Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297

jas.ankara.edu.tr

JAS

Removal of Zinc Pollution by Using Some Hyperaccumulator Plants in Sewage Sludge Treated and Untreated Soils

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ARTICLE INFO
Research Article
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Received: 3 Mar 2022 / Revised: 14 Feb 2023 / Accepted: 21 Feb 2023 / Online: 19 Sept 2023

Cite this article

BAYRAKLI B, KIZILKAYA R (2023). Removal of Zinc Pollution by Using Some Hyperaccumulator Plants in Sewage Sludge Treated and Untreated Soils. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 29(3):854-867. DOI: 10.15832/ankutbd.1082347

ABSTRACT

Soil pollution caused by heavy metals has emerged as one of the most significant environmental problems in the world. In soils, specific plant species are able to grow, adapt and absorb heavy metals. Phytoremediation is an emerging technology in which higher plants are used to reclaim the contaminated environment. In this study, the possibilities of removing the pollution caused by Zn, which is applied to the loamy soil together with and without sewage sludge at increasing levels (0, 75, 150, 300, 600 and 1200 µg g^{-1}), has been researched with certain hyperaccumulator plants such as *Brassica juncea*, *Raphanus sativus* and *Silene vulgaris* grown in Bafra ecological conditions.

In order to clean Zn added to the soil at increasing levels with or without sewage sludge by using phytoremediation technology, *Silene vulgaris* was found to remove the highest amount of Zn in the soil by producing the greatest amount of biomass in the ecological conditions of the region compared to *Brassica juncea* and *Raphanus sativus,* and other hyperaccumulator plants grown in the plots. Significant differences were determined in the development of plants and Zn removal between the sludge treated and untreated plots.

Water-soluble Zn, which was found at high levels in the cultivation of plants at 600 and 1200 µg g^{-1} Zn application doses in the sewage sludge treated plots, was determined at lower levels at the end of the harvest of the plants. In the application of increasing levels of Zn with sewage sludge, the lowest organic bound Zn was determined in the plots where *Silene vulgaris* was grown. The highest exchangeable Zn concentration was determined in soil samples taken after the harvest of the *Raphanus sativus* plant among the hyperaccumulator plants grown at all Zn application doses in the trials with and without sewage sludge application.

Keywords: Phytoremediation, *Brassica juncea, Raphanus sativus*, *Silene vulgaris,* Labile Zn fractions

1. Introduction

In this age of rapid industrialization, exposure to toxic chemicals and metals is unavoidable. In particular, heavy metal pollution has become a serious threat to the environment and food security due to the rapid growth in industry and agriculture and the rapid increase in the world population, which has a detrimental effect on the natural ecosystem. Unlike organic pollutants, heavy metals do not biodegrade but constantly accumulate in the environment. The accumulation of these heavy metals in agricultural lands and water resources poses a major threat to human health since they run the risk of entering the food chain. Zinc is an essential trace element in plant nutrition, but it becomes toxic at high doses and acts like a heavy metal (Chakroun et al. 2010; Fässler et al. 2010; Demim et al. 2013; Küçükyumuk & Erdal 2014). The bulk of research on the atmospheric accumulation of heavy metals in various European countries suggests that Zn is the most accumulated element in the soil (Shi et al. 2018). Zinc accumulation resulting from human activities comes from three activity groups; 1- mining and industrial resources, 2- agricultural activities (fertilizers and pesticides) and 3- other activities such as road traffic and incineration of waste.

Many physical, chemical and biological techniques are used to improve heavy metal contaminated soil. However, among these various methods, phytoremediation is considered to be the most economical and environmentally friendly method. (Prasad 2003; Padmavathiamma & Li 2007). This procedure is relatively inexpensive compared to other remediation techniques (Wan et al. 2016) and also leads to less environmental degradation since it produces less secondary sewage (Cunningham & Berti 2000).

Since the plant is the most important material in this technology, hyperaccumulator plants must have certain properties as follows: the ability to deposit metals in above-ground organs, the tolerance of metal accumulation, the ability to produce effective biomass, being easy to grow and harvest, developing rapidly throughout the whole season and being continuous for other seasons. In addition, the distribution and depth of the plant root must be appropriate. Proper plant selection is crucial for the success of phytoremediation. The best starting point for the selection of appropriate plant species is the use of vegetation that grows naturally in polluted areas.

Silene vulgaris is a plant commonly found in many metal-rich soils in Europe. This plant is tolerant to high heavy metal concentrations and is capable of accumulating heavy metals. It can also produce a vast amount of biomass fast, and the root system is quite large (Nadgórska-Socha & Ciepal 2009). *Raphanus sativus* is used as a model plant in laboratory toxicology studies for various pollutants and is preferred in phytoremediation due to its rapid growth, large biomass, and sensitivity to heavy metals (Hamadouche 2012). *Brassica juncea* is considered one of the most promising species for plant breeding. It is an oilseed plant with a root system known to excessively accumulate certain heavy metals (Goswami & Das 2015). *Silene vulgaris* and *Raphanus sativus* are commonly found in Black Sea region (Mumcu & Korkmaz 2018; Ozbucak et al. 2006). *Brassica juncea*, on the other hand, can spread in the ecological conditions of Turkey (Güner et al. 2012). These three plants may be preferrable for field applications due to their easy availability.

The effectiveness of phytoremediation is affected by the growth of plants, which is slow for practical applications. For this reason, the use of soil conditioners accelerates and increases the reclamation of the soil. For this purpose, many inorganic and organic materials are used. Organic materials can increase the metal uptake of plants by promoting the root growth of the plant by improving some physical and chemical properties of soils such as the soil ion exchange capacity, soil structure, water holding capacity, drainage and aeration conditions, and soil salinity. In addition, the solid form of organic matter attracts zinc to surface functional groups, reducing the solubility of zinc (Boguta & Sokolowska 2016), while complexing of zinc with dissolved organic compounds increases its solubility and mobility (Weng et al. 2002; Houben & Sonnet 2012). For this reason, it is important to reveal the effects of sewage sludge, which is an organic material, on phytoremediation. Some sewage sludges contain high concentrations of hazardous pollutants. However, sewage sludges are very rich in organic matter and contain significant amounts of N, P, K and micronutrients, so they are effective on plant growth. They also play an important role in the chelation of metals due to their various organic chelates. For these reasons, sewage sludge can be used as a suitable source of organic material in phytoremediation technology.

Heavy metal accumulation in plants is not only related to total metal concentrations in the soil but also highly dependent on other factors such as plant uptake mechanisms, soil physicochemical properties, chemical behaviour of metals, soil texture, quality and amount of nutrients, climate, and geological material (Antoniadis et al. 2017). Many researchers also note that the ecological and toxicological effects of heavy metals depend on the distribution of their fractions rather than the total concentration in the soil (Tuzen 2003; Mousavi et al. 2018). The identification of main binding sites and phase associations of trace elements in soils leads to a better understanding of geochemical processes in order to evaluate the remobilization potential and the induced risks. Speciation of the metals can help assess how strongly they are retained in soil, how easily they may be released into soil solution, and finally how they can affect environmental and human health. Therefore, knowing the metal speciation and mobility of heavy metals is crucial in order to evaluate the phytoremediation efficiency (García et al. 2005).

This study investigates the possibilities of removing the pollution caused by increasing levels of Zn, which is applied to loamy soil together with and without sewage sludge in a year with the hyperaccumulator plants *Brassica juncea, Raphanus sativus* and *Silene vulgaris* grown in Bafra (Turkey) ecological conditions.

2. Material and Methods

2.1. Materials

The research has been carried out on land belonging to the Bafra Agriculture District Directorate in Bafra District of Samsun Province, Turkey (41°34'34"N 35°53'53"E). Since the trial area soils show little pedogenetic horizon development and are located on the flood plains on the alluvials brought by Kızılırmak, they are defined as “Typic udifluvent” (Yüksel & Dengiz 1996). In the Bafra Plain, summers are generally hot, and winters are warm and rainy. The plain has a warm and temperate climate. In winter, the Bafra Plain receives more precipitation than summer. According to the Köppen-Geiger climate classification, it can be referred to as a Csa climate (Mediterranean climate). The annual average temperature is 13.6 °C, and the annual average rainfall is 730 mm (https://tr.climate-data.org/asya/tuerkiye/samsun/bafra-8522/).

The sewage sludge used in the study was obtained from Bafra Municipality Sewage Water Treatment Facility. The solid matter ratio of the cake coming out of the facility is 41.39%. The zinc required to ensure Zn pollution in the experiment was obtained from Ekmekçioğulları Incorporated Company in the form of $ZnSO_4.7H_2O$ (22% Zn). *Brassica juncea, Silene vulgaris,* and *Rapanus sativus* were used as phytoremediation plants. These plants are winter plants and are part of the natural ecology of the region. However, the seeds of *Brassica juncea* and *Silene vulgaris* were obtained from abroad (www.herbiseed.com) as certified, and for *Rapanus sativus* was obtained domestically.

2.2. Methods

2.2.1. Establishment of trials

The trials were set up with 3 replications based on the randomized block experimental design in the form of 2 trials side by side in the field on 18.10.2004. One of the experiments was created with a constant level of sewage sludge and increasing levels of Zn application while the other by only increasing the levels of Zn application. The organic matter contents of the trial area (1.53% organic matter) and the sewage sludge (52.86% organic matter) used as the material in the experiment were determined, and the amount of sewage sludge required to increase the organic matter content of the soil up to 3% was calculated on dry weight and applied equally to each plot and mixed with soil. In order to determine the Zn levels in the application, the Zinc-buffering capacity of the soil was determined by adding Zn at increasing levels under laboratory conditions to the soil samples taken from the experimental area. This level was determined to be 650 $\mu g\ g^{-1}$ in the soil sample. Based on this level, Zn pollution levels in the experiment were determined as 0-75-150-300-600-1200 $\mu g\ g^{-1}$, respectively. During the trials, the application doses determined in Zn applications were performed to cover 0-75-150-300-600-1200 $\mu g\ g^{-1}$ Zn by preparing 15 Lt aqueous solutions of zinc sulfate. After these applications, the area was fallowed for one year. In the first year of the experiment, no cultural practices were undertaken (irrigation, fertilization, spraying, etc.) after the addition of the trial materials (sewage sludge and Zn) to the plots, and the weeds grown in the plots were cleaned by hand at the beginning of their development.

At the end of the first year of the experiment (18.10.2005), the plots belonging to each application were divided into 3 sub-plots of 1 m^2 and the second year trials were established. The second year trials consisted of 108 plots. Certified seeds of *Brassica juncea, Silene vulgaris,* and *Rapanus sativus* determined as hyperaccumulator plants were planted in these sub-plots. Plants were thinned immediately after emergence so that an equal number of plants were found in each plot (25 plants/plot). In the second year of the experiment, no other cultural practices (irrigation, fertilization, spraying, etc.) but weeds grown in the plots were cleaned by hand at the beginning of their development.

Soil sampling was carried out in the last month of the first year of the trial, before planting (18.10.2005) and at the harvest (26.06.2006). In these samples, labile Zn forms (water soluble, changeable and organic bound) were determined. In addition, the underground and aboveground biomass and Zn contents of the hyperaccumulator plants grown were identified.

2.2.2. Soil and plant analysis

Water-soluble Zn was shaken in 1:10 (w/v) soil: pure water extract for 30 minutes and filtered (Kacar 1995); exchangeable Zn was shaken in 1:4 (w/v) soil: 1 M Mg $(NO_3)_2$ extract and filtered; and organic bound Zn was shaken in 1:2 (w/v) soil: 0.7 M NaOCl (pH 8.5) extract, boiled in a hot water bath for 30 minutes and filtered (Shuman 1985). The Zn contents in the soil extracts were determined using an Atomic Absorption Spectrophotometer (Perkin Elmer, Analyst 300).

At the harvest time, all hyperaccumulator plants were harvested and their dry weights were determined. The Zn contents of the underground and aboveground parts of the plants were identified individually according to Kacar (1972), and the amount of Zn that each hyperaccumulator plant removed from the soil was calculated based on the dry matter weigh of the plant and the Zn concentration in the dry matter according to Kacar (1972) and Ryan et al. (2001).

2.2.3. Statistical analysis

The statistical evaluations were made using the TARIST package program based on the predictions by Yurtsever (1984).

3. Results and Discussion

Properties of soils and sewage sludge

The soil used in the experiment was loamy in texture (17.40% clay, 34.29% silt, 48.31% sand), medium calcareous (11.08%), unsalty (0.32 dS m^{-1} EC), had low organic matter content (1.53%) and a slightly alkaline reaction (8.25 pH). Its N content was 0.10% and total Zn was 88.49 µg g^{-1}.

The Total N, organic carbon, C/N ratio and pH of the sewage sludge were found as 2.20%, 28.7%, 13.1, and 6.65, respectively. It contained 647 µg g^{-1} Zn, 45 µg g^{-1} Pb, 121 µg g^{-1} Cu, 53 µg g^{-1} Cr, 2.1 µg g^{-1} Cd and 58 µg g^{-1} Ni.

3.1. Effects of sewage sludge and zinc applications on zinc fractions of the soil

As a result of the statistical evaluations, changes caused by the application of sewage sludge and increasing levels of zinc in the water-soluble, organic bound and exchangeable Zn fractions in the soil samples taken during the harvest, and their interactions ($p<0.01$) were found to be significant (Table 1). The exchangeable Zn content was 0.1-4.7 µg g^{-1}, organic bound Zn content was 0.3-2.0 µg g^{-1} and exchangeable Zn content was 0.3-160 µg g^{-1} in post-harvest soils in both sludge treated and untreated plots (Figures 1-3). As seen from the results, the highest labile Zn fraction was changeable Zn, followed by water-soluble Zn, and the lowest was organic bound Zn. In other words, Zn is not predominantly fixed organically and similar results have been reported in other studies (Huang et al. 2020).

Table 1- Effects on Soil Labile Zn fractions (water soluble, organic bound and exchangeable)

Source of variation	***SD***	***LSD (%1)*** ***Water soluble Zn***	***LSD (%1)*** ***Organic bound Zn***	***LSD (%1)*** ***Exchangable Zn***
Sewage sludge (A)	1	0.130***	0.056***	3.484***
Plant (B)	2	0.159***	0.068***	4.267***
A×B	2	0.225***	0.097***	6.035***
Dose (C)	5	0.225***	0.097***	6.035***
A×C	5	0.318***	0.137***	8.535***
B×C	10	0.390***	0.168***	10.453***
A×B×C	10	0.551***	0.237***	14.783***

*p<0.05, **p<0.01, ***p<0.001

At all hyperaccumulator plants and Zn application doses, the labile Zn fractions (water soluble, organic bound, exchangeable) of the plots treated with sewage sludge were found to be higher than those without sewage sludge ($p<0.001$). This may be the result of the Zn content (650 µg g^{-1}) in the applied sewage sludge. In fact, Zn has been reported to be the most important pollutant in sewage sludge in other notable studies (Chen et al. 2003; Qiao et al. 2003; Liang et al. 2011). In addition, organic compounds released during the decomposition of various organic materials help the retention of various nutrients, including Zn, in the soil and their uptake by plants by preventing certain processes such as fixation, oxidation, precipitation and washing (Scheid et al. 2009; Houben & Sonnet 2012; Boguta & Sokolowska 2016). Zinati et al. (2001), Udom et al. (2004) and Nagar et al. (2006) reported that the total Zn content of the soils increased with the sewage sludge application, and as a result of this, there was a significant increase in labile Zn forms such as water-soluble. As being in the water soluble Zn, organic bound Zn levels also increased with sewage sludge application (Figure 2). Parat et al. (2005), Hseu (2006), and He et al. (2007) determined that the organic bound Zn concentrations of the soil increased with the increasing amount of sewage sludge applied to the soil. Jakubus & Czekala (2001) determined that 1-9.3% of Zn in the structure of sewage sludge is in organic bound form. In addition, organic C compounds, which are 28.7% in the structure of the sewage sludge used in the experiment, and the increasing level of Zn added to the soil merge to form organically bound Zn complexes. Thus, the organic bound Zn content of soils can increase. Similarly, higher Zn values were also found in the exchangeable Zn fraction in the plots treated with sewage sludge (Figure 3). Nogueira et al. (2010) stated that the exchangeable Zn fraction increased with the sewage sludge application at increasing doses.

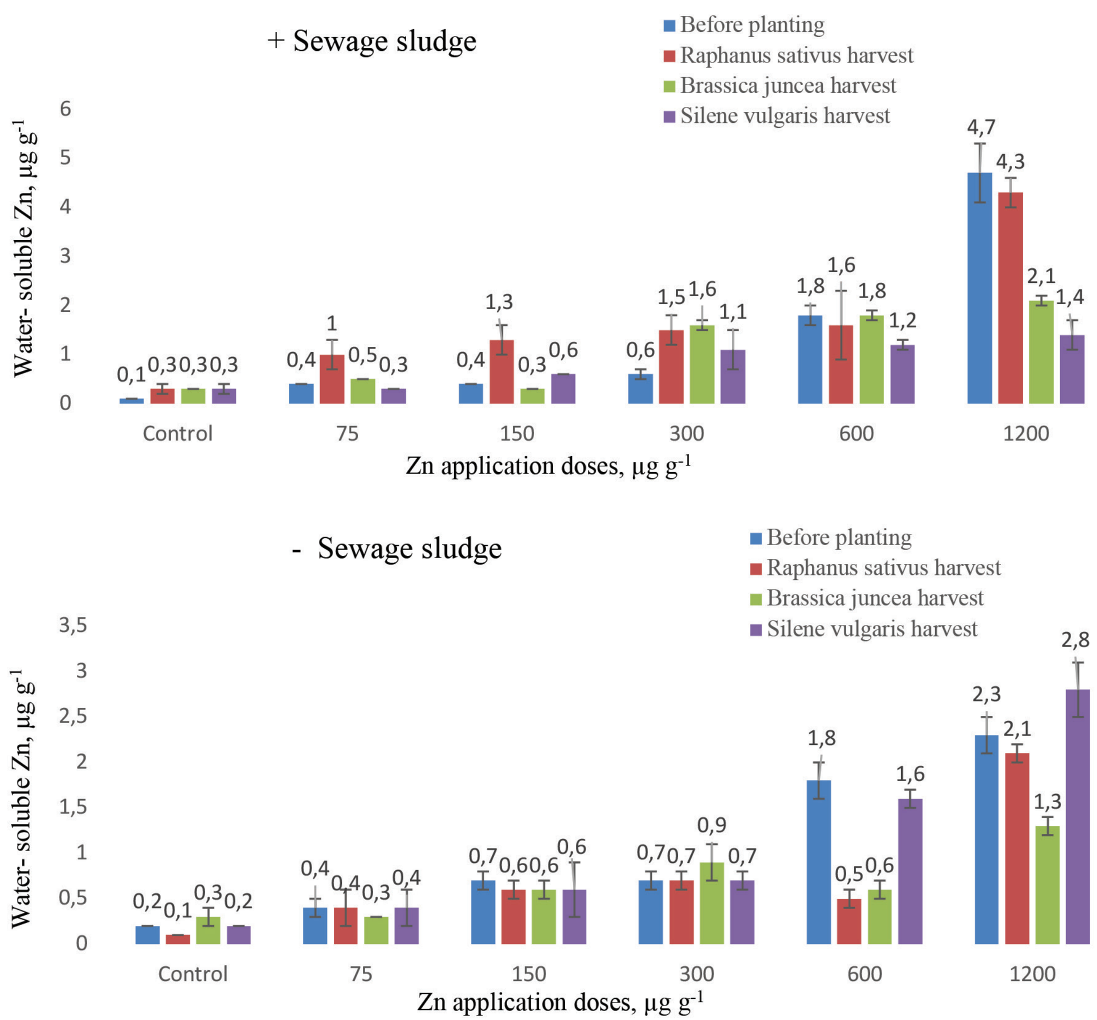

Figure 1- Changes in water soluble Zn with increasing levels of Zn application

As shown in Table 1, there were significant increases in labile Zn fractions (water soluble, organic bound, exchangeable) as the Zn application dose increased both in the plots with sewage sludge and without sewage sludge ($p<0.001$). Yadav et al. (2013) and Almendros et al. (2015) underlined that various Zn fractions in the surface soil increased significantly with increasing levels of Zn applications.

Water-soluble Zn, which was found at high levels in the planting of plants at 600 and 1200 µg g^{-1} Zn application doses treated with sewage sludge, was determined at lower levels at harvest (Figure 1). The lowest values were seen in the soil samples from the *Silene vulgaris* harvest. On the other hand, the results of the experiment without sewage sludge application showed that water soluble Zn values in the soil samples taken after harvest at all Zn application doses in the plots where *Raphanus sativus* was grown were lower than the soil samples taken before planting. As a result of the experiment where Zn was applied with increasing levels of sewage sludge, among the hyperacumulator plants grown the lowest organic bound Zn was found in the plots where *Silene vulgaris* was grown. Similar results were obtained in the trial where sewage sludge was not applied.

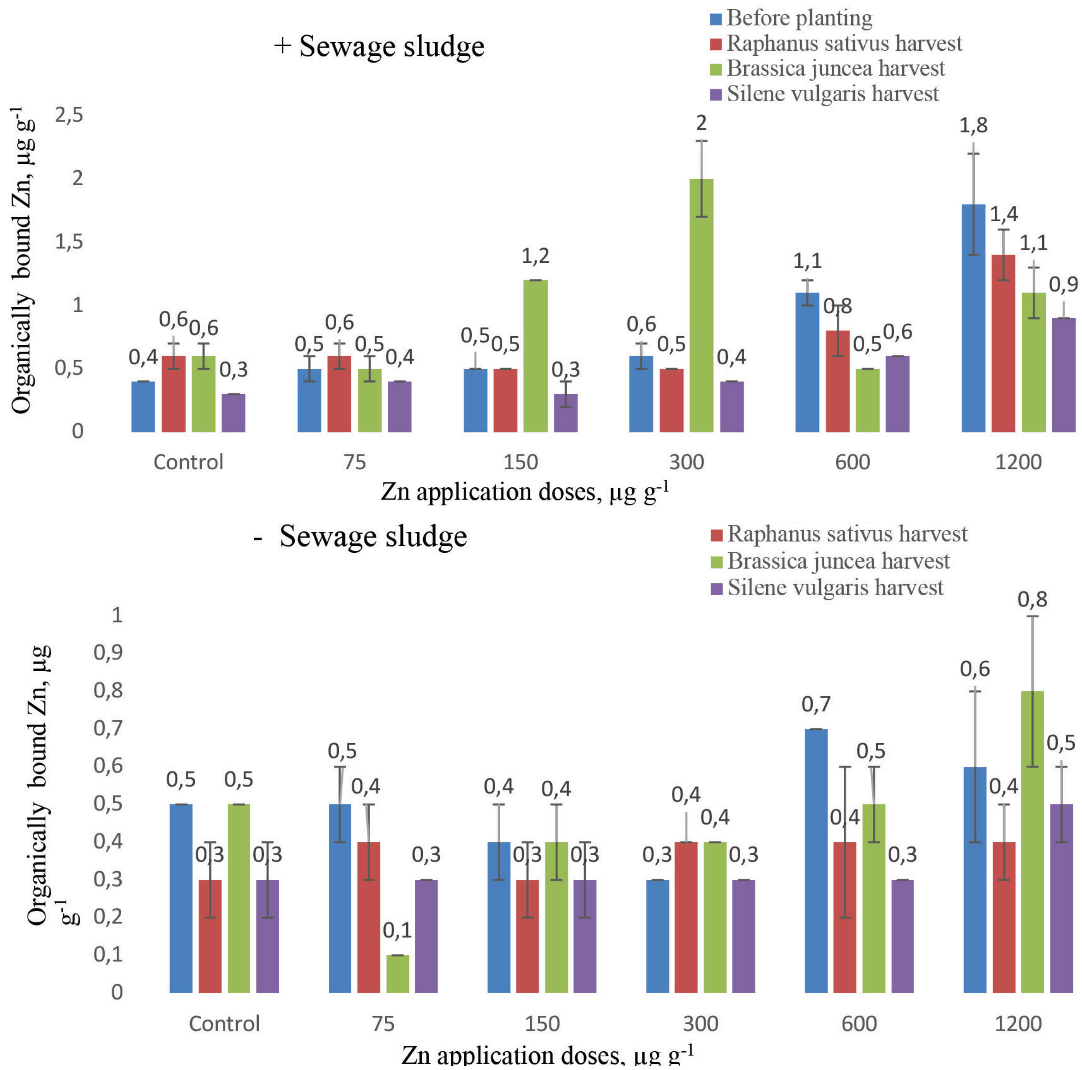

Figure 2- Changes in organic bound Zn with increasing levels of Zn application

It was found that there were significant increases in the exchangeable Zn fraction as the Zn application dose increased in the experiments with and without sewage sludge ($p<0.001$). These increases were clearer at the 600 and 1200 µg g^{-1} doses. The results of the tests, with and without sewage sludge, also showed that the highest exchangeable Zn concentration at all Zn application doses was determined in soil samples taken after the harvest of *Raphanus sativus* among the hyperaccumulator plants.

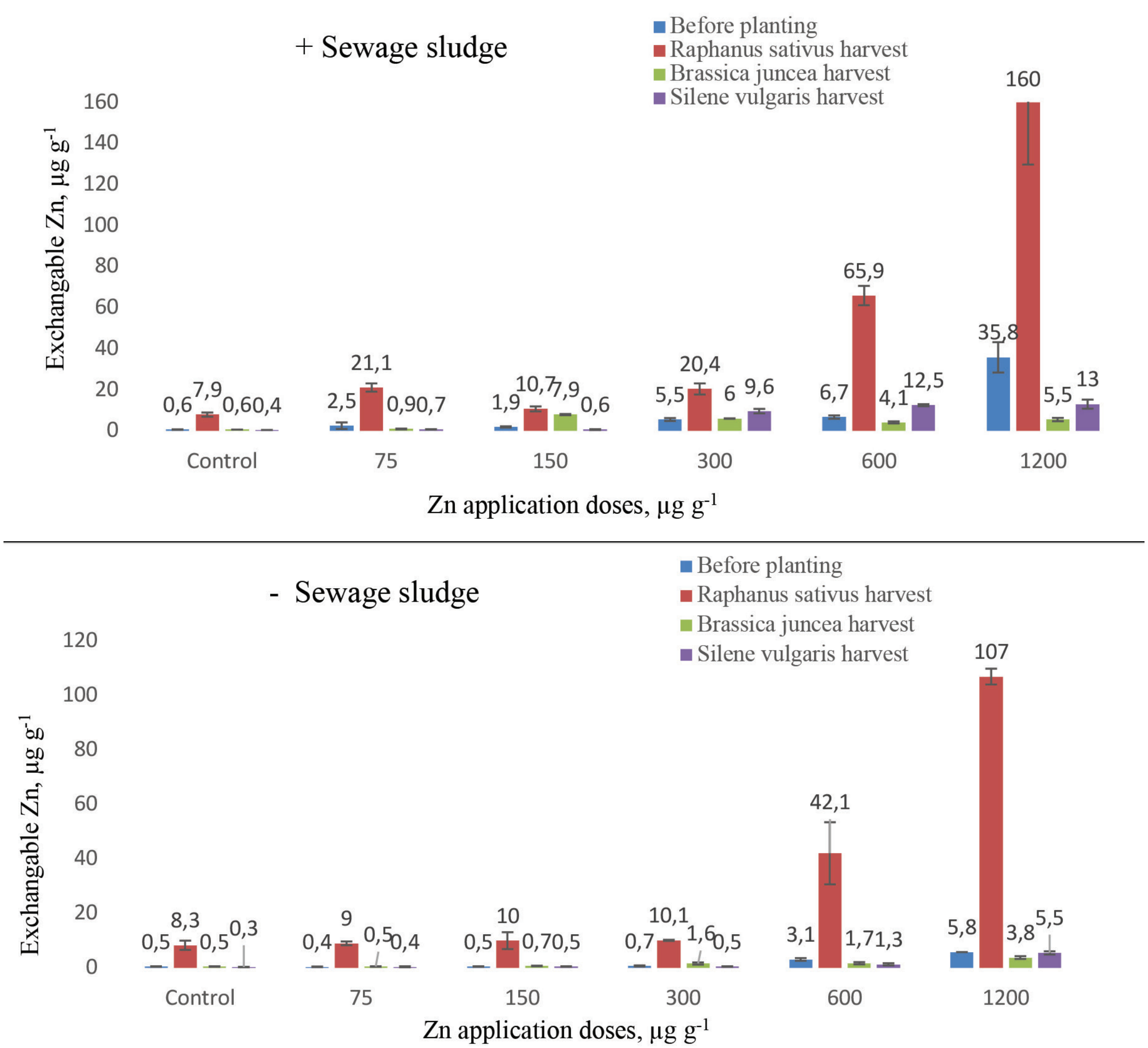

Figure 3- Changes in exchangeable Zn with increasing levels of Zn application

Although, increases in Zn doses were identical at the end of the trials, it was found that there were increases in the labile Zn contents of the soils and significant differences between the hyperaccumulator plants grown with the sewage sludge application. This may be the result of biotic factors such as the root system and the secretions secreted from the root as well as organic and inorganic compounds in the structure of the sewage sludge. McGill et al. (1986), and Huang and Schoenau (1997) found that organic carbon-containing compounds such as pollysaccharides, mucilagels and carbohydrates were synthesized from plant roots into the soil, although their amounts and types varied by plant roots. However, it was determined that the rhizosphere region was more acidic than the outside and as a result, the solubility of micro elements such as Zn in the rhizosphere region was higher. Indeed, root activity induces several modifications in the rhizospheric soil properties, like the pH, microbial activity, chemical equilibrium, mobility, and bioavailability (Clemente et al. 2010; Seshadri et al. 2015).

3.3. Zinc content of the hyperaccumulator plants and zinc amounts they removed from the soil

3.3.1. Zinc content of the hyperaccumulator plants

As a result of the statistical evaluations, with increasing levels of Zinc and sewage sludge applications, changes in the Zn content of the above-ground and underground parts of the plants and their interactions were found to be significant (Table 2). Also, a statistically significant difference was found between the plants in terms of underground and above-ground Zn contents ($p<0.001$). The Zn contents of the above-ground parts of the plants were determined to be between 7-197 µg g^{-1} and the Zn contents of the underground parts were determined to be between 45-2687 µg g^{-1} in the plots where the sewage sludge was applied. However, these values were found to be

2-186 µg g^{-1} and 41-967 µg g^{-1}, respectively, in the plots where sewage sludge was not applied (Figure 4). Zinc is an essential trace element for the mineral nutrition of plants, but at high doses it becomes toxic for these plants and behaves like a heavy metal. For most plants, 15-20 mg kg^{-1} Zn as dry matter is required for proper growth. At levels above this, it can be toxic (Tripathi et al. 2015). The toxicity of Zn in plants depends on different factors that affect the availability of the element, such as soil pH, root exudate, microbial communities and soil organic matter (Balafrej et al. 2020).

Table 2- LSD (1%) values of the underground and aboveground parts of the plants

Source of variation	***SD***	***LSD (%1) Aboveground Zn***	***LSD (%1) Underground Zn***
Sewage sludge (A)	1	10.007^{**}	5.373^{***}
Plant (B)	2	12.257^{***}	6.580^{***}
A×B	2	17.333^{**}	9.306^{***}
Dose (C)	5	17.333^{**}	9.306^{***}
A×C	5	24.513^{***}	13.160^{***}
B×C	10	30.022^{***}	16.118^{***}
A×B×C	10	42.458^{***}	22.794^{***}

*p<0.05 **p<0.01 ***p<0.001

As shown in Figure 4, as the dose of Zn content increased in the treatment, significant increases ($p<0.001$) took place in the Zn content in the underground and aboveground parts of the plants, both in the trials with and without sewage sludge. Calace et al. (2002) found that the Zn content of the plants cultivated in the soils with different quantities of Zinc differed significantly from each other, and the Zn content in the underground and aboveground parts of the plants increased depending on the increase in the Zn content in the soil. Moreover, *Silene vulgaris, Raphanus sativus,* and *Brassica juncea* cultivated in the trials, were hyperacummulator plants, and are able to accumulate Zn, which is found at high levels in the soil (Ebbs & Kochian 1998; Máthé-Gáspár & Anton 2002; Salinitro et al. 2021).

It was determined that the Zn contents of the hyperacumulator plants grown in the plots containing Zn added to the soil together with the sewage sludge and without sewage sludge differed significantly from each other, and the plant that accumulated the most Zn in their leaves was *Brassica juncea*; this was followed by *Raphanus sativus*, and the plant with the least amount of Zn in its leaves was *Silene vulgaris*. However, the highest amount of Zn was detected in the underground part of *Silene vulgaris*. When *Silene vulgaris* plant is used at a phytoremediation technology, the roots must be removed from the soil system. Muszyńska and Labudda (2020) stated that *Silene vulgaris* can survive by developing various mechanisms when grown in environments rich in heavy metal. Hanus-Fajerska et al. (2019) pointed out that *Silene vulgaris* plant is well-adapted to high metallic element levels in the rhizosphere and arid and nutrient-poor habitats; however, the translocation factor (stem Zn/root Zn) for both Zn and Cd elements is too low for phyto extraction. Ernst et al. (2000) found that the Zn concentration in different parts of *Silene vulgaris* grown on soils contaminated by heavy metals increased significantly due to the increase in Zn in the soil, but Zn removal by the roots is not possible in soils containing high Zn (100 µmol g).

The highest Zn content was obtained at the Zn application dose of 300 µg g^{-1} in the Zn doses of *Silene vulgaris* applied with sewage sludge, and at the Zn application dose of 1200 µg g^{-1}in the experiment without sewage sludge.

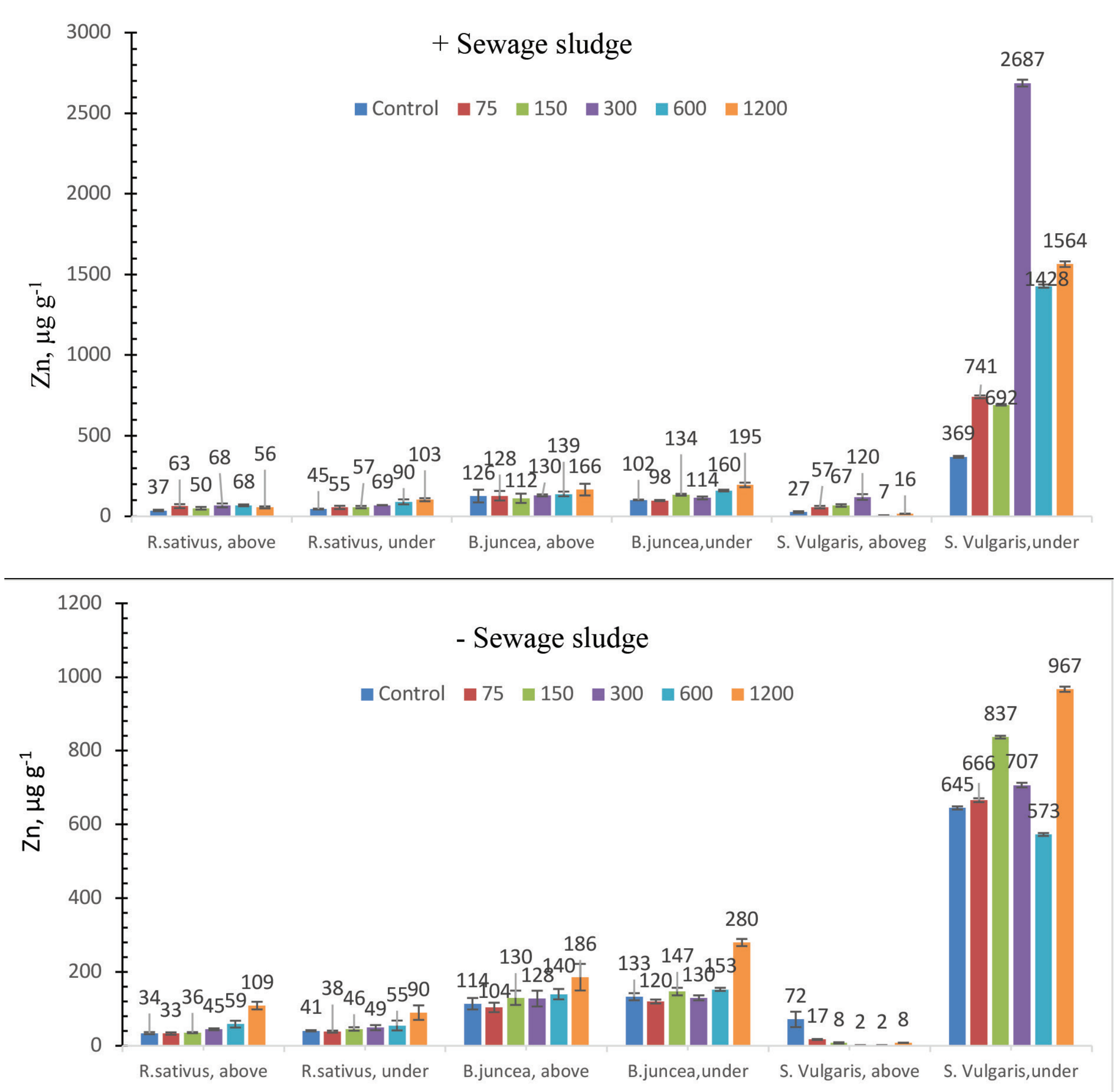

Figure 4- Zn contents of underground and aboveground parts of the hyperacumulative plants

3.3.2. Amounts of zinc removed by hyperaccumulator plants from the soil

While determining the amount of Zn removed by the hyperaccumulator plants, the Zn (88.49 µg g^{-1}) existing in the soil under natural conditions and the Zn input from the sewage sludge (647 µg g^{-1}) were also taken into consideration. The Zn removed by the above-ground part of the plants where sewage sludge was applied was determined as 0.59-5.81 g m^{-2} and the Zn removed by the underground part was determined as 0.2-31.9 g m^{-2}. The Zn removed by the above-ground and underground part of the plants was determined between 0.05-1.6 g m^{-2} and 0.1-12.3 g m^{-2}, respectively, in the plots where sludge was not applied. According to the results, it has been determined that *Silene vulgaris* was the plant removed most Zn from the soil in the ecological conditions of the region among the hyperaccumulator plants (Figure 5).

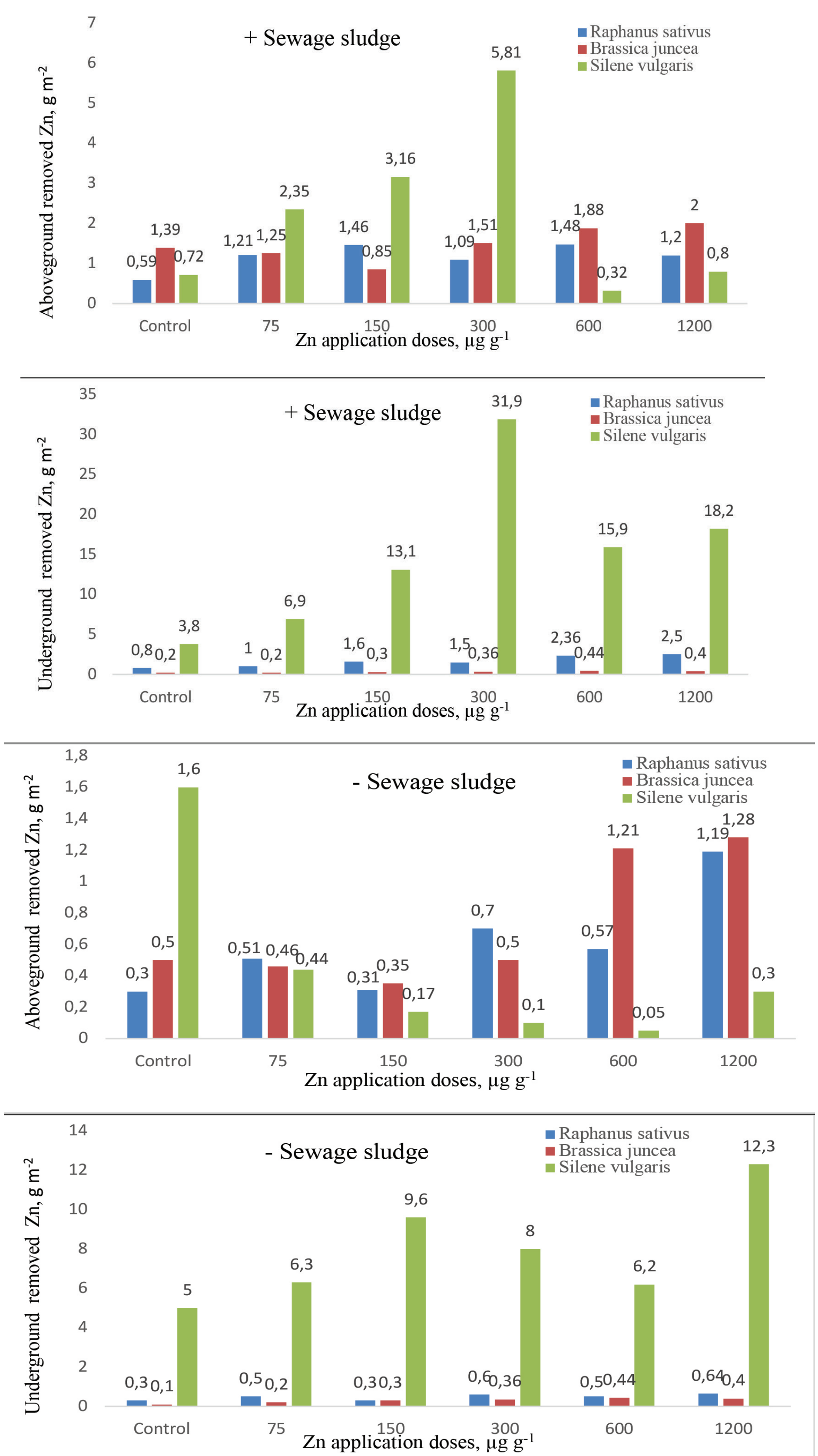

Figure 5- Zn removed from the soil by hyperaccumulator plants with their underground and aboveground parts

At the end of the trial, Zn, which was added in increasing doses in the sewage sludge treated plots, was removed from the soil by *Raphanus sativus*, *Brassica juncea* and *Silene vulgaris* at a rate of 1.18-4.82% (at the maximum Zn application dose of 75 µg g^{-1}), 1.14-3.23% (at the maximum in the control plot) and 5.51-32.23% (at the maximum Zn application dose of 300 µg g^{-1}), respectively. On the other hand, as a result of increasing Zn application without sewage sludge, *Raphanus sativus, Brassica juncea* and *Silene vulgaris* removed 0.83-3.15%, 0.74-3.08% and 3.84-30.34% of the total Zn in soils, respectively. This finding also presents that the Zn added to the soil with the sewage sludge is removed by the hyperaccumulator plants at higher rates. This may be due to the fact that the nutrients found in the sewage sludge, and necessary for the plants, are taken by the plants for a better development and more biomass, and at the same time, various organic compounds in the sewage sludge chelate with the Zn and thus facilitate the uptake of Zn by the plants. In addition, the transport of zinc applied with the sewage sludge to the plant and its concentration in the plant may be higher due to the effect of the high organic matter in the sewage sludge regulating the physico-chemical properties of the soils. Researches have shown that sewage sludge added to soils can increase vegetative production due to the nutrients it contains (Garvanska 2000; Antonkiewicz et al. 2020; Abdoli 2022) and that metals chelated with various organic chelates are taken by plants in larger amounts and accumulate more metals from the soil environment (Lombi et al. 2001; Boye 2002; Praburaman et al. 2020; Pandey et al. 2022). However, Hamlin & Barker (2002) found that N fertilizers applied to hyperaccumulator plants increased the phytoextraction potential of Zn. The 2.2% total N content that exists in the sewage sludge used in the experiment, may shown this effect. Similarly, Panwar et al. (2011) and Revathi et al. (2011) revealed that hyperaccumulator plants increased the amount of heavy metals removed from the soil by increasing their biomass with the application of farmyard manure and vermicompost. Phytoremediation can be assisted by certain practices, including organic sources (such as farmyard manure composts, biosolids, and municipal sewage, etc.) that will increase soil organic matter and fertility, as well as various inorganic substances (Park et al. 2011; Mousavi et al. 2018; Wang et al. 2019; Shaheen et al. 2019; Huang et al. 2020).

4. Conclusions

In this study, the removal possibilities of Zn added as $ZnSO_4.7H_2O$ at increasing doses (0, 75, 150, 300, 600 and 1200 µg g^{-1}) were investigated by using *Brassica juncea*, *Raphanus sativus* and *Silene vulgaris* hyperaccumulator plants in sewage sludge treated and untreated loamy soil. It was determined that among the hyperaccumulator plants, *Silene vulgaris* was the plant that removed the most Zn in the soil by producing the most plant biomass in the ecological conditions of the region. It was determined that the underground parts of all hyperaccumulator plants, including *Silene vulgaris*, accumulated more Zn than the above ground parts. This suggests that if these plants are used as phytoremediation technology in regional conditions, their roots should be removed from the soil environment. *Brassica juncea* and *Raphanus sativus* grown at the Zn doses applied with the sewage sludge produced more plant biomass and removed more Zn from the soil. Similarly, Zn application with sewage sludge in *Silene vulgaris* accumulated more Zn compared to the experiment with increasing levels of Zn in soils without sewage sludge. This is due to the fact that various organic compounds in the structure of the sewage sludge are chelated with Zn and increased uptake by the plants, and the nutrients in the sewage sludge, such as N, increase the plant biomass more.

Based on the obtained results, *Silene vulgaris* can be suggested to be the most suitable hyperaccumulator plant in the ecological conditions of the region. In addition, when there is 300 µg g^{-1} Zn in the soil, approximately 3 vegetation periods are required to clean the land by using phytoremediation technology with *Silene vulgaris*, and in case of sewage sludge application, 12 vegetation periods are required without sewage sludge application.

In the experiments, sewage sludge and increased Zn applications were considered as factors; however, no nutrients were added to the soils by fertilization. In future studies, the hyperaccumulator performance of these plants should be tested by applying a fertilization program.

Acknowledgement

This study was produced from a doctoral study named “Determining the effects of zinc contamination on biological properties of soils and removing contamination through phytoremediation method”.

Data availability: Data are available on request due to privacy or other restrictions.

Authorship Contributions: Concept: B.B., R.K., Design: B.B., R.K., Data Collection or Processing: B.B., R.K., Analysis or Interpretation: B.B., R.K., Literature Search: B.B., R.K., Writing: B.B., R.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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