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Phytoaccumulation of heavy metals (Pb and Cr) by Spinach (*Spinacia oleracea* L.) grown in different sewage irrigated soils

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ABSTRACT

The purpose of current study was to examine the phytoaccumulation capacity of Spinacia oleracea L. grown in various sewage-irrigated locations in Prayagraj, Uttar Pradesh. Three sewage-irrigated locations viz. Buxibandh, Preetam Nagar, and Mehdauri were used to collected soil and plant sample. It has been showed that Spinacia oleracea L. can accumulate a range of Pb and Cr values, from 4.36±0.35-2.09±0.20 and 3.67±0.25-2.04±0.17 mg kg^{-1} in roots to 4.32±0.38-2.05±0.15 and 3.71±0.30-2.01±0.12 mg kg^{-1} in shoots. The findings indicated that highest accumulation of heavy metals in Buxibandh in order to Pb followed by Cr, while minimum accumulation in Mehdauri. The maximum dry biomass yield of root and shoot were detected in Mehdauri, polluted with Pb and Cr ranged from 1.10±0.05-1.30±0.15 and 3.05±0.20-3.25±0.30 g plant^{-1}, While the minimum dry biomass yield was observed 0.95±0.05-1.05±0.10 and 2.80±0.15-2.85±0.18 g plant^{-1}, respectively in Buxibandh. Furthermore, their BCF and TF value of heavy metals (Pb and Cr) >1.0 demonstrated the hyper accumulation capacity of Spinacia oleracea L. which ranged from 1.040±0.10-0.950±0.07 and 1.055±0.15-1.030±0.09, 1.070±0.11-0.985±0.04 and 1.065±0.12-0.990±0.05 mg kg^{-1}, respectively. Value of EF for Pb & Cr was found 2.25±0.15 and 2.10±0.05 mg kg^{-1} in Buxibandh which denotes medium or moderate contamination while 1.05±0.02 and 0.95±0.04 mg kg^{-1} in Mehdauri which indicates the naturally weathering process increased metal concentrations in soil. Therefore, these findings highlight the need for assessing agricultural practices in sewage-irrigated soils for preventing contamination of heavy metals in food chain and safety for human health.

Keywords: Accumulation, Bioconcentration, Enrichment factor, Sewage irrigated soil, *Spinacia oleracea* L.

INTRODUCTION

The waste-water use for crop production has been increased now a day due to scarcity of water for irrigation purpose (Bougnom *et al.,* 2020, Zhang and Shen 2019). Wastewater is originated from many sources like industries, municipalities and agriculture, rich in organic matters and nutrients which takes the place of freshwater for crop irrigation (Michael-Kordatou *et al.,* 2015). As a result, presence of pollutants in the wastewater gets accumulated in the agriculture soil by irrigation. The accumulation of pollutants in soil includes heavy metals, pesticides, pathogens, etc. which are also translocated in plants by nutrient uptake (Courault *et al.,* 2017; Kumar *et al.,* 2019a; Sarwar *et al.,* 2019). Utilization of polluted agriculture commodity may cause various disease in animals and human beings. Heavy metals cause severe disease and abnormalities in human like Injury to brain, vomiting anorexia, anemia, disease related to circulatory system and neural disorder by Pb (Mahmoud *et al.,* 2019) and Diarrhea, headache, nausea, vomiting problem, carcinogen, lungs tumour etc. by Cr (Xu *et al.,* 2019). Sewage water disposal also changes the physico-chemical characteristics of soil and increase the availability of pollutants in soil and plants (Ali *et al.,* 2020). Sewage water has been the main environmental issue causing agricultural lands to become contaminated in the majority of developing nations. Enrichment factor (EF) is the measurement of how much anthropogenic activity has affected to soil for heavy metals contamination and indicate the normalized enrichment factor for concentration of heavy metals more than previously levels of contamination (Dickinson *et al.,* 1996). A value of enrichment factor is between ≤0.5 and 1.5, it indicates that the natural weathering activates increased the metal concentration in soil (Zhang

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and Liu 2002). When the EF value is nearly equal to one, it shows that the contaminants came from soil (Chiarenzelli *et al.,* 2001). If the trace metals are transported from non-crustal materials, the EF value is 1.5 (Sutherland, 2000). The man-made contamination is implies using a five category of rating system viz. low contamination is showed by EF<2, moderate contamination by EF=2-5, high contamination by EF=5-20, EF=20-40 value indicated very high contamination, and EF>40 value indicated extremely high contamination (Sutherland *et al.,* 2000).

Consequently, dietary leafy vegetable crops are susceptible to heavy metals contamination. As a result, research on soil-plant interactions based on soil characteristics and irrigation water is required. Since vegetables are high in proteins, fibre, vitamins, and minerals, they play a significant role in human nutrition (Kumar *et al.,* 2019b; Yahia *et al.,* 2019). Due to continuous use of contaminated vegetable and other related products it caused numerous health issues in human which is a major globally public concern (Kumar *et al.,* 2019a, b).

Phytoremediation is a method for using plant to accumulate, extract or neutralize heavy metal pollutants from soil and water (Gerhardt K. E. *et al.,* 2017). In phytoremediation such plant species are used which have rapid growth, high crop canopy, short life cycle, and also must be grown in various soil and climatic condition (Ranjav V. *et al.,* 2015). Plants like Spinach, Amaranth, and Lettuce are capable to accumulate high concentration of heavy metals in their root & shoot which named hyper accumulator (Ng C. C. and Boyce A. N. 2016, Sierra, B. E. G. *et al.,* 2021). Spinach is a cool season leafy vegetable crop belongs to Amaranthaceae family and grown in winter to early spring, and it is a great potential to accumulate heavy metals from soil in their root and shoot (Subhash *et al.,* 2010). In heavy metals contaminated soil, the bioconcentration factor (BCF) and translocation factor (TF) can be used to determine a plant's capacity for phytoremediation. Plants with BCF and TF values >1 regarded as hyperaccumulators. Heavy metals uptake from polluted soils has a good potential to hyperaccumulator plants.

In this study use of sewage water for irrigation purpose which increase a major concern to human health. By using these waste water in vegetable crop, they increase certain harmful heavy metals into leafy vegetable like Spinach, Amaranth, and Lettuce. Thus, the purpose of this study (1) to evaluate how sewage water affects the physico-chemical characteristics of soil under Spinach cultivation, (2) to investigate the heavy metal accumulation in sewage irrigated soils by Spinach, and (3) to evaluate the enrichment factor of various sewage irrigated soils.

MATERIALS AND METHODS

The research was conducted at different sites of Prayagraj, UP viz. Buxibandh, Preetam Nagar, and Mehdauri which is mainly belong to sub-humid regions of Indo-Gangetic plains and are elevated above 90cm of sea level. The Ganga plains containing recently developed alluvial soils which is belongs to Entisols soil order. These locations are situated between latitudes 25.39º-25.45ºN and longitudes 81.78º-81.85ºE. The average annual rainfall of Prayagraj has 850-934mm and relative humidity is (average 67%) around 42% to 87% in summer and monsoon season, respectively. In all three sites Buxibandh, Preetam Nagar, and Mehdauri various agricultural farms are situated which is irrigated with sewage water for many years more than 35 years. Above mentioned locations are mainly selected for research because continuous application of waste water for irrigation which increase heavy metals (Pb & Cr) in soils.

Representative soil samples were collected at random from different locations of sewage-irrigated by using soil auger leaving margins of 2m area at 0-20cm depth at each experimental unit. After that collected soil samples were dried in shady conditions and finally sieved with the help of 2mm sieves. One gram soil sample was taken for preparing soil extract, added 5 ml $HClO_4$ and 5 ml HNO_3 after that mixed whole content properly and heated the mixture up to dryness, added 50 ml hot distilled water (Kumar and Mani, 2010). The sample was filtered by Whatman paper No. 42. The prepared soil extract was analysed using AAS at MNIT, Prayagraj to estimation the total concentration of Pb and Cr. The plant samples *Spinacia oleracea* L. was collected from various sewage sites after 50 to 60 days of sowing because high dry biomass yield and maximum accumulation of heavy metals is found in mostly hyper accumulator plants in this growth period and this growth stage represent higher amount of phytoremediation potential of leafy vegetable similar findings also suggested by (Mani *et al.,* 2012). Separate impurities and then washing by tap water from collected plant samples after that 2% $CaCl_2$ solution was used for washing and finally dipped 2-4 times in distilled water. Plant parts were separated into root and shoot. The separated parts were shade drying for removing the moisture after that keep at 60ºC in hot air oven (1-2 hours) for weight constant, ground the sample like flour followed by passed through 2mm sieve and finally keep in jar at room temperature. Tri-acid mixture was prepared for plant extract in ratio 5:1:2 in which 1g ground plant material was digest with concentrate HNO_3, H_2SO_4 and $HClO_4$ in 100ml beaker, properly mixed the whole content by glass rod after that the mixture was heated on a hot plate at 60ºC for 30 minutes till the volume was reduced about 5ml in a dryness solution after cooling the solution added 20ml hot distilled water and then sample was filtered by using filter Paper No. 42 (Kumar and Mani, 2010). Prepared plant extract was analysed for total Pb and Cr by AAS at MNIT, Prayagraj.

The enrichment factor is calculated by mean metal content in the polluted sample divided by mean metal content in the unpolluted sample (Loska *et al.,* 1997).

$$EF = \frac{M_{polluted\ soil}}{M_{unpolluted\ soil}}$$

The bioconcentration factor is assessed by a previously established method based on the in-situ phytoremediation potential of native hyperaccumulator plants (Yoon *et al.,* 2006). The bioconcentration factor is calculated using the following equation.

$$BCF = \frac{M_{root}}{M_{soil}}$$

Where, M_{root} represents the amounts of heavy metals (mg kg^{-1} dry wt.) in roots and M_{soil} represents the total heavy metals concentration in soil. Translocation factor (TF) refers to capacity of plants to heavy metals translocation from root to shoot, following equation is used for representing translocation factor (Cui *et al.,* 2007).

$$TF = \frac{M_{shoot}}{M_{root}}$$

Where, M_{shoot} refers to metal content in shoots (mg kg^{-1} dry wt.), M_{root} refers to metal content in root (mg kg^{-1}).

Statistical analysis

All statistical analysis were performed by ICAR GOA WASP-1.0 India. The mean ±SD was applied to all represented data. The mean heavy metal (Pb and Cr) concentration in shoot and root was quantified by ANOVA; replicates are three and the level of significance various it at $P<0.05$. The Graph Pad Prism 8.0.1 (version 2.0, USA) was used for graphical work.

RESULTS AND DISCSSION

Physico-chemical properties of soil irrigated with sewage water

In present study, the textural proportion of sand, silt, and clay were represented which varied from 65.30±1.10-67.45±1.30, 16.25±1.60-20.50±1.40 and 13.10±1.10-16.30±1.20 % respectively. The pH value of all three sites varied from 7.45±0.25-7.85±0.15. The cation exchange capacity and electrical conductivity value ranged from 25.30±1.40-28.15±1.80 cmol (p^+) kg^{-1} and 0.30±0.02-0.35±0.04 ds m^{-1} respectively. Buxubandh had the highest concentration of Organic carbon (0.52±0.06%) and minimum at Mehdauri (0.49±0.05%) due to continuous and long-term use of sewage water increased organic matter in soil because sewage water contains high amount of organic waste approximately similar results associated with (Esteban *et al.,* 2000). The amount of total nitrogen was found maximum in Buxibandh (0.18±0.03 %) and minimum at Preetam Nagar (0.15±0.04 %). The available nitrogen was found maximum in Buxibandh (178.40±2.50 Kg ha^{-1}) and minimum at Preetam Nagar (175.50±2.30 Kg ha^{-1}). Mehdauri site has maximum available P_2O_5 (23.40±1.30 Kg ha^{-1}) while Preetam Nagar has minimum (22.80±1.50 Kg ha^{-1}). The amount of available K_2O has maximum in Mehdauri (618.80±3.50 Kg ha^{-1}) and minimum at (610.90±2.70 Kg ha^{-1}) Preetam Nagar. The maximum amount of Pb was found in Buxibandh (4.12±0.10 mg kg^{-1}) and minimum at Mehdauri (2.65±0.05 mg kg^{-1}). The Pb availability was found highest as compared to Cr in all three sites also similar result found by (Kumar *et al.,* 2024). The high residential and industrial area

Table 1: Physico-chemical properties of various Prayagraj sewage-irrigated soils

Soil Parameters	Sewage sites			
	Units	Buxibandh	Preetam Nagar	Mehdauri
Sand	(%)	65.30±1.50	66.50±1.10	67.45±1.30
Silt	(%)	20.50±1.40	20.40±1.50	16.25±1.60
Clay	(%)	14.20±1.25	13.10±1.10	16.30±1.20
pH	-	7.45±0.25	7.35±0.20	7.85±0.15
Electrical Conductivity	(dSm^{-1} at 25˚C)	0.35±0.04	0.30±0.02	0.33±0.06
Organic Carbon	(%)	0.52±0.06	0.50±0.04	0.49±0.05
Cation Exchange Capacity	cmol (p^+) kg^{-1}	26.30±1.20	25.30±1.40	28.15±1.80
Total Nitrogen	(%)	0.18±0.03	0.15±0.04	0.16±0.02
Available Nitrogen	(Kg ha^{-1})	178.40±2.50	175.50±2.30	176.80±2.90
Total P_2O_5	(%)	0.24±0.03	0.22±0.02	0.21±0.05
Available P_2O_5	(Kg ha^{-1})	23.15±1.15	22.80±1.50	23.40±1.30
Available K_2O	(Kg ha^{-1})	614.20±2.20	610.90±2.70	618.80±3.50
Total Lead	(mg kg^{-1})	4.12±0.10	3.05±0.15	2.65±0.05
Total Chromium	(mg kg^{-1})	3.05±0.08	2.75±0.05	1.87±0.09

near Buxibandh it was showed the maximum amounts of heavy metals in sewage water which increased the metal content (Pb and Cr) in this site. Whereas the Mehdauri site showed less contamination with heavy metals due to away from residential and industrial area as compare to remaining two sites.

Enrichment Factor (EF) of soil

The enrichment factor of soil mainly depends on the type of effluents their concentrations and the types of soil. The EF value of Pb (2.25±0.15 mg kg^{-1}) indicated moderate enrichment while value of Cr (2.10±0.05 mg kg^{-1}) indicating minimal enrichment of the soil (Gupta *et al.,* 2008). In all three sewage irrigated sites high enrichment value of heavy metals (Pb and Cr) 2.25±0.15 mg kg^{-1} and 2.10±0.05 mg kg^{-1} was found in Buxibandh, 2.15±0.10 mg kg^{-1} and 2.05±0.04 mg kg^{-1} in Preetam Nagar and minimum EF value of Pb (1.05±0.02 mg kg^{-1}) and Cr (0.95±0.04 mg kg^{-1}) was found in Mehdauri site (Fig.1). Buxibandh and Preetam Nagar site showed moderate enrichment of metals in soil while Mehdauri site showed minimal enrichment value.

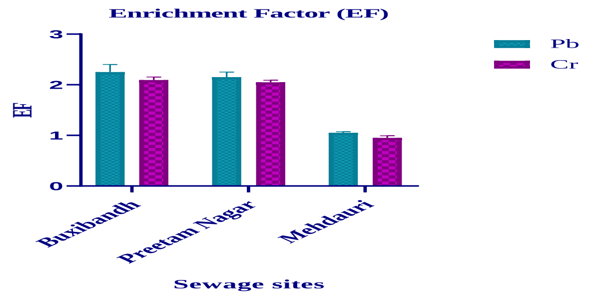

Figure 1: Enrichment factor for sewage irrigated soils

Effect of Pb and Cr on *Spinacia oleracea* L. growth and dry biomass

Since, the sewage water composed higher amount of nutrients and organic matter it increases the normal growth and biomass of Spinach plant but the noxious effect was showed at representative sites on Spinach growth due to heavy metal stress. The growth of Spinach, root and shoot was decreased by the increased levels of Pb and Cr. The Mehdauri site had the maximum root and shoot length which ranged from 3.75±0.25, 9.25±0.47 and 4.25±0.35, 10.27±0.55 cm for Pb and Cr respectively, whereas minimum root and shoot length were observed from Buxibandh site ranged from 3.05±0.10, 7.25±0.40 and 3.50±0.25, 8.50±0.45cm respectively (Fig. 2a) due to this site has highest amount of Pb and Cr causes heavy metal stress on spinach plant. The maximum Spinach's root and shoot dry biomass yield was recorded at Pb and Cr-polluted Mehdauri sewage site which ranged from 1.10±0.05, 3.05±0.20 and 1.30±0.15, 3.05±0.30g respectively. Whereas, minimum dry biomass yield of root and shoot were observed in Buxibandh site ranged from 0.95±0.05, 2.80±0.15 and 1.05±0.10, 2.85±0.18g respectively (Fig.2a, b). Kumar *et al.,* (2016) reported that when concentration of sewage sludge increases it decreased the growth of *Spinacia oleracea* L. This is because the high concentration of heavy metals in sewage sludge which decrease the vegetative growth, plant height and dry biomass of *Spinacia oleracea* L. plant. Approximately similar result was also supported by Yadav *et al.,* (2002) for growth of sorghum, wheat, rice and Egyptian clover were reduced by application of high amount of sewage waste.

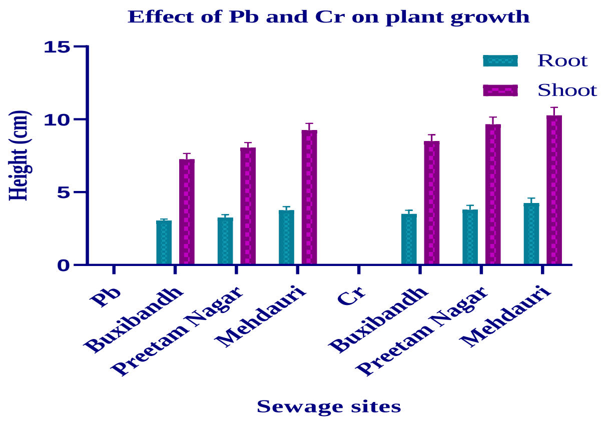

Figure 2a: Plant Height Grown in Pb and Cr Polluted Soils

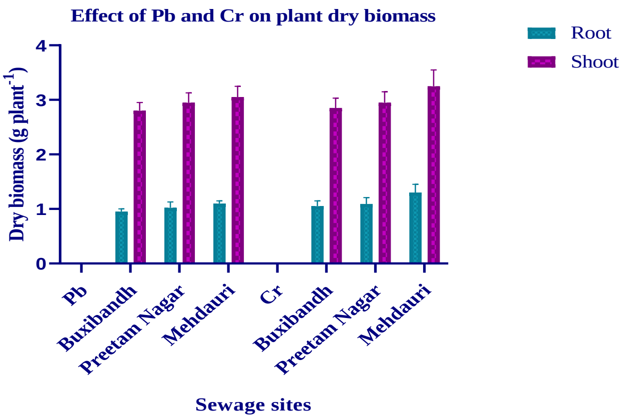

Figure 2b: Dry biomass yield of plants grown in Pb and Cr polluted soils

Phytoaccumulation of Pb and Cr by *Spinacia oleracea* L.

Figure (3) showed the phytoaccumulation pattern of Pb and Cr in *Spinacia oleracea* L. (root and shoot) which grown in various sewage sites. The plant sample were collected from different sewage site after 60 days of sowing for heavy metal analysis. The order of effective accumulation of heavy metal by *Spinacia oleracea* L. root was Pb>Cr. The maximum accumulation of Pb and Cr was found in the Spinach roots than shoots. The accumulation of Pb and Cr in root ranged from 4.36±0.35-2.09±0.20 and 3.67±0.25-2.04±0.17 and in shoot 4.32±0.38-2.05±0.15 and 3.71±0.30-2.01±0.12 mg kg^{-1} respectively.

The findings showed that *Spinacia oleracea* L. potentially accumulates the higher amount of Pb in concentration of (4.32±0.14 mg kg^{-1}) than Cr (3.71±0.12 mg kg^{-1}) in shoot, while, in root 4.36±0.18 and 3.67±0.21 mg kg^{-1} from Buxibandh sewage soil respectively (Fig.3). Mishra and Tripathi (2008) have also reported *Spinacia oleracea* L. accumulated higher amount of Pb and Cr in sewage irrigated area. Following result showed that Buxibandh sewage site had significantly higher levels of heavy metal pollution than another site. Descending order of Pb and Cr phytoaccumulation was found in all three sites like Buxibandh>Preetam Nagar>Mehduari.

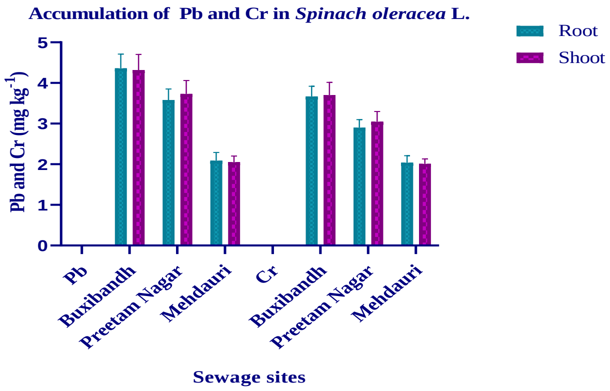

Figure 3: Accumulation of Pb and Cr in *Spinacia oleracea* L.

Enrichment, Bioconcentration and Translocation factor of Pb and Cr

The Enrichment factor value (>2) was found in Buxibandh site which ranged from 2.25±0.15 to 2.10±0.05 mg kg^{-1} indicated moderate enrichment of heavy metals in soil, while minimum enrichment value (<2) was found in Mehdauri site which ranged from 1.05±0.02 and 0.95±0.03 mg kg^{-1} indicated naturally weathering process increased heavy metals in soil (Table.2). Approximately similar results were found by Gupta *et al.,* (2008). The bioconcentration factor is the ratio of metals between soil and plants which is very important measure for crop selection in heavy metal contaminated soil (Bose *et al.,* 2008). The BCF and TF value >1 of Pb and Cr (Table 2 and Fig. 4) was showed the better phytoremediation capacity of Spinach which grown in various sewage soil. The BCF a value was found in Pb contaminated soil ranged from 1.040±0.10-0.950±0.07 mg kg^{-1} and for Cr 1.070±0.11-0.985±0.04 mg kg^{-1} in *Spinacia oleracea* L.

grown in all three sewage sites (Fig 4). The translocation factor value of Pb was ranged from 1.055±0.15-1.030±0.09 mg kg^{-1} and Cr ranged from 1.065±0.12-0.990±0.05 mg kg^{-1} in Spinach grown in sewage soil. The results showed the BCF and TF value >1 which justified the spinach is a good hyper accumulator plant for metal uptake.

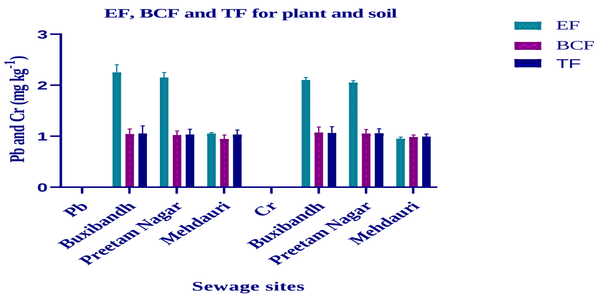

Figure 4: Enrichment, Bioaccumulation and Translocation Factor of Pb and Cr polluted soil

Table 2: Determination of EF, BCF and TF value for in different sewage irrigated soils

Sewage Sites	Pb (mg kg^{-1})			Cr (mg kg^{-1})		
	EF	BCF	TF	EF	BCF	TF
Buxibandh	2.25±0.15	1.040±0.10	1.055±0.15	2.10±0.05	1.070±0.11	1.065±0.12
Preetam Nagar	2.15±0.10	1.020±0.08	1.035±0.10	2.05±0.04	1.050±0.08	1.057±0.09
Mehdauri	1.05±0.02	0.950±0.07	1.030±0.09	0.95±0.03	0.985±0.04	0.990±0.05

CONCLUSION

The research was concluded that continuous application of sewage water for irrigation purpose increased the nutrient status besides this they pose the hazard of toxic metals like Pb and Cr in soil and plants, which in turn was responsible for uptake of Pb and Cr in *Spinacia oleracea* L. tissue. Thus, the practice of applying sewage water for irrigation may accumulate metals in vegetable crops which posing a possible risk to human health. Increasing levels of heavy metals hampered the normal growth and life cycle of plants and caused numerous physiological abnormalities. Regular monitoring is essential for buildup of excessive amounts of these metals in soil and food chain. This research is mainly focused on phytoremediation potential of *Spinacia oleracea* L. Therefore, Further investigation into the physiological processes behind the phytoremediation of heavy metal-polluted ecosystems is required to fully comprehend these facets, and also proper management and restoration of soil and water are required to prevent excessive amounts of heavy metals accumulation in plants and soil.

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