

Research Article

Microbial Diversity and Physicochemical Properties of *Eichhornia crassipes*

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ABSTRACT

Eichhornia crassipes (water hyacinth) is an invasive aquatic weed that grows rapidly and has a high lignocellulosic content. The plant has negative ecological effects but can also provide a habitat for microorganisms and serve as a potential substrate for biotechnological use. This study examined the microbial diversity and physicochemical characteristics of *E. crassipes* obtained from Sabongarin Takanebu in Miga Local Government Area, Jigawa State, Nigeria. The samples were air-dried, ground, sieved, and analysed using standard physicochemical and microbiological methods. Results showed that the plant had a pH of 5.66 ± 0.01 , temperature of 38 ± 0.3 °C, and moisture content of $5.71 \pm 0.03\%$. Organic carbon was high (49.56%), while nitrogen content was low ($0.30 \pm 0.42\%$), giving a wide C/N ratio of 55.13. Ash content was 2014.92 mg/kg. Major nutrients recorded included potassium (1234.13 ± 0.37 mg/kg), sodium (237.41 mg/kg), calcium (7115.66 ± 0.01 mg/kg), and magnesium (2945.39 ± 0.38 mg/kg). Trace elements such as copper (2.47 ± 0.01 mg/kg) and lead (18.03 ± 0.56 mg/kg) were also present. Microbial counts showed 6.8×10^5 cfu/g for bacteria and 3.0×10^3 cfu/g for fungi. Eleven bacterial species were identified, among them *Klebsiella* sp., *Streptococcus* sp., *Corynebacterium* sp., *Azotobacter* sp., and *Bacillus* sp. Fungal isolates included *Aspergillus niger*, *Alternaria alternate*, *Rhizopus* sp., and *Paecilomyces* sp. The study indicates that *E. crassipes* supports diverse microbial populations and contains valuable nutrients, suggesting its potential for use in microbial and biotechnological studies.

Keywords: Diversity; *Eichhornia crassipes*; Microbial; Nutrients; Physicochemical

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INTRODUCTION

Eichhornia crassipes (Mart.) or water hyacinth is one of the most invasive freshwater macrophytes in tropical and subtropical regions of the globe. Its rapid vegetative spread, along with its potential to form intensive floating mats, causes severe ecological and socioeconomic issues, including obstructing waterways, reducing dissolved oxygen levels, and disrupting aquatic biodiversity (Monroy-Licht *et al.*, 2024). Despite these negative impacts, the species has attracted considerable attention due to its potential to be utilized for phytoremediation, animal feed, composting, and

bioenergy production, all of which are governed by its physicochemical properties and associated microbial diversity (Moses *et al.*, 2021).

The role of *E. crassipes* in aquatic ecosystems is linked to its microbial consortia at a sophisticated level. Plant microbiota, particularly in the endosphere and rhizosphere, regulate nutrient transformations, organic matter turnover, and pollutant degradation (Ávila *et al.*, 2019). Root biofilms, for example, have been shown to be harbouring microbial populations involved in nitrogen cycling, denitrification, and carbon

metabolism, hence mediating ecosystem processes (Duraivadivel *et al.*, 2020). Moreover, microbial populations inhabiting *E. crassipes* are colonizers by nature and actively engage with the chemical constitution of the plant, driving biogeochemical processes and the ecological influence of the plant (Fan *et al.*, 2023).

The physicochemical composition of *E. crassipes* proportions of proximate nutrients (protein, fiber, and lipid), lignocellulosic components (cellulose, hemicellulose, lignin), and mineral levels plays a significant role in substrate quality for microbial colonization and biodegradation (Moses *et al.*, 2021). Notably, different parts of a plant (roots, stems and leaves) contain differing nutrient and heavy-metal content, influencing microbial function and diversity (Ávila *et al.*, 2019). Roots will fix metals and contain dense microbial biofilms that can metabolize pollutants, while nutrient-scavenging tissues may favour fermentative and plant-growth-promoting bacteria (Duraivadivel *et al.*, 2020).

The new technologies in high-throughput sequencing revealed that *E. crassipes* is diverse in microbial communities dominated by Actinobacteria and Proteobacteria, whose community structure varies with tissue type and the environment (Fan *et al.*, 2023). *Eichhornia crassipes* endophytic isolates have been found to possess promising attributes such as pollutant degradation, metal sequestration, and phytohormone synthesis, which underscore the biotechnological relevance of the plant (Ávila *et al.*, 2019; Fan *et al.*, 2023). However, comprehensive studies linking the physicochemical nature of *E. crassipes* and their microbial assemblages are still limited.

Clarification of microbial diversity in the context of plant chemistry does not only importantly accounts for explaining the ecological role of *E. crassipes*, but also helps in harnessing its potential in biomass valorisation, bioenergy, and phytoremediation (Monroy-Licht *et al.*, 2024). Biognosis encompassing both physicochemical profiling and microbial community analysis has the potential to provide insightful views of the ecological roles and applicative facets of this globally significant macrophyte.



Plate 1: *Eichhornia crassipes*

MATERIALS AND METHODS

Sampling Site/Area

Eichhornia crassipes samples were collected in March 2023 from the Sabongarin Takanebu River in the Miga Local Government Area of Jigawa State, northwestern Nigeria. The river is situated along the Gujungu–Dutse road, approximately 6 km from Sabongarin Takanebu. It lies between longitude 12°14'48.9"N 9°35'47.8"E, Figure 1 (GIS, 2025).

Collection of Sample

Fresh *Eichhornia crassipes* sample were collected from Sabongarin Takanebu within the Miga Local Government Area of Jigawa State, northwestern Nigeria. The samples were air-dried at ambient room temperature for a period of 21 days. Upon drying, the samples were crushed into coarse powder using a pestle and mortar and again sieved through a 20-mesh British Standard sieve. The fine powder recovered was preserved for subsequent analysis.

Physicochemical analysis of *Eichhornia crassipes*

Physicochemical analyses of *Eichhornia crassipes*, including measurements of temperature, ash content, pH, total nitrogen, total carbon, electrical conductivity, phosphorus, exchangeable Ca, Mg, K, Na, and Mn, as well as Pb, Zn, Cu, and Ni were carried out following standard procedures described by Oliveira *et al.* (2002), Bruce *et al.* (2009) and Eno *et al.* (2009).

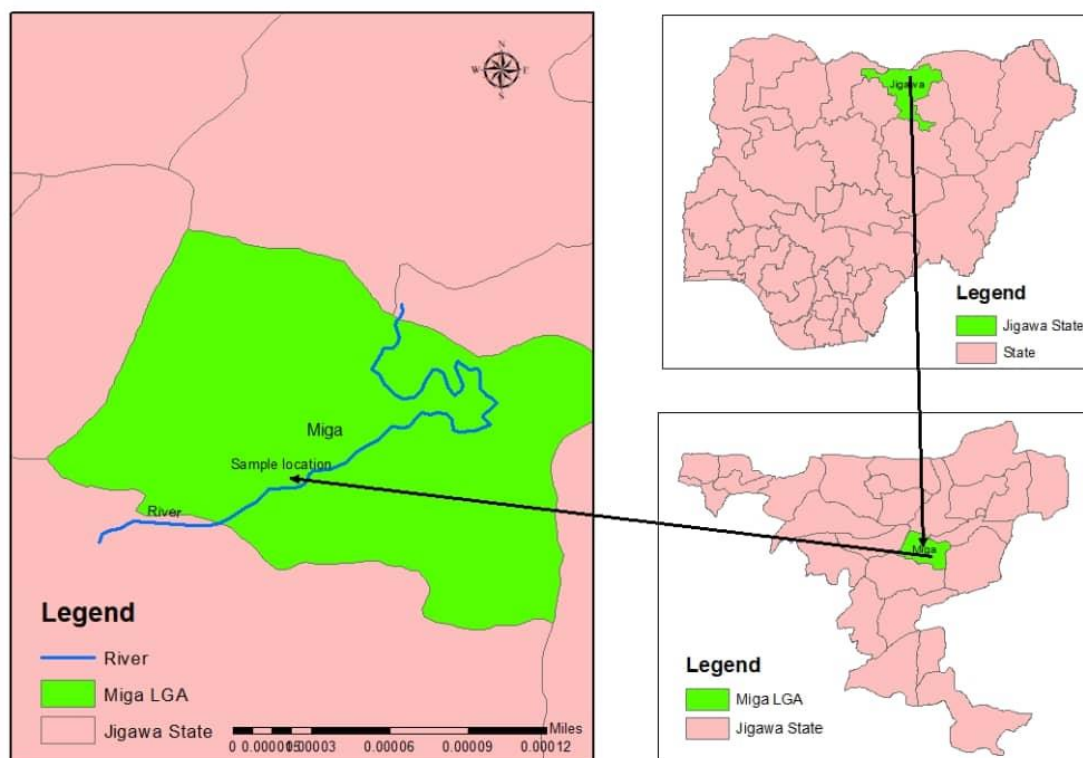


Figure 1: Map of Jigawa State showing the sampling site in Miga LGA, Jigawa State, Nigeria

Microbiological Analyses

For microbial analysis, 1g of the fine powder was mixed in 9ml of sterile distilled water. From this stock solution, 1ml was diluted serially up to 10^6 tubes. Then 0.5ml was drawn using sterile syringe from the 10^5 tube and aseptically inoculated on prepared sterile Nutrient agar plates using spread plate technique. Each sample was inoculated in triplicate and incubated at 37°C for 72 hours. Bacterial colonies that developed on the plates were counted and the average was recorded as colony forming units per milliliter (cfu/g) of the sample. The colonies were also serially subculture on fresh Nutrient Agar plates to obtain pure isolates. Pure bacterial isolates were gram-stained and also put through a series of biochemical tests including coagulase production, catalase, urease, oxidase, methyl red, Voges-Proskauer, citrate utilization test and H_2S production as described by Cheesebrough (2006).

Isolation and Identification of Fungi

Fungal isolates

For fungal isolation from *Eichhornia crassipes*, a Loopful of homogenate was inoculated on already prepared sterile Potato Dextrose Agar (PDA) plates and incubated at room temperature for 7 days Murugan *et al.* (2007). The colonies were identified by cultural and morphological characteristics. Microscopic examination of fungi (mycelium, fruiting bodies, etc.) was done using lacto-phenol Ali *et al.*

cotton blue stain technique and observed on 10x and 40x objectives of the compound light microscope (Murugan *et al.*, 2007).

RESULTS

The results of the physicochemical, macro- and micronutrient composition of *Eichhornia crassipes* are presented in Table 1. The temperature of *Eichhornia crassipes* was $38 \pm 0.3^\circ\text{C}$, while the pH value was 5.66 ± 0.01 . The percentage moisture content was 5.71 ± 0.03 . The analysis further revealed the following values: electrical conductivity ($4.51 \pm 0.02 \mu\text{S/cm}$), organic carbon $49.556 \pm 0.03\%$, nitrogen $0.30 \pm 0.42\%$, ash content 2014.92 mg/kg , C/N ratio 55.13 ± 0.03 , potassium $1234.132 \pm 0.37 \text{ mg/kg}$, sodium 237.41067 mg/kg , calcium $7115.6633 \pm 0.01 \text{ mg/kg}$, magnesium $2945.386 \pm 0.38 \text{ mg/kg}$, copper $2.47 \pm 0.01 \text{ mg/kg}$, lead $18.033 \pm 0.56 \text{ mg/kg}$, zinc $6.527 \pm 0.05 \text{ mg/kg}$, and iron $185.2 \pm 0.88 \text{ mg/kg}$.

Table 2 presents the mean microbial population on *Eichhornia crassipes*, showing higher bacterial counts ($2.4 \times 10^6 \text{ cfu/g}$) compared to fungal counts ($2.0 \times 10^3 \text{ cfu/g}$). The biochemical tests and Gram Staining yielded a wide range of bacterial isolates on *Eichhornia crassipes*, ranging from Gram-positive to Gram-negative. In Table 3, the various bacterial recovered these include *Klebsiella* sp., *Streptococcus* sp., *Corynebacterium* sp., *Azotobacter* sp., *Salmonella* sp., *Lactobacillus* sp.,

Clostridium sp., *Fusobacterium* sp., *Bacillus* sp., *Staphylococcus* sp., and numerous others.

The microscopic and macroscopic characteristics of the fungal isolates are shown in Table 4. Several species were identified, including *Aspergillus*, *Penicillium*, *Rhizopus*, *Trichoderma*, *Paecilomyces*, *Alternaria*, *Fusarium*, *Rhizoctonia*, and

Stemphylium. These fungi exhibited distinct microscopic and macroscopic features that facilitated their identification. Most isolates were isolated on Potato Dextrose Agar, whereas *Aspergillus fumigatus* and *A. candidus* were additionally isolated on Sabouraud Dextrose Agar.

Table 1. Mean Values of Physicochemical Properties, Macro and Micro-Nutrients Composition and *Eichhornia crassipies*

Parameters	Mean \pm Standard deviation SD	Standard/Optimum Range (NESREA, 2011)
Physicochemical		
Temperature °C	38 \pm 03 ^b	30-40
pH	5.66 \pm 0.01 ^a	6.5-7.5
Moisture (%)	5.71 \pm 0.026 ^b	80-90%
Electric Conductivity (ds/m)	4.51 \pm 0.021 ^b	<4ds/m
Organic carbon (%)	49.556 \pm 0.031 ^b	> 30%
Nitrogen (%)	0.30 \pm 0.42 ^b	1-3%
Phosphorus (mg /kg)	2014.92 ^b	300-1000mg/kg
Ash content (%)	24.73 ^b	< 30%
Moisture content (%)	2.360 ^b	80-90%
Carbon-Nitrogen (%)	55.13 \pm 0.03 ^b	20:1-30:1
Macro and Micro Nutrients		
Potassium (mg/kg)	1234.132 \pm 0.37 ^b	500-3000mg/kg
Sodium (kg/mg)	237.41067 ^b	<3500mg/kg
Copper (mg/kg)	2.47 \pm 0.01 ^b	10-100mg/kg
Calcium (mg/kg)	7115.6633 \pm 0.01 ^b	1000-4000mg/kg
Magnesium (mg/kg)	2945.386 \pm 0.38 ^b	500-1500mg/kg
Iron (mg/kg)	1815.2 \pm 0.88 ^b	100-1000mg/kg
Manganese (mg/kg)	420.396 ^b	10-100mg/kg
Lead (mg/kg)	18.033 \pm 0.56 ^b	<10mg/kg
Nickel (mg/kg)	6.415 \pm 0.04 ^b	1-10mg/kg
Zinc (mg/kg)	6.527 \pm 0.05 ^b	50-500mg/kg

Keys: Values with different letters within the same row are considered significantly different by analysis of variance (ANOVA) with Duncan multiple range test (P < 0.05) Values are mean \pm S.D of three independent determination.

Table 2. The Mean Values of Bacterial and Fungal Load (cfu/g)

PARAMETER	COUNNT (cfu/g)
Bacterial load	$2.4 \times 10^6 \pm 0.06$
Fungal load	$2.0 \times 10^3 \pm 0.03$

Table 3. Gram's reaction and Biochemical Characteristics of the Bacterial isolates

Growth on medium	GR	SHP	CAT	COA	IND	CIT	MR	VP	GLU	SUC	LAC	H ₂ S	GAS	Organism
MacConkey Agar	-	Rod	+	-	-	+	+	-	+	+	+	-	+	<i>Klebsiella</i> sp
Blood Agar	+	Oval	-	-	-	-	+	-	+	+	+	+	+	<i>Streptococcus</i> sp
Tryptone Soya Agar	+	Rod	+	-	-	-	-	-	-	+	+	-	-	<i>Corynebacterium</i> sp
Nutrient Agar	-	Oval	+	-	-	+	-	+	+	+	+	+	-	<i>Azotobacter</i> sp
Nutrient Agar	+	Oval	-	-	-	-	+	-	+	+	-	-	-	<i>Sarcina</i> sp
Salmonella-Shigella Agar	-	Rod	+	-	-	+	+	-	+	-	+	+	+	<i>Salmonella</i> sp
Blood Agar	+	Rod	-	+	+	-	+	-	+	+	+	-	-	<i>Lactobacillus</i> sp
Blood Agar	-	Rod	-	-	+	-	+	-	+	+	+	-	-	<i>Fusobacterium</i> sp
Blood Agar	+	Rod	-	-	+	+	+	+	+	+	-	-	-	<i>Bacillus</i> sp
Nutrient Agar	+	Rod	-	-	-	-	+	-	-	-	-	-	-	<i>Eubacterium</i> sp
Blood Agar	-	Rod	-	+	-	+	-	+	+	+	-	-	-	<i>Bacteroides</i> sp
Nutrient Agar	-	Rod	+	-	-	+	-	+	+	+	-	-	-	<i>Pseudomonas</i> sp
MacConkey Agar	-	Cocci	+	-	-	-	-	-	+	+	+	-	-	<i>Rhizobium</i> sp
Nutrient Agar	+	Cocci	+	+	-	-	+	+	+	+	-	-	-	<i>Staphylococcus</i> sp

Keys GR: Gram reaction, SHP: shape, CAT, Catalase, IND: Indole, CIT, Citrate, MR: Methyl red, VP: Voges-Proskauer, GLU: Glucose, SUC: Sucrose, LAC: Lactose, H₂S: Hydrogen sulfide, EC=*Eichhornia crassipes* and CRC=Cow Rumen Content

Table 4. Microscopic and Macroscopic Characteristics of Fungal

Mycological media	Macroscopic characteristic	Microscopic characteristic	Inference
Potato Dextrose Agar	Black, pin-like growth; powdery or granular texture	Non-branched conidiophores with bulbous ends; conidia radiate like sun-rays	<i>Aspergillus niger</i>
Potato Dextrose Agar	Green, pin-like colonies; slightly granular or velvet-like	Non-branched conidiophores; bulbous ends with terminal vesicles carrying conidia	<i>Aspergillus flavus</i>
Potato Dextrose Agar	Green colonies; powdery surface, often seen on fruits	Brush-like conidiophores (phialides in chains); hyaline and septate hyphae	<i>Penicillium sp.</i>
Potato Dextrose Agar	Cotton-like white colonies with black sporangia spots	Broad, aseptate hyphae; sporangia with spores and rhizoids present	<i>Rhizopus sp.</i>
Sabouraud Dextrose Agar	Pale yellow/brown, circular, flat or slightly raised colonies	Hyaline, septate, branched hyphae; conidiophores with short chains of conidia	<i>Aspergillus fumigatus</i>
Potato Dextrose Agar	Green colonies with powdery or velvety surface	Highly branched, irregular pyramidal conidiophores; hyaline, septate hyphae	<i>Trichoderma sp.</i>
Potato Dextrose Agar	Velvety or powdery colonies; white to yellow or pale brown, darken with age	Septate, hyaline hyphae; slender, branched conidiophores resembling a brush	<i>Paecilomyces sp.</i>
Potato Dextrose Agar	Gray to dark brown flat colonies with blackish center; velvety or woolly	Multicellular conidia with 3–10 transverse septa; club to ellipsoidal; dark brown	<i>Alternaria alternata</i>
Potato Dextrose Agar	Pink/red/purple/white colonies; cottony or powdery texture; 5–10 cm	Sickle or spindle-shaped conidia; 20–100 µm; pink to salmon-colored	<i>Fusarium sp.</i>
Sabouraud Dextrose Agar	White to creamy colonies with yellow/brown center; cottony or powdery	Spherical to sub spherical hyaline conidia (5–8 µm); septate hyphae	<i>Aspergillus candidus</i>
Potato Dextrose Agar	White/cream/light brown colonies; fluffy, woolly; irregular shape	Multinucleate septate hyphae (5–15 µm); irregular or oval conidia	<i>Rhizoctonia solani</i>
Potato Dextrose Agar	Velvety/woolly, grayish to dark brown colonies with black center	Muriform multicellular conidia with transverse and longitudinal septa; brown	<i>Stemphylium sp.</i>
Potato Dextrose Agar	Pale green to blue-green colonies; velvety to powdery; 5–7 cm diameter	Smooth-walled, spherical to ellipsoidal conidia (2.5–4 µm); pale green to blue-green	<i>Penicillium fusiculosum</i>

DISCUSSION

The physicochemical analysis of *Eichhornia crassipes* revealed 38 ± 03 °C, which falls within the ideal mesophilic range of 30–40 °C and therefore favours the best microbial growth and enzyme activity conditions. The same has also been reported by Chen *et al.* (2020), who observed that the mesophilic conditions favour

efficient degradation of lignocellulosic substrates. In contrast, the pH value of 5.66 ± 0.01 was below the recommended range of 6.5–7.5 and was therefore an acidic environment probably caused by the accumulation of organic acids during the degradation of biomass. This type of condition would inhibit activity of most microbial communities since Contreras *et al.*

(2016) testified that low pH decreases microbial diversity, which partly contradicts the range of neutrality referenced in most of the papers.

Moisture content was $5.71 \pm 0.026\%$, far below the optimal rate of 80–90%, and could be due to drying of samples in handling samples. This low moisture inhibits microbial degradation and metabolism, in line with Zhang *et al.* (2014), who demonstrated that low water content retards biodegradation efficacy. Similarly, electrical conductivity (4.51 ± 0.021 dS/m) exceeded the threshold level (<4 dS/m), indicating over excess salt accumulation that would inhibit the growth of microorganisms and nutrient uptake. This agrees with Rietz and Haynes (2003), who suggested that high Electric conductivity negatively affects microbial respiration.

Organic carbon was also excessive ($49.556 \pm 0.031\%$, $>30\%$), which indicated lignocellulosic richness of *E. crassipes* biomass. It is a nutrient which is most suitable to microbial energy provision, and the same has been reported by Singh and Bishnoi (2013). Nitrogen percentage ($0.30 \pm 0.42\%$) was very low compared to optimal 1–3% and the C/N ratio was very high (55.13 ± 0.03 , above the recommended 20–30). This difference points towards nitrogen deficiency, which inhibits microbial growth and slows biodegradation, as highlighted erstwhile by Adegunloye *et al.* (2017) and Akinbomi *et al.* (2014).

Nutrient analysis also indicated excess phosphorus (2014.92 mg/kg) compared to the required 300–1000 mg/kg. This is in agreement with Gopal (1987), who found that *E. crassipes* bioaccumulates phosphorus from polluted water bodies. Ash content (24.73%) was within the required level ($<30\%$), indicating moderate inorganic residue, which is consistent with Akinbomi *et al.* (2014).

Macro- and micronutrient analysis indicated that potassium 1234.13 mg/kg and sodium 237.41 mg/kg were safe and supportive of microbial metabolism. Yet, calcium (7115.66 mg/kg) and magnesium 2945.38 mg/kg exceeded their normal levels, owing to the capability of the plant to absorb minerals strongly. The more critical issue, though, was that manganese (420.39 mg/kg) was significantly higher than the normal 10–100 mg/kg, indicative of potential heavy-metal bioaccumulation, a finding supported by Gopal (1987). Nickel content 6.41 mg/kg was within the optimum 1–10 mg/kg, and no potential toxicity existed.

Microbial load analysis showed that *Eichhornia crassipes* had a significantly higher bacterial load (2.4×10^6 cfu/g) compared to the fungal load (2.0×10^3 cfu/g). This indicates that bacteria were the dominant microorganisms on the plant. Their greater density

could result from their fast growth rate, short generation cycle, and metabolic versatility, which enable them to break down a wide range of organic substances present in plant tissues. Fungi, by contrast, are slow colonizers and have the tendency to specialize in breaking down intricate polymers such as lignin and cellulose, and perhaps this is the reason for their relatively lower densities.

The implication of these findings is that bacteria are most likely responsible for a significant percentage of the initial degradation and cycling of nutrients of *E. crassipes*, and fungi could perhaps be more accountable for the later stages of decomposition, particularly recalcitrant plant biomass. These observations are consistent with previous observations in which bacterial dominance over fungi had been reported in aquatic weeds and organic matter due to their faster adaptability and development under optimal environmental conditions (Okoye *et al.*, 2016; O'Sullivan *et al.*, 2010). The same was noted by Thakur and Medhi (2019), who added that bacteria dominate fungi in aquatic macrophytes due to their ability to utilize easily accessible nutrients

Gram's response and biochemical description showed that *Eichhornia crassipes* contained a heterogeneous collection of bacterial isolates, including *Klebsiella* sp., *Streptococcus* sp., *Azotobacter* sp., *Salmonella* sp., *Lactobacillus* sp., *Bacillus* sp., *Staphylococcus* sp., *Proteus* sp., etc. This is a sign of the presence of both Gram-positive and Gram-negative bacteria with various morphological and metabolic features. Dominance by species such as *Klebsiella*, *Bacillus*, and *Streptococcus* can be associated with their adaptability and ability to catabolize a wide range of organic substrates present in the plant tissues. Nitrogen-fixing *Azotobacter* and *Rhizobium* were also present, which must have been maintained under nitrogen-rich conditions provided by decomposing aquatic vegetation. The implication of these findings is that *E. crassipes* is a reservoir of both pathogenic and beneficial bacteria. While nitrogen-fixing and fermentative bacteria are implicated in nutrient recycling as well as biotechnological purposes such as the production of biofertilizers and anaerobic digestion. The occurrence of potential pathogens such as *Salmonella* and *Staphylococcus* raises public health and ecological concerns. Such results conform to previous reports on diverse bacterial associations with aquatic macrophytes. For instance, Okoye *et al.* (2016) reported that *E. crassipes* contained pathogenic and beneficial bacteria in aquatic ecosystems. Similarly, O'Sullivan *et al.* (2010) reported that the richness of bacteria in aquatic weeds contributes significantly to the biodegradability of such aquatic weeds during

anaerobic digestion. Recently, Thakur and Medhi (2019) reported that *E. crassipes* harbours metabolically-varied bacterial communities, contributing further to its environmental bioremediation and bioenergy production capabilities.

The prevalent fungi isolated were the representatives of the genera *Aspergillus*, *Penicillium*, *Rhizopus*, *Fusarium*, and *Alternaria*. The prevalence of *Aspergillus* and *Penicillium* species is not unexpected since they are encountered globally in soil, water, and decaying organic matter at different environmental conditions. Their occurrence here means that *Eichhornia crassipes* provides a nutrient substrate that promotes quick-colonizing saprophytic fungi.

Fusarium, *Alternaria*, and *Rhizoctonia* are phytopathogenic fungi; their incidence can be attributed to the plant's lignocellulosic nature, which favours fungal colonization capable of breaking down complex polymers. This corresponds with findings by Leslie and Summerell (2006), who noted the frequent presence of *Fusarium* species in decaying plant material. Similarly, *Alternaria* species are often cited as common epiphytic and pathogenic fungi of aquatic and terrestrial vegetation (Thomma, 2003).

CONCLUSION

This study substantiated that *Eichhornia crassipes* possesses a very rich population of bacterial and fungal species, with bacteria as the predominant microbial population. The crop also possessed high organic carbon and essential macronutrient content, which verifies its prospect as a substrate for bioenergy production and use by microbes. However, the low nitrogen content, wide C/N ratio, and high concentration of certain heavy metals present ecologic concerns that may limit the direct use. These points place the limelight on *E. crassipes* both as a source of environmental issue, through its invasiveness and heavy metal uptake, and valuable resource for environmental management, phytoremediation, and biotechnological application if properly treated or supplemented.

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