NUTRIENT AVAILABILITY AND PHYTOREMEDIATION OF LEAD AND CADMIUM BY LILY PAD AND ALGAE IN WASTEWATER

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ABSTRACT

This study evaluated the amount of nutrients in the form of phosphate, nitrate and potassium available for lily pad and algae to carry out phytoremediation of lead and cadmium in wastewater. The analysis carried out using standard analytical procedures showed the mean macro-nutrient and heavy metal availability in the wastewater were in the descending order: phosphate > potassium > nitrate > cadmium > lead. Both lily pad and algae exhibited hyper-accumulation of all the macro-nutrient and heavy metals under review with lily pad having higher bioconcentration factor (BCF) for nitrate and phosphate while algae had higher BCF for potassium, lead and cadmium. Lily pad and algae combined, significantly remove lead from the wastewater by 0.007 mg/L (70%) and cadmium by 0.012 mg/L (11.21%) thereby revealing the use of lily pad and algae as a promising means of lead and cadmium contamination clean up.

Keywords: Lily pad, algae, macro-nutrient, heavy metals removal

INTRODUCTION

Contamination of water by inorganic and organic pollutant is of serious concern to the ecosystem due to their ability to produce one toxic form to another [1]. The quality of lots of water bodies degrades as a result of population growth, industrialization, urbanization and increasing depletion of natural water resources [2]. Water contamination occurs mainly due to the introduction of agricultural inputs, industrial and household waste waters, acid rain, heavy metals, pesticides, pharmaceuticals, oil, inorganic and organic chemical compounds [3, 4].

Contamination of water due to heavy metals such as cadmium and lead which are among the major pollutants the World Health Organization identified for special consideration for water quality owing to their high solubility and easiness to get into food chain is of concern. They are non-essential and poses' health risk. For instance, the cell membrane is mostly affected by exposure to cadmium in animals and humans while lead widespread use has caused extensive environmental contamination, human exposure and health problems in many parts of the world. It is a cumulative toxicant that can affect multiple body systems. Children are particularly vulnerable to the neurotoxic effects of lead [5, 6]. Hence, wastewater must be adequately treated prior to discharge into the environment.

Currently, conventional wastewater treatment methods are not always effective towards the complete removal of water contaminants, since residual contaminants are still found in treated water [7]. These residues may endanger habitats due to the toxic nature of the contaminants, which can interfere with many cellular functions in plants [8]. Considering the harmful effects of these contaminants to human life and aquatic ecosystems, alternative treatment methods of wastewater are required [9]. So far diverse conventional treatment methods namely: ion exchange, reverse osmosis, chemical precipitation, adsorption, electrochemical treatment among others are been employed in water purification [7]. However, sourcing a greener and environmentally sustainable means of water treatment remains a front burner in achieving the millennium sustainable goal.

Phytoremediation technique promises to be economically and environmentally favourable as it utilizes green plants to hold, sequester and detoxify contaminants from wastewater [10]. Aquatic plants have been successfully employed in wastewater treatment owing to their ability to achieve phytoremediation through rhizofiltration, phytoextraction, phytovolatilization, phytodegradation or phytotransformation techniques depending on the nature of the plant, duration of exposure, concentration of pollutants, environmental factors such as pH and temperature [9].

Swamp Lily Pad (*Nymphea odorata*) an aquatic plant regarded as the queen of Indian flowers, belonging to family Nymphaeaceae [11]. The plant has been employed to remediate total hydrocarbon content in water systems with 80.25 to 83.62% success [12]. While recently, there has been a growing interest in the use of algae for wastewater treatment owing to its high ability to accumulate heavy metals, synthesize phytochelatins and metallothioneins that can form complexes with heavy metals and translocate them into its vacuoles [13]. Also its rhizosphere is reportedly involved in various biogeochemical processes such as alleviation of biotic/abiotic stress in plants, nutrient uptake, and metal detoxification [14]. Algae restrict metal ions mobility by forming complex, once they entered its cells thereby minimizing the toxic effects of the contaminant [15].

Both algae and lily pad are classified as floating plants while algae species have been employed in the removal of heavy metals such as cadmium and lead, recording over 70% removal efficiency [16]. Likewise, water lily uptakes cadmium to a magnitude of 0.7 mg/kg to 2.1 mg/kg has been reported in a previous study [17].

However there is no report on the combine effect of algae and lily pad on phytoremediation of heavy metals even though they are both water floating plants and do coexist in some water bodies. This study seeks to determine the level of macronutrient and heavy metal levels in wastewater as well as the synergistic phytoremediation of lead and cadmium by algae and lily pad.

MATERIALS AND METHOD

Sample Collection Phytoremediation

Fresh growing lily pads (*Nymphaea sp.)* and algae from stagnant water were harvested in October, 2023, and were identified at the Department of Biological Sciences, Federal University Wukari. The lily pad and algae were transplanted in a pot containing (25 L) of wastewater inoculated with 2 g each of lead salt ($PbSO_4$) and cadmium salt ($CdCl_2$). Ten stands of the lily pad and algae were inter-planted and grown together for a period of 14 weeks to achieve Pb and Cd removal. At two weeks interval, 10 mL of the wastewater were collected to ascertain the amount of Cd and Pb removed with time over a period of 3 months. At the end of the phytoremediation experiment, the lily pad and algae tissues were harvested for onward analysis.

Plate 1: Visual of lily pad and algae effecting phytoremediation

Sample Preparation

The lily pad and algae plant tissues harvested were washed with running water, and then deionized water to remove any surface contaminants. The pre-cleaned lily pad and algae were dried thoroughly at ambient temperature (around 40 °C) to prevent nutrient loss.

The samples were ground with mortar and pestle to ensure uniform distribution of aggregates throughout the sample.

Determination of pH

For about 20 minutes, the pH meter (Jenway 2000 model) was switched on to warm up which was later calibrated with buffer 4.0, 7.0 and 9.20. The pH electrode was immersed into the water sample and the pH reading was recorded.

Determination of Macronutrient and Heavy Metals in Water and Plant Samples

Sample Mineralization

To powdered lily pad sample (2 g) in a digestion vessel, 30 mL of concentrated acids (mixture of nitric acid (HNO_3) and hydrochloric acid (HCl) in the ration of 3:1 were added to the digestion vessel. This aids the dissolution of the plant tissue and release bound elements. The digestion vessel was placed on a hotplate and heated at 75 ^oC until the acid mixture dried by about 70 percent. Afterwards, the digest was allowed to cool before diluting with deionized water to a 100 mL. Same procedure was replicated for algae plant.

Determination of Potassium, Lead and Cadmium

Potassium content in the various samples was quantified by means of a flame photometer (Jenway PFP 7). The heavy metals: lead and cadmium concentration were measured using atomic absorption spectrophotometer (Unicam 669) for the water sample before and after phytoremediation as well as lily pad and algae tissues.

Determination of Nitrate in Water

About 2% boric acid dissolves 20 g of boric acid in 1000 mL volumetric flask with mixed indicator of 0.2 g of methyl blue in 100 mL of ethanol and 0.4g of methyl red in 100 mL of ethanol which was later mixed in a 200 mL volumetric flask containing Devarda alloy, magnesium oxide and 0.025 N sulphuric acid solutions.

Procedure

About 0.20 g of MgO was added to 5-10 mL of water sample in a distillation flask after which the content was distilled into 10 mL of 2% boric acid solution till a volume of 20 mL distillate was reached. The content was titrated with 0.025 N H_2SO_4, to record titre value as against ammonium. About 0.20 g of Devarda alloy was added into the same flask after collecting enough distillate for ammonium and distilled about 20 mL distillate into 10mL volume of 2% boric acid which was titrated with 0.025 N H_2SO_4 to record the titrate value for nitrate [18].

Calculation

$$\% \ NO_3^- = \frac{0.014 \text{ x normality of acid x 100 x titre}}{\text{A Liquo taken}} \quad (1)$$

Determination of Phosphate

The concentration of phosphate in the water samples were analyzed using the ultraviolet/visible spectrophotometer (721D). This was done by adding 5 mL of the sample into a 25 mL volumetric flask, 5 mL of the molydate reagent and 1 mL of tin chloride working solution were added to it respectively and diluted to 25 mL with distilled water. The absorbance was taken using a UV/VIS spectrophotometer at wavelength 660 nm. The concentration of phosphate in the samples were calculated using the regression values from the calibration curve; $y = 0.0004x + 0.0635$, $R^2 = 0.9952$ [19, 20].

Data Analysis

Data generated from Atomic absorption spectroscopy, uv/vis spectrophotometry, titrimetric and flame photometric analyses were fitted into established models in equation 2 and 3 adopted from Yerima and Donatus [20] and Yerima et al. [21]. These were used to evaluate: Removal efficiency and bio-concentration factors of the heavy metals and macronutrients:

$$\text{Removal efficiency } (R_e) = \frac{C_o - C_e}{C_o} \times 100 \quad (2)$$

$$BCF = \frac{C_{plant}}{C_{water}} \quad (3)$$

Where C_o, C_e, C_{plant} and C_{water} represents original concentration, equilibrium concentration, concentration in plant (lily pad or algae) and concentration in water respectively of minerals and heavy metals.

RESULTS AND DISCUSSION

Table 1 displays mean pH, mean concentration of minerals and heavy metals in wastewater samples.

Table 1: Mean calculation of macronutrient and heavy metals in test water samples

SAMPLE	pH	K (mg/L)	PO_4^{3-} (mg/L)	NO_3^- (mg/L)	Pb (mg/L)	Cd (mg/L)
T0	6.945	23.00	29.21	1.68	0.010	0.107
T2	7.091	17.00	23.65	0.84	0.005	0.095
T4	7.023	17.00	20.65	0.56	0.003	0.106
T6	7.472	13.00	19.47	0.28	0.010	0.106
T8	7.001	6.30	19.47	0.28	0.004	0.100
T10	7.272	23.00	10.43	0.56	0.007	0.106
T12	7.239	11.00	12.53	0.56	0.005	0.100
Minimum	6.945	6.30	10.43	0.28	0.003	0.095
Maximum	7.472	23.00	29.21	1.68	0.010	0.107
Mean value	7.15±0.19	15.75±6.16	19.34±6.37	0.68±0.48	0.006±0.002	0.102±0.004

The pH values of the wastewater samples shown in Table 1 were within 6.945 to 7.472 implying neutral. Acidic pH will aid the availability of minerals and heavy metals for plant uptake during phytoremediation while alkaline pH favours their immobilization [6, 22].

The mean concentrations of K (15.75 mg/L) in test water samples T0, T2, T4, T6, T8, T10 and T12 with potassium concentrations ranging from 6.30 mg/L to 23.00 mg/L, align with the 2.1 mg/L to 38.7 mg/L, with an average of 12.4 mg/L of groundwater recorded by Li et al [23].

Phosphate concentrations ranged from 10.43 mg/L to 29.21 mg/L with mean concentration of 19.34 mg/L. This is more than three fold the World Health Organization recommended maximum allowable concentration of phosphate in drinking water of 5 mg/L. This level suggests possibility of the water to undergo eutrophication [24].

Nitrate level was significantly low ranging from 0.28 to 1.68 mg/L with mean content of 0.68 mg/L. This is far less than the 10 mg/L limit for drinking water set by US Environmental Protection Agency [25].

Potassium, phosphate and nitrate are macronutrients and usually components of fertilizer which are required in relatively large amount for plant growth and development.

Lead concentrations are generally low across all samples, with values ranging from 0.003 mg/L to 0.010 mg/L. The mean concentration is 0.006 mg/L which is below the World Health Organization guideline of 0.01 mg/L. Likewise, mean cadmium contents (0.102 mg/L) were also consistently low, with concentrations ranging between 0.095 mg/L and 0.109 mg/L. This is slightly higher than the World Health Organization guideline of 0.003 mg/L for drinking water [26].

Cadmium is also present as an impurity in several products, including phosphate fertilizers, detergents and refined petroleum products. In addition, acid rain and the resulting acidification of soils and surface waters have increased the geochemical mobility of cadmium and lead as a result its surface-water concentrations tend to increase as lake water pH decreases [22].

Bio-concentration Factor of Minerals and Heavy Metals

Table 2 shows the mean concentrations of mineral and heavy metal in lily pad and algae as well as their bioconcentration factor.

Table 2: Bio-Concentration Factor of Macronutrients and Heavy Metals

Metals	Test Water (mg/L)	Test Lily Pad (mg/kg)	Test Algae (mg/kg)	BCF Lily Pad	BCF Algae
K	15.75	4500	35750	285	2269
PO_4^{3-}	19.34	7750	5737	400	296
NO_3^-	0.68	113	91	166	133
Pb	0.0063	1.37	5.375	217	853
Cd	0.102	22.4	23.5	219	230

The Bio-concentration Factor shows how much macronutrient and heavy metals are concentrated in the lily pad and algae compared to the surrounding water. Higher BCF values mean greater accumulation. As shown in Table 2, algae have higher BCF for potassium (2269), lead (853) and cadmium (230) compared to lily pad with BCF of potassium (285), lead (217) and cadmium (219). The higher BCF suggest a better phytoremediation potential for lead and cadmium by algae compared to lily pad. While on the other hand, lily pad have higher BCF for phosphate (400) and nitrate (166) compared to algae with BCF of phosphate and nitrate been 296 and 133 respectively.

This study demonstrated a high BCF for potassium in both lily pads and algae, indicating efficient uptake. This is consistent with the research by Schreinemachers and Biesboer [27] who found high potassium accumulation in aquatic plants, suggesting that it is an essential nutrient readily absorbed. Similar to potassium, the high BCF for phosphate in both organisms reflects its importance for plant growth. A study by Wetzel [28] reported that aquatic plants readily take up phosphate from the surrounding water. The lower BCF for nitrate compared to potassium and phosphate is in line with findings by Downing and Jones [29] who observed that aquatic plants can discriminate between nitrogen sources, prioritizing ammonium over nitrate. The low BCF values for lead in both organisms suggest minimal bioconcentration, which is corroborated by Eisler [30] who reported limited lead uptake in aquatic plants as displayed in Table 2.

Uptake of Minerals and Heavy Metals by Lily Pad and Algae with Time

The concentration of potassium increases steadily over time for both lily pads and algae. Lily pads reach a higher concentration at around 35 mg/L after 7 weeks. Similar to potassium, the concentration of phosphate increases over time in both organisms. Lily pads reach a higher concentration at around 30 mg/L after 7 weeks. The concentration of nitrate fluctuates slightly over time for both lily pads and algae. Algae appear to have a slightly higher concentration of nitrate compared to lily pads throughout the experiment. The concentration of lead remains relatively low and stable throughout the experiment for both lily pads and algae. The concentration of cadmium appears to increase slightly over time in both lily pads and algae, with algae reaching a higher concentration at around 2.5 mg/L after 7 weeks.

Studies by Yao et al [31] reported an enhanced nutrient uptake by water hyacinths at different harvesting frequencies and that water hyacinth relates to lily pad in the efficient accumulation of potassium and phosphate from surrounding water. This assertion supports the higher bioconcentrations of potassium and phosphate in lily pads in this study. Asaeda et al [32] stated that nitrate and ammonium uptake dynamics of the freshwater macroalga (*Cladophora vagabunda*) indicated that some macroalgae species can be efficient nitrate removers. The low and stable concentrations of lead in both organisms align with expectations. Most aquatic plants are not known to readily accumulate lead [33]. The slight increase in cadmium observed in algae might warrant further exploration. Some algae species can accumulate cadmium, but the specific species and environmental factors can influence uptake levels [34].

Sorption Kinetic Data for Potassium, Nitrate and Phosphate

Potassium, nitrate and phosphate are essential macronutrients needed by plants in relatively large amount to aid structural growth and development, thereby setting the plant in a better position for phytoremediation. The synergistic uptake of these macronutrients from the water body over time by algae and lily pad follows a similar fashion except nitrate as displayed in Figure 1.

The uptake of nitrate was below detectable limit at week one, then 1.12 mg/L at week three to five which finally stabilizes at 1.4 mg/L uptake in week 6 and week 7. While phosphate uptake was least at week two as it was below detectable limit followed by a rapid uptake with maximum sorption of 18.78 mg/L at week 6. This sorption behavior was synonymous to phosphate sorption using iron–zirconium binary oxide observed by a rapid initial uptake within the first hour reported by You et al [35]. Potassium sorption was not detectable at the week 2 and week 6 week while the maximum uptake 16.7 mg/L was recorded at week 7.

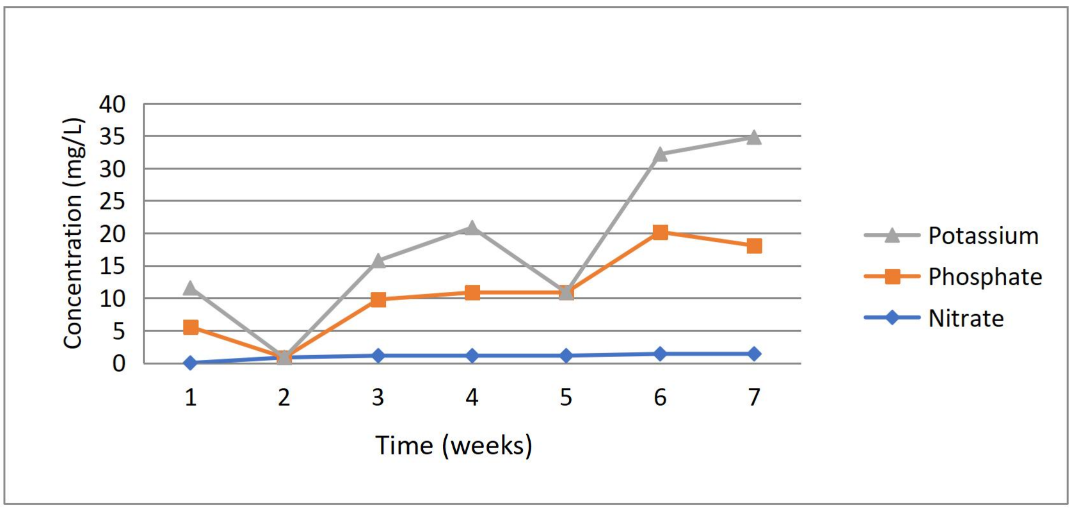

Figure 1: Uptake of minerals from wastewater by lily pad and algae with time

Sorption Kinetic for Lead and Cadmium

Lead and cadmium are non-essential elements known as heavy metals. They are not needed in biological system due to health risk associated with them. The combined uptake of these heavy metals from the water body over time by algae and lily pad is displayed in Figure 2. The uptake of lead was below detectable limit at week one and four, then 0.005 mg/L at weeks two and three which eventually improved to 0.007 mg/L in the 7th week. Cadmium uptake (0.003 mg/L) was constant through weeks three, four and five followed by a rapid uptake of 0.009 mg/L and 0.014 mg/L on the 6th and 7th week. The uptake pattern of lead and cadmium follows similar pattern reported in their adsorption studies reported by Xu et al [36] and Chen et al [37], attributing it to availability of binding sites on the material's surface.

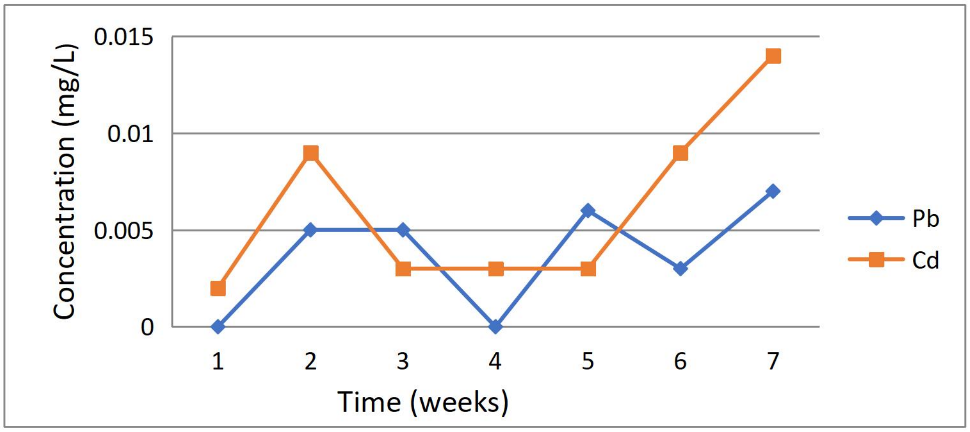

Figure 2: Uptake of heavy metal from wastewater by lily pad and algae with time

Phytoremediation Efficiency

Table 3 shows the phytoremediation efficiency of various metals and minerals.

Table 3: Phytoremediation efficiency

Metals/mineral	Before remediation (mg/L)	After remediation(mg/L)	Uptake amount (mg/L)	Uptake efficiency
K	23.00	6.30	16.7	72.60%
PO_4^{3-}	29.21	10.43	18.78	64.3%
NO_3^-	1.68	0.28	1.40	83.33%
Pb	0.010	0.003	0.007	70%
Cd	0.107	0.095	0.012	11.21%

Potassium has the highest sorption efficiency 16.7 mg/L (72.60%) followed by phosphate 18.78 mg/L (64.3%). Nitrate shows complete sorption of 1.40 mg/L (83.33%) while lead and cadmium have lower sorption efficiencies of 0.007 mg/L (70%) and 0.012 mg/L (12.84%) respectively. These fall within the 10.44 to 100% lead removal and 0.75 to 42.85% cadmium removal efficiencies effected by the combine effort of striga and maize plant [21].

The sorption values suggest that phytoremediation may be a more effective technique for removing potassium, phosphate, and nitrate from contaminated sites compared to lead and cadmium. However the 83.33% nitrate removal was less than the 98% removal achieved using titanium dioxide nanoparticles [38]. Likewise the 64.3% of phosphate removal is less than the

100% reported by thermally activated sepiolite clay [20]. Lead and cadmium have lower sorption (0.007 mg/L and 0.012 mg/L respectively).

CONCLUSION

All macro-nutrients and heavy metals investigated were present in the wastewater under consideration with mean abundance in descending order: phosphate > potassium > nitrate > cadmium > lead. Both lily pad and algae exhibited hyper-accumulation of all the macro-nutrient and heavy metals under review with lily pad having higher BCF for nitrate and phosphate while algae had higher BCF for potassium, lead and cadmium. Lily pad and algae combine significantly to phyto-remediate lead from the wastewater by 70% which was about 6 times the 11.21% recorded for cadmium removal thereby revealing them as a promising means of lead and cadmium contamination clean up in waste water.

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