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Original article

Application of aquatic plants alone as well as in combination for phytoremediation of household and industrial wastewater

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A B S T R A C T

Present study was undertaken to establish the potential of the local weeds of Pakistan (Islamabad, Rawalpindi) such as water lettuce (WL), alligator weed (AW), pennywort (PW), and duckweed (DW) as phytoremediation agents individually and in combinations (COMB) for the removal of pollutants from the wastewater. The results showed that the treatment of effluent with DW and PW resulted in 100% and 81% removal of lead (Pb) from industrial and the treatment AW resulted in 100% removal of Pb from household wastewater. Treatments PW and COMB of the industrial wastewater showed 81% removal of zinc (Zn), and treatment of household wastewater with AW resulted in 100% removal of Zn. Treatment with WL resulted in 88% and 77% of chloride removal from industrial and household wastewater, respectively. A 28% removal of ammonia was obtained with PW treatment of industrial wastewater, while AW treatment resulted in 100% ammonia removal and 85% removal efficiency for the phosphate. The COMB treatment resulted in 51% removal of sulfate from household wastewater, and WL resulted in 79% removal of potassium from industrial wastewater. In conclusion, all the weeds resulted in significant removal of contaminants from wastewater; however, alligator weed exhibited the best phytoremediation potential compared to other treatments.

1. Introduction

Water is a valuable asset on earth but unfortunately, it has been subjected to extreme misuse and has severely been contaminated due to anthropogenic activities (Pacheco and Fernandes, 2020). These contaminants are harmful and responsible for causing severe threats to the environment and human health (Kumar et al., 2016). Nalla lai is a natural stream flowing through various areas of Rawalpindi city of Pakistan. It is a rain water fed stream and contains many tributaries originating from Margalla hills, Islamabad and Rawalpindi. The household wastewater from the nalla lai contains many contaminants such as chemicals, death waste, fertilizer industry waste, heavy metals i-e Cd, Pb, Zn, Fe, NO_3, TDS, and other sewage toxic material. The waste material of this nalla is of major concern because the disposal of health hazard garbage resulted in floods and poor water quality. Similarly, industrial wastewater contains elevated levels of metals of toxicological concern (Briffa et al., 2020). Industrial area of Islamabad (I-9) contains different industries such as pharmaceutical, flour, electroplating, plastic, marble, soap and chemical industries pose a serious threat to contaminate the surrounding water bodies. Although several physico-chemical techniques are available to remove waste material and metals of toxicological concern from soil and water but these techniques are expensive and less effective when the concentrations of toxic compounds are high. Therefore, there is a need for the alternative and environmental friendly measures and one such measure is the phyto-remediation techniques which is the best alternative for cleaning up the wastewater because naturally occurring processes make it environmentally friendly, less expensive, and aesthetically pleasing (Dixit et al., 2015). Phytoremediation refers to the use of plants and associated microorganisms which work

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together wholly or partially remediate the selected pollutants from soil, wastewater and groundwater. It has been reported that aquatic plants such as duckweed showed remarkable potential for the removal of contaminant through degradation by intricate root system from the wastewater (Mustafa and Hayder, 2020). Similarly, alligator weed has been reported to remove considerable concentration of arsenic and lead from the wastewater in the laboratory scale experiment (Simmons et al., 2007). Water quality of the eutrophic ecosystem has been improved using the *Pistia stratiotes* L. (water lettuce) as a phytoremediation agent (Nahar and Hoque, 2021). A large number of plants which are called hyperaccumulator show a great potential for the removal of toxins from the wastewater using various parts such as root, shoot, leaves without showing the symptoms of toxicity. However, there are some bottlenecks regarding the efficiency of plants used in the phytoremediation such as hyperaccumulator have small biomass as well as not all plants are suitable for the removal of all types of the contaminant present in the wastewater. Therefore, it is necessary to use proper plant species which must be tolerant and remediate maximum types of the contaminant. Moreover, screening and breeding techniques must be adopted to improve plant biomass, tolerance and enhance grow rate of the plant. As the literature is scanty related to the treatment of wastewater coming from both the above mentioned sources specifically I-9 industrial area using phytoremediation process specifically using the plants mentioned in the current study, therefore, the present investigation was aimed to evaluate the potential of indigenous plants for the phytoremediation of household and industrial wastewater individually and as well as in combination.

2. Materials and methods

2.1. Sampling of wastewater

Two types of wastewater were collected, one was industrial effluent and other was household wastewater. The industrial effluent was collected from I-9 industrial area, (33°41′3.9192″ North and 73°2′52.3752″ East) Islamabad, and household wastewater was collected from Nalla lai (33°33′ and 33°46′ North and 72°55′ and 73°07′ East), Rawalpindi (Fig. 1). These samples were stored at 4 °C before using in the experiment.

2.2. Sampling of plant species

Plants used in the present study were collected from National Agricultural Research Center, Islamabad. Selection of the plant was made based on their potential to absorb waste material and the season of growth. Following medium-sized plants were collected: water lettuce (Pistia stratiotes), duckweed (*Lemna gibba* and *Lemna minor*), alligator weed (Alternanthera philoxeroides), and pennywort (Hydrocotyle umbellata). The samples were collected in the plastic bags and were transported to the lab by keeping in the ice container.

2.3. Physicochemical analysis of wastewater

Wastewater samples were filtered to remove the sludge, insects, and debris and then were autoclaved (V150, Systec, Germany) for initial analysis.

2.4. Experimental setup

For the experiment, plastic trays were arranged; five treatments with triplicate were selected for the each industrial and household wastewater Plants were thoroughly washed and individually planted in the trays containing 4000 mL wastewater. The fifth tray for each waste (household and industrial) was used for the combined plantation of all four plants to evaluate their combined effect. Growth conditions were as follows, average temperature during the experiment was 26–28 °C, plants were grown with a light and dark period of 12 hr and the relative humidity was 80%. Physical appearance of the plants was observed during the course of the experiment.

The experiment was harvested after four weeks. Water from each household and industrial waste tray was collected and filtered in clean plastic bottles.

Fig. 1. (A) I-9, Industrial area, Islamabad, Pakistan (B) Nalla lai (household area), Rawalpindi, Pakistan (Source: Google map).

2.5. Sample preparation for metals analysis

All chemicals, solvents and reagents used were of analytical grade and purchased from Sigma-Aldrich, USA. In wastewater (50 mL), concentrated HNO_3 (5 mL) was added and the volume of the mixture was reduced to 10 mL through boiling slowly. After cooling, 2.5 mL of concentrated HNO_3 was added, followed by digestion through the heating digestion process. After drying, the mixture was cooled and HCl solution (2.5 mL) was added. The mixture was warmed again (95 °C ± 5 °C), and NaOH 5 M (2.5 mL) was added. The mixture was finally filtered through Whatman No. 1 filter paper. Then the filtrate was diluted upto 50 mL with distilled water, and metals were detected by atomic absorption spectroscopy (Perking Elmer Analyst 100 Atomic Absorption Spectrophotometer, USA).

2.6. Determination of phosphate, sulfate, ammonia, chloride, and total hardness

To determine phosphate content in wastewater (Morris et al., 1966), 50 mL of the wastewater sample, 1 mL of sulfuric acid, and potassium persulphate (0.4 g) were added and the mixture was boiled for 30 min. After cooling, this mixture was transferred to the Nessler tube, and the volume was raised to 50 mL with distilled water. Then ammonium molybdate solution (2 mL) and stannous chloride (3 drops) were added and mixed, then optical density was measured at 650 nm.

To determine sulfate (APHA, 2012), in a wastewater sample (20 mL) 1 mL of HCl and conditioning reagent (1 mL) were added and mixed for 30 s. Then the sample was analyzed at 420 nm through Spectrophotometer (UV-5100, Shanghai Leewen Scientific Instrument Co., Ltd. China).

The Kjeldahl method was used to determine ammonia levels from wastewater (Dell, 1993). First, the water sample was prepared by removing the chlorine by adding a dechlorinating agent and 1 N NaOH solution to Erlenmeyer flask containing 50 mL boric acid (2%). Then the analysis of the sample was carried out.

Chloride contents were measured by Mohr Method (Kraemer and Stamm, 1924) through titration. Firstly, the pH of wastewater sample (25 mL) was measured. Then indicator (potassium chromate) was added, and titration was carried out by silver nitrate until the endpoint was obtained. The pH was measured and calculations were made.

Total hardness was measured by EDTA titration method (Betz and Noll, 1950). In 50 mL of wastewater, 2 mL buffer solution was added, followed by the addition of Eriochrome Black T solution as an indicator. Next, titration was carried out with EDTA (0.01 M) until the solution was turned sky blue from wine red. Then, titration was repeated to obtain concordant results.

2.7. Statistical analysis

Statistix software (8.1 version) was used for the analysis of variance (ANOVA), and the least significant difference (LSD) was used to compare mean values according to Steel and Torrie (1980) at $P < 0.05$.

3. Results

3.1. Physicochemical analysis of wastewater

Initial temperature of the wastewater samples collected for the experiment was 28°C for industrial and 26°C for household wastewater samples. In the beginning, the color of both the wastewater samples was light brownish-gray with unpleasant odor. However, after a couple of days of cultivation of plants in wastewater, the color of sewage started to become transparent and clear, and the unpleasant odor also vanished.

3.2. pH measurement

Initially, the pH values of BE (before experiment) samples of industrial and household wastewater were 8.6 and 6.8, respectively. At the end of the experiment, the pH values of all the treated samples of industrial and household wastewater were almost neutral (Fig. 2A). However, in industrial wastewater, a significant decrease (17%) in the pH was recorded by the treatments COMB (combined), whereas, in household wastewater, pH was significantly increased by all the treatments compared to the value of BE.

3.3. Removal efficiency of lead (Pb), zinc (Zn) and chloride (Cl)

A significant reduction in lead (Pb) concentration was recorded in both household and industrial wastewater samples by all the treatments (Fig. 2B). However, treatment DW (Duckweed) resulted in maximum removal of 100% of Pb from industrial wastewater as compared to BE. The treatment of industrial wastewater with WL (water lettuce), AW (Alligator weed), and PW (Pennywort) showed 63%, 72%, and 80% reduction in Pb concentration, respectively. Similarly, in household wastewater treatments, maximum removal efficiency (100%) was recorded by AW treatment.

All the treatments reduced the Zn concentration compared to BE; however, treatment AW of household wastewater resulted in 100% removal efficiency while DW treatment showed 83% removal of Zn compared to BE (Fig. 2C). Moreover, treatments PW and COMB resulted in a significant reduction (81%) of Zinc (Zn) concentration from industrial wastewater compared to BE.

Fig. 2D showed that treatment WL resulted in 88% and 77% maximum significant removal of chloride from industrial and household wastewater, respectively. Moreover, treatments AW and PW showed 79% and 72% significant reduction as compared to the BE, respectively.

3.4. Removal efficiency of potassium (K), calcium (Ca), ammonia (NH_3), phosphate (PO_4^{2-}), sulfate (SO_4^{2-}), and total hardness of water

Fig. 3A showed that treatments WL resulted in a maximum (79%) significant reduction in K concentration compared to BE in industrial wastewater. Treatment AW of the household sewage resulted in a maximum (77%) reduction of K concentration compared to BE.

All the treatments resulted in a significant reduction in the concentration of Ca in both types of wastewater compared to BE (Fig. 3B). However, AW resulted in a maximum decrease (47%) in calcium concentration among industrial wastewater treatments as compared to BE. Similarly, PW (36%) resulted in a maximum significant decrease in calcium concentration among household wastewater treatments compared to BE.

Significant reduction in ammonia concentration was recorded in all the treatments of both wastewaters; however, among industrial wastewater, treatment PW resulted in an appreciable decrease (73%) in the concentration of NH_3 as compared to BE followed by WL treatment (Fig. 3C). Household wastewater treated with AW showed an effective removal (100%) of NH_3 followed by PW treatment.

All the treatments resulted in a significant reduction in the phosphate concentration in both types of wastewater compared to BE (Fig. 3D). Treatment AW of both types of wastewater showed a significant (76% and 92%) phosphate removal compared to the BE treatment. Treatment COMB of household wastewater resulted in

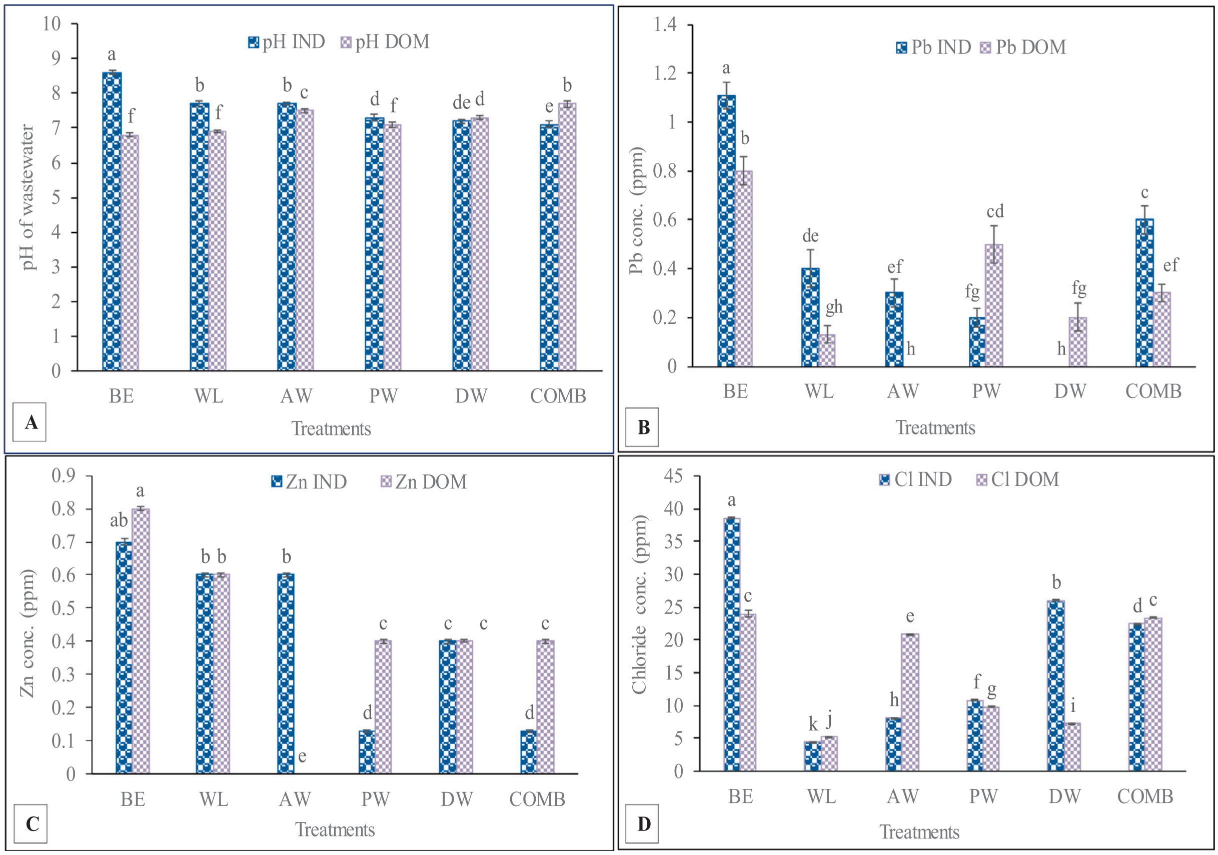

Fig. 2. pH (A), Lead (B), Zinc (C), and Chloride (D) of industrial and household wastewater samples before and after the experiment of phytoremediation. Details of the symbols used for treatments. **BE** (Before experiment), **WL** (Water lettuce), **AW** (Alligator weed), **PW** (Pennywort), **DW** (Duckweed), **COMB** (Combined), IND (Industrial wastewater), DOM (Household wastewater) All means that share a common English letter are similar, otherwise differ significantly at P < 0.05.

an appreciable amount of reduction (85%) in phosphate contents compared to BE.

A significant reduction in sulphate concentration was recorded in all the treatments of both wastewaters. However, treatment AW of industrial sewage and COMB of household wastewater resulted in maximum significant removal of sulphate concentration (61% and 78%, respectively) compared to the BE (Fig. 4A).

All the treatments of both wastewaters recorded a significant decrease in total hardness. However, maximum reduction (29% and 34%) was recorded by treating AW of industrial wastewater and PW of household wastewater, respectively (Fig. 4B).

4. Discussion

The results obtained from the current experiments showed a diverse relationship between the pollutants concentration and the absorption and leaching behavior of different plant treatments. After cultivating plants in wastewater, the color of wastewater became transparent and clear, and the unpleasant odor also vanished. The reason might be the absorption of the contaminants by the plants, which reduced the unpleasant smell and changed the wastewater's color. Similar results were reported by Ahila et al. (2021) that the treatment of wastewater by macrophytes resulted in the removal of the color of the wastewater. Before the experiment, the pH of industrial wastewater was 8.6, and at the end of the experiment, it became neutral. The decrease in water pH might be due to the uptake of metals of toxicological concern by weed plants that absorbed the heavy metal ions and thus reduced their concentration in the effluent. The reduction in pH might be due to the absorption of nutrients and other salts by plants or the simultaneous release of H^+ ions. Nutrient cations are attracted to the cortex cells of the roots due to charged surface and the cation exchange takes place over here. There is a release of H^+ as a result of cation exchange resulting in a decrease in pH of the medium.

Similar results were reported by Saha et al. (2015), who described that phytoremediation causes a gradual reduction in the pH, thus resulting in an improvement of water quality. In household wastewater, the sources of acidic contents like phosphate, sulfate, and carbonate ions come from household cleaning washing materials while small industries like paints, pharma, cosmetic, and workshop raw materials make the water acidic. The plants' uptake of phosphates and sulfates has resulted in increased pH of the treatments. Another reason for the change in pH from acidic to neutral could be that more protons (H^+) are present at low pH, which can compete with the metals at uptake sites. As the pH increases, fewer protons will be available to compete with the metals of toxicological concern at the uptake sites (2011). This is again a positive indicator of the efficiency of the treatments. Zeng et al. (2008) observed that rice roots significantly change the rhizosphere pH towards neutrality under chromium stress. The heavy metal stress inhibits the activity of H^+-ATPase (Rizvi et al., 2020) and thus increases the apoplastic pH. Such a decrease in ATPase activity results in decreased proton efflux, which ultimately would cause an increase in the pH of the rhizosphere and the surrounding water.

Lead is the inorganic environmental pollutants which can be absorbed in the soil easily and can be a part of food chain by taking up by the plants (Guedes et al., 2021). In this way, it poses a significant health hazardous effect such as hepatic, hematological,

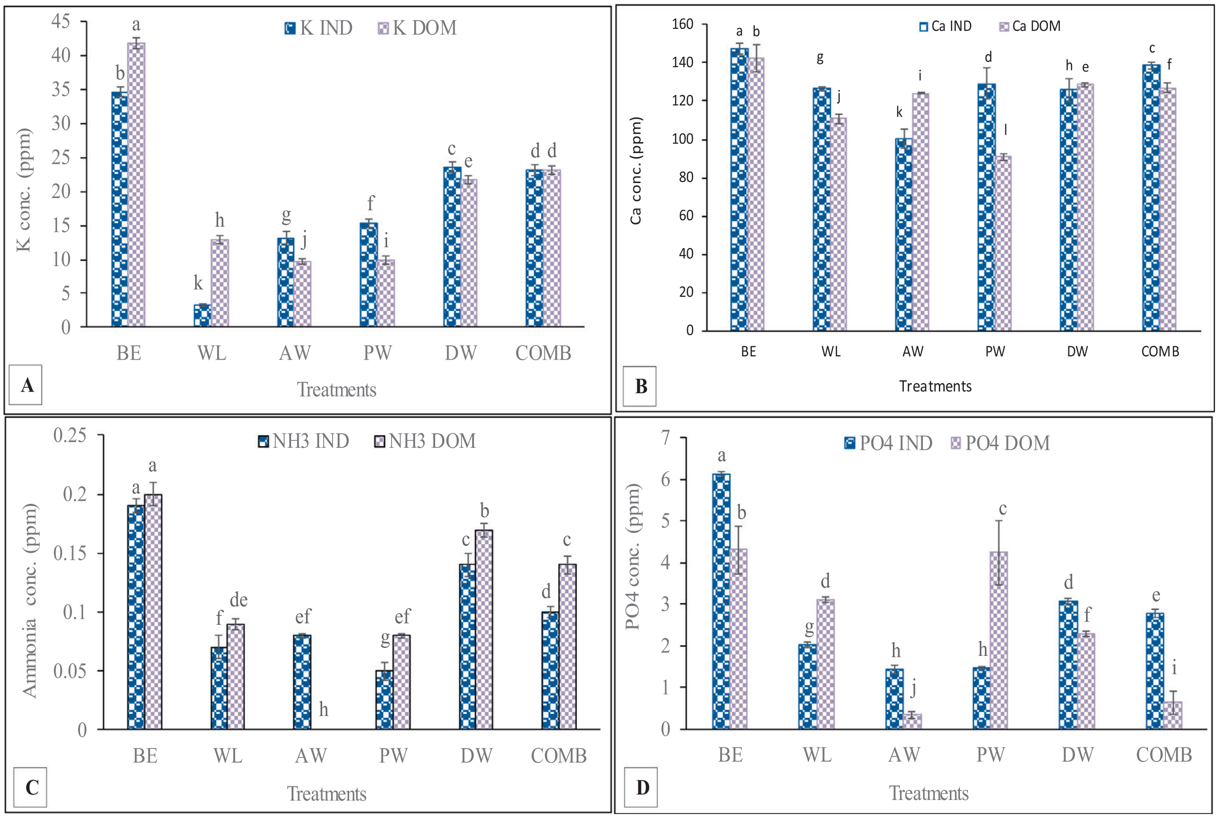

Fig. 3. Potassium (A), Calcium (B), Ammonia (C), and Phosphate (D) concentration of industrial and household wastewater samples before and after the experiment of phytoremediation. Details of the symbols used for treatments. **BE** (Before experiment), **WL** (Water lettuce), **AW** (Alligator weed), **PW** (Pennywort), **DW** (Duckweed), **COMB** (Combined), IND (Industrial wastewater), DOM (Household wastewater). All means that share a common English letter are similar, otherwise differ significantly at $P < 0.05$.

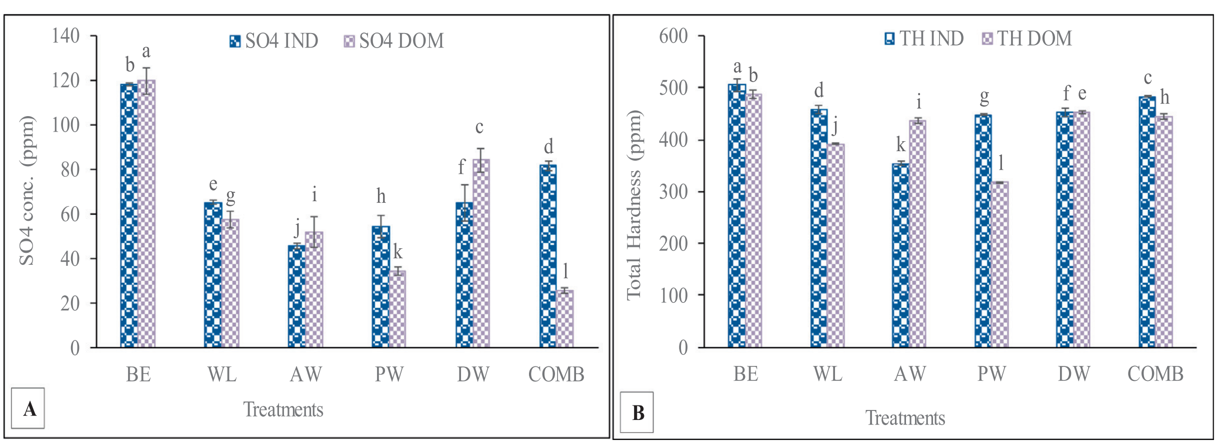

Fig. 4. Sulfate (A) and Total hardness (B) of industrial and household wastewater samples before and after the experiment of phytoremediation. **BE** (Before experiment), **WL** (Water lettuce), **AW** (Alligator weed), **PW** (Pennywort), **DW** (Duckweed), **COMB** (Combined), IND (Industrial wastewater), DOM (Household wastewater). All means that share a common English letter are similar, otherwise differ significantly at $P < 0.05$.

neurological and renal dysfunctions (Amin et al., 2021). Significant reduction of Pb from the industrial wastewater treated with PW and DW was reported in the current study because DW has been reported as a good accumulator of Pb (Verma and Suthar, 2015) in the previous study. Ahila et al. (2021) reported that freshwater macrophytes proved to be good accumulator of Pb from the wastewater. Alaboudi et al. (2018) reported that sunflower could remove lead and cadmium from the soil. Similarly, Putra et al. (2013) reported that water lettuce accumulated a high concentration of lead in its roots. As the plants use a variety of mechanisms for the remediation of waste material depending upon the plant species and type of the waste material and such mechanism has been shown in the Fig. 5. The significant reduction in Zn concentration industrial wastewater by PW treatment might be because PW has the highest potential of Zn absorption. The present study's findings are in accordance with the observations of Li et al. (2018). They reported that PW could accumulate Zn if it is present in low concentrations in the wastewater. In the case of household wastewater, treatments WL and DW effectively reduced the Zn concentration. Lu et al. (2011) reported that WL is a hyperaccumulator of Zn. The DW resulted in a 72% reduction in the concentration of Zn and can be used as biological filters for

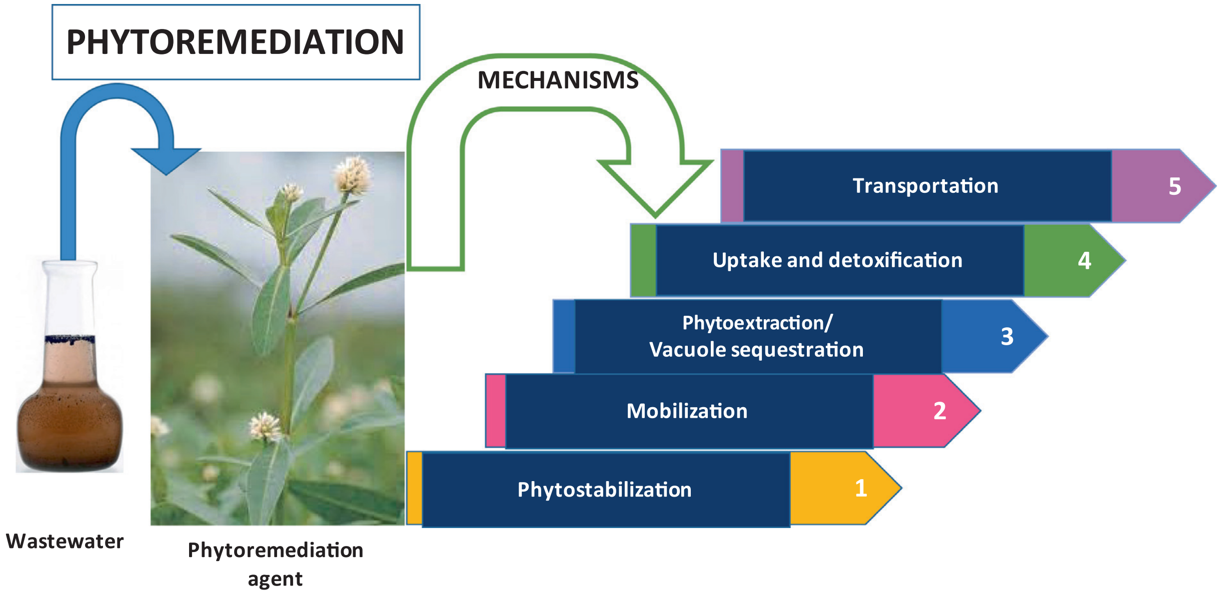

Fig. 5. Mechanisms involved in phytoremediation of wastewater.

Zn-contaminated waters and is effective in the removal and accumulation of Zn from a nutrient medium (Azeez and Sabbar, 2012).

The maximum removal efficiency of chloride contents by WL from industrial and household wastewater has been recorded in the current study and similar results were reported by Saha et al. (2015), who found that DW treatment caused 30% reduction of chloride content from wastewater. Gupta and Prakash (2014) reported that DW is specially adapted to remove chloride and sulfate from wastewater and acts as an efficient sink to harvest nutrients and metals of toxicological concern from the wastewater bodies. Treatment WL from industrial and AW from household wastewater showed maximum reduction in the K contents as compared to BE in the current experiment. Although the presence of potassium in the municipally treated wastewater has not been evident to cause any health effect but the higher concentration of K is reported to cause health effect on the susceptible individuals and can cause hypertension and heart disease to these people (Restuccio, 1992). Therefore, the optimum level is necessary for each nutrient which can be obtained by using such environmental friendly measures. Treatment AW of industrial and PW of household wastewater resulted in maximum reduction of calcium from wastewater as compared to other treatments. It is reported that calcium is an essential nutrient required by the organisms but the higher concentration of calcium resulted in the hardness of the water. The water hardness has negative effect on the availability and absorption of the other nutrients which results in the deficiency of other nutrients. Therefore, the optimum concentration is important and in the current study, the selected plants have made a contribution to reduce the level of calcium from the waste water by various mechanisms (Cotruvo and Bartram, 2009).

Ammonia is a preferential nitrogen source for plants when present at low concentrations, but it can be phytotoxic if present in high concentrations. Dias et al. (2020) reported removing ammonia from the wastewater using macrophytes and aggregates of other substances, and these results are in agreement with our findings. Similarly, Gupta and Prakash (2014) also reported removing ammonia from the wastewater about 99% by the duckweed. The biosorption is the possible mechanism through which removal of ammonia is carried out from the wastewater. It has been reported in a previous study that duckweed use ammonia from wastewater for the buildup of plant protein.

In the current study, treatment AW and COMB showed a reduction in PO_4^{2-} concentration. Phosphorus is used to form ADP and ATP and build their biochemical, structural components like nucleic acids, nucleotides, sugar phosphates, etc., especially during their growth of the plant. When plants are growing, they need a high amount of phosphorus to build up their biomass (Ng and Chan. 2017). The plants in the growing stage and needed phosphate for the growth reduced PO_4^{2-} concentration in industrial and household wastewater samples. The COMB and PW treatments showed a reduction in the SO_4^{2-} concentration in the household and AW in industrial wastewater samples. It has been reported by Gupta et al. (2015) that floating plants reduced the sulfates, nitrate, and iron significantly from the municipal waste and tap water. Sivakumar et al. (2015) reported that DW removed 80% sulfate from the textile industry effluent. The water hardness is the total concentration of calcium and magnesium ions in the water. There is a direct relationship between the concentration of calcium and the total hardness of water which is evident from the results of current study. The increase in calcium concentration increased the hardness of wastewater and the treatments AW of industrial and PW of household wastewater resulted in maximum reduction of water hardness as compared to all other treatments. The increased hardness interferes with the action of detergent and soap and also results in blockage or reduction in flow of water in the piper due to formation of the calcium sulfate or calcium carbonate deposits (Maktoof et al., 2020). Similar results were reported by Ahila et al. (2021), who observed that *P. stratiotes* L. efficiently removed 66% of total hardness from the dye-contaminated wastewater. Previous study (Maktoof et al., 2020) reported similar results that high removal efficiency of total hardness, Mg^{2+}, Na^{+}, NO_3^{-}, TDS, SO_4^{2-} and alkalinity was obtained by Schoenoplectus litoralis as compared to Hordeum vulgare in a phytoremediation experiment.

5. Conclusion

Based on the above discussion, it is inferred that indigenous plants showed a significant amount of removal efficiency of waste material from the wastewater. However, Alligator weed (AW) resulted in maximum lead, zinc, ammonia, phosphate, and potassium removal efficiency. Moreover, combination of alligator weed with water lettuce significantly reduced both effluents' chloride and potassium content. Therefore, out of all of the treatments, alligator weed showed significant removal of most of the pollutants and can be suggested as the best phytoremediation agent. However, further study is needed to evaluate the uptake capacity of each plant for each heavy metal and their synergistic effect with the microbes to remove the waste material from the wastewater.

Availability of data and materials

All data generated or analyzed during this study are included in this article.

CRediT authorship contribution statement

Mahnoor Raza: Conceptualization, Investigation, Methodology, Writing – original draft, Data curation. **Asia Nosheen:** Supervision, Conceptualization, Resources, Software, Writing – review & editing. **Humaira Yasmin:** Formal analysis, Software, Writing – review & editing, Validation. **Rabia Naz:** Software, Validation, Writing – review & editing, Visualization. **Syed Muhammad Usman Shah:** Data curation, Methodology. **Jaweria Ambreen:** Formal analysis, Funding acquisition, Validation. **Mohamed A. El-Sheikh:** Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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