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Phytoremediation potential of *Brassica juncea* L. grown in different Cd and Pb polluted sewage irrigated soils

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ABSTRACT

The current research work is mainly focused on the evaluation of the phytoremediation potential of Brassica juncea L. grown in different sewage irrigated sites in Prayagraj, Uttar Pradesh. The representative soil and plant samples were collected from different sewage irrigated sites in Prayagraj viz., Naini, Preetam Nagar, Daraganj and Rajapur. The heavy metals analyzed plant samples were showed the various concentrations of Cd and Pb ranged from 3.51±0.16-1.97±0.09,3.69±0.25-2.25±0.12 and 4.78±0.20-3.11±0.17, 5.13±0.23-3.06±0.21 mg kg^{-1} in the roots and shoots respectively. The found results were showed that the maximum accumulation of Pb 5.13 followed by Cd 3.69 mg kg^{-1} was recorded in the plant shoots at Naini sewage irrigated site. Maximum roots, shoots dry biomass yield was found at Rajapur sewage irrigated site polluted with Cd and Pb ranged from 3.10±0.07, 12.10±0.51 and 3.77±0.11, 12.81±0.32 g plant^{-1}, respectively. Whereas minimum roots, shoots dry biomass yield i.e., 1.56±0.03, 10.05±0.32 and 2.09±0.04, 10.48±0.27 g plant^{-1} was observed from Cd and Pb polluted soils at Naini sewage irrigated site, respectively. The values of BAF and TF of all Cd polluted soil ranged from 1.063±0.08-1.300±0.06 and 1.051±0.10-1.142±0.04 mg kg^{-1}, respectively. Whereas the values of BAF and TF of all Pb polluted soils ranged from 1.075±0.11-0.987±0.03 and 1.073±0.13-0.983±0.07 mg kg^{-1}, respectively. Almost all the values of BAF and TF >1 that proved Brassica juncea L. was a hyperaccumulator plant. Thus, Brassica juncea L. has greater importance in phytoremediation of heavy metal contaminated soils. This research will utilize as phytoremediation of heavy metals contaminated soils and detoxify these natural resources because of it is a cost-effective and green technique which is our future demand to avoid chemical measures.

Keywords: Bioaccumulation, *Brassica juncea* L., Heavy metal, Phytoremediation, Sewage irrigated soils, Translocation.

INTRODUCTION

Water scarcity is a major problem of the overall world caused by rapid population growth, food challenges in the agricultural sector, climate change and unavailability of fresh groundwater. The world's largest amount of freshwater is consumed by the agricultural sector which accounts 75 and 90 % of total water utilized in the developed as well as in developing countries respectively (Bougnom *et al.* 2020). To overcome such serious issue in urban areas for cultivation practices the efficient source of irrigation is sewage water. Such water originates directly from the various sources such as municipalities, industries and agriculture and is organic matter (O.M.) rich which replaces freshwater for the irrigation purpose to raising crops (Michael-Kordatou *et al.* 2015). But in other hand long-term application of wastewater to the soils pose a risk of contamination with heavy metals in higher concentration by accumulation process and enters in the food chain at different trophic levels.(Kumar *et al.* 2007; Nawaz *et al.* 2021; Singh *et al.* 2022; Tariq *et al.* 2023; Ullah *et al.* 2024; Yadav *et al.* 2023). A severe disease caused by Cd toxicity viz., 'itai-itai' in Toyama city of Japan attain health risk of heavy metals at first time (Yang *et al.* 2023). Better understanding of the heavy metals distribution as well as the sources of its contamination in the soils is critical for scientific and effectively prevention and control of pollution related to soil heavy metals (Wu *et al.* 2022).

Phytoremediation is a well-known cheapest and eco-friendly technique consisting of plants having accumulation capability to remove metals contamination from the affected soil (Madhav *et al.* 2024; Sharma *et al.* 2023; Ullah *et al.* 2 021; Wu *et al.* 2018; Yang *et al.*

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2018). Now phytoremediation is a well-known technique for decontamination of heavy metal polluted soils because it utilized various efficient mechanisms. Phytoextraction is one of the important mechanism in which uptake of toxic metals and other toxicants by plant roots from the soil or water and thereafter they transfer it into above-ground or harvestable parts like shoots where the process of accumulation takes place (Yanitch *et al.* 2020). *Brassica juncea* L. generally known as Indian Mustard is most important oilseed crop belonging to the rapeseed-mustard group. It also consumed as vegetable at early leaf stage and as condiment in pickle making. *Brassica juncea* L. considered as a good hyperaccumulator plant and grow well in contaminated soils due to its strongly resistant nature against heavy metals, strong capacity of accumulation, rapid growth pattern and high production of biomass. the plants which have capacity to accumulate the trace elements 2-3 times more than normal are falls under the category of hyperaccumulators (Cui *et al.* 2023).

The bioaccumulation factor (BAF) and translocation factor (TF) indicates the phytoremediation efficiency of certain plants which can withstand very well in heavy metals contaminated soils. The plants having the values of BAF and TF >1 is considered as hyperaccumulator. Hyperaccumulator means the plants have a good capacity to accumulate heavy metals from the contaminated soils. The significant objectives of the present research work were (i) to study the impacts of sewage irrigation on the physico-chemical characteristics of soils. (ii)to study the phytoremediation potential of *Brassica juncea* L. grown in sewage irrigated soils.

MATERIALS AND METHODS

The geographical location of all sites is between 25º37'-25º46' N latitudes and 81º78'-81º88' E longitudes and mainly belong to the sub-humid tropical region of Ganga plains at Prayagraj, U.P. India. The plains of Ganga containing some of recently originated alluvial soils belongs to the Entisols soil order. All the four sites viz., Preetam Nagar, Rajapur, Daraganj and Naini are situated in various agricultural farms irrigated with sewage water for a long time (>35 years). These sites are highly polluted with heavy metals (Cd and Pb) because of continuous irrigated with sewage water which is main reason of selecting these sites. The collection of representative soil samples was done randomly at respected place by using soil auger from different sewage irrigated sites and leaving margins of 2 m area around each experimental unit. The depth of sampling is 0-30 cm. Thereafter, collected samples dried under shady condition and finally obtaining content was sieved with the help of 2 mm sieve.

To prepare soil extract 1 gram of soil was taken from collected sample and added 5 ml HNO_3, 5 ml $HClO_4$ and mixed whole content properly after that mixture was heated till dryness then added hot distilled water (Kumar and Mani, 2010). Filtration of content was done by using standard filter paper i.e., Whatman (F.P. No. 42) and finally maintain the volume up to 50 ml by addition of distilled water. Prepared samples of soil extract were analyzed by using an AAS (Atomic Absorption Spectrophotometer) to estimate total Cd and Pb contents at Banaras Hindu University (BHU), Varanasi. The plants of Indian Mustard (*Brassica juncea* L.) were collected from sewage irrigated soils of different locations after 60 days of germination because maximum accumulation of heavy metals was found at this particular duration in any hyper-accumulator plants and this maturity stage represents higher quantity of phytochemicals reported by (Mani *et al.* 2012). To remove impurities, washing of collected samples was done by using tap water followed by 2 % calcium chloride ($CaCl_2$) solution and at final dipped with distilled water 2-4 times. Separated the whole plants into different parts i.e., roots and shoots. The moisture of separated plant parts was removed by shade drying followed by hot air oven drying at 60ºC to constant the weight, ground like flour, passed through 2 mm sieve and finally stored at room temperature in jars made up of glasses.

To prepare plant extract, 1 gram of ground material of plant was taken in 100 ml beaker and added HNO_3, H_2SO_4 and $HClO_4$ in ratio of 5:1:2 and mixed whole content properly after that mixture was heated till dryness then added hot distilled water (Kumar and Mani, 2010). Filtration of content was done by using standard filter paper i.e., Whatman (F.P. No. 42) and finally maintain the volume up to 50 ml by addition of distilled water. Prepared samples of plant extract were analyzed by using an AAS to estimate total Cd and Pb content at Banaras Hindu University (BHU), Varanasi.

Bioaccumulation factor (BAF) is the ratio of metal concentration in plant tissues (shoot and root) to the soils, which is used for measure plant effectiveness in concentrating contaminant into above-ground parts (Fayiga *et al.* 2004). The following formula is used to calculate the bioaccumulation factor.

$$BAF = \frac{M_{shoots}}{M_{soils}}$$

Where, M_{shoots} is the Content of metal in shoots (mg kg^{-1} dry weight), M_{soils} is the content of metal in soils (mg kg^{-1}).

Translocation factor (TF) refers plants capacity to translocate concentrations of heavy metal from the roots to above-ground parts, following formula is used to calculate the translocation factor (Cui *et al.* 2007).

$$TF = \frac{M_{shoots}}{M_{roots}}$$

Where, M_{shoots} is the content of metal in shoots (mg kg^{-1} dry weight), M_{roots} is the content of metal in roots (mg kg^{-1}).

Statistical analysis

All used data were represented as mean±SD. The concentrations of heavy metals (Cd and Pb) were expressed in the form of mean, replicates are thrice in number. The graphical work was carried out using Graph Pad Prism.

RESULTS AND DISCUSSION

Physico-chemical Characteristics of Different Sewage Irrigated Soils

Different samples of sewage irrigated soils were analyzed for physico-chemical characteristics and showed in Table 1.

Table 3: Physico-chemical properties and concentrations of heavy metals in form of Mean±SD in different sewage irrigated soils of Prayagraj (Uttar Pradesh), India

Soil Parameters	Sewage irrigated sites				
	Units	Naini	Preetam Nagar	Daraganj	Rajapur
Sand	(%)	68.32±2.04	66.28±1.92	65.85±2.09	64.30±2.03
Silt	(%)	17.24±1.13	18.03±1.09	21.35±1.12	20.09±1.42
Clay	(%)	14.44±1.04	15.69±1.03	12.80±1.02	15.61±1.19
pH	_	7.8±0.62	7.4±0.56	7.5±0.61	8.1±0.52
EC	(ds m^{-1} at 25ºC)	0.39±0.04	0.34±0.03	0.24±0.02	0.27±0.02
OC	(%)	0.69±0.04	0.51±0.04	0.53±0.03	0.57±0.03
CEC	Cmol (p+) kg^{-1}	27.06±1.16	25.34±1.16	23.39±1.05	22.53±1.02
Total Nitrogen	(%)	0.18±0.02	0.16±0.02	0.15±0.03	0.14±0.01
Available Nitrogen	(Kg ha^{-1})	179.23±3.09	177.39±2.93	175.42±2.72	172.03±2.68
Available P_2O_5	(Kg ha^{-1})	41.67±1.08	37.16±1.12	36.88±1.20	33.31±1.14
Available K_2O	(Kg ha^{-1})	510.63±3.28	484.59±2.92	465.28±2.76	453.47±3.41
Total Cd	(mg kg^{-1})	3.47±0.21	2.83±0.17	2.49±0.23	1.73±0.15
Total Pb	(mg kg^{-1})	4.77±0.24	4.13±0.19	3.39±0.20	2.96±0.13

Organic carbon (OC), Electrical conductivity (EC), Cation exchange capacity (CEC), Cadmium (Cd), Lead (Pb)

Textural parameter represented the amounts of sand, silt and clay ranged from 64.30±2.03-68.32±2.04, 17.24±1.13-21.35±1.12 and 12.80±1.02-15.61±1.19% respectively. Whereas pH of all four sites ranged from 7.4±0.56-8.1±0.52. The organic carbon, electrical conductivity and cation exchange capacity values ranged from 0.51±0.04-0.69±0.04%, 0.24±0.02-0.39±0.04 ds/m and 22.53±1.02-27.06±1.16 cmol (P^+) kg^{-1}, respectively. Same pattern the amount of total N (nitrogen), available N (nitrogen), P_2O_5 (phosphorus) and K_2O (potassium) ranged from 0.14±0.01-0.18±0.02%, 172.03±2.68-179±3.09, 33.31±1.14-41.67±1.08 and 453.47±3.41-510±3.28 kg ha^{-1}, respectively. The maximum and minimum concentration of Cd was found at Naini and Rajapur sewage irrigated sites i.e., 3.47±0.21 and 1.73±0.15 mg kg^{-1}, respectively whereas the maximum and minimum concentration of Pb was found at Naini and Rajapur sewage irrigated sites i.e., 4.77 ± 0.24 and 2.96±0.13 mg kg^{-1}, respectively. It means the availability of Pb was more in all representative sites as compared to Cd, approximately similar results associated with

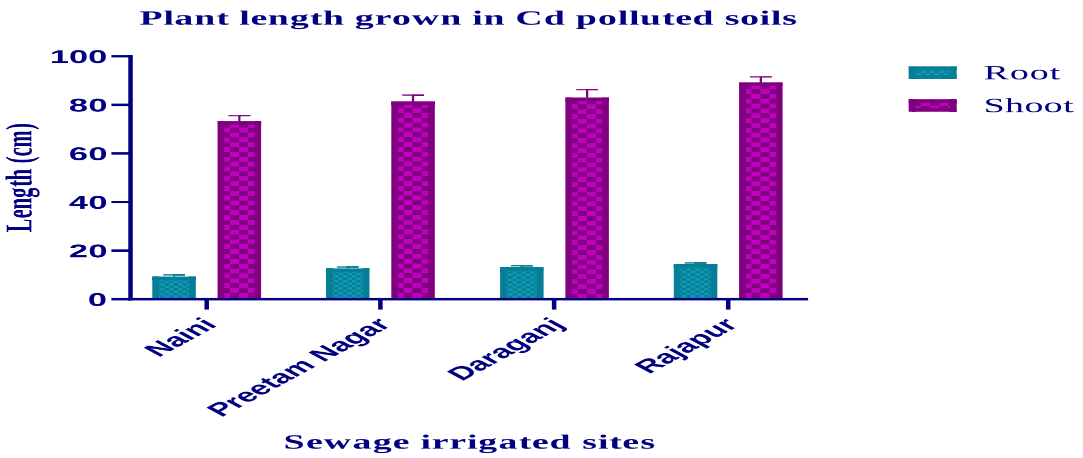

Figure 3a: Plant length grown in Cd polluted soils

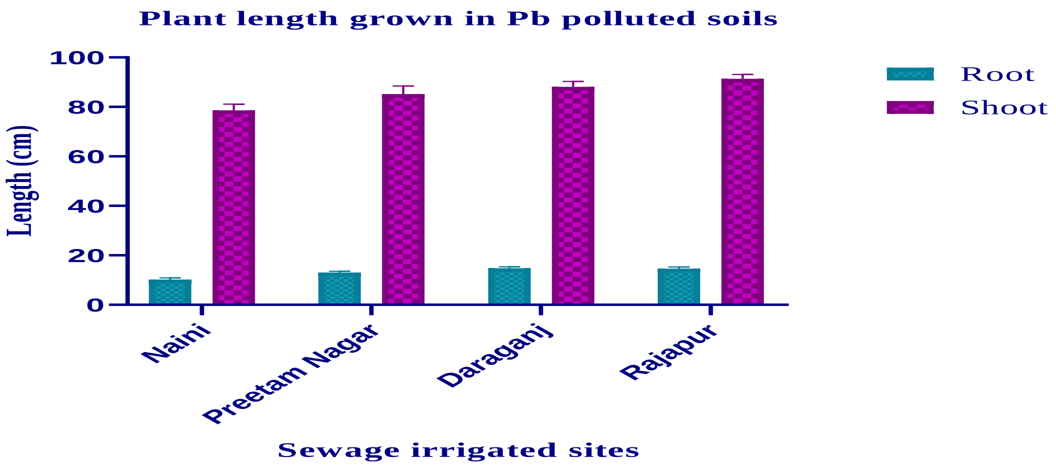

Figure 1b: Plant length grown in Pb polluted soils

(Kumar *et al.* 2024a). Naini sewage site was showed the maximum amount of heavy metals because it was highly polluted with sewage water irrigation containing toxic heavy metals (Cd and Pb) due to presence of industries near this site. Rajapur sewage site was less polluted with these metals as compare to remaining three sites. Continued sewage irrigation leads to a higher quantity of organic carbon because it contains huge amount of organic waste, thus its manurial value is high.

Effect of heavy metals (Cd and Pb) on growth and dry biomass of plants

The representative sites were showed the deleterious effect of heavy metal concentrations on the growth of *Brassica juncea* L. The higher concentrations of heavy metals (Cd and Pb) decreased the growth of root and shoot of plants. The maximum roots and shoots length were found at Rajapur sewage irrigated site polluted with Cd and Pb ranged from 14.47±0.49, 89.27±2.25 and 14.66±0.61, 91.36±1.78 cm respectively. Whereas minimum roots and shoots length i.e., 9.35±0.67, 73.41±2.17 and 10.19±0.71-78.61±2.49 cm, were observed from Naini sewage irrigated site respectively (Fig. 1 a and b). This is due to the highest amount of Cd and Pb observed at Naini site.

The maximum roots, shoots dry biomass yield were found at Rajapur sewage irrigated site polluted with Cd and Pb ranged from 3.10±0.07, 12.10±0.51 and 3.77±0.11, 12.81±0.32 g plant^{-1} respectively. Whereas minimum roots, shoots dry biomass yield i.e., 1.56±0.03, 10.05±0.32 and 2.09±0.04, 10.48±0.27 g plant^{-1}, were observed from Cd and Pb polluted soil at Naini sewage irrigated site, respectively (Fig. 2 a and b). Since the amounts of heavy metals (Cd and Pb) increased, plant height and dry biomass decreased. also reported the same. (Bhardwaj *et al.* 2009)

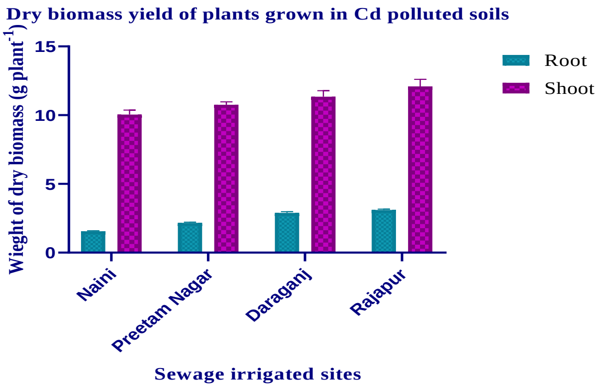

Figure 4a: Dry biomass yield of plants grown in Cd polluted soils

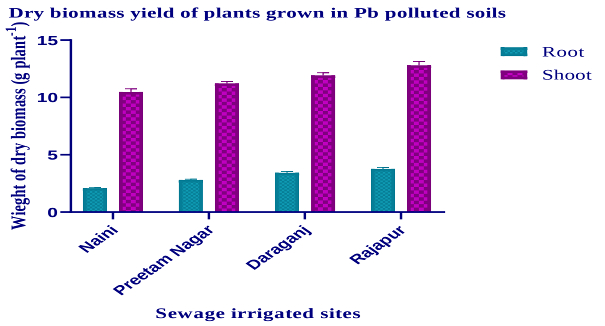

Figure 2b: Dry biomass yield of plants grown in Pb polluted soils

Accumulation of heavy metals in plants

The accumulation pattern of heavy metals Cd and Pb in roots and shoots of *Brassica juncea* L. grown in different wastewater irrigated sites at prayagraj were showed in Fig. 3a. The collection of plants was done after 60 days from all sites and plant samples were analysed for heavy metals. The phytoextraction potential of *Brassica juncea* L. grown in different sewage irrigated sites showed the various concentrations of heavy metals (Cd and Pb) ranged from 3.51±0.16-1.97±0.09,3.69±0.25-2.25±0.12 and 4.78±0.20-3.11±0.17, 5.13±0.23-3.06±0.21 mg kg^{-1} in the roots and shoots, respectively.

The results clearly showed in Fig. 3b that the plant potentially accumulates the higher amount of Pb i.e., 5.13±0.23 followed by Cd 3.69±0.25 mg kg^{-1} in shoots whereas Pb 4.78±0.20 followed by Cd 3.51±0.16 in roots from Naini sewage irrigated soil respectively. It means plants accumulate Pb to a toxic level, also reported (Singh *et al.*2015). It means Naini sewage irrigated site is highly polluted with heavy metals as compare to remaining sites. Decreasing accumulation pattern of Cd and Pb were recorded from the all four sites i.e., Naini>Preetam Nagar>Daraganj>Rajapur.

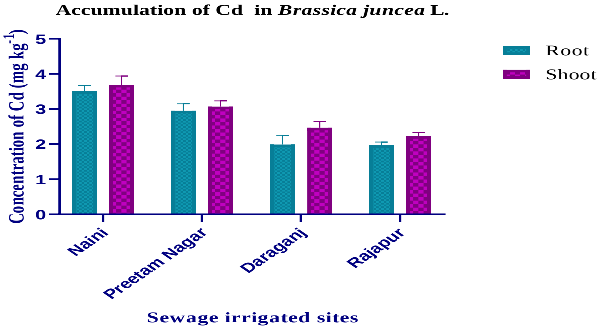

Figure 5a: Accumulation of Cd in *Brassica juncea* L.

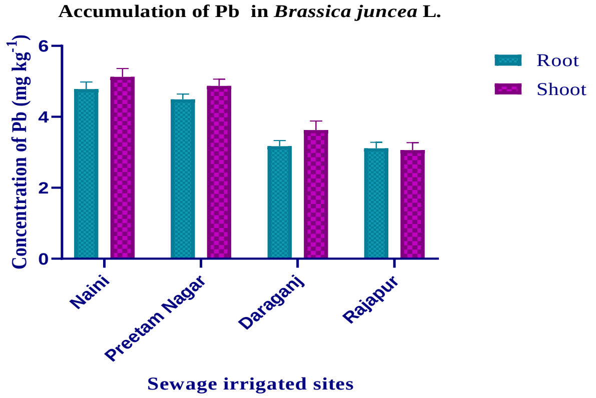

Figure 3b: Accumulation of Pb in *Brassica juncea* L.

Bioaccumulation and Translocation factors of Cd and Pb

The Bioaccumulation and Translocation factors of Cd and Pb polluted soils showed the phytoremediation efficiency of *Brassica juncea* L. grown in different sewage irrigated sites which are given in Table 2. and Fig. 4a, b. The values of BAF and TF of all Cd polluted soils ranged from 1.063±0.08-1.300±0.06 and 1.051±0.10-1.142±0.04 mg kg^{-1}, respectively. Whereas the values of BAF and TF of all Pb polluted soils ranged from 1.075±0.11-0.987±0.03 and 1.073±0.13-0.983±0.07 mg kg^{-1}, respectively. it was clearly showed that a considerable amounts of Cd and Pb accumulated by plant shoots. Mostly the values of BAF and TF was greater than >1 which justified that the *Brassica juncea* L. is a hyper accumulator plant. The values of BAF >1 was also showed the Phytoaccumulation capacity of *Brassica juncea* L. was almost equal for all sites with respected to amount of heavy metal presented. (Kumar *et al.,* 2024b) also suggested that *Brassica Juncea* L. is fit for phytoremediation of heavy metal contaminated soils.

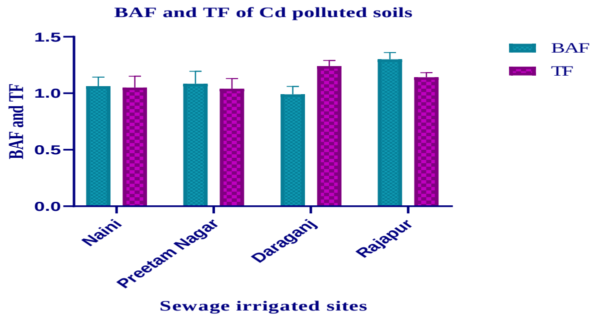

Figure 6a: Bioaccumulation and translocation factor of Cd polluted soils

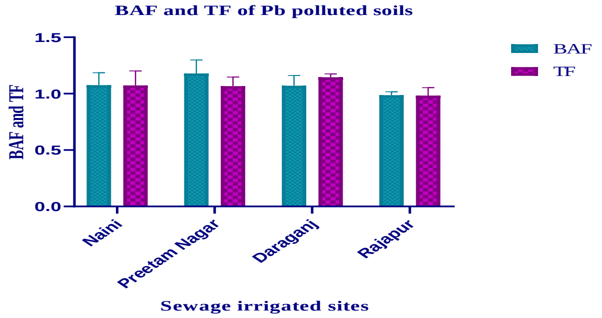

Figure 4b: Bioaccumulation and translocation factor of Pb polluted soils

Table 4: Calculated values of the bioaccumulation and translocation factors for Brassica juncea L. grown in different sewage irrigated sites

Sites	Cd (mg kg^{-1})		Pb (mg kg^{-1})	
	BAF	TF	BAF	TF
Naini	1.063±0.08	1.051±0.10	1.075±0.11	1.073±0.13
Preetam Nagar	1.084±0.11	1.040±0.09	1.179±0.12	1.067±0.08
Daraganj	0.991±0.07	1.241±0.05	1.070±0.09	1.145±0.03
Rajapur	1.300±0.06	1.142±0.04	0.987±0.03	0.983±0.07

CONCLUSION

The plants of *Brassica juncea* L. could withstand in heavy metal polluted soils resulting from continued sewage irrigation containing considerable amounts of the heavy metals (Cd and Pb). The plants of *Brassica juncea* L. were accumulated the high amounts of heavy metals (Cd and Pb) in shoots followed by roots. Various negative impacts on soil were investigated that alter the physico-chemical properties of soils. High amounts of heavy metals inhibited the growth of plants and caused various abnormalities who disturb the normal life cycle of plants. Therefore, the current research will be more useful from the phytoremediation point of view. Studies are still in the process to identify the potential of *Brassica juncea* L. to remediate the heavy metals. Further researches are needed for the development of better strategies that can help in the restoration of natural resources such as soil, water and environment.

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