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Assessment of the Potentials of Water Hyacinth (*Eichhornia crassipes*) as Raw Material for Biofertilizer

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MOI and VOO designed the study. Author MOI collected samples for the study, managed the literature searches, wrote the protocol and the first draft of the manuscript and performed the statistical analyses. Authors VOO and OPA participated in the discussion and proofread the draft manuscript. All authors read and approved the final manuscript.

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ABSTRACT

This research investigated the potentials of water hyacinth as raw material for biofertilizers. Water hyacinth was collected from Alape River in Ilaje Local Government Area of Ondo State and processed for microbiological, proximate and physicochemical analyses by standard methods. Results revealed that the total heterotrophic bacterial and fungal populations were 5.0 x 10^7 (CFU/g) and 3.5 x 10^5 (CFU/g) and 3.6 x 10^2 SFU/g and 3.0 x 10^2 (SFU/g) for root and leaf parts respectively. The population of phosphate solubilizing bacteria, *Azospirillum* spp., *Azotobacter* spp. and *Rhizobium* spp. were 3.5 x 10^4 - 1.3 x 10^6 CFU/g, 1.4 x 10^3- 2.6 x 10^4 (CFU/g), 3.4 x 10^3 – 1.7 x 10^4 (CFU/g) and 1.2 x 10^3 – 1.1 x 10^4 (CFU/g) respectively. 16S rRNA gene sequencing revealed the presence of *Enterobacter cloacae* (LT 221670.1 -53%) and *Bacillus safensis* (AB711138.1 - 99%), *Pseudomonas mendocina* (LN 881702.1 – 99%) and *Aeromonas veroni* (LC 487867.1 – 97%) from the root and leaf respectively. *Aspergillus* spp., *Fusarium* sp., *Rhizopus* sp.,

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Trichoderma sp. *Cladosporium* sp. were among the fungi from the water weed parts. The proximate, nutrient and heavy metal constituents of water hyacinth vary among the leaf, stem and root. The proximate values for moisture (65.45%), protein (12.18%) and carbohydrate (5.30%) were highest in the leaf part compared to the stem and roots. Also, the highest values of ash (18.48%), fat (3.84%) and crude fibre (12.50%) were obtained in the plant root relative to leaf and stem. Apart from magnesium and phosphorous, mineral elements such as sodium, calcium and potassium were more abundant in the leaf. Among the heavy metal, Lead, Cadmium, Nickel, Arsenic and Cobalt had undetectable values for all parts. This study concludes that the presence of microbial biofertilizer and micro and macro nutrients on water hyacinth suggests its potential as raw material for biofertilizer and can be used to promote plant growth without negative consequences.

Keywords: Biofertilizer; carrier material; chemical fertilizer; nitrogen fixation; phosphorous solubilisation.

1. INTRODUCTION

"The uncontrolled and prolonged use of chemical fertilizers has detrimental consequences on soil quality and fertility [1] and can become injurious to plants as well as animals that depend on plants for food" [2,3]. "These negative consequences due to the protracted use of chemical fertilizers lead to the introduction of biofertilizers as a viable alternative [4] to improve soil quality and integrity thereby promoting plant growth and improved crop yield for biosafety food" [5,6]. "Biofertilizers are microbial formulations constituted of beneficial microbial strains immobilized or trapped on inert carrier materials that can be employed to enhance plant growth and increase soil fertility" [7,8]. **"**Natural processes such as nitrogen fixation, phosphorus solubilization and stimulation of plant growth along with the synthesis of growth-promoting substances are among the ways through which biofertilizers promote plant growth" [9]. "Apart from biofertilizers being relatively economical, the additional advantages include longer shelf life of microbial cells, causing no adverse effects on the ecosystem" [10]. "The use of biofertilizer circumvents the need for repeated use through self - replicate and participate in nutrient cycling and contribute to crop growth and productivity [11] as well as improving soil physicochemical properties such as soil structure, texture, water holding capacity, cation exchange capacity and pH by providing several nutrients and sufficient organic matter" [12].

"Various types of materials are used as carriers for seed or soil inoculation. The quality of carrier is a vital factor in determining microbial load and shelf-life of biofertilizers. According to Somasegaran and Hoben [13] the desirable properties of a good carrier material include non-toxicity to inoculant bacterial strains and plants, good moisture absorption capacity, good pH buffering capacity, amenability to processing and sterilization and must be cheap and readily available in adequate amounts". Bacteria require different nutrients for their growth. These include an organic carbons source, a nitrogen source and a variety of other elements dissolved in water [14]. "Peat is the most frequently used carrier material for seed inoculation while granular forms of peat, perlite, charcoal or soil aggregates are suitable for soil inoculation. However, this material is not readily available in the locality and may hinder the development of biofertilizers for the use of the local farmers. Aquatic weeds are widely used to produce nutritious food for humans, (raw material and have recently been used for biofertilizer production by leaf and root" [15]. "Water hyacinths (*Eichhornia crassipes*) is a prolific, free-floating freshwater weed abundant in lakes and rivers and other water bodies causing significant economic and ecological burden to many sub-tropical and tropical regions of the world" [16]. The residents of water hyacinth infested areas use the stalk and leaves of water hyacinth to feed domestic animals, especially goats. Being very abundant and practically of no economic value in many developing countries, the freshwater weed; *Eichhornia crassipes* meets important requirements of carrier materials such as availability in adequately large amounts, nontoxicity to plant and almost cost free. There is need to explore more of its uses in order to maximize the use of water hyacinth in the locality to the benefit of the local communities living along the riverine areas. This research is therefore designed to assess the potentials of water hyacinth (*Eichhornia crassipes*) as raw material for biofertilizer production.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Fresh samples of water hyacinth were collected manually into sterile polythene bags from Alape River in Ilaje Local Government Area of Ondo State, Nigeria and transported within twenty-four (24) hours in an ice chest to the laboratory for analyses. The fresh plants were authenticated at the Herbarium of the Federal University of Technology, Akure, Nigeria.

2.2 Microbiological Analysis of Soil Samples

Dehydrated nutrient agar (NA) and Potatoes Dextrose Agar (PDA) were prepared according to manufacturers' specifications for the enumeration and isolation of total heterotrophic bacteria and fungi respectively. Yeast Extract Mannitol Agar [17], Okon Medium [18], Ashby Mannitol Agar [19] and Pikovskaya medium [20] were selective media used for the isolation of *Rhizobium* spp., *Azospirillum* spp., *Azotobacter* spp. and phosphate solubilizing bacteria respectively. All media were thoroughly mixed with the aid of a magnetic stirrer to homogenize and 20 ml of each medium was dispensed into MacCartney bottles, autoclaved at 121° C for 15 minutes and maintained at 44° C - 45° C. The nutrient agar (NA), potatoes dextrose agar (PDA), Yeast Mannitol Agar (YEMA), Okon Red Agar (ORA), Ashby Mannitol Agar (AMA) and Pikovskaya (PKV) medium each supplemented with antimicrobial agents (50µg/mL of nystatin and 75µg/mL of cycloheximide) for the enumeration of bacteria and 50mg/l of streptomycin for fungi after sterilization.

2.2.1 Enumeration of microbial population

The unwanted foreign materials in the raw water hyacinth were removed by sorting and the weed was cut into root and stalk and leaf sections and labeled PR and PL respectively. Ten (10) grams of each sample section was rehydrated with 90 ml sterile distilled water in an Erlenmeyer flask and then agitated vigorously to provide mechanical disaggregation of microbial cells from the plant parts. 1 ml aliquot was transferred with sterile pipettes to 9 ml distilled water in a test tube. A subsequent serial dilution was then prepared to 7th dilution. One (1) ml aliquot of each dilution was pour plated onto sterile petri dish and then overlaid with previously sterilized medium. Triplicate culture plates were then incubated at 30° C for 48 hrs and 28° C ± 2° C for 96 hrs for NA and PDA plates respectively while the nitrogen- fixing and phosphate solubilizing bacteria were incubated at 28° C for 7 d [21]. The plates were observed for growth and selected for count and the number of colonies expressed as colony forming unit per gram (CFU/g) or spore-forming unit (SFU/g) per gram of sample respectively for bacteria and fungi.

2.2.2 Identification of microbial isolates

Pure cultures of bacteria and fungi for identification were obtained by repeated streaking on NA and PDA respectively. Pure bacterial isolates were identified by colonial and cellular morphology as well as biochemical reactions according to Chessbrough [22] and Bergey's manual of determinative bacteriology [23]. Fungal identification was done using [24, 25, 26]

2.3 Molecular Characterization of Bacterial Isolates

2.3.1 Extraction of DNA

Bacterial isolate grown overnight was transferred to an eppendorf tube and it was spun down at 14,000 rpm for 2 minutes, the supernatant was discarded and 600 µl of 2X CTAB buffer was added to the pellet and then incubated at 65 ^{O}C for 20 mins. The sample was removed from the incubator and allowed to cool to room temperature and chloroform was added and then mixed several times by gently inversion of the tube. Thereafter, the sample was spun at 14,000 rpm for 15 minutes and the supernatant was transferred into a new eppendorf tube and an equal volume of cold isopropanol was added to precipitate the DNA. The sample was kept in the freezer for 1 hour and later spun at 14,000 rpm for 10 minutes and the supernatant was discarded and the pellet was washed with 70% ethanol and the sample was air dried for 30 mins on the bench. The pellet was resuspended in 100 µl of sterile distilled water. DNA concentration of the samples was measured on a spectrophotometer at 260 nm and 280 nm and the genomic purity was determined. The genomic purity was between 1.8 –2.0 for all the DNA samples.

2.3.2 DNA electrophoresis

Agarose gel electrophoresis was used to determine the quality and integrity of the DNA by

size fractionation on 1.0% agarose gels. Agarose gels were prepared by dissolving and boiling 1.0 g agarose in 100 ml 0.5X TBE buffer solution. The gels were allowed to cool down to about 45 ^{O}C and 10µl of 5mg/ml ethidium bromide was added, and mixed together before pouring it into an electrophoresis chamber set with the combs inserted. After the gel has solidified, 3µl of the DNA with 5µl sterile distilled water and 2µl of 6X loading dye was mixed and loaded in the well created. Electrophoresis was done at 80V for 2 hours. The integrity of the DNA was visualized and photographed on a UV light source [27,28].

2.4 Polymerase Chain Reaction (PCR) Amplification and Purification of 16S rRNA Gene

Protocol for bacteria PCR: The 16S rRNA gene was amplified using primers 5'AGAGTTTGAT CCTGGCTCAG 3' and 5'GACGGGCRGTGWGTR CA 3' forward and reverse respectively. PCR mix contained 10X buffer, 100mM dNTPs, 2.52M MgCl2, 2U*Taq*DNA polymerase, 1µl of each (forward and reverse) primers, 2µl of genomic DNA and sterilized distilled water to make a final volume of 25 ml. *Taq*DNA polymerase initiates the replication of DNA fragment by using nucleotide bases from dNTPs mixture (A, C, G, T). The PCR reaction included the following steps; initial denaturation of 2 min at 94°C (Preheating) followed by 25 cycles run on a thermal cycler, each comprising 1 min at 94°C (denaturation), 1 min at 94°C (annealing) and 1.5min at 94°C (extension), followed by a final extension of 10 min at 94°C for utilization of extra dNTPs in the PCR mixture.

2.4.1 Agarose gel electrophoresis of PCR amplicon

Agarose gel electrophoresis protocol for bacteria PCR product: A 0.8 g of agarose was weighed and taken in a 100 ml reagent bottle. To this 100 ml of 1X TBE buffer was added and it was heated in a microwave oven till agarose melts. The agarose solution was then poured into a gel-casting unit assembled with an appropriate comb and it was allowed to get polymerize. When the agarose polymerized, the comb was removed and the gel was kept in an electrophoresis tank consisting of 1X TBE buffer. About 20 µlof isolated bacterial genomic DNA from PCR product was mixed with 20 µl of loading dye (bromophenol blue) and it was loaded in 0.8 % agarose gel, 5.0 µl 1Kb DNA Ladder as a molecular marker in parallel. The gel was electrophoresed at 100 volts for about 30 minutes and it was observed on a UV transilluminator.

2.4.2 Purification of PCR products

The amplicon was further purified before the sequencing using 2M Sodium Acetate wash techniques. To 10 µl of the PCR product, 1µl 2M NaAct pH 5.2 was added, followed by 20 µl absolute Ethanol, kept at -20 ^{O}C for 1hr, spin at 10,000 rpm for 10 mins, then wash with 70% ethanol and air dried. The resulting solution was re-suspended in 5 µl sterile distilled water and kept at 4 ^{O}C for sequencing.

2.4.3 16S rRNA gene sequence, BLASTn and phylogenetic tree

Identification of strain was done by sequencing the PCR products of 16S rRNA (bacteria) using a sequencer, determined sequences were compared with sequences available in GeneBank, derived sequence aligned by Basic Local Alignment Search Tool (BLAST) algorithm, the highest S-ab value with identified species in the Sequence match search. Using the results received through BLASTn a phylogenetic tree is created using the BLASTn web page. Other genetic tool such as evolutionary distance and GC ratio will be used to determine variability in the genetic composition of bacteria.

2.5 Evolutionary Analysis by Maximum Likelihood Method

"The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model" [29]. "The tree with the highest log likelihood (-1449.96) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 25 nucleotide sequences. All positions with less than 95% site coverage were eliminated, i.e., fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option). There were a total of 777

positions in the final dataset. Evolutionary analyses were conducted in MEGA X" [30].

2.6 Analysis of the Proximate, Mineral and Heavy Metal Composition of Water Hyacinth

2.6.1 Sample preparation

The unwanted foreign materials in the raw water hyacinth were removed by sorting, draining and washing three or four times with clean water to eliminate all sand particles, The fresh water hyacinth was air-dried for 3 days and then cut into pieces of approximately 5-10 cm in length using a cutter to obtain four samples; whole plant (consisting of root, stalk and leaf), root, stalk and leaf and labeled WP, PR, PS and PL respectively.

2.7 Analyses of Proximate Composition

2.7.1 Moisture Content

Two grams (2.0 g) of each labelled part was placed in an oven maintained at 100 – 103 ^{O}C for 16 hours with the weight of the wet sample and the weight after drying noted. The drying was repeated until a constant weight was obtained. The moisture content was expressed in terms of loss in weight of the wet sample.

2.7.2 Ash Content

Two grams (2.0 g) of each of the oven-dried samples in powder form were accurately weighed and placed in a crucible of known weight. These were ignited in a muffle furnace for 8 hours at 550 ^{O}C to ash. The crucible containing the ash was then removed, cooled in a desiccator and weighed and the ash content expressed in terms of the oven-dried weight of the sample.

2.7.3 Protein

The procedures described by AOAC (2009) were used. The protein nitrogen in 1 g of the dried samples were converted to ammonium sulphate by digestion with concentrated H_2SO_4 and in the presence of $CuSO_4$ and Na_2SO_4. These were heated and the ammonia evolved was steam distilled into boric acid solution. The nitrogen from ammonia was deduced from the titration of the trapped ammonia with 0.1M HCl with Tashirus indicator (double indicator) until a purplish pink colour was obtained. Crude protein was calculated by multiplying the value of the deduced nitrogen by the factor 6.25 mg.

2.7.4 Lipid

The lipid content was determined by extracting the fat from 10 g of the samples using petroleum ether in a soxhlet apparatus. The weight of the lipid obtained after evaporating off the petroleum ether from the extract gave the weight of the crude fat in the sample

2.7.5 Fiber contents

Two grams (2.0 g) of each sample was weighed into separate beakers, the samples were then extracted with petroleum ether by stirring, settling and decanting 3 times. The samples were then air- dried and transferred into a dried 100 ml conical flask and then 200 cm^3 of 0.127 M sulphuric acid solution was added. This was then heated gently to boiling for 30 minutes. The contents were filtered to remove insoluble materials which was then washed with distilled water and then with 1% HCl, next with twice ethanol and finally with diethyl ether. Finally, the oven-dried residue was ignited in a furnace at 550 ^{O}C. The fibre contents were measured by the weight left after ignition and were expressed in term of the weight of the sample before ignition.

2.7.6 Carbohydrate

The carbohydrate content of the samples was determined as the difference obtained after subtracting the values of protein, lipid, ash and fibre from the total dry matter [31].

2.8 Mineral and Heavy Metal Analyses

The ash of each sample obtained was digested by adding 5 ml of 2 MHCl to the ash in the crucible and heated to dryness on a heating mantle. 5 ml of 2 MHCl was added again, heated to boil and filtered through Whatman No. 1 filter paper into a 100 ml volumetric flask. The filtrate was made up to mark with distilled water stoppered and made ready for reading of the concentration of Calcium, Potassium and Sodium on the Jenway Digital Flame Photometer (PFP7 Model) using the filter corresponding to each mineral element. The concentration of phosphorus was obtained by the colorimetric method.

The digest of the ash of each sample was washed into a 100 ml volumetric flask with

deionized or distilled water and made up to mark. These diluents were aspirated into the Buck 211 Atomic Absorption Spectrophotometer (AAS) through the suction tube. The concentration of each of the metals was then read at their respective wavelengths with their respective hollow cathode lamps using appropriate fuel and oxidant combination

3. RESULTS

3.1 Microbiological Analysis of Water Hyacinth Plant Parts

3.1.1 Enumeration of microbiota population

Table 1 shows the population of total heterotrophic bacteria and fungi in water hyacinth plant parts. The result revealed that the total heterotrophic bacterial and fungal populations were 5.0 x 10^7 (CFU/g) and 3.5 x 10^5 (CFU/g) and 3.6 x 10^2 SFU/g and 3.0 x 10^2 (SFU/g) for root and leaf parts respectively. Results shown in Table 1 also indicated that the population of microbial biofertilizer (phosphate solubilizing bacteria and nitrifying bacteria) also vary between the root and leaf parts of the fresh water weed with each part having considerable population of phosphate-solubilizing bacteria (PSB), *Azospirillum* (AZSP), *Azotobacter* (AZB) and *Rhizobium* (RZB) but higher population of each in the plant root than in the leaf part.

Fig. 1 shows the phylogenetic tree of indigenous plant growth promoting bacteria (PGRB) isolated from water hyacinth plant parts subjected to molecular identification using 16S universal primer. The phylogenetic tree of the bacterial isolates is the genetic relationship between 16S ribosomal RNA nucleotide sequences of the PGRB based on the alignment of partial 16S rRNA sequences while Table 2 shows the nearest relative, accession numbers and the percentage homology of the isolates. The blasting of the sequence also revealed the identities of the autochthonous plant microflora to be *Pseudomonas mendocina* (LN881702.1), *Bacillus safensis* (LR215077.1), *Aeromonas veronii* (FN432809.1) and *Enterobacter cloacae* (LT221670.1). Results in Table 2 show varying percentage homology and similarity which range 53-99%. The high percentage (97 - 99%) similarities observed between the 16S rRNA gene, partial sequences of three plant leaf bacterial isolates and previously identified bacteria in the GeneBank, indicates that they are homologous to each other.

Table 1. Population of microbe types isolated from water hyacinth (*Eichhornia crassipes*) plant parts

Microbe type	Plant Root (PR)	Plant stalk and Leaf (PL)
THF (SFU/g)	3.6 × 10^2	3. 0 × 10^2
THB (CFU/ g)	5.0 x10^7	3.5 x 10^5
PSB (CFU/ g)	1.3 x10^6	3.5 x 10^4
NFB - AMA (CFU/ g)	1.7 x 10^4	3.4 x 10^3
NFB - ORA (CFU/ g)	2.6 x 10^4	1.4 x 10^3
NFB - YEMA (CFU/ g)	1.1 x 10^4	1.2 x 10^3

Data are expressed as mean ± Standard Error

Legend: THF; Total Heterotrophic Fungi, THB; Total Heterotrophic Bacteria, PSB - PKV Phosphate Solubilizing Bacteria on Pikovskaya, NFB – AMA; Nitrifying Bacteria on Asby Mannitol Agar; NFB – ORA; Nitrifying Bacteria on Okon Red Agar; NFB – YEMA; Nitrifying Bacteria on Yeast Extract Mannitol Agar

Table 2. Complete nucleotide blast of 16S rRNA gene of phosphate solubilising bacterial isolates from the sample

Isolate code	Water hyacinth part	Organism	Ascension number	% Identity
PL5	Leaf/Stem	*Pseudomonas mendocina*	LN881702.1	99
PL3	Leaf/Stem	*Bacllus safensis*	AB711138.1	99
PR3	Root	*Enterobacter cloacae*	LT2m21670.1	53
PL6	Leaf/ Stem	*Aeromonas veroni*	(LC487867.1)	97

Legend: PL5, PL3, PL6: Water hyacinth isolates from leaf/Stem on agar, PR3: Water hyacinth isolates from the root on Pikovskaya

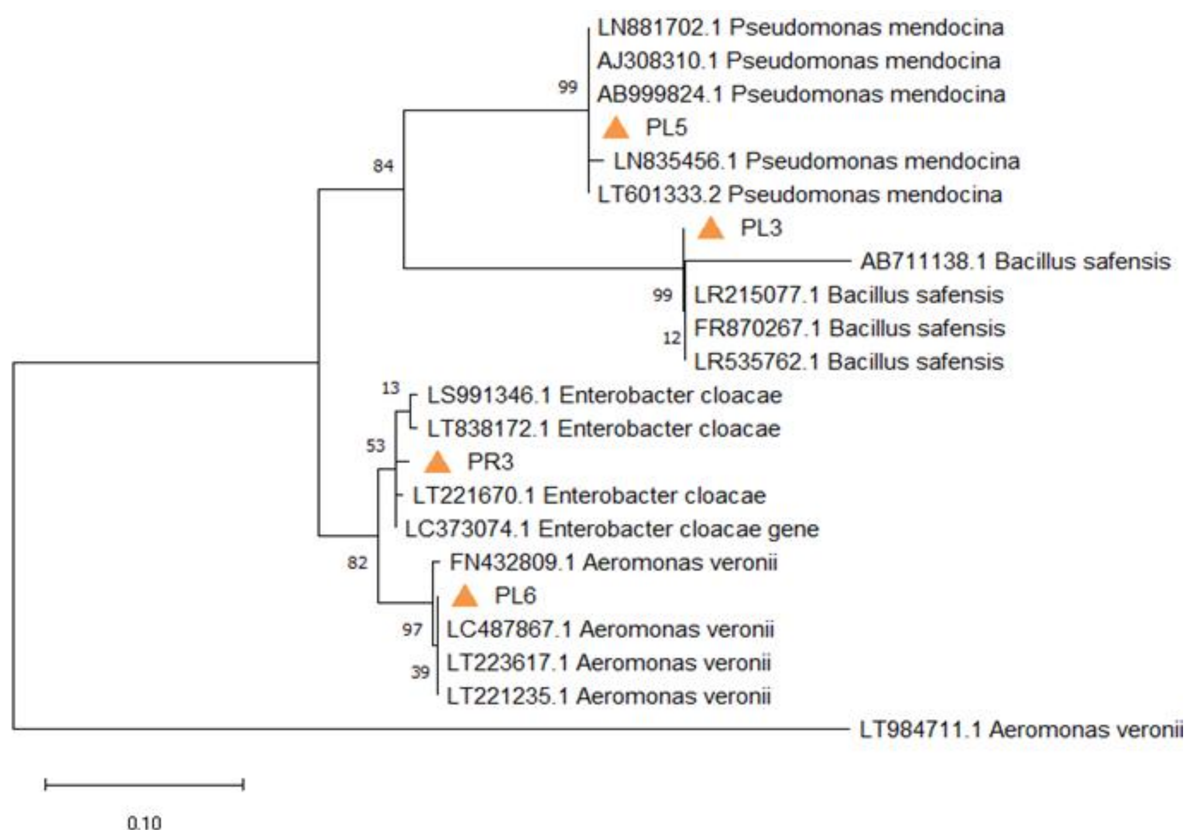

Fig. 1. Phylogenetic tree showing genetic relationship between 16s ribosomal RNA nucleotide sequences of bacteria isolated in this study. The phylogenetic tree is based on the alignment of partial 16s rRNA sequences using maximum likelihood method. The newly sequenced strains are indicated with ▲

Table 3. Identity and distribution of bacterial isolates from water hyacinth plant parts

Isolate	PL	PR
Bacteria		
Bacillus safensis (AB711138.1)	+	+
Ps. mendocina	+	-
Aeromonas veroni	+	-
Enterobacter cloacae	-	+
Azospirillum sp.	+	-
Azotobacter sp.	+	+
Rhizobium sp.	+	+

Legend: PL; Plant Leaf, PR; Plant Root, +; present, -; negative

Tables 3 and 4 show the identity and distribution of bacterial and fungal isolates on water hyacinth parts respectively. Results revealed that *Bacillus safensis*, *Azotobacter* sp. and *Rhizobium* sp. were found present in both the plant root (PR) and plant leaf (PL). Table 4 results indicated that most of the fungal isolates were common to both parts except *Cladosporium*, *Rhizoctonia* and *Pythium*. These fungal isolates belong to different genera spread across eight genera. Prominently *Aspergillus* sp., *Fusarium* sp., *Fusarium* sp, *Alternaria*, *Trichoderma* and *Rhizopus* were common genera to both plants. These fungal isolates belong to different genera spread across eight genera. Prominently *Aspergillus* sp., *Fusarium* sp., *Alternaria*, *Trichoderma* and *Rhizopus* were common genera.

Table 4. Identity and distribution of fungal isolates from water hyacinth plant parts

Isolate	PL	PR
Aspergillus sp.	+	+
Fusarium sp.	+	+
Alternaria sp.	-	+
Aspergillus flavus	+	+
Pythium sp.	+	+
Rhizopus sp.	+	+
Trichoderma sp.	+	-
Cladosprium sp	+	+

Legend: PL; Plant Leaf, PR; Plant Root, +; present, -; negative

3.1.2 Proximate and mineral content of water hyacinth (*Eichhornia crassipes*)

Tables 5 and 6 show the proximate, nutrient and heavy metal constituents of whole plant (WP), plant leaf (PL), plant stem (PS) and plant root (PR) of water hyacinth. These parameters vary among the constituent parts (leaf, stem and root). The proximate values for moisture (65.45%), protein (12.18%) and carbohydrate (5.30%) were highest in the leaf part compared to the stem and roots. Similarly, the table shows that the highest values of ash (18.48%), fat (3.84%) and crude fibre (12.50%) were obtained in the plant root relative to the leaf and stem. Apart from magnesium and phosphorous, mineral elements such as sodium, calcium and potassium were more abundant in the leaf. Among the heavy metal, Lead (Pb), Cadmium (Cd), Nickel (Ni), Chromium (Cr), Arsenic (As) and Cobalt (Co) had negligible or undetectable values for all the plant parts (leaf, stem and root).

4. DISCUSSION

The findings from this research indicated that water hyacinth had a substantial population of heterotrophic fungi and bacteria, nitrifying and phosphate - solubilizing bacteria. The population of phosphate solubilizing, *Azospirillum*, *Azotobacter* and *Rhizobium* and also vary among the weed parts. The population of the phosphate-solubilizing, *Azospirillum*, *Azotobacter* and *Rhizobium* in the root and leaf of water hyacinth represent a fraction of the total heterotrophic bacteria. The occurrence of different types of microbes suggests the diversity of microbial content of water hyacinth of which the phosphate solubilizing and nitrifying bacteria are fractions of the heterotrophic bacterial community. The bacterial and fungal genera identified from the water hyacinth were among those classified as microbial fertilizers (phosphate -solubilizing, nitrogen - fixing and mycorrhizal biofertilizers) by [32,33]. The presence of these microbes on *Eichhornia crassipes* is indicative of its potential as raw material for biofertilizers. This implies that water hyacinth harbours diverse groups of microorganisms that can enhance soil health for improved crop yield. This assertion corroborates the report of [34] that water hyacinth is a carrier of diverse groups of microbial biofertilizers. However, this study indicated the presence of *Fusarium* sp. and *Alternaria* sp which were among the fungal species considered as frequent microbiota of water hyacinth and implicated as phytopathogenic fungi of water hyacinth which can be developed into bioherbicides [35]. The implication of this study is that *Eichhornia crassipes* is not only a carrier of biofertilizer but also useful as a bioherbicide.

The proximate and chemical analyses also revealed that water hyacinth (*Eichhornia crassipes*) contain fairly large amounts of moisture content of 52.65, 58.44, 60.19 and 65.45(%) for the root, stem, whole plant and leaf respectively. These values were below the 95.5% for fresh water hyacinth plant reported by [36] and 89.20% [37]. The moisture content is an index of water activity and is used as a measure of stability and susceptibility to microbial attack. This implies that *Eichhornia crassipes* with high MC may decompose faster, having a short shelf-life due to its high moisture content and serving as organic manure. This high moisture content also suggests that the use of Water hyacinth in mulching would enhance the dissolution of soil nutrients and improve crop yield. The ash content (6.81- 18.49%) and moisture content (52.65- 65.45%) observed for the water hyacinth plants were in the range reported by [38] who reported ash content of 4.8%, 6.56%, 9.8% and 17.90% and moisture content of 65%, 56%, 57% and 45% respectively for watermelon, cow dung, food waste and sawdust respectively in their study of the characteristics of raw materials for the preparation of biofertilizer. The result of the proximate composition of water hyacinth (whole plant, leaf, root and stem) suggests that water hyacinth compares favourably with any organic resource for the production of biofertilizer. The results also suggest that the Water hyacinth sample is rich in minerals which vary among the parts. The concentration of Na, Ca, K, Mg and P obtained were higher than the values reported by [37]. The differences may have been influenced by differences in the physicochemical properties of the water body from which the weed was harvested and seasonal variation. This assertion is in agreement with the report of [39] which stated that water hyacinth can absorb nutrients from the water and this ability increases with an increase in temperature. Water hyacinth also showed varying amounts of heavy metals which may have been absorbed from the water harbouring the aquatic weed. Rango *et al*. [40] and [41] reported that water bodies receive and absorb trace elements caused by anthropogenic activities such as rapid urbanization and industrialization. Oluchukwu *et al*. [38] stated that the presence of high amounts of heavy metals in most agro-wastes is one of the most significant reasons that restricted their application in

Table 5. Proximate composition of the whole plant, leaf, stem and root of *Eichhornia crassipes* (water hyacinth)

Parameters	WP	PL	PS	PR
Moisture (%)	60.19±0.01	65.45±0.04	58.44±0.01	52.65±0.04
Ash (%)	10.31±0.05	6.81±0.01	12.26±0.01	18.49±0.01
Fat (%)	3.11±0.01	1.98±0.03	2.56±0.21	3.84±0.01
Crude Fibre (%)	12.73±0.02	8.28±0.01	11.32±0.00	12.50 ± 0.01
Protein (%)	9.65±0.00	12.18±0.01	10.18±0.02	7.45±0.03
Carbohydrate (%)	4.01±0.07	5.30±0.01	5.12±0.04	5.06±0.04

Legend: WP; whole plant, PL; Leaf, PS; Stem, PR; Root.
The data are mean value ± standard deviation of three replicates

Table 6. Minerals and heavy metal composition of the whole plant, leaf, stem and root of *Eichhornia crassipes* (water hyacinth)

Parameters	WP	PL	PS	PR
Na (mg/kg)	28.43 ± 0.17^{c}	33.40 ± 0.14^{d}	21.13 ± 0.12^{a}	22.47 ± 0.05^{b}
Ca (mg/kg)	43.90 ± 0.16^{d}	40.77±0.55c	38.37 ± 0.02^{b}	35.47 ± 0.34^{a}
K (mg/kg)	72.67 ± 0.17^{d}	65.60 ± 0.09^{c}	58.70 ± 0.08^{b}	54.73 ± 0.12^{a}
Mg (mg/kg)	12.52 ± 0.02^{a}	8.44 ± 0.00^{a}	8.19 ± 0.02^{a}	10.51 ± 0.01^{a}
Zn (mg/kg)	0.82 ± 0.01^{a}	3.08 ±0.02d	1.39 ± 0.01^{b}	2.23 ± 0.01^{d}
P (mg/kg)	56.17 ± 0.01^{c}	48.29 ± 0.02^{a}	54.27 ± 0.30^{b}	60.87 ± 0.01^{d}
Pb (mg/kg)	ND	ND	ND	ND
Cu (mg/kg)	0.23 ± 0.0^{d}	0.08 ± 0.00^{a}	0.15 ± 0.01^{b}	0.17±0.0c
Fe (mg/kg)	3.62 ± 0.01^{d}	2.86 ± 0.01^{b}	2.96 ± 0.01^{c}	2.79 ± 0.02^{a}
Cd (mg/kg)	ND	ND	ND	ND
Ni (mg/kg)	ND	ND	ND	ND
Si (mg/kg)	2.10 ± 0.03^{c}	1.85 ± 0.0^{b}	1.34 ± 0.02^{a}	3.33 ± 0.08^{d}
Cr (mg/kg)	0.02 ± 0.0^{b}	0.01 ± 0.00^{a}	0.01 ± 0.0^{a}	0.02 ± 0.00^{b}
As (mg/kg)	0.00±0.00	ND	ND	0.00±0.00
Co (mg/kg)	ND	ND	ND	0.00±0.00

Legend: WP; whole plant, PL; Leaf, PS; Stem, PR; Root, ND; Not Detected
The data are mean value ± standard deviation of three replicates

agricultural lands. The results of this study showed that the concentration of these metals is less than the maximum contaminant level established by the United States Environmental Protection Agency. This assertion implies that water hyacinth provides a better source of organic material and a safer raw material for biofertilizer production since the heavy metal contents are in relatively smaller amounts.

5. CONCLUSION

Agriculture is the second mainstay of the Nigeria economy where the people are highly dependent on the outputs of their farms for livelihood. It is therefore important to focus on the mass production of healthy food to meet the food demands of the growing populace, contributing in fighting poverty and ensuring economic growth. This study concludes that Water hyacinth is rich in mineral elements, low in heavy metal concentrations and a carrier of diverse groups of microbial fertilizer that can be harvested and applied to soil as organic fertilizer to circumvent the need for chemical fertilizer without jeopardizing environmental, plant, animal and human health. This implies that water bodies with water hyacinth problems can be managed by using the weed for biofertilizers because it can produce stable, mature and rich nutrients biofertilizers useful as a low-cost alternative source of organic fertilizer or soil improver in abundant supply.

6. RECOMMENDATION

The study recommends that government should encourage the production of organic fertilizer from water hyacinth and involve the community in their production since these will empower the community economically and help rid the river of the weed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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