

Research Article

Biosorbents Removed Copper Heavy Metal from Agricultural Land Cultivated with *Vigna radiata* (Mung Bean)

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Background. Heavy metals in excessive levels are hazardous to ecosystems as they can bioaccumulate in organisms, have toxic effects on biota, and even cause mortality in most life forms. **Methodology.** The present study consists of two studies; initially, different concentrations of copper were biosorbed by using 1 gm powder of various biosorbents such as orange peels (OP), pomegranate peels (POP), and peanut peels (PP). Furthermore, the biosorbed water was used to irrigate soil when the mung beans were cultivated. The growth parameters of plants growing under induced copper stress and treated with different bioabsorbed waters were also evaluated. **Results.** The results revealed that, among all biosorbents, the copper biosorption capacity of orange peels was maximum (90%), followed by pomegranate peel. The results of the second experiment exhibited that the plants irrigated with biosorbed water did not show metal toxicity. A remarkable increase in shoot length, shoot fresh weight, and shoot dry weight was observed (29.8 cm, 15.4 g, and 14 g, respectively) when exposed to biosorbent water with peanut peels + 200 mg kg⁻¹ (Cu²⁺). Similarly, pomegranate peel biosorbed water turned out to be an effective treatment to enhance root length, root fresh weight, and root dry weight (6.81 cm, 4.07 g, and 2.66 g, respectively) and resist against induced heavy metal stress conditions at higher concentration (200 mg kg⁻¹). Furthermore, orange peel biosorbed water elevated the total chlorophyll content and soluble sugar content in mung bean (1.56 mg/g and 1.89 mg/g). The highest tolerance index of mung bean plant grown under the stress of Cu²⁺ metal was induced by orange peel biosorbed water. **Conclusion.** Biosorption is an environmentally friendly approach to mitigate heavy metals from the water. The studies showed that agricultural wastes have enough bioabsorption potential and should be used to absorb the heavy metal present in water.

1. Introduction

Mung bean (*Vigna radiata*) belongs to the family Fabaceae (*Leguminosae*); it is one of the nutritionally rich and nitrogen-fixing summer pulse crops and is commonly grown

worldwide particularly, having a short span of the life cycle (3–4 months). Essentially, the mung bean is a leguminous plant mostly cultivated for edible seeds [1]. The application of mung is wide as fodder, food, and green manure. It authenticates as an economical source of protein which is

directly consumed by human beings; additionally, it enhances soil fertility. Mung bean seed chemical analysis revealed that, fundamentally, it possesses 24.9% protein, 1.3% fat, and 60% carbohydrates; it also comprises a fair amount of vital minerals such as phosphorous, calcium, iron, sodium, and potassium and, moreover, some vitamins such as riboflavin, thiamine, and vitamin A [2, 3]. Mung bean plant growth rate is assessed annually. The shoot of mung bean is in the form of erect or semierect, and it could gain a height of 0.15–1.25 m. The root system fabrication is composed of a slightly hairy and developed root system. However, the leaves are in alternate venation, either be trifoliate with ellipsoidal leaves to form ovate leaflets, which can grow up to 5–18 cm long and 3–15 cm wide [4].

The flowers develop in clusters, which vary from 4 to 30 at the top of the plant, and it is papilionaceous, greenish, or pale yellow. Mung bean pods are of considerable length, cylindrical, and fuzzy which might be covered with shaded layers. Their seeds may vary from 7 to 20, although it is small-sized. However, it could be cube-shaped or ellipsoidal seeds. The seeds are of many colors: generally, it is green; however, they can also be pale yellow, brown, olive, purplish brown, or black and mottled or ridged in appearance [4].

The roots of mung bean plants are narcotic and diuretic and used to treat different diseases such as abscesses, bone pain, dropsy, headache, and inflammation. The seeds of this plant are used as emollients, astringents, laxative snacks, nerve tonics, and styptics. They are useful for treating scabies, gonorrhoea, aches, hemorrhoids, heart problems, constipation, and memory weakness [4]. In some countries, including Pakistan, India, Bangladesh, Thailand, and Korea, mung bean is important as a summer legume crop. In South Asia, the demand for pulses is enhancing, but since the early 1960s, acreage and yield production have not increased [5, 6].

Heavy metals are metallic elements that have a comparatively high density and are venomous in fewer amounts; these heavy metals include arsenic (As), mercury (Hg), lead (Pb), cadmium (Cd), chromium (Cr), and thallium (Tl). Some trace elements are also known as heavy metals such as copper (Cu), selenium (Se), and zinc (Zn). They are compulsory to maintain the metabolism of the body, but they are toxic in higher concentrations [7, 8]. Excessive amounts of heavy metals are harmful as they threaten ecosystems due to their bioaccumulation in organisms, cause toxic effects in biota, and even cause death in most living organisms. Heavy metal contamination of water is caused by the addition of organic compounds, heavy metals, sludge, and sewage organisms; it also includes fungi, algae, bacteria, and hazardous material and chemicals [9]. Water pollution due to heavy metals has been recognized as a worldwide threat since the beginning of the industrial revolt and addition of hydrocarbons, heavy metals, inorganic anions, pesticides, nutrient loads, and microplastic [10]. It occurs when microorganisms and harmful chemicals from household trash and industry come into touch with water sources, run away, or seep into the water table and freshwater supplies [11]. Their high concentrations contaminate the water and ultimately living life forms. The prevalence of coliform bacteria

is indicated by the contamination of animal and human wastes. Owing to drainage in watercourses, such as rivers, lakes, and streams, bacteria development and dispersal are at their height during the rainy season. Waterborne infections spread due to inadequate treatment. In Pakistan, the potable sanitation and drainage systems run simultaneously, resulting in leaks and water quality degradation due to intermixing [12].

Copper being a metal is very toxic; it significantly affects agriculture and the environment. It comes from mining, smelting, and agricultural and industrial wastes [13]. However, it is essential for the soil, whereas its excess amount negatively affects the growth of plants. It helps plant growth by providing structural strength to the plants, and it also participates in the photosynthesis process in the electron transport chain. It also helps to catalyze the cell wall metabolism and hormone signaling. For the higher plants, copper is a well-known necessary micronutrient among other metals, but a higher amount of this metal causes a lack of other essential nutrients by competitive prohibiting at absorption sites. Besides, it reduces the enzyme of production and suppresses the functioning of the membrane [13–15]. In Pakistan, the discharge of wastes and untreated effluents of industries into freshwater bodies has become a serious problem. The number of metals, particularly copper (Cu^{2+}) included in these industrial wastes, is considerably high in amount. When these are taken up or absorbed in higher concentrations by crop plants, it creates a significant problem. They inhibit the metabolic process and reduce crop production by entering the plant's body; these metals are subsequently incorporated into the food chain and cause liver and brain disorders [16, 17].

The term biosorption implies a direct interaction between the metal sorbate and biosorbents. The diffusion of the metal ion from the bulk solution to the active sites of the microorganism, in the nonliving biomass, occurs predominantly by passive transport mechanisms. Initially, metal ions diffuse to the surface of the microbial cell where they bind to active sites on the cell surface formed by the presence of different chemical groups such as hydroxyl, carboxylate, amine, and phosphate that relate to the metal ions' affinity [18, 19].

Recently, it widely focused on various natural solid supports, which can remove contaminants from contaminated water at a low cost. These include chemical precipitation, membrane filtration, ion exchange, electrolysis, coagulation, solvent extraction, reverse osmosis, and electrocoagulation. However, these mentioned techniques have several disadvantages, such as incomplete removal of pollutants, are regarded as low selectivity, use of high reagent and chemical, energy-consuming, the production of other toxic wastes that need to be operated carefully, and mostly high cost [20]. Conversely, the use of biological methods such as bioremediation, bioaccumulation, or biosorption for the exclusion of heavy metals or utilization of microorganisms and plants for remediation function is thus achievable key to reducing the heavy metal contamination [21]. Among various remediation methods, the biosorption through agriculture waste seems to be more effective in huge

scale applications compared to the bioaccumulation process because the microbes require the addition of nutrients for their active uptake of heavy metals, which increases the biological oxygen required [22, 23]. Agricultural wastes could be assumed to be low-cost adsorbents because they are abundant in nature, low-priced, and plentiful, require a little transformation, and are also useful and convenient materials. To remove copper material, many agricultural waste materials and some peels of fruits and vegetables have been used as adsorbents (biosorbents) [24, 25]. Therefore, the main purpose of the current research work was to check three distinct biosorbents which are pomegranate, peanut, and orange peels for copper (Cu^{2+}) metal biosorption from an aqueous solution and examine biomaterial accumulation of heavy metals. Moreover, the impact of Cu^{2+} stress on morphology and biochemical profiling of the mung bean plants was also evaluated.

2. Materials and Methods

2.1. Preparation of Biosorbents. Biosorbent peels (orange, peanut, and pomegranate) were collected from various local markets in Lahore (Punjab, Pakistan). For two days, the biomaterials were kept sun-dry on the roof. By sun drying, the mass and volume of biomass were reduced. The color of the biosorbent peels was also changed after drying in the sun. Subsequently, when the sun drying is done, the biomass was decently washed with the help of tap water and once more with distilled water. After cleaning, the biomass was left for drying for a night in an oven at 60°C . After oven drying, the biomaterials (peels) were also transformed into small pieces. The biomaterials' (biosorbent peels) tiny pieces were ground in a kitchen grinder to convert them into fine powder. After crushing the biosorbent peels, a fine powder was prepared, as shown in Figure 1 [26].

2.2. Preparation of Stock Solution for Copper. Copper sulphate (CuSO_4) was purchased from Merck (Germany). Salt was used for the preparation of stock solution (s.s). The molecular weight of copper sulphate is 249.69 g per mol. For the preparation of stock solutions, 3.92 g of CuSO_4 salt was dissolved in 1000 ml distilled H_2O . These various concentrations were then measured from the stock solutions to give 0 ppm (control), 50 ppm, 100 ppm, 150 ppm, and 200 ppm copper metal concentrations. The stock solution contained in the bottle was capped and preserved in a safe place for further use in the experiment. The different concentrations (0 ppm, 50 ppm, 100 ppm, 150 ppm, and 200 ppm) were all set by diluting the stock solution (s.s) with double distilled water [27].

2.3. Experimental Trials. In the Erlenmeyer flasks (250 ml), about 100 ml of each concentration such as 0, 50, 100, 150, and 200 ppm of Cu^{2+} was taken. The pH of the solution was adjusted with a strong base and acid (0.1 M NaOH and 0.1 M HCl) by using a pH meter (model 3505, Jenway). Into each metal solution, about 1 g of each biosorbent powder (orange peel, peanut peel, and pomegranate peel powder) was added. The mixture (adsorbent + metal) holding flasks were placed on an orbital shaker for 20 minutes at 120 rpm. The filtration of the solution was done by using Whatman No. 42 after the stirring process. To find out the number of residual metal ions' concentrations, the filtrate was analyzed on an atomic absorption spectrophotometer.

2.4. Biosorption Data Evaluation. Accumulation of the quantity of Cu^{2+} by the biomaterial (peels) was calculated by distinguishing between the initial and final quantity present in the solution after equilibration with the biomaterial using the given formula. Likewise, the biosorption efficiency (E) for each experiment was calculated by using the following equation:

$$E = \left(\frac{C_i - C_f}{C_i} \right) * 100. \quad (1)$$

The concentrations of metallic ions are shown in the following formula; the initial concentration (mg L^{-1}) is represented by (C_i), whereas the final concentration (absorption) of metallic ion (mg L^{-1}) is represented by (C_f). The diagrammatic experimental flow can be seen in Figure 2 [28].

2.5. Soil Sterilization and Metal Spiking. Cotton was soaked in formalin solution, and plugs were inserted in the soil at different points for its sterilization (fumigation). After inserting the formalin-soaked cotton plugs into the soil, it is roofed with a plastic sheet and left for the next 7 days. To completely evaporate formalin fumes, all the cotton plugs and sheets were removed from the soil after 7 days to leave it open for another 7 days. Finally, the soil was sieved in pots (mung bean-sized, 2 mm) and also utilized for metal spiking.

The soil was enriched with copper metal by spraying each concentration of an aqueous solution of (V) 50, 100, 150, and 200 mg kg^{-1} and left for 15 days (followed by resieving and crushing of the soil). Furthermore, Cu^{2+} metal spiked in soil was also assessed with the following modified formula [29]:

$$\begin{aligned} \text{\% age saturation of soil} &= \frac{W_i \text{ (initial weight of soil)} - W_f \text{ (final weight of soil)}}{W_i \text{ (initial weight of soil)}} \times 100, \\ \text{required water} &= \frac{\text{total weight of soil required } (W_t) \times \text{\% age saturation of soil}}{100}. \end{aligned} \quad (2)$$



FIGURE 1: Selective biosorbents: (a) orange peels, (b) grounded powder of orange peels, (c) peanut peels, (d) grounded powder of peanut peels, (e) pomegranate peels, and (f) grounded powder of pomegranate peels.

2.6. Seed Sowing. After metal spiking into the soil, four healthy and sterilized seeds of mung bean were sown in plastic pots. In the shaded glasshouse, those soil-containing plastic pots were placed ($25^{\circ}\text{C} \pm 3$; 12-hour photoperiod). Furthermore, by irrigating with tap water, the dampness of the soil was also maintained. The details of the treatments are given in Table 1.

Furthermore, the experiment was conducted using a complete randomized design with 3 repetitions. After the tenth day of germination of the seed, all the plants were analyzed to determine the changes in morphological and height (growth) parameters, the length of the roots and shoots, and the total

biomass (fresh and dry weight). Meanwhile, antioxidant enzyme activity and the physiological changes in the plants (mung beans) were examined after 10 days of seed germination. Moreover, after the 10th day of sprouting, the accumulation of metal by the plant was also estimated.

2.7. Physiological Assays. After 7 days of germination of mung bean, various physiological parameters such as total chlorophyll content [30], soluble sugar [31], and total protein content [32] were estimated.

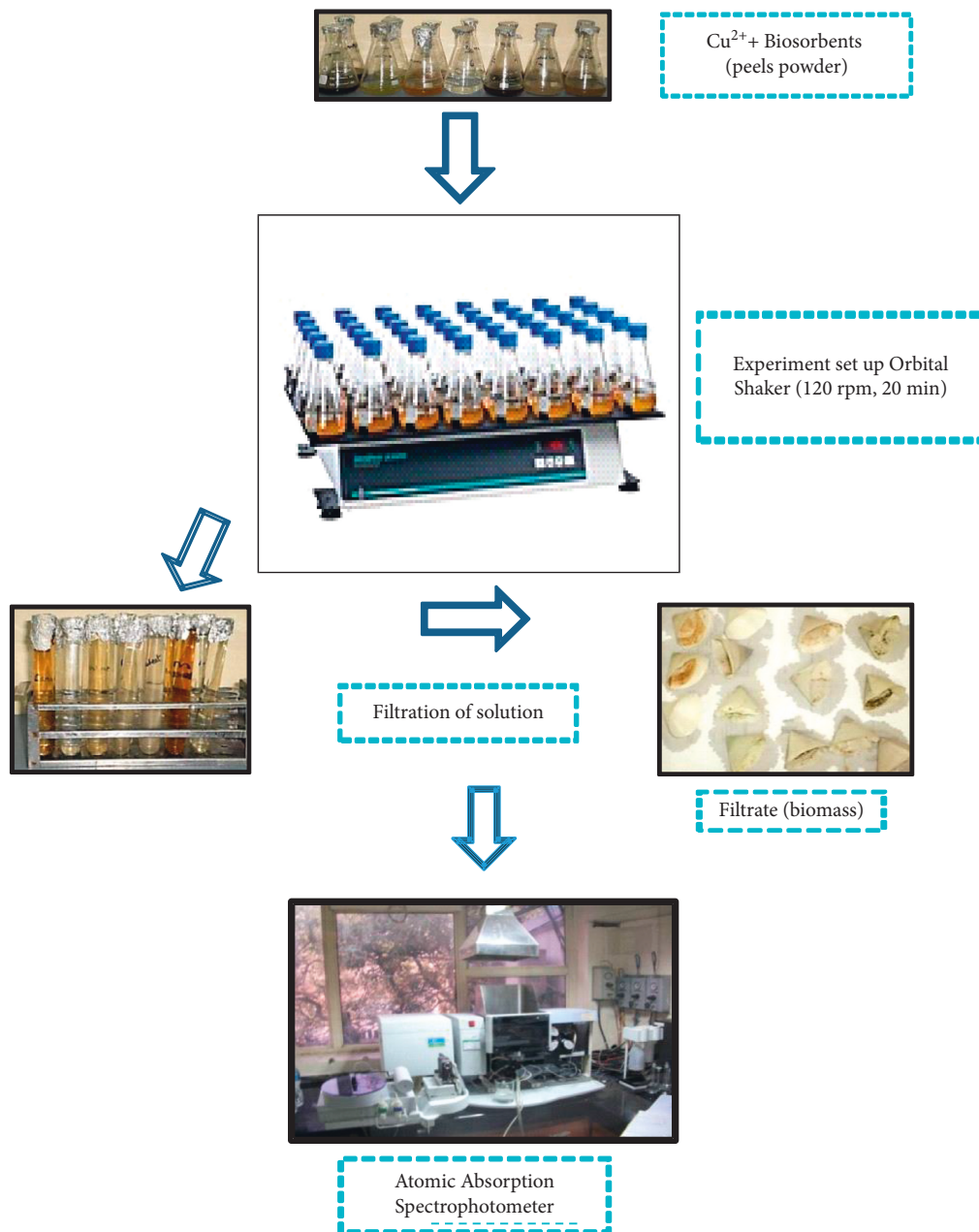


FIGURE 2: Flow diagram of biosorption technique.

2.8. Growth Assay. After 10 days, *Vigna radiata* (mung bean) plants were sampled. The plants were uprooted carefully from pots at the time of harvesting, then washed under (tap) running water, and desiccated with the help of blotting paper after uprooting and washing. Plant shoots and roots were also separated. The length and fresh weight of each plant in cm and gm were recorded, respectively. Both roots and shoots were dried in an oven for 24 hours (at 80°C) and reweighed for the dry weight measurement.

2.9. Metal Toxicity Assessment. Morphological symptoms such as the stunted growth of an entire plant, yellow patches on leaves, decrease in vegetative growth, and root reduction were also observed after 10 days of sowing mung bean plants in Cu²⁺ contaminated soils.

2.10. Statistical Analysis. The assessment of metal absorption capacity of biosorbent, toxic influence of copper and treated water with biosorbents on seed and seedling growth of mung

bean (*V. radiata*), and metal accumulation by the different parts of plants were analyzed by applying Least Significant Difference (LSD) Test and Pearson correlation. Moreover, by using computer software Statistics 8.1, all the statistical (numerical and arithmetical) analyses were done.

3. Results

3.1. Biosorption Batch. For copper (Cu^{2+}) metal biosorption from an aqueous solution, three distinct biosorbents that are pomegranate, peanut, and orange peels were tested. The examined biomaterials exhibited tremendous results for the accumulation of heavy metals.

3.2. Accumulation of Cu^{2+} by Pomegranate Peels. Adsorption experiments were carried out in batches at room temperature. Contact time, pH, initial metal ion concentration, and adsorbent concentrations (0–200 ppm) were studied. The results showed that pomegranate peels absorbed about 40–110 ppm of copper from the aqueous solution of 50 and 200 ppm copper (Cu^{2+}) metal. Moreover, the efficiency of the tested biosorbents was 80–55% from 50 to 200 ppm of copper (Cu^{2+}) metal in an aqueous (water solvent) solution, as illustrated in Table 2.

3.3. Accumulation of Cu^{2+} by Peanut Peels. It was found that the peanut peels absorbed about 20–73 ppm of copper from the aqueous solution of 50 and 200 ppm copper (Cu^{2+}) metal. Furthermore, the efficiency of the tested biosorbent was 40–37% against 50–200 ppm of copper (Cu^{2+}) metal in an aqueous solution (Table 3).

3.4. Accumulation of Cu^{2+} by Orange Peels. It was found that the orange peels absorbed about 45–155 ppm of copper from the aqueous solution of 50 and 200 ppm copper (Cu^{2+}) metal. Additionally, the efficiency of the tested biosorbents was 90–78% against 50–200 ppm of copper (Cu^{2+}) metal in an aqueous solution (Table 4).

The pot experiment was conducted in a controlled environment in which the impact of biosorbent water with different biosorbents including peanut peels (PP), pomegranate peels (POP), and orange peels (OP) was studied. Tremendous variations in shoot length parameters, root length parameters, and biomass (dry mass and fresh mass) were observed.

3.5. Metal Toxicity and Growth Parameters. The impact of Cu^{2+} stress on the morphology of the mung bean plant is depicted in Figure 3. The results revealed the deleterious effect of higher doses of copper metal (100–200 mg kg^{-1}) on the growth of mung bean plants. The stunted growths with yellowing of leaves were observed at 100–200 mg kg^{-1} of Cu^{2+} as compared to (–ve) control. The implementation of biosorbed water exhibited better growth in mung bean plants. No retarded growth symptoms were observed in mung bean plants that were treated with biosorbed water.

3.6. Shoot Parameters

3.6.1. Shoot Length. The impact of various biosorbed waters on the shoot length of mung beans is illustrated in Figure 4. After observations, it was noticed that the shoot length of the mung bean plant was reduced at 50 mg kg^{-1} in comparison with negative control, while significant reduction of shoot length was observed at 150 and 200 mg kg^{-1} concentration of Cu^{2+} (16.6 cm and 19.7 cm).

The biosorbed water has momentarily enhanced the shoot length of the plant even under induced stress of heavy metal at higher concentration. Highest shoot length was observed in the plant treated with peanut peel biosorbed water +200 mg/kg of Cu^{2+} (29.8 cm). This was followed by the plant treated with pomegranate peel biosorbed water +100 mg/kg (28 cm). The orange peel biosorbed water (OPBW) elevated the shoot length in the plant exposed to 200 mg/kg of Cu^{2+} (24.2 cm).

3.6.2. Shoot Fresh Weight. The results also revealed that the rising concentration of Cu^{2+} (50–200 mg kg^{-1}) adversely affected the shoot fresh weight of mung bean plant as compared to the negative control, whereas the biosorbed water reduced the heavy metal accumulation in plant and increased the shoot fresh weight even at 200 mg kg^{-1} concentration of heavy metal. The plant treated with peanut peel biosorbed water +200 mg kg^{-1} Cu^{2+} showed the maximum shoot biomass formation (15.4 g). Similar results were also observed in the plant treated with pomegranate peel biosorbed water +100 mg kg^{-1} Cu^{2+} (12.9 g). A moderate level of increase in shoot fresh weight of mung bean was also induced by the orange peel biosorbed water (Figure 5).

3.6.3. Shoot Dry Weight. The shoot dry weight of mung bean was considerably reduced by more than 2-folds with the exposure to a higher concentration of Cu^{2+} (100–200 mg kg^{-1}) as compared to the negative control. However, the shoot dry weight of the mung bean plant was improved (14 g) by treatment with peanut peel biosorbed water under induced Cu^{2+} stress conditions (200 mg kg^{-1}). This was followed by the plant treated with pomegranate peel biosorbed water +100 mg kg^{-1} Cu^{2+} (11.8 g). The orange peel biosorbed water also enhanced a considerable level of shoot dry biomass of mung bean (Figure 6).

3.7. Root Parameters

3.7.1. Root Length. The impact of various biosorbed waters and concentrations of heavy metal on the root length of mung bean is illustrated in Figure 7. A moderate level of reduction was observed in the root length of the mung bean plant after exposure to 50 mg kg^{-1} of heavy metal, while momentous reduction was examined at higher concentration of copper heavy metal (100–200 mg kg^{-1}). Almost 3-fold reduction was calculated in root length of plants exposed to 100 mg kg^{-1} of Cu^{2+} as compared to the negative control.

Furthermore, the results revealed that the pomegranate peel biosorbed water (POPBW) significantly inhibited the

TABLE 1: Experimental layout: experimental work was placed in two sections with 17 treatments.

T1	Negative control
T2	50 mg kg ⁻¹ (Cu ²⁺)
T3	Biosorbent water with pomegranate peels + 50 mg kg ⁻¹ (Cu ²⁺)
T4	Biosorbent water with peanut peels + 50 mg kg ⁻¹ (Cu ²⁺)
T5	Biosorbent water with orange peels + 50 mg kg ⁻¹ (Cu ²⁺)
T6	100 mg kg ⁻¹ (Cu ²⁺)
T7	Biosorbent water with pomegranate peels + 100 mg kg ⁻¹ (Cu ²⁺)
T8	Biosorbent water with peanut peels + 100 mg kg ⁻¹ (Cu ²⁺)
T9	Biosorbent water with orange peels + 100 mg kg ⁻¹ (Cu ²⁺)
T10	150 mg kg ⁻¹ (Cu ²⁺)
T11	Biosorbent water with pomegranate peels + 150 mg kg ⁻¹ (Cu ²⁺)
T12	Biosorbent water with peanut peels + 150 mg kg ⁻¹ (Cu ²⁺)
T13	Biosorbent water with orange peels + 150 mg kg ⁻¹ (Cu ²⁺)
T14	200 mg kg ⁻¹ (Cu ²⁺)
T15	Biosorbent water with pomegranate peels + 200 mg kg ⁻¹ (Cu ²⁺)
T16	Biosorbent water with peanut peels + 200 mg kg ⁻¹ (Cu ²⁺)
T17	Biosorbent water with orange peels + 200 mg kg ⁻¹ (Cu ²⁺)

TABLE 2: Accumulation of Cu²⁺ by pomegranate peels.

Concentrations (ppm)	Accumulation (ppm)	Efficiency (%)
50	40	80
100	67	67
150	100	67
200	110	55

TABLE 3: Accumulation of Cu²⁺ by peanut peels.

Concentration (ppm)	Accumulation (ppm)	Efficiency (%)
50	20	40
100	45	45
150	62	41
200	73	37

TABLE 4: Accumulation of Cu²⁺ by orange peels.

Concentration (ppm)	Accumulation (ppm)	Efficiency (%)
50	45	90
100	75	75
150	110	73
200	155	78

accumulation of Cu²⁺ in plant even at higher concentration (200 mg/kg) and promoted the root growth and development of mung bean (6.81 cm) as compared to the respective control. The peanut peel biosorbed water (PPBW) also promoted rooting (5.89 cm) in plant germinating under induced heavy metal stress conditions (200 mg kg⁻¹). Furthermore, the orange peel biosorbed water (OPBW) was effective for the plants growing under lower concentration of heavy metal (Figure 7).

3.7.2. Root Fresh Weight. Along with the length of roots, different concentrations of the heavy metals also adversely affected the root fresh biomass accumulation. The least root

fresh weight (0.11 g) was noticed in the plant grown under the stress of higher concentration of Cu²⁺ heavy metal (200 mg kg⁻¹), while these plants when grown in the presence of pomegranate peel biosorbed water had the maximum root biomass formation (4.07 g). Moreover, the soil treated with PPBW after being stressed with 200 mg kg⁻¹ concentration of Cu²⁺ enhanced the root growth and fresh biomass (3.22 g) (Figure 8).

3.7.3. Root Dry Weight. Similar to root fresh weight, the higher concentrations of the heavy metals also adversely affected the dry root biomass of mung bean plant. The results revealed that the root dry weight of the mung bean plant was reduced by 2-3-folds with the increasing concentration of Cu²⁺ as compared to the negative control, whereas the soil treated with POPBW in the presence of heavy metal at 200 mg kg⁻¹ concentration significantly improved the root dry biomass of mung bean plant by 2.5-folds (2.66 g) as compared to the plant grown under heavy metal stress only. The root dry weight of mung bean plant was also enhanced by the treatment with PPBW as compared to plant growth under the stress of copper only (150 mg kg⁻¹). Moreover, the root dry weight of mung bean was considerably increased to 2.13 g when treated with OPBW along with the presence of lower concentration of heavy metal (Figure 9).

3.8. Physiological Parameters

3.8.1. Total Chlorophyll Content. The impact of various biosorbed waters on the chlorophyll content of mung bean is illustrated in Figure 10. The heavy metal stress retarded not only the growth of the plants but also the physiological parameters significantly. A 3-fold decline in the total chlorophyll content was detected in plant stressed with higher concentration of heavy metal (200 mg kg⁻¹) as compared to the negative control. The biosorbed waters were not effective enough against higher concentration of heavy metals to promote the level of chlorophyll pigment in plants, though a moderate level of increase was detected in plants treated with biosorbed waters and low level of heavy metal stress (200 mg kg⁻¹). The maximum level of total chlorophyll content (1.56 mg/g) was detected in plants treated with orange peel biosorbed water + 50 mg kg⁻¹ of copper (Figure 10).

3.8.2. Soluble Sugar Content. The soluble sugar content of the plant was affected with induced heavy metal stress, but the treatment with biosorbed water limited the accumulation of heavy metals and their toxic effects on the mung bean plant. The biosorbed water promoted the accumulation of total soluble sugar content in the plant when treated in the presence of minimal level of heavy metal (50 mg kg⁻¹). The maximum soluble sugar content (1.89 mg/g) was detected in mung bean plant germinated under orange peel biosorbed water + 50 mg kg⁻¹ of Cu²⁺ (Figure 11).

3.8.3. Total Protein Content. It is contingent on the situation that the response of the plant to the stress state determines

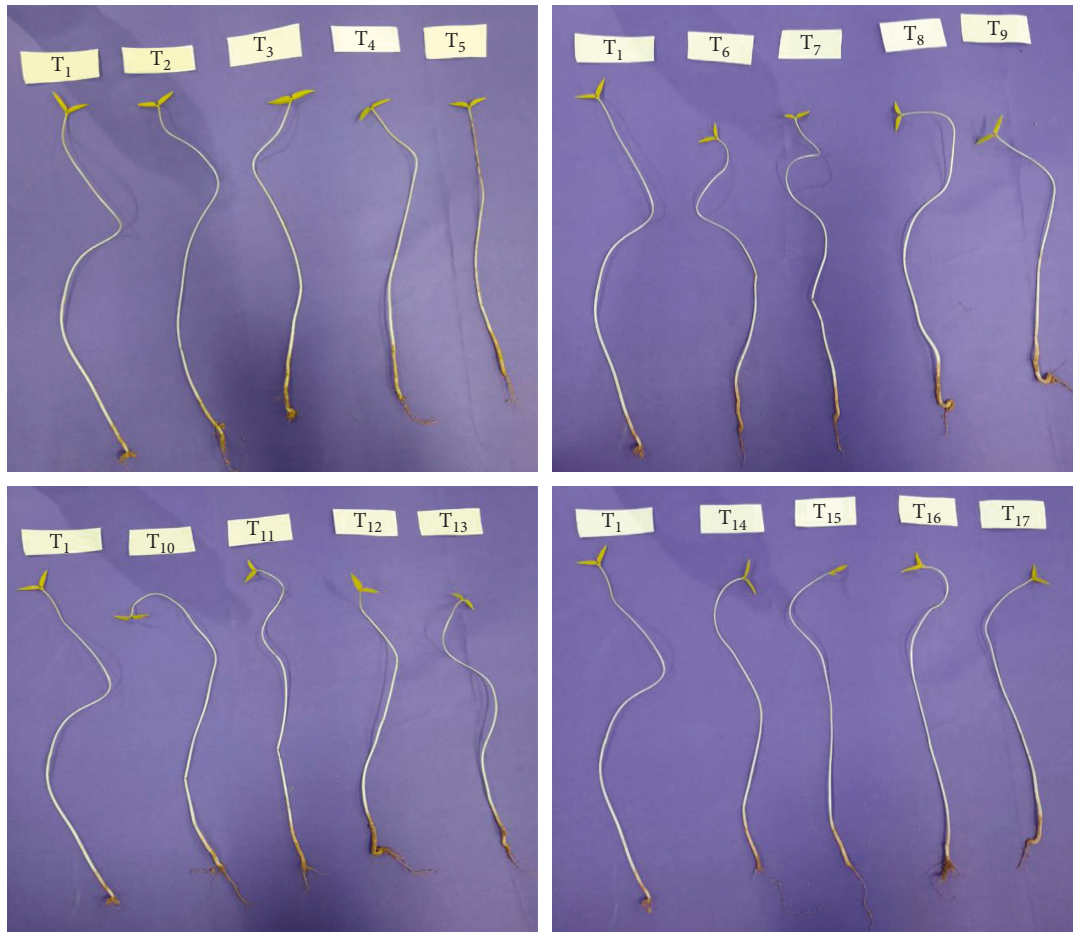


FIGURE 3: Impact of biosorbed water by various biosorbents (orange peels, pomegranate peels, and peanut peels) on the growth of mung bean after 7 days of seed germination. T1, negative control; T2, 50 mg kg⁻¹; T3, pomegranate peel biosorbed water +50 mg kg⁻¹; T4, peanut peel biosorbed water +50 mg kg⁻¹; T5, orange peel biosorbed water +50 mg kg⁻¹; T6, 100 mg kg⁻¹; T7, pomegranate peel biosorbed water +100 mg kg⁻¹; T8, peanut peel biosorbed water +100 mg kg⁻¹; T9, orange peel biosorbed water +100 mg kg⁻¹; T10, 150 mg kg⁻¹; T11, pomegranate peel biosorbed water +150 mg kg⁻¹; T12, peanut peel biosorbed water +150 mg kg⁻¹; T13, orange peel biosorbed water +150 mg kg⁻¹; T14, 200 mg kg⁻¹; T15, pomegranate peel biosorbed water +200 mg kg⁻¹; T16, peanut peel biosorbed water +200 mg kg⁻¹; T17, orange peel biosorbed water +200 mg kg⁻¹. Vertical bars (I): standard error (mean of 3 replicates). Alphabets on vertical bars: significant difference ($P \leq 0.05$) evaluated by the LSD test.

whether total protein content will be enhanced or decreased. When plants are stressed, their total protein content may rise as a result of the generation of stress-related proteins.

Similar results were also observed in the present study; the total protein content of the plant significantly increased with the raise in the concentration of induced heavy metal stress. A 2-3-fold increase of total protein content was detected in plants germinated in the presence of higher concentration of heavy metal (100, 150, and 200 mg kg⁻¹) as compared to the negative control. The lowest level of total protein content was detected in the plant developed in the presence of peanut peel biosorbed water +200 mg kg⁻¹ of Cu²⁺ (0.34 mg/g) (Figure 12).

3.9. Tolerance Index of Mung Bean Plant. It was found that the tolerance index of mung beans was 75–45% when grown in metal stress alone (50–200 mg kg⁻¹), whereas the tested index of mung bean plants by POPBW and PPBW was

122–395% and 77–213%, respectively. However, the application of OPBW increased the level of tolerance index of the mung bean plant by 136–744% (Figure 13).

4. Discussion

In the present research work for the biosorption of (Cu²⁺) heavy metal, three different biosorbents such as peanut, orange, and pomegranate peels were chosen. Three forms of these peels (powder) were used. Incredible results of capacity and effectiveness for the accumulation of heavy metals (Cu²⁺) by different biosorbents' tests are shown.

The ability of accumulation of different heavy metals and the efficiency of various biosorbents were previously studied. It was observed that the orange peels exhibited a higher biosorption capacity which is 90–78% in an aqueous metal solution. In comparison, the biosorption capacity of pomegranate and peanut peels was 80–55% and 40–37%, respectively. The current study thus revealed that the

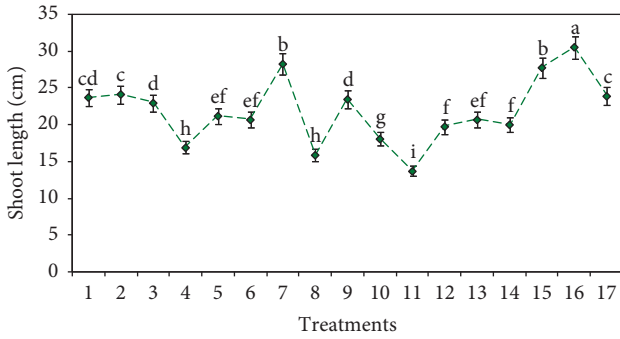


FIGURE 4: Impact of biosorbed water by various biosorbents (orange peels, pomegranate peels, and peanut peels) on the shoot length of mung bean after 7 days of seed germination. T1, negative control; T2, 50 mg kg⁻¹; T3, pomegranate peel biosorbed water +50 mg kg⁻¹; T4, peanut peel biosorbed water +50 mg kg⁻¹; T5, orange peel biosorbed water +50 mg kg⁻¹; T6, 100 mg kg⁻¹; T7, pomegranate peel biosorbed water +100 mg kg⁻¹; T8, peanut peel biosorbed water +100 mg kg⁻¹; T9, orange peel biosorbed water +100 mg kg⁻¹; T10, 150 mg kg⁻¹; T11, pomegranate peel biosorbed water +150 mg kg⁻¹; T12, peanut peel biosorbed water +150 mg kg⁻¹; T13, orange peel biosorbed water +150 mg kg⁻¹; T14, 200 mg kg⁻¹; T15, pomegranate peel biosorbed water +200 mg kg⁻¹; T16, peanut peel biosorbed water +200 mg kg⁻¹; T17, orange peel biosorbed water +200 mg kg⁻¹. Vertical bars (I): standard error (mean of 3 replicates). Alphabets on vertical bars: significant difference ($P \leq 0.05$) evaluated by the LSD test.

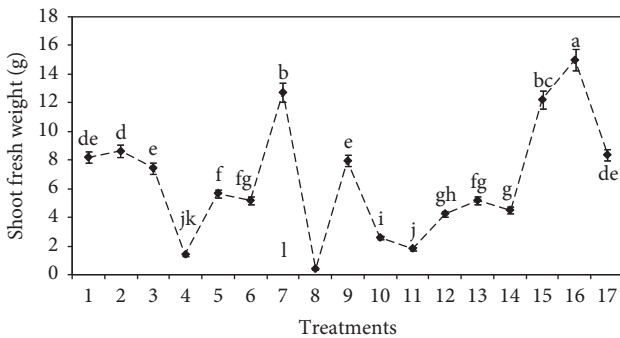


FIGURE 5: Impact of biosorbed water by various biosorbents (orange peels, pomegranate peels, and peanut peels) on the shoot fresh weight of mung bean after 7 days of seed germination. T1 negative control; T2, 50 mg kg⁻¹; T3, pomegranate peel biosorbed water +50 mg kg⁻¹; T4, peanut peel biosorbed water +50 mg kg⁻¹; T5, orange peel biosorbed water +50 mg kg⁻¹; T6, 100 mg kg⁻¹; T7, pomegranate peel biosorbed water +100 mg kg⁻¹; T8, peanut peel biosorbed water +100 mg kg⁻¹; T9, orange peel biosorbed water +100 mg kg⁻¹; T10, 150 mg kg⁻¹; T11, pomegranate peel biosorbed water +150 mg kg⁻¹; T12, peanut peel biosorbed water +150 mg kg⁻¹; T13, orange peel biosorbed water +150 mg kg⁻¹; T14, 200 mg kg⁻¹; T15, pomegranate peel biosorbed water +200 mg kg⁻¹; T16, peanut peel biosorbed water +200 mg kg⁻¹; T17, orange peel biosorbed water +200 mg kg⁻¹. Vertical bars (I): standard error (mean of 3 replicates). Alphabets on vertical bars: significant difference ($P \leq 0.05$) evaluated by the LSD test.

adsorbents such as orange peels were first-rate biosorbents for removing copper from an aqueous solution. The less dissimilarity in the biosorption capacity for the biosorbents is due to the presence of different percent of the constituents,

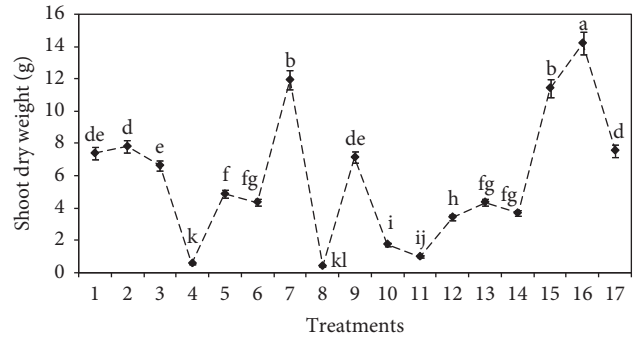


FIGURE 6: Impact of biosorbed water by various biosorbents (orange peels, pomegranate peels, and peanut peels) on the shoot dry weight of mung bean after 7 days of seed germination. T1, negative control; T2, 50 mg kg⁻¹; T3, pomegranate peel biosorbed water +50 mg kg⁻¹; T4, peanut peel biosorbed water +50 mg kg⁻¹; T5, orange peel biosorbed water +50 mg kg⁻¹; T6, 100 mg kg⁻¹; T7, pomegranate peel biosorbed water +100 mg kg⁻¹; T8, peanut peel biosorbed water +100 mg kg⁻¹; T9, orange peel biosorbed water +100 mg kg⁻¹; T10, 150 mg kg⁻¹; T11, pomegranate peel biosorbed water +150 mg kg⁻¹; T12, peanut peel biosorbed water +150 mg kg⁻¹; T13, orange peel biosorbed water +150 mg kg⁻¹; T14, 200 mg kg⁻¹; T15, pomegranate peel biosorbed water +200 mg kg⁻¹; T16, peanut peel biosorbed water +200 mg kg⁻¹; T17, orange peel biosorbed water +200 mg kg⁻¹. Vertical bars (I): standard error (mean of 3 replicates). Alphabets on vertical bars: significant difference ($P \leq 0.05$) evaluated by the LSD test.

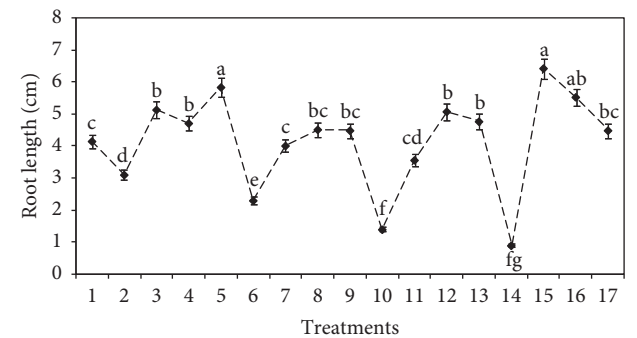


FIGURE 7: Impact of biosorbed water by various biosorbents (orange peels, pomegranate peels, and peanut peels) on root length of mung bean after 7 days of seed germination. T1, negative control; T2, 50 mg kg⁻¹; T3, pomegranate peel biosorbed water +50 mg kg⁻¹; T4, peanut peel biosorbed water +50 mg kg⁻¹; T5, orange peel biosorbed water +50 mg kg⁻¹; T6, 100 mg kg⁻¹; T7, pomegranate peel biosorbed water +100 mg kg⁻¹; T8, peanut peel biosorbed water +100 mg kg⁻¹; T9, orange peel biosorbed water +100 mg kg⁻¹; T10, 150 mg kg⁻¹; T11, pomegranate peel biosorbed water +150 mg kg⁻¹; T12, peanut peel biosorbed water +150 mg kg⁻¹; T13, orange peel biosorbed water +150 mg kg⁻¹; T14, 200 mg kg⁻¹; T15, pomegranate peel biosorbed water +200 mg kg⁻¹; T16, peanut peel biosorbed water +200 mg kg⁻¹; T17, orange peel biosorbed water +200 mg kg⁻¹. Vertical bars (I): standard error (mean of 3 replicates). Alphabets on vertical bars: significant difference ($P \leq 0.05$) evaluated by the LSD test.

which were bonded with the divalent metal ion, Cu (II). In addition, the treated forms showed a little bit lower percent removal compared to their nontreated forms. This is due to the maximal reduction of cellulosic materials during the acid

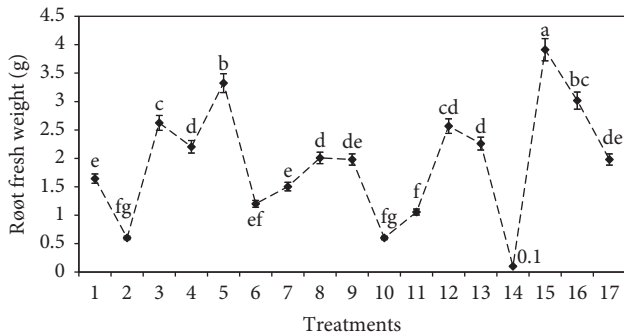


FIGURE 8: Impact of biosorbed water by various biosorbents (orange peels, pomegranate peels, and peanut peels) on root fresh weight of mung bean after 7 days of seed germination. T1, negative control; T2, 50 mg kg⁻¹; T3, pomegranate peel biosorbed water +50 mg kg⁻¹; T4, peanut peel biosorbed water +50 mg kg⁻¹; T5, orange peel biosorbed water +50 mg kg⁻¹; T6, 100 mg kg⁻¹; T7, pomegranate peel biosorbed water +100 mg kg⁻¹; T8, peanut peel biosorbed water +100 mg kg⁻¹; T9, orange peel biosorbed water +100 mg kg⁻¹; T10, 150 mg kg⁻¹; T11, pomegranate peel biosorbed water +150 mg kg⁻¹; T12, peanut peel biosorbed water +150 mg kg⁻¹; T13, orange peel biosorbed water +150 mg kg⁻¹; T14, 200 mg kg⁻¹; T15, pomegranate peel biosorbed water +200 mg kg⁻¹; T16, peanut peel biosorbed water +200 mg kg⁻¹; T17, orange peel biosorbed water +200 mg kg⁻¹. Vertical bars (I): standard error (mean of 3 replicates). Alphabets on vertical bars: significant difference ($P \leq 0.05$) evaluated by the LSD test.

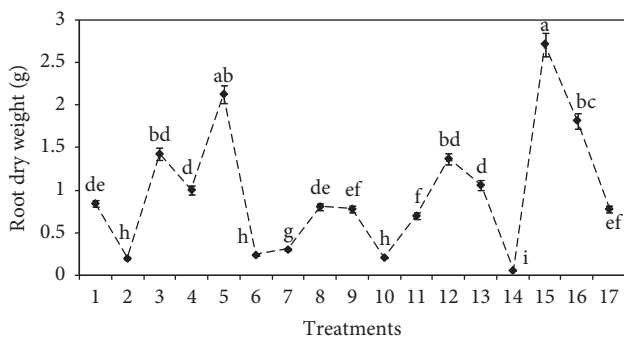


FIGURE 9: Impact of biosorbed water by various biosorbents (orange peels, pomegranate peels, and peanut peels) on root dry weight of mung bean after 7 days of seed germination. T1, negative control; T2, 50 mg kg⁻¹; T3, pomegranate peel biosorbed water +50 mg kg⁻¹; T4, peanut peel biosorbed water +50 mg kg⁻¹; T5, orange peel biosorbed water +50 mg kg⁻¹; T6, 100 mg kg⁻¹; T7, pomegranate peel biosorbed water +100 mg kg⁻¹; T8, peanut peel biosorbed water +100 mg kg⁻¹; T9, orange peel biosorbed water +100 mg kg⁻¹; T10, 150 mg kg⁻¹; T11, pomegranate peel biosorbed water +150 mg kg⁻¹; T12, peanut peel biosorbed water +150 mg kg⁻¹; T13, orange peel biosorbed water +150 mg kg⁻¹; T14, 200 mg kg⁻¹; T15, pomegranate peel biosorbed water +200 mg kg⁻¹; T16, peanut peel biosorbed water +200 mg kg⁻¹; T17, orange peel biosorbed water +200 mg kg⁻¹. Vertical bars (I): standard error (mean of 3 replicates). Alphabets on vertical bars: significant difference ($P \leq 0.05$) evaluated by the LSD test.

treatment resulting in lower binding sites of the adsorbents. The investigations are relatively valuable for developing a wastewater treatment plant. The process is economically reasonable and easy to carry out [33].

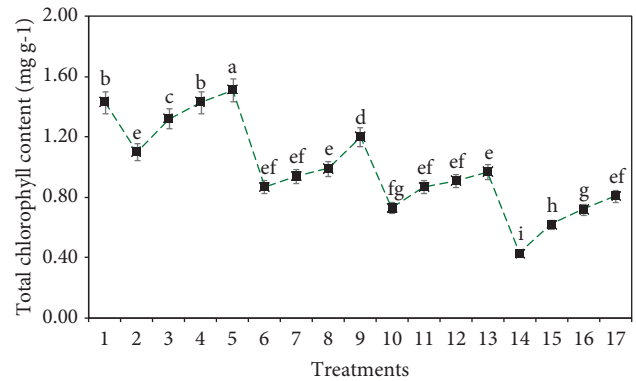


FIGURE 10: Impact of biosorbed water by various biosorbents (orange peels, pomegranate peels, and peanut peels) on total chlorophyll content of mung bean after 7 days of seed germination. T1, negative control; T2, 50 mg kg⁻¹; T3, pomegranate peel biosorbed water +50 mg kg⁻¹; T4, peanut peel biosorbed water +50 mg kg⁻¹; T5, orange peel biosorbed water +50 mg kg⁻¹; T6, 100 mg kg⁻¹; T7, pomegranate peel biosorbed water +100 mg kg⁻¹; T8, peanut peel biosorbed water +100 mg kg⁻¹; T9, orange peel biosorbed water +100 mg kg⁻¹; T10, 150 mg kg⁻¹; T11, pomegranate peel biosorbed water +150 mg kg⁻¹; T12, peanut peel biosorbed water +150 mg kg⁻¹; T13, orange peel biosorbed water +150 mg kg⁻¹; T14, 200 mg kg⁻¹; T15, pomegranate peel biosorbed water +200 mg kg⁻¹; T16, peanut peel biosorbed water +200 mg kg⁻¹; T17, orange peel biosorbed water +200 mg kg⁻¹. Vertical bars (I): standard error (mean of 3 replicates). Alphabets on vertical bars: significant difference ($P \leq 0.05$) evaluated by the LSD test.

Moreover, according to Yaneja and Kaur [34], cellulose, lignin, and hemicelluloses were the major components of agricultural residue with minor fractions of starch and water-soluble components. Cellulose is a polymer mainly composed of monomer C₆H₁₀O₅. Carbonyl, hydroxyl, and ether groups of cellulose might act as adsorbents (biosorbent) for metal ions by binding with metal ions. Agricultural residues such as orange peels were more effective for copper removal. Cu absorption ratio increased as the adsorbent particle size decreased. This can be because of the larger specific active surface of tiny particles, while most effective particles for biosorbent peels (peanut, orange, and pomegranate) were in the form of fine powder. Therefore, further experiments for biosorbent peels were done using 1.0 g of orange peels, 1.0 g of peanut peels, and 1.0 g of pomegranate peels in each solution of CuSO₄, 50 ppm, 100 ppm, 150 ppm, and 200 ppm, respectively. The accumulation efficiency of Cu by pomegranate peels was from 80% to 55%, while peanut peels were about 40%–37% and 90%–78% with the orange peel biosorption as the stock solution of Cu was increased from 50 ppm to 200 ppm. This observation was explained by the availability of less number of copper ions per unit mass of the biosorbent causing the site to remain unsaturated during the adsorption process. The maximum adsorption capacity of orange peels at optimum operating conditions was found to be 40–110 ppm [35].

The use of agricultural wastes might offer advantages for metal deletion from industrial effluents that are reported to

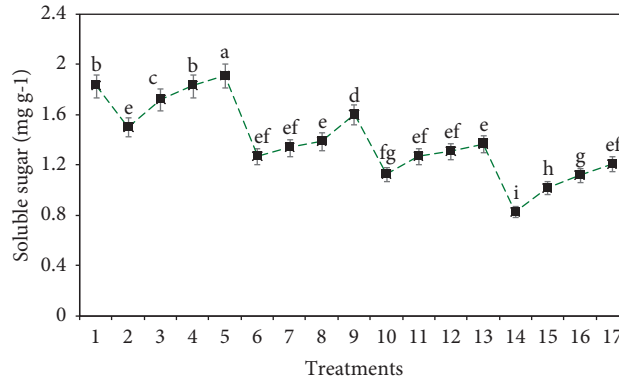


FIGURE 11: Impact of biosorbed water by various biosorbents (orange peels, pomegranate peels, and peanut peels) on soluble sugar content of mung bean after 7 days of seed germination. T1, negative control; T2, 50 mg kg⁻¹; T3, pomegranate peel biosorbed water +50 mg kg⁻¹; T4, peanut peel biosorbed water +50 mg kg⁻¹; T5, orange peel biosorbed water +50 mg kg⁻¹; T6, 100 mg kg⁻¹; T7, pomegranate peel biosorbed water +100 mg kg⁻¹; T8, peanut peel biosorbed water +100 mg kg⁻¹; T9, orange peel biosorbed water +100 mg kg⁻¹; T10, 150 mg kg⁻¹; T11, pomegranate peel biosorbed water +150 mg kg⁻¹; T12, peanut peel biosorbed water +150 mg kg⁻¹; T13, orange peel biosorbed water +150 mg kg⁻¹; T14, 200 mg kg⁻¹; T15, pomegranate peel biosorbed water +200 mg kg⁻¹; T16, peanut peel biosorbed water +200 mg kg⁻¹; T17, orange peel biosorbed water +200 mg kg⁻¹. Vertical bars (I): standard error (mean of 3 replicates). Alphabets on vertical bars: significant difference ($P \leq 0.05$) evaluated by the LSD test.

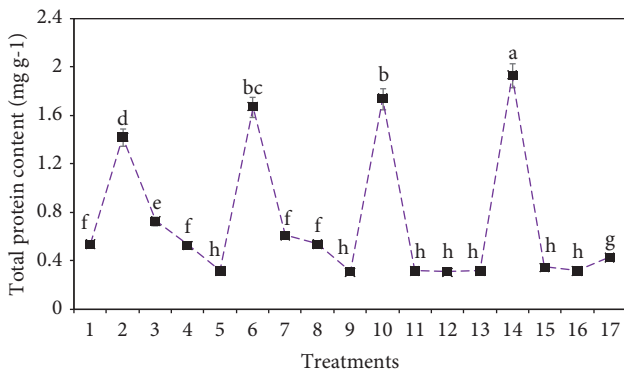


FIGURE 12: Impact of biosorbed water by various biosorbents (orange peels, pomegranate peels, and peanut peels) on total protein content of mung bean after 7 days of seed germination. T1, negative control; T2, 50 mg kg⁻¹; T3, pomegranate peel biosorbed water +50 mg kg⁻¹; T4, peanut peel biosorbed water +50 mg kg⁻¹; T5, orange peel biosorbed water +50 mg kg⁻¹; T6, 100 mg kg⁻¹; T7, pomegranate peel biosorbed water +100 mg kg⁻¹; T8, peanut peel biosorbed water +100 mg kg⁻¹; T9, orange peel biosorbed water +100 mg kg⁻¹; T10, 150 mg kg⁻¹; T11, pomegranate peel biosorbed water +150 mg kg⁻¹; T12, peanut peel biosorbed water +150 mg kg⁻¹; T13, orange peel biosorbed water +150 mg kg⁻¹; T14, 200 mg kg⁻¹; T15, pomegranate peel biosorbed water +200 mg kg⁻¹; T16, peanut peel biosorbed water +200 mg kg⁻¹; T17, orange peel biosorbed water +200 mg kg⁻¹. Vertical bars (I): standard error (mean of 3 replicates). Alphabets on vertical bars: significant difference ($P \leq 0.05$) evaluated by the LSD test.

have a lesser metal concentration (<100 mg l⁻¹) [36]. Some researchers reported that it was possible to remove various heavy metals from agricultural wastes, and the removal capacities of these agricultural wastes were significantly affected by initial metal concentration [36].

While the heavy metal removal methods are based on the conventional principle of ion exchange, coagulation, chemical precipitation, membrane filtration technology, and

biosorption, which are evolved in the earlier period, the risk of generating secondary pollutants by these methods is of great concern. Of all these methods above, the biosorption method is simple and relatively cost-effective and reduces the potential of yielding needless chemicals. It has been reported that biosorption is a promising technique for heavy metal adsorption from an aqueous environment, mainly when adsorbents are derived from biological sources: lingo-cellulose carbonaceous compounds, plant wastes, fruit and vegetable peels, natural resources, and agricultural wastes [37].

Although the toxic effects of copper were considerably adverse in plants, more uptake of Cu in plants causes significant negative effects that range from physiological and morphological to molecular levels, and these negative effects remain evident at all stages of plant growth. So, the higher concentration of Cu metal in various plants altered plant morphology root and shoot elongation. As the application of Cu increased in each treatment (10 mM and above), the shoot length and root length of the mung bean plant were similarly decreased as compared to the negative control [38].

For the optimal growth of plants, at concentrations higher than those required, Cu inhibits the growth of plants and interferes with important cellular processes such as respiration and photosynthesis. Plants grown in the presence of a higher amount of Cu²⁺ usually show a reduction in biomass and chlorotic symptoms. Lower chlorophyll content, alterations of chloroplast structure, and thylakoid membrane composition were found in leaves under such growth situations. In particular, degradation of stroma, lamellae, and grana stacking, increase in the number and size of plastoglobuli, and appearance of intrathylakoidal inclusions were observed. Cu²⁺ interferes with the photosynthetic machinery that modifies the pigments and proteins composition of photosynthetic membranes.

Furthermore, peroxidation of lipids, lipid content deduction, and changes caused by the fatty acid composition in

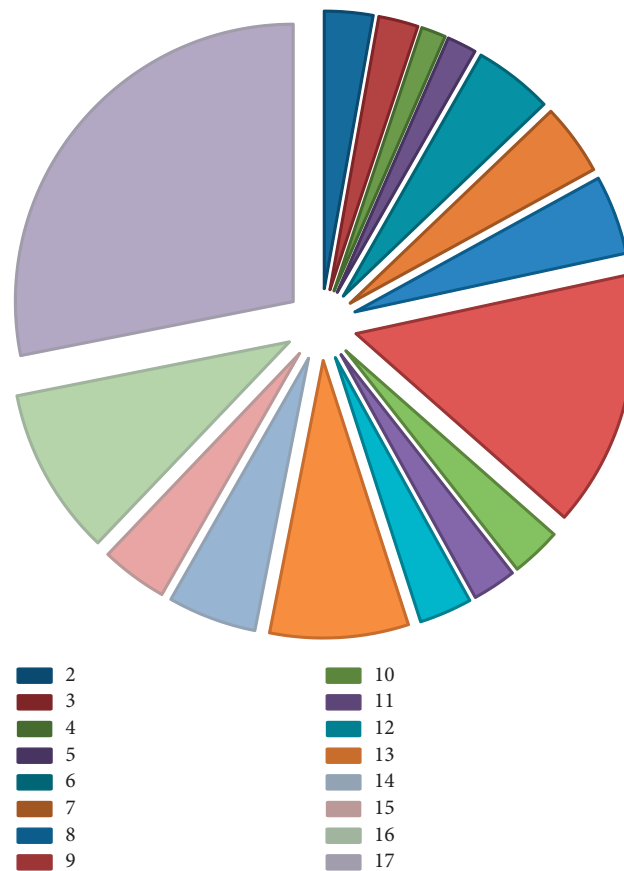


FIGURE 13: Tolerance index of mung bean plant grown under the stress of Cu^{2+} metal and biosorbed water after 15 days of seed germination. T1, negative control; T2, 50 mg kg^{-1} ; T3, pomegranate peel biosorbed water + 50 mg kg^{-1} ; T4, peanut peel biosorbed water + 50 mg kg^{-1} ; T5, orange peel biosorbed water + 50 mg kg^{-1} ; T6, 100 mg kg^{-1} ; T7, pomegranate peel biosorbed water + 100 mg kg^{-1} ; T8, peanut peel biosorbed water + 100 mg kg^{-1} ; T9, orange peel biosorbed water + 100 mg kg^{-1} ; T10, 150 mg kg^{-1} ; T11, pomegranate peel biosorbed water + 150 mg kg^{-1} ; T12, peanut peel biosorbed water + 150 mg kg^{-1} ; T13, orange peel biosorbed water + 150 mg kg^{-1} ; T14, 200 mg kg^{-1} ; T15, pomegranate peel biosorbed water + 200 mg kg^{-1} ; T16, peanut peel biosorbed water + 200 mg kg^{-1} ; T17, orange peel biosorbed water + 200 mg kg^{-1} . Vertical bars (I): standard error (mean of 3 replicates). Alphabets on vertical bars: significant difference ($P \leq 0.05$) evaluated by the LSD test.

thylakoid membranes were also observed. As a result of such modifications, variation of PSII membrane fluidity was also found. Yruela, proposed that the processes induced by Cu^{2+} could involve either the destruction of the oxygen-evolving complex polypeptide composition or the contact with ions that are essential for the proper working of the complex such as Mn, Ca, and Cl, etc. that are essential for the proper working of the complex such as Mn, Ca, and Cl [39]. It is well known that, in the nonenzymatic chemical reaction between superoxide (O_2^-) and H_2O_2 , Cu acts as a transitional metal and speeds up the configuration of hydroxyl radicals (OH^\cdot) (Haber-Weiss reaction) [40].

Hence, the presence of Cu^{2+} in excess amounts can cause oxidative stress in plants and subsequently increase the antioxidant responses due to the increased production of highly toxic oxygen free radicals. Accordingly, it was observed that excess Cu^{2+} in plants led to oxidative stress-inducing changes in the activity and content of some components of the antioxidative pathways such as ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), superoxide dismutases (SODs), and guaiacol peroxidase. The

antioxidant responses were observed in leaves and roots being both Cu-concentration dependent and time-dependent. The ascorbate-gluthathione cycle has been reported to be involved in response to excess Cu^{2+} [41].

5. Conclusion

Heavy metal contamination of water has become alarmingly more important in recent years. The focus of present research is on developing a viable solution to either avoid or decrease heavy metal contamination to a manageable level. Heavy metal pollution of water bodies may only be controlled by decreasing direct discharge into the water stream. The most extensively used traditional techniques for removing heavy metals have a number of drawbacks, including high capital and operating costs, incompatibility with small-scale industries, and inefficiency. One of the most promising methods for eliminating heavy metals from wastewater is adsorption. The adsorption technique works efficiently even at very minimal concentrations. Therefore, the use of low-cost agricultural wastes is promoted for the removal of heavy metals.

In the current research work, orange peel (OP), pomegranate peel (POP), and peanut peel (PP) biosorbed waters were initially exploited to biosorb copper heavy metal. The biosorbed water was also utilized to irrigate soil cultivated with mung beans under induced copper stress. The biosorbed waters significantly enhanced the growth and physiological parameters of the mung bean plants germinated under the stress condition of Cu^{2+} heavy metal. The peanut peel biosorbed water promoted the shoot development (29.8 cm) and shoot biomass formation, while pomegranate peel biosorbed water induced rooting in plants (6.81 cm) germinated in the presence of a higher concentration of Cu^{2+} . The orange peel biosorbed water elevated the total chlorophyll content and soluble sugar content in mung beans (1.56 mg/g and 1.89 mg/g).

It is concluded from the current research that biosorbed water made from agricultural waste can be used to absorb heavy metals in water. Heavy metals are collected by plants, which can not only affect their production but also be consumed by animals and humans. Hence, biosorbed water should be used for irrigation of agricultural land to minimise the entry of toxic heavy metals into the food chain.

Abbreviations

OP:	Orange peels
POP:	Pomegranate peels
PP:	Peanut peels
As:	Arsenic
Hg:	Mercury
Pb:	Lead
Cd:	Cadmium
Cr:	Chromium
Tl:	Thallium
Cu:	Copper
Se:	Selenium
Zn:	Zinc
CuSO_4 :	Copper sulphate
S.S:	Stock solution
NaOH:	Sodium hydroxide
HCl:	Hydrochloric acid
E:	Efficiency
LSD:	Least Significant Difference
(-ve):	Negative
(+ve):	Positive
POPBW:	Pomegranate peel biosorbed water
PPBW:	Peanut peel biosorbed water
OPBW:	Orange peel biosorbed water
APX:	Ascorbate peroxidase
GR:	Glutathione reductase
MDHAR:	Monodehydroascorbate reductase
DHAR:	Dehydroascorbate reductase
SODs:	Superoxide dismutases.

Data Availability

All the data are available within the article.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

All the authors contributed equally in performing experimental procedures, data analysis, and manuscript writing.

Acknowledgments

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