Changes in Speciation and Bioavailability of Trace Elements in Sewage Sludge after the Ozonation Process

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Abstract: This work aimed to detect changes in trace element chemical speciation in sewage sludge (SS) after the ozonation process. The modified Community Bureau of Reference (BCR) sequential extraction procedure was performed to determine the chemical speciation of trace elements in SS after the ozonation process. To assess potential soil contamination with trace elements from sewage sludge after the ozonation process, the risk assessment code (RAC) coefficient was used. The bioaccumulation factor (BAF) and translocation factor (TF) values were also calculated to characterize the efficiency of trace element accumulation in the studied plant species from soil fertilized with sludges after the ozonation process. Generally, the mean concentration of total trace elements in the SS after the ozonation process was higher, but the differences were statistically significant only in the case of Mn, Cu, Pb, and Cd. The dominant fraction of Fe, Cr, Pb, and Cd was the residual fraction F4, while the extractable/exchangeable fraction F1 was present in the smallest amount. Therefore, in the case of Mn, Zn, and Ni, the ozonation process had a significant impact on the increase in the content of these elements in the F1 fraction. The application of the SS stabilized by ozonation process for maize and wheat fertilization did not significantly affect the bioaccumulation of most of the analyzed metals in aboveground biomass. Higher values of BAF coefficients after the application of ozonated SS were found only in the case of Cu and Ni. In turn, the determined TF coefficients were lower than 1 in most cases. The obtained results showed that the slight change in the concentration of Zn, Mn, and Ni in fraction F1 causes a specific risk of their mobility in the soil environment. It should be noted that due to the variable composition of sewage sludge, an analysis of the content of individual trace elements in chemical fractions should be carried out to assess its actual impact on the environment. This can help to indicate further actions that should be undertaken to limit their negative impact on the environment.

Keywords: sewage sludge; ozonation process; trace element; crop plants; BCR procedure

1. Introduction

Sewage sludge (SS) is a byproduct of the wastewater treatment process, which threatens the environment [1]. The cost of disposal of excess sludge accounts for 25–60% of the total operating cost of the wastewater treatment plant. With its properties, sewage sludge is a waste material that must be adequately managed and disposed of due to the restrictive legal regulations regarding environmental protection [2,3].

Agricultural application of sewage sludge (SS), e.g., for fertilization of plants, is an alternative method for its management and final disposal [2–6]. It is an interesting trend due to the possibility of recycling some important components such as organic matter, nitrogen,
phosphorus, and also other plant nutrients [1,3,6]. Thus, SS can be utilized for managing the soil fertility of agricultural land instead of mineral fertilizers [7]. Municipal sewage sludge from small wastewater treatment plants is especially suitable for agricultural use [4]. In Poland, a large part of sewage sludge is used in agriculture, and this trend has been going on for many years [8]. Many studies indicate that fertilization with SS has a positive effect on the growth and development of various crop plant species [4,5,7]. As reported by Moreira et al. (2020), SS application caused a significant increase in maize physiological parameters, compared to mineral fertilization [9]. Boudjabi et al. (2015) found similar results for wheat [10]. Despite the many positive effects of SS application to soil, they may be restricted by the presence of heavy metals, organic compounds, pharmaceuticals, and pathogens which may be potentially toxic to the environment [3,6,11,12]. The use of SS in agriculture is regulated in the EU by the Sewage Sludge Directive, which establishes maximum limits for six metals in sludge [13]. Thus, it is necessary to reduce trace element content and investigate its chemical forms in SS for safe land application [9].

The ozonation of SS is used as one of the methods for its stabilization. The use of ozonation to reduce the amounts of SS was proposed in the mid-1990s [14]. The main effect of SS ozonation is the destruction of flocs and the reduction of sludge particles. Sewage sludge, after the ozonation process, yields minor toxic byproducts. Sewage sludge stabilized with ozone had a higher dry matter content than non-ozonated sludge. Higher dry matter content is directly related to the efficiency of this process [1,15–19]. Despite the effectiveness of ozone in sanitation and reduction of the amount of SS and in the improvement of many parameters, still little is known about changes in the distribution of the chemical fractions of trace elements after the ozonation process [15]. Measurements of total metal concentrations in SS are useful for evaluating the trace element burden, e.g., for determining the possibility of SS use in accordance with applicable law. However, the mobility, bioavailability, and related environmental risk of trace elements in sewage sludge depend on the chemical speciation rather than on the total concentration. For this reason, sequential extraction procedures are commonly applied because they provide information about the environmental contamination risk [20]. Assessment of the content of individual fractions of trace elements in SS, and also after the ozonation process, is essential, mainly when SS is used for agricultural purposes. Such action reduces the toxic effect caused by the excess of individual elements in the soil and, consequently, the negative impact on plants [8]. According to the Community Bureau of Reference (BCR) extraction procedure, four fractions of trace elements are present in SS [21]. The first is the exchangeable/extractable fraction (F1) characterized by the highest bioavailability. The other trace element fractions in SS are reducible fractions bound to Fe-Mn oxides (F2) and an oxidizable fraction bound to organic matter (F3). Finally, the residual fraction (F4) is stably bound in the crystal lattice and potentially not bioavailable for plants and other organisms [22–24].

As little is known about the possibilities of using sewage sludge after the ozonation process for agricultural purposes, especially for fertilization for crop plants, this work aimed to analyze the effect of ozone-stabilized SS on the bioavailability and accumulation of trace elements in crop plant biomass in pot experiment conditions. Changes in trace elements’ chemical speciation in SS after the ozonation process and their environmental risk were assessed. Induced by the ozonation of SS, a change in speciation of trace elements is an important element of novelty and allows us to estimate the impact of this material as a fertilizer.
2. Materials and Methods

2.1. Sewage Sludge Ozonation Process and Sample Preparation

The experiment was based on the use of municipal sewage sludge from a mechanical and biological wastewater treatment plant. Detailed characteristics of the SS used in the experiment were given in previous work [5]. According to the results presented in a previous paper [17], SS for the experiment was ozonated for 60 min. Sewage sludge that was not ozonated (SS_N) and sewage sludge after the ozonation process (SS_O) were used in pot experiments. To analyze changes in the concentration of trace elements in SS_N and SS_O, samples were taken after the gravitational thickening process and dried at a temperature of about 80 °C in a laboratory dryer with forced air circulation until a constant weight was obtained. The samples prepared in this way were homogenized using a laboratory mill and subjected to further analysis.

2.2. BCR Extraction Procedure for Sewage Sludge Samples

The modified Community Bureau of Reference (BCR) sequential extraction procedure was used for the fractionation of Fe, Mn, Zn, Cu, Ni, Cr, Pb, and Cd in the sewage sludge samples [22–25]. Approximately 1.0 g samples of SS_O and SS_N dry samples were used to determine the chemical speciation of trace elements (exchangeable/extractable fraction (F1); reducible fraction bound with Fe-Mn oxides (F2); oxidizable fraction bound to organic matter (F3); residual fraction (F4)) and the total concentration of trace elements [22–25]. The scheme used in the BCR procedure is described in the Supplementary Materials (S1). The concentrations of Fe, Mn, Zn, Cu, Ni, Cr, Pb, and Cd in all obtained solutions were determined with the atomic spectrometry method using the HITACHI Z-2000 apparatus (Tokyo, Japan).

2.3. Recovery Rate of Trace Elements from Sewage Sludge Samples

The recovery rate (RR) is a factor determining the precision of the method used [16]. The details of the RR calculation are described in the Supplementary Materials (S2).

2.4. Risk Assessment Code (RAC) Index

The RAC value was calculated to assess the environmental risk posed by the trace elements. It was used to assess potential soil contamination with trace elements from sewage sludge after the ozonation process. The details of the RAC index calculation are described in the Supplementary Materials (S3) [16,25,26].

2.5. Pot Experiment Design

The research also assessed the effect of ozone-stabilized sludge on the bioaccumulation of trace elements by selected crop plant species—maize and wheat. This assessment was made in a pot experiment carried out in three independent series corresponding to the dates of sewage sludge collection. All the experiment details are described comprehensively in the previous work [5] and also in the Supplementary Materials (S4).

2.6. Plant Material Samples and Analysis

At the end of the individual series of pot experiments, the plants were carefully removed from the pots. Then, aboveground parts were separated from roots. The roots were rinsed in ultra-pure water. Next, the aboveground biomass and roots were dried in the laboratory dryer at 65 °C. After that, each part was homogenized in the laboratory grinding mill. The total concentration of trace elements was analyzed in the obtained samples. About 0.5 g of dry plant samples was taken for further analysis and then 10 mL of HNO₃ and 2 mL of H₂O₂ were added. The contents were heated at 105 °C for 1 h, 120 °C for 2 h, and 160 °C for 2 h. After cooling to room temperature, the residue was dissolved in ultra-pure water with a total volume of 25 mL. The concentrations of Fe, Mn, Zn, Cu, Ni, Cr, Pb, and Cd in all the solutions were determined with the atomic spectrometry method (Hitachi, Z-2000) [5]. To characterize the efficiency of accumulation of the analyzed heavy metals in
the studied plant species from soil fertilized by sewage sludge, the bioaccumulation factor (BAF) and translocation factor (TF) values were calculated. The BAF was calculated as a ratio of the element concentration in the aboveground plant organs to their concentrations in the soil. The TF was calculated as a ratio of the concentrations of the heavy metals in aboveground biomass to their concentrations in roots [27].

2.7. Statistical Analysis

The results were statistically analyzed using the Statistica 13.3 software (StatSoft, Tulsa, Oklahoma, OK, USA). The changes in the chemical speciation and the total content of trace elements in the SS and also in plants, depending on the dose and variant of the SS applied, were assessed with the two-way analysis of variance (ANOVA) at the $p < 0.05$ significance level and the post hoc Tukey test (HDS).

3. Results and Discussion

3.1. Changes in Total Concentrations and Chemical Speciation of Trace Elements in Sewage Sludge after the Ozonation Process

As shown by available data, the total contents of Cu, Zn, Ni, Pb, Cr, and Cd undergo slight fluctuations after the ozonation process [16]. The initial treatment of sewage sludge induces an increase in the content of the total forms of trace elements, and their concentration may increase with the increasing doses of ozone [17]. This was also confirmed in our research. The average contents of Fe, Zn, Mn, Cu, Pb, Cr, Ni, and Cd in SS_N and SS_O were 6204 and 6995, 126.9 and 135.1, 726 and 735, 163 and 176, 7.73 and 8.25, 12.4 and 12.7, 12.4 and 13.8, 0.56, and 0.63 mg kg$^{-1}$, respectively (Figure 1A–H). Generally, the mean concentration of total trace elements in the SS after the ozonation process was higher, but the noted differences were statistically significant only in the case of Mn, Cu, Pb, and Cd (Figure 1A–H). However, in both analyzed sludge variants (SS_N, SS_O), the mean content of the selected elements decreased in the following order: Fe > Zn > Mn > Cu > Pb > Cr > Ni > Cd, which means minor changes (Figure 1A–H). As reported by He et al. (2021), the increase in the total content of trace elements after the ozonation process confirms the effectiveness of ozone in the disintegration of sludge flocs, as a result of which its properties are improved [17]. However, the mobility, bioavailability, and related environmental risk of trace elements in sewage sludge depend on the chemical speciation rather than on the total concentration [28]. Therefore, changes in the environmental risk will depend on BCR extraction results [21,29,30].

Regardless of the sewage sludge stabilization method, the dominant fraction of Fe, Cr, Pb, and Cd was the residual fraction F4, and the F1 fraction was present in the smallest amount. The opposite trend was observed in the case of Mn, Zn, and Ni. In the case of Cu, the F3 fraction had the largest share, while the F2 fraction accounted for the smallest percentage. Copper mainly creates complex connections with organic matter; hence, it is usually most abundant in the F3 fraction [16]. In turn, the smallest share of Cr was found in fraction F2, while the largest amount was detected in fraction F4.
Figure 1. Total concentration and chemical speciation of trace elements Fe (A), Mn (B), Zn (C), Cu (D), Ni (E), Cr (F), Pb (G), Cd (H), and percentage share of individual fraction (I) in non-ozonated (SS_N) and ozonated (SS_O) sewage sludge. Mean values ($n = 18$) ± standard error. Identical letters denote no significant ($p < 0.05$) differences between the analyzed experimental variants according to the post hoc Tukey HSD test.
As a result of the ozonation process, there were some changes in the content of individual fractions of the analyzed trace elements determined with the BCR method (Figure 1A–I). In SS_O, compared to SS_N, there was an increase in the Fe content in fractions F2 and F4 (by 1 and 19%, respectively) with a slight decrease in its content in fractions F1 and F3 (by 3 and 5.5%, respectively) (Figure 1A,I). Similar relationships were also found for Cu, Cr, Pb, and Cd. In SS_O, the share of the Cu fraction F1 decreased by 18%, while the F2, F3, and F4 fractions increased by 2, 8, and 15%, respectively (Figure 1D,I). In turn, the Cr content in SS_O compared to SS_N increased only in fraction F4 (by 39%), while its share in the other isolated fractions, F1, F2, and F3, decreased (by 10, 12, and 2%, respectively) (Figure 1F,I). In turn, the content of Pb and Cd in SS_O, compared to SS_N, decreased in the F1 fraction by 12 and 3.7%, respectively, and increased in the other determined fractions (Figure 1G–I). In SS_O, compared to SS_N, the content of fraction F1 of Mn, Zn, and Ni increased by 11, 4, and 5%, respectively. In the case of Mn and Zn, an increase in the content of fractions F2 and F3 was found after ozonation (by 16 and 12% as well as 28 and 26%, respectively), while the content of fraction F4 decreased (by 13.6 and 32%). In most cases, however, the changes described were statistically insignificant, and statistically significant differences were found only in the case of Mn, Zn, and Ni (Figure 1B,C,E). The applied ozonation process had a significant impact on the increase, especially in the content of the F1 fraction of Mn, Zn, and Ni, as mentioned above. The metal ion existing in this form is in a weak state and is easily affected by environmental conditions; hence, the mobility of the acid-soluble/exchangeable fraction (F1) is strong and the ozonation process may increase the solubility [16].

The observed changes in the content of the individual fractions of elements probably result from the conversion of organic matter contained in sewage sludge caused by the ozonation process [17].

The recovery rate is a factor determining the precision of the BCR method used [19]. Good recoveries in the range of 94.66–99.43% were obtained (Figure 2). This means that the sum of the concentrations of the four separated fractions was consistent with the total concentration of trace elements determined in the sewage sludge. Based on these results, it was shown that the BCR procedure used in these studies for the separation of individual fractions of trace elements in sewage sludge (SS_O and SS_N) was adequate, which is also confirmed in studies conducted by other authors [16,31].

![Figure 2. Recovery rate of trace elements from sewage sludge samples (%).](image-url)
3.2. Influence of the Process of Sewage Sludge Ozonation on the Risk Assessment Code (RAC) Index

The RAC indices determined based on the average content of the F1 fraction and the total content of trace elements in the sewage sludge used in the experiment varied (Figure 3).

![Figure 3. Risk assessment code (RAC) for sewage sludge used in the experiment. <1 no risk; 1–10 low risk; 11–30 medium risk; 30–50 high risk; >50 very high risk [16,26,27].](image)

The total content of heavy metal forms in the sewage sludge used in the experiment was well below the lower limit of concentrations specified by law [32]. According to this document, the sludge used in the experiment could be used for agricultural purposes, including food and feed production. Despite the relatively low concentrations of the determined metals, the RAC factors clearly show a potential risk to the environment associated with the agricultural use of the sewage sludge, regardless of the form of stabilization used. The RAC coefficients were different for particular metals and resulted mainly from the percentage share of individual fractions, including the main F1, in the total content of these elements. In the case of Fe, Cu, Cr, and Pb, the RAC values were in the range of 1–10, which indicated that the sewage sludge used in the experiment was characterized by low risk for the environment. In the case of these metals, a decrease in the RAC value was also observed after the ozonation process, and the determined coefficients for SS_N and SS_O were Fe 9.01 and 7.76, Cu 8.14 and 6.21, Cr 9.63 and 8.39, and Pb 6.66 and 5.32, respectively (Figure 3). The most significant risk of using the sewage sludge in the experiment was associated with introducing such metals as Mn, Zn, Ni, and Cd into the soil. The RAC values in SS_N and SS_O were 47.6 and 41.4, respectively, for Cd and 48.9 and 48.0, respectively, for Ni, indicating a potentially high environmental risk. In the case of Mn and Zn, the RAC values for SS_N and SS_O were 70.3 and 73.4 as well as 54.4 and 55.8, respectively, indicating a very high risk of environmental contamination. In turn, the ozonation process slightly increased the RAC in the case of Mn and Zn and decreased the value of this indicator in the case of Cd. However, in the case of Ni, these values were at a similar level. Despite the differences in the RAC values and the share of the F1 fraction of the analyzed elements, the potential environmental risk associated with the natural use of SS_N and SS_O was similar. This suggests that the ozonation process used in our experiments significantly improves the properties of sewage sludge [17]. At the same time, it does not cause significant changes in the solubility of trace elements. The agricultural use of such sludge, provided that specific legal regulations are met, should not increase the migration of trace elements. However, as shown in other studies, the use of sewage sludge after the ozonation process increases...
the environmental risk due to increased mobility, especially of Mn, Ni, Zn, and Cd. It is therefore suggested that sewage sludge after the ozonation process should be pretreated before other applications [16].

3.3. Bioaccumulation and Translocation of Fe, Mn, Zn, Cu, Ni, Cr, Pb, and Cd in Maize and Wheat after Application of Sewage Sludge

The concentrations of Fe, Mn, Zn, Cu, Ni, Cr, Pb, and Cd in the aboveground biomass and roots of maize and wheat are presented in Figures S1–S4 in the Supplementary Materials. In the present study, the concentration of most analyzed trace elements was higher in the roots than in the aboveground biomass both in maize and wheat (Figures S1–S4). It is well known that trace elements are retained to a greater extent within the root zone of plants, and their translocation to various parts of plants depends on their chemical form or the plant species [33,34].

The evaluation of the amount of trace elements transferred to plants is of fundamental importance for risk assessment and environmental regulation [27,35]. It was reported to indicate the trace element contamination status of the site and revealed the abilities of various plant species to take up and accumulate elements originating from soil amendment with sewage sludge [34]. It is well known that the use of sewage sludge causes increased bioaccumulation of trace elements in various plant tissues, as indicated by many studies conducted in laboratory and field conditions [2–4,6,11,34]. The risk associated with the natural use of sewage sludge is mainly related to the introduction of large amounts into the soil, especially Zn and Cd, which are present in sewage sludge in the most mobile forms and are thus bioavailable [28]. Due to the high bioavailability of these elements, they easily enter the food chain, in which humans are the last link. As mentioned earlier, little is known about the effect of the sewage sludge ozonation process on changes in the fractional composition of trace elements contained therein, and thus on their bioaccumulation in plants. It is also worth mentioning that changes in the mobility and solubility of various trace elements contained in sewage sludge after their introduction into soils result from many of their properties, such as pH or organic matter content [28]. Since the conducted research involved the application of sewage sludge in the surface layer of sandy soil characterized by low content of organic matter and the period of the experiment was short, it was concluded that the obtained results reflect the direct impact of sewage sludge on the accumulation of metals in the aboveground and underground parts of plants. The bioaccumulation factors (BAF) and translocation factors (TF) were determined from the average content of Fe, Mn, Zn, Cu, Ni, Cr, Pb, and Cd in the aboveground biomass of maize and wheat (Figures S1–S4) and the content of these elements in the soil (Tables S1 and S2) exhibited considerable variation (Figures 4 and 5).

The values of the BAF coefficients were dependent mainly on the species of the cultivated plant and on the dose of sewage sludge used in the experiment, which is consistent with studies conducted by other authors [36]. Generally, a higher accumulation of the analyzed metals was found in the aboveground parts of wheat than in maize (Figure 4A–H). Similar results were obtained by Yang et al. (2018), who found that wheat was a more sensitive indicator of sewage sludge amendment than maize [37]. Jalali et al. (2022) found that among elements present in soil, Pb, Cd, and Cu were most easily absorbed by wheat roots, which was explained by the content of individual fractions of these metals in the soil [6]. The BAF determined in the present study for the aboveground biomass of maize and wheat had the highest values for Cu, Cd, Zn, Mn, and Ni (BAF > 1) and the lowest values for Fe, Cr, and Pb (BAF < 1) (Figure 4A–H). BAF values > 1 indicate intensive accumulation of Cu, Cd, Zn, Mn, and Ni, while BAF < 1 indicates low accumulation of Fe, Cr, and Pb [34]. In general, Pb is characterized by low mobility and is hardly available to plants. Nevertheless, in the case of significant soil contamination, it has a stimulating effect on the uptake of Cd, Ni, Mn, and Zn, most likely due to the secondary effect of damaging the selective function of biological membranes. The use of sewage sludge contributes to
an increase in lead content both in underground and aboveground parts of plants [11,12], which was also confirmed in our research.

Figure 4. Bioaccumulation factors (BAFs) of Fe (A), Mn (B), Zn (C), Cu (D), Ni (E), Cr (F), Pb (G), and Cd (H) in aboveground biomass of maize and wheat plants relative to the dose and type of sewage sludge. Mean values ($n = 9$) ± standard error. Identical letters denote no significant ($p < 0.05$) differences between the experimental objects according to the post hoc Tukey HSD test. Explanation: SS_N—non-ozonated sewage sludge; SS_O—ozonated sewage sludge; D1, D2, D3—dose of sewage sludge—3, 6, 9 Mg ha$^{-1}$, respectively.
Figure 5. Translocation factors (TFs) of Fe (A), Mn (B), Zn (C), Cu (D), Ni (E), Cr (F), Pb (G), and Cd (H) in maize and wheat depending on the dose and type of sewage sludge used in the experiment. Mean values (n = 9) ± standard error. Identical letters denote no significant (p < 0.05) differences between the experimental objects according to the post hoc Tukey HSD test. Explanation: SS_N—non-ozonated sewage sludge; SS_O—ozonated sewage sludge; D1, D2, D3—dose of sewage sludge—3, 6, 9 Mg ha⁻¹, respectively.

The values of the BAF coefficients were dependent mainly on the species of the cultivated plant and on the dose of sewage sludge used in the experiment, which is consistent with studies conducted by other authors [36]. Generally, a higher accumulation of the analyzed metals was found in the aboveground parts of wheat than in maize (Figure 4A–H). Similar results were obtained by Yang et al. (2018), who found that wheat was a more sensitive indicator of sewage sludge amendment than maize [37]. Jalali et al. (2022) found that among elements present in soil, Pb, Cd, and Cu were most easily absorbed by wheat roots, which was explained by the content of individual fractions of these metals in the soil [6]. The BAF determined in the present study for the aboveground biomass of maize and wheat had the highest values for Cu, Cd, Zn, Mn, and Ni (BAF > 1) and the lowest values for Fe, Cr, and Pb (BAF < 1) (Figure 4A–H). BAF values > 1 indicate intensive accumulation of Cu, Cd, Zn, Mn, and Ni, while BAF < 1 indicates low accumulation of Fe, Cr, and Pb [34]. In general, Pb is characterized by low mobility and is hardly available to plants. Nevertheless, in the case of significant soil contamination, it has a stimulating effect.
The application of the ozonation process did not significantly affect the bioaccumulation of most of the analyzed metals. Higher values of BAF coefficients in the SS_O variant were found only in the case of Cu and Ni (Figure 4D,E). The accumulation of individual elements in the aboveground parts of plants and in the root zone was undoubtedly influenced by the content of the individual fractions of the analyzed metals in the applied sewage sludge. In most cases, no increase in the mobility of the determined elements was found after the ozonation process. An increase in the mobile F1 fraction was noted only for Mn, Zn, and Ni, but only Ni was characterized by increased bioaccumulation in both tested plants, compared to SS_N (Figure 1B–E).

In turn, the determined TF coefficients were lower than 1 in most cases. They reached a value above 1 only in the case of Fe and Mn. TF > 1.0 indicates that the plant is effective in the translocation of trace elements from the root to the shoot [32]. Differences in TF values may be related to the interaction of trace elements. The synergism and antagonism reactions of metals with other elements can affect the bioavailability of metals in soil [28,34]. These processes may affect the efficiency of trace element uptake by plant roots and their further distribution to the aboveground parts. Various translocations of trace elements from the soil are also associated primarily with the form occurrence of metals in the soil and the physiological processes of plants. In the case of trace elements contamination, plant processes controlling metal contents in the aboveground parts of plants are activated [34]. As mentioned earlier, trace elements contained in sewage sludge mostly accumulated in the roots of both tested plants. It also should be assumed that, with the progressing decomposition of organic matter contained in sewage sludge, the rate of metal release will be higher, which may increase both bioaccumulation and translocation coefficients.

4. Conclusions

The obtained results revealed the following findings:

1. The use of the ozonation process to stabilize sewage sludge did not cause significant changes in the content of individual fractions of the analyzed trace elements determined by the BCR method. Generally, the dominant fraction in SS after the ozonation process was the residual fraction F4, while the extractable/exchangeable fraction F1 was present in the smallest amount.

2. It should be noted that the ozonation process had a significant impact on the increase in the content of Mn, Zn, and Ni in the F1 fraction in SS, which may pose a specific risk of their increased mobility after introduction to the soil environment.

3. The bioaccumulation of the trace elements in the aboveground biomass of analyzed plants was much more dependent on the crop plant species and the dose of sewage sludge used in the experiment rather than on the ozonation process used for sewage sludge stabilization.

4. The mobility, bioavailability, and related environmental risk of trace elements in sewage sludge depend on the chemical speciation rather than the total concentration. For this reason, sequential extraction procedures should be applied because they provide information about the environmental contamination risk. Assessment of the content of individual fractions of trace elements in SS, and also after the ozonation process, is essential, mainly when SS is used for agricultural purposes. This may be used to reduce the toxic effect caused by the excess of individual elements in the soil and, consequently, the negative impact on plants.
Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13040794/s1, The BCR procedure scheme (S1); The recovery rate of trace elements from sewage sludge samples (S2); Risk assessment code (RAC) index calculation (S3); Pot experiment designed (S4); Content of Fe, Mn, and Zn in the aboveground biomass of maize (A) and wheat (B) plants relative to the dose and type of sewage sludge (Figure S1); Content of Cu, Ni, Cr, Pb, and Cd in the aboveground biomass of maize (A) and wheat (B) plants relative to the dose and type of sewage sludge (Figure S2); Content of Fe, Mn, and Zn in the roots of maize (A) and wheat (B) plants relative to the dose and type of sewage sludge (Figure S3); Content of Cu, Ni, Cr, Pb, and Cd in the roots of maize (A) and wheat (B) plants relative to the dose and type of sewage sludge (Figure S4); The mean concentration of heavy metals in soil under maize cultivation relative to the dose and type of sewage sludge (Table S1); The mean concentration of heavy metals in soil under wheat cultivation relative to the dose and type of sewage sludge (Table S2).

Author Contributions: M.S., conceptualization, investigation, data curation, writing—original draft preparation, writing—review and editing, methodology, data curation, visualization; N.M., investigation, data curation, writing—review and editing; P.K., investigation; A.I., visualization, formal analysis; M.B., investigation, methodology. All authors have read and agreed to the published version of the manuscript.

Funding: The present research and open access fees were partially funded with a subsidy to maintain the Department of Food and Agriculture Production Engineering research potential of the University of Rzeszów for 2023.

Data Availability Statement: The entire set of raw data presented in this study is available on request from the corresponding author.

Acknowledgments: The authors would like to thank anonymous reviewers for the thorough assessment of the present paper and their many valuable and helpful suggestions.

Conflicts of Interest: The authors declare no conflict of interest.

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