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# The Effect of Compost and Fly Ash Treatment of Contaminated Soil on the Immobilisation and Bioavailability of Lead

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**Abstract:** The study discusses changes in lead contents in soil and crops after application of compost and fly ash. A three-year experiment was conducted on narrow-leaved lupine (*Lupinus angustifolius* L.), camelina (*Camelina sativa* L.) and oat (*Avena sativa* L.), which were grown on medium soil fertilised with compost or fly ash (FA). The lead amounts in various combinations were evaluated using sequential (BCR analysis) and single (DTPA solution) methods. The total contents of lead and amounts of selected macronutrients were assessed in the biomass of plants. The Pb contents in the soil and plants were used to calculate the risk assessment code (RAC), individual contamination factor (ICF), bioconcentration factor (BCF) and contamination coefficient level (CCL). Lower amounts of bioavailable Pb in the soil were observed as a result of compost and FA application. Fly ash immobilised Pb more effectively, as evidenced by the BCF and CCL values. The increase in the Pb contents in the sequentially separated fractions and in plant biomass were caused by applied compost or FA. The Pb contents in exchangeable forms decreased, whereas its contents in residual forms increased.

**Keywords:** sequential and single methods; stabilising agents; plant uptake; risk assessment code (RAC); individual contaminated factor (ICF); bioconcentration factor (BCF); coefficient of contamination level (CCL)

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## 1. Introduction

In recent decades heavy metal contamination has become a global problem and was listed as a threat to soil in The Thematic Strategy for Soil Protection—a document published by the European Commission in 2006 and updated in 2012 [1]. Oil drilling, mining, smelting, improper disposal of sewage sludge and other human activities have heavily polluted soil with various heavy metals and considerably reduced its fertility [2]. Lead is a well-known heavy metal. This element is considered as the most problematic and toxic heavy metal among toxic threats among all the substances on earth because of its risk of global health impact [3]. Authors of recent studies have emphasised its negative impact on the quality of crops, its easy incorporation in the food chain and the resulting threats to human health [4,5]. Although recent findings suggest that trace amounts of Pb (approx. 29 ng/g diet) are essential for enzyme activity and proper functioning of the cellular system, even very low amounts of this element consumed by humans and animals can be dangerous for their health [4,6]. Soil contamination with lead mostly results from smelting and mining processes, the use of fertilisers and pesticides. Moreover, excessive amounts of lead enter the soil environment as a result of emissions and immission of atmospheric dust and exhaust gases [4]. In view of the need to protect the soil environment and produce uncontaminated food, it is necessary to control agricultural soils for their Pb content. The natural soil background Pb level is 25–40 $mg \cdot kg^{-1}$ [7]. According to the Regulation of the Minister of the Environment [8], in Poland the admissible Pb content in agricultural soils is 100 to 500 mg·kg$^{-1}$, depending on the soil texture and pH. It is well-known that soils contaminated with heavy metals should be remediated; thus, various remediation techniques are proposed for soils contaminated with heavy metals, including Pb. According to [4,5], physical, chemical and biological processes reducing the mobility and bioavailability of Pb have been developed. One of them is in situ chemical immobilisation/stabilisation through the application of inexpensive inorganic and/or organic amendments such as lime, phosphates, clays, fly ash, organic matter and activated carbon to maintain long-term stability of heavy metals and reduce their toxicity in soils [2,9]. Chen et al. [2] also stressed the fact that stabilisation technology is particularly useful for soil remediation because of its strong compatibility with pollutants, cost effectiveness and lack of adverse effects on the environment.

The mobility of hazardous metals such as Pb strongly depends on their chemical form rather than total concentration. The redistribution and bioavailability of potentially toxic elements in soils can be estimated with several analytical tests, including single and sequential extraction procedures [10]. In general, selective sequential extraction methods have been used extensively to separate metals in various chemical pools, including soluble, adsorbed, organic and residual forms [9]. According to [11], sequential extraction provides information on the association of heavy metals with different soil phases and helps to determine their distribution in different fractions as well as their mobility and toxicity. Metal amounts found in water-soluble and exchangeable forms are readily available to plants, whereas heavy metals incorporated into crystal lattices of clays are inert. Other forms precipitated as carbonate bound, Fe-Mn oxides bound and organic matter bound are considered relatively less active or firmly bound, depending on the physical and chemical properties of soils [11]. Despite many advantages, sequential methods are more time-consuming than one-step (single) methods. Single methods enable estimation of the amounts of bioavailable or available metals. Bioavailability is a very complex concept, but generally refers to the dissolved metal fraction which can be taken up by plant roots and soil organisms. Bioavailability is connected with the physical dispersion and chemical mobility of metals as well as the organism's exposure level, biological characteristics and individual sensitivity. The issue of metal bioavailability was discussed in detail in a review study [12]. Among the single extraction procedures, DTPA and EDTA are used to estimate the bioavailability of metals in soil. Although the results of sequential analyses differ from those obtained by single extraction, they complement each other, especially in a comprehensive interpretation of the transformations of heavy metals in the soil–plant relationship. In this context the response of plants is particularly important, as they constitute an essential link in the food chain, into which metals can be incorporated. The effect of lead on morphological, physiological and biochemical processes in plants has been investigated by various authors [13,14].

Although plants have various defence mechanisms against metals, they take them up and accumulate and this process is controlled by various soil factors (pH, organic matter content, quality and quantity of mineral components) as well as the plant's susceptibility. The application of various amendments into soil may change the natural soil conditions and as a consequence disturb both the natural biogeochemistry of lead and its uptake by plants [4]. Therefore, this fact should be taken into account in the interpretation of data related to Pb changes in soil as well as their potential effect on plant nutrition. So far interactions between Pb and essential nutrients for plants have not been thoroughly investigated. There is only fragmentary information on the effect of Pb in soil on nutrient uptake by plants in the literature [15,16]. Therefore, apart from routine determinations of Pb content in plants, this study also analysed the potential effect of Pb on selected macronutrients. In view of such assumptions, the aims of this study are to provide a comprehensive analysis of:

1. Quantitative changes of Pb content in sequentially separated soil fractions;
2. Variability of the amounts of bioavailable Pb to plants;

3. Plant responses expressed as Pb accumulation and its potential interaction with selected macronutrients.

## 2. Materials and Methods

### 2.1. Experimental Design

The design of a pot experiment was described in detail in an earlier study [10]. Here, general information is provided to clarify the context of the research. A three-year outdoor pot experiment was conducted on medium agricultural soil (clay loam) classified as *Haplic cambisol* according to WRB [17]. Compost made from biowaste and manure mixed at a 1:1 ratio and fly ash (FA) formed as a by-product of lignite combustion were used as immobilising agents. Dry soil samples of 10 kg were weighed in eight replications and thoroughly mixed with the compost or fly ash two weeks before plant cultivation. The compost and fly ash were applied into the soil in a single dose at an equivalent amount of 40 $t\cdot ha^{-1}$. Compost and FA in given amount were applied once at the beginning of the experiment. Each mixture was wetted to 60% of the field capacity. The design of the experiment included: T0—control soil (without compost or fly ash added), TI—soil with compost added, TII—soil with fly ash added. Three crops, i.e., narrow-leaved lupine (*Lupinus angustifolius* L.), camelina (*Camelina sativa* L.) and oat (*Avena sativa* L.), were planted in the same pot (10 plants per pot) in three consecutive years after harvest. Supplementing mineral fertilisers were applied at balanced doses corresponding to the amounts of N, P, K applied with the compost and fly ash. The fertiliser doses covered the nutritional needs of cultivated plants, which per hectare was 60 kg N, 50 kg P and 60 kg K for oats; 70 kg N, 40 kg P and 60 kg K for camelina and 60 kg P and 80 kg K for lupine. For this purpose, ammonium nitrate, triple superphosphate and potassium salt were used. The vegetation period of individual plants, as well as the climatic conditions prevailing at that time were typical of Polish conditions.

The basic parameters of the soil, compost and fly ash are listed in Table 1. The pH of the compost and fly ash was measured in $H_2O$ and in 1 $mol\cdot dm^{-3}$ KCl for soil. The soil/compost/fly ash solution ratio was 1:2.5 ($w/v$). The total organic carbon (TOC) and total nitrogen ($N_{Tot}$) contents in the soil, compost and fly ash were measured using a Vario Max CNS analyser. The total lead contents ($Pb_{Tot}$) in the soil, compost and fly ash were determined spectrophotometrically according to the ISO procedure [18]. Levels of K, Ca, Mg and Na were determined in the extracts of 6 $mol\cdot dm^3$ HCl by atomic absorption spectrophotometry, while total phosphorus ($P_{Tot}$) content was measured colorimetrically applying the vanadium–molybdenum method [19].

**Table 1.** Basis properties of soil, compost and fly ash (data for composite samples).

| Parameter | Soil | Compost | Fly Ash |
|---|---|---|---|
| pH | 7.0 | 6.8 | 13.7 |
| TOC ($g\cdot kg^{-1}$) | 16.8 | 181.3 | n.d. [1] |
| $N_{Tot}$ ($g\cdot kg^{-1}$) | 1.8 | 2.7 | 0.5 |
| $P_{Tot}$ ($g\cdot kg^{-1}$) | 1.8 | 3.8 | 10.8 |
| $K_{Tot}$ ($g\cdot kg^{-1}$) | 2.9 | 3.5 | 3.7 |
| $Mg_{Tot}$ ($g\cdot kg^{-1}$) | 3.5 | 1.5 | 8.4 |
| $Ca_{Tot}$ ($g\cdot kg^{-1}$) | 13.4 | 18.4 | 18.4 |
| $Na_{Tot}$ ($g\cdot kg^{-1}$) | 0.2 | 1.2 | 8.8 |
| $Pb_{Tot}$ ($mg\cdot kg^{-1}$) | 120.9 | 14.8 | 17.6 |

[1] n.d.—not determined.

### 2.2. Analysis of Soil and Plant Samples

As was mentioned above, detailed information on the analytical procedures can be found in an earlier study [10]. Soil samples were collected each time after the harvest of individual plants. Before soil sample collection from the individual pots, the entire contents of the pots were thoroughly mixed and the plant roots were removed. The sample taken was approximately 0.5 kg. The loss of soil and compost or FA was properly replenished, so that the quantitative proportions are the same as at the beginning of the experiment. Owing to this the next plant had the same growing conditions. The samples were air dried at room temperature for the analyses. The amount of bioavailable Pb ($Pb_{DTPA}$) was assessed by the organic chelating compound DTPA extraction [20]. The Pb distribution in individually separated fractions of the soil samples was assessed by the BCR method [21]. According to this protocol, the following fractions were separated: Fr. 1—exchangeable, water and acid soluble, Fr. 2—reducible, e.g., bound to iron and manganese oxyhydroxides, Fr. 3—oxidisable, e.g., bound to organic matter and sulphides, and Fr. 4—residual, non-silicate bound metals. Pb concentrations in the extracts were measured by flame atomic absorption spectrometry (FAAS) in a Varian Spectra AA 220 FS apparatus. The amounts of Pb in the separated soil fractions were used to calculate two independent indexes: the risk assessment code (RAC) and individual contamination factor (ICF). The details of the RAC calculations are given by [22]. This index expresses a percentage share of metals in the exchangeable fraction (Fr. 1). The resulting values were interpreted on the basis of five classes as follows:

RAC < 1%—no risk (NR, safe to the environment)
RAC 1–10%—low risk (LR, relatively safe to the environment)
RAC 11–30%—medium risk (MR, relatively hazardous to the environment)
RAC 31–50%—high risk (HR, hazardous to the environment)
RAC >50%—very high risk (VHR, highly hazardous to the environment).

The individual contamination factor (ICF) was calculated according to the method described in [23]. It is the quotient of the sum of metal amounts in fractions 1–3 and the content of the metal in fraction 4.

$$\mathrm{ICF} = \frac{\mathrm{Fr.}\ 1 + \mathrm{Fr.}\ 2 + \mathrm{Fr.}\ 3}{\mathrm{Fr.}\ 4}.$$

The obtained values were interpreted on the basis of four classes as follows:

ICF < 1—low contamination (LC)
1 < ICF < 3—moderate contamination (MC)
3 < ICF < 6—considerable contamination (CC)
ICF > 6—very high contamination (VHC).

The plant material (aboveground parts) was dried at 60 °C, ground and ashed in a furnace at 450 °C for 6 h. The ash was dissolved in 5 mL of 6 $\mathrm{mol{\cdot}dm^3}$ HCl and diluted to a constant volume with distilled water [19]. The resulting extracts were analysed to measure the Pb content by atomic absorption spectrophotometry (ASA) in a Varian Spectra AA 220 FS apparatus.

To express the plants' ability to take up and transport the most mobile and bioavailable Pb forms and to assess the actual site contamination with heavy metals the specified factors were calculated. The Pb contents in the soil and crops were used to calculate the bioconcentration factor for the Pb content in Fr. 1 ($BCF_{Fr.1}$), the bioconcentration factor for bioavailable Pb ($BCF_A$) and the coefficient of contamination level (CCL) [24].

The $BCF_{Fr.1}$ is the ratio of Pb content in plant shoots to its content in fraction 1 of the soil.

$$\mathrm{BCF_{Fr.1}} = \frac{\text{metal in plant shoots}}{\text{total metal content in fraction 1}}$$

The $BCF_A$ is the ratio of Pb content in plant shoots to the bioavailable metal content in the soil.

$$\mathrm{BCF_A} = \frac{\text{metal in plant shoots}}{\text{bioavailable metal amount}}$$

The CCL is the ratio of Pb content in plant shoots cultivated at Pb-contaminated sites to Pb concentrations in plant shoots cultivated at control sites

$$\mathrm{CCL} = \frac{\text{metals in plant shoots in amended site}}{\text{metals in plant shoots in the control}}$$

There were three replicates of all assays analysing the content of nutrients in the samples.

### 2.3. Statistical Analysis

One-way analysis of variance was used to compare the soil fractions depending on the experiment conditions, separately for each fraction and soil conditions, while the Shapiro–Wilk test was used as the test of normality [25]. Moreover, for this analysis the homogeneous groups were determined. Dependencies between the mineral elements are shown on dendrograms based on Ward's hierarchical agglomerative clustering method. Relationships between the elements under analysis were determined with the Pearson correlation coefficient in the form of heatmaps. The same method was applied to analyse the relationships between elements in the plants. Relationships between the plants and contents of elements were determined using the analysis of variance and the non-parametric Kruskal–Wallis test due to the lack of normal distribution for each plant. The Dunn test was applied for post hoc analysis to determine the homogenous groups. The BCF and CCL were compared by one-way analysis of variance. The relationships between various Pb forms, separately for each amendment in the soil and for each plant, were determined based on multiple regressions. The coefficient of determination $R^2$ is a measure of matching multiple regression to data [26]. The data were statistically analysed at a significance level $\alpha = 0.05$ with the R, software version 4.0.4 (15 February 2021) using the stats, graphics, agricolae, dplyr, ggplot2, dynlm packages and software available in these packages.

## 3. Results

Under each set of experimental conditions the amount of Pb is compared between years. The amount of Pb in Fr. 1 was significantly greater in the first year of the study than in the last (Table 2 and Figure 1). In all the research years the Pb content in Fr. 2 was the smallest in the control soil and the ones with the compost added (Table 2 and Figure 1). The Pb content in Fr. 2 in the soil with FA was lowest in the first year of the study, but it increased in the subsequent years. The Pb content in Fr. 3 in the control soil was the lowest in the second year of the experiment, whereas the soil with compost and FA had the lowest Pb content in the last year of the experiment. The highest Pb content in Fr. 4 was found in the last year. In the last year of the study the highest $\mathrm{Pb_{Tot}}$ content was recorded in the control soil, whereas in the soil with the FA the lowest $\mathrm{Pb_{Tot}}$ content was noted. The $\mathrm{Pb_{DTPA}}$ showed that the lowest content of bioavailable Pb was observed in the first year of the experiment in the soil with the compost and fly ash added. There were no significant differences in the next two years. Regardless of the year of study, there were no differences in the $\mathrm{Pb_{DTPA}}$ content in the control soil. The same observation was made for $\mathrm{Pb_{Tot}}$ in the soil enriched with the compost (Figure 1).

**Table 2.** *p*-values from one-way ANOVA determined separately for different Pb fractions: Fr. 1–4, Total and DTPA in soil depending on amendment (compost or fly ash): T0—control soil, TI—soil with compost addition and TII—soil with FA addition. Comparison between years.

| Pb | T0 | TI | TII |
|---|---|---|---|
| Fr. 1 | $1.99 \times 10^{-6}$ *** | $2.79 \times 10^{-5}$ *** | $4.75 \times 10^{-6}$ *** |
| Fr. 2 | $6.68 \times 10^{-14}$ ** | $2.82 \times 10^{-5}$ *** | $5.55 \times 10^{-7}$ *** |
| Fr. 3 | 0.041 * | 0.001 *** | $3.12 \times 10^{-6}$ *** |
| Fr. 4 | 0.028 * | $9.5 \times 10^{-8}$ *** | $4.69 \times 10^{-5}$ *** |
| Total | 0.005 ** | 0.185 | 0.013 * |
| DTPA | 0.762 | 0.003 ** | 0.022 * |

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

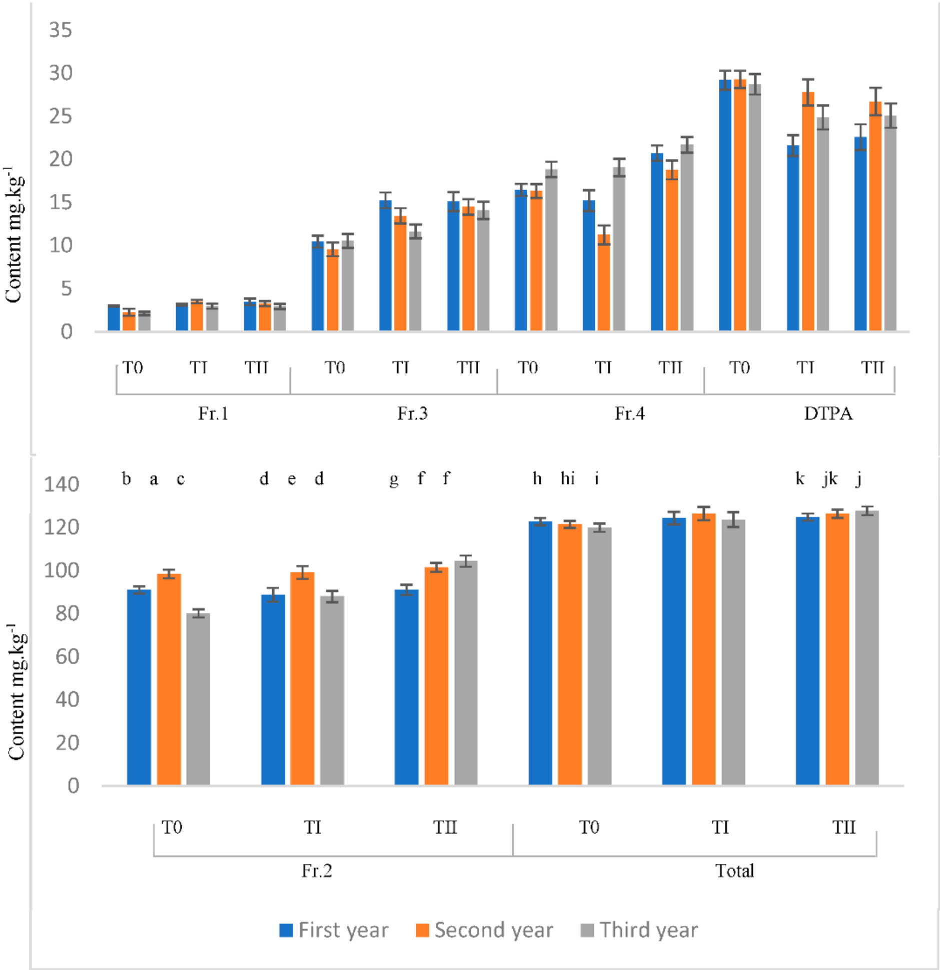

**Figure 1.** The effect of treating the soil with two amendments on mean Pb amounts in fractions Fr. 1-4, Total and $Pb_{DTPA}$ in soil samples: T0—control soil, TI—soil with compost addition and TII—soil with FA addition. In the figure, the letters denote homogenous groups and numbers mean SD values.

The dendrograms in Figure 2 present the hierarchical clustering of observations for the three years of the experiment and the investigated Pb fractions. As can be seen, the same pattern is appropriate for the combinations of years and fractions. In the first two years of research, the Pb content in Fr. 3 and total amounts in soil with amendments were comparable. The same trend was noted for the last year of research for Pb contents in Fr. 1, Fr. 4 and $Pb_{DTPA}$. There was a different trend in the second year of the research in the case of Pb contents in Fr. 2 and Fr. 4. The amounts of this element were comparable in the control and soil enriched with FA. On the other hand, the content of Pb fractions obtained in FA differed significantly from those obtained for the control soil as well as that amended with composts for Pb in Fr. 1 and Fr. 4 in the first year and for Pb in Fr. 2, Fr. 3 and the total amount in the last year of the study.

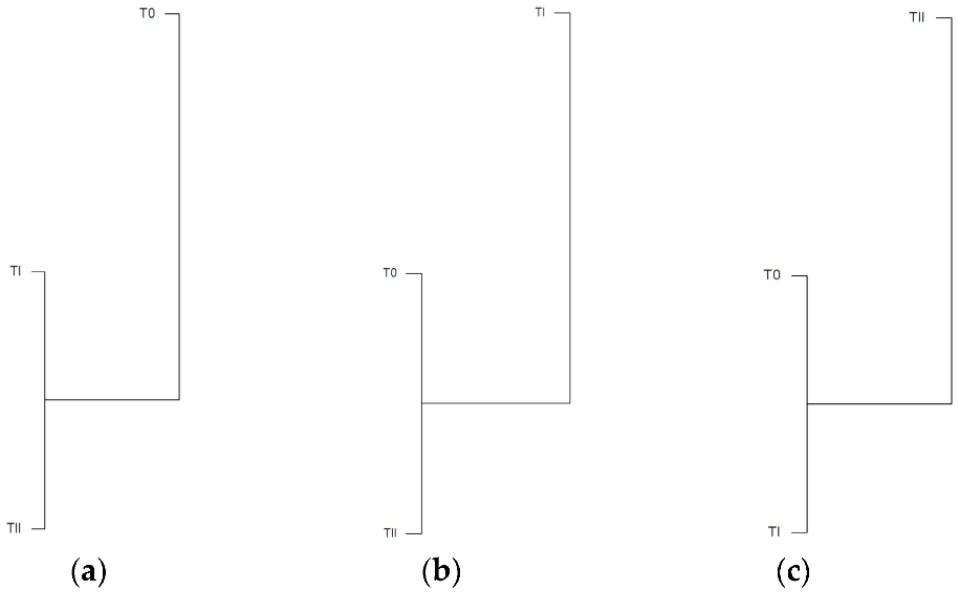

**Figure 2.** Dendrograms for Pb fractions in soil. Dendrogram (**a**) is suitable for Fr. 1 in the 2nd year and in the 3rd year, Fr. 3 in the 1st year and in the 2nd year, Fr. 4 in the 3rd year, $Pb_{Tot}$ in the 1st year and in the 2nd year, $Pb_{DTPA}$ in the 1st year and in the 3rd year. Dendrogram (**b**) is suitable for Fr. 2 in the 1st year and in the 2nd year, Fr. 4 in the 2nd year. Dendrogram (**c**) is suitable for Fr. 1 in the 1st year, Fr. 2 in the 3rd year, Fr. 3 in the 3rd year, Fr. 4 in the 1st year, $Pb_{Tot}$ in the 3rd year, $Pb_{DTPA}$ in the 2nd year.

The data in Figure 3 are presented in the form of heat maps with Pearson's correlation coefficients between amounts of Pb in sequentially separated soil fractions as well as total and bioavailable amounts of the metal. The analysis was performed separately for the treatments and the years of the study. The experimental factors influenced the mutual relationships. A negative correlation was observed between $Pb_{DTPA}$ and Pb in the residual fraction (Fr. 4) in the control and between Pb in Fr. 2 in the soil compost application in the first year of the study. On the other hand, a negative correlation was found in the soil with the compost amendment between Pb in Fr. 2 and Fr. 3, whereas the correlation between these two fractions was positive in the soil amended with FA. Moreover, in the above-mentioned cases a positive correlation was observed between $Pb_{Tot}$ and Pb in Fr. 3 and Fr. 4, which was not found in the other variants. The dependencies between Pb amounts in the individual fractions became most visible in the second year of the study in the case of soil with compost addition. A positive correlation was observed between $Pb_{DTPA}$ and metal amounts in fractions 1–3. Only in these variants it was noted that Pb in Fr. 2 was positively correlated with $Pb_{Tot}$ and negatively correlated with Pb in Fr. 4. Only for the soil with the FA addition, a negative correlation was observed between Pb in Fr. 1 and Fr. 4 as well as $Pb_{Tot}$. In the last year of the study a positive correlation was found between $Pb_{DTPA}$ and Pb in Fr. 2 and Fr. 4 in the control and between $Pb_{DTPA}$ and Pb in the other fractions in soil with compost addition. Moreover, the greater the $Pb_{DTPA}$ content, the greater the $Pb_{Tot}$ and Pb levels in the residual fraction in the soil with FA application. The dependencies between Pb in Fr. 2 and 4 were positive in the control and negative in the soil with compost addition. A similar trend was observed for the dependence between Pb in Fr. 3 and Fr. 4, which was negative in TI and positive in TII (Figure 3).

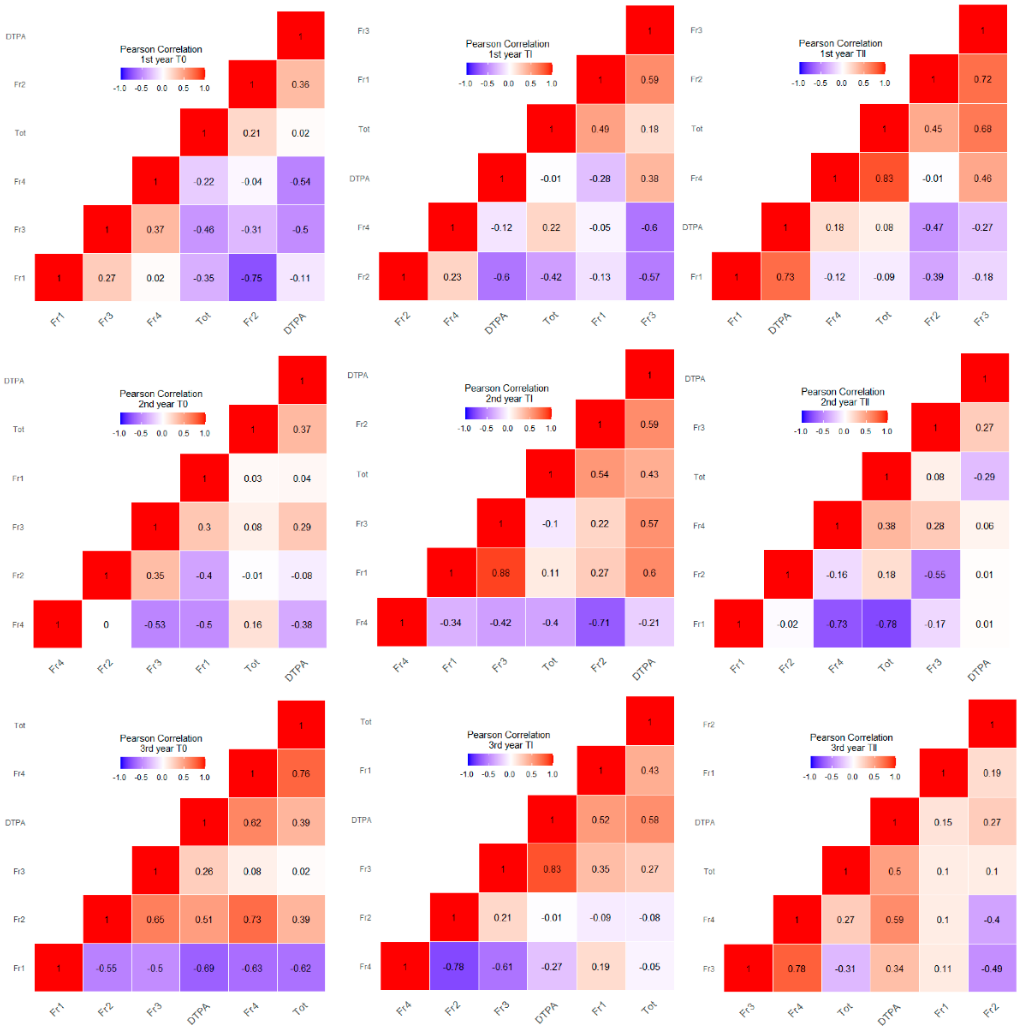

**Figure 3.** Heat maps presenting Pearson's correlation coefficient between Pb in Fr. 1–Fr. 4, $Pb_{Tot}$ and $Pb_{DTPA}$ for soils: T0—control soil, TI—soil with compost addition and TII—soil with FA.

The RAC and ICF were also used to assess the effect of the stabilising agents on the potential Pb immobilisation in the soil. As can be seen in Table 3, the values of both factors were higher in the soil fertilised with either compost or FA. Regardless of the year of the study, the mean RAC values ranged from 2.23% to 2.77% for the soil with compost addition and the soil enriched with FA, and from 1.78% to 2.42% for the control soil. The ICF values ranged from 4.93 to 6.34 for the control soil and from 5.06 to 10.33 for the soil with compost and FA. Regardless of the amendments applied, both the RAC and ICF values decreased over the years of the study, and the differences were significant (Table 3).

**Table 3.** Risk assessment codes (RAC) and individual contamination factors (ICF) assessed for soil depending on treatment and year of study (mean values).

| **Treatment** | **Year of the Study** | | | | | |
|---|---|---|---|---|---|---|
| | **1st** | | **2nd** | | **3rd** | |
| | **RAC (%)** | **ICF** | **RAC (%)** | **ICF** | **RAC (%)** | **ICF** |
| T0 | 2.42 ± 0.103 | 6.34 ± 0.257 | 1.84 ± 0.302 | 5.89 ± 1.001 | 1.78 ± 0.194 | 4.93 ± 0.408 |
| TI | 2.48 ± 0.067 | 10.33 ± 0.529 | 2.36 ± 0.701 | 7.04 ± 1.276 | 2.23 ± 0.596 | 5.38 ± 0.605 |
| TII | 2.77 ± 0.371 | 6.75 ± 0.208 | 2.57 ± 0.274 | 6.35 ± 0.908 | 2.42 ± 0.266 | 5.06 ± 0.513 |
| *p*-value | 0.0133 * | $3.08 \times 10^{-6}$ *** | 0.0021 ** | $1.34 \times 10^{-5}$ *** | 0.0062 ** | 0.0014 ** |

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

The Kruskal–Wallis test, which was conducted to find the relationships between the plants in terms of K, Ca, Mg, Pb and Na amounts, revealed significant differences in the concentrations of this metal for each plant depending on the experimental factors (Table 4). Due to the fact that this study analysed the influence of Pb on the uptake of selected macronutrients (K, Ca, Mg and Na), also their contents in the studied plants are presented as Supplementary material (Figure S1). Regardless of the plant, the highest amounts were found for Ca (from 29,639 $\mathrm{mg{\cdot}kg^{-1}}$ to 11,474 $\mathrm{mg{\cdot}kg^{-1}}$), while the lowest for Na (from 2022 $\mathrm{mg{\cdot}kg^{-1}}$ to 233 $\mathrm{mg{\cdot}kg^{-1}}$). Dunn's test generally confirmed the differences in the amounts of these macronutrients for individual plants resulting from the additives used.

**Table 4.** *p*-values obtained for combinations of factors levels from the Kruskal–Wallis test for plants. Small *p*-values indicate that treatment had a significant effect on content of considered elements separately in each plant.

| Plant | Lupine | Camelina | Oat |
|---|---|---|---|
| *p*-value | $2.268 \times 10^{-16}$ | $3.432 \times 10^{-12}$ | $2.743 \times 10^{-10}$ |

Regardless of the plant, the highest Pb content was recorded in plants cultivated on the soil with compost added (Figure 4), although for oats there were no significant differences in soils with amendments. Pb content in the plants after compost treatment was the highest in the case of lupine and camelina, and it was significantly different from that in the control. However, even though in absolute values camelina resulted to have a higher uptake than lupine, in relative terms the situation was similar, with about 24% or 33% more Pb with respect to the control for compost or FA, respectively. These results show that the plants cultivated on the soil enriched with the FA took up lower amounts of Pb than those grown on the soil with the compost added (Figure 4).

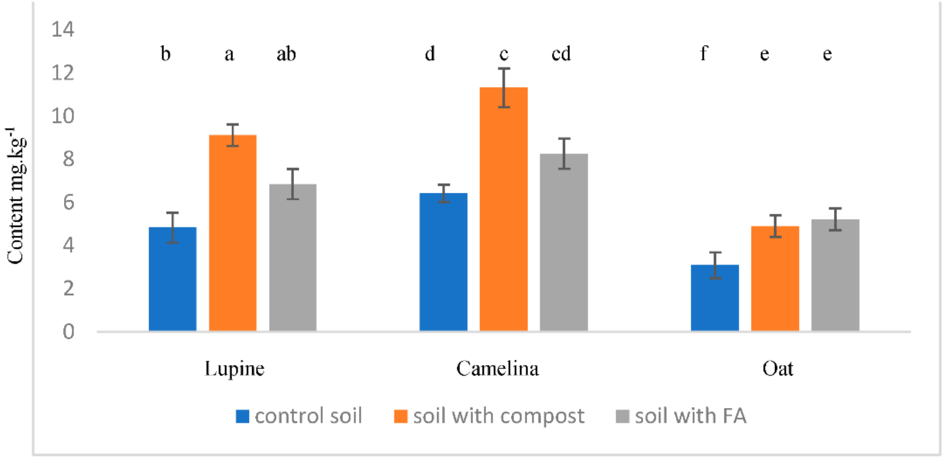

**Figure 4.** The average content and SD (for three years) of Pb amounts in plants for treatments. Homogeneous groups were determined using the Dunn test. In the figure the letters denote homogenous groups and numbers mean SD values.

In order to analyse how the stabilising agents affected the differences in the Pb concentration in the plants, the bioconcentration factors ($\mathrm{BCF_{Fr{\cdot}I}}$ and $\mathrm{BCF_A}$) and the coefficient of contamination level (CCL) were calculated (Table 5). Regardless of the plant, the $\mathrm{BCF_{Fr.1}}$ values ranged from 1.45 to 3.43 and were greater than the $\mathrm{BCF_A}$ values, which ranged from 0.11 to 0.42. The $\mathrm{BCF_{Fr.1}}$ and $\mathrm{BCF_A}$ values were the lowest for oat and comparable for lupine and camelina. The CCL values ranged from 1.29 to 1.93 (Table 5).

**Table 5.** Bioconcentration factors ($BCF_{Fr.1}$ and $BCF_A$) and coefficient of contamination level (CCL) for plants depending on treatment (mean values).

| **Treatment** | **Lupine** | | | **Camelina** | | | **Oat** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **$BCF_{Fr.1}$** | **$BCF_A$** | **CCL** | **$BCF_{Fr.1}$** | **$BCF_A$** | **CCL** | **$BCF_{Fr.1}$** | **$BCF_A$** | **CCL** |
| T0 | 1.63 c | 0.17 c | - | 2.92 a | 0.22 bc | - | 1.45 a | 0.11 b | - |
| TI | 2.95 a | 0.42 a | 1.93 a | 3.43 a | 0.42 a | 1.76 a | 1.78 a | 0.22 a | 1.78 a |
| TII | 2.01 b | 0.30 b | 1.45 b | 2.57 a | 0.32 b | 1.29 b | 1.69 a | 0.20 a | 1.66 a |
| *p*-value | $2.23 \times 10^{-9}$ *** | $1.23 \times 10^{-14}$ *** | 0.009 ** | 0.0522. | $1.26 \times 10^{-14}$ *** | 0.004 ** | 0.2031 | 0.0002 *** | 0.619 |

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. a–c denote belonging to the homogeneous groups.

The results of one-way ANOVA for $BCF_{Fr.1}$ and $BCF_A$ showed that for camelina and oat the highest values were noted for TI, followed by TII (Table 5). The values of the CCL for lupine and camelina grown in the soil fertilised with the compost were significantly higher than the CCL for these crops grown on the soil with the FA. There were no statistically significant differences in the CCL for oat (Table 5).

As the aim of this study was to analyse lead contents in different plants grown under different conditions, only the relationship between the content of this element and selected macronutrients was analysed (Figure 5). The correlation between Pb and Na contents was positive in lupine under control conditions and negative for camelina cultivated in soil with compost addition. The correlation between Pb and Mg amounts was negative for lupine grown in the soil with FA addition and positive for camelina grown in the control variant. The applied additions and their lack had a significant impact on the dependencies between Pb amounts and other elements for oat, because a negative correlation was observed between Pb and Ca in soil TI and TII and between Pb and K in the control variant.

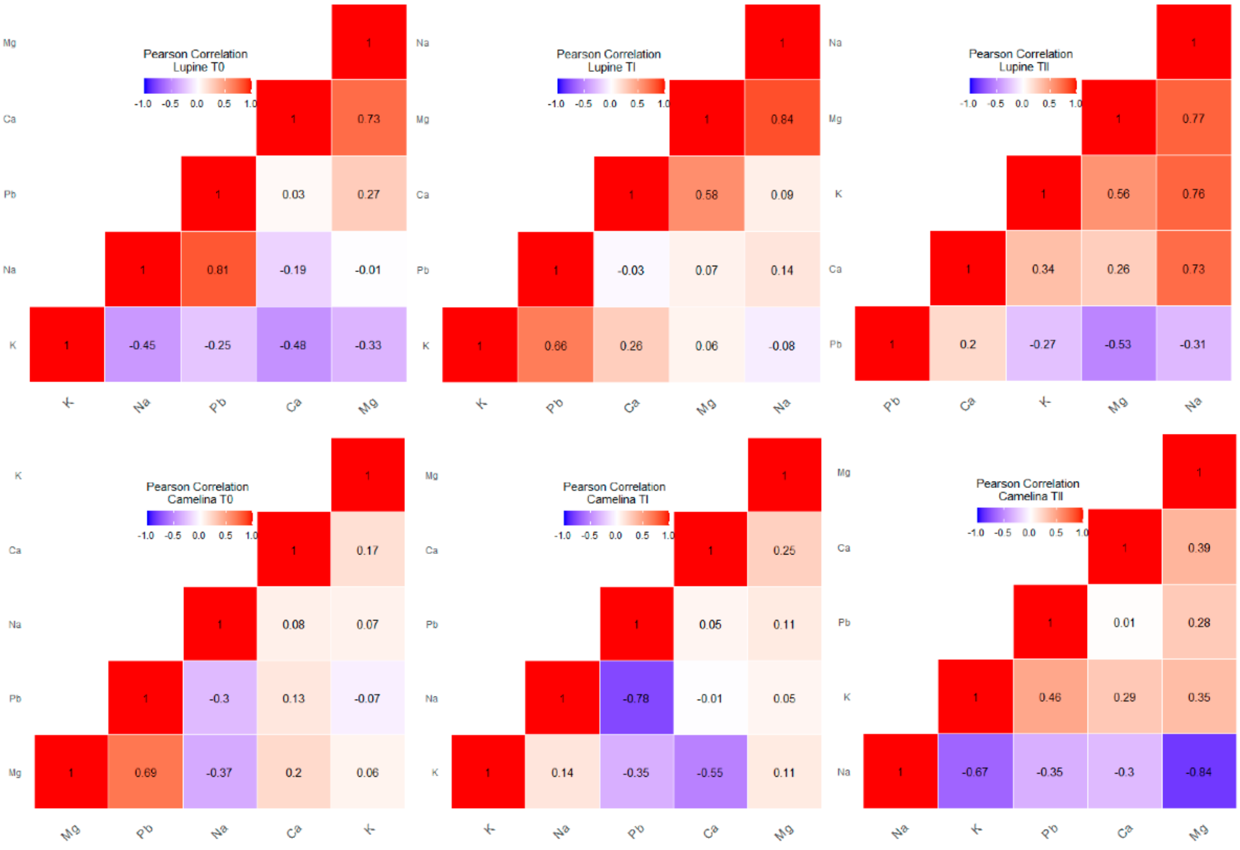

**Figure 5.** *Cont.*

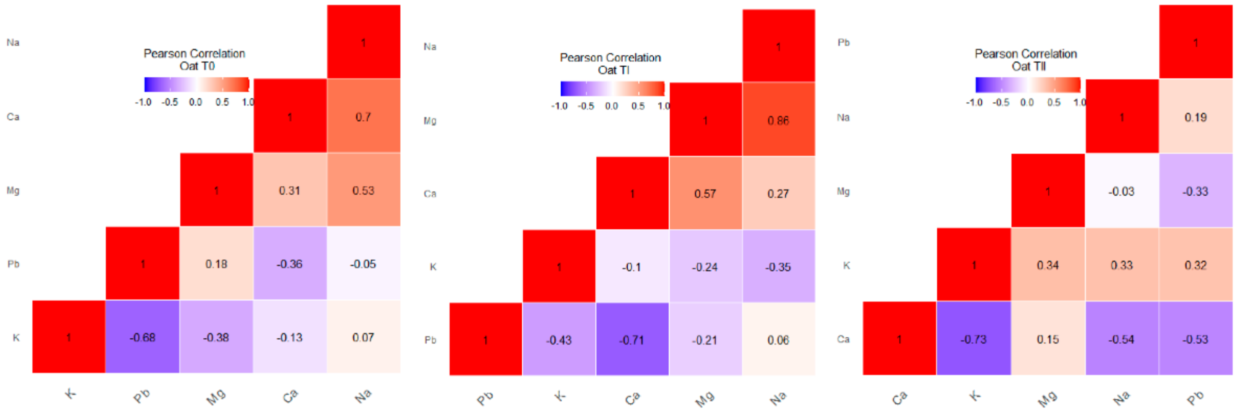

**Figure 5.** Heat map for Pearson's correlation coefficients between Ca, K, Mg, Na and Pb for plants. (lupine, camelina and oat) for different treatments (T0, TI, TII).

Multiple regression analysis was used to determine a linear relationship between the Pb content in soil and its content in the crops. The first step involved the multiple regression equation for the variables that had a significant influence on the Pb content. The best-fit multiple regression equations are listed in Table 6.

**Table 6.** Multiple regression equations for relations between Pb contents in plants and in Pb forms for soils with applied amendments.

| Plant | Treatment | Equation | $R^2$ |
|---|---|---|---|
| Lupine | T0 | Pb content = 43.554 + 0.166·Fr.2 + 0.282·Fr.4 + 0.084·$Pb_{DTPA}$ | 0.68 |
| | TI | Pb content = −8.174 + 0.049·Fr.4 + 0.053·$Pb_{DTPA}$ | 0.61 |
| | TII | Pb content = 2.932 + 0.109·Fr.2–0.278·Fr.4 | 0.58 |
| Camelina | T0 | Pb content = 11.943 + 0.473·Fr.1–0.059·Fr.2–0.154·Fr.3 + 0.045·Fr.4 | 0.60 |
| | TI | Pb content = 11.136 + 0.938·Fr.1–0.067·Fr.2–0.029·Fr.3 + 0.938·Fr.4 | 0.64 |
| | TII | Pb content = 3.847–0.771·Fr.1 + 0.341·Fr.3 + 0.106·Fr.4 | 0.59 |
| Oat | T0 | Pb content = −27.656 +2.949·Fr.1–0.044·Fr.2 + 0.886·Fr.3 +0.106·Fr.5 | 0.58 |
| | TI | Pb content = 4.716 + 0.297·Fr.3–0.126·Fr.4 + 0.035·$Pb_{DTPA}$ | 0.67 |
| | TII | Pb content = −5.775 + 1.087·Fr.1–0.011·Fr.2–0.123·Fr.3 | 0.61 |

The data in Table 6 show that the Pb content in different plants was determined by the Pb content in various bound forms. Irrespective of the experimental treatments the Pb content in plants was determined in one of the largest impacts by Pb in Fr. 4 for lupine and camelina, and by Pb in Fr. 3 in the case of camelina and oat. The Pb content in camelina for all the considered treatments and in oat for T0 and TII soils was determined on the basis of the Pb content in the first fraction. Moreover, excluding lupine and oat grown on the soil with compost and camelina on soil with FA, the Pb content in all the plants under all experimental conditions was determined on the basis of the Pb content in Fr. 2. Regardless of the experiment conditions, the Pb content in camelina and oat was influenced by the Pb content in organic bonds (Fr. 3), which was evidenced by changes in the Pb content in the plants within −0.154–0.886, caused by an increase of 1 mg·kg$^{-1}$ in the Pb content in Fr. 3. The Pb content in lupine and camelina for all treatments was influenced by the Pb content in the residual fraction (Fr. 4), as was evidenced by the changes in the Pb content in the plants within −0.278–0.938, caused by an increase of 1 mg·kg$^{-1}$ in the Pb content in Fr. 4 (Table 6). Moreover, the Pb content in camelina grown in soil T0- TII and in lupine as well as oat cultivated in the soil with compost addition was influenced by water- and acid-soluble forms (Fr. 1) and bioavailable amounts of Pb ($Pb_{DTPA}$), respectively.

## 4. Discussion

The significant mobility of heavy metals contaminating anthropogenic soils results in a potential risk of their rapid incorporation into the food chain. Therefore, such soils must undergo remediation through environment-friendly, rapid and cost-effective techniques. Among other things, organic and inorganic amendments meet these requirements because they immobilise heavy metals, reduce their mobility and bioavailability by creating stable complexes. Therefore, composts and fly ash were used in our study to reduce lead mobility. It should be noted that the use of these materials is considered a potent eco-remediation strategy to alleviate Pb contamination in soil. Additionally, it is an ecological solution improving soil fertility by increasing the content of organic matter, nutrients and CEC [2,27]. The influence of organic and mineral amendments on changes in Pb bioavailability has been analysed by various authors [2,9,11,28–30], who generally observed positive effects, expressed by reduced Pb mobility and its lower uptake by plants. The reduced Pb contents in the exchangeable fraction (Fr. 1) [9,11,28] and the contents of bioavailable Pb extracted with DTPA [2,9,29] were particularly emphasised. The findings from this study also confirmed such interactions of compost and fly ash. DTPA-extractable heavy metals mainly exist in water-soluble and ion-exchangeable states, which can be directly adsorbed by plants [2], so it is particularly important to extend the knowledge concerning this form of lead. Regardless of the year of the study, the contents of bioavailable Pb in the soil enriched with the compost and FA were 10–25% lower than the contents of bioavailable Pb in the control soil. The alkaline treatment (FA) particularly significantly decreased the DTPA extractable Pb amounts, as compared with the control soil. This effect was caused by an increase in pH, which enhanced metal precipitation/adsorption. It is noteworthy that organic materials such as compost can effectively remediate contaminated sites by transforming exchangeable/soluble Pb bound forms into organically bound ones, which are less available to plants [29]. This effect was also proven by the obtained outcomes. According to [2,31], mature compost can achieve the metal immobilisation by creating stable complexes with the functional groups of organic polymers such as OH and COOH. In addition, organic matter incorporated with compost may limit Pb solubility due to the formation of insoluble salts.

The effect of the amendments used in the experiment was manifested less spectacularly in the fractional distribution of Pb. Regardless of the year of the study, the Pb content in the separated fractions increased in the following series Fr. 2 > Fr. 4 > Fr. 3 > Fr. 1, which is consistent with the data in other scientific publications [28,30]. It has been proved that Pb preferentially binds to mineral oxides in soil, e.g., manganese oxides (Fr. 2). The FA applied into soil significantly increased the amount of Pb in reducible bound forms, e.g., those bound to iron and manganese oxides, as compared with the Pb content in Fr. 2 in the control soil and the soil enriched with the compost. This effect was caused by an increase in pH value and Fe and Mn oxides introduced with the FA. Kumar et al. [6] particularly underlined these chemical properties of FA, because it is composed of aluminium, iron, silicon and calcium oxides in 95–99% and the pH of FA usually ranges from 4.5 to 12.0 (in this study the pH of the FA was 13.7, see Table 1). Shahkolaie et al. [9] and Murtaza et al. [14] stressed the significance of the alkaline pH and the presence of sorption mineral colloids, such as Fe and Mn oxides, for increased Pb adsorption. Moreover, both the compost and FA resulted in an increase of Pb contents in soil compared to the control soil, which was the most pronounced in the exchangeable, water- and acid-soluble (Fr. 1) and organic bound forms (Fr. 3). The studies [5,31,32] indicated that the Pb content in various combinations with the soil solid phase may increase under the influence of organic and mineral amendments. From the practical point of view it is crucial to assess the mitigation of metal mobility in soil after stabiliser application. In this study the compost and FA immobilised Pb, although their effectiveness did not differ significantly. The Pb contents in the exchangeable, water- and acid-soluble forms (Fr. 1) decreased, whereas the Pb contents in the residual fraction increased, which was particularly noticeable in the FA-fertilised soil. The study done by [9,11,30] also emphasised that mineral additives stabilised Pb in soil more effectively.

The risk assessment code (RAC) is a useful tool in assessing the negative environmental impact of heavy metals. The RAC value indirectly indicated reduced Pb mobility in the conducted experiment. The RAC values in the research years decreased, although the drop was the most noticeable in the control soil. Nevertheless, the RAC values indicated a low environmental risk of Pb and relatively safe conditions for plant cultivation. The individual contamination factor (ICF) is another tool that helps to assess the influence of lead on the soil environment. In the last year of the experiment there were slightly lower ICF values in the soil treated with the compost or FA compared to the first year. This indicated considerable Pb contamination, whereas the immobilisation effect of the compost and FA was not pronounced.

It is interesting to note the influence of the amendments used in the experiment on the interrelations between the Pb contents in different combinations, described by Pearson's correlation coefficients. In the soil fertilised with the compost there was a positive effect of the Pb contents in Fr. 3 on the contents of this metal in Fr. 1 and Fr. 2. Simultaneously, there was an inversely proportional relationship between the Pb contents in Fr. 2 and Fr. 4. On the other hand, in the soil enriched with the FA the residual amounts of Pb had a positive effect on its total contents and its contents in organic forms. These dependencies were confirmed by the effect of organic matter applied with the compost as well as secondary components in the form of silicon, aluminium and iron oxides introduced to the soil with the FA. The amounts of Pb in the compost and FA were more or less active sources of this element (due to the presence of different bound forms), which had a diverse influence on its contents in the fractions. It is assumed that the Pb pool that is theoretically directly available to plants reflects the amounts of this metal obtained with the DTPA solution and in Fr. 1. The results from this study partly confirmed this statement, because the amounts of DTPA were positively correlated only with the Pb contents in Fr. 1 in the first year of the study in soil TII and with the Pb contents in Fr. 3 in the third year of the study in soil TI. Moreover, the regression equations showed that the Pb amounts present in reduced, organic and residual bound forms were a much more important source of this element for the plants cultivated in the experiment. It may indirectly prove the stabilising effect of the amendments, expressed by the immobilisation of mobile Pb forms, described by the amount of bioavailable ($Pb_{DTPA}$) and exchangeable bound forms (Pb in Fr. 1), which are considered to be a readily accessible source of the element for plants.

The amendments used in this experiment also determined the Pb amounts in the plants, which was particularly noticeable in those fertilised with the compost. The Pb contents in lupine and camelina cultivated in the first and second years of the study were significantly higher (almost 2.0 times) in soil TI than the Pb contents in the control plants. This effect may have been caused by chelates, which organic amendments may have formed with Pb in the soil solution, thus increasing its availability to the plants [14]. It is possible that along with the progressive humification of organic matter applied to the soil more stable organic complexes were formed, which resulted in low Pb contents in the oat grown in the third year of the experiment. These Pb amounts were comparable (no significant differences) to the Pb amounts in the oat cultivated in soil TII. The observed differences in the Pb accumulation in the plants were also caused by genetic, physiological and morphological differences between them. The fact that plants differ in root morphology, requirements for fertilisers and the possibility to control the bioavailability of the metal [14] should also be taken into account. The cited authors emphasised the ability of legume plants to effectively generate $H^+$ ions during the nitrogen fixation process that may result in a higher uptake of metal ions, such as Pb, by plants roots. On the other hand, camelina, which belongs to the *Brassicaceae* family, abounds in a cysteine (Cys)-rich protein, which is a crucial antioxidant and chelator of heavy metals in plants [33] and also can explain higher Pb amounts in this plant.

Scientific publications provide very little data on interactions between the amounts of Pb and macronutrients, such as those analysed in this study. The amounts of Pb contained in lupine had a positive effect on the content of Na (T0) and K (TI), but a negative effect on the content of Mg (TII). There was a positive relationship between Pb and Mg in the camelina grown in the control soil and the one fertilised with the compost. The Pb content in oat was negatively correlated with the contents of K (T0) and Ca (TI, TII). He et al. [34] observed no effect of Pb content in Chinese cabbage on the concentration of K. Negative correlations between the amount of Pb in plants or in soil and the content of K in plants are indicated by [15,16]. At the same time, Siedlecka [16] emphasised the increase in the amount of Mg in various vegetables observed under the influence of lead. Moreover, Alle et al. [13] found that Pb caused an increase in Mg content, but a decrease of K and Ca contents in crops. This observation was partially confirmed by the findings in this study. The shown relationships should be interpreted on the basis of cation competition at the root entrance. According to [4], Pb competes with Ca for calcium channels, so Pb absorption is inhibited by Ca. In turn, magnesium is directly involved in photosynthesis, so its increase or decrease should be interpreted as the plant's adaptive response to protect and enhance photosynthesis under Pb stress [13,35].

The bioconcentration factor (BCF) is widely used to assess the toxicity of heavy metals as well as their translocations from soil to plants [5,24,36–38]. According to [39], the bioaccumulation values rather than the total contents of heavy metals should be taken into consideration. As was shown above, bioavailability is a more important indicator of heavy metal toxicity in soil and plants. Therefore, the $BCF_A$, $BCF_{Fr.1}$ and CCL factors were used in this study. Moreover, there is another concept to ensure a reliable assessment of the plant contamination level. One can hypothesise that both bioconcentration factors ($BCF_A$, $BCF_{Fr.1}$) and coefficient of contamination level (CCL) may precisely describe the migration of heavy metals from soil to plants, their uptake and the potential toxic effect [24]. Scientific publications provide very little data on this issue. Popova [40] reported that the BCF in plants varied from 0.7 to 1.0, where a range of 0.1–1 indicated average metal accumulation. Kumar et al. in [6] noted a BCF of 0.4 for rice, which indicated low mobility of Pb from soil to plants. The authors calculated the BCF on the basis of the total metal contents, so their values do not fully correlate with the results presented in this study. Regardless of this fact, the $BCF_A$ values ranged from 0.17 to 0.42 for lupine, from 0.22 to 0.42 for camelina and from 0.11 to 0.22 for oat and they were similar to the data cited in reference publications [6,24,40]. The $BCF_{Fr.1}$ values were much higher, i.e., from 1.63 to 2.95 for lupine, from 2.57 to 3.43 for camelina and from 1.45 to 1.78 for oat. At the same time these values were very comparable and they do not differ significantly for camelina and oat. Regardless of this fact, application of both compost and FA caused higher values of $BCF_{Fr.1}$ and $BCF_A$ in comparison to data presented for control plants. This was a consequence of differences in the Pb contents in the soil and plants. However the FA application resulted in a better immobilisation effect, as evidenced by the CCL values. The CCL values calculated for lupine and camelina cultivated in the soil amended with FA were significantly lower.

## 5. Conclusions

The research results confirmed the complexity of remediation of lead-contaminated soils. The compost and fly ash used in the experiment as stabilising agents proved their partial practical suitability for Pb immobilisation. Their stabilising effect was significantly manifested by the amounts of bioavailable Pb. Simultaneously the FA was more effective than the compost, as evidenced by the values of the $BCF_A$, $BCF_{Fr.1}$ and CCL coefficients. Less evidently, a decrease in the Pb contents was statistically confirmed in the exchangeable bound forms described as Fr. 1 and an increase in the residual fraction of Pb (Fr. 4). The RAC and ICF indices confirmed this trend. Regardless of the amendment applied to the soil, the Pb distribution in the sequentially separated soil fractions was not affected. The highest Pb contents were found in reducible bonds, while the lowest in exchangeable ones. The statistical analysis confirmed the significance of bioavailable amounts of Pb as well as water- and acid-soluble forms in the plants' uptake of the metal under the experimental conditions. This may indicate the effect of the immobilisation of readily mobile Pb compounds by the stabilising agents applied to the soil. Although both stabilising agents were slightly burdened with the amount of lead, their application resulted in an increase in the content of this metal in the biomass of plants and in the soil in the first two years of the study.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/agronomy11061188/s1. Figure S1: The effect of treating the soil with two amendments on mean Ca, K, Mg and Na amounts in plants. On the figure, the letters denote belonging to homogenous groups and numbers mean SD values.

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