Availability of Trace Elements in Soil with Simulated Cadmium, Lead and Zinc Pollution

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Abstract: The research was based on a pot experiment in which the impact of increasing Cd, Zn and Pb doses on the content of available trace elements in soil was compared. Seven series of trials were designed: 1 (Cd), 2 (Pb), 3 (Zn), 4 (Cd + Pb), 5 (Cd + Zn), 6 (Pb + Zn), 7 (Cd + Pb + Zn). Aside from the control one (without the metals), three increasing levels of contamination were considered within each series. Mobile forms of trace elements (Cd, Pb, Zn, Fe, Mn, Cu, Ni, Co, and Cr) in soil were determined, in addition to which selected physicochemical soil properties—reaction (pH), salinity (EC), hydrolytic acidity (HAC), total exchange bases (TEB)—were identified while cation exchange capacity (CEC), base saturation (BS) and availability factor (AF) were calculated. The application of Cd and Pb to soil resulted in an increase in the share of potentially available forms of these metals in their total content. The availability factor (AF) in the pots polluted with these metals was higher than in the control, in the range 17.5–20.0% for Cd, and 62.8–71.5% for Pb. In turn, the share of Zn mobile forms was comparable in most experimental objects, oscillating around 30%. Moreover, addition to soil of Cd, Pb and Zn usually caused a significant decrease in the content of available forms of Fe, Mn and Cu, and resulted in significantly higher content of available forms of Cr in the soil.

Keywords: soil pollution; trace elements; soil properties

1. Introduction

Heavy metals are among the most persistent soil pollutants, demonstrating lasting effects and toxicity [1,2]. This negative impact of trace elements is compounded by their continuous emissions into the natural environment from many anthropogenic sources [3–6]. In many areas exposed to intense anthropogenic activity, there is an increased content in heavy metals such as Cd, Pb and Zn [7–9]. Contamination of soil with Cd, Pb and Zn poses high threats to soil quality [7]. It may significantly decrease the metabolic activity of bacteria and other soil microorganisms [10], disrupt plant development and thus pose risks to humans and ecosystems [1], which is a serious environmental problem [10]. According to Anju and Banerjee [11], total metal content is a useful preliminary indicator of areas where the risks of metals excess are high. However, the total concentration of metals in soils gives little information on their toxicity and mobility [12]. The increased availability of the metals indicates continuous soil enrichment in these elements [2]. According to Wierzbowska et al. [13] and Jaafar et al. [14], an elevated content of heavy metals in soil may not pose a threat depending on soil conditions. The mobility of trace elements in soil is controlled by numerous biochemical and chemical processes, and the strength of mutual relationships depends on a given metal [15,16]. Under natural conditions, lead is one of the least mobile elements in the soil [17]. The soil content of available trace metals depends on different factors, including soil reaction [8,18–23], organic matter content [19,20,23,24], soil sorption complex capacity and cation exchange capacity [12,18,19,25], soil salinity [19], grain-size composition [26,27], content of carbonates [20] content of organic carbon and total nitrogen [25], iron and manganese compounds [17,28,29], the occurrence and activity of soil microorganisms [30], or the content of sulphate and phosphate [31,32] or aluminium.
compounds in the soil [33]. In addition, the content of available trace metals in the soil may be affected by fertilisation [13,31] or the application of mineral [32] and organic substances to soil [7,32]. Moreover, the content of metal soluble forms in the topsoil is closely correlated with the soil content of the total forms of these elements [8,33], which may become bioavailable when soil conditions change [1,34]. Soils richer in organic matter, characterised by higher reaction (pH) [8,20,22] and greater cation exchange capacity (CEC) can immobilise heavy metals more effectively [28].

The mobility and availability of heavy metals depend on the form in which they occur in soil [29]. In soils polluted due to anthropogenic activities, metals are most often adsorbed to inorganic soil particles and insoluble organic matter, or else they appear in the soil solution as precipitated pure or mixed solid bodies [15,33]. For environmental safety reasons, when soil is polluted with heavy metals, it is important to determine not only their total content but also the concentrations of their potentially available forms [2,28,35]. By extracting soil with mild extractants, such as ammonium nitrate, it is only possible to obtain information about the correct quantities of the phytoavailable forms of metals. Estimation of the total activation of these elements is possible when 1 M HCl is applied as an extractant [12]. According to Wieczorek et al. [8], there is a strong positive correlation between the total Cd and Pb in the soil content and their bioavailability. Moreover, increased content of some heavy metals of soil may increase the bioavailability of other trace elements [3]. However, the interrelationships between metals are quite complex and not fully understood, therefore it is necessary to conduct further research in this field. Knowledge of the content of heavy metals in soil and their forms can enable us to limit their negative impact on the ecosystem in which they occur [1,2,12,35].

Therefore, the research hypotheses were as follows: (1) increased content of Cd, Pb and Zn in the soil increases their mobility and bioavailability, and (2) changes in the content of available forms of Fe, Mn, Cu, Ni, Co, and Cr. Therefore, the purpose of this study was to determine the effect of the increasing doses of metals (Cd, Pb and Zn) on the content of potentially available forms of some trace elements (Cd, Pb, Zn, Fe, Mn, Cu, Ni, Co, and Cr) in the soil.

2. Materials and Methods

2.1. Description of the Subject of the Study

The research comprised assays of the soil obtained from a pot experiment in which the test plant was perennial ryegrass (Lolium perenne L.), grown for 138 days. The aboveground mass was collected twice during the cultivation of ryegrass. At the end of this experiment, after separating the grass roots, the remaining soil in the pot was mixed thoroughly and a representative sample was taken for further analysis.

2.2. Analytical Methods

The soil collected after the experiment was air-dried and passed through a = 2 mm mesh sieve. Then, the soil samples were submitted to chemical analyses and the following parameters were determined: pH of soil, EC, HAC, TEB, and the content of available trace elements (Cd, Pb, Zn, Cu, Mn, Fe, Co, Ni and Cr).

The analyses of the starting soil and the soil obtained after the experiment were carried out with the same methods. The soil pH was determined with the potentiometric method [36] in the soil /1 M KCl solution in a ratio of 1:2.5 (w/v), using a pH SenTix61 electrode (WTW, Wroclaw, Poland) and a pH 538 WTW meter. EC was determined in a mixture of soil/deionised water in a 1:2 (w/v) ratio, using a portable EC meter by EC Hanna Instruments HI 8733 (Hanna Instruments, Bangladesh, Romania). HAC and TEB were determined with Kappen’s method [36]. The HAC and TEB determinations served to calculate CEC and BS. CEC and BS were calculated from the following formulas: CEC = TEB + HAC and BS = (TEB/CEC) × 100.

In the starting soil, the total organic carbon (TOC) content was determined with a Shimadzu TOC-L analyser (Shimadzu Corporation, Kyoto, Japan) coupled with a solid
sample module SSM-5000A, the content of N-total by the Kjeldahl method, available P and K by the Egner-Riehm method [37] and Mg by the Schachtschabel method [38].

In order to determine the total of heavy metals, the soil was mineralised in a MARS 6 microwave stove (CEM Corporation, Matthews, NC, USA), in MARS Xpress Teflon® vessels, in a mixture of the acids: 65% HNO₃ + 38% HCl, in a 4:1 ratio [39]. To determine the content of the forms of these metals potentially available to plants, soil extraction was conducted in 1 M HCl solution [12,13,16,18,40,41]. Extraction of soil with the use of 1 M HCl allows us to determine metal fractions: water-soluble, exchangeable, related to carbonates, oxides and organic compounds, and partially residues [40]. Total forms of trace elements in the input soil, as well as their available content in soil after the plant harvest, were determined on an atomic absorption spectrophotometer SpectrAA-240FS, using reference models by MERCK (Darmstadt, Germany), according to the protocol US-EPA3051 [42]. For quality control of the determination of the total metals content of samples, certified reference materials (CRM020-50) were also analysed.

2.3. Properties and Chemical Composition of the Starting Soil

The input soil for the trials was collected from the humic-arable horizon under an arable field and had the texture of loamy sand (granulometric composition: 73.9% sand > 0.05 mm, 24.1% silt 0.002–0.05 mm and 2.0% clay < 0.002 mm). The soil had an acidic reaction (pH_KCl = 4.42). EC of the soil was 0.140 dS m⁻¹, its HAC equalled 33.80 mmol, TEB reached 33.24 mmol, CEC was determined at 71.64 mmol kg⁻¹, while the BS equalled 46%. The soil content of nutrients was as follows: TOC was 0.61%, N-total—0.74 g kg⁻¹, and available forms of P, K and Mg: 62.00, 84.00 and 54.00 mg kg⁻¹, respectively. The total content of trace elements determined in the input soil equalled: 0.16 mg Cd, 9.2 mg Pb, 28.19 mg Zn, 4.80 mg Cu, 1.23 mg Co, 5.46 mg Cr, 2.92 mg Ni, 230.42 mg Mn, and 5942.16 mg Fe kg⁻¹. These amounts fall within the natural content of trace elements determined for Polish soils [43].

2.4. Experiment Description

The experiment was performed in order to compare the influence of increasing levels of soil pollution with Cd, Pb and Zn. Seven series were designed: 1 (Cd), 2 (Pb), 3 (Zn), 4 (Cd + Pb), 5 (Cd + Zn), 6 (Pb + Zn) and 7 (Cd + Pb + Zn). The first three series focused on the impact of each metal separately, while the other series demonstrated their mutual effects on soil and the test plant. Within each series, aside from the control variant (without the metals), three increasing levels of contamination were considered. The applied doses of the elements were the following: 2.0, 4.0 and 6.0 mg Cd; 50, 100 and 150 mg Pb; and 150, 300 and 450 mg Zn kg⁻¹ of soil. Cadmium was applied as cadmium sulphate (3CdSO₄·8H₂O), lead in the form of lead acetate (Pb(CH₃COO)₂·3H₂O), while zinc was used as zinc sulphate (ZnSO₄·7H₂O). The three metals as aqueous solutions were added to soil while setting up the experiment. Additionally, the soil was enriched with urea (CH₄N₂O), potassium phosphate (K₂HPO₄) and potassium sulphate (K₂SO₄), in amounts corresponding to 192 mg N, 48 mg P and 111 mg K kg⁻¹ of soil. All additives (metals and fertilizers) were thoroughly mixed with soil, which was transferred to properly numbered pots. Liquid fertiliser Florowit was also added to soil during the experiment, supplying the following quantities of elements: 0.005 mg N, 0.002 mg K, 0.005 mg Cu, 0.030 mg Fe, 0.013 mg Mn, 0.002 mg Mo and 0.011 mg Zn kg⁻¹ as well as trace amounts of Ca, Mg, S and B. The experiment was carried out in three replications. Cd, Pb and Zn were used in the experiment due to the high risk of these elements from anthropogenic sources. The doses of metals applied to the soil were based on the applicable Polish national standards [44,45]. The doses of Cd, Pb and Zn used in the experiment were determined based on the toxicity and mobility of these elements. According to national standards [44], the first two doses of each metal are the permissible content for arable land soils, and the third dose is the level proving soil contamination with these elements. Tests performed in areas exposed to
continuous emission of pollutants often show an increased content of Pb, Cd and Zn in soils [5,9].

2.5. Calculation and Statistical Methods

Based on the determination of available forms and total content of trace elements in soil, following other authors [2,16], the availability factor (AF) was calculated from the formula: 

\[ AF(\%) = \frac{\text{available content} \times 100}{\text{total content}}.\]

The total content of heavy metals in the soil was the sum of the contents of these elements in the starting soil, the fertilizers used (Florowit) and solutions of cadmium sulphate, lead acetate and zinc sulphate based on the accepted scheme.

The results were submitted to statistical analysis in Statistica 12 programme. By applying an ANOVA test for factorial sets at significance \(p = 0.05\), LSDs were calculated. Homogenous groups within the analysed factors (I—increasing doses of metals, and II—kind of metal ) were identified with Duncan’s test. The Pearson’s simple correlation coefficient \((r)\) was also calculated between the analysed features and relative to the increasing levels of pollution. Its significance was based on statistical tables [46] of threshold values at n-2 degrees of freedom.

3. Results and Discussion

3.1. The Content of Mobile Forms of Trace Elements in Soil

Considering all the series, the average content of determined available forms of elements (Tables 1–3) can be arranged in the decreasing order: Fe (646.7 mg kg\(^{-1}\) of soil) > Mn (76.89 mg kg\(^{-1}\) of soil) > Zn (47.80 mg kg\(^{-1}\) of soil) > Pb (36.92 mg kg\(^{-1}\) of soil) > Cr (2.821 mg kg\(^{-1}\) of soil) > Cu (1.224 mg kg\(^{-1}\) of soil) > Ni (0.601 mg kg\(^{-1}\) of soil) > Co (0.438 mg kg\(^{-1}\) of soil) > Cd (0.419 mg kg\(^{-1}\) of soil).

The concentrations of the Cd, Pb and Zn mobile forms increased highly significantly alongside the growing contamination in the series where these elements were added to soil (Table 1). The significant increase in the available forms of these metals was evidenced by the high correlation coefficient \((r)\), which ranged from 0.994** to 1.000** for Cd (series with Cd, Cd + Pb, Cd + Zn, Cd + Pb + Zn), from 0.971** to 0.997** for Pb (series with Pb, Cd + Pb, Pb + Zn, Cd + Pb + Zn) and from 0.999** to 1.000** for Zn (series with Zn, Cd + Zn, Pb + Zn, Cd + Pb + Zn). Each metal dose in these series resulted in a significant rise in the amounts of the mobile forms of these metals. The highest concentrations of soluble forms were noted at the highest contamination level, where they reached: 1.337–1.579 mg Cd, 106.29–139.88 mg Pb and 140.15–142.45 mg Zn kg\(^{-1}\) of soil. These quantities were from 95- to 132-fold higher in the case of Cd, from 22- to 29-fold higher for Pb and from 15- to 16-fold higher for Zn than their concentrations determined in the control samples in these series. Furthermore, higher Cd and Pb mobility was observed in the samples where Zn was another soil pollutant. Moreover, a significant increase in the mobility of Zn \((r = 0.727^*)\) was observed in series with Cd + Pb, where this element was not applied to soil. However, the application of Cd and Pb to soil in the samples of this series caused an elevated mobility of Zn. The highest content of zinc mobile forms (23.00 mg kg\(^{-1}\) of soil) was noted in this series at the second dose of pollutants, where it was over 2.5-fold higher than in the control.

The research results obtained from the series with Cd and Pb are supported by the literature data [8,11,12,14,33], which suggest a correlation between the total content and concentrations of bioavailable forms of these metals, whose may be easily uptake by the plants [33]. In the by Lemanowicz and Bartkowiak [31] studies, greater variability of available Cd content than Pb and Zn content in soil was also observed against the background of changing soil conditions. The presence of phosphates, zinc and silicon in the soil reduces the availability of Cd [47]. Zinc is very mobile in the soil environment, especially in low pH conditions [6,31]. In the studies by Wyszkowski and Modrzewska [32], the application of increasing doses of Zn in the form of ZnCl\(_2\) resulted in an increase in the total content of this element and Pb in the soil and in a decrease in the content of Cd in the soil. In turn, lead has low mobility in the environment and becomes immobilized at an
alkaline pH [6]. However, the increase in the content of mobile Pb forms in soil may be enhanced by anthropogenic factors [17]. According to Lamb et al. [35], the bioavailability of Pb is relatively high in soils contaminated with Pb. Higher bioavailability of Pb, Cd and Zn was also demonstrated by Kashem et al. [40] in soils contaminated with these elements. In soils contaminated with heavy metals, the Cd content in the mobile fraction increases. On the other hand, Zn and Pb are mainly associated with the oxide and residual fractions. Thus, Cd was potentially more bioavailable than Zn and Pb [33]. Moreover, on agricultural soils, the lack of phosphorus and potassium fertilization may increase the availability of Zn, and the lack of magnesium and sulfur fertilization may increase the availability of Cd. On the other hand, fertilization without sulfur reduces the content of available Zn and Pb forms in the soil [31].

Table 1. Content of available forms of Cd, Pb and Zn, in mg kg\(^{-1}\) of soil.

| Metal Doses in Soil* | Series of Experiment | Means for Doses |
|----------------------|----------------------|-----------------|
|                      | Cd                   | Pb              | Zn              | Cd + Pb | Cd + Zn | Pb + Zn | Cd + Pb + Zn |
| 0                    | 0.012\(^{a}\)        | 0.012\(^{a}\)   | 0.012\(^{a}\)   | 0.012\(^{a}\) | 0.012\(^{a}\) | 0.012\(^{a}\) | 0.012\(^{a}\) |
| I                    | 0.477\(^{b}\)        | 0.029\(^{a}\)   | 0.012\(^{a}\)   | 0.012\(^{a}\) | 0.494\(^{b}\)   | 0.301\(^{a}\)   | 0.505\(^{b}\) |
| II                   | 0.024\(^{a}\)        | 0.014\(^{a}\)   | 0.012\(^{a}\)   | 0.825\(^{c}\)   | 0.994\(^{e}\)   | 0.013\(^{a}\)   | 0.505\(^{b}\) |
| III                  | 1.446\(^{a}\)        | 0.013\(^{a}\)   | 0.012\(^{a}\)   | 1.337\(^{d}\)   | 1.495\(^{e}\)   | 0.011\(^{a}\)   | 1.579\(^{b}\) |

Means for series 

\(r\) 0.708** -0.192n.s. 0.135n.s. 0.994** 1.000** -0.264n.s. 0.999** 0.569**

LSD\(_{0.05}\) for: metal dose = 0.019; kind of metal = 0.025; interaction = 0.050

|                  | Cd                   | Pb              | Zn              | Cd + Pb | Cd + Zn | Pb + Zn | Cd + Pb + Zn |
|------------------|----------------------|-----------------|-----------------|---------|---------|---------|--------------|
| 0                | 4.82\(^{a}\)        | 4.82\(^{a}\)   | 4.82\(^{a}\)   | 4.82\(^{a}\) | 4.82\(^{a}\) | 4.82\(^{a}\) | 4.82\(^{a}\) |
| I                | 5.21\(^{a}\)        | 38.26\(^{b}\)  | 6.91\(^{a}\)   | 44.29\(^{b}\) | 6.16\(^{a}\)   | 49.32\(^{c}\)   | 42.87\(^{b}\) |
| II               | 5.41\(^{a}\)        | 71.68\(^{d,e}\) | 5.63\(^{a}\)   | 68.08\(^{d}\)   | 5.40\(^{a}\)   | 79.03\(^{f}\)   | 81.05\(^{f}\) |
| III              | 5.12\(^{a}\)        | 107.97\(^{a}\) | 6.02\(^{a}\)   | 106.29\(^{a}\) | 5.36\(^{a}\)   | 120.10\(^{h}\)  | 139.88\(^{h}\) |

Means for series 

\(r\) 0.708** -0.192n.s. 0.135n.s. 0.994** 1.000** -0.264n.s. 0.999** 0.569**

LSD\(_{0.05}\) for: metal dose = 2.939; kind of metal = 3.888; interaction = 7.777

|                  | Cd                   | Pb              | Zn              | Cd + Pb | Cd + Zn | Pb + Zn | Cd + Pb + Zn |
|------------------|----------------------|-----------------|-----------------|---------|---------|---------|--------------|
| 0                | 9.16\(^{a}\)        | 9.16\(^{a}\)   | 9.16\(^{a}\)   | 9.16\(^{a}\) | 9.16\(^{a}\) | 9.16\(^{a}\) | 9.16\(^{a}\) |
| I                | 9.04\(^{a}\)        | 7.67\(^{a}\)   | 55.26\(^{f}\)  | 15.60\(^{a}\) | 54.87\(^{f}\)  | 54.94\(^{f}\)   | 54.48\(^{f}\) |
| II               | 7.67\(^{a}\)        | 9.05\(^{a}\)   | 95.30\(^{f}\)  | 23.00\(^{a}\)  | 95.03\(^{f}\)  | 96.40\(^{g}\)   | 94.55\(^{g}\) |
| III              | 8.33\(^{a}\)        | 11.85\(^{b}\) | 140.15\(^{h}\) | 18.06\(^{d}\)  | 140.21\(^{h}\) | 142.45\(^{h}\)  | 140.48\(^{h}\) |

Means for series 

\(r\) -0.447n.s. 0.598n.s. 1.000** 0.727** 1.000** 0.999** 0.999** 0.586**

LSD\(_{0.05}\) for: metal dose = 0.862; kind of metal = 1.140; interaction = 2.280

* dose size explained in the methodology; correlation coefficient (\(r\)) *—significant for \(p = 0.05\); **—highly significant for \(p = 0.01\); n.s —not significant; n = 12; the letters from “a” to “i” differentiate homogeneous groups, means followed by the same letter do not differ at \(p = 0.05\) by the LSD test.
Heavy metals in uncontaminated soils and sediments are mainly immobile as they are bound to silicates and to primary minerals [20]. According to Chibuike and Obiora [3], the increased content of some heavy metals in the soil may increase the availability of other trace elements. However, in the presented research, the application of Cd, Pb and Zn affected differently the content of available forms of Fe, Mn, Cu, Ni, Cr and Co (Tables 2 and 3). The application of Cd (series 1) to soil resulted in a significant decrease in the content of available Fe \( (r = -0.688^*) \) while highly significantly decreasing Mn \( (r = -0.875^{**}) \) and Co \( (r = -0.771^{**}) \). The Pb application (series 2) caused a highly significant decline in amounts of mobile forms of Fe \( (r = -0.834^{**}) \), Mn \( (r = -0.813^{**}) \) and Cu \( (r = -0.805^{**}) \). On the other hand, the soil contamination with Zn was significantly negatively correlated with the soil content of mobile forms of Mn \( (r = -0.697^*) \) and Cu \( (r = -0.744^*) \), being significantly positively correlated with the content of Ni \( (r = 0.702^*) \). Moreover, in the series with Cd + Pb, Cd + Zn, Pb + Zn, Cd + Pb + Zn, where interactions of the analysed metals were tested, a significant or highly significant decrease in the content of available forms of Fe \( (-0.861^{**} < r < -0.652^*) \) and Mn \( (-0.875^{**} < r < -0.755^*) \) was noted, alongside a highly significant increase in the content of Cr \( (0.765^{**} < r < 0.874^{**}) \). Finally, soil pollution with Pb and Zn (series 6) led to a significant decrease in the quantities of Cu available forms \( (r = -0.700^*) \), while the application of Cd and Zn (series 5) or Cd, Pb and Zn (series 7) caused a significant rise in the content of mobile Co \( (0.712^* < r < 0.731^*) \). In contaminated soils, Zn shows mainly an affinity for Fe-Mn oxides [33,34], which may

Table 2. Content of available forms of Fe, Mn and Cu, in mg kg^{-1} of soil.

| Metal Doses in Soil* | Series of Experiment | Means for Doses |
|----------------------|----------------------|-----------------|
|                      | Cd       | Pb       | Zn       | Cd + Pb  | Cd + Zn  | Pb + Zn  | Cd + Pb + Zn |
| 0                    | 728.3^h,i| 728.3^h,i| 728.3^h|i| 728.3^h| 728.3^h| 728.3^h| 728.3^h| 728.3^h| 728.3^h| 728.3^h|
| I                    | 731.0^h  | 673.8^h,e| 690.1^h| 664.0^d-f| 640.2^d-e| 542.1^e| 480.1^a,b| 629.0^b| 615.4^a|
| II                   | 708.7^h,i| 646.3^d-e| 688.6^h| 663.3^d-f| 645.3^e| 490.2^a,b| 465.1^a| 615.4^a|
| III                  | 689.6^h,g| 648.5^d-e| 691.8^h,d| 627.4^d| 650.5^d-f| 502.6^d-f| 487.4^a,b| 614.0^a|
| Means for series     | 714.4^b  | 674.2^a| 699.7^b| 670.8^a| 666.1^d| 561.3^d| 540.2^e| 646.7 |
| r                    | -0.688^* | -0.834^{**} | -0.463^{n.s.} | -0.861^{**} | -0.652^* | -0.809^{**} | -0.750^* | -0.450^{**} |

LSD_{0.05} for: metal dose = 12.031; kind of metal = 15.915; interaction = 5.131

| 0                    | 91.26^h  | 91.26^h  | 91.26^h  | 91.26^h  | 91.26^h  | 91.26^h  | 91.26^d |
| I                    | 87.36^h  | 73.24^c-f| 72.93^c-f| 75.92^c-e| 70.13^c  | 74.27^c  | 65.76^d  | 74.23^c |
| II                   | 78.83^g  | 70.22^b-c| 73.53^b-c| 79.60^b  | 71.24^c  | 68.34^d-b| 62.70^b  | 72.07^b |
| III                  | 75.44^c  | 70.78^b-e| 74.12^c  | 69.48^d-e| 70.47^b  | 68.15^b  | 61.56^a  | 70.00^a |
| Means for series     | 83.22^d  | 76.38^a,b| 77.96^b  | 75.79^b  | 75.51^a  | 70.32^c  | 76.89   |
| r                    | -0.876^{**} | -0.813^{**} | -0.697^{*} | -0.800^{**} | -0.755^{*} | -0.875^{**} | -0.825^{**} | -0.741^{**} |

LSD_{0.05} for: metal dose = 1.939; kind of metal = 2.566; interaction = 5.131

| 0                    | 1.872^e  | 1.872^e  | 1.872^e  | 1.872^e  | 1.872^e  | 1.872^e  | 1.872^b |
| I                    | 1.352^{d} | 1.005^{a-d} | 1.002^{a-d} | 1.007^{a-d} | 0.940^{d-a} | 0.737^{a} | 0.995^{d-a} | 1.005^{a} |
| II                   | 0.894^{c} | 0.859^{a,b} | 1.067^{d}  | 1.190^{d}  | 0.736^{a}  | 1.002^{d-a} | 1.018^{d-a} | 0.967^{a} |
| III                  | 1.375^{d} | 0.743^{a}  | 0.981^{a-d} | 1.018^{d}  | 1.188^{a-d} | 0.814^{a-b} | 1.255^{b-d} | 1.053^{a} |
| Means for series     | 1.373^{b} | 1.120^{a}  | 1.231^{a,b} | 1.272^{a,b} | 1.184^{a,b} | 1.106^{a}  | 1.285^{a,b} | 1.224 |
| r                    | -0.552^{n.s.} | -0.805^{**} | -0.744^{*} | -0.610^{n.s.} | -0.534^{n.s.} | -0.700^{*} | -0.473^{n.s.} | -0.619^{*} |

LSD_{0.05} for: metal dose = 0.149; kind of metal = n.s.; interaction = n.s.

* dose size explained in the methodology; correlation coefficient (r) —significant for \( p = 0.05 \); \(^*—highly significant for \( p = 0.01 \); \(^{n.s.}—not significant; n = 12; the letters from “a” to “i” differentiate homogeneous groups, means followed by the same letter do not differ at \( p = 0.05 \) by the LSD test.
explain the decrease in the content of mobile forms of Fe and Mn shown in our experiment. Most of Mn is related to the residual fraction of soil. In turn, Cu is bound to Fe-Mn oxides and the residual fraction. However, the most durable combination of Cu is with the organic fraction, which makes it relatively non-mobile in soils. It is especially noted in organic soils [34]. Moreover, the increased availability of Mn, Cu, Ni and Zn is observed in agricultural land as a result of the abandonment of phosphorus and potassium fertilization. Content of available Cu forms in the soil was also increased in the absence of magnesium. On the other hand, the lack of sulfur fertilization results in a decrease in the available Cu forms in the soil [31]. The ability of soil to retain and immobilize heavy metals is determined by the amount and type of clay minerals, the content of iron (Fe), aluminum (Al) and manganese (Mn) oxides, and the redox potential. When their contents are exceeded, the amount of heavy metals available to plants is increased [28].

Table 3. Content of available forms of Ni, Cr and Co, in mg kg\(^{-1}\) of soil.

| Metal Doses in Soil \(^{a}\) | Series of Experiment | Means for Doses |
|-----------------------------|----------------------|-----------------|
|                             | Cd       | Pb       | Zn       | Cd + Pb | Cd + Zn | Pb + Zn | Cd + Pb + Zn |
| Ni                          |          |          |          |          |          |         |              |
| 0                           | 0.571\(^{a-c}\) | 0.571\(^{a-c}\) | 0.571\(^{a-c}\) | 0.571\(^{a-c}\) | 0.571\(^{a-c}\) | 0.571\(^{a-c}\) | 0.571\(^{a-c}\) |
| I                           | 0.560\(^{a-c}\) | 0.554\(^{a-c}\) | 0.634\(^{b-d}\) | 0.645\(^{b-c}\) | 0.703\(^{d}\) | 0.565\(^{a-c}\) | 0.543\(^{b}\) |
| II                          | 0.487\(^{a}\)  | 0.594\(^{a-d}\) | 0.680\(^{d-e}\) | 0.761\(^{e}\) | 0.703\(^{d}\) | 0.571\(^{a-c}\) | 0.520\(^{b}\) |
| III                         | 0.611\(^{a-d}\) | 0.600\(^{a-d}\) | 0.680\(^{d-e}\) | 0.697\(^{d-e}\) | 0.622\(^{b-d}\) | 0.543\(^{a-b}\) | 0.566\(^{a-c}\) |
| Means for series            | 0.557\(^{a}\)  | 0.580\(^{a}\)  | 0.641\(^{b}\)  | 0.669\(^{b}\)  | 0.650\(^{b}\)  | 0.563\(^{a}\)  | 0.550\(^{a}\)  |
|                             | 0.075\(^{a,ns}\) | 0.250\(^{a,ns}\) | 0.702\(^{b}\)  | 0.612\(^{n.s.}\) | 0.236\(^{n.s.}\) | -0.151\(^{n.s.}\) | -0.074\(^{n.s.}\) |

\(LSD_{0.05}\) for: metal dose = n.s.; kind of metal = 0.054; interaction = n.s.

| Cr                          |          |          |          |          |          |         |              |
| 0                           | 1.864\(^{a-b}\) | 1.864\(^{a-b}\) | 1.864\(^{a-b}\) | 1.864\(^{a-b}\) | 1.864\(^{a-b}\) | 1.864\(^{a-b}\) | 1.864\(^{a-b}\) |
| I                           | 1.782\(^{a-b}\) | 1.705\(^{a-b}\) | 1.751\(^{a-b}\) | 2.102\(^{d}\)  | 4.200\(^{d}\)  | 4.562\(^{d-e}\) | 4.874\(^{a}\)  |
| II                          | 1.766\(^{a-b}\) | 1.609\(^{a}\)  | 1.729\(^{a-b}\) | 2.752\(^{e}\)  | 4.791\(^{e}\)  | 4.783\(^{a}\)  | 4.814\(^{a}\)  |
| III                         | 1.666\(^{a-b}\) | 1.679\(^{a-b}\) | 1.745\(^{a-b}\) | 3.089\(^{d}\)  | 4.991\(^{e}\)  | 4.587\(^{d-e}\) | 4.960\(^{c}\)  |
| Means for series            | 1.770\(^{a}\)  | 1.714\(^{a}\)  | 1.772\(^{a}\)  | 2.452\(^{e}\)  | 3.962\(^{b}\)  | 3.949\(^{b}\)  | 4.128\(^{b}\)  |
|                             | -0.516\(^{a,ns}\) | -0.504\(^{n.s.}\) | -0.288\(^{n.s.}\) | 0.874\(^{**}\)  | 0.873\(^{**}\)  | 0.765\(^{**}\)  | 0.781\(^{**}\)  |

\(LSD_{0.05}\) for: metal dose = 0.050; kind of metal = 0.066; interaction = 0.131

| Co                          |          |          |          |          |          |         |              |
| 0                           | 0.346\(^{c}\)  | 0.346\(^{c}\)  | 0.346\(^{c}\)  | 0.346\(^{c}\)  | 0.346\(^{c}\)  | 0.346\(^{c}\)  | 0.346\(^{c}\)  |
| I                           | 0.301\(^{c-e}\) | 0.154\(^{b}\)  | 0.330\(^{b}\)  | 0.390\(^{i}\)  | 0.827\(^{k}\)  | 0.766\(^{b}\)  | 0.683\(^{b}\)  |
| II                          | 0.224\(^{b-d}\) | 0.108\(^{i}\)  | 0.215\(^{c}\)  | 0.523\(^{b}\)  | 0.794\(^{k}\)  | 0.699\(^{b}\)  | 0.608\(^{g}\)  |
| III                         | 0.206\(^{c}\)  | 0.206\(^{c}\)  | 0.272\(^{c}\)  | 0.387\(^{g}\)  | 0.780\(^{b}\)  | 0.645\(^{e}\)  | 0.710\(^{b}\)  |
| Means for series            | 0.269\(^{a}\)  | 0.204\(^{c}\)  | 0.291\(^{a}\)  | 0.412\(^{d}\)  | 0.687\(^{e}\)  | 0.614\(^{b}\)  | 0.587\(^{b}\)  |
|                             | -0.771\(^{**}\) | -0.474\(^{n.s.}\) | -0.576\(^{n.s.}\) | 0.287\(^{n.s.}\) | 0.712\(^{n.s.}\) | 0.565\(^{n.s.}\) | 0.731\(^{*}\)  |

\(LSD_{0.05}\) for: metal dose = 0.039; kind of metal = 0.051; interaction = 0.102

\(^{a}\) dose size explained in the methodology; correlation coefficient (\(r\)) —significant for \(p = 0.05\); \(^{**}\)—highly significant for \(p = 0.01\); \(^{n.s.}\)—not significant; \(n = 12\); the letters from “\(^{a}\)” to “\(^{c}\)” differentiate homogeneous groups, means followed by the same letter do not differ at \(p = 0.05\) by the LSD test.

3.2. Availability Factor (AF)

The calculated average availability factor (AF) suggested higher mobility of Pb, Co, Zn and Mn, but lower one of Cr, Cu, Ni, Cd and Fe, calculated at 64, 36, 35, 34 and 27, 26, 21, 15 and 11%, respectively (Figure 1). Similar results concerning the percentages of mobile forms in the total content of trace elements in soil were achieved by Wierzbowska et al. [13] who demonstrated higher shares of Zn, Mn, Pb and Cu, but lower ones of Fe, Ni and Cr. Other researchers indicated a higher contribution of the mobile forms of Pb, Cd
and Zn [12,16] but a lower one of Cu and Ni [16]. The different availability of the elements found by the cited authors could be caused by different soil types and properties in these investigations. The application of Cd and Pb to soil caused an increase in the share of potentially available forms of these metals in their total content, the consequence that was also indicated by the values of AF (Figure 1). Concerning Cd, the AF value nearly trebled in the polluted samples in series with Cd, Cd + Pb, Cd + Zn and Cd + Pb + Zn, where the average value of this factor ranged from 18 to 20%. For Pb, the rise of AF was lower and occurred in series with Pb, Cd + Pb, Pb + Zn and Cd + Pb + Zn. In the samples within these series, the value of AF was within 63 and 72%. The highest AF for both Cd and Pb was determined in the series where Zn was another soil contaminating element. On the other hand, the application of Zn to soil did not induce a rise in the share of the mobile forms of this element in its total content as determined in the control variant. In most of the designated series, the average AF value oscillated around 30%. An increase up to 42% was only noted in the object with the highest dose of Pb in series 2 (Pb) and in all samples within series 4 (Cd + Pb), where the AF values were in a range of 55 to 82%. Similar shares of bioavailable Pb forms were reported by Lamb et al. [35] and Wierzbowska et al. [13], while those of Zn were approximately the same in the study by Senila et al. [18]. Much higher contributions of Cd, Pb and Zn mobile forms were noted in the experiment conducted by Baran et al. [12], while that of Cd was determined to be higher by Senila et al. [18]. A possible explanation of these differences lies in the origin of soil and its source of heavy metals and different extraction methods [12,18] or in the type of substances applied to soil [13]. According Massas et al. [2] the high availability of metals may indicate a relatively recent enrichment of soil with metals that have not yet been sequestered and heavily absorbed by soil colloids. The availability of metals depends on the fraction of free metals in the soil solution in relation to total metal content in the solid phase [48].

Figure 1. Availability factor (AF %). Control (0); first metal dose (I); second metal dose (II); third metal dose (III).

Series of experiment: 1—Cd, 2—Pb, 3—Zn, 4—Cd + Pb, 5—Cd + Zn, 6—Pb + Zn, 7—Cd + Pb + Zn.
Regardless of the type and level of soil contamination, a similar decline in the AF value of Mn and Cu was noted in all series (Figure 1). With respect to Fe, a decrease in AF was only noted in series 6 (Pb + Zn) and 7 (Cd + Pb + Zn). On the other hand, the mobility of Cr increased considerably under the influence of the combined Cd, Pb and Zn soil pollution, which was evident in series 4 (Cd + Pb), 5 (Cd + Zn), 6 (Pb + Zn) and 7 (Cd + Pb + Zn). In the same series, the AF values for Co increased distinctly. Concerning Co, the metals applied to soil separately caused decreased AF values. In turn, the AF values for Ni were approximately the same in all experimental samples. In a study by Wierzbowska et al. [13], despite using the same extraction method, higher shares of Fe (26–37%), Mn (53–61%) and Cu (38–61%) were determined while those of Ni (10–14%) and Cr (5–6%) were lower than in this study. Likewise, Senila et al. [18], having extracted soil with 1 M HCl, noted a much higher share of Cu (64%) than that determined in the experiment reported herein. These differences most probably are a result of using fertilisers with a high contribution of organic matter [13] or else arise from soil properties [18]. The changes in the content of mobile forms of heavy metals and their shares in the total content induced by the simulation increase in the soil contamination with Cd, Pb and Zn, determined in our experiment, implicate easy transformation of chemical forms of these metals in soil, which was also underlined by Osakwe et al. [34]. According to Nunes et al. [19], the mobility of heavy metals is closely correlated with soil properties. In polluted soils, these elements are less strongly attached to soil particles and are therefore more easily transformed into mobile forms.

3.3. Correlations between Content Available Forms of Trace Elements in Soil

Fe, Mn and Cu show a positive correlation with each other and a negative correlation with the rest of the elements in the soil (which in turn show a positive correlation with each other), with the exception of Ni (Table 4). A positive correlation between concentrations of the mobile forms of Cd, Pb and Zn was found by Baran et al. [12], while Wieczorek et al. [8] reported such correlation between Cd and Pb, and in turn, Anju and Banerjee [11] between Cd and Zn. Massas et al. [2], who analysed the content of available heavy metals in soils in Greece, also reported several correlations similar to the ones demonstrated in our study. Other authors [19,23], pointing to numerous and positive correlations between the analysed elements, explain the same similar type of pressure to which soils were exposed and that anthropogenic activity may increase the mobility of these elements.

Table 4. Correlations (r) between the content of available trace elements and selected properties of the soils.

| Elements | Zn  | Cd  | Pb  | Mn  | Fe  | Ni  | Cr  | Cu  | Co  |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cd       | 0.269* |     |     |     |     |     |     |     |     |
| Pb       | 0.311** | 0.235* |     |     |     |     |     |     |     |
| Mn       | -0.612** | -0.434** | -0.604** |     |     |     |     |     |     |
| Fe       | -0.587** | -0.269* | -0.707** | 0.786** |     |     |     |     |     |
| Ni       | 0.114p.s. | 0.151p.s. | -0.046n.s. | -0.125p.s. | 0.131n.s. |     |     |     |     |
| Cr       | 0.679** | 0.440** | 0.437** | -0.601** | -0.800** | -0.012n.s. |     |     |     |
| Cu       | -0.391** | -0.204n.s. | -0.418** | 0.730** | 0.504** | -0.083n.s. | -0.363** |     |     |
| Co       | 0.583** | 0.346** | 0.225* | -0.377** | -0.608** | 0.103n.s. | 0.907** | -0.211n.s. |     |

| EC       | 0.962** | 0.206n.s. | 0.242* | -0.462* | -0.531** | -0.026n.s. | 0.646** | -0.263* | 0.559** |
| pH       | 0.428** | -0.011n.s. | 0.128p.s. | 0.074n.s. | -0.373** | -0.322** | 0.562** | 0.182p.s. | 0.539** |
| HAC      | 0.432** | 0.363** | 0.513** | -0.760** | -0.527** | -0.013n.s. | 0.274* | -0.527** | 0.067n.s. |
| TEB      | -0.085n.s. | -0.022n.s. | -0.310** | 0.326** | 0.286* | 0.020p.s. | -0.042n.s. | 0.205p.s. | 0.072n.s. |
| CEC      | 0.206n.s. | 0.218p.s. | 0.045p.s. | -0.193n.s. | 0.079n.s. | 0.021n.s. | 0.137p.s. | -0.137n.s. | 0.109n.s. |
| BS       | -0.282* | -0.201n.s. | -0.485** | 0.619** | 0.483** | 0.482** | -0.184n.s. | 0.411** | 0.003n.s. |

HAC—hydrolytic acidity; TEB—total exchangeable bases; CEC—cation exchange capacity; BS—base saturation; correlation coefficient (r) *—significant for \( p = 0.05 \); **—highly significant for \( p = 0.01 \); n.s.—not significant; \( n = 84 \).
3.4. Physico-Chemical Properties against the Background of Soil Pollution

3.4.1. Sorptions Properties of Soils

In this research, the soil application of Cd, Pb and Zn solutions also caused a significant or highly significant increase in HAC, which was observed in series with Cd, Pb, Zn, Pb + Zn and Cd + Pb + Zn \((0.753^{**} < r < 0.939^{**})\) (Table 5). In turn, a rise in HAC led to a decrease in the BS in series with Cd, Zn and Pb + Zn \((-0.760^{*} < r < -0.694^{*})\). Moreover, HAC correlated highly significantly and positively with the mobile forms of Zn, Cd, Pb, significantly with Cr and negatively and highly significantly with Mn, Fe and Cu (Table 4). In turn, TEB correlated negatively with Pb, and positively with Mn and Fe. The CEC in our study did not reveal any significant correlations with the content of the mobile forms of trace elements. However, BS was highly significantly and positively correlated with the content of mobile Mn, Fe and Cu, while being negatively correlated with Zn and Pb (Table 4). The obtained high values of correlation coefficients indicate significant relationships between the content of heavy metals and the considered physicochemical properties of soils [6].

The study by Józefowska et al. [25] demonstrated positive relationships between the total content of Cd and Pb with HAC. In turn, Wierzbowska et al. [13] showed negative correlations of HAC with Cu, Mn and Pb, in addition to positive correlations of CEC with Cu, Pb and Cr. The study by Baran et al. [12] demonstrated positive relationships between the content of Cd, Pb and Zn and CEC, while Józefowska et al. [25] between the total content of Zn and CEC, and Nunes et al. [19] between CEC and Cd, Cr, Cu, Ni, Pb and Zn. In the study of Wyszkowski and Modrzewska [21], increasing Zn soil contamination resulted in an increase in soil HAC and a decrease in TEB, in CEC and in the saturation degree of the degree of BS. The chemical properties of metals in soil and their retention in the solid phase of soil are influenced not only by the amount of metal but also by the CEC of the soil [28,48]. According to Senila et al. [18], CEC has a stronger influence on the availability of Cu than that of Cd or Zn. Moreover, the soil sorption complex may also adsorb the heavy metals contained in soil via exchange. The soil sorption increases with increasing CEC [49], which depends on the content of organic matter [33] and the type and content of clay [28]. The type and amount of clay determine the CEC, which increases with the clay content, especially when it contains a high proportion of lattice-type minerals (e.g., montmorillonite) [28]. Changes in the chemical properties of soils affect the concentration of metals in soil solution and it causes changes in their availability to plants [48].

3.4.2. Soil Reaction (pH)

Besides raising HAC, the application of solutions of heavy metals to soil caused a significant decrease in pH (Figure 2). Consequently, a change in soil acidity may contribute to a change in the mobility of trace elements [31,48]. When the pH is increased, the solubility of most metals decreases [28,48].

This effect was particularly manifested when the elements Cd and Pb were applied singly. The lowest soil reaction \((4.46 < \text{pH} < 4.52)\) was determined in series 1 (Cd), 2 (Pb), 3 (Zn) and 4 (Cd + Pb) while significantly elevated soil reaction \((4.69 < \text{pH} < 4.87)\) appeared in series 5 (Cd + Zn), 6 (Pb + Zn) and 7 (Cd + Pb + Zn). Soil reaction plays a pivotal role in immobilisation of trace elements [16,22,23,28]. According to Sungur et al. [20], Navarro-Pedreño et al. [22] and Wieczorek et al. [8] higher soil pH tends to induce a decline in the mobility of toxic metals due to their adsorption, occlusion, and precipitation of hardly soluble metal salts. In turn, Nunes et al. [19] prove that under certain conditions, increased pH may promote the release of metals by mobilizing the components of the soil in which they are retained. As a rule, however, it is assumed that low pH is conducive to the mobility of trace elements in soil [8,11,22]. Elements such as Cd, Co, Cu, Mn, Ni, Pb, and Zn, become more soluble and mobile under acidic conditions [11]. The generally low soil reaction noted in our experiment correlated positively and highly significantly with the mobility of Zn \((r = 0.428^{**})\), Cr \((r = 0.562^{**})\) and Co \((r = 0.539^{**})\) but negatively and likewise highly significantly with the content of mobile forms of Fe \((r = -0.373^{**})\).
and Ni ($r = -0.322^{**}$) (Table 4). There are reports on positive correlations between soil reaction and mobile forms of Cd [12,19,30], Pb [12,13,16,19] and Zn [12,19,22] as well as Cr, Cu [15,19], Mn, Fe [13] and Ni [19]. On the other hand, Senila et al. [18] demonstrated a stronger effect of soil reaction on the bioavailability of Cd than that of Zn and Cu. Other researchers showed a negative relationship between soil reaction and the content of mobile forms of Zn [2,20,48], Mn [2,22], Ni [20] as well as Fe and Cu [22]. However, according to Jaafar et al. [14], there were no significant correlations between soil pH and the content of available forms of Cd, Pb and Ni. Such discrepancies between research results could be a consequence of applying organic matter to soil [13], using different methods to determine mobile forms of metals [14,20,48], the type and duration of soil contamination [2,14] and the level of the reaction in analysed soils [14].

Table 5. Some physicochemical properties of soil.

| Metal Doses in Soil $^a$ | Series of Experiment | Means for Doses |
|-------------------------|----------------------|-----------------|
|                         | Cd | Pb | Zn | Cd + Pb | Cd + Zn | Pb + Zn | Cd + Pb + Zn |
| 0                      | 37.00$^{a,b}$ | 37.00$^{a,b}$ | 37.00$^{a,b}$ | 37.00$^{a,b}$ | 37.00$^a$ | 37.00$^b$ |
| I                      | 38.50$^{b,d}$ | 41.50$^{e,f}$ | 40.50$^{d-f}$ | 39.50$^{a,e}$ | 41.00$^{d-f}$ | 40.50$^{d-f}$ | 41.76$^{d-g}$ | 40.47$^a$ |
| II                     | 40.00$^{c-e}$ | 42.00$^{f-g}$ | 42.00$^{f-g}$ | 38.50$^{a-d}$ | 38.50$^{a-d}$ | 38.50$^{a-d}$ | 39.86$^{b-e}$ | 41.76$^{d-g}$ | 40.23$^a$ |
| III                    | 44.50$^{g,h}$ | 43.00$^{f-h}$ | 42.00$^{f-g}$ | 42.00$^{f-g}$ | 42.00$^{f-g}$ | 42.00$^{f-g}$ | 43.03$^{c-h}$ | 45.56$^{b}$ | 42.94$^c$ |

Means for series 0.00$^{a,b}$ 0.00$^{a,b}$ 0.38$^{a-c}$ 0.00$^a$ 0.39$^{a,b}$ 0.40$^{10,a,b}$ 41.52$^b$ 40.16

r 0.783$^{**}$ 0.753$^*$ 0.853$^{**}$ 0.576 0.522 0.815$^{**}$ 0.939$^{**}$ 0.715$^{**}$

LSD$_{0.05}$ for: metal dose = 0.94; kind of metal = 1.24; interaction = 2.48

|  |  |  |  |  |  |  |
|-------------------------|----------------------|-----------------|
| 0 | 39.33$^c$ | 39.33$^c$ | 39.33$^c$ | 39.33$^c$ | 39.33$^c$ | 39.33$^c$ |
| I | 40.67$^{e-g}$ | 39.33$^c$ | 38.50$^{a-b}$ | 38.50$^{a-b}$ | 38.50$^{a-b}$ | 39.33$^c$ |
| II | 38.67$^{b-c}$ | 36.75$^{e-f}$ | 36.75$^{e-f}$ | 36.75$^{e-f}$ | 36.75$^{e-f}$ | 36.75$^{e-f}$ |
| III | 38.00$^{a-b}$ | 36.75$^{e-f}$ | 36.75$^{e-f}$ | 36.75$^{e-f}$ | 36.75$^{e-f}$ | 36.75$^{e-f}$ |

Means for series 39.17$^a$ 36.17$^{b-c}$ 37.50$^{a-c}$ 37.33$^{a-c}$ 39.30$^{a-c}$ 37.69

r $-$0.282 $-$0.264 $-$0.445 $-$0.185 0.488 $-$0.472 $-$0.198 $-$0.179

LSD$_{0.05}$ for: metal dose = n.s.; kind of metal = 2.67; interaction = 5.35

|  |  |  |  |  |  |  |
|-------------------------|----------------------|-----------------|
| 0 | 76.33$^{b-d}$ | 76.33$^{b-d}$ | 76.33$^{b-d}$ | 76.33$^{b-d}$ | 76.33$^{b-d}$ | 76.33$^{b-d}$ |
| I | 79.17$^{d}$ | 76.17$^{d}$ | 78.17$^{d}$ | 77.00$^{d}$ | 82.30$^{d}$ | 74.03$^{d}$ | 77.81$^a$ |
| II | 78.65$^{d}$ | 75.33$^{a-c}$ | 78.65$^{d}$ | 75.00$^{a-d}$ | 80.66$^{b-d}$ | 75.89$^{b-d}$ | 76.75$^a$ |
| III | 82.50$^{d}$ | 80.33$^{b-d}$ | 78.65$^{d}$ | 81.33$^{c-d}$ | 82.50$^{d}$ | 77.09$^{b-d}$ | 81.16$^{c-d}$ | 80.51$^b$ |

Means for series 79.17$^a$ 77.04$^a$ 77.88$^a$ 76.33$^a$ 78.58$^a$ 79.10$^a$ 76.85$^a$ 77.85

r 0.502 $-$0.440 0.368 $-$0.113 $-$0.813$^{**}$ $-$0.006 0.332 0.302$^{**}$

LSD$_{0.05}$ for: metal dose = 2.13; kind of metal = n.s.; interaction = n.s.

|  |  |  |  |  |  |  |
|-------------------------|----------------------|-----------------|
| 0 | 51.50$^a$ | 51.50$^a$ | 51.50$^a$ | 51.50$^a$ | 51.50$^a$ | 51.50$^a$ |
| I | 51.41$^{a}$ | 47.92$^{d}$ | 49.48$^{d}$ | 46.73$^{a-e}$ | 50.71$^{d-e}$ | 43.38$^a$ | 47.87$^a$ |
| II | 49.05$^{a-e}$ | 44.23$^{b}$ | 46.60$^{a}$ | 45.85$^{a-d}$ | 50.94$^{d}$ | 50.53$^{d-e}$ | 44.93$^{a-e}$ | 47.45$^a$ |
| III | 46.10$^{a-c}$ | 46.48$^{a}$ | 46.58$^{a}$ | 48.38$^{b}$ | 50.89$^{a-e}$ | 44.16$^{a}$ | 43.37$^{a}$ | 46.57$^a$ |

Means for series 49.52$^a$ 46.92$^{b}$ 48.15$^{b}$ 48.80$^{a,b}$ 50.02$^a$ 49.23$^a$ 45.80$^a$ 48.35

r $-$0.760$^*$ $-$0.529 $-$0.740$^*$ $-$0.524 0.111 $-$0.694$^*$ $-$0.536 $-$0.481$^{**}$

LSD$_{0.05}$ for: metal dose = 1.56; kind of metal = 2.07; interaction = 4.14

\* dose size explained in the methodology; correlation coefficient ($r$) $^*$—significant for $p = 0.05$; $^{**}$—highly significant for $p = 0.01$; $^{a,b}$—of significant; n = 12; the letters from “$^{a}$” to “$^{b}$” differentiate homogeneous groups, means followed by the same letter do not differ at $p = 0.05$ by the LSD test.
Figure 2. Values of electrolytic conductivity (EC) and reaction (pH) of soils. ■ control (0); ■ first metal dose (I); ■ second metal dose (II); ■ third metal dose (III); Series of experiment: 1—Cd, 2—Pb, 3—Zn, 4—Cd + Pb, 5—Cd + Zn, 6—Pb + Zn, 7—Cd + Pb + Zn; correlation coefficient (r) **—significant for p = 0.05; ***—highly significant for p = 0.01; n.s.—not significant; n = 12; the letters from "a" to "d" differentiate homogeneous groups, means followed by the same letter do not differ at p = 0.05 by the LSD test.

3.4.3. Soil Salinity (EC)

Retention of various contaminants by soil modifies its physicochemical properties, including not only pH, but also conductivity [4]. Soil salinity has a direct impact on the solubility and mobility of heavy metals in soil [23]. The solutions of metals added to soil had significant albeit different effects on soil salinity (Figure 2). Cadmium sulphate and lead acetate significantly depressed the salinity of soil (−0.80** < r < −0.65*), unlike zinc sulphate, which effected a highly significant increase in soil salinity (0.96** < r < 0.97**). In series 3 (Zn), 5 (Cd + Zn), 6 (Pb + Zn) and 7 (Cd + Pb + Zn), where Zn was another soil contaminating metals, soil salinity was 3-fold higher on average than in series 1 (Cd), 2 (Pb) and 4 (Cd + Pb), without zinc as a soil pollutant. The growing soil pollution with Zn significantly raised the salinity of soil. On the other hand, high EC was positively and highly significantly connected with the content of the mobile forms of Zn (r = 0.962**), Cr (r = 0.646**), and Co (r = 0.559**), while being significantly connected with mobile Pb (r = 0.242*) (Table 4). Additionally, a highly significant but negative correlation was noted between soil salinity and the content of the mobile forms of Mn (r = −0.462**) and Fe (r = −0.531**). The increased mobility of heavy metals demonstrated in own research with increasing soil salinity is confirmed by the work of other authors [19,24]. Cited authors [19,24] showed significant correlations between salinity and mobility of Cd [19,24] and Pb [19]. Similarly, the Kirkham [47] study found that adding chlorides to soil increases the availability of cadmium. In contrast, Jaafar et al. [14] did not note any significant relationship between the mobility of Cd, Pb and Ni versus EC. According to Yan et al. [23], higher salt concentrations in soil can lead to an increase in soil pH, which was observed in our own studies in series with Cd + Zn, Pb + Zn and Cd + Pb + Zn, in which a higher EC value coincided with a much higher soil response.

4. Conclusions

According to the assumed hypothesis, the application to the soil of increasing doses of Cd, Pb and Zn significantly stimulated a rise in the content of mobile forms of these elements. Higher mobility of Cd and Pb was reported in series where zinc sulphate was added. Moreover, the application of Cd and Pb to soil resulted in a rise in the share of
potentially available forms of these metals. The AF in those series was significantly higher than in the control and was in the range 18–20% for Cd and 63–72% for Pb. The percentage of mobile forms of Zn in most samples oscillated around 30%.

The effect of the application of Cd, Pb and Zn to the soil on the content of available forms of Fe, Mn, Cu, Co, Cr and Ni was more complex. The results of the research indicate that in objects with increased content of Cd, Pb and Zn, one can expect a reduced availability of Fe, Mn and Cu in the soil, which is important from the point of view of nutrition, and an increased mobility of Cr. The excessive content of Cr in soil may turn out to be harmful.

The observed dependencies may be related to the used form of metal solutions, among which sulphates (cadmium sulphate and zinc sulphate) were a major part. The application of these solutions to the soil determined the increase in soil EC and HAC and the decrease in soil pH in most objects of the experiment. These properties were generally positively correlated with the content of the available forms of Zn, Cd, Pb, Co and Cr, and negatively with the content of the available forms of Fe, Mn and Cu in the soil.

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