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Phytoremediation potential of *Pistia stratiotes, Eichhornia crassipes,* and *Typha latifolia* for chromium with stimulation of secondary metabolites

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ABSTRACT

Anthropogenic activities have significantly polluted the natural environments all over the world. Leather processing industries release toxic heavy metals through their effluents posing a great threat to the environment. Chromium (Cr) is the major component of tannery effluents. We designed this experiment with the aim to remediate Cr from effluents of tanneries through phytoremediation. We selected three native macrophytes i.e. Pistia stratiotes, Eichhornia crassipes, and Typha latifolia to grow in a set of Constructed Wetland systems (CWs) with a continuous supply of tannery wastewater. T. latifolia was the most efficient phytoremediator of these macrophytes as it reduced the Cr content by 96.7%. The effluent after passing through the CWs containing T. latifolia showed only 0.426 mg/L Cr content. All macrophytes showed an enhanced phytochemical activity such as total antioxidant activity (TAA), total reduction potential (TRP), total phenolic content (TPC), total flavonoid content (TFC), and DPPH radical scavenging activity (DPPH) substantially. The activation of antioxidant mechanism may have contributed towards robust defense system of these plants for survival in excessive Cr contaminated media. Also, these macrophytes showed a positive relationship in reducing Cr content from tannery wastewater. Results of this study could help in effective sustainable management of aquatic environments contaminated with metal pollutants from human activities.

1. Introduction

Sialkot is a well-known industrial city of Pakistan and is one of the major exporters of leather around the world. The processing of leather generates large quantities of waste materials that may cause contamination of the soil and water [1]. The effluents produced by tanneries are released into the environment. About 250 tanneries are present in Sialkot city and its suburbs. In a single day, 215,036.1 gallons of effluents are released here from the tanning industry alone [2,3]. Several harmful chemicals are used in tanneries in various processing applications and it is very tough to overcome contamination by conventional means [4]. Currently, the most sources of

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irrigation water in urban areas in Sialkot are contaminated with tannery effluents. The emission of these effluents may cause substantial damage to the environment and could be a threat to all life forms [5].

Chromium (Cr) is a toxic metal which is mainly released from tanning industries [3]. It is both a toxic and a non-essential element [6]. Plants have specific transporters for the uptake and transport of mineral ions required for various metabolic processes. Cr enters the plants through these transporters; rate of uptake, transport and accumulation depends upon its metal speciation and plant species thus determining the overall toxic effects such as decline in growth and productivity of the plant [7]. It is among the most hazardous chemicals hostile to all living things including humans [8]. Different physiochemical approaches such as soil replacement, chemical application etc. have been used for the treatment of water pollutants, but the individual setups needed for these techniques are often costly [9]. On the other hand, biological approaches are cost-effective and human friendly [10]. The use of phytoremediator plants against metal toxicity has gained significant interest because of its ecologically friendly nature, minimal cost, and natural potential to absorb metals [11,12].

Constructed wetlands (CWs) are human made structures more or less like natural wetlands used for holding water for various purposes but specially for the remediation of water pollutants. CWs could be an efficient remediation unit for heavy metals and a promising solution to cleaning up of wastewater before it gets discharged into the environment. In CWs, macrophytes and/or microorganisms are used to absorb pollutants from the contaminated water [13]. Floating plants are the most significant part of marine and wetland ecosystems [10]. The have been proven for their adaptation to water toxicity [11]. CWs for the decontamination of contaminated wastewaters have been highly recommended especially in the current situation of rising demand for water and energy sources in agricultural and industrial fields [14]. In CWs, these floating plants may perform several roles such as stabilization, retention of nutrients, enhancement of microbial community, removal of toxic substances, and tolerance for complex wastewater effluents for effective phytoremediation [15].

Water lettuce (*Pistia stratiotes*) is an aquatic plant also known as a bioaccumulator. Its highly absorbing roots offer a wide surface area for the growing population of microbes which may further enhance its ability to absorb pollutants [16]. Water hyacinth (*Eichhornia crassipes*) also has long roots that penetrate deep into the water for an efficient ion accumulation [17]. Some studies have proved its ability to act as a phytoremediator to extract metals from the metal contaminated sites [18]. The third plant used in this experiment, the cattail (*Typha latifolia*), is emergent and most productive natural plant species in temperate aquatic ecosystem [1]. *T. latifolia* can easily survive in saline soil and moist conditions [19]. All these plants have proved to be good phytoremediators. Although the phytoremediation and bioaccumulation abilities of these plants are reported by various studies previously, no research has performed yet on the phytochemical activities of these macrophytes in response to their absorption and accumulation of heavy metals particularly Cr. Thus, this study was designed with the aim to: 1) evaluate and assess the phytoremediation potential of three macrophytes *E. crassipes*, *P. stratiotes*, and *T. latifolia* for Cr metal from tanneries' wastewaters; 2). phytochemical activities of Cr.

2. Materials and methods

2.1. Experimental setup

A pilot scale experiment was performed in the leather field industry located at Wazirabad Road near Sahoala stand, Sialkot. Long steel tanks were constructed consisting of 12 feet in length, 4.5 feet in width and the 2.8 feet in depth covering the surface area of 54 ft² and a volume of 152.5 ft³ each, further divided into three equal parts named as A, B, and C. These three chambers were designed at the flow rate of 0.72 m³/day. The flow rate was controlled with the help of small motor pump and the treated wastewater was discharged back into the drain from the outlet of CWs. All these three chambers in each CW have their inlets and outlets where tannery wastewater moved in and out. Overall, the capacity of each CWs setup was 140 L. The young seedlings of *E. crassipes, P. stratiotes*, and *T. latifolia* were collected from a pond nearby the selected tanning industry. Three different plant species were placed in each chamber for two months in the CWs. Therefore, the arrangement of each CW was like section 1 (*P. stratiotes*), section 2 (*E. crassipes*), and section 3 (*T. latifolia*). Plant samples were collected from inlet and outlet of the experimental system with the help of glass cylinders and was analyzed for the concentration of Cr present in it; more detail about the procedure is given in the following sections. The pilot-scale inlets and outlets were checked twice a week for proper functioning. The main aim of this inspection was to make sure that the effluent flowed directly from the pump to the tank to avoid any blockage due to the suspended solids in the wastewater.

2.2. Determination of phytochemical activities

2.2.1. Sample preparation

Air dried leaf samples of 100 mg from each plant species were suspended in dimethyl sulfoxide (DMSO) for 24 h. The samples were then centrifuged at 4000 rpm for 5 min. Supernatant of the solution was decanted and used for further analysis to determine various phytochemicals described in the proceeding sections.

2.2.2. Total flavonoid content (TFC)

Total flavonoid content was determined using the aluminum chloride method [20]. Briefly, 1 ml of the sample and 4 ml of distilled water were added into a volumetric flask (10 ml). To this solution, 0.3 ml of 5% sodium nitrile solution was added followed by the addition of 0.3 ml of 10% aluminum chloride solution. The solution mixture was incubated for 6 min following which 1 ml of 1 M

solution of sodium hydroxide was added. The final volume was made up to10 ml with the addition of distilled water. Absorbance was measured at 450 nm wavelength by using spectrophotometer (Model U2001, Tokyo, Japan).

2.2.3. Total phenolic content (TPC)

For the determination of total phenolic contents, the supernatant (0.2 ml) was mixed with 0.6 ml of distilled water and 0.2 ml of Folin-Ciocalteu's phenol reagent (1: 1). About 1 ml of saturated sodium carbonate solution (8% w/v aqueous solution) was added after 5 min. Total volume was marked up to 3 ml with distilled water. The solution was kept in dark for 30 min. Absorbance was recorded at 630 nm wavelength by using spectrophotometer (Model U2001, Tokyo, Japan) [21].

2.2.4. DPPH radical scavenging activity

DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity was analyzed using the method described by Clarke et al. [22]. For that, 20 μ l of the sample was diluted in DMSO and then mixed with 180 μ l of DPPH in methanol. The solution was kept in the dark for 15 min. Absorbance of the solution was measured at 515 nm wavelength by using spectrophotometer (Model U2001, Tokyo, Japan).

2.2.5. Total antioxidant activity (TAA)

Total antioxidant activity was determined following by Clarke et al. [22]. The relative capacity of antioxidants to scavenge 2, 2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS) radical was measured as compared to antioxidant potential of Trolox as



Fig. 1. Phytochemical activities in *E. crassipes, P. stratiotes* and *T. latifolia* under the influence of tannery wastewater. a). Total Phenolic Content (TPC: µg AAE/100 mg Dry wt.), b). Total Reduction Potential (TRP: µg AAE/100 mg Dry wt.), c). Total Flavonoid Content (TFC: µg QE/100 mg Dry wt.), d). Total Antioxidant Activity (TAA: µg AAE/100 mg Dry wt.), and e). DPPH radical scavenging activity (DPPH: %).

standard. The absorbance was measured at 630 nm at spectrophotometer (Model U2001, Tokyo, Japan).

2.2.6. Total reduction potential (TRP)

The reducing potential of the sample was determined by the method described by Jafri et al. [23]. 200 μ l of extract was prepared in DMSO and mixed with 0.5 ml of 2 M phosphate buffer solution at 6.6 pH. 0.5 ml of 1% potassium ferricyanide [K₃Fe (CN)₆] solution was added and the mixture was incubated at 50 °C for half an hour. 10% trichloroacetic acid (0.5 ml) was added to the mixture and then centrifuged at 4000 rpm for 5 min. The supernatant was mixed with equal volume of distilled water and 100 μ l of ferric chloride (FeCl₃) solution. The absorbance was noted at 630 nm on spectrophotometer (Model U2001, Tokyo, Japan). 200 μ l of DMSO was used for the preparation of blanks instead of the extract. The total reducing power of the samples which may be equivalent to ascorbic acid content were assumed as total reduction potential (TRP).

2.3. Determination of Cr content in plant and water sample

Plants were harvested for the determination of Cr from all three CWs. Triplicate samples of all the three plants were taken for this purpose. Fresh plant leaves were selected randomly from the middle of each plant and were placed in an oven for 72 h at 65 °C for drying. Cr was determined by following Asfaw et al. [24]. Oven dried plant leaf samples were homogenized and 0.5 g (DW) were weighed into a round bottom flask (250 ml volume). About 7 ml solution of HNO₃ (70%), HCLO₄ (70%) and H₂O₂ (30%) in the ratio 4:2:1 was added into the sample. The material was then digested on a hot plate. The solution was then allowed to cool for 15 min and then 15 ml of distilled water was added. The solution was then filtered using filter paper (Whatman no 1). To this solution, 2% HNO₃ solution (5 ml) was added and the solution was incubated in the dark for 12 h. Then total Cr was determined using an Atomic Absorption Spectrophotometer (AAS) (Model: Savant AA, Australia).

For the analysis of Cr in wastewater, 100 ml of each water sample was collected from three different sites of the pilot scale's inlet and outlet. The samples were digested with aqua regia (HNO₃ 67%: HCl 37% = 3:1). By using a Bergh of MWS-2 microwave digester, the mineralization of digested samples was performed. Three samples were collected per site. Flame Atomic Absorption Spectrometry (FAAS) was used to determine the concentration of Cr in water samples [25].

2.4. Statistical analysis

Data were tested for normality before statistical analysis. The analysis of variance (ANOVA) was then employed based on variable levels of CWs and intervals on all the measure attributes. The statistical analysis used in the figures (e.g., standard errors, mean, analysis of variance (ANOVA) was determined by using Co-stat computer program v.6.303 (CoHort Software, 2021). The bar-graph of various plant attributes were generated by Origin Pro v.2021 (OriginLab Corporation, 2021). Pearson's correlation was drawn using R studio v 4.0.4 (R Development Core Team, 2020).

3. Results

3.1. Determination of phytochemical activities

The phytochemical activities of *E. crassipes, T. latifolia*, and *P. stratiotes* were studied to investigate the impact of Cr stress caused by the tannery effluent. These biochemical activities showed activation of oxidative stress mechanism in those species caused by the Cr toxicity. Fig. 1(a–e) depicts variations in different phytochemical activities of *E. crassipes, T. latifolia*, and *P. stratiotes*. Application of industrial effluents through CWs considerably enhanced the biochemical activities of macrophytes. The lowest total phenolic contents were observed in *P. stratiotes* (98.2 µg AAE/100 mg dry wt.) compared to other plant species.

Antioxidant activities triggered by the Cr toxicity were calculated by determining the values of TAA, TRP and DPPH. The obtained values of total reducing power among these free-floating plants were as follows: *T. latifolia* > *E. crassipes* > *P. stratiotes*. *T. latifolia* had the highest DPPH content as well among these macrophytes, followed by *E. crassipes* and *P. stratiotes*. As a result, the activity of DPPH-based free radical scavenging in *P. stratiotes* was generally lower. In general, levels of DPPH were low in *P. stratiotes* compared to other free-floating species in each CWs. Furthermore, the plants were continuously grown in the effluents that caused variations in free radical scavenging activity in selected macrophytes. *T. latifolia* and *E. crassipes* had significantly higher total reducing power and antioxidant activity than *P. stratiotes*. It might be the more accumulation of Cr content in *T. latifolia* or *E. crassipes* which caused stress.

3.2. Determination of chromium in CWs

The level of Cr accumulation in all aquatic macrophytes was assessed at all three inlet and outlet points. Since each section has an inlet and outlet, samples were obtained from both points. Fig. 3 depicted a slight difference in Cr content in the influent level of all three sections. This experiment indicated that the level of Cr in influents moving through the inlet in each section was approximately equal. On the other hand, a significant difference was observed in the tannery effluent released from each section. The lowest level of Cr (0.426 mg/L) in tannery effluent was recorded where *T. latifolia* was grown. Whereas, the highest was in *E. crassipes* at 1.953 mg/L. *T. latifolia* is the most efficient phytoremediator of all of these macrophytes where it reduced the Cr content by about 97% followed by *P. stratiotes* (92%) and *E. crassipes* (88%).

3.3. Determination of chromium in macrophytes

Cr was found to be significantly accumulated in leaves of selected macrophytes as presented in Fig. 4 (a – d). This study indicated that Cr-containing tannery wastewater resulted in enhanced Cr concentration in selected plants. According to these findings, generally the first interval had the lowest Cr concentration in all three plants compared to subsequent intervals, while the last interval had the highest. Also, intervals differed significantly from each other for Cr concentration in all three plants. The highest concentrations of Cr among all three macrophytes were observed during Interval 4 in comparison to the other intervals. These highest Cr concentrations during the last interval might be due to the prolonged period of plant species grown in tannery polluted water. It is important to mention that the tannery wastewater flow rate remained constant during the entire time of the experiment. Among three species, *T. latifolia* had the highest concentration of Cr which is $4113 \pm 13.86 \text{ mgkg}^{-1}$ in Interval-3. Fig. 4 shows a clear distinction between different periods of Cr concentration in different species with the passage of time. Moreover, the content of Cr increased with the continuity of the study period for two months. Generally, the Cr accumulation in plant species was following the trend as: *T. latifolia* > *P. stratiotes* > *E. crassipes*.

A correlation of Cr content on all the three plant species with phytochemicals in these plants revealed that total phenolic content had a highly significant positive correlation with Cr concentration in all the three species (Fig. 5). But total antioxidant activity and DPPH were found negatively correlated with Cr content in these plants.

4. Discussion

Outcomes of the present study revealed that there is no massive effect on the phytochemical activities of *E. crassipes, T. latifolia* and *P. stratiotes* by the exposure of these plants to Cr toxicity in tannery wastewater (Fig. 1). Plants die under stress but the natural phenomenon of bioaccumulator plants allows them to survive. During stressful environments, plants have evolved various enzymatic and non-enzymatic defense systems for regulating reactive oxygen species at steady levels [22].

A correlation of various phytochemicals in all the three macrophytes revealed various responses (Fig. 2). Generally, total antioxidant activity indicated significant positive correlations with other parameters. However, a significant negative correlation of DPPH was noted for the other phytochemicals in all the three plant species. Plant's secondary metabolites have serious consequences against oxidative stress caused by any environmental activity [25]. Because of radical scavenging activity, phenol compounds are much more essential in plants [26]. The variability of phytochemical activities in *E. crassipes* and *P. stratiotes* indicated that the enhancement of secondary metabolites in these macrophytes toward tannery effluent stress could be beneficial. As a response to metal ion accumulation, antioxidants facilitate the production of reactive oxygen species within plant tissues [12]. This research support previous findings indicating that the high concentration of phenolic compounds appears in plants under stressed conditions [27,28]. The increasing level of antioxidant activity in all sites might be due to the stimulation of the defense mechanism in these plants in response to Cr toxicity.

The intriguing aspect is the tannery effluent had a marginal impact on *T. latifolia*, which resulted in a higher degree of its survival in Cr toxicity [1]. This study highlighted the enhancement of antioxidant activities that work against oxidative stress caused by the environmental biotic and abiotic stress factors. These aquatic macrophytes assumed to be feasible in constructed wetlands for treating tannery effluents. This research found that there was no massive impact on phytochemical activities by the application of tannery wastewater on these macrophytes.

The result clearly indicated that these three macrophytes significantly accumulated Cr from the tannery wastewater. Cr toxicity



Fig. 2. Correlation matrix (Pearson's) for different phytochemicals in all the three macrophytes.



Fig. 3. Concentration of Cr in tannery influent (a) and effluent (b) of each CW. Values are given as average.

shows a deterioration effect on morphological and structural components of plants [3,18]. Perhaps because of the inadequate translocation mechanism, P. stratiotes accumulates an average Cr level [29,30]. Our findings about of T. latifolia as Cr accumulator correspond to other studies suggesting that rooted macrophytes from polluted areas can easily absorb different metals [19]. Previous studies revealed strong metal accumulation ability of T. latifolia especially in its roots compared to other plant parts [31]. According to our results, T. latifolia had higher removal efficiency than P. stratiotes and E. crassipes. Similar findings have stressed the use of T. latifolia for decontamination of metals from industrial wastewaters [32]. On the other hand, E. crassipes and P. stratiotes can also be good accumulators of Cr. Our results of these two plants for the accumulation of Cr also conform with other studies [29]. E. crassipes and P. stratiotes have evolved their metabolism to survive in such polluted and harsh environmental conditions [29]. Macrophytes have an efficient root system which help them in the removal of metal pollutants from water [33]. Untreated wastewater from leather factories may adversely affect the essential ecological health of an aquatic ecosystem [3]. But the use of such plants for the decontamination of wastewater can be used before the release of polluted water into the environment. Wastewater from tanneries could be a rich point source of various toxic organic and inorganic pollutants. But it is mostly famous because of having excessive quantities of Cr which is largely used during the processing of leather goods and tanning of hides. However, the plants under study could be utilized in an effective natural sustainable way for the cleaning up of wastewater released from tanneries. Other studies have also demonstrated the abilities of these species for the decontamination of salts from saline aquatic environments [34,35]. The results of Cr decontamination from tannery effluents are in accordance with the results of Rozema et al. [36] who reported the enhanced metal removal efficiency of T. latifolia in constructed wetland systems. Tannery wastewaters are highly toxic to agricultural land and can also disturb plant's structural and functional activities [25,36]. Effluent water of these industries may cause contamination of agricultural lands of the city after being used for irrigation purposes.

The negative and positive correlations of phytochemicals with in plants revealed a specific pattern (Fig. 5). Certain phytochemicals tend to positively correlated with each other in all three plants such as total antioxidant activity. TRP also found to be highly significantly positively correlated with TPC. Correlation of TFC and TRP was also significantly positive. However negative correlation of DPPH was seen with all the other metabolites. Other species have also demonstrated the similar enhanced concentrations of antioxidant cell metabolites linked to increased metal toxicity in the environment, for example, *Zygophyllum fabago* in Pb contaminated soil [37], Coriandrum sativum L. in Cd contaminated soil [38], and *Zantedeschia aethiopica* and *Anemopsis californica* in As contaminated ground water [39]. This robust defense mechanism of these plants which is stimulated in harsh environmental conditions might be attributed to plants' ability to maintain cell membrane integrity (electrolyte leakage) [37,40]. Thus, plants in our study might also cope with excessive Cr concentration in the environment through activating their defense system.

Likewise, as shown in Fig. 5, the correlation analysis of phytochemicals with Cr content indicated that certain parameters were higher and lower with increased concentration of Cr in plants such as total phenolic content and DPPH, respectively.

A significantly reduced Cr content in effluents collected from the end points of CWs compared to their influents showed the efficacy of these three plants for the remediation of this pollutant. These aquatic macrophytes grown in the hypersaline wastewater have the ability to accumulate Cr content because of their high resistance toward this contaminant. These free-floating plants could act as great phytoremediators. Additionally, according to our research, tannery polluted water does not significantly affect these plants; they may enhance their phytochemical activities to cope with the toxic effects of Cr. The stimulation of strong defense systems in these plants facilitated their survival in such harsh environment. This experiment indicated that CWs can be used in an environmentally sustainable way by utilizing native aquatic macrophytes for the remediation wastewater.

5. Conclusion

The macrophytes in these CWs were able to significantly reduce the level of Cr generated by tanneries. This pilot-scale research indicated that CWs planted with *T. latifolia, E. crassipes,* and *P. stratiotes* cannot only survive but also help alleviating contaminants



Fig. 4. Cr concentration (mg kg⁻¹) in *E. crassipes, P. stratiotes,* and *T. latifolia* for a period of 2 months followed by four consecutive intervals of two weeks each: a) interval-1, b) interval-2, c) interval-3, d) interval-4.

from hypersaline tannery effluents. *E. crassipes* and *T. latifolia*, notably showed significant increases in antioxidant activities under metal stress, which increases their survival rate against stress circumstances. This finding demonstrated that, under local climate conditions, *T. latifolia* was generally superior to the other two macrophytes in terms of Cr accumulation. But *E. crassipes* and *P. stratiotes* also have the potential to uptake Cr from the tannery wastewater and remediate it. Furthermore, this pilot-scale experiment recommends these macrophyte species for the remediation of Cr from wastewaters. This study also recommends use of these plants for the phytoremediation of the other toxic heavy metals in different wastewaters.

Ethics approval and consent to participate

Not applicable.



Fig. 5. Correlation matrix (Pearson's) for different phytochemicals with Cr in all the three macrophytes.

Availability of data

Data will be provided on request.

CRediT authorship contribution statement

Zarrin Fatima Rizvi: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Muqaddas Jamal:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Haseena Parveen:** Software, Methodology, Formal analysis, Data curation, Conceptualization, Investigation. **Wajiha Sarfraz:** Writing – review & editing, Visualization, Validation, Software, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Syeda Nasreen:** Writing – review & editing, Writing – original draft, Visualization, Software, Resources, Methodology, Investigation, Formal analysis. **Noreen Khalid:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Project administration, Funding acquisition, Conceptualization.

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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