (mg·kg^{-1} d.m.**)*								
		Zn		Pb		Cd		Cu		Content series heavy metals
		**	%	**	%	**	%	**	%	
0	Light	180.1	100	3.42	100	2.80	100	8.9	100	Cd > Zn > Cu > Pb
	Average	140.9	78	3.00	88	1.35	48	7.7	86	
I	Light	199.3	100	3.71	100	3.60	100	11.3	100	Cd > Zn > Cu > Pb
	Average	170.5	85	3.23	87	2.34	65	9.7	86	
II	Light	288.0	100	4.64	100	4.00	100	12.9	100	Cd > Pb > Cu > Zn
	Average	270.7	94	3.90	84	2.80	70	11.1	86	
III	Light	344.9	100	5.44	100	4.42	100	13.1	100	Cd > Pb > Zn = Cu
	Average	317.3	92	5.20	95	3.72	84	12.0	92	

**For average pH and Corg.*

Table 8.
Heavy metal content of serradella according to soil species at different levels of contamination.

On the other hand, the average heavy metal content in barley grain was 14–40% lower compared to that on light soil, and the greatest reductions occurred in the amount of Cd, by 40% at pollution level I and Pb by 43% at pollution level III, respectively (**Table 9**).

In barley straw, the average heavy metal content on medium soil compared to light soil was lower by 19–42%, and the greatest reduction in content was for Cd and Pb at pollution level III by 41% and 36%, respectively (**Table 10**).

Conclusion.

> Soil species significantly influenced the heavy metal content of the forage crops tested. Serradella, rye forage, and barley contained significantly more heavy metals at a given level of contamination on light soil, compared to the contents found on medium soil. An increase in the content of soil runoff parts significantly reduced the content of all heavy metals tested, with Cd and Pb being the most abundant.

Level pollution	Species soils	Heavy metal content (mg·kg^{-1} d.m.**)*								
		Zn		Pb		Cd		Cu		Content series heavy metals
		**	%	**	%	**	%	**	%	
0	Light	26.2	100	0.32	100	0.12	100	2.7	100	Cu > Pb > Cd > Zn
	Average	22.1	84	0.24	75	0.10	83	1.9	70	
I	Light	46.1	100	0.44	100	0.25	100	3.60	100	Cd > Pb > Cu > Zn
	Average	37.2	81	0.33	75	0.15	60	2.74	76	
II	Light	64.6	100	0.74	100	0.60	100	5.5	100	Cd > Pb = Cu > Zn
	Average	49.0	76	0.54	73	0.43	72	4.0	73	
III	Light	82.2	100	1.50	100	0.90	100	7.2	100	Pb > Cd > Cu = Zn
	Average	70.8	86	0.85	57	0.70	78	6.2	86	

**For average pH and Corg.*

Table 9.
Heavy metal content of barley grain according to soil species at different levels of contamination.

Level of pollution	Soil types	Heavy metal content (mg·kg^{-1} d.m.**)*								
		Zn		Pb		Cd		Cu		Content series heavy metals
		**	%	**	%	**	%	**	%	
0	Light	30.3	100	0.53	100	0.21	100	3.1	100	Pb > Cd > Zn = Cu
	Average	24.6	81	0.31	58	0.13	62	2.5	81	
I	Light	72.7	100	0.80	100	0.50	100	4.4	100	Cd > Pb > Zn = Cu
	Average	55.8	77	0.60	75	0.35	70	3.4	77	
II	Light	99.9	100	1.05	100	0.85	100	6.6	100	Cd > Cu > Pb > Zn
	Average	80.1	80	0.81	77	0.60	71	4.9	74	
III	Light	220.0	100	3.20	100	1.70	100	10.2	100	Cd > Pb > Zn > Cu
	Average	160.4	73	2.05	64	1.00	59	7.7	75	

**For average pH and Corg.*

Table 10.
Heavy metal content of barley straw according to soil species at different levels of contamination.

4.3 Effect of soil contamination on heavy metal content of light and medium soil profile

4.3.1 Physicochemical characteristics of soils

The actual pH of light soils was 4.0, 4.97, and 5.96, while of medium soils, it was 4.03, 5.08, and 6.05, respectively. The actual Corg. concentration in the light soil was 6.6, 9.2, and 11.9 g–kg^{-1}, while in the medium soils, it was 6.2, 8.9, and 1.8 g–kg^{-1}, respectively.

4.3.2 The relocation of heavy metals in soil profiles

Metal contamination of the soil surface layer suggested an investigation into the migration of heavy metals in soil profiles that was necessary to determine the potential contamination of the lower soil horizons. After 14 years, from the introduction of different rates of metals into the top layer (0–30 cm) of the two soils, a relatively large movement of heavy metals in the soil profile occurred (**Table 11**). The amount of leached metals depended mainly on the rate of a given element. The more contaminated the soil was, the heavier metals leached to lower genetic levels. Contaminated soils always had a higher concentration of individual metals in Et than in Bt level. In the Et layer, the content of the metals tested determined in HCl (1 mol–d.m.$^{-3}$) was comparable to the humus level. Only at the depth below 50 cm (Bt), the content of the studied metal form was much lower than at the surface levels. It indicates that within a few years after the contamination of soils with heavy metals, the largest amounts of them moved to the subsoil layer. However, in the layer below 50 cm, significant amounts of the heavy metals studied were also obtained, which indicates the possibility of their movement into deeper soil layers.

The calculated mobility coefficients of the tested metals determined in 1 M HCl indicate a larger movement of the tested metals in lighter soils than in medium soils (**Table 6**). The highest displacement coefficients were obtained for cadmium, while the lowest was for lead. An increase in mobility was obtained alongside the increase in soil contamination with the heavy metals. By analyses of the mobility coefficients

Soil texture	Level of contamination	Cd		Cu		Zn		Pb	
		Mobility factor CM							
		Et	Bt	Et	Bt	Et	Bt	Et	Bt
Strong loamy sand	0								
	I	2.3^{a}	2.0^{a}	2.1^{a}	1.2^{a}	2.1^{a}	1.4^{a}	1.7^{a}	1.0^{a}
	II	4.6^{b}	5.0^{b}	4.0^{b}	2.7^{b}	4.4^{b}	2.7^{b}	4.0^{b}	2.5^{b}
	III	8.3^{c}	7.0^{c}	9.5^{c}	6.5^{c}	8.3^{c}	7.2^{c}	8.5^{c}	4.8^{c}
	Mean	5.1	4.7	5.2	3.5	4.9	3.8	4.7	2.8
Light silty loam	0								
	I	2.0^{a}	1.3^{a}	1.5^{a}	1.5^{a}	2.2^{a}	1.8^{a}	1.6^{a}	1.2^{a}
	II	4.7^{b}	4.3^{b}	3.2^{b}	2.1^{b}	4.4^{b}	2.3^{b}	3.2^{b}	2.0^{b}
	III	6.8^{c}	5.8^{c}	6.3^{c}	5.2^{c}	6.7^{c}	6.2^{c}	7.8^{c}	4.8^{c}
	Mean	4.5	3.8	3.6	2.9	4.4	3.4	4.2	2.7

a, b, and c—Treatments with the same letter are not significantly different ($p \leq 0.05$), Et: the subsurface horizon of eluvial loss of silt and clay, Bt: the subsurface horizon of illuvial accumulation of clay and humic substances.

Table 11.
The mobility coefficients (CM) for Cd, Cu, Zn, and Pb are determined in HCl (1 mol d.m.$^{-3}$) in Et and Bt levels in light soil depending on the degree of contamination of the soils with these elements.

(heavy metal increase in the Bt and Et layers), they can be ranked in the following decreasing sequence: on light soils: Cd > Cu > Zn > Pb and on medium soils: Cd > Zn > Pb > Cu.

5. Discussion

In the study, the heavy metal content was determined only in the parts of the plants used for animal feed. Plants contained the heaviest metals on sites at pollution level III in an acidic environment, on light soil with the lowest organic matter content. Serradella accumulated the most Zn and Cd, which is confirmed by the high values of the bioaccumulation index for these elements, 1.11 and 1.30, respectively. A similar trend occurred in the case of rye green fodder with a Zn bioaccumulation index of 1.12. The value of these indices indicates that the increase in heavy metal concentration in plants may be greater than the content in the soil. Similar results were obtained by Piesak [73, 74] and Curyło and Jasiewicz [75] for vegetables. Also, Chłopecka [76] reports that a 1-unit increment in soil Cd content resulted in a 1.2-unit increase in its content in wheat straw. Literature contains information that the ratio of Cd content in plants to its amount in the soil is between 1 and 10 [44]. In contrast, the lowest values of the bioaccumulation index for Cd and Zn were found for barley grain, 0.36 and 0.17, respectively. In all the plants studied, the lowest values of bioaccumulation indices were obtained for Pb and Cu, 0.007 and 0.15, respectively, indicating that these metals are less well taken up by plants than Cd and Zn. This is confirmed by the research of Alloway and Ayres [44], who report that the value of the bioaccumulation index for Pb does not exceed 0.1. This is also consistent with the thesis that the concentration of heavy metals in the soil depends on the dynamics of the movement of metals from the roots to other plant organs, where accumulation follows the following sequence: grain (seed) < leaf < stem < root, and on the mobility of the metal and its chemical properties. Consequently, lead is immobilized easily in the roots [77]. The metal is retained in cell membranes and easily binds to proteins and fats [78]. According to Horubala [79], the highest concentration of lead in the potato is found in the skin, with a slightly lower concentration in the flesh. The low permeability of Pb from the soil to the plant is related to the strong sorption of this metal in the soil [80]. Wallace and Romney [77] report that zinc accumulates mainly in aboveground parts, as it can move very easily into stems, leaves, and even seeds. A similar trend occurred in our study of serradella and rye greens, with serradella accumulating higher amounts of Zn.

According to Strączyński [81, 82], the distribution and content of copper in plants depend not only on the degree of soil contamination with this element but also on the species and parts of the plant. In his studies, the concentration of copper in generative organs is generally lower (except for cereals and oilseed rape) than the amount accumulated in vegetative parts, and soil contamination with copper significantly differentiated the content of this element in crop plants. As soil quality deteriorates, the copper content in plants increases. Maximum concentrations of this metal in plants were found on sites with severe soil contamination. The highest accumulation is found in hemp leaves, stalks, potato stalks, and flax bags. In contrast, Kucharski et al. [83] report that Cu, at doses of 0 to 80 mg/kg soil, has no toxic effect on faba bean, and the growth of this plant is only inhibited by a dose of 100 mg Cu per kg soil. In our study, Cu content in plants increased with increasing soil contamination, as evidenced by high correlation coefficients (0.70 for serradella, 0.85 for barley straw, 0.68 for barley grain, and 0.77 for rye green fodder), but no exceedances of the permissible Cu content were found in plants. Most likely, significant amounts of it were accumulated by the roots. According to Kabaty-Pendias [28], plant roots retain significant amounts of Cu, both in excess and deficiency. In addition, compared to other elements, it is not very mobile in plants. The low uptake of Cu by plants could also be due to antagonisms between Cu and Zn and Ca. Increased concentrations of these elements in the plant habitat reduce Cu uptake. This is due to their chemical properties because Zn can occupy the same positions as Cu in organic carriers and substitute each other [21]. Also, Chaudhry et al. [84] report that high doses of zinc can cause copper deficiency in plants. The antagonistic effect of Zn with respect to Cu is generally coupled to Cd. Excessively high doses of Cd result in lower uptake of Cu by plants, due to its property of substituting Cu for Cd in enzymes and proteins. In contrast, Traynor and Knezek [85] report that an increase in soil Cd content results in increased uptake of Cu and Fe by plants. On the other hand, studies by Lagerwerff [86] indicate that Cu reduces Cd uptake, while Pb increases its concentration in plants. When considering the Zn and Cd content of the plants studied, it was found that rye greens and serradella accumulated particularly high levels of heavy metals. This is consistent with the hypothesis that plants during their slow growth period, that is, early spring and autumn, accumulate large amounts of cadmium and lead [87, 88]. Also, studies carried out by IUNG showed that the Cd and Pb content in cereals, legumes, and grasses harvested in spring exceeded the threshold levels proposed by IUNG [13, 14] for feed purposes. Also, research carried out in the area of Huta, Częstochowa, showed that buckwheat harvested in spring contained more Zn compared to the content found in buckwheat harvested in autumn. Buckwheat in spring accumulated particularly high amounts of Cd (6.6 mg/kg d.m.) on light and acid soils. Serradella reacted similarly in our study to even low levels of heavy metal contamination in soils. It contained high amounts of Zn and Cd even on metal-free soil, which may be due to its species' predisposition to accumulate heavy metals, as shown, for example, by spinach or lettuce. Similar relationships were shown in a study by Gębski et al. [89], which determined the effect of soil contamination with heavy metals on their content in radish, wheat grain and straw, Chinese cabbage, and potatoes. It was found that the average increase in heavy metals in cabbage as soil contamination increased was greater than in the other plants. In this crop, an increase in soil metal content increases lead and cadmium the most and copper the least. Warda [90] also notes the relationship between plant species and cadmium and nickel content in pasture plants. She reports that timothy growing on black soil accumulates only minimal amounts of cadmium, while cocksfoot, under the same conditions, contains almost four times as much of this element. Also, white clover, which makes up a significant proportion of the pasture sward, accumulates small amounts of cadmium, while common dandelion accumulates more than twice as much cadmium as common cocksfoot and perennial ryegrass growing on organic soil. The increased uptake of cadmium by common dandelion under conditions of low concentrations of this metal in the soil may therefore indicate a danger of its inclusion in the trophic chain as its concentration in the soil increases. Therefore, when selecting plant species for grazing mixtures, the predisposition of individual plant species to uptake heavy metals must be taken into account.

In the literature, statements can be found that cadmium and zinc are more phytotoxic, while lead causes less harm [38, 39, 91]. Our own research did not confirm, unequivocally, which metal was the most toxic, as plants were affected by a total of four heavy metals.

In our study, the Pb content in plants increased with increasing levels of contamination, but, as in the case of Cu, there were no exceedances of its permissible content in forage. Larger amounts of Pb were found in rye and serradella greens and the lowest in barley grain.

Piotrowska and Kabata-Pendias [92, 93] and Kabata-Pendias and Pendias [94] report that lead is a low-mobility element, and under conditions of contamination, it increases mainly in the roots and to a lesser extent in the aboveground parts. A study by Gębski et al. [89] showed that in radish roots, zinc, and lead content increased the most and copper the least. Similarly, it was the case with potatoes, but lead and cadmium content increased the most and Cu content the least. In wheat grain, on the other hand, zinc and copper content increased the most and lead content the least. This is due to the fact that zinc and cadmium are among the mobile elements that accumulate most rapidly in the plant. It is generally believed that dicotyledonous plants are more susceptible to the harmful effects of heavy metals compared to monocotyledonous plants [31, 95]. On the other hand, many authors [29, 30] report that, from the point of view of plant use, vegetables are found to be more sensitive to the toxic effects of heavy metals than agriculturally used plants.

According to some scientists [96], the phytotoxicity of heavy metals is largely genetically determined, which is why there are strong species and cultivar variations among plants. This is related, among other things, to the non-uniform action of resistance mechanisms in individual species and cultivars. In lead-tolerant plants, one such mechanism is to reduce the movement of lead from the roots to the aboveground parts. Lead in such plants is stored in the cell walls of the roots in the form of organic compounds that are difficult to dissolve [25]. In the literature [97], attention is drawn to the antagonism between cadmium and zinc, which is usually expressed as a decreased uptake of cadmium by plants when the amount of zinc in the environment is increased, and a decrease in zinc concentration in plants that take up a lot of cadmium. The phenomenon of parallel uptake of both elements under conditions of high accumulation in the soil is also known. This relationship occurred in our study in the case of serradella, on sites where the soil contained higher amounts of zinc and cadmium, respectively (pollution levels I. and II. and at "0" pollution level, in an acidic environment, with low soil organic matter content). Cadmium concentration in plants, according to many researchers, is a characteristic of species and even their cultivated varieties [97, 98]. Some species can show considerable tolerance to the presence of cadmium in the habitat and are able to take up large amounts of this component. Grasses vary in their ability to take up cadmium. Considering the concentration of this element in dry matter, they can be ranked as follows: Festuca pratensis >Alopecurus pratensis > Festuca arundinacea > Lolium perenne > Dactylis glomerata > Phleum pretense. In all grass species tested, there was an inhibition of growth and development with an increase in cadmium uptake.

When considering the content of tested elements in plants, it should be noted that the content of Zn and Cd in serradella grown on light and medium soil on all combinations including those with natural heavy metal content exceeded the values limiting their use for fodder according to the Regulation of the Minister of Agriculture and Rural Development of 23 January 2007 [99]. Also, too high concentration of Zn in green fodder on light soil at I, II, and III levels of contamination, on medium soil at II and III levels of contamination, and Cd on light soil at III level of contamination disqualifies it for fodder. In barley straw, on the other hand, exceedances of standards occurred only for Zn on combinations at pollution level III on light and medium soil and Cd on light soil at pollution level III. Of the plants tested, only barley grain can be used for animal feed without reservation.

Soil species, regardless of the level of heavy metal contamination, significantly influenced their content in forage crops. On medium soil, the heavy metal content in fodder plants compared to light soil was 13–58% lower in rye forage, 5–52% lower in serradella, 14–40% lower in barley grain and 19–42% lower in barley straw. On medium soil, the Cd and Pb contents decreased the most in plants compared to light soil. This is mainly due to the sorptive properties of medium soil, which immobilizes heavy metals, acting as a barrier preventing plants from being poisoned by them, and it confirms the thesis put forward by Niemyska-Łukaszuk [100] that as the content of floating parts in the soil increases, the mobility of heavy metals decreases, so plants do not take up harmful elements. In soils with a higher content of clay minerals, a significant reduction in the solubility of heavy metals is found, mainly as an effect of their non-specific adsorption [65]. The binding strength of heavy metals by clay minerals decreases in the direction: Pb > Cu > Zn > Cd [65]. Light soils, due to their low content of floating parts, structure, and granulometric composition, have a limited capacity to retain incorporated substances due to their low sorption capacity. In contrast, heavy and medium soils act as a kind of buffer, retaining substances introduced into the soil, including heavy metals. This property allows the medium soil to fix and sorb nutrients and heavy metals. The heavier the soil, the richer it is in clay and silt particles and the greater its sorption capacity, which leads to blocking the uptake of harmful elements by plants [6, 13, 14].

In ore search, the movement of PCE in the soil profile was detected. The highest leaching took place at low soil pH and relatively low organic matter content [62]. In the more contaminated soil, the heavier metals leached deep into the soil profile. The most susceptible to leaching was Cd, while the least was Cu. In the literature, it can be found that more leaching of cadmium and nickel occurs from sand soil than from loam soil [101]. Comparing the results of the research with the data of other authors, it can be stated that higher leaching of heavy metals deep into the soil profile occurs on lighter soils compared to the medium. In Gębski's study [51], heavy metals (Zn and Cd) introduced into the arable layer were easily moved to deeper layers of the soil profile. The mobility of these elements, and thus, their leaching intensity, increased together with the decrease in soil pH. A similar tendency was observed in our own research. However, at high soil pH, the movement of Cd deep into the soil profile is small [102]. In turn, Borowiec et al. report that Cd can accumulate mainly at the accumulation level, although most studies indicate that this element can easily move deep into the soil profile. Many researchers have observed a significant reduction in the leaching of Zn and Cd metals under the influence of an increase in soil pH value and a lack of Pb response to a change in pH [103]. This thesis is confirmed by our own research. There is also no evidence of the movement deep into soil profiles of Zn, Cd, and Pb in soils with a similar pH to the neutral one and having a higher content of floatable parts [104]. The finding that higher movement of Zn and Cd occurs in sandy soils with strong acidity [105] is consistent with the results of our research.

Most literature reports that lead does not leach into the soil profile, but Bowen [105] states that this element does move in the soil, yet very slowly. Kabata-Pendias and Pendias [94], on the other hand, draw attention to the possibility of a serious change in the rate of lead movement in the soil profile due to a decrease in soil sorption capacity or an increase in acidification. Under such conditions, this element can migrate to groundwater, posing a serious toxicological risk. Other researchers from the Czech Republic who determine the mobility of Pb, Zn, and Cd in soils from the Příbram region (Czech Republic) heavily contaminated by metallurgy-two profiles of alluvial soils were closely studied-report that the profile distribution of lead (the least mobile metal) is characterized by a gradual decrease with depth [106].

The literature also states that heavy metals do not leach deep into the soil profile but accumulate in the topsoil layer [107, 108]. In the arable layer of Lublin soils, higher amounts of cadmium are found compared to their subsoil [109]. In their study, a higher Cd concentration was found in the layer of 25–50 than 0–25 cm. This shows that the leaching of heavy metals in natural conditions is lower than in the microplot experiment, where this movement is strong.

6. Conclusions

The heavy metal content of forage plants depended on soil and plant species. The average increase of heavy metals in fodder plants as contamination increased was greater on light soil than on medium soil. As the level of soil contamination increased, their content in forage plants increased. The highest contents of the heavy metals tested were found at soil contamination level III and were many times higher compared to sites with natural contents. Under the influence of low pollution, that is, at level I of soil contamination with heavy metals, the content of Cd and Zn increased the most in fodder plants, and the content of Cu increased the least. In contrast, at pollution level III, Pb content increased in plants. It is noteworthy that already pollution level I, that is, a relatively low contamination of soils with heavy metals, caused a significant increase in the heavy metal content of the tested fodder plants, especially in the case of rye green fodder. The highest average values of heavy metal bioaccumulation rates in plants were obtained for Zn and Cd, indicating a high propensity of plants to assimilate these elements. On the other hand, lower values of bioaccumulation indices for Pb and Cu indicate lower predisposition of plants to uptake of these elements. However, there was no effect of soil species on the value of the heavy metal bioaccumulation index. Individual heavy metals also differed in their indicators of the degree of plant contamination. In terms of this characteristic, they can be ranked in the following order, according to the decreasing value of the index: Cd > Pb > Zn > Cu, which means that under conditions of soil contamination with toxic metals, the relative content of Cd increases the most in plants and Cu the least). Of the plants tested, serradella proved to be the most sensitive to high heavy metal content. Even at "0" contamination level, it accumulated large amounts of Zn and Cd, 180 and 2.8 mg/kg, respectively.

According to the Regulation of the Minister of Agriculture and Rural Development of 23 January 2007 [99] on the permissible content of undesirable substances in feed (Journal of Laws No. 162, item 1704 [110]), the content of Cd in feed should not exceed 1 mg/kg d.m., Zn—100 mg/kg d.m., Pb—10 mg/kg d.m. and Cu—30 mg/kg d.m.

The high Cd and Zn content of serradella and rye green fodder on most combinations, especially on light soil, disqualifies them for use as fodder. Barley grain and straw (with the exception of crops harvested on combinations at contamination level III, as there was an exceedance of the standards in terms of Zn and Cd content there) can be used for animal feed.

The leaching of the studied metals depended on the degree of contamination and soil species. The more contaminated the soil was, the more PCE determined in 1 M HCl leached to lower genetic levels, more to Et than Bt. The highest displacement factors were obtained for cadmium, and the lowest were for lead. By analyzing the mobility coefficients (heavy metal growth in the Bt and Et layers), they can be ranked in the following decreasing sequence: on light soils: Cd > Cu > Zn > Pb and on medium soils: Cd > Zn > Pb > Cu.

Source of results: Own study and doctoral thesis entitled: "Influence of the selected physicochemical properties of the soil on the content of heavy metals in fodder plants" Warsaw University of Life Sciences, Department of Agriculture and Biology, Department of Agricultural Chemistry, 2006.

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