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Phytochemistry and the Use of Benin *Water Hyacinth* Leaf Extract as a Biosorbent in the Bioremediation to Cu, Pb, Cd from Surface Water

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Abstract: Eichhornia crassipes (water hyacinth) is an aquatic plant that threatens aquatic biodiversity. Currently, research is exploring the use of plant-based biosorbents for the remediation of water resources. In this work, we utilized extracts of Eichhornia crassipes in water treatment for its valorization as a biosorbent in the bioremediation of certain metals and its application for treating surface water from the reservoir of the coastal lagoon of Ouidah in the village of Agouin. The physicochemical parameters and the Trans Metallic Element (TME) content were measured using a multi-parameter analyzer and an atomic absorption spectrophotometer, respectively. The phytochemistry of hydroethanolic (70°) and ethanolic (96°) extracts of $Eichhornia\ crassipes$ indicates the presence of flavonoids, leucoanthocyanins, coumarins, reducing compounds, proteins and that they are rich in alkaloids and catechic tannins only. The bicinchoninate method revealed that the ethanolic extract had the highest absorbency (70%) among the other extracts. Treatment of the sample (surface water) with the ethanolic extract of Eichhornia crassipes showed a high absorption rate of Cu (95.35%), Pb (93.46%), and Cd (49.97%) with a low variation in salinity (1.60-1.46)PSU; TDS (1550-1405) mg/L; pH (8.54-8.39), and a decrease in dissolved O₂ (2.45-0.5). It is concluded that this plant is a candidate for biosorbent of TME in water treatment.

1. Introduction

Eichhornia crassipes, commonly known as water hyacinth, is an aquatic plant that live and reproduce in floating freely on the surface of freshwater or anchored in mud. Its asexual reproduction, occurring at a rapid pace under favorable conditions (Herfjord *et al.*, 1994), threatens aquatic biodiversity worldwide. It prevents the diversity of local plants by altering the physical and chemical composition of the aquatic environment, but also constitutes obstacles to development activities (Ghabbour *et al.*, 2004), (Isebe, 2016). This plant, primarily found in tropical and subtropical regions,

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also thrives in certain freshwater areas of Benin, notably the shores of Sô-Ava, Lake Nokoué, and the shores of Porto Novo. The literature mentions *Eichhornia crassipes* in exploratory studies of biosorbent plants for water treatment. It has been noted as an excellent bioindicator of water polluted by organic pollutants such as endocrine disruptors and neonicotinoids (De Laet *et al.*, 2019). Other studies have shown that it also can phytoaccumulate metallic pollutants and is capable of bioconcentrating toxic metals such as Cr, Cu, Co, Ni, Zn, Pb, Cd and As in its root system (Ugya *et al.*, 2016), (Shiomi, 2018), (Goswami and Das, 2018). Heavy and toxic metals pose significant threats to human health, animals, aquatic life, and the environment. These metals can accumulate in living organisms and disrupt various biological processes, leading to a range of adverse effects (X). By phytoremediation, Yovo *et al.* (2017) demonstrated that a combination of *Eichhornia crassipes* and *Thalia geniculata* has a high capacity for sequestrating nitrogen in domestic wastewater within a few days, thereby reducing nutrients in graywater. Furthermore, studies have shown that *Eichhornia crassipes* is a natural source of molecules with antioxidant and antimicrobial properties (Isebe, 2016), (Rufchaei *et al.*, 2021).



Photo 1. Eichhornia crassipes

Recently, researchers reoriented their focus to use materials derived from biological sources called biosorbents, which are increasingly utilized for water treatment due to their cost-effectiveness, environmental friendliness, and ability to remove a wide range of contaminants. These materials, including agricultural waste, plant residues, and microbial biomass, offer a sustainable alternative to traditional methods (Blaga *et al.*, 2025; Gouledehi *et al.*, 2025; Salahat *et al.*, 2023; Nematollahzadeh *et al.*, 2022; Akartasse *et al.*, 2022). We recently published a study on the phytochemistry and biosorbent potential of *Euphorbia prostrata* extract to remove Cu, Pb, and Cd from Ouidah coastal lagoon waters in Benin (Agnimonhan *et al.*, 2025). It is noted that the extract, particularly the ethanolic extract (70°) of *Euphorbia prostrata* possesses interesting absorbent properties, making this plant a candidate for biosorbent of MTEs in water treatment. As an extension of our work on the use of plants in water treatment, we presented the phytochemistry and absorbent potential of extracts from the leaves of *Eichhornia crassipes* acclimated in Benin. We sought to identify the most interesting extract for application to surface water.

2. Bibliometric analysis

Various databases, such as Publish or Perish, Web of Science, and Scopus, provide bibliometric analyses on specific topics by utilizing keywords. This opportunity provides access to indicators universally used for both research assessment practices and daily tasks, as well as enhanced

visualization of countries, laboratories, authors, and their collaborations (Passas, 2024; Laita *et al.*, 2024; Chakir *et al.*, 2023). In other words, bibliometric studies become crucial for evaluating various aspects of research, including the impact of scholarly work, research trends, and the structure of academic fields. They provide quantitative data to assess the influence of publications, authors, and institutions, helping to map research landscapes and identify key contributors (Öztürk, 2024; Nandiyanto *et al.*, 2024; Hammouti *et al.*, 2025). Searching Scopus using "*Eichhornia crassipes*" indicates more than 4300 documents. **Figure 1** illustrates a stagnation in publications until 2000, followed by an increase in recent years, with more than 270 articles published in the last few years. **Figure 2** provides more information about the nature of published documents (98.6% are articles, conference papers, reviews, and book chapters). The various studies on *Eichhornia crassipes* cover 28.5% of Environmental science, 22.2% of Agriculture and biological sciences, 6.3% of Biochemistry, and Genetics and Molecular biology, 5.5% of Chemical Engineering... (**Figure 3**).

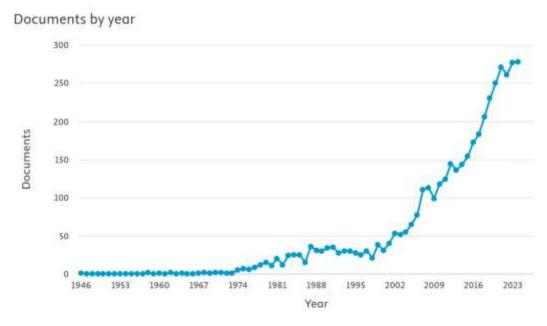


Figure 1: Evolution of the publications from 1946 to 2024

Documents by type

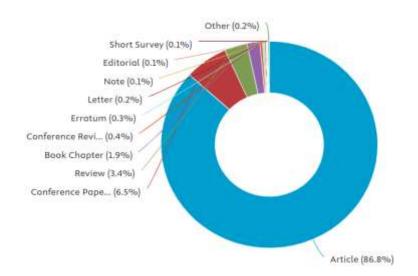


Figure 2: Percentages of the publication nature from 1946 to 2024

Documents by subject area

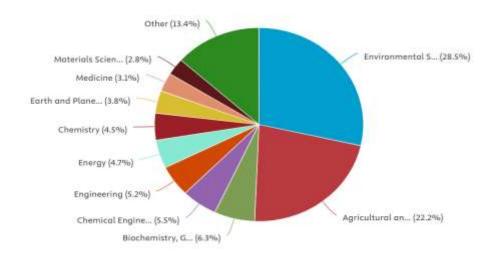


Figure 3: Distribution of the publications by area from 1946 to 2024

Authors target journals to publish their work in Scopus-indexed journals like Aquatic Botany (IF of 2.6 and Cite score 4.6), Environmental Science and Pollution Research, Water Science and Technology, Bioresource Technology, etc., as shown in **Figure 4**. The collected data also indicated 13925 authors, and 2252 having more than two articles, and 738 with more than three. **Figure 5** presents the top ten published authors. Hill M.P. from Rhodes University, Grahamstown, South Africa, has a total of 48 articles on *Eichhornia crassipes*, as well as 239 articles and an H-index of 34, which has garnered over 4,300 citations across 2,533 documents. The others are shown in **Figure 5a**. The most cited paper is a review by Villamagna and Murphy, published in Freshwater Biology, which examines the ecological and socio-economic impacts of the invasive water hyacinth (*Eichhornia crassipes*), garnering over 500 citations (Villamagna and Murphy, 2010). Visualization VOS viewer was used to gain a comprehensive understanding of the most authors and countries in this topic. **Figure 5b** depicted 737 authors from 13925 who published more than 3 articles.

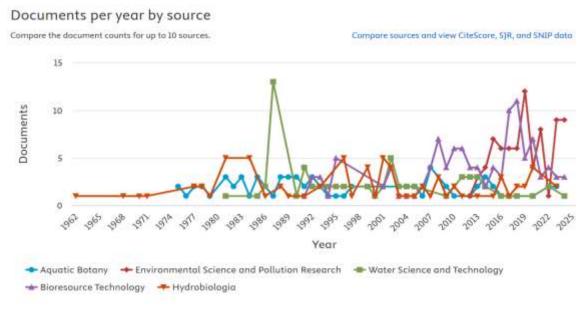


Figure 4: Major Journals of the published articles from 1946 to 2024

The authors presented by nodes (circles) with different colors and sizes. The diameters reflected the quantity of papers to Prof Hill Martin P. of green node, Reddy K.R. purple one and Kalamhad Ajay S. with a Yellow-green circle. The results indicate more than 160 countries and only 99 published ≥3 and 114 99 published ≥2 articles. The study also shows that India is the most productive country, accounting for more than 1020 articles, i.e, over a quarter of the total publications. China is the second one and the US in the third position. The overlay visualization presented in **Figure 6** provides more information about the time of publications: the dark purple color to show the years around 2010 and yellow for recent years (2024), as shown for Indonesia, Malaysia, Saudi Arabia, etc.

Documents by author

Compare the document counts for up to 15 authors.

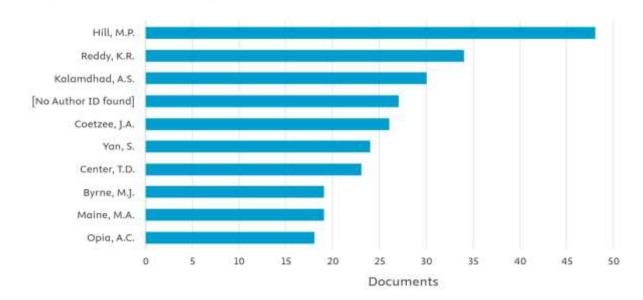


Figure 5a: Top ten published authors from 1946 to 2024

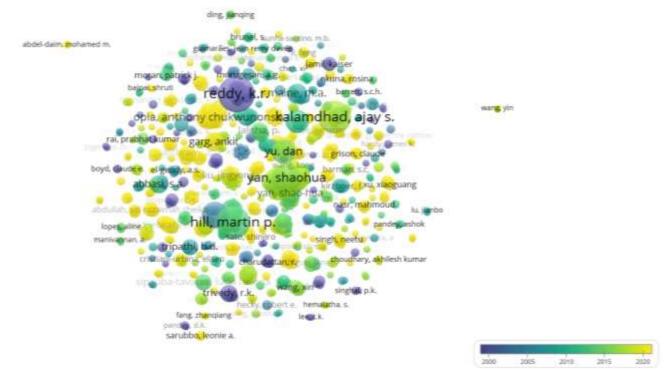


Figure 5b: Top ten published authors from 1946 to 2024

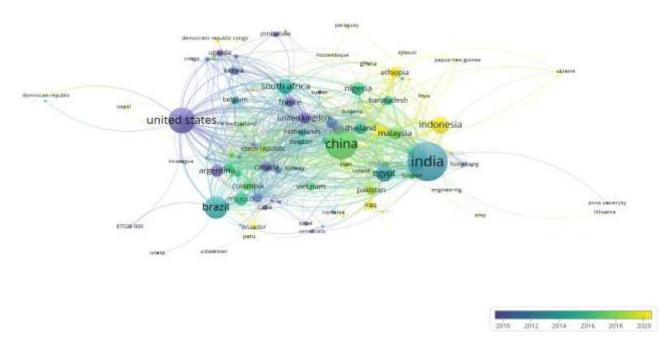


Figure 6. Overlay visualization VOS viewer

3. Materials and Methods

3.1 Plant material and preparation of the plant extracts

The leaves of *Eichhornia crassipes* were harvested from the Ouémé River in the commune of So-Ava. The leaves were washed and dried in the laboratory at 18°C for several days and then heated in an oven at 60°C for one hour before being ground. The aqueous extracts were prepared by decoction and the organic extracts by maceration according to the method described by Agnimonhan *et al.* (2025). The extracts were stored in a refrigerator at -4°C before use.

3.2 Collection of surface water sample

A surface water sample was collected from the Ouidah coastal lagoon reservoir in Agouin village, located at 6°20' North - 2°10' East, using a 1.5 L plastic container immersed in water 20 cm from the surface and filled to the brim to prevent air bulb entry. The lagoon is in the western wetland complex of southern Benin (Ramsar site no. 1017) and located between parallels 6°27'01" and 6°19'40" and between meridians 2°20'48" and 2°04'57" and covers an area of 4000 hectares (Viaho *et al.*, 2020).

3.3 Phytochemical screening

The screening analysis was conducted according to the methods described by Houghton and Raman (1998) and Shaikh and Patil (2020), with revisions and updates.

3.4 Copper absorption experiments

The Bicinchoninate method described by Nakano & Yakugaku (1962). Adapted to laboratory conditions, this method is done as reported by Agnimonhan *et al.* (2025). The readings indicating the amount of copper (maj C indice 0) in the reference solution and the amount of copper (maj C indice f) present in each tube were taken using the DR/890 colorimeter, 2 minutes after addition. The percentage of Cu absorption was calculated from the following formula:

$$R (\%) = 100 \left(\frac{C_0 - C_f}{C_0} \right)$$

 C_0 : amount of copper in the control solution; C_f : amount of uncomplexed copper present in the extract tube.

3.5 Processing and analysis of water samples

Use of extract for absorption experiment was done in laboratory condition according to the methods described by Assaad (2006) and Siew-Teng *et al.* (2012) and reported by Agnimonhan *et al.* (2025). The copper, zinc, lead, and cadmium content were analyzed by a flame Atomic Absorption Spectrophotometer (AAS).

4. Results and Discussion

4.1 Screening phytochemical

The phytochemical composition of the extracts recorded in Table 1 revealed that all plant extracts contain polyphenolic compounds, tannins, flavonoids, leucoanthocyanins, alkaloids, coumarins, reducing compounds, and proteins. Furthermore, only the aqueous extract of *Eichhornia crassipes* contains saponins. This result was also observed by Hamid *et al.* (2013) during his work on *Eichhornia crassipes* acclimated in Iran, although an absence of reducing compounds was observed in his ethanolic extracts and the aqueous one studied by Ponnusamy *et al.* (2011). Our phytochemical study showed that the aqueous, hydroethanolic, and ethanolic extracts of *Eichhornia crassipes* acclimated in Benin are rich in reducing compounds. This could be explained by biological and environmental factors (Gouvea *et al.*, 2012), that influence the chemical compositions according to the collection region (da Cunha, 2010).

Table 1. Phytochemical composition of *Eichhornia crassipes* extracts

Phytochemical composition		Aqueous extract (Eaq)	Ethanol 70° (E _{HE})	Ethanol 96° (E _{ET})		
Alcaloïdes		+	+	+		
	Catéchic tannins	+	+	+		
Tannins	Gallics tannins	-	-	-		
Flavonoïds	'	+	+	+		
Anthocyanins		-	-	-		
Anthraquinon	es	-	+	+		
Leuco-anthocy	vane	+	+	+		
Saponosides		+	-	-		
Reducing com	pounds	+	+	+		
Coumarines		+	+	+		
Terpenoïdes		-	+	+		
Stérols		-	+	+		
Glycosides car	diaques	-	-	-		
Protéines		+	+	+		

(+): Present; (-): Absent; Eaq: Aqueous extract; E_{HE}: ethanolic extract (70°); E_{ET}: Ethanolic extract (96°)

4.2 Surface water of Agouin village

The MTE analysis of water samples reported in Table 3, reveals the presence of certain heavy metals as Cu (13.05 mg/l), Pb (14.59 mg/l), and Cd (8.7 mg/l). These content of heavy metals at the Agouin site are higher than the results obtained by Bocodaho (2020), which vary for the short season: Cu (0.31 to 2.45 mg/l), Pb (0.26 to 0.89 mg/l), and Cd (0.01 to 0.17 mg/l). Furthermore, no traces of Zn were found in our sample, unlike the work of Bocodaho (2020). This increasing variation could be explained by demographics and the intensification of agricultural and household activities, as well as the construction of road infrastructure. The values of the physicochemical parameters of the recorded in Table 2, are not significantly different from those of Bocodaho (2020), whose pH is between 7.2 and 8.9 and the temperature between 25.6°C and 33.2°C. The dissolved O₂ values of our sample are well below the general water quality standard and also lower than those estimated by Bocodaho (2020). This low O₂ level characterizes the very poor water quality of the Ouidah coastal lagoon in Agouin village. During our field surveys, we observed floating aquatic plants and agricultural activities around the lagoon, which could contribute to the low dissolved O₂ content.

Table 2. Physicochemical parameters of the sample before and after treatment

	Temperature (°C)	Turbidity (FNU)	Salinity (PSU)	TDS (mg/l)	Conductivity (ms/cm)	dissolved O2 (mg/l)	$\mathbf{P}^{\mathbf{H}}$	ORP (mv)
T_0	29.6	2.2	1.60	1550	3101	2.45	8.54	165.8
T_{EC}	27.10		1.46	1405	2795	0.51	8.39	62.3

Table 3. Content heavy metals in samples before and after treatment

	Cu (mg/l)		Zn (mg/l)		Pb (mg/l)			Cd (mg/l)				
	T_0	T_{EC}	P%	T_0	T_{EC}	P%	T_0	T_{EC}	P%	T_0	T_{EC}	P%
Content	13.05	0.606			00			0.516	93.46	8.71	4.357	49.97
$P(0)$, the counting respective $P(0) = 100 \left(\frac{T_0 - T_{EC}}{T_0} \right)$												

P%: absorption percentage, P (%) = $100 \left(\frac{T_0 - T_{EC}}{T_0} \right)$

4.3 Eicchornia crassipes extracts, heavy metals - water quality

The evaluation of the copper absorption by the bicinchoninate method which the results are reported in Table 4, reveals that all the extracts complexed copper. The ethanolic extract of *Eichhornia crassipes* (E_{ET}) proved to be more interesting with a yield of 70.10%. However, this copper absorbing potential is low compared to the extracts of *Euphorbia prostata* reported in our previous work (Agnimonhan *et al.*, 2025). The treatment of water samples with extracts of *Eicchornia crassipes* showed an absorbing of its extracts on Cu, Pb and Cd present in the sample.

Fitsum *et al.* (2022) showed the ethanolic extract of the leaves has an absorbing effect of Cr⁶⁺, Cr³⁺ ions during the treatment of tannery influent Our study showed that the ethanolic extract of *Eichhornia crassipes* acclimated in Benin has an absorbing capacity for Cu, Pb and Cd with a high absorption rate of Cu (95.35%), Pb (93.46%) and Cd (49.97%). This low absorbing capacity of the different extracts of *Eichhornia crassipes* compared to that of *Euphorbia prostata* could be explained by the presence of catechic and gallic tannins contained in the extract of Euphorbia prostata (Slabbert *et al.*, 1992), (Agnimonhan *et al.*, 2025). Tannins have been accepted as biocoagulants and biosorbents in wastewater treatment (Leiviskä and Santos 2023). Regarding the physicochemical parameters of the treated water, a low variation was observed except for the dissolved O₂ value which decreased considerably. The ability of certain chemical compounds to chelate the metal cations in medium,

making it weakly oxidizable. Therefore, the absorbent power of our extracts could explain the decrease in the value of dissolved oxygen.

Table 4. Copper test by the bicinchoninate method

Extracts	Remaining copper content: Cf (mg/l)	Yield (%)		
Eaq	1.53	16		
$\mathbf{E}_{\mathbf{HE}}$	1.58	14.13		
$\mathbf{E}_{\mathbf{ET}}$	0.55	70.10		
Witness (Co, mg/l)	1.84			

Eaq: Aqueous extract; E_{HE}: Hydroethanolic extract (70°); E_{ET}: ethanolic (96°)

Conclusion

Water hyacinth (*Eichhornia crassipes*), an invasive aquatic that is a serious threat to aquatic ecosystems worldwide, is attracting considerable attention in wastewater treatment.

This study showed that the ethanolic extract of *Eichhornia crassipes*, which has an adsorbent effect on copper, has demonstrated significant adsorbent potential in the bioremediation of trans-metallic elements (TMEs) such as Pb, Cd, and Cu.

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