Innovations

Floating Filters: Harnessing Duckweed (*Lemna paucicostata*) for the Phytoremediation of Heavy Metal in Industrial Wastewater, Calabar, Nigeria

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Abstract: *This study explores the potential of Duckweed (Lemna paucicostata) as a natural phytoremediation agent for heavy metal removal from industrial wastewater in Calabar, Nigeria, aiming to enhance treatment efficiency and ensure compliance with national discharge standards. Duckweed samples of 100g, 300g, and 500g were introduced into 20L of wastewater, and controls were maintained, in monthly trials from May to August 2023. Metals tested included lead (Pb), cadmium (Cd), copper (Cu), nickel (Ni), iron (Fe), and zinc (Zn) before and after a 28-day treatment period. In the Niger Mills effluent, the 500g duckweed treatment achieved reductions in Pb (87.1%), Cd (83.4%), Cu (71.8%), Ni (77.0%), Fe (72.8%), and Zn (84.2%), while in the Iron and Steel effluent, reductions included Pb (72.4%), Cd (90.1%), Ni (93.1%), Fe (77.64%), Cu (88.86%), and Zn (84.75%). Treatments reduced Cd concentrations to levels below National Environmental Standards and Regulations Enforcement Agency (NESREA) acceptable discharge limits in both effluents, with 500g achieving discharge limits for Ni, Cu, Fe, and Zn in Iron and Steel effluent. This study concludes that L. paucicostata holds substantial potential for industrial wastewater remediation, achieving significant reductions in heavy metal levels. To optimize its effectiveness, further research is recommended to investigate the impact of higher duckweed biomass densities and extended treatment durations. Additionally, integration of duckweed with other treatment methods could be explored to enhance removal efficiency for metals like Pb and Ni that exceeded limits, supporting a more sustainable and cost-effective approach to wastewater management in industrial settings.*

Keywords: *Heavy metals, phytoremediation, duckweed, industrial effluent, environmental regulations*

1. 1. Introduction

2. Introduction

Improper waste management, resulting from various human activities, is one of the biggest issues facing emerging nations. In recent years, there has been an increasing concern over the relationship between man and his environment. Globally, anthropogenic waste has done a lot of damage to life forms and the environment **(Kanu and Achi, 2011)**. There is no doubt that the world today is different from primitive times when man was one with nature and his environment. He had no problem generating and disposing his wastes. Since man's massive technological and industrial advancement, coupled with his attitude towards exploitation of nature, nature's mechanism for wastes disposal disappeared. This left man with the task of not only to manufacture his food and convenience but also to dispose of the wastes he generates. Undoubtedly, domestic and industrial waste water is produced in a large quantity daily; proper treatment and disposal has become a challenge **(Patel and Kanungo, 2010)**. Heavy metals, such as lead (Pb), cadmium (Cd), chromium (Cr), and mercury (Hg), are prevalent pollutants in industrial effluents due to their widespread use in various manufacturing processes **(Zhang *et al.,* 2020)**. These contaminants are non-biodegradable, persist in the environment, and can bioaccumulate in aquatic organisms, leading to toxic effects throughout the food chain **(Ali *et al.,* 2013)**.

Wastewaters are produced by numerous industries and homes as consequences of their operation and processing. Based on its usage, the wastewater contains suspended materials that are both bio-degradable and non-biodegradable such as oils and greases, heavy metals ions, organics, dissolved inorganics, acids and bases and colouring compounds **(Kanu and Achi, 2011)**. Treatment of waste water has posed great challenge to mankind since the discovery that industrial wastewater discharged into environment without appropriate handling, could interrupt the ecological stability of such environment **(Udiba *et al.,* 2012a)**

There are several technologies available for recovery of contaminated media. Most of these technologies are however, expensive to implement and have the potential to worsen the already created environmental problems. Evaluation of the efficacy, financial implications, and public acceptability of numerous technologies led to the discovery and development of phytoremediation as an innovative, cost-effective, and environmentally friendly cleanup technique not only for contaminated water but also for other environmental media **(Saxena *et al.*, 2019)**. Phytoremediation is and emerging green technology which entails the use of naturally occurring or genetically engineered plants for the clean-up of contaminated environmental media **(Saxena *et al.*, 2019)**. Naturally, substances generated by nature are filtered and metabolized by plants in the ecosystem. The technology mainly depends on the intricate chemical, physical, and biological interactions that occur in the medium adjacent to plant roots to clean up the contaminated medium using a variety of phytoremediation methods. Several contaminated waters such as sewage and municipal waste water, agricultural run-off, industrial effluent, landfill leachates, coal pile run off and ground plumes can be Phyto remediated **(Olgiun & Galvan, 2010)**.environmental media **(Saxena *et al.*, 2019)**. Phytoremediation is and emerging industrial effluent, landfill leachates, coal pile run off and ground plumes can be Phyto remediated **(Olgiun& Galvan, 2010)**.

Phytoremediation also referred to as, green remediation, agro-remediation and botano-remediation has gain recognition as an environmentally friendly, energy proficient and cost effective strategy for the remediation of sites with low contamination level **(Mojiri, 2012)**. It involves continuum of processes and each process going on at dissimilar degrees for different settings, pollutants and plants. It embraces different methods that can lead to contaminants removal, degradation or immobilization. Surface water can be remediated by rhizofiltration or phytodegradation techniques while soil and sediments can be treated using phytovolatilization, phytostabilization, and phytoextraction **(Bruce, 2001)**.

Vegetation-based remediation shows potentials for altering a low level of persistent contaminants **(Karim, 2013)**. Reports show that some plants (e.g., duckweeds, water lettuce and water hyacinth) can remediate nutrients and heavy metals in water bodies. Among the various plants investigated for phytoremediation, duckweed species, particularly *Lemna paucicostata*, have shown significant potential in absorbing and accumulating heavy metals from contaminated water bodies **(Dwivedi *et al*., 2019)**. Duckweed species are fast-growing, ability to blossom in different habitat, and have a high capacity for metal uptake due to their simple structure, intrusive nature, wide dispersal, extensive root surface area, making them suitable for treating contaminated water **(Khan *et al.,* 2022)**. Duckweeds are tiny aquatic plant. They are members of the vascular plant family Lemnaceae. Four genera have been identified in this family; these are *Lemna*, *Wolffia*, *Spirodela*, *Wolffiela*. The giant species of duckweed *Spirodela polyrhiza* belongs to the genus *spirodela* while the smallest duckweed *Wolffia arrihiza* belongs to the genius *wolffia* **(Ekanem, 2001)**. According to **Ekperusi *et al.,* (2019)** duckweed particularly the *Lemna* genera have been very efficient in removal of nutrients and heavy metals waste water, rendering the water free from pollution. Duckweeds have tremendous potential in nutrient recovery and recycling from municipal and agricultural waste water but its greatest potential is in livestock feed.

Normally, the industrial wastewater is diverted into low lying areas and emptied in to surface water bodies, thus posing serious ecotoxicological health threat. Several methods are already being used to clean up contaminated waste water, but most of these methods are either far from best performance, expensive or environmentally unfriendly. Thermal and chemical methods are technically difficult, environmentally unfriendly and expensive. Hence, the needs for an inexpensive means of treating industrial wastewater before disposal to reduce adverse impacts on water bodies. The aquatic plant *Lemna paucicostata* (duckweed) shows significant potential for phytoremediation of heavy metals due to its efficient pollutant uptake and accumulation (**Landesman, 2020**). Studying duckweed's role in heavy metal remediation addresses critical environmental concerns and offers sustainable benefits to aquatic ecosystems, particularly in industrial regions like Calabar, Nigeria. Duckweed's capacity to remove heavy metals from wastewater before discharge can protect aquatic biodiversity and support fish populations dependent on clean water, positively impacting local food security (**Xu *et al*., 2020**). Optimizing duckweed's remediation efficiency could also aid industries in meeting environmental discharge regulations, supporting safer, ecosystem-friendly practices.

Despite extensive research on duckweed's phytoremediation potential, its specific efficacy in industrial wastewater treatment, particularly in Calabar, remains underexplored. Most existing studies emphasize overall water quality enhancement rather than the precise mechanisms of heavy metal uptake, translocation, andaccumulation. No study has also tested the ability of this plant cultured under different density conditions or explore its optimization potentials. Furthermore, limited research bridges phytoremediation outcomes with regulatory discharge standards, leaving uncertainties about its viability for industrial compliance. This study addresses these gaps by generating localized data on *Lemna paucicostata* as a targeted remediation solution for industrial wastewater in Calabar. By benchmarking its performance against national and international discharge limits, this research provides critical insights to inform policy recommendations and sustainable wastewater management strategies.

2. Materials and Methods

2.1: Study Location

Calabar is the capital of Cross River State. It lies between latitude 4° 5"30" and 5° 30"N and longitude 8°18" 0" and 8° 22"30" E. The city is surrounded by the Calabar River to the West, the Great Kwa River to the East and creeks of the Cross River estuary to the South. Calabar has two Local Government. Areas, Calabar Municipality and Calabar South L.G.A. It has an area of 406km^2.This study was carried out in Calabar Municipality Local Government Area (Figure 1). Weather condition affects the city of Calabar due to its coastal location and high rain fall related with the tropical rain forest. The rainfall in Calabar starts from the month of April and ends in October with its peak in June and September. Temperatures are relatively constant throughout the year, ranging from 25 to 34 degree Celsius; with little variation between day and night time **(Osang *et al.*, 2013)**. The vegetation of Calabar is characterized by mangrove and rain forest ecosystem and the vegetation is mainly fresh water swamp forests with few savanna vegetation and ornamental/avenue tree/shrub species. The foremost soil nature is the clayey-loamy soils **(Osang *et al.*, 2013)**. The two industries studied: Niger Mill Company and Iron and Steel Company are located in Calabar municipality. Niger mill is located at the popular Flour Mill junction. It is a popular industry that produces baking flour and animal feeds, Iron and steel company is located at Calabar Free Trade zone, an industrial hub in Calabar. The company manufactures metals and aluminum.

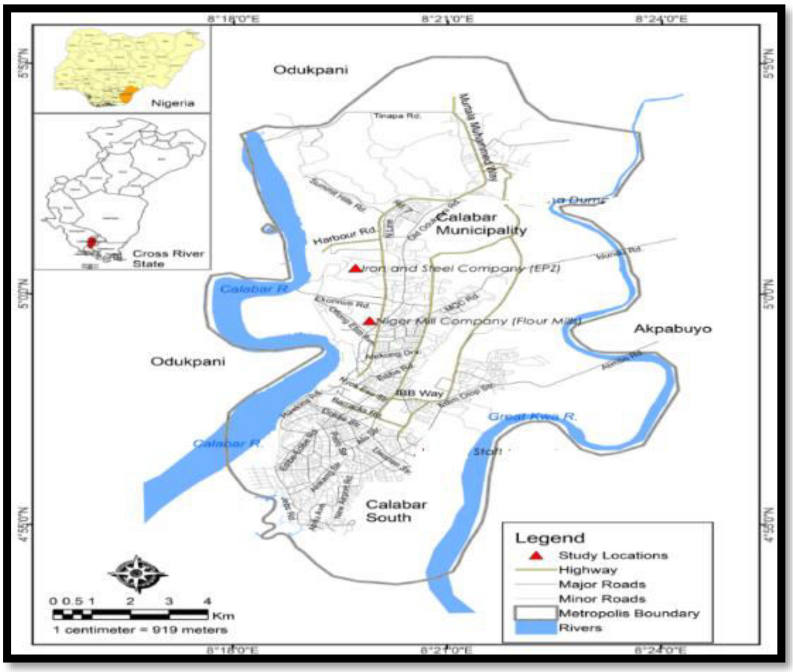

Figure 1: Map of Calabar showing the study sites

2.2: Sample collection

Industrial wastewater was collected from each industry using a plastic bucket by simple scoop and emptied into a 50-liter gallon respectively.

2.3: Duckweed collection

A simple strainer was made with mosquito netting (Plate 1) and used to harvest colonies of *L. paucicostata*from where it was cultured (Plate 2). Duckweed was tempered to get used to the sampled waste water before introduction into experimental setup (Plate 3).

PLATE 1: Net used for harvesting duckweed

PLATE 2: Duckweed culture

2.4: Experimental set-up

To determine the detoxification effects of duckweed (*L. paucicostata*) in industrial wastewater, an ex-situ cultured experiment was conducted. 500g fresh biomass of tempered duckweed (*L. paucicostata*) was stocked in triplicate in basins that contain 20L of the waste water from each of the sampling sites. Another stocking density of tempered 300g of duckweed was stocked in triplicates in basins that contain 20L of waste water from each of the sampling sites, also a stocking density of 100g of duckweed was stocked in triplicates in basins that contain 20L of waste water from each of the sampling site. A control was setup in triplicate in basins that contain 20L of waste water from each of the sampling site but with no biomass of tempered duckweed**(Ajibade*et al.*, 2013;Raju*et al.*, 2010)** (Plate 4). Each experimental set-up was cultured for a period of 28 days (Plate 5). Environmental conditions such as temperature (25 to 33 °C) and relative humidity (55 to 62%) were observed. The experiment was conducted once a month for four months (May to August 2023).

Plate 3: Colonies of tempered duckweeds **Plate 4:** Wastewater without duckweed (control)

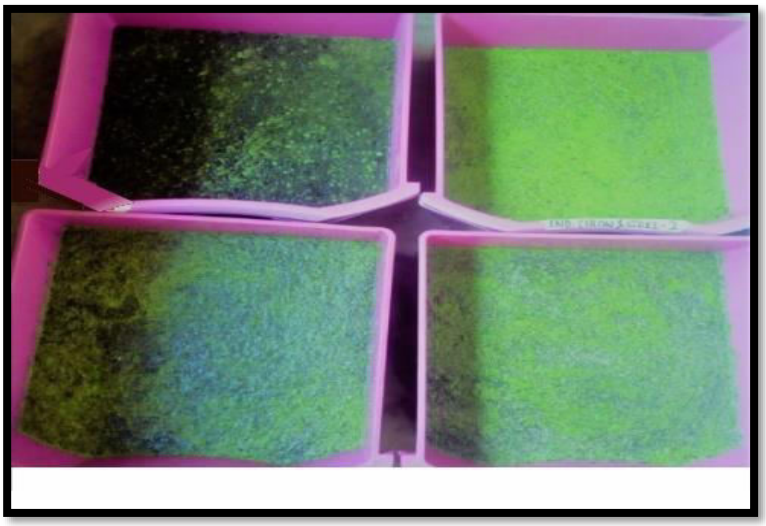

Plate 5: Duckweed mat in the waste water.

2.5: Heavy Metals Analysis

Wastewater samples from the experimental setup were each digested in 250 ml acid washed conical flasks. Concentrated nitric acid (20 ml) was added and brought to slow boiling before being evaporated on a hot plate to almost dryness. As needed,concentrated nitric acid was added to the mixture until the clear solution indicated that digestion was complete. The digest was filtered into a 50 ml volumetric flask and topped off with distilled deionized water.Heavy metals (Pb, Cd, Ni, Cu, Fe, and Zn) content of the wastewater was analyzed at Cross River State Water Board laboratory using atomic absorption spectrophotometer (model AA-6800, Japan) before and after 28 days period in each month.

2.6: Statistical analysis

A statistical test of significance was performed on the data collected. Independent t-test was used to compare metals levels in wastewater before and after phytoremediation. Analysis of variance (ANOVA) was used to compare metals levels between the three treatments (500g, 300g and 100g). Probabilities less than 0.05 were considered to be significant. Statistical analysis was carried out using IBM SPSS version 23 for windows.

2.7: Reduction efficiency (RE)

Contaminant reduction efficiency (RE) expressed in percentages was calculated following equation **(Ekperusi, *et al.*, 2020).**

$$R(\%) = \frac{Co-Ct}{Ct} X\ 100 \quad \text{.......} \quad (1)$$

Where Ris reduction efficiency of contaminant (%), $C_o$is the initial contaminant level (mg/L), C_t final contaminant level (mg/L)

3. Result

3.1: Heavy metal concentration in Effluent from Niger Mill Company

Results of the analysis of heavy metals in effluent from Niger Mill Company before and after 28 days phytoremediation across the different duckweed concentration culture (500g, 300g and 100g) are presented in Table 1. Comparison of metals concentrations before and after remediation for the different treatments and the control are presented in Figure 2.

The mean concentrations of lead, cadmium and copper before remediation were: 1.22±0.44mg/L, 1.04±0.24mg/L and 0.55±0.54 mg/L respectively. Mean concentrations of the metals after 28 days of phytoremediation were: 0.12±0.07mg/L, 0.18±0.13mg/L and 0.08±0.02mg/L, 0.21±0.07mg/L, 0.36±0.25mg/L and 0.10±0.10 mg/l, and 0.23±0.09mg/L, 0.16±0.06mg/L and 0.18±0.15 for 500g, 300g and 100g of duckweed culture respectively (Table 1). The difference in concentration of Pb, Cd and Cu before and after 28 days remediation was significant ($p \leq 0.05$) for each treatment. The difference in each of the metal's concentration between the different treatments (500g, 300g and 100g) after 28 days were however not significant (ANOVA, $p \geq 0.05$, Figure 2).

Mean concentration of nickel before phytoremediation was 2.67±0.65mg/L and after remediation 0.56±0.12mg/L, 0.80±0.14mg/l and 1.13±0.29mg/L for 500g, 300g and 100g duckweed culture respectively. The difference in concentration of Ni before and after remediation was significant ($p \leq 0.05$) for each treatment. The difference in Ni concentration between the different treatments (500g, 300g and 100g) was significant (ANOVA, $p \leq 0.05$), the concentration of nickel in 500g treatment being significantly lower than 100g. The difference between 300g and 100g, and between 500g and 300g were however, not significant at 95% confidence level.

Mean iron and zinc concentration before remediation was 0.83±0.89mg/L and 5.96±1.38mg/L. Their mean content after phytoremediation were 0.08±1.38mg/L and 0.91±0.50, 0.27±±0.29 mg/l and 0.19±0.28 and 0.13±0.06mg/l and 1.95±0.7for 500g, 300g and 100g duckweed culture respectively. The difference in the concentration of Fe and Zn before and after 28 days remediation for each treatment was significant ($p \leq 0.05$). The difference in Fe concentrations between the different treatments (500g, 300g and 100g) was also significant (ANOVA, $p \leq 0.05$).

There was no reduction in the concentration of each metal in the control experiment before and at the end of the 28 days remediation.

3.2: Heavy metal concentration in Iron and Steel industrial effluent

Results of the analysis of heavy metals in effluent from Iron and Steel Company effluent before and after 28 days phytoremediation across the different duckweed concentration culture (500g, 300g and 100g) are presented in Table 2. Comparison of metals concentrations before and after 28 days remediation for the different treatments and the control are presented in Figure 3.

The mean concentration of Pb before remediation was 6.73± 1.00mg/L and the mean content after 28 day of phytoremediation was 1.80±0.12mg/L, 2.28±0.29mg/L and 2.19±0.35mg/L for 500g, 300g and 100g of duckweed culture respectively (Table 2). The mean percentage reductions were 72.42% for 500g, 64.77%% for 300g and 66.13% for 100g treatments respectively. The difference in concentration of Pb before and after 28 days remediation was significant ($p \leq 0.05$) for each treatment. The difference in Pb concentration between the different treatments (500g, 300g and 100g) after 28 days was however not significant (ANOVA, $p \geq 0.05$, Figure 3).

Mean Cd and Ni concentration before remediation was 0.85±0.33mg/L and 2.73±0.69mg/L. Their mean content after phytoremediation were 0.07±0.04 mg/L and 0.16±0.07, 0.04±0.02mg/L and 0.59±0.13 and 0.11±0.06mg/l and 0.71±0.21for 500g, 300g and 100g duckweed culture respectively. Mean percentage reductions were 90.0% and 93.0%, 93.4% and 78.1%, 85.6% and 74.2% for 500g, 300g and 100g treatments respectively. The difference in the concentration of Cd and Ni before and after 28 days remediation for each treatment was significant ($p \leq 0.05$). Only nickel displayed significant difference in concentrations between the different treatments (500g, 300g and 100g) was also significant (ANOVA, $p \leq 0.05$), with 500g being significantly lower than 300g and 100g treatments. The difference between 300g and 100g was however not significant.

Mean Cu, Fe and Zn concentration before remediation was 8.75±2.83mg/L, 56.1±12.3 and 10.2±1.95mg/L. Their mean content after phytoremediation were 0.82±0.22 mg/L, 12.2±1.60 and 0.55±0.26, 3.91±0.22mg/L, 20.6±3.87 and 2.13±0.29, and 3.39±1.22mg/L, 20.7±6.15 and 2.94±0.35 for 500g, 300g and 100g duckweed culture respectively. Mean percentage reductions were 88.8%, 77.6% and 84.7%, 45.6% 62.6%, and 78.3%, 61.2% 62.8% and 70.3% for 500g, 300g and 100g treatments respectively. The difference in the concentration of the metals before and after 28 days remediation for each treatment was significant ($p \leq 0.05$). The three metals displayed significant (ANOVA, $p \leq 0.05$) difference in concentrations between the different treatments (500g, 300g and 100g), with 500g being significantly lower than 300g and 100g treatments. The difference in concentration between 300g and 100g was also significant for iron and zinc.

There was no reduction in concentrations of all the heavy metals in the control experiment values obtained before and at the end of the remediation

Table 1: Heavy metal concentration in Niger Mill waste water before and after phytoremediation

Metals	Remediation Potential	500gDuckweed Concentration					300gDuckweed Concentration					100gDuckweed Concentration				
		May	June	July	Aug.	Mean/STD	May	June	July	Aug.	Mean/STD	May	June	July	Aug.	Mean/STDev
Lead	BeforePhytoremediation	0.74	0.82	1.70	1.62	1.22±0.44	0.74	0.82	1.70	1.62	1.22±0.44	0.74	0.82	1.70	1.62	1.22±0.44
	Average after remediation	0.23	0.11	0.10	0.02	0.12±0.07	0.21	0.14	0.15	0.33	0.21±0.07	0.20	0.09	0.32	0.29	0.23±0.09
	%Reduction	68.9	86.5	94.1	98.8	87.9	71.62	82.9	91.	79.6	81.3	72.97	89.0	81.1	82.09	81.3
Cadmium	BeforePhytoremediation	0.95	0.72	1.13	1.37	1.04±0.24	0.95	0.72	1.13	1.37	1.04±0.24	0.95	0.72	1.13	1.37	1.04±0.24
	Average after remediation	0.03	0.10	0.36	0.24	0.18±0.13	0.07	0.26	0.37	0.75	0.36±0.25	0.08	0.12	0.24	0.18	0.16±0.06
	%Reduction	96.84	86.1	68.1	82.5	83.3	92.63	63.8	67	45.2	67.2	91.57	83.3	78.7	86.86	85.1
Nickel	BeforePhytoremediation	3.12	3.01	2.98	1.55	2.67±0.65	3.12	3.01	2.98	1.55	2.67±0.65	3.12	3.01	2.98	1.55	2.67±0.65
	Average after remediation	0.73	0.46	0.44	0.60	0.56±0.12	0.71	0.96	0.62	0.89	0.80±0.14	1.21	1.21	1.45	0.65	1.13±0.29
	%Reduction	76.60	84.7	85.2	61.3	76.9	77.24	68.1	79.0	42.5	66.7	61.21	61.2	51.3	58.06	57.9
Copper	BeforePhytoremediation	0.32	0.15	1.48	0.24	0.55±0.54	0.32	0.15	1.48	0.24	0.55±0.54	0.32	0.15	1.48	0.24	0.55±0.54

	Average after remediation	0.10	0.09	0.07	0.04	0.08±0.02	0.07	0.09	0.14	0.11	0.10±0.02	0.12	0.09	0.44	0.09	0.18±0.15
	%Reduction	68.75	40.0	95.2	83.3	71.8	78.12	40.0	99.	54.1	67.8	62.50	50.0	70.2	62.50	61.3
Iron	BeforePhytoremediation	0.52	2.35	0.36	0.10	0.83±0.89	0.52	2.35	0.36	0.10	0.83±0.89	0.52	2.35	0.36	0.10	0.83±0.89
	Average after remediation	0.03	0.42	0.09	0.06	0.15±1.38	0.11	0.77	0.11	0.08	0.27±0.29	0.23	0.11	0.08	0.09	0.13±0.06
	%Reduction	94.23	82.1	75.0	40.0	72.8	78.84	67.2	69.	20.0	58.8	55.76	95.3	77.7	10.00	59.7
Zinc(Zn)	BeforePhytoremediation	7.31	5.48	3.92	7.14	5.96±1.38	7.31	5.48	3.92	7.14	5.96±1.38	7.31	5.48	3.92	7.14	5.96±1.38
	Average after remediation	0.45	0.55	0.89	1.73	0.91±0.50	0.96	1.19	1.65	0.97	1.19±0.28	2.22	2.99	1.03	1.57	1.95±0.73
	%Reduction	93.84	89.9	77.2	75.77	84.2	86.85	78.2	57.	86.4	77.3	69.63	45.4	73.7	78.01	66.7

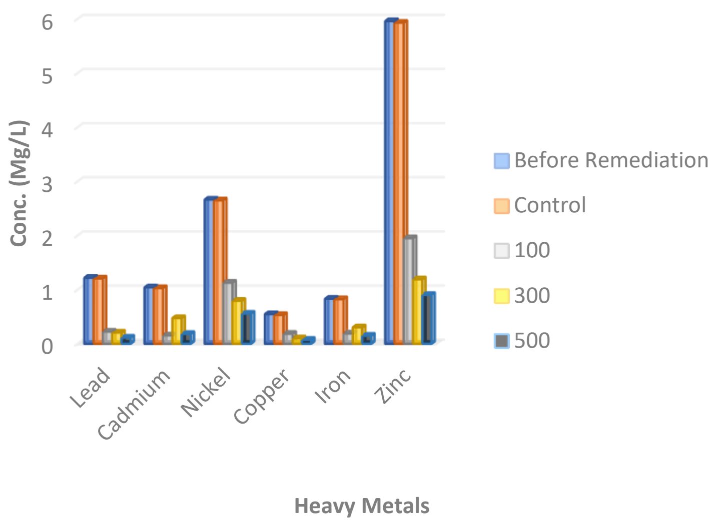

Figure 2: Comparison of heavy metals levels in Niger Mill industrial effluent before and after 28 days of remediation using different concentrations of duckweed.

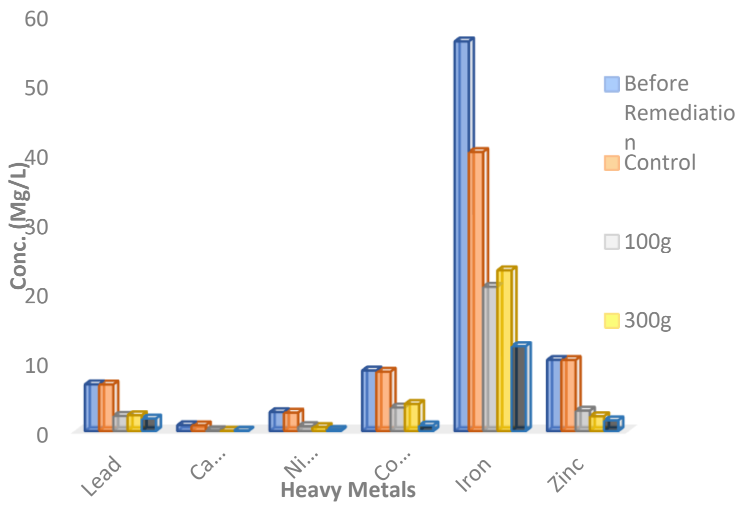

Figure 3: Comparison of heavy metal levels in Iron and Steel industrial effluent before and after 28 days of remediation using different concentrations of duckweed

Table 2: Heavy metal concentration (mg/L) in Iron and Steel Company waste water before and after phytoremediation

Metals	Remediation Potential	500gDuckweed Concentration					300gDuckweed Concentration					100gDuckweed Concentration				
		May	June	July	Aug.	Mean/STD	May	June	July	Aug.	Mean/STD	May	June	July	Aug.	Mean/STDev
Lead	BeforePhytoremediation	7.34	6.05	8.01	5.50	6.73±1.00	7.34	6.05	8.01	5.50	6.73±1.00	7.34	6.05	8.01	5.50	6.73±1.00
	Average after remediation	1.62	1.97	1.78	1.84	1.80±0.12	1.98	2.26	2.13	2.75	2.28±0.29	2.27	2.05	1.73	2.70	2.19±0.35
	%Reduction	77.9	67.4	77.7	66.54	72.4	73.02	62.6	73.4	50.0	64.7	69.07	66.1	78.4	50.9	66.1
Cadmium	BeforePhytoremediation	1.03	0.50	1.30	0.56	0.85±0.33	1.03	0.50	1.30	0.56	0.85±0.33	1.03	0.50	1.30	0.56	0.85±0.33
	Average after remediation	0.03	BDL	0.07	0.12	0.07±0.04	0.05	0.05	0.01	0.06	0.04±0.02	0.21	0.07	0.09	0.08	0.11±0.06
	%Reduction	97.1	-	94.6	78.57	90.0	95.14	90.0	99.2	89.3	93.4	79.61	86.0	91.2	85.7	85.6
Nickel	BeforePhytoremediation	3.70	2.10	2.06	3.05	2.73±0.69	3.70	2.10	2.06	3.05	2.73±0.69	3.70	2.10	2.06	3.05	2.73±0.69
	Average after remediation	0.10	0.20	0.26	0.09	0.16±0.007	0.76	0.39	0.56	0.64	0.59±0.13	1.01	0.53	0.49	0.82	0.71±0.21
	%Reduction	97.3	90.5	87.4	97.05	93.0	79.45	81.4	72.8	79.0	78.1	72.70	74.7	76.2	73.1	74.2
Copper	BeforePhytoremediation	11.6	9.20	4.08	10.10	8.75±2.83	11.60	9.20	4.08	10.1	8.75±2.83	11.60	9.20	4.08	10.1	8.75±2.83
	Average after remediation	0.83	0.49	0.86	1.11	0.82±0.22	3.55	4.16	3.96	3.95	3.91±0.22	3.56	3.63	1.49	4.89	3.39±1.22
	%Reduction	92.8	94.6	78.9	89.01	88.8	63.41	54.7	3.41	60.9	45.6	69.31	60.5	63.4	51.5	61.2
Iron	BeforePhytorem	43.	55.	76.	49.	56.1±12	43.5	55.	76.4	49.	56.1±12	43.5	55.	76.	49.6	56.1±12.

	ediation	5	0	4	60	.3	0	0		6	.3	0	0	4		3
	Average after remediation	9.4 7	13. 0	13. 0	13. 33	12.2±1. 60	20.3 4	18. 7	26.9	16. 6	20.6±3. 87	21.3 3	18. 9	30. 0	12.8	20.7±6.1 5
	%Reduction	78. 2	76. 3	82. 9	73. 13	77.6	53.2 4	65. 9	64.7	66. 6	62.6	50.9 6	65. 5	60. 7	74.1	62.8
Zinc(Z n)	BeforePhytorem ediation	8.0 5	10. 4	13. 3	9.2 5	10.2±1. 95	8.05	10. 4	13.3	9.2 5	10.2±1. 95	8.05	10. 4	13. 3	9.25	10.2±1.9 5
	Average after remediation	1.3 7	1.7 8	1.8 2	1.2 2	1.55±0. 26	2.20	2.5 3	1.74	2.0 4	2.13±0. 29	3.02	3.4 5	2.7 8	2.50	2.94±0.3 5
	%Reduction	82. 9	82. 8	86. 3	86. 81	84.7	72.6 7	75. 6	86.9	78. 0	78.3	62.5 0	66. 8	79. 1	72.9	70.3

4. Discussion

Waste water treatment is essential before discharge to prevent any substantial adverse effect **(Mishra*et al.,* 2012; Alkhateeb & Asker, 2005; Miller *et al.,* 2002)**. Every industry should ideally install pollution abatement equipment based on the best available technology (B.A.T.) or best practical technology (B.P.T.) approach to detoxify its effluents. With emergence of phytoremediation as a cost effective and eco-friendly clean-up strategy, systematic screening of plants with bio-accumulating potentials is of great importance. Duckweed species *L. paucicostata*have previously been reported to be effective in industrial wastewater remediation. In this study, its potentials for the remediation of lead, cadmium, nickel, copper, and zinc in effluent was evaluated. Different stock densities (500g, 300g and 100g) of duckweed species *L.paucicostata* was used in the treatment of 20 liters of industrial effluents obtain from Niger Mills Company and Iron and steel Company, Calabar for 28 days.

4.1: Heavy Metals Remediation in Niger Mill Effluent

The study demonstrates that, *L. paucicostata* has significant potentials for the remediation of lead cadmium and copper. This inference was drawn from the significant difference observed between the concentrations of the metals before and after the 28 days for effluents treated with Duckweed species *L. paucicostata* and validated by the fact that the control that was not treated with duckweed did not display reduction in the metal's levels. The significant reduction in metals concentrations observed only basins treated with duckweed suggests that the duckweed could be responsible for the reduction. These conform to the findings of **Tariq*et al.,* (2006)** who reported a related finding of 0.27 mg/L for Pb concentrations in wastewater from Pepsi industry (carbonated drink effluent) in Pakistan. The Nigeria Environmental Standard Regulation and Enforcement Agency (NESREA) standards for discharge of industrial waste water from food beverages and tobacco manufacturing and processing industries into land or surface water is 0.05 mg/L, 1 mg/L, and 0.5 mg/L for Pb, Cd, and Cu, respectively **(NESREA, 2011)**. The concentrations of lead after remediation period were found to be higher than the established NESREA effluent permissible limit. Cadmium and copper were within the acceptable limits. The average percentage reductions of 87.09%, 83.39% and 71.8% for 500g, 81.33%, 67.25%, 67.8% for 300g and 81.31%, 85.13% and 61.3% for 100g treatments respectively suggests the effectiveness of the plant in the remediation process. **Ucuncu*et al*., (2013)**reported that 90% of Pb, was extracted by *L. paucicostata* from effluents and is in line with the finding of this study. The difference in the metals' concentration between the different treatments (500g, 300g and 100g) after 28 days were not significant (ANOVA, $p \geq 0.05$). This may suggest that for, 20L of effluent from Niger mills, even the lowest treatment (100g) could be sufficient to effectively remediate the metals. The rate at which the remediation proceeded between day one and the day 28th which may be a function of duckweed density was however not assessed. To this end, the study could not ascertain which of the duckweed treatment proceeded at a faster rate.

Significant reduction in nickel, iron and zinc content of effluent obtained from Niger mills was also observed after 28 days treatment with different densities (500g, 300g and 100g) of duckweed species *L. paucicostata*. No reduction in the concentrations of these metals was observed in the control that had no duckweed. This also suggest that *L. paucicostata* can effectively be employed in the clean-up of nickel, iron and zinc in Niger mills effluent. At the end of the 28 days treatment, nickel concentrations in the different treatments were still higher than NESREA acceptable limits (0.05 mg/L) for nickel in effluents from food, beverages and tobacco production and processing industries. Zinc concentrations after the treatments were reduced to acceptable limit. Iron is not regulated by NESREA in effluents from food, beverages and tobacco producing and processing industries. The average percentage reductions (76.96%, 72.8% and 84.2% for 500g, 66.78%, 58.8% and 77.3% for 300g and 57.96%, 59.7% and 66.7% for 100g treatments respectively) were however lower for the different treatment when compared to lead cadmium and copper suggesting that, though *L. paucicostata* is also effective in the remediation of nickel, iron and zinc in effluent, it is more effective in the remediation of the clean-up of lead, cadmium and copper. The significant difference in Ni concentration between the different treatments (500g, 300g and 100g), with the concentration of nickel in 500g treatment being significantly lower than 100g, suggests that clean-up of nickel in a 20L effluent from Niger Mills is density dependent. The higher the density the more effective the clean-up. The implication of these findings is that treating 20litres effluent from Mill Company with 100g-500g of duckweed can effectively reduce cadmium, copper and zinc concentration in the effluent to NESREA acceptable limits for discharge into land and surface water in Nigeria. The phytoremediation carried out in the present study was for 28 days with *L. paucicostata* densities ranging from 100mg/L-500mg/L. Extending the remediation period or increasing the density of *L. paucicostata* may yield better results and is hereby recommended.

4.2: Heavy Metals Remediation in Iron and Steel Industrial Effluents

Practically every industry contributes to the pollution of water with major contributors being the manufacturing and construction industries **(McKinney *et al*., 2013)**. The pollution of water is a major cause of infections and deaths worldwide. **Ogbu*et al.,* (2016)** reported that more than 14,000 deaths are recorded globally on daily basis. Hence the need for remediation of waste water before introduction into surface or inland waters. Studies reveal that several species of duckweed plant have proven capable and efficient in the remediation of contaminants in waste water. In this study, the ability of *L. paucicostata* (a tiny floating aquatic plant)to remediate lead, cadmium, nickel, copper, iron, and zinc in industrial effluent obtained from Iron and steel was assessed. The study shows that, *L. paucicostata* has a great deal of promise for lead, cadmium, and nickel clean-up. The metals concentration of effluents after 28 days treatment with different densities of the plant were significantly (ANOVA, $p \leq 0.05$) lower than the concentrations before treatment. Metals levels in the control (not treated with duckweed) did not display reduction in the metals levels (Figure 3). This observation suggests that, *L. paucicostata* was responsible for the reduction of the metals levels except for iron. The percentage reductions efficiency for lead cadmium and nickel were: 72.42%, 90.09%, 93.41%, 93.07% for 500g, 64.77%, 93.41%, 78.17% for 300g, and 85.65%, 66.13%, 74.20% for 100g treatments, respectively. Copper, iron and zinc showed the following percentage reductions efficiency: 77.64%, 88.86%, 84.75%, for 500g, 62.63%, 45.62%, 78.30% for 300g, and 62.82%, 61.23%, 70.35% for 100g treatments respectively. The highest percentage reductions were recorded by 500g of duckweed concentration treatment and lowest by 100g concentration treatment. This observation implies that, the remediation of the metals increases as the concentration of the duckweed increases. The Nigerian Environmental Standards and Regulations Enforcement Agency (NESREA) effluents limit for metal manufacturing (Metallurgical) industries is 0.2 mg/L, 0.1 mg/L, 0.5 mg/L, 0.1 mg/L, 19 mg/L, 2.0 mg/L for Pb, Cd, Ni, Cu, Fe, and Zn respectively. Cadmium concentrations in the effluents after the 28 days treatment with duckweed were found to be below the NESREA limit for discharge of industrial effluent into surface and inland water, suggesting that even the lowest density of duckweed (100g) could effectively cadmium concentration in 20L effluent form iron and steel industry. Nickel, copper, iron and zinc were reduced to NESREA acceptable limit only by the highest density of duckweed treatment (500g) in the study. 300g and 100gs also showed significant reduction in the metals concentrations, however, the acceptable limits for discharge into Nigerian environment was exceeded. Significant reduction in lead concentration was observed in all the treatment but the levels at the end of the 28 day treatment were still above NESREA acceptable limits for discharge. The remediation period in this study was within 28 days. It is believed that extending the remediation period or increasing the duckweed density may yield better results and is hereby recommended.

There are a few possible reasons for the slight decrease in iron concentration in the control experiment (where duckweed was not used to treat the effluent): (1) since the experiment was carried out in an open system, some iron compounds may volatilize or interact with the surrounding air and reduce in concentration. (2) Even under control conditions, microorganisms naturally present in the effluent might contribute to the reduction of iron. (3) In the presence of oxygen, iron, particularly ferrous iron (Fe^{2+}), can oxidize to ferric iron (Fe^{3+}). Iron ferric is less soluble. (4) When exposed to air (oxygen) or when the pH of the effluent changes, iron in aqueous solutions may precipitate out of solution as iron hydroxides or iron oxides. The concentration of soluble iron is decreased by this process of spontaneous precipitation. (5) Over time, gravity may cause iron particles, particularly those in suspended forms (such as iron oxides), to sink to the bottom of the container. The amount of iron in the water that is measured may drop as a result of this sedimentation.

5. Optomization Potential for Regulatory Compliance

1. **Increase Biomass Density**

Higher biomass densities (like the 500g treatment) generally showed better metal reduction rates by providing more surface area for absorption, using biomass amounts above 500g as opined by **Banerjee *et al.*, (2018)** may enhance metal absorption. Testing with 700g to 1,000g in the same wastewater volume (20L) could yield higher reduction percentages for Pb, Ni, and other metals still above regulatory limits**(Yan *et al.*, 2020; Ma *et al.*, 2018)**. These heavier biomasses may allow duckweed to handle higher contaminant loads more effectively in industrial settings.

2. **Optimizing Exposure Time**:

Extending the treatment period beyond 28 days could increase the exposure time and metal uptake by duckweed, especially for metals that did not meet regulatory limits in shorter time frames. Trials with durations of 35, 45, and even 60 days could be valuable to determine optimal exposure for maximum metal removal. Prolonging treatment durations can further reduce metal concentrations, as shown in research on longer remediation periods yielding significant reduction in metal content **(Li *et al.*, 2021)**

3. **Periodic Nutrient Supplementation**

To support duckweed growth and enhance metal uptake efficiency, adding low concentrations of essential nutrients periodically (such as nitrogen and phosphorus) may help prevent nutrient deficiencies and sustain healthy biomass. Managing nutrient supplementation in wastewater properly to avoid eutrophication can improves duckweed growth, boost plant vitality, and potentially improve metal absorption rates**(Sarwar *et al.*, 2010)**.

4. **Sequential Harvesting and Replanting**

Harvesting duckweed periodically (e.g., every two weeks) and replacing it with fresh biomass could maintain high absorption rates. Replanted duckweed has greater metal absorption potential, reducing the risk of saturation and potentially enhancing overall metal uptake within the same treatment period**(Xu *et al.*, 2022)**.

5. **Biostimulation and Bioaugumentation**

Supplementing with Chelating Agents such EDTA can boost metal bioavailability for duckweed uptake, which accelerates remediation **(Linger *et al.*, 2002)**. Introducing Biochar as a Substrate could be effective in reducing metal mobility and enhancing plant growth, thereby improving overall phytoremediation efficacy **(Liu *et al.*, 2018)**. Determining and adjusting pH to optimal levels can enhance metal solubility and uptake in plants, increasing the remediation efficiency of duckweed **(Yan *et al.*, 2020)**. Determining and consistently maintaining temperature within an optimal range supports better metabolic activity and metal absorption in duckweed **(Liu *et al.*, 2013)**. Beneficial microbes that promote plant growth can also increase metal tolerance, enhancing Lemna's uptake efficiency **(Backer *et al.*, 2019)**. Genetic modifications of duckweed to enhance heavy metal tolerance and uptake capacity also holds significant promise **(Lyu *et al.*, 2019)**.

6. **Scaling Up to Industrial Applications**:

Large-scale trials can provide insights into the economic viability and practical challenges in using duckweed for industrial effluent treatment **(Chen *et al.*, 2016)**. Scaling can reveal operational difficulties such as nutrient requirements and maintenance costs, helping to refine processes for broader applications **(Linger *et al.*, 2002)**.

Conclusion:

Treating 20 litres effluent from Mill Company with 100g-500g of duckweed effectively reduce cadmium, copper and zinc concentration in the effluent to The National Environmental Standards and Regulations Enforcement Agency (NESREA) standards for discharge of industrial wastewater from food beverages and tobacco manufacturing and processing industries into Nigerian environment. Significant reduction was also recorded in lead and nickel concentrations but the values exceeded NESREA limits for discharge. Cadmium concentrations in the effluents from iron and steel industry after the 28 days treatment with duckweed were found to be below the NESREA limit for discharge for all the treatments. Nickel, copper, iron and zinc were reduced to NESREA acceptable limit only by the highest density of duckweed treatment (500g) in the study. 300g and 100gs also showed significant reduction in the metals concentrations, however, the acceptable limits for discharge into Nigerian environment was exceeded. The study concludes that *L. paucicostata* has significant potentials to reduce metals levels in industrial effluents to NESREA acceptable limits for discharge. Further studies with higher incubation periods and increased *L. paucicostata* densities is here-by recommended for optimization.

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Conflict of Interest Statement:

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