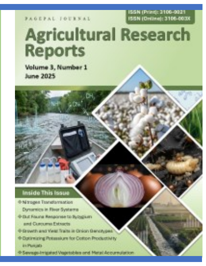
Agricultural Research Reports

https://journals.pagepal.org/index.php/ARR

ISSN (Online), 3106-003X; (Print), 3106-0021

https://doi.org/10.54219/arr.03.1.2025.418



Effects of sewage water irrigation on heavy metals accumulation in vegetables in peri-urban areas of Faisalabad, Pakistan

Muhammad Abubakar[1], Basit Haider[2]*, Hadeeqa Arshad[2], Tufail Shah[3], Ameer Khan[1], Hummera Nawaz[1], Maryam Batool[4]

[1]Department of Botany, University of Education, Faisalabad Campus, Faisalabad-38000 Pakistan.
[2]Nuclear Institute for Agriculture and Biology College (NIAB-C), Pakistan Institute of Engineering and Applied Sciences, Faisalabad-38000, Pakistan.
[3]College of Land Science and Technology, China Agricultural University, Beijing-100193, China.
[4]University of Sahiwal, Sahiwal-57000, Pakistan.

Article Info

Keywords:
Food chain contamination, Heavy metals, Wastewater irrigation, Vegetables

Date:
Received: 12 April 2025
Accepted: 1 June 2025
Published: 30 June 2025

Volume: 03 (01), 2025

Abstract

Untreated sewage effluents are frequently used for irrigation, posing a risk of heavy metal accumulation in edible crops. The present study aimed to assess the concentrations of heavy metals in water, soil, and two commonly consumed vegetables, Spinach (Spinacia oleracea L.) and Cauliflower (Brassica oleracea var. botrytis), collected from Ghulam Muhammad Abad (Site-I) and Chokera (Site-II) in Faisalabad, Pakistan. Water and soil samples were analyzed for pH, electrical conductivity (EC), and heavy metals, while plant samples were evaluated for biomass, photosynthetic pigments, antioxidant enzyme activities, and metal accumulation. Heavy metals, including Cd, Co, Cr, Pb, Cu, Ni, and Hg, were quantified using inductively coupled plasma optical emission spectrometry (ICP-OES). Results showed that water EC exceeded safe limits (Site-II: 29.86 NTU), while soil EC remained below 1 dS m^{-1}, indicating no salinity issues. Heavy metal concentrations in water exceeded WHO permissible limits, particularly Cd (0.50 mg L^{-1} at Site-II) and Hg (1.92 mg L^{-1} at Site-II). Soil samples had elevated Co and Pb levels, with Site-II showing 6.09 mg kg^{-1} Co and 5.39 mg kg^{-1} Pb. In vegetables, Cd, Co, Ni, and Pb exceeded safe limits, with spinach showing Cd (3.58 mg kg^{-1}) and Co (4.71 mg kg^{-1}), and cauliflower showing Cd (3.92 mg kg^{-1}) and Co (5.55 mg kg^{-1}). Heavy metal exposure significantly reduced root and shoot lengths (up to 74% decrease in fresh biomass and 59% in dry biomass) and photosynthetic pigments, while antioxidant enzyme activities (CAT: 142 Unit g^{-1} F. wt, POD: 125 Unit g^{-1} F. wt) were elevated, indicating oxidative stress. These findings demonstrate that the continued use of untreated sewage water for irrigation poses substantial health risks through heavy metal accumulation in food crops. The study highlights the urgent need for monitoring and remediation strategies to ensure food safety.

1. Introduction

The Wastewater is used for irrigation practices in some developing countries, such as Pakistan. There are several harmful metals in the wastewater, which have become components of many agricultural crops (Khan *et al.,* 2022; Harsonowati *et al.,* 2024). Sewage water has poor quality, but it is still considered an essential supply to irrigate the agricultural lands (Santos *et al.,* 2023). Wastewater has been used for irrigation practices for a long time due to the shortage and accessibility of freshwater (Younas & Younas,

Corresponding Author: Basit Haider Email: basit769@gmail.com

2022). Heavy metals are found in significant amounts in wastewater (Agoro *et al.,* 2020). According to a study, when wastewater containing heavy metals is used to irrigate agricultural lands, toxic heavy metals accumulate in the soil and eventually become accessible for crops (Vasilachi *et al.,* 2023).

Since vegetables are an excellent source of many essential elements, including vitamins, minerals, and dietary fibers, they have been crucial in supplying people's nutritional needs worldwide (Slavin & Lloyd, 2012). Many assessments of the health risks that are associated with heavy metals in polluted vegetables have been conducted in industrialized nations (Rahim *et al.,* 2024); however, in developing nations, there is little to no data on the exposure of people to toxic or heavy metals (Angon *et al.,* 2024). Regarding heavy metal contamination in Pakistani vegetables, not much information has been reported (Ashraf *et al.,* 2021). Heavy metals become hazardous and poisonous when they accumulate in humans, animals, and plants (Kumari & Mishra, 2021; Okereafor *et al.,* 2020). Heavy metal interference disrupts a number of physiological processes, including photosynthesis, gaseous exchange, and nutrition absorption. According to Mulenga *et al.* (2020), heavy metals may also hinder plant development and production. The reactive oxygen species (ROS) overproduction might induce oxidative stress in plants, which is regarded as an indirect impact of metal toxicity (Hafeez *et al.,* 2023; Mulenga *et al.,* 2020). ROS may be directly produced by Haber-Weiss reactions in a variety of metals. These potentially harmful metals re-accumulate in agricultural soils as a result of plant death and decomposition (Kiran *et al.,* 2022).

High levels of heavy metals consumed from vegetable consumption may cause toxicity and major illness because they interfere with several metabolic processes in the human body (Abuzed Sadee & Jameel Ali, 2023; Baffa *et al.,* 2022). Some heavy metals used for commercial purposes, such as Pb, As, Hg, Cd, and Cr, are highly toxic. When they seep into agricultural soils, they are absorbed by plants and eventually by humans, where they can lead to diseases of the kidneys, liver, and nervous system (Balali-Mood *et al.,* 2021; Manwani *et al.,* 2022). Consumption of fruits and vegetables that contain dangerous heavy metals, including Al, Cu, Pb, Hg, As, and Zn, may cause pneumonia, depression, vomiting, and gastrointestinal issues. Several lung problems, including bronchitis, emphysema, alveolitis, and subchronic breathing, have been linked to cadmium (Singh, 2022; Srivastava *et al.,* 2024).

Manures from animals fed diets containing metals were found to contain substantial amounts of As, Cu, and Zn (Duan & Feng, 2021; Zheng *et al.,* 2022). Heavy metals accumulate on agricultural grounds when these manures are administered regularly (Guimaraes *et al.,* 2022). Sewage sludge is an organic bio-solid created during the wastewater treatment process and may be recovered for its own purposes (Fijalkowski *et al.,* 2017).

Various factors, including the specific metallic element, the species of plant, and the soil's physical characteristics, influence the efficiency of metal transfer (Jogawat *et al.,* 2021; Shen *et al.,* 2022). Many mechanisms, including adsorption, complexation, ion exchange, inorganic micro-precipitation, oxidation, and reduction, are thought to be involved in the absorption of metals (Ali *et al.,* 2021; Shah *et al.,* 2024). In addition to contaminating agricultural soils, heavy metal buildup may have a negative impact on the safety and quality of food. Thus, it is important to monitor food quality (Qin *et al.,* 2021).The primary pathway for heavy metals to gradually enter the food chain in plants is through root absorption (Pasricha *et al.,* 2021).

There are two types of water; ground water with poor quality and sewage water, both kinds of waters negatively affect the crops (Asli *et al.,* 2022). It is because both the waters adversely affect the growth of plants. The need for this research is to inform our farmers about the presence of toxic metals in sewage water. There is extensive data regarding the accumulation of toxic metals in various crops and the utilization of sewage water for irrigation purposes in Pakistan, but there is still lack of comprehensive data that may address cauliflower and spinach. Therefore, the present study was conducted to assess the levels of heavy metals in water, soil, and vegetable samples collected from sites irrigated with sewage water.

2. Materials and Methods

2.1. Site description

Two specific peri-urban sites of Faisalabad were selected for this study, where sewage water was utilized for irrigation purposes. These sites were Ghulam Muhammad Abad (Site I: 31.46262˚ N, 73.04837˚ E) and Chokera (Site II: 31.46509˚

N, 73.00476˚ E). A research study was conducted to evaluate the toxic heavy metals, physio-chemical parameters of water, soil and morpho-biochemical parameters of vegetables such as Spinach (*Spinacia oleracea* L.) from Site I and Cauliflower (*Brassica oleracea* var. botrytis) from Site II.

2.2. Sampling, physicochemical characterization, and elemental analysis

Sewage water samples were collected from Site I and Site II in pre-cleaned, high-density polyethylene (HDPE) bottles. Before sampling, the bottles were cleaned with detergent, rinsed with tap water, and then rinsed three times with deionized water to avoid contamination. The soil samples (0-30 cm depth) were collected using a pre-cleaned, inert plastic scooper made of high-density polyethylene (HDPE) to avoid contamination with metals. The scooper was washed thoroughly with treated with detergent, then rinsed with tap water, and subsequently rinsed three times with deionized water before sampling. The edible leafy portions of *Spinacia oleracea* L. and *Brassica oleracea* var. botrytis were collected from Site I and Site II, respectively. At each site, three representative plants of each vegetable were randomly selected to account for variability within the field. For spinach, the whole above-ground portion was harvested, while for cauliflower, both edible heads and associated leaves were collected. Approximately 1 kg of fresh plant material per species per site was collected. The plants were carefully excavated with their roots using a clean, inert plastic spatula to avoid contamination. Samples were placed in pre-labeled polyethylene bags, transported to the Plant Physiology and Biochemistry Laboratory, University of Education Lahore, Faisalabad campus, in an icebox at 4°C, and processed immediately for physiological, biochemical, and heavy metal analyses.

Prior to the physicochemical analysis, the soil samples were air-dried, followed by sieving with a 2-mm sieve to remove stones, organic debris, and pebbles. The collected soil and water samples were tested for EC and pH using a Milwaukee multimeter (Milwaukee 2217-20, Milwaukee Tool, USA). Turbidity was measured in nephelometric turbidity units (NTU) using a nephelometric turbidity meter (Turb® 430 IR, WTW, Germany). Total dissolved solids (TDS) were determined utilizing a microprocessor-controlled conductivity meter (HI-2003-2, HANNA Instruments, Romania), whereas the alkalinity of the water samples was assessed by the titration technique. Alkaline potassium permanganate method was used to determine the available nitrogen (Subbiah & Asija, 1956); phosphorus was assessed using an extraction method with ammonium molybdate solution (Olsen, 1954); concentration of available potassium was analyzed using the ammonium acetate extraction method (Merwin & Peech, 1951) and analyzed by using a flame photometer (PFP-7, Jenway, UK).

2.3. Morphological parameters

After washing with water to remove dirt and dust, the vegetable samples were dried and gently wiped with tissue paper. The lengths of shoots and roots were measured from base to tip and tip to base, respectively, measurements were taken using a measuring tape. The fresh weight of the leaves was recorded while they were on the electronic balance (Vario EL Cube, Germany). To calculate dry weights, the leaf samples were allowed to air dry before being baked for 24 hours at 60°C. These leaf samples were dried and then put on an electronic balance, and their dry weight was measured.

Concentration of heavy metals in vegetables, soil and water samples Cauliflower and spinach dried leaf samples (0.2 g) ground material was accurately weighed using an electronic scale. 5 mL of H_2SO_4 was taken and each digestion tube was prefilled, followed by adding the leaf sample. The digestive tubes were kept incubated overnight at ambient temperature. temperature. The subsequent day, 1 mL of H_2O_2 was added to every digestion tube and placed in digestion block for heating at 200°C until visible fumes appeared. The heating was continued for another thirty minutes to ensure complete digestion. After digestion, the tubes were removed from the digesting block and allowed to cool. Subsequently, 1 mL of hydrogen peroxide (H2O2) was added to each tube and placed back in digestion block. The digestion procedure was continued until a transparent liquid was obtained followed by filtration with Whatman No. 42 filter paper, once the samples were cooled down. The filtered solution was then diluted with distilled water to the desired volume in a 50 mL volumetric flask. Similar procedure was followed for heavy metal determination in soil and water samples. Working standard solutions of Co, Cd, Cr, Pb, Cu, Ni, and Hg were prepared from the stock standard solutions containing 1000 ppm of each element. The elemental analysis was subsequently performed using the Inductively Coupled Plasma Emission Spectrometer (ICAP-6300 Duo, Thermo Fisher Scientific, USA).

2.4. Estimation of photosynthetic pigments

The chlorophyll contents in plant samples were calculated using Arnon technique (1949). Fresh foliage leaves (0.2 g) were scraped, extracted, and homogenized in 10 mL of 80% acetone. The resulting solution was kept in the dark at room temperature for 12 hours. Chlorophyll concentrations at 663nm and 645nm were determined using a visible Ultra Violet Spectrophotometer.

2.5. Estimation of antioxidants in plants

Catalase (CAT) and peroxidase (POD) activities were determined using the method described by Chance and Maehly (1955).At first, 4 mL enzyme extract, 5.9 mM H2O2 and a phosphate buffer (pH=7.0) was mixed and then the decrease in absorbance at 240 nm was recorded to determine the catalase activity. This decrease in absorbance represented the consumption of Hydrogen peroxide for every 15 seconds. To estimate the activity of POD, 0.1 mL enzyme extract, 20 mM phosphate buffer (pH=7.0), 2mM H_2O_2 and 0.05% guaiacol were mixed. The mixture was centrifuged to remove residual. An increase in absorbance was recorded at 470 nm for 03 minutes. Peroxidase enzyme catalyzed the oxidation of guaiacol, which represented the increase in absorbance. The extinction coefficient of 26.6 nm M^{-1} cm^{-1} was utilized for calculating the guaiacol concentration.

2.6. Statistical Analysis

The data in this experiment were analyzed using the ANOVA (Statistical Analysis of Variance) approach using the STATISTIX 8.1 software. At the 5% level of probability, the "Least Significant Difference" (LSD) test was also used to determine the differences between the significant means (Steel *et al.,* 1997).

3. Results and discussion

3.1. Physicochemical properties of water and soil samples

The water samples from Site I exhibited the lowest pH value, whereas those from Site II showed the highest pH value (Table 1). The pH of the water samples from the two locations differs by nearly 3%. The release of domestic or industrial effluents into sewage water channels from nearby places may be the cause of the small pH fluctuation seen at various locations. Rabbani *et al.* (2024) stated that the pH of the irrigation water cannot be regarded as an indicator for the quality of water, as numerous different crops can tolerate a broad pH range and the soil frequently buffers irrigation water. The soil samples from Site I exhibited the highest pH level, whereas those from Site II demonstrated the lowest pH level. Compared to water samples, soil samples were more alkaline.

The water samples taken from Site I had the lowest EC value as compared to Site II. The EC of the water samples from the two locations differs by over 21%. High EC values were observed in the water sample from Site II and might be due to the direct release of domestic effluents and detergents into sewage water channels. Similar results were obtained on EC values for soil samples. For instance, Site II exhibited a higher EC value (0.70 ± 0.09 dS m^{-1}) than Site-I (0.48 ± 0.15 dS m^{-1}). A 37% difference was noted in the electrical conductivity of soil samples collected from the two locations. All samples had EC values below 4.0 dS m^{-1}, indicating no salinity issues. The turbidity of the water samples from Site I and Site II was measured at 24.16 NTU and 29.86 NTU, respectively. The elemental analysis showed that the contents of nitrogen, phosphorus, and potassium in the soil samples from Site-I were 247.33 kg ha^{-1}, 10.35 kg ha^{-1}, and 127.33 kg ha^{-1}, respectively, while from Site-II the concentrations were 290 kg ha^{-1}, 8.54 kg ha^{-1}, and 134.1 kg ha^{-1}, respectively.

3.2. Morphological results

The results showed that the presence of heavy metals in the soil samples affected the root and shoot lengths of *Brassica oleracea* var. botrytis and *Spinacia oleracea* L (Figure 1). Roots have more sensitivity than shoots, partly due to being the initial plant components that meet hazardous chemicals. Liu *et al.* (2004) indicated that diminished cell development resulting from exposure to Cd and Pb can lead to shortened lengths of shoots and roots. Elevated levels of Pb in spinach led to toxicity, as indicated by diminished plant biomass and growth length. Comparable results were seen by Naz *et al.,* (2013). Both Bakhshayesh *et al.,* (2014) and Devi *et al.,* (2007) reported findings that were comparable to the findings that were obtained by the present study.

It was found that heavy metals had adverse effects on the fresh and dry weights of plants. There was a significant difference found in the fresh and dry weights of Spinacia oleracea L. and *Brassica oleracea* var. botrytis. A difference of nearly 74% was seen in fresh weights, whereas a 59% difference was noted in dry weights. Elevated concentrations of Co, Cr, Cd, and Pb were observed to diminish plant biomass (both fresh and dry weight). These findings closely correspond with the results documented by Naz *et al.* (2013). gymnosperms (Picea abies) and some angiosperms (Acer rubrum, Quercus palustris and Zea mays) (Siedlecka, 1996). Sunflower also showed a decline in chlorophyll levels

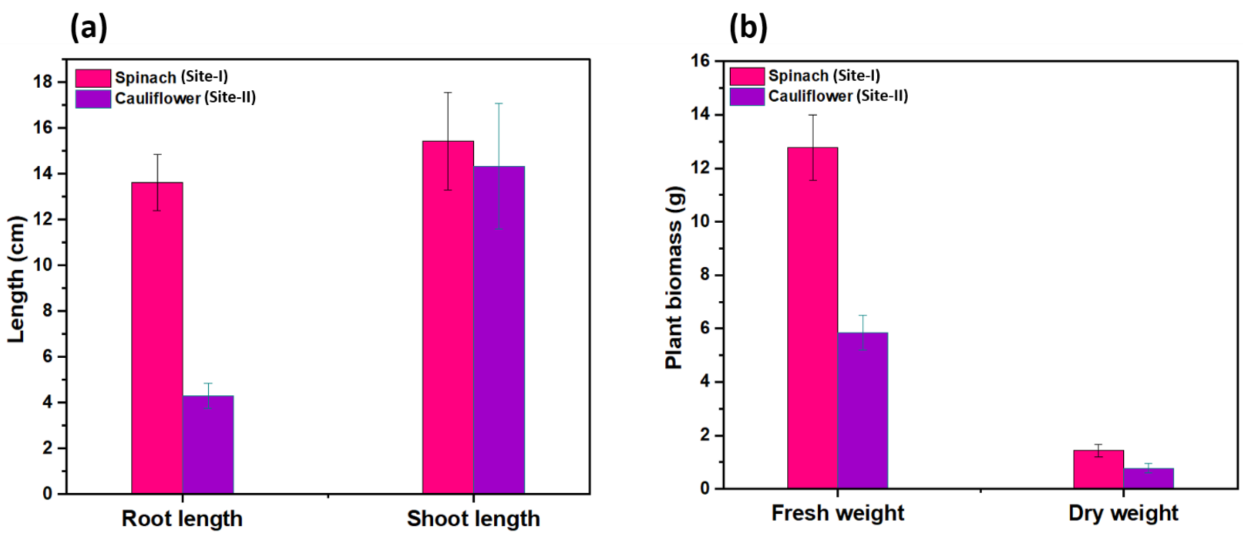


Figure 1. Morphological parameters (a) root and shoot lengths (cm) and (b) fresh & dry weights (g) of vegetables (spinach and cauliflower) from Site I and II, respectively.

Error bars indicate standard deviations from replicate measurements. The values are the means of three replicates (n=3±SD).

3.3. Photosynthetic pigments

The presence of heavy metals affected all photosynthetic pigments, including chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids (Figure 2). The raised redox potential of some heavy metals is believed to be the source of the reduction of photosynthetic pigments. This is because the elevated redox potential has a negative impact on the reductive phases of several biosynthetic pathways. Heavy metals inhibit the enzyme protochlorophyllide reductase, essential for the conversion of protochlorophyll to chlorophyll (Rathi *et al.,* 2022). The accumulation of copper results in the decline of chlorophyll level in plants, which is associated with the copper-induced peroxidation of chloroplast membranes (Farid *et al.,* 2021; Huang *et al.,* 2020).

Higher levels of Cr and Pb in plant leaves lowered the concentrations of chlorophyll a, b and carotenoids which suggests the decline in chlorophyll synthesis pathway and chlorophyllase activity (Salavati *et al.,* 2021; Singh *et al.,* 2023). Previous research studies have also described a Diminished chlorophyll content because of toxic metal stress in many organisms such as Chlorella, Cyanobacteria,

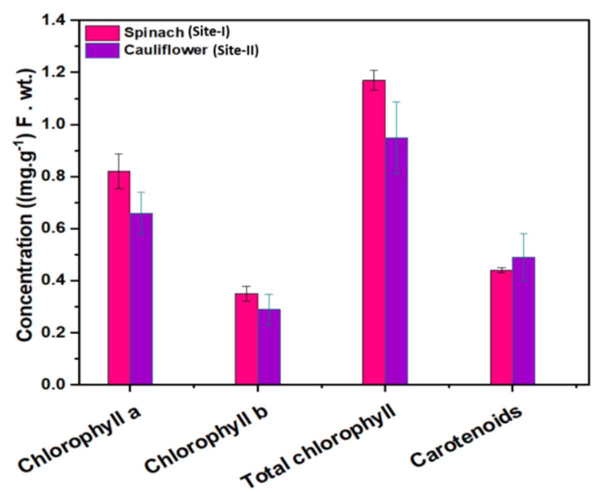


Figure 2. Different Photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids) concentrations (mg g^{-1} Fresh weight) in spinach and cauliflower from Site I and II, respectively.

Error bars indicate standard deviations from replicate measurements. The values are the means of three replicates (n=3±SD).

(Zengin & Munzuroglu, 2006). The toxicity induced by cadmium stress may limit the biosynthesis of chlorophyll and photosynthesis, which ultimately results in a decrease in biomass (Ivanov & Kosobryukhov, 2020).

3.4. Antioxidant activities

The activities of CAT and POD were found to be elevated in the leaf samples due to the presence of toxic metals (Figure 3). It showed that the level of POD (125 Unit g^{-1} F. wt) was exceeding the CAT (81 Unit g^{-1} F. wt). Cho & Seo, (2005); Lombardi & Sebastiani, (2005) have also reported the higher values of POD and lower values of CAT due to the presence of toxic metals. The accumulation of hydrogen peroxide (H_2O_2) has been reported to induce oxidative stress as a result of hazardous metal concentrations (Cho & Seo, 2005). The tetrameric heme-containing enzymes referred to as CATs are crucial for the detoxification of reactive oxygen species (ROS) during stressful conditions, as they may directly dismutate H_2O_2 into H_2O and O_2 (Garg & Manchanda, 2009). CAT plays a crucial role in eliminating hydrogen peroxide (H_2O_2) which is formed in peroxisomes through the activity of oxidases participating in fatty acid β-oxidation, photorespiration, and purine catabolism(Gill & Tuteja, 2010).

Similarly, the presence of heavy metals in cauliflower dramatically elevated the levels of antioxidants CAT (142 Unit g^{-1} F. wt) and POD (60 Unit g^{-1} F. wt). Hydrogen peroxide synthesis is extremely detrimental to plants; however, it can be transformed into water and oxygen by the antioxidant enzymes CAT and POD (Chaudhury, 2023). Antioxidants serve as protective agents against oxidative stress (Radotić *et al.,* 2000). According to Ashraf (2009), antioxidants transform H_2O_2 into water and oxygen. The results correspond with the previous studies (Ahmad *et al.,* 2017; Wang *et al.,* 2022). Pyrogallol (PPX), glutathione (GPX), cytochrome c (CcP), and ascorbate peroxidase (APX) are examples of substrates for peroxidases, a category of enzymes that facilitate the oxidation of substrates using hydrogen peroxide (Garg & Manchanda, 2009). Similar results were observed in C. vulgaris by Kebeish *et al. (*2014).

3.5. Heavy metal concentrations in sewage water, soil, and vegetable samples

The sewage water samples from Site I and Site II showed toxic metals above the permissible limits of WHO. The observed values of toxic metals at Site I were Cd (0.17 mg kg⁻¹), Co (0.72 mg kg⁻¹), Pb (1.07 mg kg⁻¹), Cr (1.79 mg kg⁻¹), Hg (1.38 mg kg⁻¹), Cu (2.66 mg kg⁻¹) and Ni (0.55 mg kg⁻¹) respectively. Whereas at Site-II, the concentrations were Cd (0.50 mg kg⁻¹), Co (0.66 mg kg⁻¹), Pb (0.87 mg kg⁻¹), Cr (1.23 mg kg⁻¹), Hg (1.92 mg kg⁻¹), Cu (3.21 mg kg⁻¹) and Ni (0.87 mg kg⁻¹), respectively (Figure 4). All the

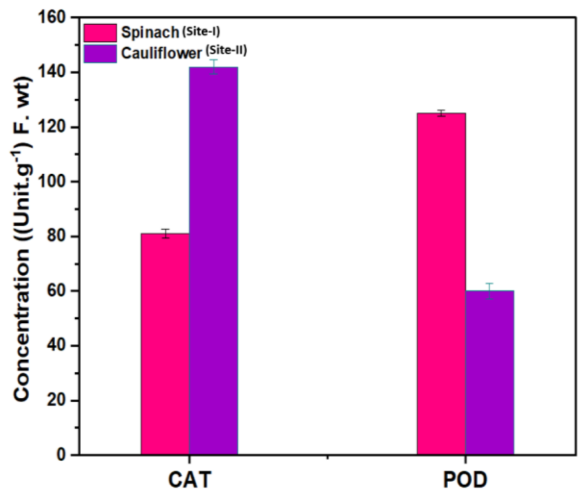


Figure 3. Different antioxidants (CAT and POD) concentrations (Units g^{-1} fresh weight) in spinach and cauliflower from Site-I and Site-II, respectively. *Error bars indicate standard deviations from replicate measurements. The values are the means of three replicates (n=3±SD).*

toxic metals showed a significant difference in the sewage water samples. Sewage water analysis of Site-I revealed the higher levels of Cr, Cu, Pb and Hg while lower levels of Co, Cd and Ni. Sewage water from Site II exhibited elevated concentrations of Cr, Cu and Hg but lower concentrations of Co, Cd, Pb and Ni. There were many industries for paint production, fabric printing, battery manufacturing and textile along the sewage water channels, which release toxic metals into these sewage water bodies. Water channels for irrigation purposes are contaminated due to discharge of toxic metals such as Cu, Co, Cd, Cr and Hg by these industries (Singh *et al.,* 2010). The study exceeded the Findings from previous studies (Ashraf *et al.,* 2021; Kinuthia *et al.,* 2020).

The soil metal concentrations at both sewage-irrigated sites were within USEPA (1997) guidelines, except for Co and Pb. The heavy metal concentrations at Site I were Cd (1.67 mg kg^{-1}), Co (3.94 mg kg-1), Pb (2.96 mg kg^{-1}), Cr (4.39 mg kg^{-1}), Hg (5.61 mg kg^{-1}), Cu (4.10 mg kg^{-1}) and Ni

(7.43 mg kg⁻¹), respectively. Whereas at Site II, the concentrations were Cd (2.36 mg kg^{-1}), Co (6.09 mg kg^{-1}), Pb (5.39 mg kg^{-1}), Cr (5.85 mg kg^{-1}), Hg (4.64 mg kg^{-1}), Cu (2.93 mg kg^{-1}) and Ni (5.56 mg kg^{-1}) respectively. All toxic metals showed significant soil composition changes between the two locations. Site-I soil samples had high concentration of Cu, Cr, Ni and Hg but low contents of Pb, Co and Cd. Site II soil samples were having high contents of Cr, Co, Ni and Pb but low Cu, Cd and Hg. Industrial waste and sewage effluent may explain Site II high Co, Ni, Cr, and Pb concentrations. Constant wastewater irrigation may change the soil physicochemical properties, causing food crops, especially vegetables, to absorb heavy metals. Oxidation, form, and phase greatly affect heavy metal bioavailability (Lei *et al.,* 2008). Lead pollution changes soil microorganisms and their activities, reducing soil fertility, physiological markers, and crop yields (Majer *et al.,* 2002).

Figure 4 shows the heavy metal content in cauliflower and spinach. The results revealed that vegetables grown at both sewage-irrigated sites exhibited heavy metal levels below the WHO (1996) permissible limits, except for Cd, Co, and Ni. The observed values for different heavy metals in Spinach were Cd (3.58 mg kg^{-1}), Co (4.71 mg kg^{-1}), Pb (6.13 mg kg^{-1}), Cr (9.43 mg kg^{-1}), Hg (3.83 mg kg^{-1}), Cu (3.11 mg kg^{-1}), and Ni (4.77 mg kg^{-1}), respectively. Similarly, in Cauliflower, the concentrations were Cd (3.92 mg kg^{-1}), Co (5.55 mg kg^{-1}), Pb (7.76 mg kg^{-1}), Cr (5.71 mg kg^{-1}), Hg (1.94 mg kg^{-1}), Cu (2.51 mg kg^{-1}), and Ni (2.68 mg kg^{-1}), respectively.

This study showed that cauliflower exhibited elevated levels of Cd, Co, Cr, and Pb. Spinach exhibited significant accumulation of Co, Ni, Cr, and Pb. The increased concentrations of these metals might result from the direct release of untreated effluents by adjacent industries into sewage water bodies. Crops irrigation with sewage effluents have increased levels of Cd and Co than those documented by Murtaza *et al.,* (2003). The increased levels of the specified metals may be linked to various small-scale industries beyond textile manufacturing, such as soap, plastic, and clothing production. The textile industry, along with dyes and pigments utilized in clothing, insecticides, plastics, and fertilizers, has the potential to increase the concentrations of Cr and Cd in vegetables (Ahmed *et al.,* 2019). Increased Cd levels can cause oxidative stress, which includes alterations in the antioxidant defense system's enzymes, decreased food absorption, and suppression of several enzyme activities (Vitória *et al.,* 2001).

4. Conclusion

This study demonstrated that sewage-irrigated soils and vegetables from Site I and Site II contained elevated concentrations of heavy metals, with levels in spinach and cauliflower exceeding WHO safe limits. The metals accumulated in edible plant tissues, indicating potential health risks for human consumption. These findings underscore the need for proper wastewater treatment and regular monitoring of heavy metal contamination in irrigation water and crops. Future studies should investigate the long-term effects of heavy metal accumulation in food crops and explore mitigation strategies, such as soil amendments or alternative irrigation practices, to reduce health risks.

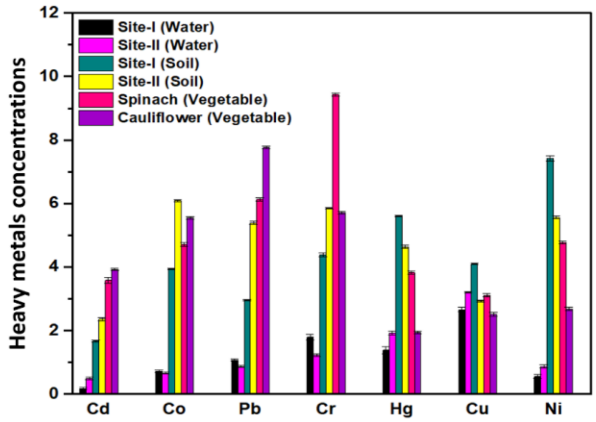


Figure 4. Different heavy metals; Cadmium (Cd), cobalt (Co), lead (Pb), chromium (Cr), mercury (Hg), copper (Cu), and nickel (Ni) concentrations in water (mg L^{-1}), soil (mg Kg^{-1}) and spinach and cauliflower (mg Kg^{-1}) from Site I and II, respectively.

Error bars indicate standard deviations from replicate measurements. The values are the means of three replicates (n=3±SD).

5. Funding

No funding was received for this study.

6. Acknowledgment

Authors are thankful to Department of Botany, University of Education Lahore, Faisalabad Campus, for providing space for the experimentation.

7. Conflict of Interest

The authors declare no conflict of interest.

8. Authors' Contribution

Muhammad Abubakar: Data curation, Investigation, Methodology, Project administration, Formal data analysis, Writing-Original Draft. Basit Haider: Writing- Reviewing and editing of original draft. Hadeeqa Arshad: Writing-Reviewing and Editing. Tufail Shah: Resources, Writing-Reviewing and Editing. Ameer Khan: Resources, Writing-Reviewing and Editing. Hummera Nawaz: Resources, Writing- Reviewing and Editing. Maryam Batool: Writing-Reviewing and Editing.

9. SDG Goals

This study aligns with *SDG 6 (Clean Water and Sanitation)* by highlighting the risks of using untreated sewage water for irrigation and the need for effective wastewater treatment. It also supports *SDG 3 (Good Health and Well-being)* and *SDG 2 (Zero Hunger)* by focusing on heavy metal contamination in food crops and its implications for public health and food safety. The work further contributes to *SDG 15 (Life on Land)* through its focus on soil quality deterioration and the need for sustainable environmental management.

References

Abuzed Sadee, B., & Jameel Ali, R. (2023). Determination of heavy metals in edible vegetables and a human health risk assessment. *Environmental Nanotechnology, Monitoring & Management, 19,* 100761.

Agoro, M. A., Adeniji, A. O., Adefisoye, M. A., & Okoh, O. O. (2020). Heavy metals in wastewater and sewage sludge from selected municipal treatment plants in Eastern Cape Province, South Africa. *Water, 12*(10), 2746.

Ahmad, R., Ali, S., Hannan, F., Rizwan, M., Iqbal, M., Hassan, Z., Akram, N. A., Maqbool, S., & Abbas, F. (2017). Promotive role of 5-aminolevulinic acid on chromium-induced morphological, photosynthetic, and oxidative changes in cauliflower (*Brassica oleracea* botrytis L.). *Environmental Science and Pollution Research, 24*(9), 8814-8824.

Ahmed, M., Matsumoto, M., Ozaki, A., Thinh, N. V., & Kurosawa, K. (2019). Heavy metal contamination of irrigation water, soil, and vegetables and the difference between dry and wet seasons near a multi-industry zone in Bangladesh. *Water, 11*(3), 583.

Ali, A., Shah, T., Ullah, R., Zhou, P., Guo, M., Ovais, M., Tan, Z., & Rui, Y. (2021). Review on recent progress in magnetic nanoparticles: Synthesis, characterization, and diverse applications. *Frontiers in Chemistry, 9,* 629054.

Angon, P. B., Islam, M. S., Kc, S., Das, A., Anjum, N., Poudel, A., & Suchi, S. A. (2024). Sources, effects and present perspectives of heavy metals contamination: Soil, plants and human food chain. *Heliyon, 10*(7), e28357.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts: Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology, 24*(1), 1-15.

Ashraf, I., Ahmad, F., Sharif, A., Altaf, A. R., & Teng, H. (2021). Heavy metals assessment in water, soil, vegetables and their associated health risks via consumption of vegetables, District Kasur, Pakistan. *SN Applied Sciences, 3*(5), 552.

Ashraf, M. (2009). Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnology Advances, 27*(1), 84-93.

Asli, S., Massalha, N., Diab, M., & Hugerat, M. (2022). Phytotoxic effects of treated wastewater used for irrigation on root hydraulic conductivity and plant growth of maize seedlings (*Zea mays* L.). *Irrigation Science, 40*(6), 817-828.

Baffa, A., Waziri, M., & Uttu, A. (2022). Dietary intake of heavy metals from the consumption of fruits and vegetables in Gashua, Nigeria. In *Proceedings of the 45th Annual International Conference, Workshop, and Exhibition of the Chemical Society of Nigeria (CSN)*. Chemical Society of Nigeria.

Bakhshayesh, B. E., Delkash, M., & Scholz, M. (2014). Response of vegetables to cadmium-enriched soil. *Water, 6*(5), 1246-1256.

Balali-Mood, M., Naseri, K., Tahergorabi, Z., Khazdair, M. R., & Sadeghi, M. (2021). Toxic mechanisms of five heavy metals: Mercury, lead, chromium, cadmium, and arsenic. *Frontiers in Pharmacology, 12,* Article 643972.

Chance, B., & Maehly, A. C. (1955). Assay of catalases and peroxidases. In S. P. Colowick & N. O. Kaplan (Eds.), *Methods in enzymology* (Vol. 2, pp. 764-775). Academic Press.

Chaudhury, P. (2023). Role of secondary metabolites of *Momordica charantia* in combating copper-induced stress. *American Journal of Applied Bio-Technology Research, 4*(1), 5-29.

Cho, U.-H., & Seo, N.-H. (2005). Oxidative stress in *Arabidopsis thaliana* exposed to cadmium is due to hydrogen peroxide accumulation. *Plant Science, 168*(1), 113-120.

Devi, R., Munjral, N., Gupta, A. K., & Kaur, N. (2007). Cadmium induced changes in carbohydrate status and enzymes of carbohydrate metabolism, glycolysis and pentose phosphate pathway in pea. *Environmental and Experimental Botany, 61*(2), 167-174.

Duan, B., & Feng, Q. (2021). Comparison of the potential ecological and human health risks of heavy metals from sewage sludge and livestock manure for agricultural use. *Toxics, 9*(7), 145.

Farid, M., Farooq, M. A., Fatima, A., Abubakar, M., Ali, S., Raza, N., Alhaithloul, H. A. S., & Soliman, M. H. (2021). Copper-induced responses in different plant species. In M. Hasanuzzaman (Ed.), *Approaches to the remediation of inorganic pollutants* (pp. 259-280). Springer Singapore.

Fijalkowski, K., Rorat, A., Grobelak, A., & Kacprzak, M. J. (2017). The presence of contaminations in sewage sludge-The current situation. *Journal of Environmental Management, 203,* 1126-1136.

Garg, N., & Manchanda, G. (2009). ROS generation in plants: Boon or bane? *Plant Biosystems, 143*(1), 81-96.

Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry, 48*(12), 909-930.

Guimaraes, O., Wagner, J. J., Spears, J. W., Brandao, V. L. N., & Engle, T. E. (2022). Trace mineral source influences digestion, ruminal fermentation, and ruminal copper, zinc, and manganese distribution in steers fed a diet suitable for lactating dairy cows. *Animal, 16*(4), 100500.

Hafeez, A., Rasheed, R., Ashraf, M. A., Qureshi, F. F., Hussain, I., & Iqbal, M. (2023). Effect of heavy metals on growth, physiological and biochemical responses of plants. In A. Husen (Ed.), *Plants and their interaction to environmental pollution* (pp. 139-159). Elsevier.

Harsonowati, W., Nurrahma, A. H. I., Shah, T., Hashem, A., Abd_Allah, E. F., & El-Beltagi, H. S. (2024). Bioremediation of heavy metals contaminated industrial effluents by endophytes and their mechanisms. In A. Husen et al. (Eds.), *Bio-organic amendments for heavy metal remediation* (pp. 57-67). Elsevier.

Huang, W.-L., Wu, F.-L., Huang, H.-Y., Huang, W.-T., Deng, C.-L., Yang, L.-T., Huang, Z.-R., & Chen, L.-S. (2020). Excess copper-induced alterations of protein profiles and related physiological parameters in citrus leaves. *Plants, 9*(3), 291.

Ivanov, A. A., & Kosobryukhov, A. A. (2020). Ecophysiology of plants under cadmium toxicity. In M. Hasanuzzaman (Ed.), *Plant ecophysiology and adaptation under climate change* (Vol. 1, pp. 429-484). Springer Singapore.

Jogawat, A., Yadav, B., Chhaya, & Narayan, O. P. (2021). Metal transporters in organelles and their roles in heavy metal transportation and sequestration in plants. *Physiologia Plantarum, 173*(1), 259-275.

Kebeish, R., El-Ayouty, Y., & Husain, A. (2014). Effect of copper on growth, bioactive metabolites, antioxidant enzymes and photosynthesis-related gene transcription in *Chlorella vulgaris*. *World Journal of Biology and Biological Sciences, 2*(2), 34-43.

Khan, M. M., Siddiqi, S. A., Farooque, A. A., Iqbal, Q., Shahid, S. A., Akram, M. T., Rahman, S., Al-Busaidi, W., & Khan, I. (2022). Towards sustainable application of wastewater in agriculture: A review on reusability and risk assessment. *Agronomy, 12*(6), 1397.

Kinuthia, G. K., Ngure, V., Beti, D., Lugalia, R., Wangila, A., & Kamau, L. (2020). Levels of heavy metals in wastewater and soil samples from open drainage channels in Nairobi, Kenya: Community health implication. *Scientific Reports, 10*(1), 8434.

Kiran, Bharti, R., & Sharma, R. (2022). Effect of heavy metals: An overview. *Materials Today: Proceedings, 51,* 880-885.

Kumari, S., & Mishra, A. (2021). Heavy metal contamination. In *Soil contamination-Threats and sustainable solutions.* IntechOpen.

Lei, M., Liao, B. H., Zeng, Q. R., Qin, P. F., & Khan, S. (2008). Fraction distributions of lead, cadmium, copper, and zinc in metal-contaminated soil before and after extraction with disodium EDTA. *Communications in Soil Science and Plant Analysis, 39*(13-14), 1963-1978.

Liu, W.-J., Zhu, Y.-G., Smith, F. A., & Smith, S. E. (2004). Do iron plaque and genotypes affect arsenate uptake and translocation by rice seedlings (*Oryza sativa* L.) grown in solution culture? *Journal of Experimental Botany, 55*(403), 1707-1713.

Lombardi, L., & Sebastiani, L. (2005). Copper toxicity in *Prunus cerasifera*: Growth and antioxidant enzyme responses of in vitro grown plants. *Plant Science, 168*(3), 797-802.

Majer, B. J., Tscherko, D., Paschke, A., Wennrich, R., Kundi, M., Kandeler, E., & Knasmüller, S. (2002). Effects of heavy metal contamination of soils on micronucleus induction in *Tradescantia* and on microbial enzyme activities. *Mutation Research, 515*(1), 111-124.

Manwani, S., Vanisree, C., Jaiman, V., Awasthi, K. K., Yadav, C. S., Sankhla, M. S., Pandit, P. P., & Awasthi, G. (2022). Heavy metal contamination in vegetables and their toxic effects on human health. In *Sustainable crop production: Recent advances* (p. 181). Agri FOSS.

Merwin, H., & Peech, M. (1951). Exchangeability of soil potassium in the sand, silt, and clay fractions as influenced by the nature of the complementary exchangeable cation. *Soil Science, 71*(6), 425-430.

Mulenga, C., Clarke, C., & Meincken, M. (2020). Physiological and growth responses to pollutant-induced biochemical changes in plants: A review. *Pollution, 6*(4), 827-848.

Murtaza, G., Ghafoor, A., Qadir, M., & Rashid, M. (2003). Accumulation and bioavailability of Cd, Co and Mn in soils and vegetables irrigated with city effluent. *Pakistan Journal of Agricultural Sciences, 40*(1-2), 18-24.

Naz, A., Khan, S., Qasim, M., Khalid, S., Muhammad, S., & Tariq, M. (2013). Metals toxicity and its bioaccumulation in purslane seedlings grown in controlled environment. *Natural Science, 5*(5), Article 32071.

Okereafor, U., Makhatha, M., Mekuto, L., Uche-Okereafor, N., Sebola, T., & Mavumengwana, V. (2020). Toxic metal implications on agricultural soils, plants, animals, aquatic life and human health. *International Journal of Environmental Research and Public Health, 17*(7), 2204.

Olsen, S. R. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *US Department of Agriculture Circular, 939,* 1-19.

Pasricha, S., Mathur, V., Garg, A., Lenka, S., Verma, K., & Agarwal, S. (2021). Molecular mechanisms underlying heavy metal uptake, translocation and tolerance in hyperaccumulators. *Environmental Challenges, 4,* 100197.

Qin, G., Niu, Z., Yu, J., Li, Z., Ma, J., & Xiang, P. (2021). Soil heavy metal pollution and food safety in China: Effects, sources and removing technology. *Chemosphere, 267,* 129205.

Rabbani, Z., Afroz, K., Baloch, M. K., Rehman, W., Nawaz, M., Tul Muntaha, S., & Bibi, I. (2024). Evaluation of spinach (*Spinacia oleracea*) quality depending on various combinations of soil and water attributes. *International Journal of Environmental Science and Technology, 21*(1), 63-72.

Radotić, K., Dučić, T., & Mutavdžić, D. (2000). Changes in peroxidase activity and isoenzymes in spruce needles after exposure to different concentrations of cadmium. *Environmental and Experimental Botany, 44*(2), 105-113.

Rahim, N., Noor, A., Kanwal, A., Tahir, M. M., & Yaqub, A. (2024). Assessment of heavy metal contamination in leafy vegetables: Implications for public health and regulatory measures. *Environmental Monitoring and Assessment, 196*(8), 684.

Rathi, S., Mittal, N., & Kumar, D. (2022). Photosynthetic response of plants against heavy metals. In *Heavy metals in plants* (pp. 149-162). CRC Press.

Salavati, J., Fallah, H., Niknejad, Y., & Barari Tari, D. (2021). Methyl jasmonate ameliorates lead toxicity in *Oryza sativa* by modulating chlorophyll metabolism, antioxidative capacity and metal translocation. *Physiology and Molecular Biology of Plants, 27*(5), 1089-1104.

Santos, A. F., Alvarenga, P., Gando-Ferreira, L. M., & Quina, M. J. (2023). Urban wastewater as a source of reclaimed water for irrigation: Barriers and future possibilities. *Environments, 10*(2), 17.

Shah, T., Irshad, M. K., Javed, W., Sheraz, M., Muhmood, A., Noman, A., Alomran, M. M., Lee, S. S., & Shang, J. (2024). Potential of iron oxide-modified biochar in simultaneous mitigation of lead and nickel toxicity in wheat (*Triticum aestivum* L.). *South African Journal of Botany, 175,* 32-43.

Shen, X., Dai, M., Yang, J., Sun, L., Tan, X., Peng, C., Ali, I., & Naz, I. (2022). A critical review on the phytoremediation of heavy metals from environment: Performance and challenges. *Chemosphere, 291,* 132979.

Siedlecka, A. (1996). Interaction between cadmium and iron and its effects on photosynthetic capacity of primary leaves of *Phaseolus vulgaris*. *Physiologia Plantarum, 97*(2), 335-340.

Singh, A., Sharma, R. K., Agrawal, M., & Marshall, F. M. (2010). Health risk assessment of heavy metals via dietary intake of foodstuffs from the wastewater irrigated site of a dry tropical area of India. *Food and Chemical Toxicology, 48*(2), 611-619.

Singh, I. (2022). Impacts of heavy metals on aquatic life, soil, plants, and human health. Thanuj International Publishers.

Singh, R., Kumar, V., Tewari, R. K., Pratap, S. G., & Singh, P. K. (2023). Hazardous waste leachates induced changes in plant water relations, photosynthetic pigments, heavy metal accumulation and yield of mustard (*Brassica juncea* L.). *Journal of Hazardous Materials Advances, 10,* 100306.

Slavin, J. L., & Lloyd, B. (2012). Health benefits of fruits and vegetables. *Advances in Nutrition, 3*(4), 506-516.

Srivastava, H., Saini, P., Singh, A., & Yadav, S. (2024). Heavy metal pollution and biosorption. In P. Saini (Ed.), *Biosorption processes for heavy metal removal* (pp. 1-38). IGI Global.

Steel, R. G., Torrie, J. H., & Dickey, D. A. (1997). *Principles and procedures of statistics: A biometrical approach* (3rd ed.). McGraw-Hill.

Subbiah, B., & Asija, G. (1956). A rapid procedure for the estimation of available nitrogen in soils. *Current Science, 25*(8), 259-260.

Vasilachi, I. C., Stoleru, V., & Gavrilescu, M. (2023). Analysis of heavy metal impacts on cereal crop growth and development in contaminated soils. *Agriculture, 13*(10), 1983.

Vitória, A. P., Lea, P. J., & Azevedo, R. A. (2001). Antioxidant enzyme responses to cadmium in radish tissues. *Phytochemistry, 57*(5), 701-710.

Wang, X., Cai, D., Ji, M., Chen, Z., Yao, L., & Han, H. (2022). Isolation of heavy metal-immobilizing and plant growth-promoting bacteria and their potential in reducing Cd and Pb uptake in water spinach. *Science of the Total Environment, 819,* 153242.

WHO. (1989). *Evaluation of certain food additives and contaminants* (Report No. 776). World Health Organization.

WHO. (1996). *Permissible limits of heavy metals in soil and plants*. World Health Organization.

Younas, H., & Younas, F. (2022). Wastewater application in agriculture: A review. *Water, Air, & Soil Pollution, 233*(8), 329.

Zengin, F. K., & Munzuroglu, O. (2006). Toxic effects of cadmium (Cd++) on metabolism of sunflower (Helianthus annuus L.) seedlings. *Acta Agriculturae Scandinavica, Section B-Plant Soil Science, 56*(3), 224-229.

Zheng, X., Zou, D., Wu, Q., Wang, H., Li, S., Liu, F., & Xiao, Z. (2022). Review on fate and bioavailability of heavy metals during anaerobic digestion and composting of animal manure. *Waste Management, 150,* 75-89.