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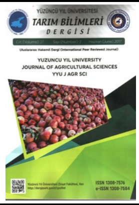

Araştırma Makalesi (Research Article)

Effects of Cadmium and Zinc Applied to the Soil Enriched with Sewage Sludge on Plant Growth and Antioxidative Enzyme Activity of Lettuce (*Lactuca sativa* L. *var. longifolia*)

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Abstract: The aim of this study is to investigate the effects of a constant rate of sewage sludge (SS) together with cadmium (Cd) and zinc (Zn) at varying levels on the growth of lettuce and antioxidative enzyme activity. In the pot experiment, a fixed ratio of 10%SS with varying doses of Cd (Cd1:50 mg Cd kg^{-1}; Cd2:100 mg Cd kg^{-1}) and Zn (Zn1:250 mg Zn kg^{-1}; Zn2:500 mg Zn kg^{-1}) was applied. According to experiment results, compared to the control, 10% SS + Cd1 and 10% SS + Cd2 applications significantly reduced fresh and dry shoot weights, plant height, Zn content and SOD (superoxide dismutase) enzyme activity in the root and shoot of lettuce. However, 10%SS +Cd1 and 10%SS+Cd2 applications significantly increased shoot and root Cd contents, GPX (glutathione peroxidase) enzyme activity in plant and the amount of Cd in soil. On the other hand, 10%SS+Zn1+Cd1 application increased fresh shoot weight, Zn content, SOD and GPX enzyme activities in the shoot of lettuce and decreased the amount of DTPA-Cd in comparison with 10%SS+Cd1 application. Compared to the Cd1 application, 10%SS+Zn2+Cd1 application increased SOD and GPX enzyme activities in the root of lettuce. In comparison with 10%SS +Cd2 application, 10%SS+Zn2+Cd2 application increased fresh and dry shoot weight, Zn content, SOD and GPX enzymes in both shoot and root of lettuce and decreased shoot Cd content and the amount of DTPA-Cd in soil. These results indicate that the Zn application could be beneficial for reducing the toxic effects of Cd in lettuce.

Arıtma Çamuru ile Zenginleştirilmiş Toprağa Uygulanan Kadmiyum ve Çinkonun Marulun (*Lactuca sativa* L. *var. longifolia*) Gelişimi ve Antioksidatif Enzim Aktivitesine Etkisi

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Anahtar kelimeler
Antioksidatif enzimler,
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Marul *(Lactuca sativa* L. *var. longifolia),*
Çinko,

Öz: Bu çalışmanın amacı sabit oranda arıtma çamuru ile birlikte kadmiyum ve çinko düzeylerinin marul bitkisinin gelişimine ve antioksidatif enzim aktivitesine etkisini araştırmaktır. Saksı çalışmasında sabit oranda, arıtma çamuru (%10AÇ), değişen oranlarda kadmiyum (Cd1:50 mg Cd kg^{-1};Cd2:100 mg Cd kg^{-1}) ve çinko (Zn1:250 mg Zn kg^{-1}; Zn2:500 mg Zn kg^{-1}) uygulanmıştır. Elde edilen sonuçlara göre,%10 AÇ+Cd1 ve %10AÇ+Cd2 uygulamaları, kontrole göre, marul bitkisinin kuru ve yaş sürgün ağırlığını, bitki boyu, çinko içeriğini ve kök ve sürgün bölgesinde SOD (süperoksit dismutaz) enzim aktivitesini önemli düzeyde azaltmıştır. Bununla birlikte marul bitkisinin kök ve sürgün kısımlarında ve toprakta kadmiyum konsantrasyonu ile GPX (glutatyon peroksidaz) enzim aktivitesi önemli düzeyde artış göstermiştir. Ancak 10%AÇ +Zn1+Cd1 uygulaması ile 10%AÇ +Cd1 karşılaştırıldığında, marulun sürgün kısımlarında

SOD ve GPX enzim aktiviteleri, çinko içeriği ve sürgün yaş ağırlığında artışa neden olurken toprakta DTPA-Cd (yarayışlı-Cd) içeriğinde düşüşe neden olmuştur. 10%AÇ +Zn2+Cd1 uygulaması ile 10%AÇ +Cd1 karşılaştırıldığında, marul kök bölgesinde GPX ve SOD enzim aktiviteleri artış göstermiştir. 10%AÇ +Zn2+Cd2 uygulaması ile 10%AÇ +Cd2 karşılaştırıldığında bitkinin sürgün yaş ve kuru ağırlığı, Zn içeriğinde ve hem kök hem sürgün SOD ve GPX enzim aktivitesinde artışa neden olurken sürgün Cd içeriğinde ve toprak DTPA-Cd (yarayışlı-Cd) içeriğinde düşüşe neden olmuştur. Bu sonuçlar göstermektedir ki, marul bitkisinde çinko uygulaması kadmiyum toksisitesini azaltmada faydalı olabilir.

1. Introduction

Cadmium (Cd) is a non-essential and toxic heavy metal (Grant et al., 1998). Cd is very easily taken up by plant roots and accumulates in plants due to its much more mobile nature relative to other heavy metals. Various crops such as lettuce and spinach, grown in heavy metal polluted soils accumulate large amounts of Cd. This causes heavy metals to join the food chain (Monterio et al., 2009). Cd causes visible symptoms such as browning of roots, necrosis, chlorosis and slow growth in many plant species (Gülser and Sönmez, 2012; Tran and Popova, 2013). Cd has been found to induce oxidative stress in cells leading to the accumulation of reactive oxygen species (ROS) (Liu et al., 2008). Plant cell which is exposed to Cd stress activates enzymatic defence mechanism including superoxide dismutase (SOD), glutathione-S-transferase (GST), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) (Hana et.al., 2008; Monterio et al., 2009; Lin and Aarts, 2012; Viehweger, 2014).

Zinc (Zn) is an essential micronutrient for plants due to a component of many enzymes needed for the normal development of plants (Aravind et al., 2005). Zn ions in soil lessen the effects of Cd toxicity by reducing Cd uptake (Köleli et al., 2004). In lettuce and spinach, Zn has a synergistic interaction with Cd at the root surface (Kabata-Pendias and Pendias, 1984). However many studies show that the relationship between Zn and Cd is antagonistic because both elements have overlapping features (Rizwan et al., 2017). Zn helps the reduction of Cd concentration both in plants and in soil solution (Saifullah et al., 2014). Mckenna et al. (1993) reported that Zn application reduces the accumulation of Cd in roots and transportation of Cd from roots to young leaves in lettuce and spinach. The relationship between Cd and Zn depends heavily on plant type, tissue type and cadmium and zinc concentration (Zhao et al., 2011). Zn has an important role in the anti-oxidative defence system against the destructive effects of reactive oxygen species. Zn is a necessary metal component of key enzymes such as Cu-Zn SOD, APX, and GR (Cakmak, 2000; Wu and Zhang, 2002). Zn application improves growth and yield in wheat grown in Cd-contaminated soil (Abbas et al., 2017). Rizwan et al. (2017) reported that foliar application of Zn-lysine (Zn-lys) complex decreased Cd contents as well as reduced the oxidative stress in wheat.

Sewage sludge (SS) contains high levels of heavy metal. Heavy metal toxicity is an important factor in limiting agricultural production. In this case, the source of the SS is important. However, the application of SS results in an increase in organic matter, nitrogen, phosphorus and microbiological activity in the soil (Castro et al., 2009). Erdogan et al. (2011) reported that the SS application increased heavy metal concentration in ornamental plants. Hossain et al. (2015) reported that compared to biochar and sewage sludge, biochar application reduced the accumulation of heavy metals in cherry tomatoes. Although SS is a source of heavy metals, it is an important organic complex that controls the solubility of heavy metals, due to its high organic matter content.

The aim of this study is to investigate the effects of Cd and Zn in the soil added with SS on plant development and antioxidative enzyme activity in lettuce. We also investigated Cd solubility in soil and preventive effects of Zn application to mitigate Cd toxicity in lettuce.

2. Materials and Methods

2.1. Characterization of soil and SS

The soil used in the experiment was taken from the experimental area of the Faculty of Agriculture of Van Yüzüncü Yıl University. The soil characteristics are presented in Table 1. The experiment soil was characterized by alkaline pH, unsalted, medium calcareous, low organic matter and low nitrogen. SS was taken from the Van Municipality Wastewater Treatment Plant. Cd, Pb, Cu and Zn contents in SS were under the threshold values recommended in the soil pollution control guideline (Anonymous, 2010) (Table 1).

Table 1 Characteristics of soil and SS

	Units	**Soil**	**SS**
Texture		Sandy Clay Loam	
pH (1/2.5)		8.42	6.91
Salt,	dS/m	0.10	1.0
Lime,	%	12.22	-
Organic Material,	%	1.97	27.4
Total N	%	0.095	1.42
Available P,	mg/ kg	7.1	
Extractable with DTPA	mg/kg		
Fe	mg/kg	9.18	
Zn	mg/kg	1.52	
Cu	mg/kg	0.72	
Mn	mg/kg	8.99	
Cd	mg/kg	0.01	
Pb	mg/kg	0.285	
Total elements	%		
P	%	-	0.58
K	%	-	0.19
Fe	%	-	0.79
Zn	mg/kg	-	762
Cu	mg/kg	-	111
Mn	mg/kg	-	645
Cd	mg/kg	-	0.73
Pb	mg/kg	-	40.3

2.2. Pot experiment

The pot experiment was carried out in the growth chamber in the Faculty of Agriculture, Van Yüzüncü Yıl University. The temperature of the growth chamber was set to 18±2^{0}C because lettuce is a cool climate vegetable crop. In the present study, completely randomized design with four-replication trial was carried out. After eight weeks of the development period, plant samples were taken from each parcel, both from the root and above-ground parts. In each application, 4 plots and 15 pots were formed in each parcel. Each pot was filled with 300 g soil and planted with 5 seeds. After germination, the seedling was checked and noted for 8 days. Dilution was made in the way that a plant remains in each pot. In the experiment, at a fixed ratio of 10% SS, two different levels of Cd and Zn were applied. These applications were 50 mg Cd kg^{-1}, 100 mg Cd kg^{-1}, 250 mg Zn kg^{-1} and 500 mg Zn kg^{-1}. Ten applications consisted of combinations of SS, Cd, and Zn are as follows:
1-. Control 2- 10% (SS) 3-10%SS+Cd1: 50 mg Cd kg^{-1}4-10%SS+Cd2: 100 mg Cd kg^{-1} 5-10%SS +Zn1: 250 mg Zn kg^{-1}6-10%SS +Zn2: 500 mg Zn kg^{-1}7-10%SS+Zn1+Cd1 8-10%SS+Zn1+Cd2 9-10%SS+Zn2+Cd1 10-10%SS+Zn2+Cd2.

2.3. Chemical analyses of soil and SS

The soil samples and SS were air-dried in a shadowy place and passed through a 2 mm sieve. In line with the guidelines, soil texture was determined by Bouyoucous hydrometer method (Bouyoucous, 1951). In 1:2.5 soil-water mixture and 1:5 SS-water mixture, pH was measured (Jackson, 1958). Lime content was calculated using Scheiblercalcimeter (Hızalan and Ünal, 1966). Soil organic matter was determined using Walkley Black method (Walkley, 1947). Total N was measured using the Kjeldahl method (Kacar, 1994). Available Fe, Cu, Zn, Mn, and Cd were extracted with DTPA and then measured using Atomic Absorption Spectrophotometer (AAS) technique (Kacar and Katkat, 1999). The amount of organic matter in SS was determined using the combustion method suggested by Kacar, 1994. Air-dried samples were digested with a mixture of HNO_3-$HClO_4$ acids and analysed for the concentration of P,K, Fe, Cd, Pb Fe, Zn, Cu, and Mn by using atomic absorption spectrophotometer (Kacar, 1994).

2.4. Enzyme analysis in plant sample

Enzymatic measurements were carried out at 4 ^{0}C. Fresh samples (1 g) were homogenized in 6 ml 0,1 M potassium phosphate buffer (pH 7.0). After being homogenized, 200 mg Polyvinylpolipidolidone (PVPP) and 0.1 M EDTA (Etilen diaminetetraacetic acid) was added. Homogenates taken into the tubes were centrifuged at +4 °C at 21000 g for 15 minutes. The supernatant was used as a crude enzyme extract for SOD, GPX, GR, GSH enzyme analyses. Superoxide dismutase (SOD EC 1.15.1.1) activity was measured with calculating inhibition percentage of formazan formation using the Randox-Ransod enzyme kit at 505 nm spectrophotometrically (McCord and Fridovich, 1969). Glutathione-S-transferase (GST) was measured at 25 ^{0}C spectrophotometrically by following the conjugation of glutathione with 1-chloro-2, 4-dinitrobenzene (CDNB) at 340 nm (Mannervik and Guthenberg, 1981). Glutathione peroxidase was measured by the Randox-Ransel enzyme kit at 340 nm using spectrophotometry (Shimadzu UV/VIS-1201). This enzyme activity was determined by the change in absorbance values during the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) (Fhole and Günzler, 1984). GR was measured at 340 nm by following the oxidation of NADPH by GSSG, oxidized glutathione, (Beutler, 1984). CoStat software package was used for statistical analyses. Significant differences were determined by Duncan's Multiple Range tests (Düzgüneş et al., 1987).

3. Results and Discussion

3.1. Plant growth

The application of 10% SS alone did not have a significant impact on shoot length, shoot fresh weight and shoot dry weight in lettuce compared to the control due to low Cd content. 50 mg Cd kg^{-1}(Cd1) and 100 mg Cd kg^{-1} (Cd2) additions decreased shoot length, shoot fresh and dry weight compared to the control in lettuce (Table 2). Visual symptoms of Cd toxicity were necrotic areas spreading from the edges to centre in the oldest leaf in lettuce. Another symptom of Cd applications was a noticeable decline in biomass and development compared to the control (Figure1). In comparison with the control, 100 mg Cd kg^{-1} decreased root fresh and dry weight. It has been determined that Cd stress inhibits plant growth in various studies on different plant species (Benavides et al 2005). This may affect only young leaves, only old or both young and old leaves (Tran and Popova, 2013). 10%SS +Zn2+Cd1 and 10%SS +Zn2+Cd2 applications increased shoot length, shoot fresh and dry weight compared to 10%SS+Cd1 and 10%SS +Cd2 applications. However, 10%SS+Zn1+Cd1 and 10%SS+Zn1+Cd2 applications did not have a significant impact on the yield parameters in lettuce (Table 2). We found that high levels of Zn had significant positive effects on yield parameters of lettuce under Cd toxicity conditions in SS amended growth medium.

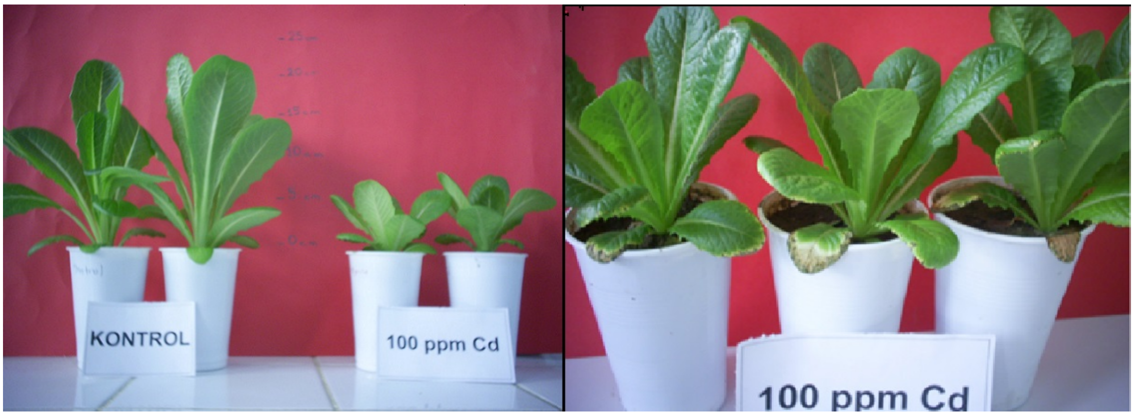

Figure1: Effects of, 100 mg/kg Cd applications (right), 100 mg kg^{-1} Cd and control applications (left) on the shoot of lettuce.

Hassan et al. (2005) identified that Zn applications increased growth in two rice cultivars under Cd toxicity. Wu and Zhang (2002) reported that the addition of Zn in Cd-contained solutions was related to an increase in shoot and root biomass relative to Cd alone treatment in barley.

Table 2. Effects of SS, Cd (50,100 mg kg^{-1}) and Zn (250, 500 mg kg^{-1}) applications on plant growth of lettuce.

Applications	Shoot length (cm)	Shoot fresh weight (g/ pot)	Shoot dry weight (g/ pot)	Root fresh weight (g/ pot)	Root dry weight (g/ pot)
Control	16.90±1.60b	11.97±1.57ab	0.75±0.045b	3.61±0.35a	0.185±0.046 a
10%SS	17.11±1.84b	13.14±1.53a	0.68±0.095bc	2.31± 0.36b	0.113±0.029 cde
10%SS+Cd1	12.70±0.64cd	8.62±1.78c	0.50±0.105 de	3.25± 0.61a	0.165± 0.021ab
10%SS+Cd2	10.48±0.45e	5.31±1.30d	0.37±0.027 e	2.31±0.53 b	0.130± 0.037cde
10%SS +Zn1	17.57±1.39b	12.40±0.70ab	0.64±0.050 bc	2.13±0.35 b	0.108±0.022 e
10%SS +Zn2	18.26±1.49ab	14.02±1.63a	0.92± 0.134a	2.58±0.59 b	0.147±0.028 bcd
10%SS +Zn1+ Cd1	12.70±0.82cd	6.86±1.92d	0.45±0.027 e	2.02±0.38 b	0.117±0.010cde
10%SS +Zn1+Cd2	11.75±1.48de	6.64±1.30d	0.38±0.053 e	2.10 ±0.079b	0.108±0.014 e
10%SS +Zn2+Cd1	19.61±0.82a	13.77±2.86a	0.70±0.146 bc	2.34± 0.16b	0.112±0.013 de
10%SS +Zn2+Cd2	14.52±1.03c	9.58±2.37bc	0.60±0.042 cd	2.52 ±0.55b	0.149± 0.022bc

*a,b,c,d,e: Statistically significant mean differences are indicated with different letters in the same column ($p<0.05$).

3.2. Antioxidative enzyme activity in lettuce

10%SS alone application did not change SOD, GST and GR enzymes activity in shoot and root compared to the control. However,10 % SS +Cd1 and 10 % SS +Cd2 applications decreased SOD enzyme activity and increased GPX activity in shoot and root as compared to the control. 10 % SS +Cd1+Zn1 application increased SOD and GPX enzyme activity in shoot and increased GST and GR enzyme activity in root compared to Cd1 alone application (Table 3,4). In root 10 % SS+Zn2+ Cd1 application increased SOD and GPX enzymes activity compared to Cd1 alone (Table 4). 10 % SS+Zn1+ Cd2 and 10 % SS + Zn2+ Cd2 applications increased SOD and GPX enzymes activity in shoot compared to Cd2 alone (Table 3). We found that applications did not result in a significant change of GST and GR enzyme activity but increased SOD and GPX enzyme activity in the shoot and root of lettuce plant. Jibril et al. (2017) reported that Cd toxicity may prevent cell in two different ways through binding to specific groups of proteins and lipids thus it may cause free radical formation inducing oxidative stress. At the same time, nutrient imbalances and reduction in the amount of vitamin C resulting from the toxic effect of Cd can alone trigger oxidative stress, nutrient deficiencies, and reduction in plant growth and development of agricultural crops. Plant cells activate anti-oxidative protection systems in motion to deal with the harmful effects of reactive oxygen species (Hana et al., 2008; Shahabivand and Aliloo, 2016). In a similar study, Cd treatment decreased SOD and CAT enzymes activity in bean (*Phaseolus vulgaris)* (Chaoui et al., 1997). However, Cherif et al. (2011)

found that Cd application increased SOD enzyme activity whereas APX, GR enzyme activity decreased in tomato plants.

Table 3. Effects of SS, Cd (50, 100 mg kg^{-1}) and Zn (250, 500 mg kg^{-1}) applications on anti-oxidative enzyme activities in the shoot of lettuce.

	Shoot (U g^{-1} tissue)			
Applications	SOD	GST	GR	GPX
Control	867±15.45 bc	0.177±0.046 a	0.174±0.013a	1858±124 e
10%SS	873± 4.97abcde	0.161± 0.042a	0.127±0.046ab	7052± 512ab
10%SS+Cd1	837±16.59 d	0.162±0.017 a	0.129± 0.028ab	4427± 1176cd
10%SS+Cd2	836± 10.39d	0.154±0.013 a	0.131± 0.050ab	4015± 488 d
10%SS +Zn1	875± 2.22ab	0.177± 0.031a	0.108± 0.036b	5921± 876bcd
10%SS +Zn2	881± 1.26a	0.151±0.017 a	0.098± 0.017b	7148± 2603ab
10%SS +Zn1+ Cd1	881±1.26 a	0.186± 0.020a	0.104±0.025 b	7956± 2071ab
10%SS +Zn1+Cd2	861±3.65 c	0.190±0.005 a	0.118± 0.027b	6550± 1989bc
10%SS +Zn2+Cd1	878±3.40ab	0.164±0 .025a	0.125±0.030ab	9063±1743 a
10%SS +Zn2+Cd2	876± 2.50ab	0.174±0.029 a	0.123±0.025b	6540± 597bc

*a,b,c,d,e: Statistically significant mean differences are indicated with different letters in the same column ($p<0.05$).

Table 4. Effects of SS, Cd (50, 100 mg kg^{-1}) and Zn (250, 500 mg kg^{-1}) applications on anti-oxidative enzyme activities in the root of lettuce

	Root (U g^{-1} tissue)			
Applications	SOD	GST	GR	GPX
Control	832±9.43 a	0.115±0.012 e	0.038±0 .007cd	287± 102e
10%SS	831±20.82 a	0.144±0.023 de	0.035±0.007 d	422± 119e
10%SS+Cd1	739± 19.55c	0.164± 0.049bcde	0.045± 0.013bcd	1723± 255bc
10%SS+Cd2	753± 31.35d	0.158 ±0.016cde	0.056 ±0.011ab	1759 ±92.6bc
10%SS +Zn1	834± 11.84a	0.128±0.020 e	0.042±0.004 bcd	455±89.4 e
10%SS +Zn2	848± 11.63a	0.210± 0.056abc	0.044±0.006 bcd	1005±293 d
10%SS +Zn1+ Cd1	789± 28.13bc	0.227± 0.038a	0.068±0.011 a	1777±271 bc
10%SS +Zn1+Cd2	771± 23.02cd	0.220±0.047 ab	0.048±0.004 bcd	2046 ±296b
10%SS +Zn2+Cd1	828± 7.05a	0.191±0.039 abcd	0.051± 0.015bc	2818± 874a
10%SS +Zn2+Cd2	820± 9.54ab	0.207± 0.034abcd	0.047± 0.004bcd	1440±233cd

*a,b,c,d,e: Statistically significant mean differences are indicated with different letters in the same column ($p<0.05$).

Zn as a metal component of SOD and as an inhibitor of NADPH-oxidase preserves cell membranes from damage by $O_2^{\bullet-}$ radicals (Hajiboland, 2000). Zn containing SOD (Cu, Zn -SOD)and catalase enzymes activity reduced in insufficient Zn content in plants. However, under Zn sufficient condition, oxidative stress was alleviated with the enzymatic defence mechanism including SOD, GR, APX (Cakmak, 2000). In a similar study under Cd toxicity conditions, Fe applications influenced SOD enzyme activity in lettuce (Canal and Bozkurt, 2017; Canal and Bozkurt, 2018). The experiment conducted with tomato under Cd stress conditions demonstrated that anti-oxidative enzyme activities were associated with Zn levels (Cherif et al., 2011). Rizwan et al. (2017) found that the application of Zn-lysine (Zn-lys) complex caused an increase in SOD, APX, CAT and POD enzyme activities in wheat plant under cadmium stress.

3.3. Cd and Zn contents in different parts of lettuce

The application of 10%SS alone did not increase Cd content compared to the control due to low Cd content of SS. However 10% SS+Cd1 and 10% SS+Cd2 applications increased Cd content in shoot and root compared to the control. In 10% SS+Cd1 treatment, root Cd content was 8,65 times higher than shoot Cd content. In the 10% SS+Cd2 treatment, root Cd content was 8,0 times higher than shoot Cd content (Table 5). Generally, Cd content reduced in the following order roots> stems>leaves (Zhao et al 2011). 10% SS+Zn1+Cd1 application decreased Cd content in shoot compared to 10%SS+Cd1 application. 10%SS+Zn2+Cd2 application decreased Cd content in shoot compared to 10%SS+Cd2 application (Table 5). 10%SS+Zn1 and 10%SS+Zn2 applications increased Zn content compared to the control in root and shoot. 10%SS+Zn1+Cd2 and 10%SS+Zn2+Cd2 applications increased Zn content compared to 10%SS+Cd2 treatment in root and shoot (Table 5). This result indicated that Zn treatments increased Zn and decreased cadmium uptake in different plant parts of lettuce. In the growing environment with Cd, Zn application reduces the Cd concentration in the above-ground parts of the lettuce plant and also reduces the transport from the roots to the upper organs. Cd concentration is higher in the root region of lettuce. It is thought that cadmium accumulates more in roots as a plant defence mechanism. In a similar study, Rajapaksha and Amarakoon, (2011) found that the presence of Zn decreased Cd accumulation in shoots of lettuce**.** Zn and Cd with a common transfer membrane protein go into the root cell. Many investigators have suggested that Zn inhibits Cd uptake (Sharma and Agrawal, 2006; Benakova et al., 2017). However Green et. al. (2017) reported that Zn did not have an impact on Cd uptake in root and translocation to shoot in rice. Zareet. al. (2018) reported that Cd: Zn ratio was to be very important in cadmium uptake by lettuce plant. Results were consistent with the previous studies reportingan antagonistic relationship between Zn and Cd (Cakmak, 2000; Zhao et al., 2005; Murtaza et al., 2017).

Table 5. Effects of SS, Cd (50,100 mg kg^{-1}) and Zn (250, 500 mg kg^{-1}) applications on Cd and Zn contents of lettuce

Applications	Cd (mg kg^{-1})		Zn (mg kg^{-1})	
	Shoot	Root	Shoot	Root
Control	0.10±0.026e	0.47±0.026 e	39.5± 1.81g	97.8± 15.0gh
10%SS	0.19±0.039e	0.84± 0.036e	76.7± 5.85e	170.6±9.37f
10%SS+Cd1	114.0± 3.55c	985.9± 8.91d	61.3±1.64f	109.1± 5.62g
10%SS+Cd2	186.3±1.32a	1487± 18.82b	56.7±0.44f	85.7±3.21h
10%SS +Zn1	0.84±0.076e	5.81±0.128 e	162.8±5.83b	485.2± 6.31c
10%SS +Zn2	0.57±0.051e	3.15±0.098e	217.7±7.28a	822.1± 13.80a
10%SS +Zn1+ Cd1	103.3±0.863d	1070.1±8.98 c	119.2±2.00d	384.1± 5.02d
10%SS +Zn1+Cd2	180.9±3.59ab	1654± 36.51a	122.3± 3.35d	339.6± 8.83e
10%SS +Zn2+Cd1	119.8±14.22c	1057± 13.42c	150.8± 2.28c	554.9 ±25.60b
10%SS +Zn2+Cd2	178.5 ±1.995b	1499±27.92 b	164.0± 5.69b	491.8 ±8.71c

[*]a,b,c,d,e: Statistically significant mean differences are indicated with different letters in the same column ($p<0.05$).

3.4. Cd and Zn content of experimental soil

10%SS alone application did not increase DTPA-Cd compared to the control due to low Cd content of SS. However 10%SS+Cd1 and Cd2 applications increased DTPA-Cd content compared to the control. 10 % SS+ Zn+ Cd1 application decreased DTPA-Cd content compared to Cd1 alone application. Similarly in 10%SS amended growth medium, increased Zn application with Cd2 decreased DTPA-Cd content compared to Cd2 alone application (Table 6). A reduction of the extractable Zn in soil by the application of Cd may be due to the exchange of Zn by Cd from soil sorption sites and non-absorbable complexes in the soil solution (Kabata-Pendias, 2010).

10%SS alone application increased DTPA-Cd compared to the control due to high Cd content of SS. Increased levels of Zn application with Cd1 increased DTPA-Zn content compared to Cd1 alone. Similarly, increased levels of Zn application with Cd2 application increased DTPA-Zn content compared to Cd2 alone (Table 6).We found that 10% SS +Zn applications with Cd reduced Cd concentration and increased Zn concentration in soil. Köleli et al. (2004) reported that Cd application in Zn deficient soil reduced Zn uptake in wheat, whereas the application of Cd to soil with adequate Zn did not reduce Zn uptake in wheat. The decrease in grain Cd concentration by Zn application in Cd-contaminated and uncontaminated soil might be due to the antagonistic effect of Zn (Saifullah et al., 2014).

Table 6. Effects of SS, Cd (50,100 mg kg^{-1}) and Zn (250, 500 mg kg^{-1}) applications on DTPA- Cd and Zn contents of soil

Applications	Cd (mg kg^{-1})	Zn (mg kg^{-1})
Control	0.012±0.001 g	1.65± 0.034i
10%SS	0.043±0.001g	6.72± 0.19h
10%SS+Cd1	109.8± 1.31d	12.45±0.56g
10%SS+Cd2	202.5± 5.51a	12.02±0.15g
10%SS +Zn1	0.075± 0.024g	66.1±1.79e
10%SS +Zn2	0.133±0.092g	114.4±1.08a
10%SS +Zn1+ Cd1	93.6±1.15e	63.83±0.82f
10%SS +Zn1+Cd2	196.7± 1.17b	67.56± 1.074d
10%SS +Zn2+Cd1	86.8±4.95f	104.36±0.92b
10%SS +Zn2+Cd2	165.6±4.51c	93.53±0.95c

*a,b,c,d,e: Statistically significant mean differences are indicated with different letters in the same column ($p<0.05$).

4. Conclusion

According to these results, the low cadmium content of the used sewage sludge did not cause toxic effects. However, 10% SS +Cd applications had significant toxicity effects in the lettuce plant. However, 10% SS +Zn applications along with Cd was found to decrease soil Cd concentrations and increase Zn availability. It was found that this situation reduced Cd uptake in lettuce plant and resulted in an improvement of yield criteria. Anti-oxidative enzyme activity, particularly SOD and GPX enzymes, increased with Zn applications under 10% SS+ Cd conditions. These results indicate that a 10% SS+Zn application could be beneficial for ameliorating the toxic effects of Cd in lettuce.

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