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Experimental Study on Phytoremediation of Heavy Metal from Mine Wastewater by *Rumex nepalensis* Spreng.

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ABSTRACT

Tailings pond is considered as the main source of heavy metal pollution in gold mining areas. These heavy metals are directly released into fresh water without proper treatment. Phytoremediation process with the selected terrestrial plants may be an alternative solution for the mine wastewater treatment. In the current study, an experimental investigation found that *Rumex nepalensis* Spreng. has found a good accumulator of multi-metals in 15 days of experimental period. The results revealed that the removal efficiencies for Zn, Cu, Ni and Pb were 100%, 92%, 87%, and 67%, respectively. These indicate the plant showed its maximum accumulation of multi-metals. However, Pb reached saturation at the end of the 10^{th} day, which makes its removal efficiency only in the first 10 days of the experimental period. The experiment revealed Pb and Ni which were above WHO standard for drinking water in the mine wastewater were made to permissible limit for these metals after the treatment.

Keywords: Mine wastewater, phytoremediation, *Rumex nepalensis* Spreng., tailings pond

INTRODUCTION

Rapid urbanization and development have considerably increased the demand for water, as it is a basic necessity of all living organisms (Mustafa & Hayder, 2021). However, some industrial activities introduce toxic pollutants directly into fresh water without proper treatment (Gupta & Shukla, 2020). Industrial wastewater is a major problem due to high concentrations of toxic pollutants, particularly heavy metals (Razzak *et al*., 2022). Heavy metal pollution has become a major global concern due to its toxicity (Demková *et al*., 2017; Sey and Belford, 2019; Agarwal *et al*., 2022). The presence of heavy metals in wastewater poses a significant risk as it can contaminate surface water and soil, ultimately finding its way into the food chain (Ahmad *et al*., 2022; Tong *et al*., 2022).

Gold processing often leads to the production of substantial quantities of tailings and wastewater, both of which can be sources of heavy metal contaminants (Manyuchi *et al*., 2022). Mine tailings are a mixture of finely powdered rock and water residual after gold has been extracted from a mine (Chen *et al.*, 2018). Mine tailings are the main heavy metal pollution source in gold mining areas (Zhang *et al*., 2020). A previous study has shown that the mine wastewater of the study area contains several heavy metals. Moreover, the mine wastewater is discharged to the nearby water bodies without proper treatment of heavy metals (Getaneh & Alemayehu, 2006). The release of toxic heavy metals from mine tailings can contaminate surface water, groundwater and agricultural soils (Bempah & Ewusi, 2016). Heavy metal contaminants are bio-accumulative and non-biodegradable (Dayal *et al*., 2016; Bouzekri *et al*., 2020) and have a potential to pose health risks to humans and aquatic life in the vicinity of mining areas (Liang *et al*., 2017; Adewumi & Laniyan, 2020).

Various conventional physicochemical and green biological techniques are applied to remove heavy metals. Conventional treatment methods including adsorption, coagulation, flocculation, chemical precipitation, membrane separation, ion exchange, flotation, and electrochemical technologies produce swift results, although they generate contaminated slurry and more costly compared to bioremediation of heavy metals from wastewater (Razzak *et al*., 2022). Phytoremediation technique is a branch of bioremediation that employs the application of plants for the remediation of wastewater (Mustafa & Hayder, 2021). It is a cost-effective and environmental friendly technology of wastewater treatment (Kumar *et al.*, 2017). Heavy metal pollution and its repercussion for human health have increased research in developing low cost and sustainable remediation technology (Razzak *et al*., 2022). Therefore, the use of plants to remediate water pollution is currently attracting scientific community, as it provides a sustainable, cost-effective, less harmful and eco-friendly process (Kumar & Chopra, 2018; Razzak *et al*., 2022).

Rumex nepalensis is an herbaceous plant belongs to Polygonaceae family which has been used as traditional herbal medicine (Yadav *et al*., 2011). The plant has the antibacterial property against some strains of bacteria (Pal & Saha, 2003). The leafy vegetable of the *R. nepalensis* has antioxidant potential (Anusuya *et al*., 2016; Kumar & Singh, 2020) and its consumption will prevent aging related diseases (Anusuya *et al*., 2016). In Ethiopia, the plant is traditionally used for the treatment of stomach ache, tonsillitis, ascariasis, and uterine bleeding (Dabe *et al*., 2020). Moreover, some studies have shown the plant's potential for phytoremediation (Ahmad *et al*., 2022; Aras, 2022). A study undertaken in Uranium Mine of Southwestern China revealed the highest accumulation of uranium metal by the plant (Li *et al*., 2019).

Currently, various research results have been achieved in the phytoremediation of heavy metals from wastewater by using aquatic plant (Chaudhary & Sharma, 2019; Abbas *et al*., 2021; Panneerselvam & Priya, 2021). Aquatic plants are plants that grow in or near water and are either emergent, submersed, or floating (Balamoorthy *et al*., 2022). Aquatic plants have potential to absorb pollutants such as heavy metals found in domestic, agricultural and industrial wastewaters (Mustafa & Hayder, 2021). However, limited research has been done on the treatment of wastewater using terrestrial plants. Terrestrial plants are those plants which are largely dominating land surfaces (Beraldi-campesi, 2013). *Rumex nepalensis* was selected among other terrestrial plants based on previous research by Mengistu *et al*. (2023). In this study, the translocation factor which is the plant's ability to transport Cu, Ni and Pb in the upper part of the plant were reported 2.84, 1.5, and 3.0 respectively. These values can be considered as indicators for the plant's potential for phytoextraction (Waris *et al.*, 2022; Mengistu *et al*., 2023). Furthermore, there were few studies which used terrestrial plants to treat particularly using real wastewater. Among the few reported studies, most of them used synthetic wastewater prepared in laboratories. Therefore, this work is an experimental investigation of heavy metal removal efficiency of *R. nepalensis* from mine wastewater at laboratory scale.

MATERIALS AND METHODS

Sample Collection

Wastewater sample was collected from Legadembi Gold Mine which is located at about 500 km south of the Capital, Addis Ababa. The longitude and latitude of tailing pond is 38.899453° and 5.7208514°. The wastewater sample was taken using polyethylene bottles, previously rinsed with mine water from the sampling site and brought to the laboratory for experimental investigation.

The herbaceous plant species, *R. nepalensis*, which was abundantly grown around the tailing pond was uprooted and packed in polyethylene bags and then transported to laboratory for the purpose of mine wastewater treatment. The plant was selected among other terrestrial plants based on literature review, its production of densely branched roots and high above-ground biomass (Ali *et al.* 2013). Moreover, the plant was selected among other plants based on previous research by Mengistu *et al.* (2023). In their study, the plant has showed efficient translocation of heavy metals especially, Cu, Ni and Pb in the upper part of the plant.

Experimental Setup

The experiment was performed at laboratory scale with adequate amount of sunlight and air by using *R. nepalensis* in order to investigate the percentage removal of heavy metals from mine wastewater (Figure 1). The plants were first cleaned of dirt and then the acclimatisation was undertaken. Acclimatisation process was carried out for three days, which is a process of adapting the plants to the new planting medium, and the surrounding conditions (Ratna & Slamet, 2020). Plants with equal height and weight were used for each treatment pots containing 1 L of the mine wastewater which was filled up to the mark. One fresh and healthy plant was kept in each of the treatment pots. The wastewater sample without the plant species was used as control. The experiment was carried out for 15 days. This time was selected based on the work of Kothari *et al*. (2022) and Singh *et al*. (2021). Duplicate treatments were designed for each of the three variations of time (5 days, 10 days, and 15 days). Plants were removed from two containers in each of the respective time duration, and then the water samples kept for laboratory analysis. Distilled water was added daily to each container to compensate for water loss through plants transpiration and evaporation (Abbas *et al*., 2021). Hoagland solution of 5 mL (Wu *et al*., 2022) was added to the treatment pots each day. The Hoagland nutrient was prepared based on (Buta *et al*., 2014).

Analysis

The wastewater was examined before and after phytoremediation experiments for the selected heavy metals. Water samples were collected from the treatment pots on days: 5, 10, and 15 for analysis. The wastewater was digested according to EPA method 3051A (EPA, 2007) with modification, in a High Performance Microwave Digestion System (ETHOS UP milestone). 50 mL of wastewater was digested with mixtures of concentrated solutions of 5 mL of HNO_3 and 1 mL of HCl. The chemical reagents used were analytical or guaranteed reagent grade. Finally, the solution was transferred into a 50 mL volumetric flask and diluted to 50 mL using distilled water. The solution was then ready for the quantitative analysis of the heavy metals. Hence, the dilution factor becomes 1. Metal concentrations in water sample were calculated as follows Eq. (1):

$$C = \frac{Cs.Vs}{V} \qquad \textbf{Eq. (1)}$$

Where: C = Metal concentration (mg/L) in water sample

C_s = Concentration of metal in digested dilute sample (mg/L)

V_s = Final volume of the digested sample solution (mL)

V = Volume of digested sample (mL)

For quality control purpose the same samples were analysed in duplicates and after every five samples, a calibration standard was analyzed to verify the response and efficiency of the analytical instrument. The accuracy of the method was confirmed by using known standard and the recovery of the tested samples using the following Eq. (2). The percentage recovery varied between 83.3% and 116.7%. These values are acceptable recovery which lies within a range of 80% - 120% as shown in Table 1.

$$\%\ Recovery = \frac{[\text{Spiked sample}] - [\text{Unspiked sample}]}{[\text{Amount added}]} x100 \qquad \textbf{Eq. (2)}$$

Where, [ ] = concentrations

Table 1. Results of the recovery analysis for the wastewater samples

Heavy metal	Concentration before spiking	Concentration after spiking	Amount added	% Recovery
Cu	0.085±0.005	3.62±0.021	4 mg/L	88.3%
Zn	0.12±0.007	4.13±0.015		100.25%
Pb	0.025±0.005	4.09±0.082		101.6%
Ni	0.05±0.014	4.72±0.250		116.7%
Cd	0.00±0.000	3.96±0.014		99.0%

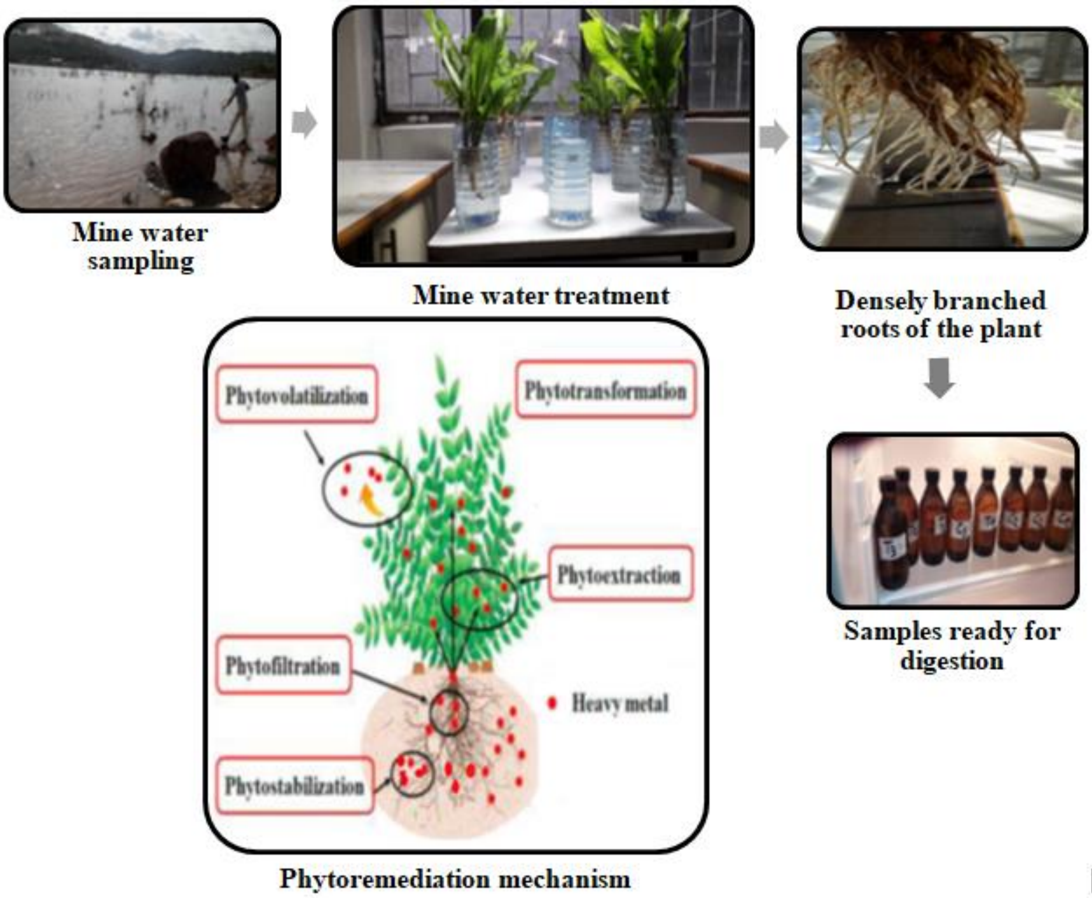

Figure 1. The experimental procedures and heavy metal removal mechanisms

In addition, Blank samples were digested following the same techniques employed for digesting the wastewater samples. Each blank were analysed for the metal contents (Cu, Cd, Zn, Pb and Ni) by MP-AES. The standard deviation (Std) of replicate blanks was calculated to determine method detection limit (MDL) using Eq. (3) (Butcher & Sneddon, 1998; Techane *et al.*, 2019).

MDL= blank mean + Std **Eq. (3)**

The method detection limits of the microwave assisted digestion for the wastewater samples in mg/L were 0.006, 0.010, 0.005, 0.001, and 0.020 for Cu, Cd, Zn, Pb and Ni metals, respectively.

The water samples were analysed for heavy metals Pb, Ni, Zn, Cu and Cd using Agilent Technologies 4200 Microwave Plasma Atomic Emission Spectrometer (MP-AES). Moreover, the pH of water samples was measured using sensION^{TM} + MM150, HACH. All statistical analyses were performed with Microsoft Excel and STATA 14.1. The removal efficiency of the heavy metals (Pb, Ni, Zn, Cu and Cd) was calculated using Eq. (4).

Removal efficiency (%) = $\frac{Ci-Cf}{Ci}$ **Eq. (4)**

Where, C_i= the initial concentrations of the heavy metals in the mine wastewater and C_f = the final concentrations of the heavy metals in the treated mine wastewater.

RESULTS AND DISCUSSION

The mechanism of removing heavy metals could be either by phytoextraction, phytostabilization, or phytovolatilization (Chen *et al*., 2023) as showed in Figure 1. Phytoextraction is the removal of heavy metals by aboveground tissues of the plant while phytostabilization is the plant's ability to resist high metal concentrations and halt them within their roots (Priya *et al.*, 2023). Metals are absorbed by the roots and enter the stem through xylem vessels, which are then passed to the stems and then to the leaves through photosynthetic mechanism (Ratna & Slamet, 2020). Real wastewater from tailings pond of gold mining industry was used to test the phytoremediation potential of *R. nepalensis*. Time dependent removal efficiency of the plant is presented. Table 2 shows the results of the phytoremediation potential for 15 days in five days intervals. The mine waste water was analysed for Cd, Cu, Zn, Pb, and Ni before treatment, and also after five days, 10 days and 15 days of treatments with *R. nepalensis*. The analysis result of Cd was below detection limit both before and after treatments of the mine waste water. The contamination levels of the collected mine wastewater for Pb and Ni metals were beyond the safe limit of WHO for drinking water. The level of Pb and Ni in the tailings pond is about 1.5 and 3.4 times, respectively higher than the standard limit for drinking water (WHO, 2011). Moreover, the level of Ni in the initial mine wastewater is still slightly above the permissible limit set for wastewater discharge (WHO, 2006). Generally, the removal efficiencies exhibited an increasing trend as treatment days increases.

Table 2. The pH and heavy metal concentration (mg/L) before and after treatment

Parameters	Before phytoremediation	After phytoremediation			WHO limit drinking water* *	WHO limit wastewater*
	Co (Initial)	C1 (5th day)	C2 (10th day)	C3 (15th day)		
pH	8.84	8.53	7.98	7.72	6.5-8.5	6.5-8.5
Cu	0.13	0.1	0.025	0.01	2	0.2
Zn	0.05	0.03	0.02	ND	3	2
Pb	0.015	0.01	0.005	0.005	0.01	0.5
Ni	0.24	0.17	0.05	0.08	0.07	0.2

(WHO, 2006*; WHO, 2011**)

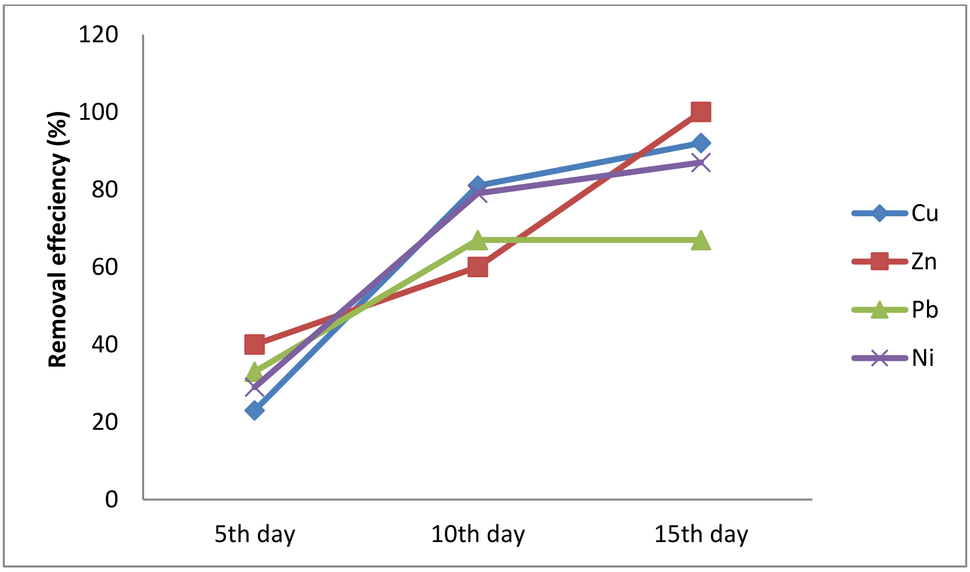

Figure 2. Removal efficiencies of varous metals at regular time interval

The pH values of the mine wastewater were decreased significantly during the phytoremediation using *R. nepalensis*. In this study, the pH in the three intervals of treatment: (5^{th}, 10^{th}, and 15^{th} day) was reduced by 3.51%, 9.73%, and 12.67% respectively. That means at the end of 15 days of treatment, the maximum pH reduction of 12.67% was recorded. In previous studies, (Singh *et al.*, 2021) were reported the highest pH reduction of 10.75% in the treatment of paper mill effluent using *P. stratiotes* which was the decrease of pH values from 8.56 to 7.64. In addition, Azeez (2021) has also showed a maximum pH reduction of 7.6% from the sewage treatment unit using *Utricularia vulgaris* L. The variation of the pH might be due to the formation of transitional compounds during the microbial degradation of dissolved organic substances in the wastewater (Singh *et al.*, 2021).

The initial concentration of Cu, Zn, Pb, and Ni in the untreated mine wastewater were 0.13

mg/L, 0.05 mg/L, 0.015 mg/L, and 0.24 mg/L, respectively. In other studies, Kothari *et al*. (2022) reported high concentrations of Cu (4.4 mg/L), Zn (7.0 mg/L), Pb (2.6 mg/L), and Ni (3.8 mg/L) in tannery effluents, which are all higher than the levels of the heavy metals in the untreated mine wastewater of this study. However, George *et al*. (2017) used initial concentrations of Cu (0.008 mg/L), Zn (0.05 mg/L), Pb (0 mg/L), and Ni (0.06 mg/L) in municipal wastewaters which are lower than the levels of heavy metals in this study. The concentration of heavy metals in our study decreased to 0.01 mg/L for Cu, 0.01 mg/L for Pb and 0.08 mg/L for Ni on the 15th day of the treatment, while Zn was below detection limit at this final treatment day. The maximum removal efficiencies for Zn, Cu, Ni, and Pb were 100%, 92%, 87%, and 67%, respectively, indicating the highest removal efficiency for Zn, followed by Cu, Ni, and finally Pb (**Error! Reference source not found.**). In other study Balamoorthy *et al*. (2022) has used the synthetic wastewater containing Cd, Pb, and Cu with initial concentrations of 0.25 mg/L, 0.5 mg/L, and 2 mg/L respectively, by introducing *Mimosa pudica* and treating them for 16 days. Their results showed that there was a reduction in Cd, Pb, and Cu to a concentration of 0.02 mg/L, 0.21 mg/L, and 0.4 mg/L level of heavy metals from the wastewater, respectively which showed the plant's ability to accumulate up to 92% of Cd, 58% of Pb, and 80% of Cu.

The concentration of Cu was 0.10 mg/L on the 5th day, 0.025 mg/L on the 10th day, and 0.01 mg/L at the end of the 15th day. The removal efficiency of *R. nepalensis* for Cu from the mine wastewater was enhanced from 23% on the 5th day to 81% on the 10th day and finally became 92% at the end of the treatment schedule. The rate of percentage removal of Cu metal increased as the uptake time increased (Abbas *et al*., 2021). The rate of absorption of Cu was 81% in the early days of treatment indicates at the first 10 days the accumulation process was achieved by the roots only and later the translocation of Cu was carried by the shoots of the plant (Balamoorthy *et al*., 2022). Abbas *et al*. (2021) has showed the highest percentage removal efficiency of Cu with 87.78% at 16 days experiment from industrial wastewater using *Typha latifolia*. Other study by Balamoorthy et al. (2022) which used a terrestrial plant, *Mimosa pudica*, also showed the improvement in the removal of Cu from wastewater from 40% on the 4th day to 80% at the end of the 16th day. Ahmad *et al*. (2022) have reported the potential of *R. nepalensis* which able to accumulate greater than 0.05 g/kg of Cu in its shoots. Furthermore, Aras (2022) has investigated the accumulation of 13.6 mg/L of Cu metal in *R. nepalensis* plant.

The concentration of Zn was recorded 0.03 mg/L on the 5th day, 0.02 mg/L on the 10th day and below detection limit at the end of the 15th day. The removal efficiency of *R. nepalensis* for Zn from the mine wastewater was enhanced from 40% on the 5th day to 60% on the 10th day and finally became 100% on the 15th day. Similar to Cu, the uptake of Zn by the plant has increased as time progressed. In other studies, Abbas *et al*. (2021) have showed the highest percentage removal efficiency of Zn with 75.81% at 16 days experiment from industrial wastewater using *T. latifolia.* Similarly, Azeez (2021) has reported the reduction of Zn in wastewater from 2.92 mg/L to 0.69 mg/L using *U. vulgaris* plant in 21 days of treatment, which is a percentage removal of about 76%. A laboratory-scale experimental investigation on phytoremediation of industrial effluent using *Nelumbo nucifera* Gaertn has also showed a reduction of Zn from 10.14 mg/L to 4.92 mg/L (Al-huqail *et al*., 2022). Moreover, in an investigation undertaken to determine the elemental content of heavy metals by Aras (2022) has reported a total of 45.5 mg/L of Zn in *R. nepalensis* plant.

Lead is among non-essential toxic metals. A continuous consumption of Pb polluted water leads to severe effects on human health such as brain diseases, cognitive and productivity problems, carcinogenic effects, kidney and bones diseases (Panneerselvam & Priya, 2021). The level of Pb in our study reduced to 0.01 mg/L on the 5th day, 0.005 mg/L on the 10th and 15th days. Unlike other heavy metals, the removal efficiency of *R. nepalensis* for Pb from the mine wastewater was recorded 33% on the 5th day to 67% on the 10th day and 15th day. This might be due to a low translocation of Pb to the shoot, which makes Pb immobile compared to the other heavy metals studied (Fritioff *et al*., 2010). From test results, it can be noticed that the root of *R. nepalensis* reached saturation at the end of the 10th day of treatment. This indicates the effective removal of Pb is on the 10th day. This finding is in agreement to a study undertaken by using a terrestrial plant known as

M. pudica which showed, the percentage of removal of Pb on the 16th day was found to be very less in amount compared to the percentage of removal of Cd and Cu (Balamoorthy *et al*., 2022). However, Ratna and Slamet (2020) have showed effective removal of Pb from industrial wastewater containing 0.42 mg/L using *Pistia stratiotes* in six days experiment. Similar to Zn and Cu of our study, the percentage removal of Ni has also enhanced as the treatment days increased.

CONCLUSION

Phytoremediation process with the terrestrial plant may be an alternative solution by putting the plants on a floating bed and keeping the roots in the wastewater. The study results evidenced that the removal efficiency of Zn, Cu, Ni, and Pb were 100%, 92%, 87%, and 67% respectively. The pH values of the wastewater were decreased significantly during the phytoremediation. The plant showed maximum accumulation of multi-metals. However, Pb reached saturation at the end of the 10th day, which makes its effective removal on the first 10 days of experimental period. From the present study, the mine wastewater should be treated by *R. nepalensis* for at least 10 days to reduce the toxic heavy metals especially Ni and Pb to their WHO standard limits. As this phytoremediation experiment has used a real type of wastewater with slightly lower concentrations of heavy metals, further studies are recommended with higher levels of heavy metals using synthetic wastewater or theoretical concentrations in laboratories.

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