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Heavy Metal Detection and Antibiotic Resistance in Bacteria Isolated from Water Hyacinth Compost

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Abstract: Identified bacteria isolates were subjected to antibiotics sensitivity test using the Kirby Bauer disc Bacterial isolates exhibit synergistic resistance to heavy metals and antibiotics through comparable mechanisms. Antibiotic resistance genes in the environment may be amplified by this synergy and then transmitted to clinical settings. Assessing antibiotic resistance and heavy metals in bacteria isolated from water hyacinth compost was the goal of this investigation. sample taken from new Calabar River, Nigeria. Using established techniques, the microbiological analysis, physicochemical analysis, antibiotic sensitivity test, and total heterotrophic bacteria count (THBC) were all performed. diffusion method, and the resulting multidrug resistant (MDR) isolates were tested for heavy metal tolerance using the agar dilution method with increasing doses of the heavy metals under study (50, 100, 150, 200, and 250 µg/ml). 2.31± 0.43 x10^8 was the THBC. Seven isolates were tested for antibiotic sensitivity. Among them, WHC 1 (Bacillus sp), WHC 2 (Vibrio sp), WHC 3 (E. coli), WHC 6 (Pseudomonas sp), WHC 7 (Bacillus sp), WHC 9 (Aeromonas sp), and WHC 10 (Staphylococcus sp) were 100% resistant to numerous treatments. WHC 3 and WHC had the lowest level of antibiotic resistance (r=3), but WHC 6 and WHC 9 had the highest level (r=6). With the exception of cadmium and lead, all test isolates were 100% susceptible to chromium, vanadium, and cobalt at 250 µg/ml. At the lowest dose (50 µg/ml), all MDR isolates were able to withstand all heavy metals. The control isolate was sensitive to all concentrations of heavy metals but only resistant to cobalt and chromium at 50 µg/ml. According to this finding, bacteria that were separated from water hyacinth compost are resistant to both heavy metals and antibiotics.

Keywords: *Antibiotics, Resistance, Sensitivity, Microbiological, Techniques, Bacteria Count.*

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I. INTRODUCTION

In many parts of the world, especially in tropical and subtropical areas, the floating aquatic plant known as water hyacinth (*Eichhornia crassipes*) is regarded as an invasive species (López et al., 2017). Its quick spread in bodies of water frequently has detrimental ecological effects, including obstructing water flow, upsetting aquatic ecosystems, and interfering with irrigation and hydropower production. Notwithstanding these difficulties, water hyacinth has drawn interest due to its potential use in a number of ways, such as a filter for wastewater treatment, a source of biomass for composting, and the generation of biofuel (Sreenivasan et al., 2018). Because of its high nutrient content, water hyacinth compost—which is made from decomposed water hyacinth material—is seen as a useful resource for organic farming. However, there have been worries raised regarding possible contamination from environmental pollutants, including heavy metals and antibiotics.

Persistent environmental contaminants known as heavy metals present serious hazards to human and environmental health. Due to industrial discharges, agricultural runoff, and other human activities, common heavy metals like lead (Pb), mercury (Hg), cadmium (Cd), arsenic (As), and chromium (Cr) can build up in the environment, especially in water bodies (Zhou et al., 2020). Water hyacinth is a useful tool for phytoremediation because it has been demonstrated to

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bioaccumulate heavy metals from contaminated water (Sreenivasan et al., 2018).

Nevertheless, the buildup of these metals in the plant's tissues may contaminate compost made from water hyacinth, which would raise questions regarding its safety for use in agricultural contexts (Mahapatra et al., 2018). According to studies, water hyacinth has the ability to collect and concentrate a variety of heavy metals from contaminated environments, such as lead, copper, nickel, and zinc (Prasad et al., 2018). These metals have the ability to linger and build up in plants and soil during composting, which could lead to their entry into the food chain (Rai et al., 2020). This study aims to identify antibiotic and heavy metal resistance in bacteria isolated from composted water hyacinth.

II. MATERIALS AND METHODS

➢ *Preparing the Sample:*

A weighing balance was used to weigh approximately 1g of water hyacinth compost, gotten from New Calabar River in Port Harcourt, Nigeria, which was then dissolved in 10ml of regular saline. After serially diluting the sample, 0.1 ml of the dilution was plated on nutrient agar (NA) using the spread plate method. To aid in colony development, the plates were meticulously labeled and incubated for 24 hours at 370C.

➢ *Sterilization of Glassware:*

Clean water was used to rinse all glassware after it had been thoroughly cleaned with detergent. To guarantee total sterility, pipettes, test tubes, conical flasks, and Petri dishes were then autoclaved for 15 minutes at 121°C and 15 pressure.

➢ *Examination of Microbes*

The total number of heterotrophic bacteria To create a pure culture of isolates, colonies were counted and sub cultured on Nutrient Agar (NA) following a 24-hour incubation period. Colony Forming Unit Calculation (CFU/g) The formula will be used to calculate the CFU/g. Number of Colonies x Dilution Factor x Culture Plate Volume = CFU/g.

➢ *Biochemical Characterization*

- *Morphological Identifications of Bacteria:*

The organisms were cultivated on different media plates in order to undertake morphological characterization, which enabled the isolation of unique colonies. Specific traits like size, shape, pigmentation, elevation, and texture were used to choose pure colonies. Each isolate's morphology was then immediately inspected under a microscope to ascertain its microscopic properties.

➢ *Antibiotic Susceptibility Testing:*

Freshly made Mueller Hinton Agar was subjected to the disc diffusion procedure. Ciprofloxacin (30µg), Ceftriaxone (10µg), Cefotaxime, Erythromycin (30µg), Imipenem (10µg), Gentamicin (10µg), Aztreonam (300µg), Nitrofurantoin (300µg), Amoxicillin (30µg), Cefixime (10µg), and Levofloxacin (30µg) were the test antibiotics utilized in the study. The NCCLS guideline was used to evaluate the zone of inhibition diameters as sensitive and resistant.

➢ *Heavy Metal Resistant Test:*

For the heavy metal resistance test, isolates exhibiting multiple drug resistance (i.e., resistance to at least two antibiotics) were chosen. Mueller-Hinton Agar plates with varying concentrations (50, 100, 150, 200, and 250 µg/ml) of the test heavy metal (chromium, vanadium, cobalt, cadmium, and lead) were infected with a loopful of bacteria cultured in tryptic soy broth for 12 to 16 hours. For 24 hours, incubation was conducted at 37°C. Following incubation, plates exhibiting growth were considered resistant, and those that did not were considered sensitive.

III. RESULT AND DISCUSSION

➢ *Total Heterotrophic Bacteria Counts Obtained from Water Hyacinth Compost*

The total heterotrophic bacteria obtained from water hyacinth compost studied

Table 1 Total Heterotrophic Bacteria Counts Obtained from Water Hyacinth Compost

Bacterial count	
THBC (CFU/g)	$2.31 \pm 0.43 \times 10^8$

Values are mean ± standard deviation (M±S.D) of duplicate Determinations (n=2) Concentration (Mean ± S.D)

➢ *Morphological Characteristics of Bacteria Isolates from Water Hyacinth Compost*

The morphological characterization of the organisms isolated from water hyacinth compost is as detailed in Table 2 and Table 3. The predominant species isolated were *Bacillus* sp, *Vibrio* sp, *Escherichia coli*, *Pseudomonas* sp, *Aeromonas* sp, and *Staphylococcus* sp.

Table 2 Morphological Characteristics of Bacteria Isolates from Water Hyacinth Compost

Sample (WHC)	Margin	Color	Elevation	Texture	Shape	Size	Opaque
WHC 1	Entire	Milky	Convex	Moist	Round	2mm	Opq
WHC 2	Wavy	Milky	Flat	Brittle	Round	2mm	Opq
WHC 3	Filamentous	Milky	Flat	Brittle	Filamentous	5mm	Opq
WHC 4	Entire	Milky	Raised	Moist	Round	2mm	Opq
WHC 5	Entire	Golden brown	Flat	Moist	Round	0.5mm	Opq
WHC 6	Filamentous	Milky	Flat	Moist	Rhizoid	4mm	Opq

WHC 7	Filamentous	Milky	Flat	Moist	Filamentous	5mm	Opq
WHC 8	Entire	Golden brown	Raised	Moist	Filamentous	2mm	Opq
WHC 9	Entire	Dirty white	Flat	Mucoid	Round	2mm	Opq
WHC 10	Entire	Dark brown	Flat	Moist	Round	2mm	Opq

Table 3 Biochemical Characteristics of Bacteria Isolates from Water Hyacinth Compost Compost

Sample	Lactose	Glucose	Sucrose	Catalase	Oxidase	Indole	MP	VP	Starch	citrate	Morphology	Gram rxn	Possible genera
WHC 1	-	A	A	+	+	-	-	+	+	+	Rod	+ve	*Baccilus* sp
WHC 2	-	A	-	+	+	+	-	-	-	+	Rod	-ve	*Vibrio* sp
WHC 3	A	AG	-	+	-	+	+	-	-	-	Rod	-ve	*Escherichia* sp
WHC 6	-	-	-	+	+	-	-	-	-	-	Rod	-ve	*Pseudomonas* sp
WHC 7	A	A	A	+	-	-	+	+	+	+	Rod	+ve	*Aeromonas* sp
WHC 9	A	A	A	+	+	+	-	+	-	+	Rod	-ve	*Baccillus* sp
WHC 10	A/G	A/G	A/G	+	-	-	-	+	-	+	cocci	+ve	*Staphylococcus* sp

➢ *Antibiogram of Gram's Positive and Negative Isolates Obtained from Water Hyacinth Compost*

The gram's positive and negative isolates showed varies sensitivity patterns to the various antibiotics tested as detailed on the antibiogram in table 4 and table 5. The gram positive isolates showed resistance to (CTX, ZEM, IMP, CXM, AUG, CRO, ERY) and gram negative isolates showed resistance to (CTX, AUG, ZEM, IMP, CXM, ACX). All isolates were sensitive to (LBC, OFX, GN).

Table 4 Antibiogram of Gram Positive Isolates Obtained from Water Hyacinth Compost (Zone of Inhibition in mm)

Test samples	CTX	ERY	CRO	AUG	ZEM	LBC	CIP	AZN	IMP	CXM	OFX	GN
WHC 1	0mm	19mm	17mm	11mm	0mm	23mm	23mm	21mm	0mm	0mm	21mm	14mm
WHC 2	0mm	14mm	0mm	0mm	0mm	18mm	25mm	19mm	13mm	0mm	21mm	20mm
WHC 3	9mm	15mm	0mm	0mm	0mm	27mm	25mm	15mm	12mm	0mm	24mm	21mm
WHC 7	0mm	20mm	0mm	0mm	0mm	17mm	31mm	18mm	17mm	0mm	23mm	18mm
WHC 9	0mm	0	0mm	0mm	0mm	22mm	22mm	18mm	14mm	0mm	20mm	11mm
WHC 10	10mm	C	13mm	0mm	0mm	21mm	21mm	C	0mm	14mm	C	18mm

Key: Ceftriaxone (CRO) 30 µg; Gentamicin (GN), Imipenim (IMP), ciprofloxacin (CIP), Cefixime (ZEM), Amoxicillin (AUG), Erythromycin(ERY), Cefotaxime(CXT), levofloxacin (LBC), cefuroxime (CXM), oflaxacin (OFX)
C: All zone cleared , 0mm: Resistance

Table 5 Antibiogram of Gram Negative Isolates Obtained from Water Hyacinth Compost

Test sample(s)	CTX	ACX	CRO	AUG	ZEM	LBC	NF	OFX	IMP	CXM	GN	NA
WHC 6	0mm	0mm	16mm	0mm	0mm	25mm	14mm	20mm	0mm	0mm	25mm	17mm

Key: Nitrofurantoin (NF); Cefuroxime(CXM); Ceftriaxone(CRO); Ampiclox(ACX); Cefixime(ZEM); Levofloxacin(LBC); Cefotaxime(CTX) ; Imipenem (IMP)10 µg; Ofloxacin(OFX); Gentamicin(ZEM) ; Nalidixic acid (NA); Augmentin (AUG)

➢ *Physicochemical and Heavy Metals Analysis of Water Hyacinth Compost*

The results of the physicochemical and heavy metal analyses are presented in Table 6.

Table 6 Physicochemical and Heavy Metals Analysis of Water Hyacinth Compost

Parameters	
Nitrogen, mg/kg	14.83 ±0.22
Phosphorus, mg/kg	1.92 ± 0.00
Nitrate, mg/kg	8.38 ± 0.05
Phosphate, mg/kg	0.57 ± 0.02
Potassium, mg/kg	6.51 ± 0.10
Sodium, mg/kg	17.70 ± 1.20
Magnesium, mg/kg	3.48 ± 0.16
Total organic carbon%	40.00 ± 0.53
TPH mg/kg	69,657± 0.20
pH	7.2± 0.53
Total organic Nitrogen%	2.43± 0.11
Heavy Metals	
Manganese mg/kg	0.13 ± 0.00

Iron , mg/kg	22.73 ± 0.16
Nickel, mg/kg	0.01 ± 0.00
Copper , mg/kg	0.15 ± 0.03
Vanadium, mg/kg	0.21 ± 0.00
Selenium, mg/kg	0.03 ± 0.00
Lead , mg/kg	1.0 ± 0.00
Zinc, mg/kg	0.50 ± 0.00

- *Heavy Metal Resistances among Bacteria Isolated from Water Hyacinth Compost*

The isolates were screened for heavy metals resistance.

Table 7 Heavy Metal Resistance among Bacteria Isolated from Water Hyacinth Compost

Sample (s)	Cr 50, 100, 150,200,250	V 50, 100, 150,200,250	Cd 50, 100, 150,200,250	Co 50, 100, 150,200,250	Pb 50, 100, 150,200,250
***Bacillus* sp(WHC 1)**	R R R R S	R S S S S	R R R S S	R R S S S	R R R R S
***Vibrio* sp (WHC 2)**	R R R S S	R R R S S	R R R R S	R R R S S	R R S S S
***E.coli* (WHC3)**	R R S S S	R R S S S	R R R S S	R R S S S	R R R S S
***Pseudomonas* sp (WHC 6)**	R R R S S	R R R S S	R R R R S	R R R R S	R R R R S
***Bacillus* sp (WHC 7)**	R R R S S	R R S S S	R R R R S	R R R S S	R R R R S
***Aeromonas* sp (WHC 9)**	R R S S S	R R R S S	R R R S S	R S S S S	R R S S S
***Staphylococcus* sp (WHC 10)**	R R R S S	R R R R S	R R R S S	R R S S S	R R R S S

Key: R= Resistance, S= Sensitive, V=Vanadium, Cr= Cadmium, Co=Cobalt and Pb=Lead

IV. DISCUSION

The current study assessed antibiotic and heavy metal resistance in bacteria that were isolated from compost made from water hyacinth. A total of 2.31± 0.43 x108 heterotrophic bacteria were found. Numerous species were represented in the bacterial isolates derived from the investigation, but the most common ones were Bacillus, Staphylococcus sp., Pseudomonas sp., Vibrio sp., Aeromons sp., and Escherichia coli. Since Escherichia coli are markers of fecal contamination, their presence indicated that agricultural runoff—animal waste—was the source of the contamination. Gram-negative isolates were susceptible to ceftriaxone, levoflaxacine, oflaxacin, and gentamicin, according to the results of the antibiotic sensitivity test. However, they shown variable resistance to cefotaxime, amoxicillin, cefixime, imipenim, cefuroxime, and augmentin. The findings of Okoh et al. (2021), who documented widespread resistance among Gram-negative bacteria, especially in communal contexts where antibiotics may have been overused, are consistent with this pattern of resistance. Gram-positive isolates' susceptibility to LBC, OFX, and GN indicates that these antibiotics are still effective against these pathogens. However, as Akinmoladun et al. (2020) point out, the resistance seen against other antibiotics raises the possibility that regular exposure to these medications in the environment may have aided in the emergence of resistant bacteria. The isolates that showed the greatest resistance to CTX, AUG, ZEM, IMP, CXM, ACX, and CTX, ERY, CRO, AUG, CXM, and ZEM were WHC 6 and WHC 9 (identified as Pseudomonas sp and Aeromonas sp). To ascertain the isolates' resistance, they were tested against the following heavy metals: V stands for vanadium, Cr for cadmium, Co for cobalt, and Pb for lead. Growth-confirmed zones were susceptible to heavy loads, while growth-free zones were confirmed to be resistant. At varying concentrations, all isolates exhibited tolerance to heavy metals. E. Coli and Vibrio sp. demonstrated the lowest resistance to heavy metals at varying concentrations, whereas Pseudomonas sp. and Bacillus sp. demonstrated the highest resilience. At varying concentrations, Staphylococcus and Aeromonas bacteria demonstrated a modest level of tolerance to heavy metals.

V. CONCLUSION

The bacteria found in water hyacinth compost are resistant to both heavy metals and antibiotics, which poses a complex problem for the environment and public health. In water hyacinth habitats, the buildup of contaminants produces selective pressures that promote bacterial resistance and adaptation. These results underline the necessity of more all-encompassing environmental management techniques, such as monitoring, pollution prevention, and sustainable composting methods, in order to reduce the dangers of antimicrobial resistance spreading and the possible negative effects on the environment and human health.

In order to safeguard the environment and public health from the growing threat of resistant bacteria, scientists, environmentalists, public health experts, and legislators must work together to address this issue. The particular geographic location and source of contamination may be the cause of variations in the bacterial counts seen in this study's sample when compared to others. Significant environmental and health risks are shown by the investigation of heavy metals and antibiotic resistance in bacteria isolated from water hyacinth compost. A number of suggestions can be made to reduce the hazards connected to these problems, with an emphasis on management techniques, policy implementation, and research.

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