Asian Journal of Chemistry; Vol. 36, No. 12 (2024), 2961-2966

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2024.32542

Phytoextraction of Heavy Metals: Assessing the Potential of *Brassica juncea* in Heavy Metals (Copper and Cadmium) from Contaminated Soils

T.E. Haribabu[1], Srinivasan Latha[2], G. Lavanya[3], A. Rekha[4], K. Sudhakar[5], R. Suresh[6], Z. Ansar Ali[7] and P.N. Sudha[8,*]

[1]Department of Chemistry, Government Polytechnic College, Cheyyar-604407, India
[2]Department of Chemistry, VIT University, Vellore-632014, India
[3]Department of Chemistry, Department of Chemistry, Saveetha Engineering College, Tandalam, Chennai-602105, India
[4]Department of Microbiology, DKM College for Women (Autonomous), Vellore-632001, India
[5]P.G. Department of Zoology, Kalaignar Karunanidhi Government Arts College, Tiruvannamalai-606603, India
[6]Department of Chemistry, Government Polytechnic College, Nagapadi, Tiruvannamalai-606705, India
[7]Department of Chemistry, C. Abdul Hakeem College (Autonomous), Melvisharam-632509, India
[8]Department of Physiology, Saveetha Dental College & Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-602105, India

*Corresponding author: E-mail: drparsu8@gmail.com

Received: 20 August 2024; *Accepted*: 23 November 2024; *Published online*: 30 November 2024; AJC-21841

This study investigated the phytoremediation potential of *Brassica juncea* in copper and cadmium contaminated soils. *B. juncea*, a fast-growing, high-biomass-producing plant, has been shown to tolerate and accumulate heavy metals. This study aimed to assess the effectiveness of *B. juncea* in removing copper and cadmium from contaminated soils and to evaluate the morphophysiological and biochemical responses of the plant to metal stress. Soil samples with varying concentrations of copper and cadmium were prepared and *B. juncea* was grown in these soils under controlled conditions. Plant growth parameters, metal accumulation in plant tissues and biochemical stress markers were analyzed. The results showed the potential use of *B. juncea* for the phytoremediation of copper and cadmium contaminated soils. The presence of heavy metals had a direct effect on the biochemical parameters, growth performance, and the antioxidant enzymes of the plant. At the same time, the plant seemed to tolerate the metal stress and accumulate, which helped in the extraction of metals from soils. The current study contributes to the development of sustainable strategies for the remediation of the metal-polluted sites.

Keywords: Phytoremediation, Copper, Cadmium, *Brassica juncea*, Bioaccumulation.

INTRODUCTION

Pollution from toxic metals poses an imminent threat to both humans and other ecosystem components. Environmental pollutants are substances that disrupt habitats and life on earth. These pollutants can originate from several sources, including natural and human-caused processes such as industry, agriculture, waste disposal, shipping and farming which have contributed to this contamination, especially in the last three centuries [1]. Such pollution is becoming more pervasive in all parts of life and poses a serious threat to the possibility of achieving sustainability [2]. Cadmium, lead, copper and zinc are toxic chemicals that play major roles in several environmental and health problems [3]. Plants can take up heavy metals *via* the root system in addition to water and the surface of the soil acts as a storage area for these metals. It is possible for these metals to be transported through the veins of the plant, which could be harmful since it could cause heavy metals to accumulate within the parts of plants that humans or animals can eat and ingest, which could have negative health effects. Thus, it is essential to monitor and control heavy metal contamination in soil to maintain food sustainability and the health of the environment [4]. When heavy metals are ingested more than the recommended limits, they can have detrimental effects on the body, which are known as biotoxic effects. The general symptoms related to metal poisoning include vomiting, nausea, tremors, headaches, dizziness, numbness, memory loss, cardiovascular & respiratory problems and renal damage [5].

Heavy metals such as Cd, Ni and Zn, which have soluble soil fractions that typically contribute more than Cu and Pb, have a greater potential for soil-plant mobility than Cu and Pb [6]. According to Nazir *et al.* [7], plants under high copper stress are typically characterized by a loss in biomass. Exposure to high levels of cadmium, (where the acceptable limit is 3-8 mg/Kg) can indeed have detrimental effects on plants, including reductions in photosynthesis, water uptake and nutrient uptake.

Copper is an essential element for plant growth, with plant tissues typically requiring 5-20 mg/kg Cu to be considered adequate [8]. Plants require copper as a micronutrient, but when plant concentrations of copper exceed the threshold, various detrimental consequences occur, including leaf chlorosis, increased cytotoxicity and oxidative stress and can also affect plant growth. Kumar *et al.* [9] reported that the recommended permissible limit for copper in a soil sample is 20 mg kg^{-1}. The excessive accumulation of copper in plants, results in the production of reactive oxygen species (ROS) which can lead to oxidative damage. The superoxide radicals and hydrogen peroxide (H_2O_2) present in ROS, can damage proteins, lipids and DNA and affect plant growth [10]. Because of their ubiquitous prevalence in polluted sites and toxicity, two heavy metals that have received attention for remediation are copper and cadmium.

Remediation of metal contaminated soil is primarily performed to either fix or remove toxic metals. Using plants to absorb, stabilize or degrade pollutants from the soil lowers the concentration of pollutants to tolerable levels [11]. In present study, *Brassica juncea* (mustard plant) was selected for the phytoextraction of heavy metal-contaminated soil. This plant was chosen due to its local availability, fast growth, high biomass, tolerance to metals and effectiveness in transferring metals from the root to the plant system [12]. The cells of plants possess an antioxidant defense system that includes enzymatic antioxidants such as glutathione reductase (GR), ascorbate peroxidase (APX), peroxidase (POX), superoxide dismutase (SOD), catalase (CAT) and polyphenol oxidase (PPO), which reduce the damaging effects of free radicals [13]. While previous research has explored the capacity of *Brassica juncea* to hyperaccumulate the heavy metals, this study focuses on the potential of mustard plants to extract and recover metals like copper and cadmium for the purpose of achieving sustainability.

EXPERIMENTAL

Collection of soil samples: The top layer of soil, ranging in depth from 10 to 15 cm, was taken from the farmlands of Kaniyambadi hamlet (N12º 48′ 42.6384″, E79º 8′ 8.61″), which is near Vellore city, India. Following air drying, the samples were ground into a fine powder and sieved through a 0.2 mm screen. Polythene bags were used to preserve the soil samples for further experiments. The 15 cm of surface topsoil, polluted by tannery industrial effluents in Ranipet Tannery Industrial Town was collected as polluted soil. The collected soil samples were crushed, allowed to dry naturally and then sieved through a 0.2 mm mesh screen. The sieved soil samples were kept in airtight polythene bags.

Using a magnetic stirrer, 50 g of different soil samples and 500 mL of distilled water were mixed and stirred for 2 h. The aqueous extract was used to analyze the physico-chemical properties of both the collected soil samples, including their pH, electrical conductivity, porosity, specific gravity, organic content, overall salinity and nutrients such as N, P, K, Na and Ca ions. Following treatment with 2 mL of perchloric acid and 5 mL of conc. HNO_3, 1 g of each soil sample was digested in a microwave digestion system and analyzed for heavy metals using AAS (Varian 240FS).

Copper chloride and cadmium chloride were used to conduct lethality tests with different concentrations of $CuCl_2$ and $CdCl_2$ on saplings of *B. juncea* grown in fertile control soil. The sublethal and 1/2 sublethal concentrations of $CuCl_2$ and $CdCl_2$ spiked soils such as 200 mg/Kg and 100 mg/Kg, respectively were used for the phytoremediation studies (600 mg/Kg was the IC_{50} values, 1/3rd of IC_{50} is sub-lethal *i.e.* 200mg/Kg and ½ of sub-lethal value 100 mg/Kg). Studies on the effects of metals at sublethal and half-sublethal concentrations and the effect of polluted soil on the various parameters and antioxidant capabilities of *B. juncea* after six weeks of exposure was carried out.

Experimental setup: The following experimental setup was used for the different exposures of the *B. juncea* plants to soils spiked with different concentrations of the heavy metals copper and cadmium and polluted soil. All the experiments were conducted in triplicate to ensure the reproducibility.

1. Garden soil + *Brassica juncea* plant
2. Garden soil + 200 mg/kg Cu + *B. juncea* plant
3. Garden soil + 100 mg/kg Cu+ *B. juncea* plant
4. Garden soil + 200 mg/kg Cd + *B. juncea* plant
5. Garden soil + 100 mg/kg Cd + *B. juncea* plant
6. Polluted soil + *B. juncea* plant

The plants were subjected to various examinations after 42 days following germination. After chopping the shoots off the soil's surface, the plants were cleaned with deionized water. Plant roots were extracted from the soil and rinsed three times with deionized water. The biomass was measured after the plant parts were drained of water. The fresh plant was used for the preparation of plant extract. The shoot with leaves and root parts was completely dried at 70 ºC for 48 h. After that the samples were finely powdered and used for the analysis of metal accumulation content.

Plant sample analysis

Preparation of plant extract: Each fresh green plant material (10 g) was homogenized in 100 mL of distilled water for 25 min to produce cold water-based extracts. After boiling, cold-based and macerated solutions of prepared extracts were filtered through Whatman No. 1 paper and the filterate were dried at 50 ºC in an oven and finally stored at 4 ºC. Chlorophyll and all the biochemical factors such as total carbohydrates (anthrone method), total proteins (Lowry assay), total lipids (spectrophotometric method) and vitamins such as β-carotene and thiamine by spectrophotometric method, ascorbic acid by titration method and minerals such as Na, Ca, K and Fe by flame photometric method were measured using the procedures described by Sadasivam & Manickam [14]. Total phenolics were measured using the Folin-Ciocalteu reagent, the activity of DPPH in scavenging free radicals was measured using the Blosi method [15], the amino acid proline was measured using the ninhydrin procedure and catalase was measured using the Beers & Seizer method [16]. SOD was measured through the Misra & Fridovich [17] technique and glutathione was measured using the Ellman method [18].

Extraction of metals from plant samples: The dried powdered plant sample was incinerated at high temperatures. The ash obtained from the incinerated plant samples was digested with conc. HCl and perchloric acid and the metals were analyzed using an atomic absorption spectrometer (AAS) [19].

RESULTS AND DISCUSSION

Physico-chemical characteristics of soil: The physico-chemical characteristics of the control and polluted soils are displayed in Table-1. Compared to garden soil (pH of 7.5), the pH of polluted soil (6.4) was acidic (Table-1). The findings demonstrated that the contaminated soil had lower levels of porosity, specific gravity, organic matter, total nitrogen and phosphorus than the control soil. The polluted soil had the highest levels of pH, electrical conductivity, potassium, sodium, calcium and salinity, while the garden soil had the lowest levels. This may be due to tannery wastewater contaminating the already polluted terrain.

TABLE-1
PHYSICO-CHEMICAL CHARACTERISTICS OF THE CONTROL AND POLLUTED SOIL SAMPLES

Physico-chemical factors	Garden soil	Polluted soil
pH	7.5 ± 0.012	6.4 ± 0.026
Electrical conductivity (mmho/cm^2)	0.348 ± 0.002	1.2 ± 0.003
Porosity	2.08 ± 0.017	0.83 ± 0.0002
Specific gravity	2.2 ± 0.063	1.0 ± 0.003
Organic matter (%)	5.6 ± 0.07	5.1 ± 0.02
Total nitrogen (mg/kg)	6527 ± 4.94	6251 ± 3.85
Phosphorus (mg/kg)	418.8 ± 1.52	301 ± 1.58
Potassium (mg/kg)	120 ± 0.78	252 ± 1.12
Sodium (mg/kg)	420 ± 1.9	1241±2.16
Calcium (mg/kg)	380 ± 1.33	410 ± 0.72
Salinity (mg/kg)	21.6 ± 0.33	126.2 ± 0.54
Cu (mg/kg)	0.02 ± 0.0007	42.2 ± 0.03
Cd (mg/kg)	ND	12.3 ± 0.01
Cr (mg/kg)	ND	106 ± 0.04
Pb (mg/kg)	ND	77.4 ± 0.01

ND: Not detectable. Values are expressed as the mean ± SE of six individual values.

The polluted soil included high concentrations of heavy metals including lead, cadmium, chromium and copper, while copper was only present at 0.02 mg/kg and the amount of lead, chromium and cadmium in the garden soil was undetectable. This demonstrated the significant concentrations of lead, copper, cadmium and chromium in the extremely salty contaminated soil. As a result, the elements in the contaminated soil indicate a high degree of contamination caused by several industrial effluents. The current research aims to use phytoremediation, a technique that uses plants to purify soil. The plant, *B. juncea* has been assessed for its potential to remediate soil contaminated with metals.

Biochemical factors of *B. juncea*: Fig. 1 illustrates the effects of exposure to the heavy metals copper and cadmium on the biochemical properties of *B. juncea*. The biochemical characteristics of *B. juncea* leaves growing in sublethal, half-sublethal copper and cadmium mixed fertile garden soil. Compared with those in the control soil, the total amounts of proteins, carbohydrates, fats and amino acids decreased by 20.7%, 18.4%, 25% and 25.6% at 100 ppm of Cu and 17.2%, 17%, 15.4% and 7.7%, respectively at 100 ppm Cd. These values further decreased as the concentration of heavy metals increased, increasing by 35.6%, 25.1%, 34.6% and 38.5% at 200 ppm Cu and 20.7%, 22%, 21.2% and 20.5% at 200 ppm Cd, respectively.

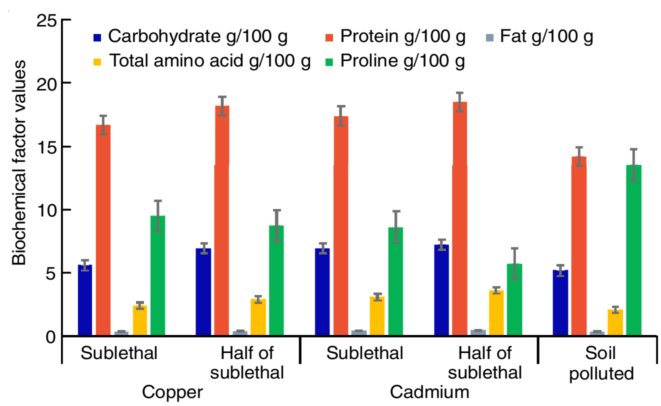

Fig. 1. Biochemical factors of the plant *Brassica juncea*

The total amount of carbohydrates, proteins, lipids and amino acids in *B. juncea* exposed to contaminated soil decreased significantly by 40.2%, 36.3%, 40.4% and 46.2%, respectively, whereas the proline value increased by 264% compared to the control soil. Among other protective mechanisms, plants subjected to oxidative stress exhibit increased production of flavonoids, polyphenols and total carbohydrates [20,21]. Proline is a protective agent for proteins and membranes against the damaging effects caused by elevated inorganic ion levels and strong temperatures. It can also act as a radical hydroxyl scavenger and a protein-compatible hydrotrope [22].

Present analyses of the total protein content were in agreement with those of Singh & Sinha [23], who reported that when *B. juncea* was grown on different amendments of heavy metal containing tannery waste, the amount of soluble protein decreased. The observed decrease in protein content in *B. juncea* at higher concentrations of Cd and Cu could perhaps be attributed to an accelerated protease activity-driven process of protein breakdown [24]. Heavy metals induce reactive oxygen species (ROS), which causes enzyme inhibition, chlorophyll degradation, cell wall damage and chelation of essential nutrients as described by Das & Roychoudhary [25].

Vitamin and mineral content of *B. juncea*: The mineral and vitamin contents of *B. juncea* cultivated in sublethal, half-lethal copper and cadmium contaminated fertile garden soil and the tannery effluent contaminated soil are shown in Fig. 2. The findings demonstrate that, in comparison to those in the control soil, the amounts of vitamins and minerals in the contaminated and spiked soils were considerably lower.

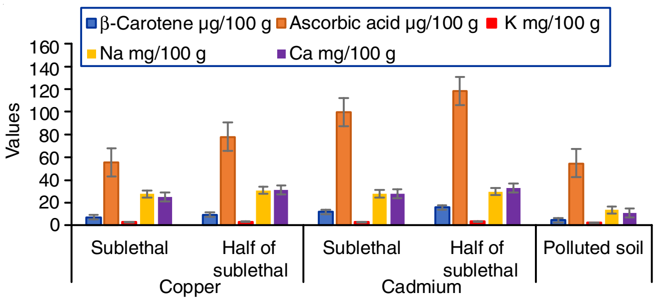

Fig. 2. Vitamins and minerals of the plant *Brassica juncea*

Compared to those in the control soil, the maximum reductions in total phenolics, β-carotene, ascorbic acid, thiamine, K, Na and Ca were 43.4%, 83.6%, 64.1%, 71.4%, 50%, 61.5% and 71.8%, respectively in the polluted soil and that of iron was 39% at 200 ppm copper. *B. juncea* was exposed to sublethal and half of sublethal concentrations of copper, cadmium and polluted soil. *B. juncea* is a plant that is rich in antioxidant phytochemicals, as evidenced by the much higher levels of vitamins A, B and C, total phenolics and other minerals in the control soil. In plant cells, vitamins function as catalysts for biological reactions. Vitamins are also among the organic dietary elements needed for the ongoing growth and metabolic processes of living organisms. These substances have seldom been tested for their ability to mitigate some of the harmful consequences of heavy metal stress [26].

By scavenging active oxygen species and interrupting radical chain events during lipid peroxidation, phenolics are usually believed to prevent oxidative damage. These antioxidative activities require the reduced form of phenolics, whereas, in the oxidized form, they act as prooxidants. The redox characteristics of phenol, which enable them to function as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators, are primarily responsible for their antioxidant action [27,28].

Phenolics have a strong propensity to attach to metals since they contain carboxyl and hydroxyl groups. The chelation process reduces the toxicity of heavy metals, such as cadmium and copper in plants [29]. Many plants exposed to heavy metals release large amounts of phenolics from their roots [30]. Phenol chelates heavy metals such as copper and cadmium, which reduces the toxicity. This is indicated by the lower phenolic content with increasing concentrations of these heavy metals. Ascorbic acid is a naturally occurring antioxidant that is vital for pollution tolerance. There is a direct correlation between endogenous ascorbic acid levels and plant susceptibility to pollutants [31]. Moreover, it functions as a cofactor for a variety of enzymes, including those that produce cell walls and, most importantly, hydroxylate proline residues [32]. Thus, the vitamins and mineral contents are affected by the metal toxicity and also due to high vitamin contents, the plant can tolerate the metal toxicity.

Antioxidant properties: The antioxidant characteristics of *B. juncea* cultivated in rich garden soil, sub-lethal and half-sublethal concentrations of soil laced with copper and cadmium and contaminated soil are displayed in Fig. 3. The plants exposed to the control soil exhibited the highest level of antioxidant activity, which was 88%. Compared with those in the control soil, the plant antioxidant activity decreased by 15% at 100 ppm of Cu, 26% at 200 ppm Cu, 7% at 100 ppm Cd, 28% at 200 ppm Cd and 22% in the polluted soil. The proportion of antioxidant activity was reduced to its minimum at 100 ppm and to its maximum at 200 ppm of Cd metal. This demonstrates that plants exposed to heavy metal contaminated soils have significantly less free radical scavenging activity.

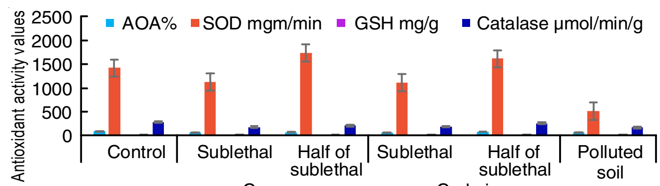

Fig. 3. Antioxidant properties of the plant *Brassica juncea*

In comparison to those in the control soil, the levels of superoxide dismutase and reduced glutathione increased by 22.6% and 26.8%, respectively at 100 ppm Cu, 12.1% and 7.4% at 100 ppm Cd and decreased by 21% and 11.2% at 200 ppm of Cu and 21.6% and 19.2% respectively at 200 ppm Cd. However, the levels of catalase decreased by 26.4%, 9.4%, 36.5% and 33.9% at 100 ppm Cu, 100 ppm Cd, 200 ppm Cu and 200 ppm Cd, respectively. When comparing the polluted soil to the control soil, the levels of reduced glutathione, catalase and superoxide dismutase decreased by 63.9%, 24.8% and 40.4%, respectively (Fig. 3). In response to cadmium exposure, Kaur *et al.* [33] reported alterations in the antioxidant defense system of *B. juncea* seedlings. The activities of superoxide dismutase (SOD) and glutathione (GSH) were notably reduced by cadmium. However, the levels of several enzymes such as catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR), increased.

These investigations showed that *B. juncea* plants overproduce reactive oxygen species (ROS) in response to cadmium exposure. The above results show that the heavy metals copper and cadmium significantly affect the biochemical factors, vitamins and mineral factors and antioxidant enzymes in *B. juncea*. Among the two metals, copper showed greater toxicity than cadmium. This may be due to the detoxification of cadmium by phytochelatins (PCs) and metallothioneins (MCs).

Remediation of heavy metals from polluted soils using *B. juncea*: The impact of the heavy metals *viz.* copper and cadmium on the biomass and chlorophyll content of *B. juncea* was investigated. Fig. 4 shows the growth performance (biomass) and chlorophyll content of *B. juncea* exposed to fertile garden soil, sublethal and half-sublethal copper and cadmium spiked soils and polluted soil.

A maximum of 198 g of biomass and 54.4 mg/kg of chlorophyll was recorded in the control soil. Compared with those of the control soil, the percentage of plant biomass in the polluted

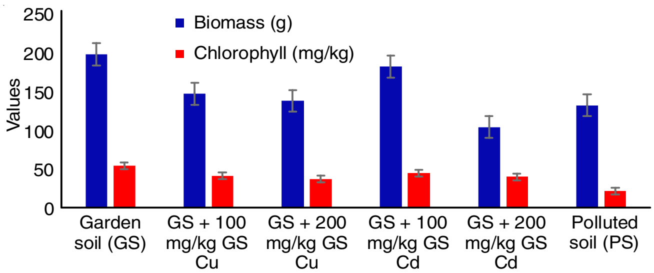

Fig. 4. Biomass and chlorophyll content of the plant *Brassica juncea*

soil decreased by 25.8% at 100 ppm Cu, 30.3% at 200 ppm Cu, 8% at 100 ppm Cd, 47.5% at 200 ppm Cd and 33.3%. Compared with those in the control soil the percentages of plants whose chlorophyll content decreased were 24.6% in the 100 ppm of Cu treatment, 32% in the 200 ppm Cu treatment, 18.4% in the 100 ppm of Cd treatment, 26.7% in the 200 ppm Cd treatment and 60.7% in the polluted soil.

The biomass of *B. juncea* was strongly affected by cadmium at a concentration of 200 ppm and chlorophyll content was affected by the polluted soil. These results showed that the growth performance in terms of biomass and chlorophyll content of *B. juncea* exposed to heavy metal spiked soils and polluted soil was drastically lower than that of *B. juncea* exposed to control soil. Fig. 5 shows the percentages of the bioaccumulation of copper and cadmium metals by *B. juncea* plants. Many Brassica species are known to accumulate metals and *B. juncea*, or Indian mustard, in particular, has been the subject of years of study on the accumulation of various metals in its shoots [34].

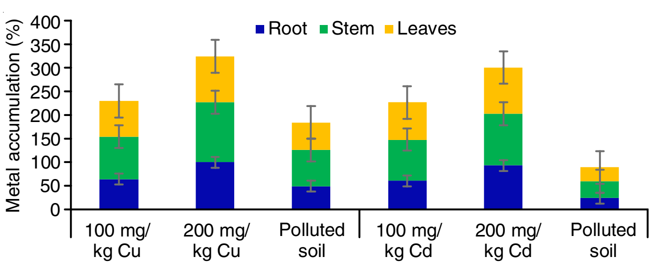

Fig. 5. Percent bioaccumulation of copper and cadmium by *Brassica juncea*

The percentage of copper bioaccumulated in the plant was 77.5% at 100 ppm Cu, 109.4% at 200 ppm Cu and 62.2% in polluted soil. The percentage of cadmium bioaccumulated in the plants was 76.5% at 100 ppm Cd, 101.3% at 200 ppm Cd and 30.1% in polluted soil. By comparing the percentage bioaccumulation of different concentrations of copper and cadmium metals by *B. juncea*, it was observed that the order of bioaccumulation was stem > leaves > root. Among the two metals, copper was found to have greater bioaccumulation than cadmium in the polluted soil, because the bioavailability of copper was greater than that of cadmium.

Fig. 6 shows the proportions of heavy metals extracted from *B. juncea* plants. Toxic metals must be removed from the soil to protect people and the environment. According to the findings of several studies on metal hyperaccumulating plants, the phytoextraction of metals is a workable remediation technique for the decontamination of soil contaminated with metals [35]. Recent investigations on the viability of phytoextraction have shown that high biomass yields and metal hyperaccumulation are necessary for this method to be successful [36]. The percentage of recovered copper was 67% at 100 ppm of Cu, 89% at 200 ppm Cu and 53% at polluted soil. The percentage of recovered cadmium was 64% at 100 ppm of Cd, 81% at 200 ppm of Cd and 23% in polluted soil. Among the two metals, copper is recovered more than cadmium, due to the greater accumulation of copper compared to cadmium in *B. juncea*. Therefore, this method not only removes metals from the soil but can also be regenerated by proper extraction processes.

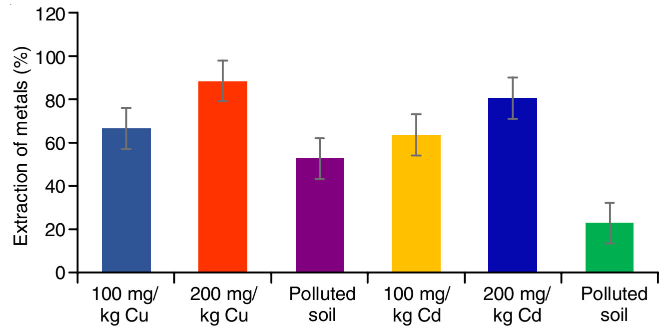

Fig. 6. Percentage extraction of heavy metals from *Brassica juncea*

The soluble metals in the soil are taken up by the roots which are transferred to the shoots through the xylem of the roots and the metals are further distributed and accumulated through the phloem of the shoots and further extracted. The data obtained confirm the efficiency of *B. juncea* in removing these two metals from contaminated environments and potentially for assessing their suitability for phytoremediation purposes.

Conclusion

This study investigated the phytoremediation potential of *Brassica juncea* in copper and cadmium contaminated soils. The plant responses to metal stress were investigated by changes in biochemical parameters, vitamins and minerals and antioxidant enzyme analysis. The plants were evaluated for metal bioaccumulation capacity and the accumulated Cu and Cd were phytoextracted using conc. HCl. The bioaccumulation percentages of various amounts of copper and cadmium demonstrated a decreasing sequence as: stem > leaves > root. Due to its higher bioavailability in contaminated soil compared to cadmium, it was found that copper exhibited a greater level of bioaccumulation in the soil than cadmium. The results provide insights into the potential use of *B. juncea* for phytoremediation and phytoextraction and contribute to the development of sustainable strategies for the remediation of metal-polluted sites. Hence, through this studies, it is proved that the heavy metals in the soil can be efficiently extracted safely through growing plants and recovered from the same.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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