Asia-Pacific Journal of Science and Technology: Volume: 29. Issue: 03. Article ID.: APST-29-03-08. Research Article

APST

Asia-Pacific Journal of Science and Technology

https://www.tci-thaijo.org/index.php/APST/index

Published by the Research and Graduate Studies,
Khon Kaen University, Thailand

Mass balance study of an integrated phytoremediation system using different types of constructed wetlands for chromium removal

Anca A. Sembada[1,2,*] and Yohanes Theda[1,3]

[1]Bioengineering Study Program, School of Life Sciences and Technology, Bandung Institute of Technology, Bandung, Indonesia
[2]Graduate School of Engineering, Tokyo University of Agriculture and Technology, Koganei, Tokyo, Japan
[3]Department of Biochemical Engineering, University College London, London, United Kingdom
[*]Corresponding author: anca@st.go.tuat.ac.jp

Received 28 February 2023
Revised 25 April 2023
Accepted 29 June 2023

Abstract

Phytoremediation is a biological waste treatment technique with highly promising prospects compared to other treatment systems. Constructed wetlands are one of the most widely applied phytoremediation methods. This study aimed to evaluate the phytoremediation process of chromium (Cr) using an integrated phytoremediation system. In this study, three different constructed wetland systems-free-floating, vertical subsurface flow, and horizontal subsurface flow-were used in sequence with three different plant species-*Eicchornia crassipes*, *Typha augustifolia*, and *Catharanthus roseus*. Each system had a residence time of three days. The Cr concentration used as the initial input was 0.5 mg/g. *C. roseus* had the highest bioconcentration factor (0.44±0.03) and translocation factor (1.27 ± 0.24) when compared to other species ($p < 0.05$). This integrated system had an overall process efficiency for Cr removal of 73.8 ± 1.07%. Based on this study, the integrated system is confirmed to have the potential for the removal of Cr from contaminated water when using these three plants and the further development of this integrated system on a larger scale is recommended.

Keywords: *Catharanthus roseus*, Chromium, Constructed wetlands, *Eicchornia crassipes*, Mass balance, Phytoremediation, *Typha augustifolia*

1. Introduction

The volume of heavy metal waste continues to grow with increased industrial activity [1]. One particularly concerning heavy metal is chromium (Cr), which has a devastating impact on the environment, living organisms, and even humans. Cr waste has become a global threat due to its high toxicity, which can lead to several serious diseases, especially skin and respiratory diseases [2]. To date, various treatment methods have been developed to mitigate the hazard posed by Cr. Conventional treatments include chemical and physical techniques, such as precipitating agents and membrane filtrations [3]. Most of these methods are expensive, energy-intensive, and not environmentally friendly [4]. Phytoremediation, which employs plants with the ability to absorb and accumulate heavy metals in their tissues, is a highly promising alternative [5].

Amongst different phytoremediation systems, constructed wetland (CW) systems are more widely applied in industry [6]. Constructed wetlands is an umbrella term that encompasses several phytoremediation systems mimicking natural wetland processes. In general, CW systems can be divided into three types: horizontal subsurface flow (HSSF), vertical subsurface flow (VSSF), and free water surface (FWS) [7]. Most phytoremediation endeavours utilise only one of these CW systems despite past studies showing that the integration of two or more phytoremediation systems can significantly increase the process efficiency and optimise the use of plants [8]. A study by Guarino and Sciarrilo [9] integrating plant, fungal, and bacterial remediation systems showed high removal of arsenic, cadmium, lead, and zinc in unsaturated soils. A more recent study by Qi, et al. [10] combined three plants with *Bacillus* sp. to decontaminate soil, finding that the integrated system could increase the efficiency of phytoextraction. The integrated phytoremediation system (IPS) used in this present study consisted of three types of CW systems—HSSF, VSSF, and FWS—and three plant species—

Eicchornia crassipes, *Typha augustifolia*, and *Catharanthus roseus*—that were used to remove Cr as a model heavy metal. These three plant species were chosen due to their ability as heavy metal accumulators based on the findings of several previous studies. *E. crassipes* was shown to be able to accumulate 90 mg and 53 mg of Cr in its roots and leaves, respectively [11]. *T. augustifolia* also showed a good ability to accumulate Cr. *C. roseus* was chosen for use in this study due to its low water requirements and similarity to *H. annuus* which has previously been shown to be compatible with wetland systems [12].

A study conducted by Mant, et al. [13] showed that constructed wetlands containing three plants (*Penisetum purpureum*, *Brachiaria decumbents,* and *Phragmites australis*) were able to remediate Cr with a removal efficiency of 97–99.6% at 24 hours. Wastewater treatment with a single phytoremediation system was only able to remediate certain contaminants with varying times [14]. These limitations indicate that an integrated system capable of treating various types of wastewater with different characteristics is needed. The purpose of this study is to demonstrate the ability of a constructed wetland-based integrated phytoremediation system to remediate Cr.

2. Materials and methods

2.1 Integrated phytoremediation system

The integrated phytoremediation system used in this study consisted of three types of CW systems and three different plant species including a free-floating (FF) system with *E. crassipes*, a vertical subsurface flow (VSF) with *T. augustifolia*, and horizontal subsurface flow (HSF) with *C. roseus*. The three IPS subsystems were arranged in the sequence depicted in Figure 1. The plants used were taken from the greenhouse of the School of Life Sciences and Technology at the Bandung Institute of Technology in Jatinangor, Sumedang, West Java and are shown in Figure 2. All plants used were in the mature plant category (age range from one to three months after planting). A 0.5 mg/g potassium dichromate ($K_2Cr_2O_7$) solution was used as a waste model [15].

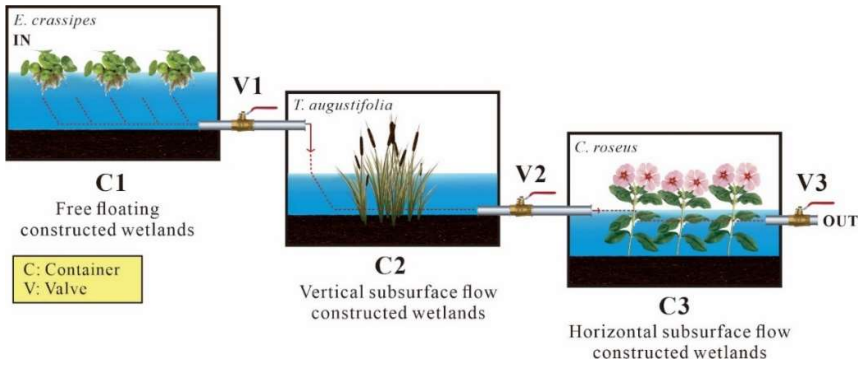

Figure 1 The integrated phytoremediation system (IPS) used in this study consists of three constructed wetlands systems: horizontal subsurface flow, vertical subsurface flow, and free water flow, each with a different plant species.

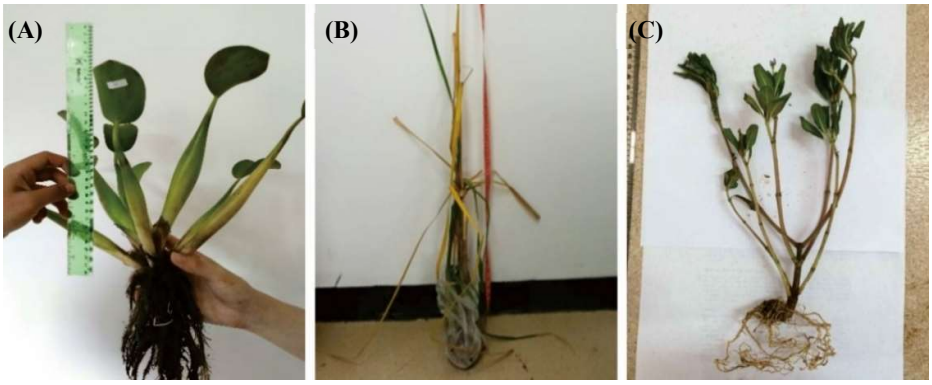

Figure 2 Examples of the three plant species used as phytoremediators in this study, in which (A) = *E. crassipes*, (B) = *T. augustifolia*, and (C) = *C. roseus.*

The subsystems were individually contained in three containers with dimensions of 42×60×28 cm (W×L×H) that were connected by PVC pipes with ball valves to control the flow. The containers were filled with soil up to

± 6 cm from the top of the container [16]. While the valves were closed, container C1 containing the FF system was filled with a 10% strength Hoagland's solution [17]. The model $K_2Cr_2O_7$ solution was then added to container C1 and the system was incubated for three days. Valve V1 was then opened to transfer the mixture from container C1 to C2 and the system was incubated for another three days. Valve V2 was then opened to transfer the mixture from container C2 to C3, and the system was incubated for a final three days, making the whole process last for 9 days.

2.2 Sample preparation and chromium measurement

Sample preparation was done by wet ashing prior to Cr measurement [18-20]. Three samples from each medium (growth medium, soil, and plant biomass) were taken from each container at the end of its incubation period. Five mL of 65% nitric acid (HNO_3) was added to fifty mL of growth medium and heated until it became clear. The mixture was then diluted with 50 mL of distilled water and used for Cr measurement. Soil and plant biomass samples were oven-dried at 50°C for two days [21]. The *C. roseus* and *E. crassipes* samples were separated into roots, stems, and leaves, while the *T. augustifolia* sample was separated into roots and aerials. The samples were oven dried until they reached constant weights and ground using mortar and pestle.

One mL of 65% HNO_3 was then added to test tubes that each contained 0.1 g of a soil and plant tissue sample. The test tubes were then heated in a water bath at a water temperature of 50°C and 1-mL additions of 30% hydrogen peroxide (H_2O_2) were made until the samples became clear. The resulting clear mixtures were then diluted using 10 mL of distilled water in volumetric flasks. Cr concentrations in the samples were measured via atomic absorption spectrophotometry (AAS; Shimadzu AA-630, Japan) at a wavelength of λ = 357.9 nm [18]. Three samples were analysed for each variable.

2.3 Calculation of bioconcentration and translocation factors

Bioconcentration factor (BCF) is the ratio between the concentration of a metal in the tissue of a plant C_{PLANTS} to that in its medium C_{MEDIUM} [22]. It is used to measure the effectiveness of a plant in absorbing metals. The larger the value of BCF, the more effective a plant is at absorbing the specific metal within a growth medium. BCF is calculated using Equation 1.

$$\mathrm{BCF} = \frac{C_{PLANTS}}{C_{MEDIUM}} \tag{1}$$

The ability of a plant to distribute metals absorbed through its roots to its upper part is measured by the translocation factor (TF) [23]. TF is defined as the ratio between the concentration of a metal in a plant's aerial parts ($C_{AERIALS}$) and that in its roots (C_{ROOTS}). A TF value >1 implies that more heavy metal has been translocated to the aerials than remains accumulated in the roots and thus the plant has a high ability to translocate metals. TF is calculated using Equation 2.

$$\mathrm{TF} = \frac{C_{AERIALS}}{C_{ROOTS}} \tag{2}$$

2.4 Calculation of process efficiency

The process efficiency (PE) of a system is defined as the ratio between the amount of metal absorbed by the plant and the amount input into the system. The calculation of the PE values of the CW subsystems in this research is done through a mass balance analysis, measuring the Cr concentrations at the input and output of a system [24]. By comparing the different PE values of the systems, the most efficient integrated system can be determined. PE can be calculated by Equation 3.

$$\mathrm{PE} = \frac{C_I - C_O}{C_I} \times 100\% \tag{3}$$

where C_I and C_O are the metal concentration in the input and the output of the system, respectively.

2.5 Statistical analysis

Data obtained in this study were evaluated using Duncan's multiple range test (DMRT) with a *p*-value of 0.05. The software package used in the statistical analysis was IBM SPSS Statistics 26 (IBM Corporation, USA).

3. Results and discussion

3.1 Concentrations of chromium in growth medium, soil, and plant biomass

To ensure that any measured Cr came only from the $K_2Cr_2O_7$ used as the model metal, the concentrations of Cr in the plants, growth medium, and soil were measured. No Cr was detected in any of the plant samples, growth medium, or soil. The detection limit of the AAS was 1.47 µg/mL [25]. Cr concentrations of the growth medium and soil (Figure 3), as well as of the plant biomass (Figure 4) were measured. The results show that the CW system allowed for the binding of heavy metals by the soil even in very small concentrations. However, there were no statistically significant differences in the concentrations of Cr in the soil across the subsystems ($p > 0.05$).

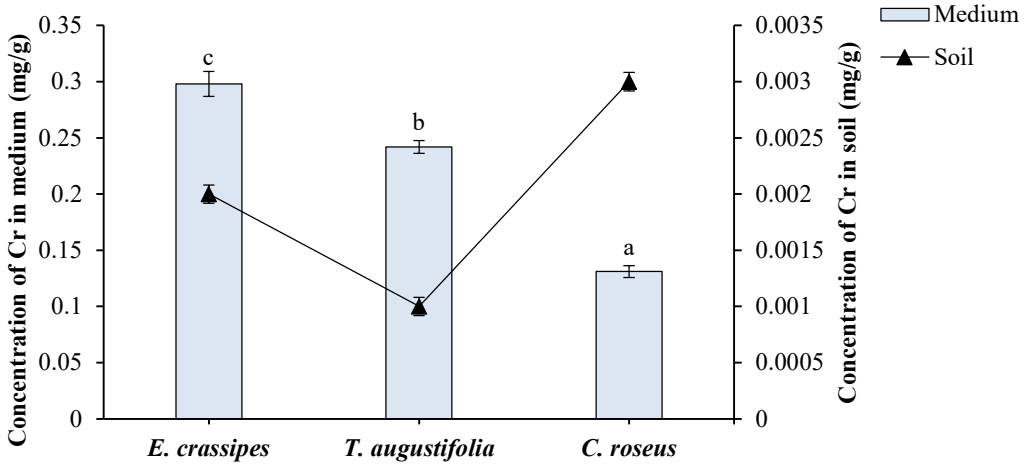

Figure 3 Chromium concentration in the growth medium and soil. Letters indicate significant differences in Cr concentrations ($p < 0.05$) according to Duncan's test**.**

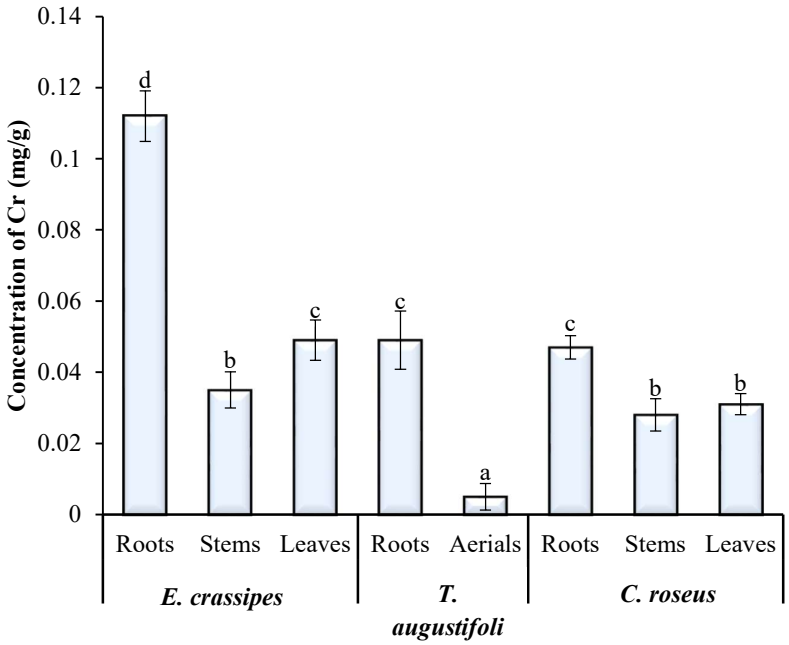

Figure 4 Chromium concentration detected in the plant biomass. Letters indicate significant differences in Cr concentrations ($p < 0.05$) according to Duncan's test.

In all plant species employed in the study, the highest Cr concentration was found in the roots, due to their direct exposure to Cr. Cr concentration in the roots of *E. crassipes*, *T. augustifolia*, and *C. roseus* were 0.112 ± 0.007, 0.049 ± 0.008, and 0.047 ± 0.003 mg/g, respectively. This is in alignment with a study conducted by

Tabinda, et al. [11] which showed that the roots of *E. crassipes* accumulated more Cr than the leaves. A study conducted by Sudarsan, et al. [26] also demonstrated that the roots of *T. latifolia* can store 3-15 times more Cr than the leaves. However, other past studies have shown that there are plants that accumulate more Cr in their shoots than in the roots, including *Allium griffithianum*, *Himalaiella heteromalla*, *Stellaria media*, *Rosularia adenotricha*, and *Wulfeniopsis amherstiana* [27].

3.2 Bioconcentration factor and translocation factor

The BCF and TF values of the system are shown in Figure 5. *C. roseus* had the highest BCF value at 0.44±0.03 compared to *E. crassipes* (0.39±0.02) and *T. augustifolia* (0.18±0.03). *C. roseus* also had the highest TF value (1.27 ± 0.24) followed by *E. crassipes* (0.75 ± 0.08) and *T. augustifolia* (0.11 ± 0.08). The differences between the three TF values were statistically significant ($p < 0.05$). The TF value of *C. roseus* was greater than one (>1), implying that total Cr accumulation in the aerial parts (stems and leaves) was higher than in the roots. The high accumulation of Cr in aerial parts was likely caused by stronger aerial structures of *C. roseus* compared to the other two species, resulting in a higher metal transportation potential from roots to shoots [28]. This is consistent with the results obtained by Sajad, et al. [27] which showed that Cr accumulation in the shoots of *C. roseus* was more than 17 times higher than accumulation in the roots. In addition, the TF value for *E. crassipes* in this study was consistent with the TF range obtained by Hasan, et al. [29] of 0.2 to 0.78.

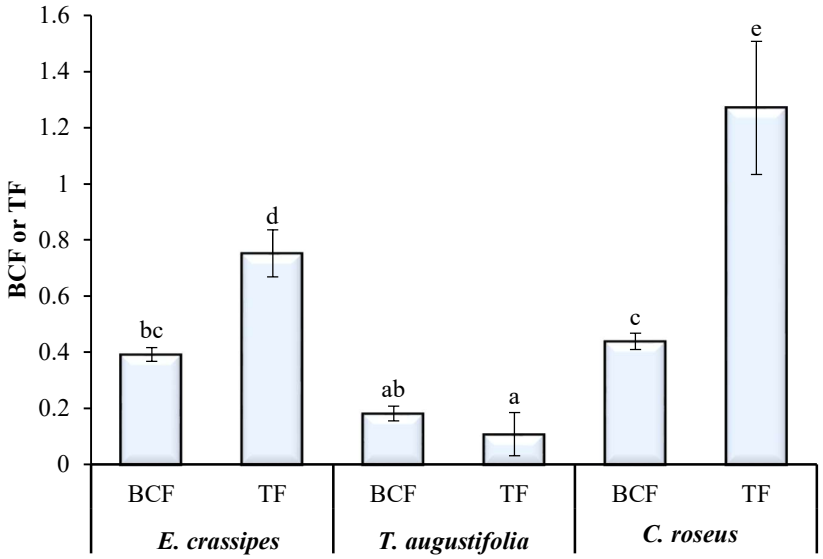

Figure 5 Bioconcentration and translocation factors calculated for all species**.** Letters indicate significant differences ($p < 0.05$) according to Duncan's test**.**

Phytoremediation can be achieved via several mechanisms, including degradation, accumulation, dissipation, and immobilisation of heavy metals. Examples of degradation mechanisms are rhizodegradation and phytodegradation, while accumulation mechanisms include phytoextraction and rhizofiltration. Dissipation mechanisms include phytovolatilization and immobilisation mechanisms include hydraulic control and phytostabilisation [30]. The TF value is indicative of the mechanism through which a phytoremediation system processes heavy metals. Phytostabilisation occurs when the TF value of a system is less than one (TF < 1), while phytoextraction occurs when the TF value is greater than one (TF > 1) [31]. Therefore, it can be inferred from the calculated TFs that *E. crassipes* and *T. augustifolia* utilise the phytostabilisation mechanism to remediate Cr, while *C. roseus* utilises phytoextraction.

3.3 Mass balance analysis and process efficiency

A mass balance analysis was carried out in order to track the flow of Cr from the input to the final output of the integrated system (Figure 6). Process efficiency values were calculated to determine the effectiveness of each subsystem, as well as the overall efficiency of the integrated system (Table 1). At the input to the system, the growth medium had a concentration of 0.5 mg/g Cr. At the output of the third subsystem, the Cr concentration in the growth medium was 0.131 mg/g, which translates to a PE value of 73.8±1.07%, suggesting that nearly three-

quarters of the Cr was removed by the system. This overall PE value was higher than the PE values of individual subsystems, indicating that the integrated system removed Cr better than any single subsystem.

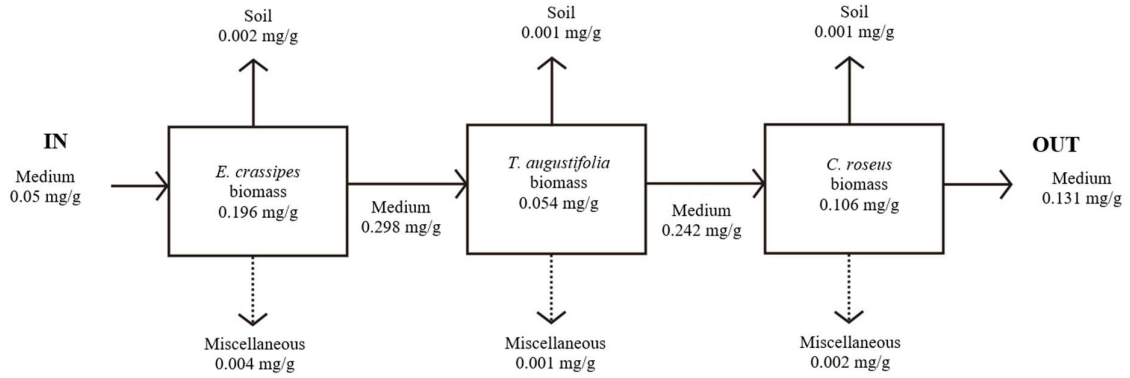

Figure 6 Mass balance analysis of the integrated phytoremediation process.

Table 1 Process efficiency of the integrated phytoremediation process.

Container	System	Process efficiency (%)
C1	Free-floating constructed wetlands	40.40±2.20
C2	Vertical subsurface flow constructed wetlands	18.69±3.40
C3	Horizontal subsurface flow constructed wetlands	45.84±2.54
Integration	Integrated phytoremediation system (overall process)	73.80±1.07

Several factors affect the PE value, including the structure of a plant's roots, the water-use efficiency of the system, the affinity of transporter proteins on the root surface, and the xylem loading capacity [32]. The age and growth stage of a plant can also affect the uptake of heavy metals [33]. Furthermore, residence time also contributes to the level of absorption. In this study, a longer residence time would have likely resulted in a higher Cr absorption.

Integrated phytoremediation systems have advantages over a single phytoremediation system. IPS are able to treat wastewater containing more than one type of contaminant. Wastewater containing multiple contaminants is difficult to treat with only one phytoremediation system as the physical and chemical properties of each contaminant are different [14]. By combining several phytoremediation systems with multiple types of plants, integrated phytoremediation systems are expected to remediate various types of contaminants at one time.

4. Conclusion

As shown in past studies, the integration of multiple constructed wetlands systems has the potential to be a more sustainable and environmentally friendly waste treatment technique. In this study, three CW systems were combined into an integrated phytoremediation system for Cr removal: a free-floating system with *Eicchornia crassipes*, a vertical subsurface flow system with *Typha augustifolia*, and a horizontal subsurface flow system with *Catharanthus roseus*. The results of this study suggest that integrated phytoremediation systems have better metal-removing performance compared to individual CW systems used separately.

5. Acknowledgements

We thank Dr Taufikurahman (Bandung Institute of Technology, Indonesia) for valuable discussions about phytoremediation and constructed wetlands. We also thank Teguh Adhitia Suyadi (Tokyo City University, Japan) for assistance in conducting the experiment.

6. References

[1] Joseph L, Jun BM, Flora JR, Park CM, Yoon Y. Removal of heavy metals from water sources in the developing world using low-cost materials: a review. Chemosphere. 2019;229:142-159.

[2] Neghab M, Azad P, Honarbakhsh M, Zarei F, Ghaderi E. Acute and chronic respiratory effects of chromium mists. J Health Sci Surveill Syst. 2015;3(3):119-124.

[3] Peng H, Guo J. Removal of chromium from wastewater by membrane filtration, chemical precipitation, ion exchange, adsorption electrocoagulation, electrochemical reduction, electrodialysis, electrodeionization, photocatalysis and nanotechnology: a review. Environ Chem Lett. 2020;18:2055-2068.

[4] Chai WS, Cheun JY, Kumar PS, Mubashir M, Majeed Z, Banat F, et al. A review on conventional and novel materials towards heavy metal adsorption in wastewater treatment application. J Clean Prod. 2021;296:126589.

[5] Sembada AA, Suyadi TA. Ability of greater duckweed (*Spirodela polyrrhiza*) to absorb cadmium and its potency as phytoremediator. J Edubiotik. 2022;7(2):160-166.

[6] Said NSM, Abdullah SRS, Ismail NI, Hasan HA, Othman AR. Phytoremediation of real coffee industry effluent through a continuous two-stage constructed wetland system. Environ Technol Innov. 2020;17:100502.

[7] Gorgoglione A, Torretta V. Sustainable management and successful application of constructed wetlands: a critical review. Sustainability. 2018;10(11):3910.

[8] Manzoor M, Gul I, Manzoor A, Kallerhoff J, Arshad M. Optimization of integrated phytoremediation system (IPS) for enhanced lead removal and restoration of soil microbial activities. Chemosphere. 2021;277:130243.

[9] Guarino C, Sciarrillo R. Effectiveness of in situ application of an Integrated Phytoremediation System (IPS) by adding a selected blend of rhizosphere microbes to heavily multi-contaminated soils. Ecol Eng. 2017;99:70-82.

[10] Qi X, Hao X, Chen X, Xiao S, Chen S, Luo X, et al. Integrated phytoremediation system for uranium-contaminated soils by adding a plant growth promoting bacterial mixture and mowing grass. J Soils Sediments. 2019;19:1799-1808.

[11] Tabinda AB, Irfan R, Yasar A, Iqbal A, Mahmood A. Phytoremediation potential of *Pistia stratiotes* and *Eichhornia crassipes* to remove chromium and copper. Environ Technol. 2018;41(12):1514-1519.

[12] Garcıa-Pérez A, Harrison M, Grant B. Sunflowers (*Helianthus annuus* L.) on top of a constructed wetland as an engineered ecosystem to clean sewage onsite. Open J Water Pollut Treat. 2014;1(2):83-91.

[13] Mant C, Costa S, Williams J, Tambourgi E. Phytoremediation of chromium by model constructed wetland. Bioresour Technol. 2006;97(15):1767-1772.

[14] Yuliasni R, Kurniawan SB, Marlena B, Hidayat MR, Kadier A, Ma PC, et al. Recent progress of phytoremediation-based technologies for industrial wastewater treatment. J Ecol Eng. 2023;24(2):208-220.

[15] Razavi-Mehr M, Fekri MH, Omidali F, Eftekhari N, Akbari-Adergani B. Removal of chromium (VI) from wastewater by palm kernel shell-based on a green method. J Chem Health Risks. 2019;9(1):75-86.

[16] Huang J, Cao C, Yan C, Liu J, Hu Q, Guan W. Impacts of silver nanoparticles on the nutrient removal and functional bacterial community in vertical subsurface flow constructed wetlands. Bioresour Technol. 2017;243:1216-1226.

[17] Sembada AA, Faizal A. Characterization of starch from duckweeds and its conversion into reducing sugars via enzymatic saccharification. Asian J Plant Sci. 2023;22(1):130-137.

[18] Vidayanti V, Choesin DN, Iriawati I. Phytoremediation of chromium: distribution and speciation of chromium in *Typha angustifolia*. Int J Plant Biol. 2017;8(1):6870.

[19] Buscaroli A. An overview of indexes to evaluate terrestrial plants for phytoremediation purposes. Ecol Indic. 2017;82:367-380.

[20] Diarra I, Kotra KK, Prasad S. Application of phytoremediation for heavy metal contaminated sites in the South Pacific: strategies, current challenges and future prospects. Appl Spectrosc Rev. 2022;57(6):490-512.

[21] Faizal A, Sembada AA, Priharto N. Production of bioethanol from four species of duckweeds (*Landoltia punctata*, *Lemna aequinoctialis*, *Spirodela polyrrhiza*, and *Wolffia arrhiza*) through optimization of saccharification process and fermentation with *Saccharomyces cerevisiae*. Saudi J Biol Sci. 2021;28(1):294-301.

[22] Soubasakou G, Cavoura O, Damikouka I. Phytoremediation of cadmium-contaminated soils: a review of new cadmium hyperaccumulators and factors affecting their efficiency. Bull Environ Contam Toxicol. 2022;109(5):783-787.

[23] Buscaroli A. An overview of indexes to evaluate terrestrial plants for phytoremediation purposes. Ecol Indic. 2017;82:367-380.

[24] He W, Long A, Zhang C, Cao M, Luo J. Mass balance of metals during the phytoremediation process using *Noccaea caerulescens*: a pot study. Environ Sci Pollut Res. 2021;28:8476-8485.

[25] Prasetyo E. Monosodium glutamate for simple photometric iron analysis. IOP Conf Ser Mater Sci Eng. 2018;285(1):012009.

[26] Sudarsan J, Annadurai R, Kumar KS, Nithiyanantham S. Heavy metal removal using different parts of *Typha latifolia*. J Bionanosci. 2018;12(1):87-91.

[27] Sajad MA, Khan MS, Bahadur S, Naeem A, Ali H, Batool F, et al. Evaluation of chromium phytoremediation potential of some plant species of Dir Lower, Khyber Pakhtunkhwa, Pakistan. Sheng Tai Xue Bao. 2020;40(2):158-165.
[28] Oh K, Li T, Cheng H, He X, Yonemochi S. Study on tolerance and accumulation potential of biofuel crops for phytoremediation of heavy metals. Int J Environ Sci Dev. 2013;4(2):152.
[29] Hasan SMM, Akber MA, Bahar MM, Islam MA, Akbor MA, Siddique MAB, et al. Chromium contamination from tanning industries and phytoremediation potential of native plants: a study of savar tannery industrial estate in Dhaka, Bangladesh. Bull Environ Contam Toxicol. 2021;106(6):1024-1032.
[30] Kafle A, Timilsina A, Gautam A, Adhikari K, Bhattarai A, Aryal N. Phytoremediation: mechanisms, plant selection and enhancement by natural and synthetic agents. Environ Adv. 2022;8:100203.
[31] Rana V, Maiti SK. Municipal wastewater treatment potential and metal accumulation strategies of *Colocasia esculenta* (L.) Schott and *Typha latifolia* L. in a constructed wetland. Environ Monit Assess. 2018;190:1-15.
[32] Laghlimi M, Baghdad B, El-Hadi H, Bouabdli A. Phytoremediation mechanisms of heavy metal contaminated soils: a review. Open J Ecol. 2015;5(8):375.
[33] Souri MK, Hatamian M, Tesfamariam T. Plant growth stage influences heavy metal accumulation in leafy vegetables of garden cress and sweet basil. Chem Biol Technol Agric. 2019;6(1):1-7.