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The Potential of *Pistia stratiotes* in the Phytoremediation of Selected Heavy Metals from Simulated Wastewater

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Abstract. The pollution of heavy metals in aquatic environments is a major concern for human beings. The present study demonstrates the phytoremediation potential of the aquatic macrophyte *Pistia stratiotes* for removal of Cr, Pb and Ni from simulated wastewater. *Pistia stratiotes* was grown in Faculty of Science & Natural Resources (FSNR) lake water and spiked with different concentrations of heavy metals at 1 mg/L, 2 mg/L and 3 mg/L of Cr, Pb and Ni, respectively. The experiment was conducted within a 14-day period in laboratory conditions. The study investigated the percentage of removal of heavy metals by *P. stratiotes* as well as determining the distribution of heavy metal patterns in plant tissues, the bioconcentration factor (BCF), translocation factor (TF) and relative treatment efficiency index (RTEI). The results showed that *P. stratiotes* is efficient in removing single Pb at 1 mg/L and 3 mg/L and single Cr at 1 mg/L, with a removal efficiency of 99.31%, 79.86% and 76.25%, respectively. It was found that *P. stratiotes* managed to concentrate Pb in its roots up to 15,000 mg/kg in plant tissue. Data on bioconcentration factor (BCF) showed that *P. stratiotes* managed to reach BCF values over 6,000 each for single chromium at 2 mg/L and lead at both 2 mg/L and 3 mg/L. It was found that the plant can consistently translocate nickel from the roots to the shoots, while chromium and lead tend to concentrate in the root tissues. The results revealed that *P. stratiotes* uses rhizofiltration as its phytoremediation uptake mechanism. This study helps significantly to increase knowledge regarding the potential of *P. stratiotes* in the phytoremediation of heavy metal-polluted wastewater.

Keywords: Bioconcentration factor; Phytoremediation; Pistia stratiotes; Relative treatment efficiency index; Translocation factor

1. Introduction

Heavy metals are hazardous, as they can affect human health, plant growth and biodiversity. Heavy metals can be classified into non-essential and essential metals. Heavy metals such as cadmium (Cd) and lead (Pb) are considered non-essential heavy metals that are not necessary for humans and animals (Tamele and Loureiro, 2020). Zinc (Zn) and nickel (Ni) are essential heavy metals for human metabolism; nevertheless, they can cause unfavorable effects when they exceed certain threshold levels (Edelstein and Ben-Hur, 2018). Water pollution by heavy metals is non-biodegradable, as they can exist in diverse oxidation states that can last long in the environment (Yadav et al., 2018).

As Malaysia is a fast-growing country with rapid industrialization and urbanization, this encourages an increase in the generation of heavy metals (Firmawan et al., 2012). The

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improper disposal of these contaminants can be transported to water bodies through the runoff of untreated metals introduced into open water bodies, which eventually causes water pollution (Suwartha and Pujiastuti, 2017; Li et al., 2019). In 2015, Malaysia spent RM 2.6 billion to protect these sectors from polluting the environment: 73.6% for manufacturing industries, 11.3% for public services, 6.9% for mining and quarrying, 6.4% for construction and another 1.8% for agriculture, forestry and fishing (Mahidin, 2018). From these statistics, the manufacturing industry is the sector of most concern for environmental pollution, and specifically water pollution. There are some treatment technologies that have been established to isolate heavy metals from water, such as membrane filtration, coagulation-flocculation, precipitation, ion exchange and adsorption (Hudaya et al., 2018). These conventional or physico-chemical techniques for treating heavy metals are time-saving, but they require a high operational cost as well as extra costs for sludge disposal (Barakat, 2011). Phytoremediation is an eco-friendly and cost-effective technique for utilizing plants in removing heavy metals from polluted water bodies without generating sludge during the treatment process (Zhang et al., 2018). In this study, *Pistia stratiotes*, a type of free-floating aquatic macrophyte that belongs to the Araceae family, was chosen based on its special characteristics, such as its high rate of growth, high tolerance to heavy metals and ease of cultivation. This plant can easily be found in tropical and sub-tropical areas, where the abundance of sunlight and warm temperatures encourage its growth to the point where it can be considered an invasive species and a nuisance (GISD, 2005). A laboratory experiment was conducted in a 40L reactor tank cultivated with *P. stratiotes* for 14 days to treat selected heavy metals taken from simulated wastewater. The accumulation of heavy metals in *P. stratiotes* tissues was examined by the end of the treatment. The present study also highlighted the relative treatment efficiency index (RTEI) and the feasibility for *Pistia stratiotes* to thrive in high concentrations of heavy metals up to 3 mg/L. This work can provide information on the defense mechanism of the plant in coping with heavy metals, namely Cr, Pb and Ni. This data can be used in pilot aquatic plant-based wastewater treatment plants. As *Pistia stratiotes* can easily be found in Sabah Malaysia, the use of this plant for remediation is an appealing green technology for treating the accumulation of different heavy metals.

2. Methods

2.1. *Sampling Area*

Mature *Pistia stratiotes* was collected along the drain near the Pekan Kimanis Lama, Papar, Sabah Malaysia. It was used to assess its heavy metal removal capacity for single heavy metals, which were chromium, lead and nickel, from simulated wastewater. The samples were washed with running tap water to remove debris and other small biota. The rinsed plants were placed into a 40 L glass tank and cultivated for three weeks in a tank filled with lake water from the Faculty of Science and Natural Resources (FSNR), Universiti Malaysia Sabah (UMS), Malaysia. Light was provided using Philip brand 2×4 ceiling lighting (fluorescent lamp) in a 12–12-hour light/dark cycle at room temperature.

2.2. *Experimental Set-up for Removal of Cr, Pb and Ni by P. stratiotes*

Approximately 100 g (fresh weight) of *P. stratiotes* was cultivated in a 40 L reactor tank of FSNR's Lake water. Standard solutions of three heavy metals, Cr, Pb, and Ni (Scharlau), were prepared using 1000 mg/L of standard stock (99% purity) and added in the form of single metals to form simulated wastewater with initial heavy metal concentrations of 1 mg/L, 2 mg/L, and 3 mg/L, respectively. A separate set of controls (with no plants) was created to obtain the control values of the heavy metal concentrations in simulated wastewater. The experiment was performed in duplicate to obtain accurate data. Figure 1 below illustrates the experimental design of the study and a schematic drawing of the reactor tank used.

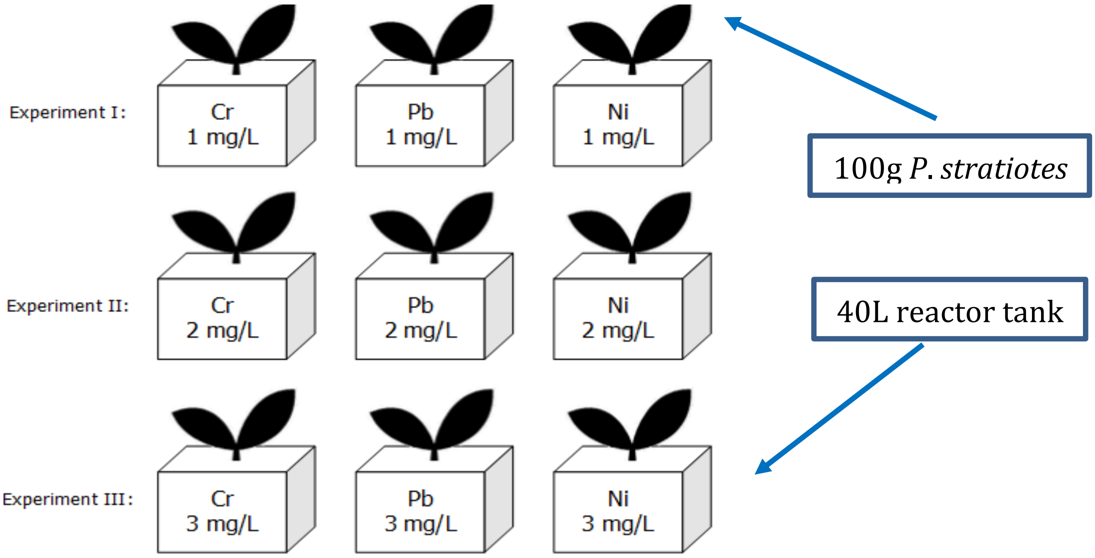


Figure 1 Schematic representation of experimental setup

2.3. *Sampling and Preparation of Simulated Wastewater & P. stratiotes*

The simulated wastewater was sampled at 24-hour intervals throughout the experiment. The samples were collected in duplicate at 10 AM each day using clean 50 mL polyethene bottles (Unisampler). Prior to sampling, the simulated wastewater was gently stirred to ensure the collection of a homogenous sample. The sampling bottles were closed securely and stored in a fridge at 4°C until analysis. For *P. stratiotes*, the plant samples were collected at Day 0 and Day 14 in duplicate. During sampling, aerial and root parts of the plant were chosen from random plants in each reactor in order to produce a representative sample of the entire population of plants per reactor. Care was taken to ensure no plants were excessively sampled to the point where they could possibly die. The samples obtained were placed into a clean aluminum container and dried in an oven at 75°C for 24 hours. The samples were taken out and cooled before being stored in a resealable Ziploc bag until further use.

2.4. *Determination of Cr, Pb and Ni in Simulated Wastewater and P. stratiotes*

The heavy metal analysis of water samples was performed in accordance with American Public Health Association (APHA) 030 B (Baird et al., 2017). The water sample was filtered through a 0.45 µm membrane filter, transferred to an acid-washed 40 mL polyethene bottle and placed in refrigeration at temperatures below 4°C prior to analysis. The heavy metal analysis of plant samples was performed in accordance with U.S. EPA Method 3050B with modifications (Tighe et al., 2004). The dried plant samples were cut into small pieces using clean scissors. Two g of dry weight of plant samples were digested using 25 mL of concentrated nitric acid (Merck) (65%) in a 50 mL conical flask on a hot plate (Thermo Scientific Cimarec SP88850107) at 120°C for 2 hours until only 5 mL of solution was left in the flask. The solution was left to cool, and the walls of the flask were rinsed with distilled water. 5 mL of concentrated HNO_3 was added into the solution, and the solution was heated until a gentle reflux action was achieved. Heating was stopped when the digestate was light in color and did not change in appearance with continued refluxing. The digestate was left to cool. Ten mL of hydrochloric acid (Merck) (50%) and 15 mL of deionized water were added to the digestate. The digestate was heated for an additional 15 minutes before cooling. The sides of the flask were rinsed with deionized water, and the digestate was filtered using a 0.45 μm membrane filter (Whatman). The filtrate was transferred to a 100 mL volumetric flask and deionized water was added until the final volume reached the 100 mL mark. The final solution was poured into an acid-washed polyethene bottle (Unisampler) and placed in refrigeration at below 4°C prior to heavy metal analysis using ICP – OES (Agilent 5800).

2.5. Removal Efficiency, Uptake of Heavy Metals and Relative Treatment Efficiency Index (RTEI) of P. stratiotes

The potential and performance of *P. stratiotes* for phytoremediation of heavy metals was determined by examining the removal efficiency, the uptake of heavy metals in plant parts and the relative treatment efficiency index (RTEI). Heavy metal removal efficiency was used to determine how well *P. stratiotes* performed in the removal of heavy metals (Mahardika et al., 2018). The formula used to calculate heavy metal removal efficiency is shown below:

$$Heavy\ metal\ removal\ efficiency = \frac{C0-C1}{C0} \times 100\% \tag{1}$$

where $C0$ is the concentration of heavy metals in simulated wastewater (ppm) at Day 0 and $C1$ is the concentration of heavy metals in simulated wastewater (ppm) at Day 14.

For uptake of heavy metals in plant parts, the initial and final concentrations of heavy metals in plants were calculated based on the formula below:

$$Uptake\ of\ heavy\ metals\ in\ plant\ parts\ \left(\frac{mg}{kg}\right) = \frac{Cp \times Tv}{Dw} \tag{2}$$

where Cp is the concentration in plant parts (mg/L), Tv is the total volume (L) and Dw is the weight of biomass (kg).

Thus, for the relative treatment efficiency index (RTEI) which is based on comparing control treatment metal removal with influent metal concentration and effluent treatment metal removal, the formula for calculating the RTEI index is shown below (Marchand et al. 2011):

$$Relative\ Treatment\ Efficiency\ Index\ = \frac{T-C}{T+C} \tag{3}$$

where T is the removal efficiency of heavy metals in plants (%) and C is the removal efficiency of heavy metals in control reactors.

2.6. Phytoremediation Mechanism Uptake of P. stratiotes by Bioconcentration Factor (BCF) and Translocation Factor (TF)

The bioconcentration factor (BCF) is the ratio of the concentration of a heavy metal in an organism to the concentration of the heavy metal in the surrounding environment (Franco et al., 2009). BCF was used to determine the potential of *P. stratiotes* to absorb varying concentrations of heavy metal from the simulated wastewater. Meanwhile, translocation factor (TF) was used to determine the mobilization of heavy metals to other parts of the plant (Bello et al., 2018). The formulae used to calculate BCF and TF are shown below:

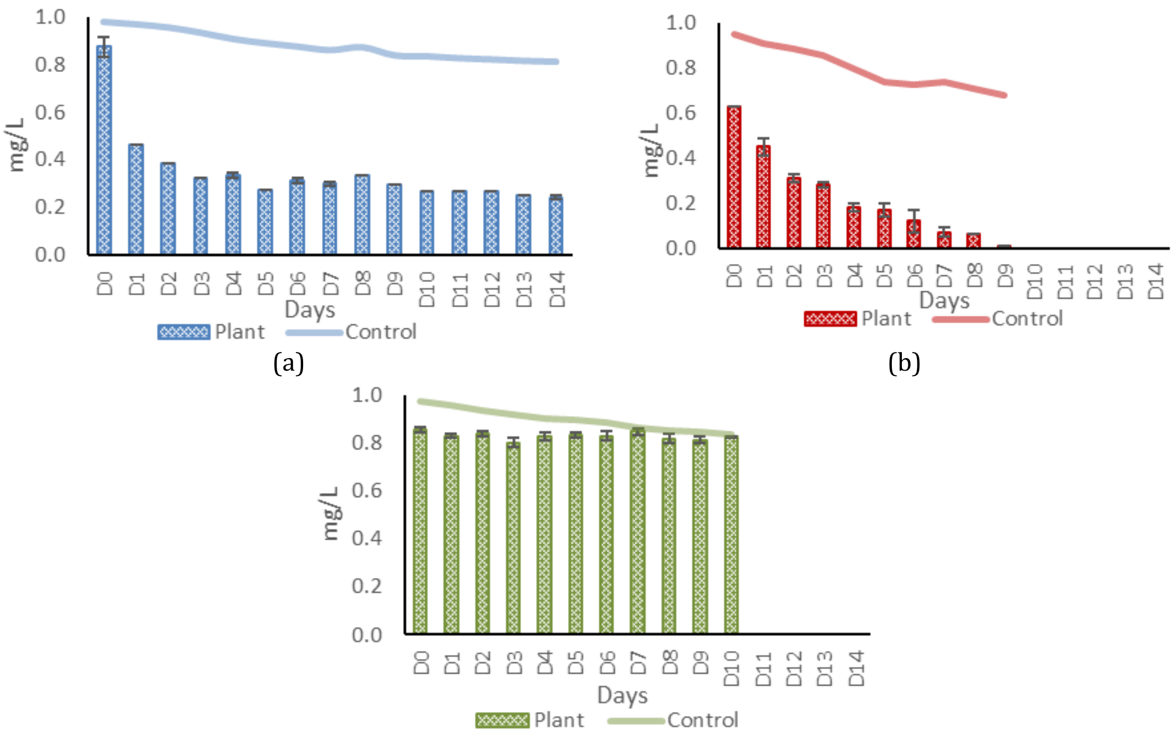
$$BCF\left(\frac{L}{kg}\right) = \frac{Heavy\ metal\ concentration\ in \frac{root}{shoot}\left(\frac{mg}{kg}\right)}{Heavy\ metal\ concentration\ in\ water\ \left(\frac{mg}{L}\right)} \tag{4}$$

$$TF = \frac{Heavy\ metal\ concentration\ in\ shoots\ \left(\frac{mg}{kg}\right)}{Heavy\ metal\ concentration\ in\ roots\ \left(\frac{mg}{kg}\right)} \tag{5}$$

3. Results and Discussion

3.1. Removal Efficiency of Cr, Pb and Ni by P. stratiotes at 1mg/L, 2 mg/L and 3 mg/L Concentration Levels

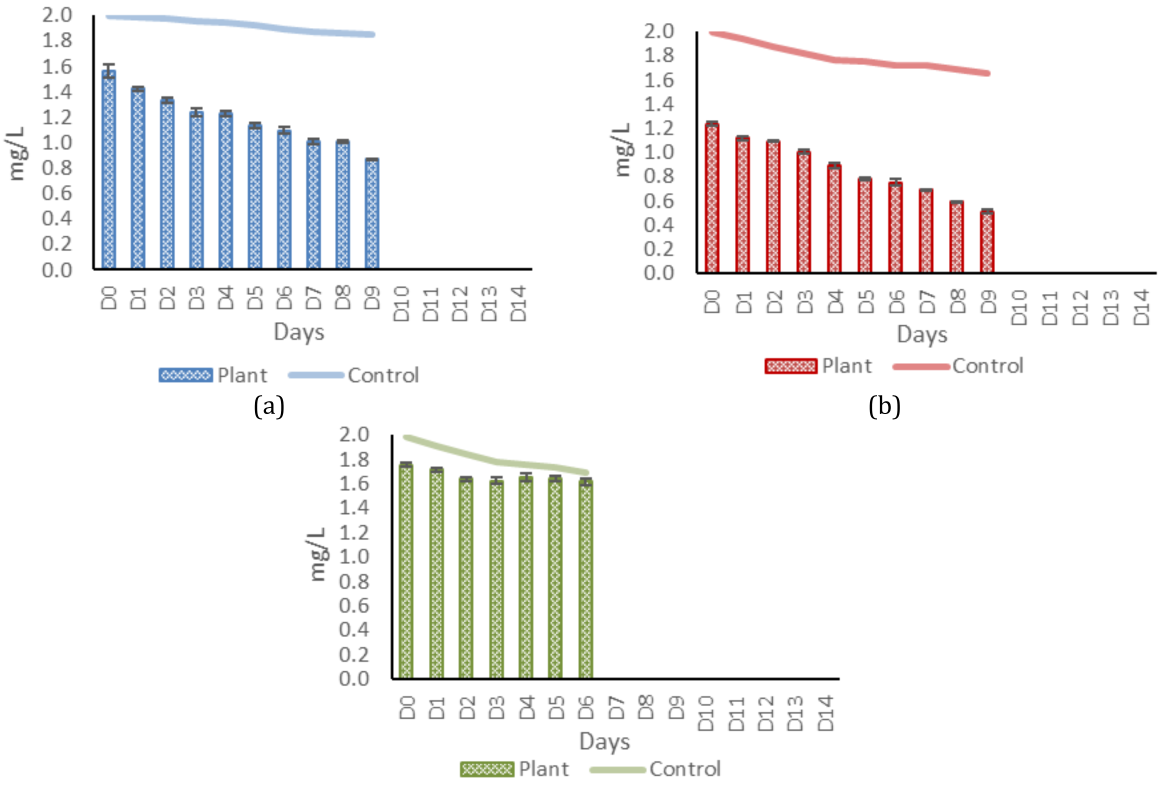
Figures 2a–2c shows the removal efficiencies of Cr, Pb, and Ni by *P. stratiotes* at 1 mg/L concentration. Different heavy metals are represented by different colors, while their respective control values are represented by the straight lines in the graph below.



(a) (b) (c)

Figure 2 Metal concentration (mg/L) against time (days) for removal of: (a) chromium; (b) lead; and (c) nickel by *P. stratiotes* at 1.0 mg/L concentration level

As shown in Figures 2a-2c, *P. stratiotes* is most efficient at removing lead, with a removal efficiency of 99.31%, followed by chromium at 76.25%. The removal efficiency of nickel is the lowest, with 20.50% on Day 9 of cultivation. The concentration of heavy metal for the control dropped by 7.83% for chromium, 6.86% for lead and 5.73% for nickel. The loss of heavy metal can be attributed to several factors, such as sedimentation, adsorption to clay particles and organic matter, or co-precipitation with secondary minerals (Mishra and Tripathi, 2008). The slow removal of nickel from Day 0 up until the death of *P. stratiotes* at Day 10 suggests that this plant is not a hyperaccumulator of that particular element. On the other hand, *P. stratiotes* reacted very favourably upon lead, as it managed to remove over 99% of lead over a span of 9 days, while with chromium, it managed to remove over 75% in 14 days. It is also worth noting that for both chromium and lead, most of the removal occurred within the first 48 hours of the experiment. This suggests that the initial stages of heavy metal removal occurred in the form of the binding of heavy metals to root exudates, ion exchange sites, and extracellular precipitation (Sharma and Dubey, 2005). This was followed by the gradual movement of heavy metals into the root tissue and translocation to the shoots of *P. stratiotes.* It should be noted that out of the three heavy metals, *P. stratiotes* managed to tolerate chromium for the full duration of the experiment (14 days), while the plant displayed signs of heavy metal toxicity, such as darkening and detachment of roots, as well as chlorosis for lead and nickel, on days 9 and 10, respectively.



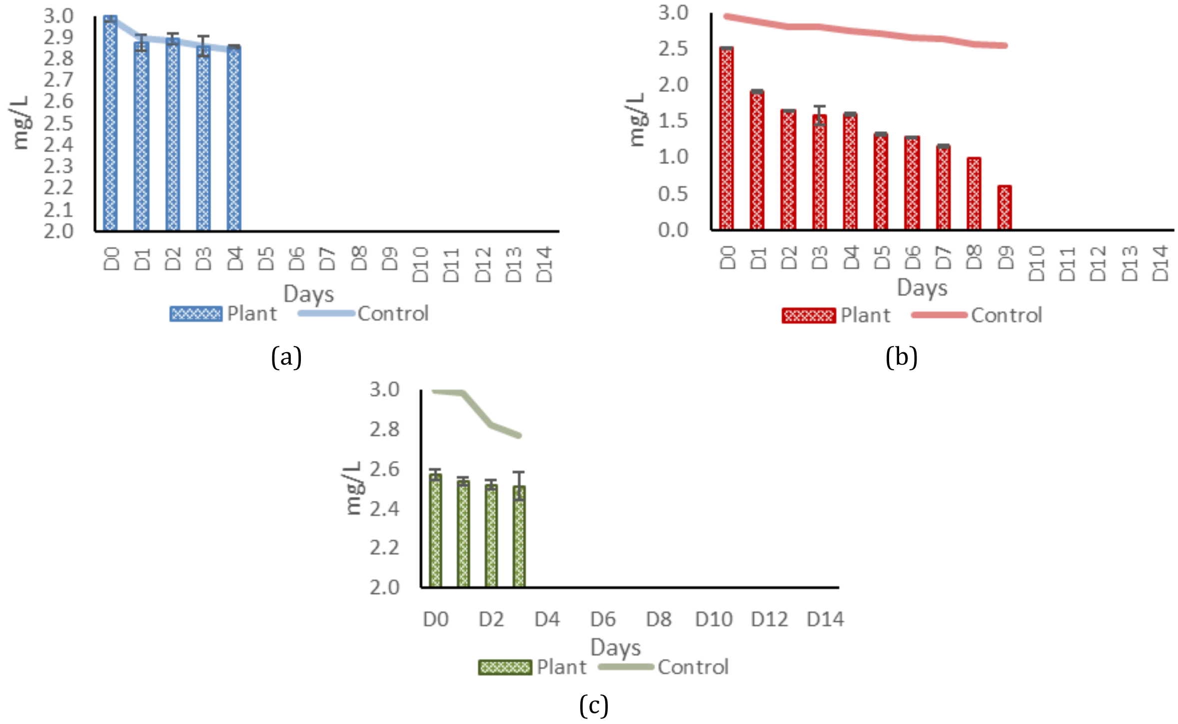
(a)

(b)

(c)

Figure 3 Metal concentration (mg/L) against time (days) for removal of: (a) chromium; (b) lead; and (c) nickel by *P. stratiotes* at 2.0 mg/L concentration level

An experiment containing 2 mg/L showed that lead was observed to have the highest removal efficiency with 75.02%, while chromium recorded 55.86%, followed by nickel with 23.63% (Figures 3a-3c). The concentration of heavy metal for the controls dropped by 7.37% for chromium, 17.28% for lead and 14.54% for nickel. It is important to note that the removal efficiency pattern for single metals was similar to the experiment with 1 mg/L, where removal efficiency was shown in descending order: Pb > Cr > Ni. The poor results of nickel accumulation by *P. stratiotes* are echoed by another study, indicating that nickel is not a preferential metal of the plant (Odjegba and Fasidi, 2004). Figures 4a-4c shows that at 3 mg/L, lead was observed to have the highest removal efficiency with 79.86%, while nickel recorded 17.17%, followed by chromium with 5.34%. For the control treatment, the concentrations were 4.82% for chromium, 13.59% for lead and 7.52% for nickel. Based on these findings, it can be reported that chromium at 2 and 3 mg/L is detrimental to the health of *P. stratiotes*, as its tolerance duration dropped from 9 days at 2 mg/L to only 4 days at 3 mg/L. Meanwhile, lead remained a good accumulator even with a constant tolerance duration of 9 days throughout all three concentrations (1, 2, and 3 mg/L). At 3 mg/L, the uptake of nickel was greater than chromium; this may be because chromium is not an essential nutrient, while nickel is a micronutrient needed by plants.



(a) (b) (c)

Figure 4 Metal concentration (mg/L) against time (days) for removal of: (a) chromium; (b) lead; and (c) nickel by *P. stratiotes* at 3.0 mg/L concentration level

3.2. Comparable Performance on Removal Efficiency of Cr, Pb and Ni by Pistia stratiotes with Previous Study

Table 1 shows the comparable removal efficiency of related studies of *P. stratiotes* at 1.0 and 2.0 mg/L concentration levels. Interestingly, the results obtained from the present study revealed that Pb removal percentages were highest, at 99% within nine days of treatment. This was 1.05-fold higher than the figure reported by Zhou et al. (2013). However, the removal efficiency of Cr was reported as highest by Mishra and Tripathi (2008), with 81% and 75% efficiency at 1.0 and 2.0 mg/L, whereas the present work recorded 76% and 55%. Mishra and Tripathi (2008) stated that Cr is one of the most difficult metals to remove from water since this macrophyte does not require this element for any physiological purposes. Moreover, most aquatic plant species have a low mobility of Cr due to the barriers to transporting this metal from roots to shoots (Kleiman and Cogliatti, 1998).

Table 1 Comparative studies on removal efficiency of Cr, Pb and Ni by *Pistia stratiotes*

Heavy Metals	Initial Concentration (mg/L)	Removal Efficiency (%)	Duration (Days)	References
Cr	1.0	81%	15 days	Mishra and Tripathi (2008)
	2.0	75%		
Pb	1.0	94%	10 days	Zhou et al. (2013)
	2.0	92%		
Ni	0.05	41.5%	Not stated	Lu et al. (2011)
Cr	1.0	76%	14 days	Present work
	2.0	55%	9 days	
Pb	1.0	99%	9 days	
	2.0	75%	9 days	
Ni	1.0	20%	10 days	
	2.0	23%	6 days	

Among the heavy metals studied, Ni shows the lowest removal performance at 41%, as stated by Lu et al. (2011). These results are in line with our findings, which showed that the removal of Ni was lowest, with 20% and 23% efficiency, at 1.0 and 2.0 mg/L, respectively. This might be due to the inhibitory effect of high levels of Ni, which affect the chlorophyll content and create a nutrient imbalance for the biosynthesis process (Gautam and Pander, 2008). Overall, it can be summarized that the removal efficiencies vary for different heavy metals with varying concentrations studied. However, it is important to highlight that *P. stratiotes* proved equally efficient in the cleaning of heavy metals across the different experiments.

3.3. Uptake and Distribution of Cr, Pb, and Ni in Plant Tissue of P. stratiotes at Different Concentration Levels

In Figure 5, it can be observed that the concentration of chromium is greater in the root than shoot at 1, 2, and 3 mg/L. The highest concentration of chromium in the root can be found in samples of 2 mg/L (12068 mg/kg), while the highest concentration of chromium in the shoot can be found in 3 mg/L (304 mg/kg). Lead has the highest root and shoot concentration of all concentration levels, at 18,932 mg/kg and 617 mg/kg, respectively. For nickel, the accumulation is concentrated in the root tissues of *P. stratiotes*. However, the highest nickel concentration in the root (1050 mg/kg) is less than 0.1-fold of chromium's concentration in the root. It can be stated in general that more heavy metal is accumulated in the roots as compared to the shoot. This suggests that the root of the plant acts as a barrier against the translocation of heavy metals to other parts of the plant.

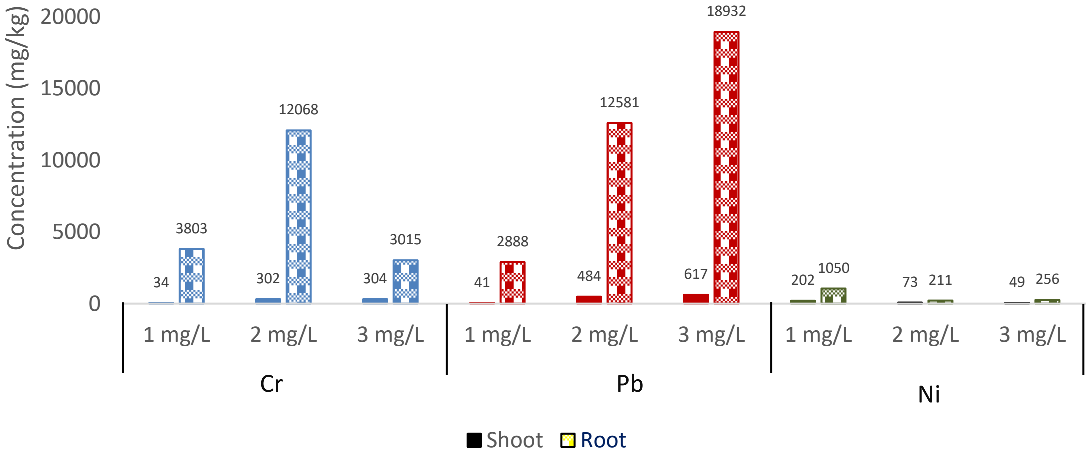


Figure 5 Concentration of: (a) Cr; (b) Pb; and (c) Ni in shoot and root of *P. stratiotes* at 3 different concentrations studied

The binding of lead to the carboxyl group of mucilage uronic acids, ion exchangeable sites, extracellular precipitation and others help to secure and localize heavy metals in the root of *P. stratiotes* (Sharma and Dubey, 2005). When the heavy metals enter the root, their movements can be limited by several processes or factors, such as immobilization by negatively charged pectins in the cell wall, precipitation in the form of insoluble salts in intercellular spaces, and sequestration in the vacuoles of rhizodermal and cortical cells (Seregin et al., 2004; Islam et al., 2007; Kopittke et al., 2007). It is important to note that there is less accumulation of nickel in roots and shoots of *P. stratiotes* at all concentrations studied. A possible explanation for this phenomenon might be toxicity symptoms such as chlorosis, withering of roots and decaying of shoots (Rolli et al., 2017). All these will adversely affect the uptake of heavy metals into the plant, thus reducing the quantity of accumulated heavy metals in the tissues.

3.4. *Relative Treatment Index of Cr, Pb and Ni by P. stratiotes*

The relative treatment efficiency index is a method to quantify how well a plant or constructed wetland can remove a specific pollutant from the environment while taking into account factors such as metal removal efficiency due to organic matter, gravel, and metal removal efficiency that occurs in plant-less controls. The values of RTEI range from 1 to -1, where values closer to 1 indicate strong effects of metal removal, while values closer to -1 show otherwise. Based on Figure 6, the value of RTEI was observed in the range of 0.05–0.766. The highest RTEI value was shown in Cr at a 2 mg/L concentration, while at 3 mg/L, the RTEI was recorded lowest (0.05) among the others. It is interesting to report that a higher removal efficiency does not equate to higher RTEI. This can be observed with lead at 1 mg/L achieving a removal efficiency and RTEI of 99.31% and 0.56, respectively. The reason for this inconsistent occurrence lies in the fact that RTEI is influenced not only by removal efficiency but also by the removal of heavy metals in the control reactors. In control reactors, events such as sedimentation, adsorption of heavy metals to clap particles and organic matter, and co-precipitation with secondary minerals can result in a decrease in heavy metal concentration (Mishra and Tripathi, 2008).

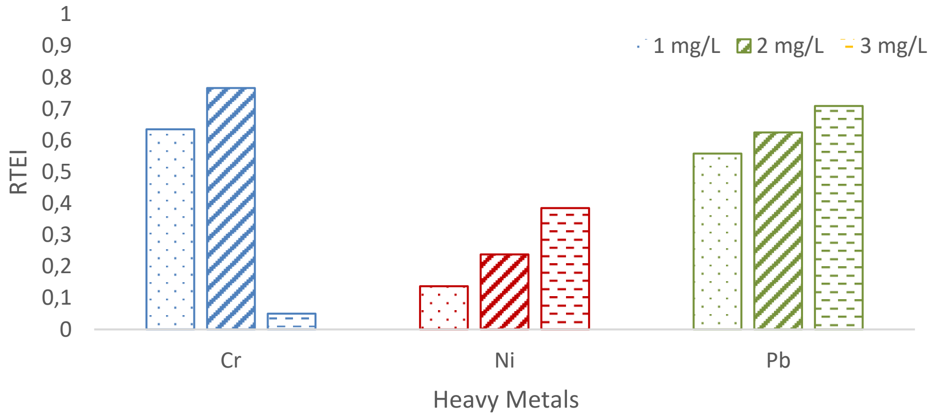


Figure 6 RTEI of *P. stratiotes* at (a) 1 mg/L, (b) 2 mg/L and (c) 3 mg/L concentration

3.5. *Bioconcentration Factor and Translocation Factor of Heavy Metals (Cr, Pb and Ni) by P. stratiotes*

Bioconcentration factor is an index that classifies a plant's ability to accumulate heavy metals relative to the available heavy metal in the environment (Zhou et al., 2013). Based on Figure 7, the highest BCF of chromium occurred in the roots at 2 mg/L with a value of 6,034.22, while in roots it was 151.37. For lead, the highest BCF value for roots is 6,310.97 at 3 mg/L, while in shoots, it was 242.15 at 2 mg/L. Finally, the BCF of nickel in the root and shoot is highest at 1 mg/L, with 1050.34 and 202.65, respectively. Previous studies have stated that a plant can be considered a good accumulator of a specific element if it has BCF values of over 1,000; thus, it can be concluded that *P. stratiotes* is a good accumulator of all three heavy metals tested at varying concentration levels (Zhou et al., 2013). On the subject of translocation factors, it can be found that the TF values for all concentrations studied are relatively low, in the range of 0.01–0.35. Based on the BCF and TF values of *P. stratiotes*, it can be concluded that *P. stratiotes* uses rhizofiltration as its phytoremediation mechanism, as most of the heavy metal detected in plant tissues is concentrated in the root, with relatively low quantities of heavy metal being transported from the root to shoot tissues. The findings of this study coincide with the findings of Galal et al. (2017). They investigated the water pollution in Egyptian wetlands and found that the bioconcentration factor (BCF) of Pb was greater than 1,000, while the translocation factor (TF) did not exceed one for Cr.

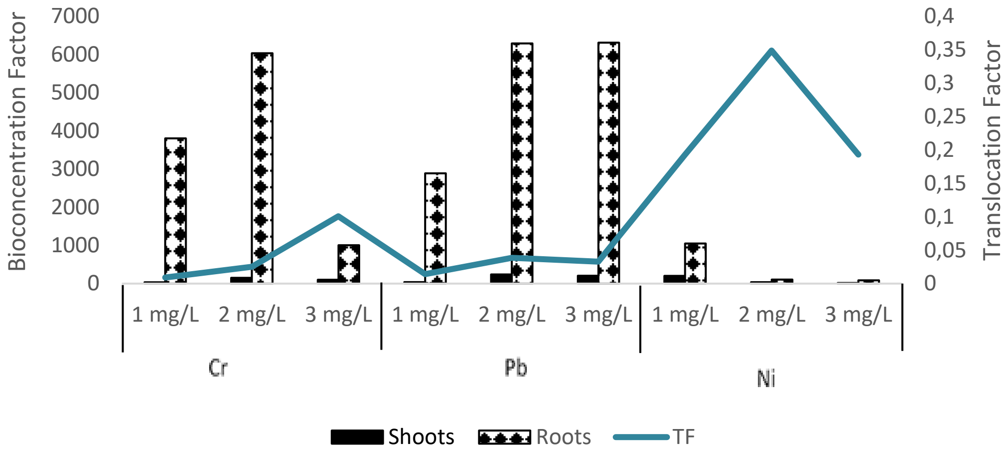


Figure 7 Bioconcentration factor and translocation factor of: (a) chromium; (b) lead; and (c) nickel in *P. stratiotes* at different concentration levels

4. Conclusions

The obtained results show that *P. stratiotes* has the highest tolerance and removal efficiency for lead, with a removal percentage of 99.31% at 1 mg/L. This plant can accumulate chromium and nickel, although it may not survive at higher concentrations of heavy metals nor achieve a removal efficiency greater than that of lead. The RTEI values proved that *P. stratiotes* is capable of removing all three heavy metals tested in this study. For BCF and TF values, it was found that the phytoremediation mechanism uptake of *P. stratiotes* is rhizofiltration. The ability of *P. stratiotes* to remove heavy metals especially lead had indicated their potential in treatment of metal polluted water. It is important to note that the plants must be harvested regularly to avoid it releasing heavy metals back into the environment.

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