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The Effect of Immobilizing Agents on Zn and Cu Availability for Plants in Relation to Their Potential Health Risks

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Abstract: Soil contamination with heavy metals is one of the most important threats to the environment because they are easily incorporated into the food chain, threatening the health of plants, animals, and humans. In this study, the effectiveness of the introduced substances (compost and fly ash) was assessed in terms of its influence on the content of Cu and Zn in the soil, potential accumulation of these metals in the cultivated plants (camelina and oat), and thus in food products prepared from these plants. Therefore, the following indicators were used: bioconcentration factors calculated for the total amount (BCF_T) and bioavailable amount of metals (BCF_B) as well as gender-estimated daily intake (EDI) and health risk index (HRI). Regardless of gender, the EDI values ranged from 0.31 $\mu g \cdot kg^{-1}$ to 0.49 $\mu g \cdot kg^{-1}$ for Cu and from 0.9 $\mu g \cdot kg^{-1}$ to 1.8 $\mu g \cdot kg^{-1}$ for Zn in oat. For camelina, the calculated values were as follows: 4.1–8.5 $\mu g \cdot kg^{-1}$ for Cu and 7.1–12.1 $\mu g \cdot kg^{-1}$ for Zn. The HRI values were very low (in general 0.03–0.2), indicating no health risk connected with potential consumption of oat or camelina food products. The amounts of Cu and Zn in the crops grown on the soil amended with compost or fly ash were significantly lower (by 21–37% for oat and 14–34% for camelina) compared to the content of these metals in the control plants. Moreover, the levels of bioavailable metals decreased in soil as a result of the applied immobilizing agents. The study showed that the immobilization efficiency of compost and fly ash was comparable, and therefore the choice of either of these substances for the chemical remediation of soil contaminated with heavy metals is justified.

Keywords: compost; fly ash; camelina; oat; bioconcentration factors (BCF); estimated daily intake (EDI); health risk index (HRI)

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1. Introduction

Global agricultural soil pollution with heavy metals represents one of the greatest challenges for sustainable development. It is closely connected to the fact that the accumulation of heavy metals in agricultural soils is an obstacle to achieving global food safety and security [1]. According to cited authors, the presence of heavy metals in the environment is connected with natural processes (weathering processes, volcanic eruption) and anthropogenic factors (mining and smelting activities and the resulting wastes, the use of agrochemicals, fossil fuel burning, vehicle use, and electronic waste processing). The latter impacts predominate today, causing undesirable concentrations of metals in soils. This is especially noticeable for agricultural land, including pastures and arable soils, either marginally or significantly influenced by agricultural activity. Also, the proximity of urban and industrialized areas has such an impact due to waste landfill and atmospheric deposition [2,3]. The presence of heavy metals in soils reduces productivity and interferes with the basic functions of soil. Moreover, it is well documented that heavy metals can pose a significant and serious health risk to plants, animals, and humans [4]. Soils heavily contaminated with heavy metals are excluded from the cultivation of plants intended for consumption or fodder, reducing the area used for plant production. This should be considered an unfavorable trend, especially when forecasting world population growth and the need to feed a large population. The importance of threats resulting from soil contamination with heavy metals was emphasized in the Thematic Strategy for Soil Protection [5]. As mentioned before, there are many sources from which heavy metals can get into the soil, and this transport can be direct as a result of soil application of agrochemicals or indirect as an impact of metal emissions from industrial sources. Regardless of the way in which metals are introduced into the soil, it is necessary to underline the high potential mobility and bioavailability of metals introduced in these ways. According to Hou et al., Nag et al., and Gupta et al. [1,3,4], the mobility of metals is largely influenced by soil geochemistry, including, among other things, soil pH, organic matter amount, redox conditions, cation exchange capacity (CEC), clay, and moisture contents. Some of these properties can be controlled and modified by atmospheric factors resulting in the limitation and/or reduction of metal bioavailability and their uptake with the crop yield. For this purpose, various remediation techniques are proposed to remove heavy metals from the environment or make them inactive [6]. Remediation techniques utilize physical, chemical, and biological technologies such as capping, encapsulation, landfilling, soil flushing, soil washing, electrokinetic extraction, stabilization, immobilization, solidification, vitrification, phytoremediation, and bioremediation. The choice of remediation methods depends on many factors, including the geographical location, soil properties (pH, soil texture, organic matter content, water content) and type and level of contamination. Remediation efficiency, the economic aspect, and environmental security are also important factors when selecting such techniques. In practice, cheap, cost-effective, and eco-friendly remediation techniques are used [7]. Such requirements are met by in situ chemical immobilization or phytoremediation methods. These methods are recommended for areas moderately contaminated with heavy metals, where the techniques used can give noticeable and measurable results. The principle of operation for both methods is different. Phytoremediation allows us to remove contaminations from a polluted area with the plantation of specific plants (crop-producing plants must be avoided as the edible parts may hold a higher concentration of metals), wherein phytoextraction, phytostabilization, phytovolatilization, phytodegradation, and phytofiltration are used [3]. Biological treatment is considered advantageous thanks to its being environmentally friendly and economically feasible. However, it simultaneously has some limitations, such as its long duration and low efficiency of the remediation process, application of selected plant species, and formation of toxic byproducts in the ash form after the incineration of plant residues.

In general, chemical methods are simple operations and provide quick results; however, the production of insoluble precipitates can limit applicability of these methods [8]. The group of chemical methods includes immobilization, stabilization, and solidification. According to Awathasi et al. [7], in situ immobilization has gained growing attention because of its potential effectiveness, the short duration of the treatment, the low cost, extensive time of action, and the less adverse influence on the environmental health. Hence, in recent years many immobilizing agents, such as red mud, lime biochar, composts, and fly ash have been applied to treat and mitigate heavy metal contamination in soils. Regardless of the used materials, the final effect is similar—reduction in heavy metal mobility and bioavailability for plants. The potential application of organic or alkali substances for remediation processes of soils contaminated with heavy metals has been underlined by various authors [7,9–15]. Binding of metals by organic matter or formation of insoluble compounds under alkali conditions leads to restricted metal uptake by plants and consequently prevents the bioaccumulation of heavy metals in the food chain. Bioaccumulation of metals from soil to crops varies across soil-cropping systems and depends on plant variety and metal concentration in soil [3]. The uptake of heavy metals from soil by plants is a significant path to harm human health. It is extremely important in the case of heavy metal-contaminated soils, because it promotes the actual state of metal mobility and the potential uptake by plants. Therefore it is important to control and limit the accumulation of heavy metals both in soil and in plants. Soil assessment of heavy metal contamination has to be based on total amounts of metals. Despite the total metal amounts being a basic criterion, when assessing the heavy metal contamination in soil it may inadvertently overestimate the potential risk [16]. Hence, more adequate and reliable information is provided by the mobile amounts of metals, indicating a risk of potential exposure for the soil–crop–human transfer, which is associated with metal bioavailability. Bioavailability is a very complex concept, but it generally refers to the dissolved metal fraction, which can be taken up by plant roots and soil organisms. Bioavailability is associated with the physical dispersion and chemical mobility of metals as well as the organism's exposure, biological characteristics, and individual sensitivity [16]. Evaluation of bioavailability must be based on more sensitive methods than in the case of total amounts; as a result, greater applicability is ascribed to the chelate DTPA. DTPA-extractable heavy metals mainly exist in water-soluble and ion-exchangeable states, which can be directly adsorbed by plants [9], so it is particularly important in the evaluation of the potential risk of their incorporation into the food chain. Moreover, it is recommended by the International Organization for Standardization [17]. Additionally, the DTPA test has been indicated as the best extractant method for Cu and Zn bioavailability in soil [18].

Based on the amount of metals in the soil and in the plant, the transfer of heavy metals from soil to plants should be assessed, with the bioconcentration factors (BCF) being useful coefficients [11,19]. Furthermore, the probable human risk associated with heavy metals should be calculated on the basis of two indices, such as dietary intake and health risk. These parameters are commonly used in practice as useful and reliable tools when assessing potential negative effects of heavy metals present in soils and plants in relation to their possible influence on human health [2].

Heavy metals constitute a group of elements characterized by high density and high toxicity even at low concentrations in the environment. According to the United States Environmental Protection Agency (EPA) compilation, 7 heavy metals—Pb, Cr, Zn, Cu, Cd, Hg, Ni and metalloid As—are listed to be most widespread heavy metals in the environment [8]. Simultaneously it should be underlined that Cu and Zn are essential micronutrients for plants needed for normal plant development [4]. These metals are also important for an adequate state of human health, because they play many functions in metabolic and physiological processes [20]. Therefore, often these metals are not perceived as a real threat to living organisms. However, an assessment of the potential transfer of Cu and Zn from soil to plants of consumer importance should be carried out even when the soil has an elevated metal content. According to the authors' knowledge, the current literature related to human health risk appraisal of Cu and Zn is concentrated mainly on analyses of commonly consumed food products of both plant (fruit and vegetables) and animal (meat, dairy) origin. The knowledge of metal dietary intake and potential health risks in the case of plant-origin products is limited, especially for oat and camelina—plants which are not so popular in comparison to fruits and vegetables. These plants are not directly consumed (only plant seeds are processed into food as oil or flakes), so their cultivation on soils with elevated amounts of heavy metals is allowed. Although such soils are used for agricultural purposes, simultaneously they undergo remediation processes. Consequently, the objectives of the present study were to: (1) measure contents of Cu and Zn in cultivated crops such as camelina and oat; (2) determine the total and bioavailable amounts of Cu and Zn in soil; (3) calculate the bioconcentration factors (BCF), estimated dietary intake (EDI), and health risk (HRI) indices; and (4) evaluate the effect of applied immobilizing agents (compost and fly ash) on Cu and Zn contents both in plants and soil, as well as their influence on the calculated factors and indices.

2. Materials and Methods

2.1. Experiment Description

This work presents a fragment of a long-term experiment conducted on medium soil (clay loam) classified as haplic cambisol according to WRB [21]. Detailed information concerning the experimental conditions is given by Jakubus and Graczyk [13]. According to the data presented in Table 1, in comparison to the thresholds established by the Directive [22], the tested soil was characterised by an elevated level of Cu and a permissible level of Zn. Compost (C) (a 1:1 mixture of biowaste and manure) and fly ash (FA) formed as a byproduct of lignite combustion were used as the immobilizing agents, and they represented a source of organic matter and alkali substances, respectively. Compost and fly ash were applied as dry mass substances into the soil at an equivalent amount of 40 $t \cdot ha^{-1}$ two weeks before plant cultivation. Each of the substances was thoroughly mixed with soil, placed in PVC pots (10 kg) and wetted to 60% of the field water capacity. Thus the design of the experiment included 3 treatments: T0—control soil (without compost or fly ash added), TI—soil with compost added, and TII—soil with fly ash added, and each one comprised eight replications.

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
Cu_{TOT} ($mg \cdot kg^{-1}$)	200 *	33	44
Zn_{TOT} ($mg \cdot kg^{-1}$)	100 *	15	20

* According to [22], the permissible content for Cu is up to 140 $mg \cdot kg^{-1}$ and for Zn up to 300 $mg \cdot kg^{-1}$.

Both crops grown in the crop rotation system adopted in the experiment, i.e., camelina and oat, are of nutritional importance, because they are used both as forage and for human nutrition. It is therefore reasonable to assess the accumulation of agricultural metals, as well as their probable uptake by humans along with the consumed products. Camelina (*Camelina sativa* L.) as a plant from the Brassicaceae family is characterized by the content of an oil with health-promoting properties, which is recommended for everyday consumption. On the other hand, oat (*Avena sativa* L.), is one of the most popular cereals, and it is intended for the production of oat flakes. Both plants at a density of 10 plants per pot were cultivated year by year in crop rotation in the same pot. After the harvesting of each plant from the pots, soil samples were taken for analysis. Next, the pots were cleaned of the remaining roots and the soil, and the compost and fly ash were replenished. This way, the next crop had the same growing conditions and the amounts of Cu and Zn in the substrate were theoretically always the same. Depending on the nutritional requirements of plants, adequate supplemental mineral fertilization was applied. The applied doses of fertilizers (ammonium nitrate, triple superphosphate, and potassium salt) were balanced in such a way that they took into account the amounts of N, P, and K introduced with compost and fly ash.

2.2. Analysis of Plant and Soil

The contents of Cu and Zn in plant and soil samples were assessed separately by using individual methods. In the case of plants, after they were dried at 60 °C, they were ground and ashed in a furnace at 450 °C for 6 h according to the method described by Ostrowska et al. [23]. Briefly, ash was dissolved in 5 mL of 6 $mol \cdot dm^3$ HCl and diluted to a constant volume (15 mL) with distilled water. In the case of soil, the total and bioavailable amounts were determined by using the *aqua regia* procedure [24] and DTPA complexing solution [17], respectively. The concentrations of metals in an ionic form in obtained extracts of plants and soil were determined by flame atomic absorption spectrometry (FAAS) by using a Varian Spectra AA 220 FS apparatus. All the assays identifying the amounts of nutrients in the tested samples were performed in three replications.

On the basis of Cu and Zn, total (Cu_{TOT}, Zn_{TOT}) and bioavailable amounts (Cu_{DTPA}, Zn_{DTPA}) in the soil and, in cultivated plants, two separate bioconcentration factors were calculated according to the ratio of metal concentration in plant seeds (only plant seeds are included because they are processed for consumption as stated above) and total or bioavailable amounts of metals as follows [11]:

$$BCF_T = \frac{\text{metal in plant seeds}}{\text{total metal content}}$$

$$BCF_B = \frac{\text{metal in plant seeds}}{\text{bioavailable metal amount}}.$$

The estimated daily intake (EDI) and the health risk index (HRI) were calculated. For the estimated daily intake (EDI) of metals the following equation was used [25]:

$$\text{EDI}\ [\mu g \cdot kg^{-1}] = \frac{\text{metal in plant seeds} \cdot \text{average daily consumption}*}{\text{body weight} **},$$

where $*$ is based on WHO data [26] on adults in Europe who have an average daily consumption of 2.0 g oat per person per day. Due to the fact that WHO does not give the daily consumption of camelina oil, considering that this plant is an oil crop, the same dose as provided by WHO for rapeseed was adopted, i.e., 7.3 g per person per day. Furthermore, $**$ is based on Polish statistics where the average body weight of adult women is 65 kg and that of men is 83 kg.

The health risk index (HRI) was calculated by using the following equation given by Jan et al. [27], cited after [20]:

$$\text{HRI} = \frac{\text{EDI}}{\text{RfD}*},$$

where $*$ RfD is the reference oral dose; the values for Cu and Zn amounted to 0.04 and 0.30 (mg/kg/day), respectively (values given according to US-EPA [28]).

2.3. Statistical Analysis

In order to compare the influence of compost and fly ash on the tested parameters, Cu and Zn levels in plants, total and bioavailable amounts of metals, BCF_T, BCF_B, EDI_W, EDI_M, HRI_W, and HRI_M, were compared for the following pairs: T0 and TI, T0 and TII, as well as TI and TII with respect to the amounts of Cu and Zn. In the first step, it was checked whether the samples came from the normal distribution. Normality was verified with the Shapiro–Wilk Test (Shapiro–Wilk Normality Test). If the sample showed a normal distribution, the appropriate Student's *t*-test was selected for analysis in order to compare the mean Cu and Zn amounts in plants and soil, as well as mean values of the parameters BCF_T, BCF_B, EDI_W, EDI_M, HRI_W, and HRI_M. On the other hand, when testing normality if it was concluded that the sample did not come from a normally distributed population, the Wilcoxon test was used to compare the two samples. Regardless of the soil or the additive used, or the cultivated plant, all analyzed samples had the same number of replications, $n = 8$. Therefore, the design of the considered experiment was treated as a completely random design, and appropriate statistical tools were applied. All the analyses were performed in the R environment (R version 4.1.2). The violin charts were used for the visualization of Cu and Zn contents in plants and soil. These graphs show all the basic statistics from the samples. The bottom line of each violin chart represents the minimum metal amount in plant and soil (total and bioavailable amounts), whereas the top value of the violin is the maximum metal amount. The lower range of the black box represents the lower quartile of Q1, below which 25% of the sample observations (in terms of metal content) are found. The upper range of the black box represents the upper quartile of Q3, below which 75% of the sample observations are found. The white dot in the black box represents the median Q2 (middle value), below which 50% of the observations in terms of metal amount are found.

3. Results

3.1. Camelina

The average Cu content in camelina grown on the control soil was the highest and amounted to 75.5 mg·kg^{-1}; the lowest was found for the plant grown on compost-enriched soil (47.5 mg·kg^{-1}), and the addition of fly ash contributed to the amount of Cu in the plants at 52.5 mg·kg^{-1} (Figure 1). According to the data in Table 2, the amount of Cu in camelina grown in the soil enriched with compost differed statistically significantly from the amount of Cu in camelina cultivated in the control soil. Significant differences can also be underlined when comparing the Cu content in plants grown in the soil with FA addition with that in camelina cultivated in the control soil. Moreover, in terms of Cu content in camelina, the use of compost or fly ash resulted in quantitative changes at a similar level (they did not differ statistically significantly).

Table 2. The *p*-values from the comparisons of Cu and Zn contents in camelina, soil, and other parameters depending on the applied amendments (compost—TI and fly ash—TII) and their absence (control—T0).

	Cu								
	Plant	**Total Content**	**Bioavailable Content**	**BCF_T**	**BCF_B**	**EDI_W**	**EDI_M**	**HRI_W**	**HRI_M**
T0–TI	<0.001 *	<0.001 * W	<0.001 *	<0.001 *	0.002 * W	<0.001 *	<0.001 *	<0.001 *	<0.001 *
T0–TII	<0.001 *	<0.001 *	<0.001 *	<0.001 *	0.002 * W	<0.001 *	<0.001 *	<0.001 *	<0.001 *
TI–TII	**0.098**	0.038 * W	**0.063**	**0.452**	**0.051**	**0.098**	**0.098**	**0.098**	**0.098**
	Zn								
	Plant	**Total Content**	**Bioavailable Content**	**BCF_T**	**BCF_B**	**EDI_W**	**EDI_M**	**HRI_W**	**HRI_M**
T0–TI	<0.001 *	**0.077**	0.016 *	<0.001 * W	0.006 *	<0.001 *	<0.001 *	<0.001 *	<0.001 *
T0–TII	<0.001 *	**0.203**	<0.001 *	<0.001 * W	**0.261**	<0.001 *	<0.001 *	<0.001 *	<0.001 *
TI–TII	**0.334**	**0.664**	**0.389**	**0.161** W	**0.197**	**0.334**	**0.334**	**0.334**	**0.334**

*—denotes statistically significant differences at $\alpha = 0.05$; W—denotes comparisons made with the Wilcoxon test for data, for which normality was not met. The other comparisons were made with Student's *t*-test. The *p*-values for pairs, which do not differ statistically significantly, are marked in bold.

On average (for all the samples), the highest total Cu level was observed in the soil with the addition of fly ash, where it amounted to 243.3 mg·kg^{-1}; the lowest mean value was recorded in the control soil at 199.5 mg·kg^{-1}, as a result of the application of compost where the total Cu content was 233.4 mg·kg^{-1} (Figure 1). The total Cu content in soil TII was statistically significantly higher than the total content of Cu in T0. Moreover, Cu_{TOT} in the soil enriched with compost was statistically significantly higher than Cu_{TOT} in the control soil (Table 2). Additionally, a significantly lower amount of Cu was found in the soil enriched with compost compared to the soil with the addition of fly ash (*p*-value = 0.038) (Table 2).

The average Cu_{DTPA} for the control soil was the highest and amounted to 105.9 mg·kg^{-1}, the lowest mean bioavailable amount of this metal (86.78 mg·kg^{-1}) was determined in the soil with fly ash addition, whereas for the soil fertilized with compost, the average Cu_{DTPA} was 92.7 mg·kg^{-1} (Figure 1). Cu_{DTPA} in the soil enriched with compost was statistically significantly lower in comparison to the data showed for the control soil. Similarly, a significant difference was observed between the bioavailable Cu amount in the control soil and that found for the soil fertilized with fly ash (Table 2).

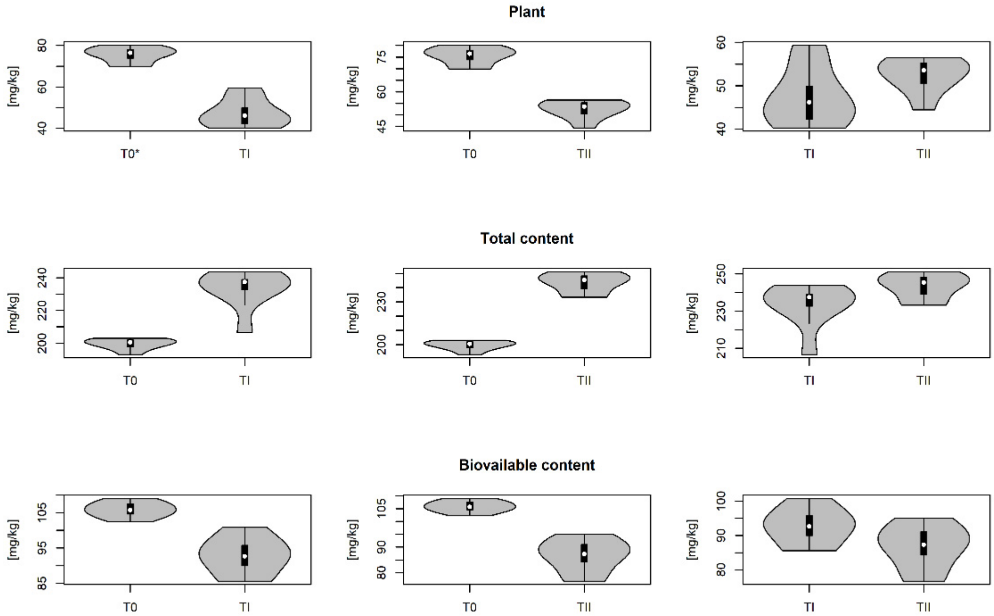

Figure 1. Cu contents in camelina and soil (total and bioavailable amounts) * descriptions see at Material and Methods, statistically significant differences and *p*-value are presented in Table 2.

The highest mean amount of Zn in camelina was determined for plants grown in the control soil, where it was 107.4 mg·kg^{-1}, and the lowest was in plants grown in the soil fertilized with compost (80.3 mg·kg^{-1}). On the other hand, the addition of fly ash contributed to the average amount in camelina at 84.6 mg·kg^{-1} (Figure 2). The highest mean total Zn content (for all the samples) was recorded for the soil enriched with compost (106.0 mg·kg^{-1}), and the lowest mean total Zn amount was found for the control soil (101.2 mg·kg^{-1}), whereas in soil with the fly ash addition the total Zn content amounted to 104.6 mg·kg^{-1} (Figure 2). The average Zn_{DTPA} amount found for the control soil was the highest and amounted to 17.10 mg·kg^{-1}. The lowest mean bioavailable amount of this metal (14.4 mg·kg^{-1}) was observed in the soil with the fly ash addition, whereas in the soil enriched with compost the mean amount of Zn_{DTPA} was 15.01 mg·kg^{-1} (Figure 2). In general, Zn levels in camelina and soil, as well as the calculated parameters, showed a similar tendency as was indicated for Cu. Bioavailable amounts of Zn as well as Zn contents in camelina grown in soil amended with compost or fly ash were significantly lower in comparison to data evaluated for the control plants. An exception was found for the Zn_{TOT} amount, because the compost or fly ash addition into the soil did not significantly affect the total metal contents (Table 2).

On the basis of the metal amounts found in camelina and soil, the bioconcentration factors were calculated by taking into account the total amounts of Cu and Zn and their bioavailable amounts. According to data in Table 2, it can be seen that the applied compost and fly ash influenced the calculated bioconcentration factors (BCF_T, BCF_B) for both metals. The values of these parameters in the compost-enriched soil were statistically significantly lower. A similar tendency can be indicated in relation to the control soil and soil enriched with fly ash (Table 2), where the difference between values was significant. The exception was for the BCF_B values calculated for Zn, because a lack of differences was observed between the control soil and that fertilized with fly ash (*p*-value = 0.261, Table 2). The values of bioconcentration factors for camelina are presented in Figure 3. According to these data, BCF_T for Cu averaged from 0.2 (TI, TII) to 0.4 (T0), whereas for Zn it was from 0.8 (TI, TII) to 1.1 (T0). Slightly higher average values were found for BCF_B, which for Cu ranged from 0.5 (TI) to 0.7 (T0). In the case of Zn, these parameters showed higher average values ranging from 5.4 (TI) to 6.3(T0). For Cu under the control conditions, the values were higher by 43% (BCF_T) and 15–27% (BCF_B) compared to those found for soils fertilized with compost or fly ash. In the case of Zn, the above bioconcentration factors were also higher in the control soil by 24–28% (BCF_T) and by 14% (BCF_B) (Figure 3).

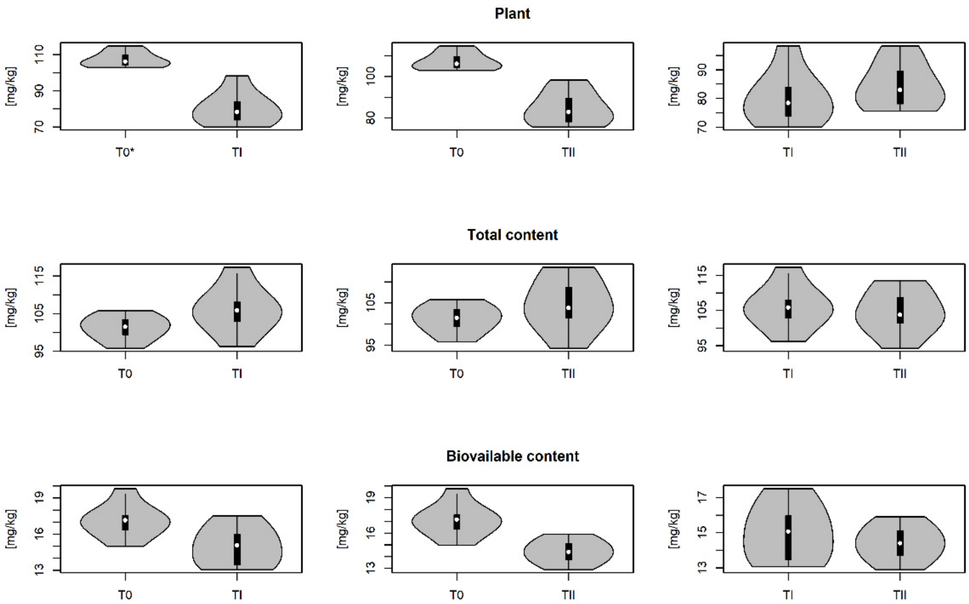

Figure 2. Zn contents in camelina and soil (total and bioavailable amounts) * descriptions see at Material and Methods, statistically significant differences and *p*-value are presented in Table 2.

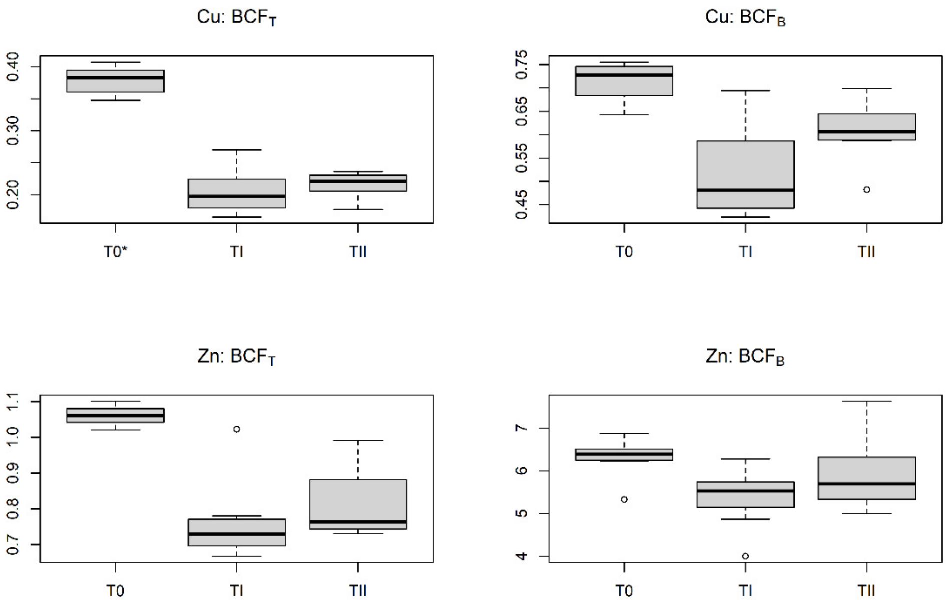

Figure 3. Bioconcentration factors for total (BCF_T) and bioavailable amounts (BCF_B) calculated for Cu and Zn in camelina; * for descriptions see Material and Methods. Statistically significant differences and *p*-value are presented in Table 2.

When assessing the influence of the applied additives on the estimated daily intake (EDI) and health risk index (HRI), it was marked more strongly in the case of the first parameter, with the calculated values of EDI and HRI for Cu and Zn being greater for women than for men. Based on the data in Table 2, it can be seen that the applied compost and fly ash also influenced the estimated daily intake (EDI) and health risk index (HRI). The values of these parameters in the compost-enriched soil were statistically significantly lower. A similar tendency can be indicated in relation to the control soil and soil enriched with fly ash (Table 2), where the difference between values was significant. As presented in Figure 4, the average values of EDI for women ranged from 5.3 $\mu g \cdot kg^{-1}$ (TI) to 8.5 $\mu g \cdot kg^{-1}$ (T0), whereas for HRI it was from 0.13 (TI) to 0.21 (T0). In the case of men, the lowest average EDI and HRI values were determined for TI (4.2 $\mu g \cdot kg^{-1}$ and 0.10, respectively, for EDI and HRI) and the highest for T0 (6.6 μg·kg^{-1} and 0.16, respectively, for EDI and HRI) (Figure 4). The data in Figure 5 show the calculated parameters for Zn. Under the control conditions (T0), the highest average values of EDI_W, EDI_M, HRI_W, and HRI_M were 12.1, 9.45, 0.04 and 0.03, respectively. In turn, the lowest values of these parameters were calculated for TI and TII, whereas the average values were comparable (EDI_W = 9.5 μg·kg^{-1}, EDI_M = 7.4 μg·kg^{-1}, HRIw = 0.03; HRI_M = 0.02) (Figure 5).

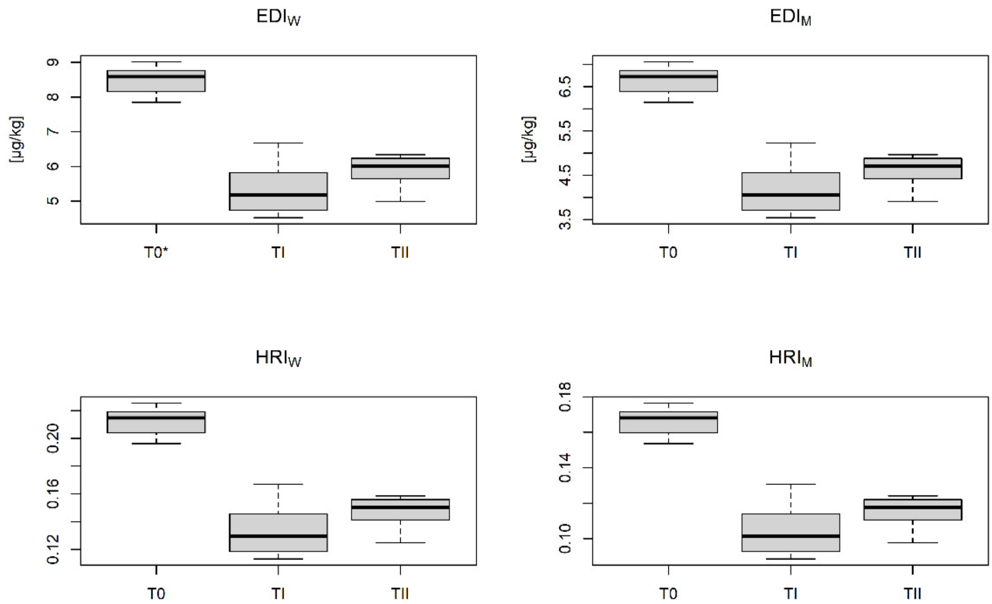

Figure 4. Estimated daily intake (EDI) and health risk index (HRI) calculated for women (W) and men (M) for Cu in camelina; * for descriptions see Material and Methods. Statistically significant differences and *p*-value are presented in Table 2.

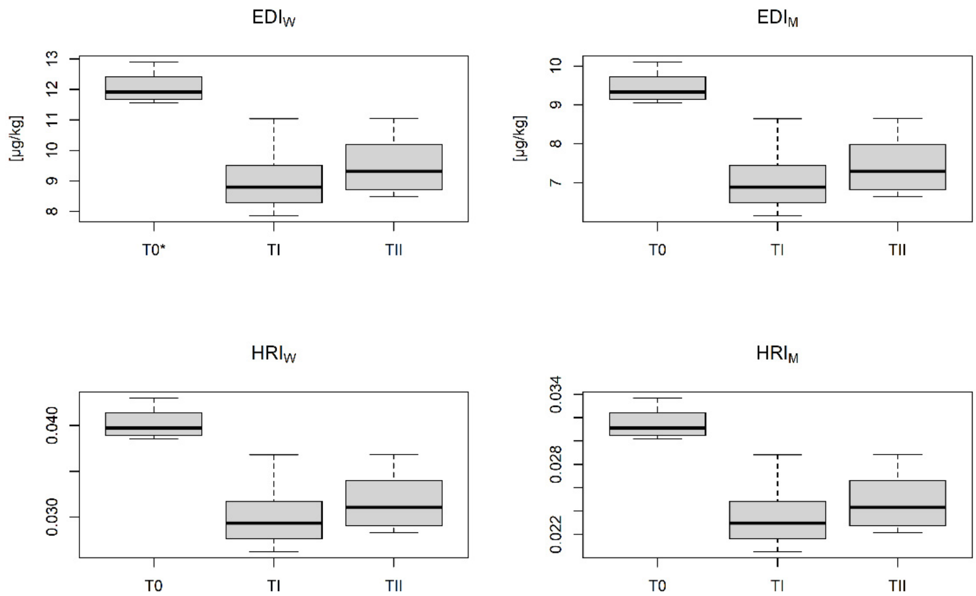

Figure 5. Estimated daily intake (EDI) and health risk index (HRI) calculated for women (W) and men (M) for Zn in camelina; * for descriptions see Material and Methods. Statistically significant differences and *p*-value are presented in Table 2.

3.2. Oat

As for camelina, the average Cu amount in oat grown in the control soil was the highest and amounted to 16.0 mg·kg^{-1}. Average values for plants grown in soil enriched with compost and with the addition of fly ash were comparable amounting to 13.1 mg·kg^{-1} (TI) and 13.8 mg·kg^{-1} (TII) (Figure 6). On average (for all the samples), the total amount of Cu in soil was the highest in the case of applied fly ash (TII) and amounted to 231.2 mg·kg^{-1}, whereas the lowest average value was found for the control soil (192.4 mg·kg^{-1}). In turn, the soil enriched with compost showed the total mean Cu content at 215.9 mg·kg^{-1} (Figure 6). The average bioavailable Cu amount (103.7 mg·kg^{-1}) was the highest in the control soil, whereas the average Cu_{DTPA} levels for the soil with compost and fly ash were significantly lower and for TI amounted to 89.3 mg·kg^{-1}, whereas for TII it was 85.7 mg·kg^{-1} (Figure 6). The data presented in Table 3 showed a strong, statistically confirmed influence of compost and fly ash on Cu amounts in both plants and soil. The amount of Cu in oat grown in the soil enriched with compost differed statistically significantly from that in plants cultivated in the control soil. On the other hand, Cu_{TOT} in soil TI was significantly higher than Cu_{TOT} in T0. Moreover, Cu_{DTPA} in the soil enriched with compost was statistically significantly lower than the amount in the control soil (Table 3). As was indicated in the case of camelina, the highest average Zn amount was also found for oat grown in the control soil, in which it was 58.3 mg·kg^{-1}. In oat cultivated in soil enriched with compost, the mean Zn content amounted to 38.6 mg·kg^{-1}. With the addition of fly ash, the average Zn amount in plants was 41.6 mg·kg^{-1} (Figure 7). On average (for all the samples), the total amount of Zn was the highest in the soil with the fly ash addition (117.0 mg·kg^{-1}), the lowest mean total amount of metal was recorded for the control soil at 105.4 mg·kg^{-1}, whereas for the soil enriched with compost it was 114.4 mg·kg^{-1} (Figure 7).

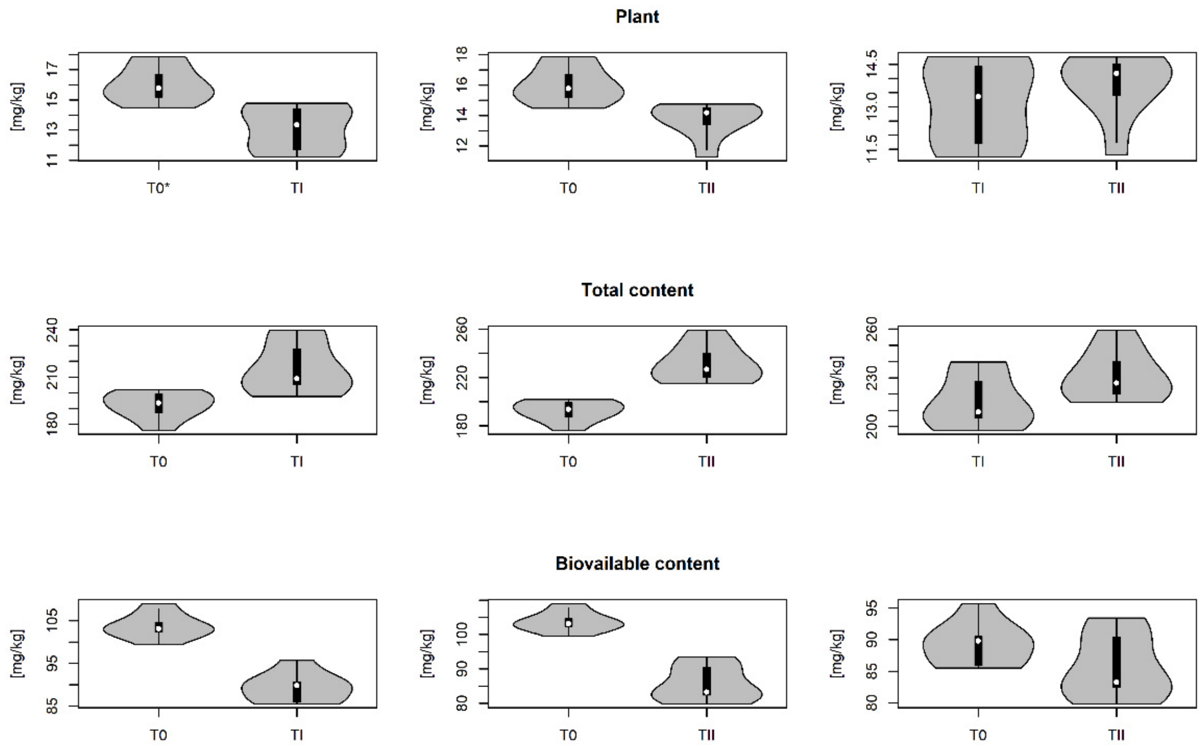

Figure 6. Cu contents in oat and soil (total and bioavailable amounts). * for descriptions see Material and Methods. Statistically significant differences and p-value are presented in Table 3.

Table 3. The *p*-values from the comparisons of Cu and Zn contents in oat, soil and other parameters depending on the applied amendments (compost, TI and fly ash, TII) and their absence (control, T0).

				Cu					
	Plant	**Total Content**	**Bioavailable Content**	**BCF_T**	**BCF_B**	**EDI_W**	**EDI_M**	**HRI_W**	**HRI_M**
T0–TI	<0.001 *	0.003 *	<0.001 *	<0.001 *	**0.388**	<0.001 *	<0.001 *	<0.001 *	<0.001 *
T0–TII	0.001 * W	<0.001 *	<0.001 *	<0.001 *	**0.383**	0.001 * W	0.001 * W	0.001 * W	0.001 * W
TI–TII	**0.574** W	**0.075**	**0.116**	**0.682**	**0.122**	**0.574** W	**0.574** W	**0.574** W	**0.574** W
				Zn					
	Plant	**Total Content**	**Bioavailable Content**	**BCF_T**	**BCF_B**	**EDI_W**	**EDI_M**	**HRI_W**	**HRI_M**
T0–TI	<0.001 *	0.009 *	0.005 *	<0.001 *	<0.001 *	<0.001 *	<0.001 *	<0.001 *	<0.001 *
T0–TII	0.001 *	0.001 *	<0.001 *	<0.001 *	**0.978**	0.001 *	0.001 *	0.001 *	0.001 *
TI–TII	**0.256**	**0.407**	0.029 *	**0.439**	0.023 *	**0.256**	**0.256**	**0.256**	**0.256**

* denotes statistically significant differences at α = 0.05; W denotes comparisons made with the Wilcoxon test for data, for which normality was not met. The other comparisons were made with Student's *t*-test. The *p*-values for pairs, which do not differ statistically significantly, are marked in bold.

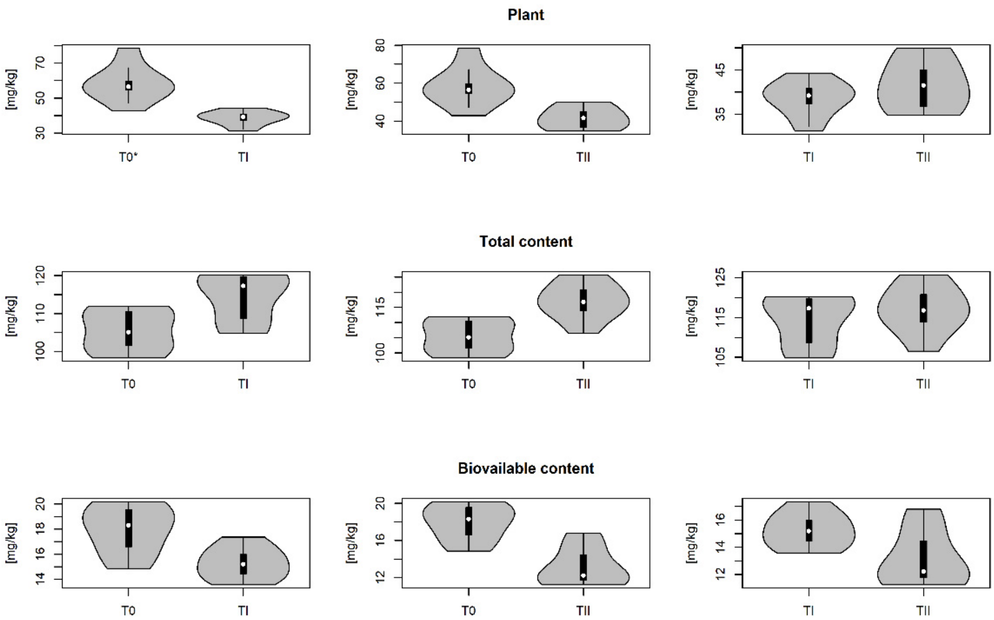

Figure 7. Zn contents in oat and soil (total and bioavailable amounts); * for descriptions see Material and Methods. Statistically significant differences and *p*-value are presented in Table 3.

Similar to camelina, the highest (17.9 mg·kg^{-1}) average bioavailable amount of Zn was recorded for the control soil, whereas in soils enriched with compost and fly ash it was 15.3 mg·kg^{-1} (TI) and 13.2 mg·kg^{-1} (TII), respectively (Figure 7). Cultivation of oat did not change the general Zn behavior and the potential influence of applied amendments. Thus, the amount of Zn in oat grown in the soil enriched with compost differed statistically (it was significantly lower) than that in plants grown in the control soil (Table 3). On the other hand, Zn_{TOT} in the soil amended with compost was statistically significantly higher than the total amount of zinc in the control soil. Moreover, Zn_{DTPA} in the soil enriched with compost was significantly lower than the amount of bioavailable zinc in the control soil (Table 3).

Moreover, for oat the bioconcentration factors were calculated by taking into account the total contents of Cu and Zn, as well as their bioavailable amounts. Considerably lower values of these indicators were found compared to camelina, although the tendency and the effect of the additives used were the same (Figure 8). Thus BCF_T and BCF_B were higher under the control conditions. According to the data presented in Table 3, the observed influence of the applied immobilizing agents was confirmed statistically. As was proven for camelina, in the case of oat the influence of compost and fly ash can be seen, because the differences between values of bioconcentration factors, calculated for Cu and Zn and those obtained for T0 and TI as well as T0 and TII differed significantly. An exception in this respect was found for BCF_B of copper. Enriching the soil with compost or fly ash did not significantly change the value of this parameter for Cu (Table 3). Application of compost or fly ash decreased the Cu values by 24–26% (BCF_T) and by 14–17% (BCF_B), respectively. For Zn the values of bioconcentration factors were lower by 35–39% (BCF_T) and by 22% (BCF_B) in comparison with the control. At the same time, the stronger influence of compost should be underlined here. The average BCF_T values for Cu ranged from 0.06 (TI, TII) to 0.08 (T0), whereas the average BCF_B values for this metal ranged from 0.15 (T0, TI) to 0.16 (TII). In the case of Zn, BCF_T ranged from 0.34 (TI) to 0.55 (T0), whereas BCF_B ranged from 2.53 (TI) to 3.24 (T0, TII), respectively (Figure 8).

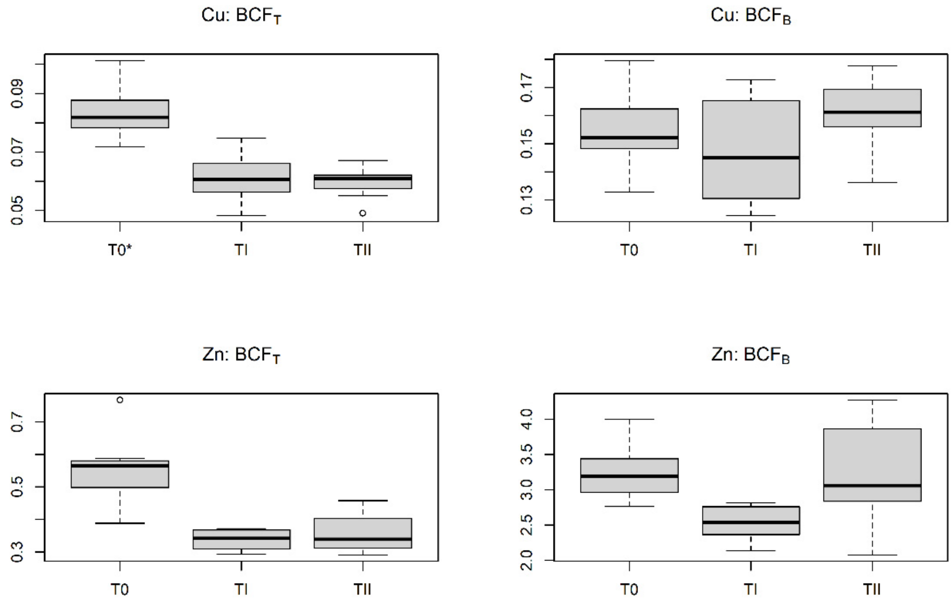

Figure 8. Bioconcentration factors for total (BCF_T) and bioavailable amounts (BCF_B) calculated for Cu and Zn in oat; * for descriptions see Material and Methods. Statistically significant differences and *p*-value are presented in Table 3.

The influence of compost and fly ash was proven statistically because the differences between values of EDI_W, EDI_M, HRI_W, and HRI_M calculated for Cu and Zn for T0 and TI as well as T0 and TII differed significantly (Table 3). Simultaneously, the effect of both compost and fly ash on the calculated parameters (EDI_W, EDI_M, HRI_W, and HRI_M) for both metals was the same, because the values did not differ statistically. The data in Figure 9 shows the distribution of EDI and HRI values depending on gender of consumers and the additives used. A similar tendency may be observed as shown earlier for camelina, with higher values of these parameters determined for women, whereas at the same time under control conditions they were significantly higher. For women the calculated average EDI value for Cu ranged from 0.40 $\mu g \cdot kg^{-1}$ (TI) to 0.49 $\mu g \cdot kg^{-1}$ (T0), whereas HRI, regardless of the applied amendments, showed comparable values ranging from 0.010 to 0.012. The EDI_W values calculated for TI and TII were 14–18% lower in comparison to these obtained for T0. In the case of men, the average values of both parameters calculated for Cu ranged from 0.32 $\mu g \cdot kg^{-1}$ (TI, TII) to 0.39 $\mu g \cdot kg^{-1}$ (T0) for EDI and from 0.008 (TI, TII) to 0.009 (T0) for HRI (Figure 9). Referring to the calculated EDI values for Zn, they were significantly higher for both women and men, whereas the HRI values were significantly lower compared to those discussed above for Cu. As it results from the data presented in Figure 10, the highest values of EDI_W, EDI_M, HRI_W, and HRI_M calculated for Zn were determined under the control conditions. In turn, for the soil fertilized with compost or fly ash, they were lower. The application of immobilizing agents resulted in a reduction of EDI_W and EDI_M by 34% and 29%, respectively. For women, average EDI values for Zn ranged from 1.19 μg·kg^{-1} (TI) to 1.79 μg·kg^{-1} (T0), whereas for HRI these average values ranged from 0.004 (TI, TII) to 0.006 (T0). For men, the average EDI values for Zn ranged from 0.93 μg·kg^{-1} (TI) to 1.41 μg·kg^{-1} (T0), and the average HRI ranged from 0.003 (TI, TII) to 0.005 (T0), respectively (Figure 10).

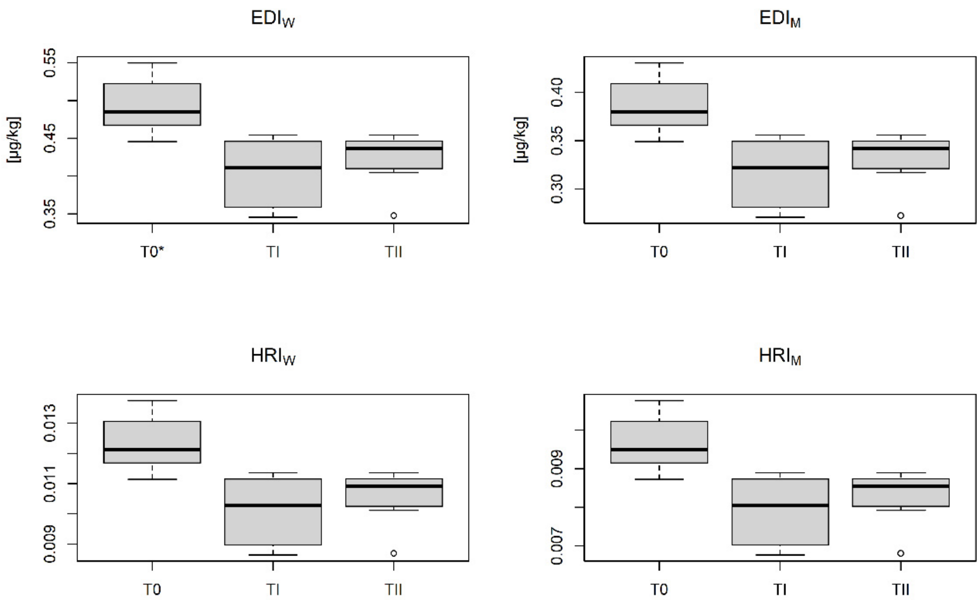

Figure 9. Estimated daily intake (EDI) and health risk index (HRI) calculated for women (W) and men (M) for Cu in oat; * for descriptions see Material and Methods. Statistically significant differences and *p*-value are presented in Table 3.

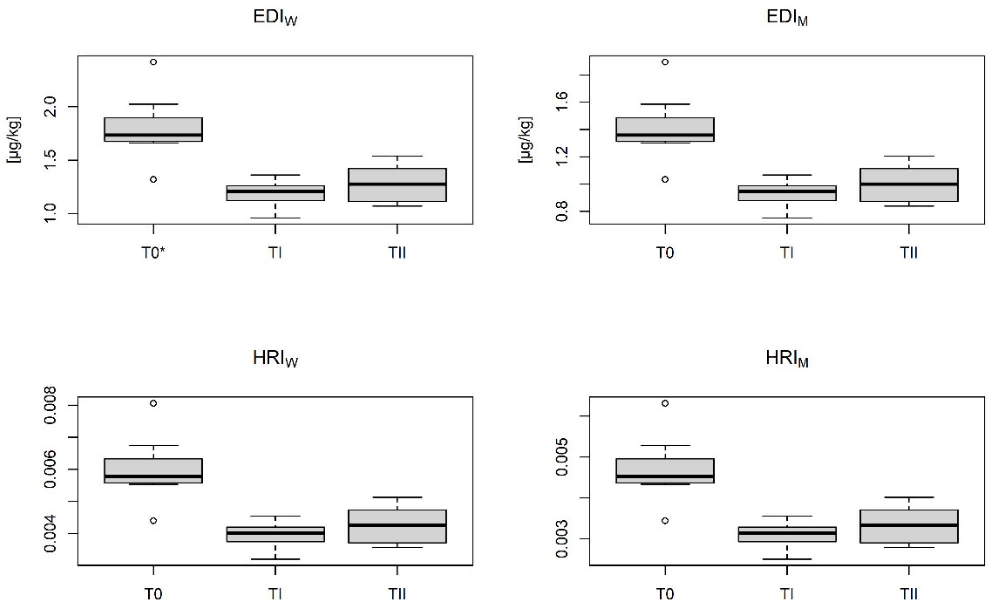

Figure 10. Estimated daily intake (EDI) and health risk index (HRI) calculated for women (W) and men (M) for Zn in oat; * for descriptions see Material and Methods. Statistically significant differences and *p*-value are presented in Table 3.

4. Discussion

4.1. Effect of Immobilizing Agents on Bioconcentration Factors (BCF)

Despite the fact that some metals perform important metabolic functions, their excessive amounts pose a significant health hazard for humans [29]. Thus, the need to improve soil conditions and mitigate heavy metal mobility through the use of remediation methods is indicated. Especially inexpensive, natural and/or industrial byproducts may be an effective alternative to conventional immobilization methods [30]. In practice, organic and inorganic substances are usually used as immobilization agents. The most popular organic immobilizing agent are composts, manure, and biochar [7,9,11,13–15]. In turn, mineral substances proposed for remediation purposes include natural zeolites, lime, phosphorus [15,30], and fly ash [13,14,31]. It is worth emphasizing that the abovementioned substances not only immobilize metals, but also fertilize (especially compost and manure), modifying the physicochemical properties of fertilized soils, first of all pH, OM, TOC and CEC, which consequently mitigates the bioavailability of heavy metals for plants [7,9].

The bioconcentration factor (BCF) is widely used to assess the toxicity of heavy metals as well as their translocation from soil to plants [11,19,32–34]. Additionally, Hu et al. [35] stated that the bioaccumulation values rather than the total contents of heavy metals should be taken into consideration. In practices, the bioconcentration factor is usually applied based on the total amount of heavy metals (BCF_T) in soil and plant tissue. According to Jakubus et al. [11], the bioaccumulation values rather than the total contents of heavy metals should be taken into consideration, and the authors proposed an additional bioconcentration factor by taking into account the bioavailable amounts of metals. As a result, in this study two separate bioconcentration factors were calculated: BCF_T and BCF_B. Generally, the applied amendments caused only an increment of Cu and Zn total contents in soil, whereas the other analyzed parameters show a downward tendency in comparison to the control conditions. The applied compost and fly ash similarly influenced most of the tested indices, which resulted in non-significant differences between the values of individual parameters recorded in this study. Heavy metal concentrations in plants are crucial, because they greatly influence the nutritional value. According to Guo et al. [36], the accumulation ability of heavy metals varies among plants, which may be affected by factors related to soil properties as well as differences in the morphology and physiology of plants. Generally, leafy vegetables accumulate greater amounts of metals in comparison to non-leafy vegetables [4,35]. It is in agreement with findings published by Guo et al. [36], who for leafy vegetables recorded the highest values of BCF for Cu (0.018) and Zn (0.024). Greater values of BCF for Zn (0.1–0.7) and BCF for Cu (0.2–1.0) in vegetables irrigated with different water sources were indicated by Leblebici and Kar [37]. Comparable values of BCF for Zn in young (0.7) and mature (0.2) tea leaves were found Zhang et al. [38]. On the other hand, in the abovementioned paper BCF for Cu ranged from 0.1 (mature tea leaves) to 0.16 (young tea leaves). In this study, regardless of the analyzed metal, the addition of both substances reduced their mean amounts in camelina by 21–37% and by 14–34% in oat in relation to metal levels found in plants cultivated in the control soil. Despite the fact that the addition of both organic and inorganic amendments into soil resulted in a small (12–20%), but statistically significant increase of Cu and Zn amounts in the soil (except for Zn in the soil where camelina was grown), the BCF_T coefficients for Cu and Zn were significantly lower (on average by 20–40%, regardless of the plant) compared to the values specified for the control conditions. BCF_T for camelina ranged from 0.2 (TI, TII) to 0.4 (T0) for Cu and from 0.8 (TI, TII) to 1.1 (T0) for Zn. In this study, for oat as a cereal plant BCF_T was from 0.06 (TI, TII) to 0.08 (T0) for Cu and from 0.3 (TI, TII) to 0.6 (T0) for Zn. Luo et al. [39] reported for another cereal (wheat) a BCF of 0.23 and 0.20 for Cu and Zn, respectively. Taking into consideration the threshold (above 1) given by the cited authors for BCF, in this study the probability of metal accumulation may be indicated only in the case of Zn in camelina cultivated in the control soil. This may be due to the fact that, in general, camelina was characterized by higher contents of Cu and Zn compared to oat and although the content of Cu was greater in the soil, more Zn was found in the cultivated plants. The immobilization effect of the applied additives was also recorded and confirmed statistically in the case of Cu_{DTPA} and Zn_{DTPA} as well as BCF_B for these metals. Generally, the bioavailable amounts of Cu account for 36–53% of Cu_{TOT}, wherein the highest percentage share was recorded for the control soil. On the other hand, Zn_{DTPA} in total Zn content accounted for 11–17%

and similarly the highest percentage share was found in the control soil. Regardless of the cultivated plant, the application of these amendments caused an increase in the total contents of these metals and a reduction of their bioavailable amounts in the analyzed soils. However, the effect of the applied immobilizing agents was weaker, because, regardless of the cultivated plant, the differences between bioavailable amounts of Cu and Zn found for TI and TII were lower by 12–18% (Cu) and by 12–26% (Zn) in comparison to values determined for T0. A similar range of differences was found for BCF_B. The presented study confirmed earlier reports [7,11,13–15,30,31] concerning the immobilization effects of compost and fly ash on heavy metals in soil and plants, especially in relation to their bioavailable amounts. As was indicated by Chen et al. [9], a compost addition can increase bioavailability of Cu in soil. In this study, a lack of significant differences between compost and fly ash in their immobilizing activity was confirmed, but a stronger effect was observed for fly ash. However, the differences of pH values were not confirmed statistically; fly ash caused an increase in soil pH, which was expressed as a slightly alkaline soil reaction. According to Nag et al. [3], such pH conditions show low mobility of heavy metals, especially Cu and Zn solubility is distinctly diminished. Apart from alkaline pH, the most important factor controlling the mobility of heavy metals is organic matter because of the presence of huge amounts of humic substances, which could associate with heavy metals, immobilize them, and then mitigate their availability for plants. However, it needs to be remembered that the input of fresh organic matter (for example as compost) can result not only in the formation of tight chelates with humic substances, but also in an increase of the bioavailability of metals due to bonding with organic acids [3]. This can explain the weaker effect of applied composts in this study and the lack of the immobilizing effect for Cu reported by Chen et al. [9].

4.2. Effect of Immobilizing Agents on Estimated Daily Intake (EDI) and Health Risk Index (HRI)

Studies related to the accumulation of heavy metals in plants and their further impact on the human diet are mainly focused on vegetables and fruit, so it is difficult to compare the results of this study with those presented in the literature. Nevertheless, the cited literature data constitute grounds for discussion, especially because the presented values of EDI or HRI, regardless of plants, are similar in some cases. Sultana et al. [40] analyzed various vegetables and fruit and recorded EDI values for Cu amounting from 5 to 40 $\mu g \cdot kg^{-1}$ and for Zn from 20 to 110 $\mu g \cdot kg^{-1}$. In turn, Amer et al. [41] for various fruits obtained very high EDI for Cu ranging from 95 to 990 $\mu g \cdot kg^{-1}$. Remarkably lower values for EDI (Cu from 0.05 to 0.15 $\mu g \cdot kg^{-1}$ and Zn from 0.2 to 0.5 $\mu g \cdot kg^{-1}$) were found by Leblebici and Kar [37] for vegetables. Latif et al. [20] also tested vegetables and obtained higher EDI values for Cu (1.66 $\mu g \cdot kg^{-1}$) and Zn (13.9 $\mu g \cdot kg^{-1}$). Vegetables analysed by Guo et al. [36] showed greater amounts of Cu and Zn, which was expressed in EDI values of 4.48 and 23.7 $\mu g \cdot kg^{-1}$, respectively. In this study, the estimated daily intake for camelina and oat was calculated separately for adult men and women and, regardless of the metal, the mean values which were obtained were higher for women (Cu: 5.3 to 8.5 $\mu g \cdot kg^{-1}$ for camelina and 0.4 $\mu g \cdot kg^{-1}$ for oat; Zn: 9.0 to 12.1 $\mu g \cdot kg^{-1}$ for camelina and 1.2 to 1.8 $\mu g \cdot kg^{-1}$ for oat) because of their lower body weight. Regardless of the observed differences between men and women for the EDI values, the effect of applied compost and fly ash was the same and led to a decrease in these amounts by 18 to 34% in comparison to the data found for the control. In order to realistically assess the obtained EDI_W and EDI_M values for Cu and Zn calculated for camelina and oat, they should be referred to the provisional tolerable daily intake (PTDI) values. It needs to be underlined here that EDI provides information concerning the daily intake of heavy metals (here Cu and Zn) with food products. On the other hand, tolerable daily intake refers to the daily amount of heavy metals in food that can be tolerated by humans over a long period with no adverse health effects. According to Latif et al. [20], PTDI amounts to 60 mg and 3 mg per day for Zn and Cu, respectively. Taking into account the above information and confronting it with the EDIw and EDI_M values obtained in this study for both metals, it can be stated that they were considerably lower than those given for PTDI. Therefore, it may be stated that the amounts of Cu and Zn that could be theoretically consumed by adults with oat or camelina products do not pose any health risk.

Similarly, although they are weaker, effects may be observed in the case of HRI values, which were significantly higher for the control samples. Regardless of gender, the HRI values were comparable and for Cu amounted on average to 0.008–0.01 for oat and 0.11–0.21 for camelina. On the other hand, for Zn the average HRI values ranged from 0.003 to 0.006 for oat and from 0.002 to 0.004 for camelina. The values shown above are significantly lower than those reported in the literature [20], where HRI for Zn and Cu reached 0.46 and 0.041, respectively. In light of the HRI threshold given by Guo et al. [36], amounting to less than 1, neither camelina nor oat tested in this study represent any adverse effect on human health, and the consumption of products originating from these plants (oat flakes and camelina oil) pose no threats connected with heavy metals.

5. Conclusions

Soils with low heavy metal pollution (as in this study) are approved for agricultural use in the production of crops, but not those directly consumed. Following the principles of human and animal health safety, constant monitoring of such soils and plants grown there is recommended. It is closely connected with food security and the potential transport of heavy metals via the food chain to humans. The presented findings underlined the potential utilization of soils with elevated amounts of Cu for agricultural purposes to produce crops, which are not directly consumed by humans. However, continuous monitoring of the actual heavy metal contamination in soil, as well as metal concentrations in crops, is essential and necessary for the simultaneous application of chemical immobilizing methods. The conducted studies confirmed the applicability of immobilizing agents (compost and fly ash) introduction into the soil with elevated Cu contents. In this study, Cu and Zn were analyzed and their contents in camelina and oat were significantly reduced in soils enriched with either compost or fly ash. The positive influence of compost and fly ash was expressed in the case of bioavailable amounts of Cu and Zn as well as their BCF_B, because these values significantly decreased in comparison to the control conditions. Despite the fact that under the influence of the applied soil additives the total content of Cu and Zn increased in relation to their amounts in the control soil, the calculated concentration coefficients (BCF_T) for the soil fertilized with compost (TI) and fly ash (TII) were lower compared to the values calculated for the control soil (T0). The EDI and HRI values calculated for Cu and Zn individually for camelina and oat decreased significantly in the case of plants grown in the soil with the applied immobilizing agents compared to the values obtained for the control plants. The findings connected with EDI and HRI data showed that camelina and oat are safe as potential food products. The Cu and Zn amounts theoretically consumed with oat flakes or camelina oil by humans do not pose any potential health risk. The lack of significant differences between compost and fly ash in relation to the efficacy of Cu and Zn immobilization indicates that the practical usefulness of these substances is similar, and in practice the selection of either of the two depends only on their local availability. In light of the obtained findings, the utilization of bioconcentration factors, estimated daily intake, and health risk indices should be considered helpful tools in assessing the influence of Cu and Zn on their accumulation in plants as a result of probable transfer from soil, as well as qualitative appraisal of food products in terms of potential health risks.

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