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## Research Article

### Investigation of Bacteria Biostimulation Strategy for Heavy Metal Bioremediation in Wupa Wastewater Treatment Plant, Abuja

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#### ABSTRACT

Heavy metal pollution in water systems disrupts ecosystems and poses serious public health risks. Bioremediation remains the most cost-effective and eco-friendly, relying on naturally occurring microorganisms to degrade or transform toxic contaminants. This study was aimed at stimulating microbial isolates from Wupa Wastewater Treatment Plant (WWTP) with nutrients to remove heavy metals from effluents obtained. Wastewater samples collected from Wupa WWTP were analyzed for physicochemical parameters, including pH, electrical conductivity, turbidity, temperature, BOD, COD, TSS, TDS, and concentrations of zinc (Zn), manganese (Mn), and iron (Fe), using standard methods. Bacteria and fungi were isolated microbiologically, and a nine-day container experiment was conducted with four nutrient setups containing different concentrations of peptone (150 ml, 75 ml) and glucose (9 g, 4.5 g) to enhance microbial degradation capability. Biomass growth was monitored every three days using a UV spectrophotometer. The results revealed that physicochemical parameters of the treated and untreated wastewater exhibit significant differences ( $p < 0.05$ ), except for pH and temperature. All physicochemical parameters showed significant differences ( $p < 0.05$ ) between treated and untreated wastewater, except pH and temperature. Heavy metal concentrations also differed significantly ( $p < 0.05$ ), with Mn decreasing from  $0.37 \pm 0.20$  mg/L (untreated) to  $0.31 \pm 0.10$  mg/L (treated), Fe from  $1.58 \pm 0.2$  mg/L to  $0.91 \pm 0.3$  mg/L, and Zn from  $0.501 \pm 0.10$  mg/L to  $0.501 \pm 0.10$  mg/L. Eight bacterial species were isolated. The biostimulated microbial consortium effectively remediated Mn, Fe, and Zn by 66.8%, 60.5%, and 70.2%, respectively. This study demonstrates promising potential for heavy metal removal from wastewater treatment plants and industrial effluents.

**Keywords:** Aquatic pollution; Biostimulation; Bioremediation; Heavy metals

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## INTRODUCTION

Freshwater ecosystems across Africa are increasingly endangered by human-driven activities such as land-use modification, pollution, hydrological disturbances, and climate variability (Edegbene *et al.*, 2025). Among these threats, heavy metal contamination has emerged as a significant global environmental concern since the onset of the industrial era (Adamu *et al.*, 2022). Industrial processes have substantially contributed to the elevated release of heavy metals into the environment (Bahiru *et al.*, 2019). These metals are recognized as highly toxic pollutants commonly detected in contaminated water bodies and have had profound adverse effects on human health worldwide (Ibrahim *et al.*, 2023).

Although heavy metals occur naturally and play essential roles in the production of materials that enhance human living standards, their high density and resistance to degradation contribute to their persistence and toxicity. Lead, cadmium, mercury, and arsenic are notable examples used in various industrial tools and equipment. However, their introduction into aquatic systems—where they are neither needed nor beneficial—renders the water harmful to aquatic organisms and other life forms dependent on it (Ibrahim *et al.*, 2024a). Even at low concentrations, heavy metals in polluted water can disrupt ecosystem balance and pose serious health risks to both humans and animals (Maishanu *et al.*, 2022).

Wastewater, a product of anthropogenic activities, is composed of a mixture of heavy metals, coliforms, and other toxic substances that when left untreated pose a lot of health risks to all life forms on the planet (Chowdhary *et al.*, 2018). This liquid waste is an accumulation of all fluids generated as byproducts from hospitals, industries, homes, and farms, and as such the degree of contamination is at a large scale (Ibrahim *et al.*, 2023). The treatment plan for wastewater depends on the source of contamination, for instance, wastewater from mines, dyes, hospital waste, and electronic factories because different pollutants have different removal methods (Ibrahim *et al.*, 2025). Many methods have been adopted to reduce the number of heavy metals present in the

environment such as nitrification, and thermal desorption. Out of all these techniques, the most cost-effective and eco-friendly approach is bioremediation, it has been gaining a lot of attention due to its use of microorganisms (Singh *et al.*, 2020a; Ibrahim *et al.*, 2025).

Bioremediation is a common biological technique that has previous works attached to its name in remediating polluted environments (Ibrahim *et al.*, 2024b). A common approach under bioremediation is biostimulation which calls for enhancing the degradation potential of indigenous microorganisms' present in polluted sites (Turek *et al.*, 2019; Brown *et al.*, 2025). Biostimulation is a common strategy employed for the in-situ bioremediation of contaminated water bodies and involves the supply of growth-limiting nutrients such as nitrogen and phosphorus to facilitate the interaction of native microorganisms in the degradation of polluted environments (Andreolli, *et al.*, 2015; Ibrahim *et al.*, 2024c). When microorganisms are repeatedly exposed to toxic elements, they develop tolerance and resistance to the stress of the variations in their environment (Kumar *et al.*, 2017). Compared to wastewater treatment facilities, this is a better option for water remediation as it is not limited by the fact that it focuses mainly on organic matter and is not influenced by power outages.

## MATERIALS AND METHODS

### Study Area

Abuja is the largest and fastest growing city in Nigeria. Located at the center of the country, it covers about 275sqm, it lies on longitude coordinates of 7.491302 and latitude coordinates of 9.072264 (Figure 1). It has six Area Councils namely Abaji, Bwari, Gwagwalada, Kuje, Kwali and Abuja Municipal (AMAC) (Chukwu & Oranu, 2018). The Federal Capital Territory is in Abuja where the government runs the affairs of the country. The Federal Capital Territory is planned and built with current residential buildings. The research will be carried out in Abuja. The Wupa treatment plant is in Abuja and the wastewater was collected from the treatment plant and sent immediately to the biotechnology/microbiology laboratory of the Nile University of Nigeria which is located in Abuja for further analysis.

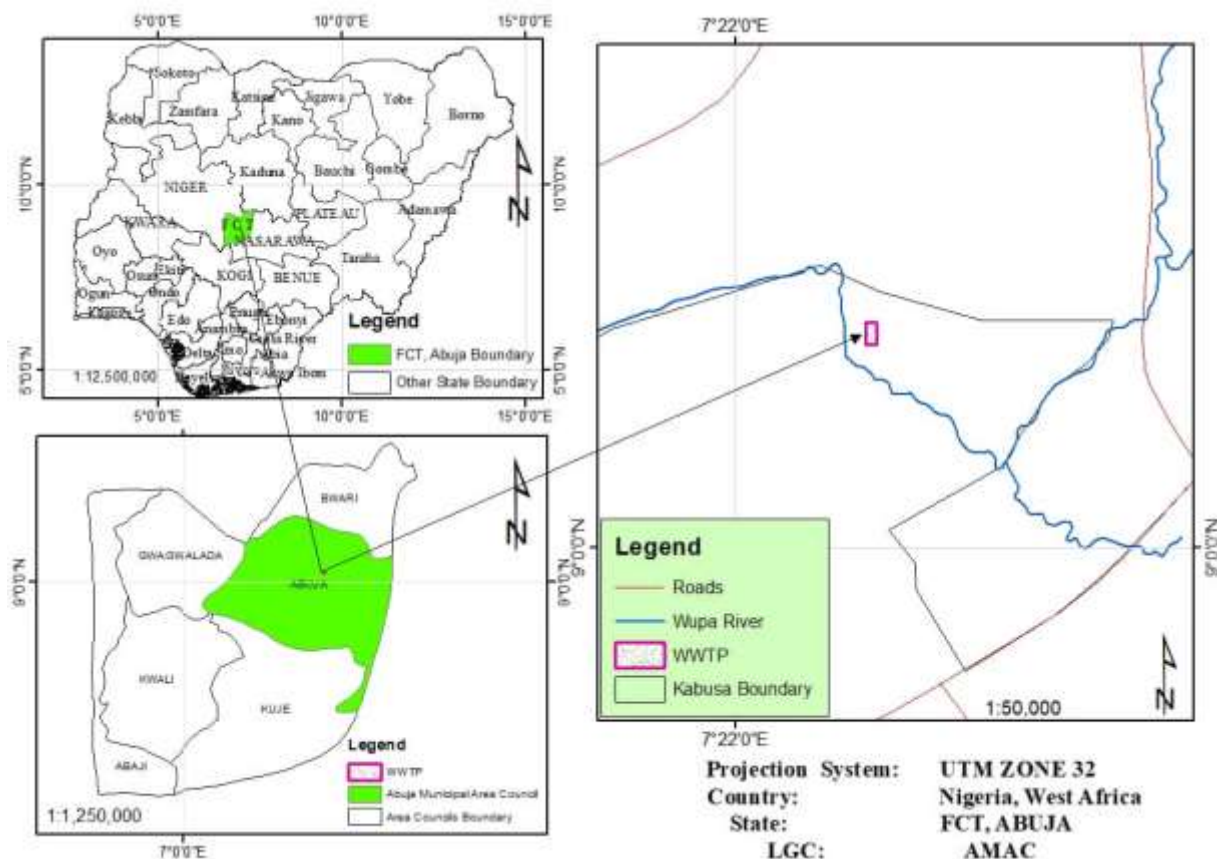


Figure. 1 Map of the Federal Capital Territory showing the location of the Wupa Wastewater Treatment plant.

### Collection of Samples

The method adopted by Ezra *et al.* (2021) was employed for Collection of samples. Effluent (treated wastewater) was collected from the WUPA treatment place at the discharge sampling point while the Influent (untreated wastewater) was collected from the influent tank. The wastewater was collected aseptically using a sterile 1500ml sample bottle and corked immediately to avoid further contamination. Samples were collected in different bottles for physiochemical analysis, determination of heavy metal content and for isolation of microorganisms.

### Determination of Physiochemical Parameters of Wastewater

The physiochemical parameters of the wastewater collected from WUPA treatment plant were analyzed using their respective analyzer and according to the manufacturer instructions. Apart from temperature, this was measured using digital thermometer and heavy metals using AAS. The other parameters include pH, Turbidity, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Dissolved Oxygen (DO),

Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), manganese (mg), iron (Fe) and zinc (Zn) level were all determined using the standard methods and procedures of American Public health association (APHA, 2017).

### Wastewater Sample Preparation for Microbial Analysis

The method employed by Ezra *et al.*, (2021) was used to process wastewater for serial dilution. About 1 ml of wastewater was dispensed aseptically into a sterile test tube to which 9 ml of sterile distilled water had been previously added. The mixture was shaken to homogenize and a dilution factor of  $10^{-1}$  was obtained. Then 1 ml of this dilution ( $10^{-1}$ ) was pipetted and dispensed aseptically into another sterile test tube containing 9 ml of sterile distilled water this makes a mixture of one in hundred dilutions i.e.,  $10^{-2}$ . The process was repeated until a dilution of  $10^{-5}$  was obtained.

### Isolation of Bacteria from the Wastewater Samples

Exactly 10 ml of the molten and sterile Nutrient agar for bacteria isolation were dispensed into clean and

sterile petri dish. The petri dish was rotated carefully to allow even distribution of the inoculum within the medium. This was then allowed to set and solidify. This was followed by the addition of 0.1 ml each of the two dilutions ( $10^{-3}$  and  $10^{-4}$ ) into the plates. The petri dish (plates) was incubated at 35 – 37 °C anaerobically for 24 hours. Distinct colonies were picked and subcultured severally into Nutrient agar. Pure cultures for bacteria were further inoculated into sterilized Bijou bottles containing media to avoid contamination (Ochei *et al.*, 2000).

#### **Molecular Characterization and Phylogenetic Analysis**

The genomic DNA of axenic cultures of bacterial isolates was extracted using the ZR Fungal/Bacterial DNA Kit™ (Zymo Research, Irvine, CA) following the manufacturer's instructions. The obtained DNA was then amplified using the 16S rRNA universal gene primer set (27F and 1492R) as described by Ijoma, Selvarajan, and Oyourou (2019) under the following cycling conditions: an initial denaturation at 98 °C for 3 min, followed by 32 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. The final PCR products were then purified and sequenced in the forward and reverse directions on the ABI PRISM™ 3500xl Genetic Analyzer. The obtained sequences were subjected to BLAST analysis for the identification of bacterial taxa and submitted to NCBI GenBank for the generation of accession numbers. The accession numbers of the submitted sequences are KX641888 and KX641889. Phylogenetic analysis was performed using the Molecular Evolutionary Genetics Analysis version 7 (MEGA7) software (Kumar, Stecher, & Tamura, 2016), using an alignment created with SINA Aligner.

#### **Bioremediation of Wastewater using Stimulants**

Biostimulation of isolates in combating both organic and inorganic contaminants was conducted in a container experiment. Thirteen containers were utilized for this experiment, and they were first carefully cleaned with 10% diluted nitric acid and twice deionized distilled water. Nine containers were used as treatment containers, while three containers were used as control containers holding wastewater. The wastewater from Wupa WWTP (1500mL of wastewater) was contained in containers with the designation C. Peptone (75mL), Peptone (150 mL),

Glucose (4.5g) and glucose (9g) (15 mL) in Wupa WWTP influent (1500 mL each) were found in the treatment containers A1, A2, B1, and B2. To create a total of 12 containers, each setup was carried out three times (Khosro *et al.*, 2011).

The growth and the remediation potential were monitored at three days interval for nine days. The remediation potential of the selected organisms was evaluated by monitoring the growth of biomass using ultraviolet (UV) spectrophotometer at 600 nm (PG instruments T80+). The residual heavy metal in the container was determined using AAS (SHIMADZU AA-7000 model) (Khosro *et al.*, 2011).

Bioremoval efficiency (%) was calculated using the equation below. Where R represents the removal percentage,  $C_i$  is the initial concentration of the metal in the water samples, and  $C_f$  is the final concentration of the metal in the water samples (Ali *et al.*, 2023).

$$R = \frac{C_i - C_f}{C_i} \times 100$$

## **RESULTS**

### **Physicochemical characteristics of wastewater**

Table 1 shows the mean values of some physicochemical parameters of effluent and influent water samples from Wupa wastewater treatment plant Abuja. All measured physicochemical parameters of the influent and effluent samples with exception of water pH and temperature show significant differences ( $p < 0.05$ ) between the effluent and the influent. The mean value of the heavy metal studied of Wupa wastewater treatment plant presented in table 1. showed mean value manganese ranged from  $0.31 \pm 0.10$  mg/L in the effluent to  $0.37 \pm 0.20$  mg/L in the influent. Manganese mean value shows significant difference ( $p < 0.05$ ) between the influent and effluent as the effluent record low values after treatment. Iron mean value ranged from  $0.91 \pm 0.3$  mg/L in the effluent to  $1.58 \pm 0.2$  mg/L in the influent. Iron mean value shows significant difference ( $p < 0.05$ ) between the influent and effluent as the effluent record low values after treatment. Zinc mean value ranged from  $0.501 \pm 0.10$  mg/L in the influent to  $0.501 \pm 0.10$  mg/L in the effluent. Zinc mean value shows significant difference ( $p < 0.05$ ) between the influent and effluent as the effluent record low values after treatment. All the measured heavy metal in both influent and effluent exceeds the WHO standard.

**Table 1: Some physicochemical parameters of wastewater from Wupa WWTP**

Parameters	Influent (Mean±S.D)	Effluent (Mean±S.D)	WHO Standard
pH	7.14±0.02	7.25±0.03	6.5 – 8.5
Temperature	27.3±0.01	29.4±0.02	<40
Conductivity (µs/cm)	53.4±0.02*	35.5±0.01	1250
Turbidity (NTU)	181.0±0.01*	24.0±0.03	<40
TDS (mg/L)	320.0±0.01*	213.1±0.01	300
BOD (mg/L)	41.0±0.01*	3.0±0.03	10
COD (mg/L)	77.9±0.01*	5.70±0.01	<5.0
TSS (mg/L)	149±0.03*	22.0±0.02	30
Manganese (mg/L)	0.37±0.10	0.31±0.020	0.05
Iron (mg/L)	1.58±0.20	0.91±0.30	0.3
Zinc (mg/L)	0.50±0.10	0.5±0.10	0.1

**S.D = Standard Deviation**

The result showed higher bacterial load in influent (raw wastewater) samples with higher bacterial concentration that are too numerous to count (TNTC). On the other hand, the effluent (treated wastewater) sample has an average bacterial load of  $7.14 \times 10^6$  showing that the wastewater treatment was effective in reducing bacterial load as presented in Table 2. The result presented in Table 3 showed the molecular identification of bacterial isolates obtained

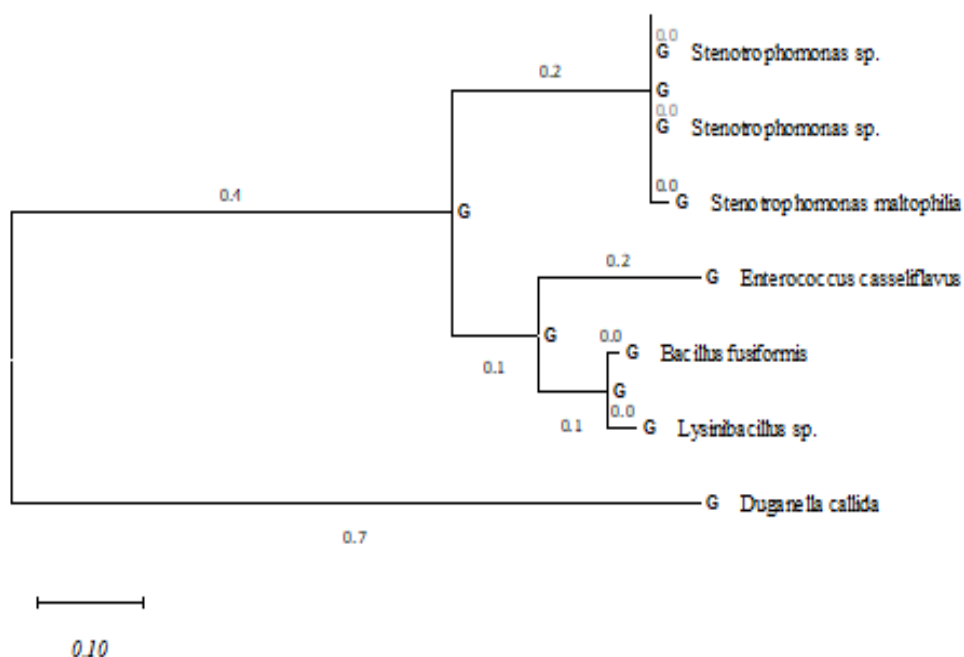
from wastewater samples collected at the WWTP. The study identified five bacterial species, including *Stenotrophomonas*, *Bacillus fusiformis*, *Lysinibacillus sp.*, *Enterococcus casseliflavus*, and *Duganella callida* among which *Stenotrophomonas* was most frequently detected in wastewater samples (Table 2). The phylogenetic tree showed that isolates belong to their respective closest similarities as shown in Figure 2.

**Table 2: Total bacterial count**

Samples	Dilution factor	Number of colonies	Cfu/ml	Mean Bacterial Count (Cfu/ml)
Effluent	$10^3$	148	$1.48 \times 10^5$	$7.14 \times 10^6$
	$10^4$	123	$1.28 \times 10^6$	
Influent	$10^3$	TNTC	TNTC	TNTC
	$10^4$	132	$1.32 \times 10^6$	

**Table 3: Molecular identification of Bacterial isolates of wastewater from Wupa WWTP**

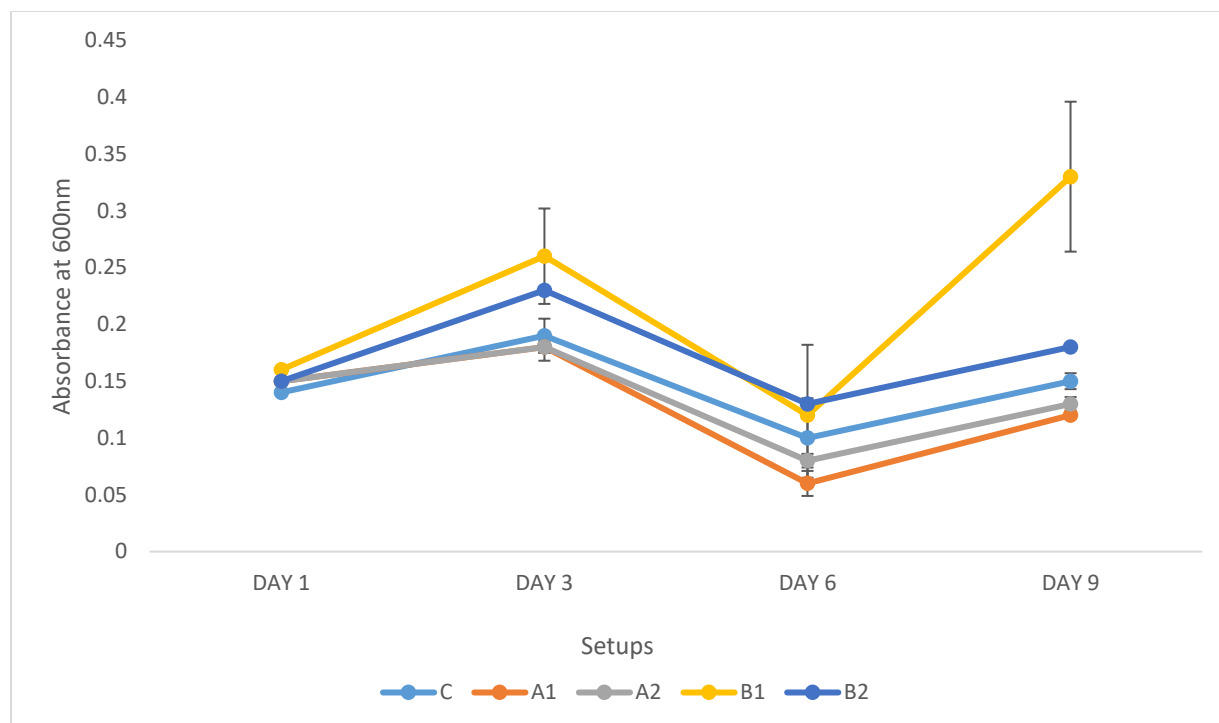
Isolate code	Accession number	Percentage	Organism
S1	MN493876.1	98.17%	<i>Stenotrophomonas sp.</i>
S2	MG674373.1	98.43%	<i>Stenotrophomonas sp.</i>
S3	AY548954.1	91.84	<i>Bacillus fusiformis</i>
S4	CP040436.1	88.76	<i>Stenotrophomonas maltophilia</i>
S5	MF465572.1	96.12%	<i>Lysinibacillus sp.</i>
S6	MH899231.1	90.54%	<i>Enterococcus casseliflavus</i>
S7	MN208463.1	98.82%	<i>Stenotrophomonas sp</i>
S8	NZ_SPVG01000119.1	87.67 %	<i>Duganella callida</i>



**Figure 2: Phylogenetic tree of bacteria isolates identified from wastewater samples collected from Wupa treatment plant Abuja**

The mean biomass growth of the bacterial isolate in different experimental setups was measured in three days interval for nine days experimental setup, as presented in Figure 3. At day three (3), higher growth was observed with Treatment for Glucose 9g (B1) with value of 0.26, followed by Treatment for Glucose 4.5g (B2) which recorded 0.23 while least growth observed in Treatment for Peptone 150mg and Peptone 75mg with value of 0.18. Control (C) had 0.19. At day six (6), the absorbance showed decrease in growth for all the growth nutrients with B2 having the highest growth with absorbance of 0.26 followed by B1, C, A2 and A1 with absorbance of 0.12, 0.10, 0.08 and 0.06 respectively. At day nine (9), the absorbance revealed increase in bacterial growth with B1 having highest absorbance value of 0.33 followed by B2, C, A2 and A1 with the absorbance of 0.18, 0.15, 0.13 and 0.12 respectively. The percentage reduction of physicochemical parameters of the water samples used for the

experiment was presented in Table 4. The results showed that the influences of microorganisms on the pH were insignificant, with the changes ranging from 0.27-1.25% with all the pH reduction above WHO accepted limit (6.5 – 8.5). At the initial stage, the observed Conductivity value for Wupa WWTP was 53.4 $\mu$ S/cm. The Conductivity values were significantly reduced (0.6–22.1%) in the container experiment. The TSS value at the initial stage observed for Wupa WWTP was 149.0 mg/L, reduced to a range of 40.4–82.8%. Similarly, the TDS values at the initial stage for Wupa WWTP samples were observed as 320 mg/L. The initial value of TDS of wastewater from Wupa WWTP is slightly above WHO accepted limit (300 mg/L) for wastewater before discharge; thus, a significant reduction was observed (1.6-22.8%). A reduction in the values of BOD and COD was noted in the range of 19.0–94.9% and 63.0–97.3%, respectively.



**Figure 3: Mean Biomass growth of bacteria isolates in different experimental setup**

Keys: C= Container for control; A1= Treatment for Peptone 150mg; A2= Peptone 75mg; B1=Treatment for Glucose; 9g B2= Treatment for Glucose 4.5g

**Table 4: Mean physicochemical parameters of water samples of bioremediated wastewater samples**

Parameters		C1	A1	A2	B1	B2	WHO Standard
pH	I	7.14	7.19	7.19	7.20	7.22	6.5 – 8.5
	F	7.22	7.21	7.23	7.29	7.31	
Temperature	I	27.3	27.3	27.3	27.3	27.3	<40
	F	21.2	20.4	19.7	21.7	21.2	
Conductivity (µs/cm)	I	53.4	52.4	53.4	53.4	53.4	1250
	F	53.1	84.5	92.1	65.2	72.4	
TDS (mg/L)	I	320.0	320.0	320.0	320.0	320.0	300
	F	315.0	281.2	262.5	247.0	247.4	
BOD (mg/L)	I	41.0	41.0	41.0	41.0	41.0	10
	F	33.2	2.1	4.4	3.2	3.3	
COD (mg/L)	I	77.9	77.9	77.9	77.9	77.9	<5.0
	F	28.8	2.2	2.9	2.7	2.1	
TSS (mg/L)	I	149.0	149.0	149.0	149.0	149.0	30
	F	88.8	27.7	25.7	28.1	27.1	

Key: C= Container for control; A1= Treatment for Peptone 150mg; A2= Peptone 75mg; B1= Treatment for Glucose; 9g B2= Treatment for Glucose 4.5g; I= Initial; F= Final

Bioremoval efficiency of Peptone and Glucose biostimulation for Mn was presented in Figure 4. The highest value was recorded in Peptone (150mg) for treatment water with bioremoval efficiency of 66.80%. This is then followed by 66.47, 65.10, 57.70

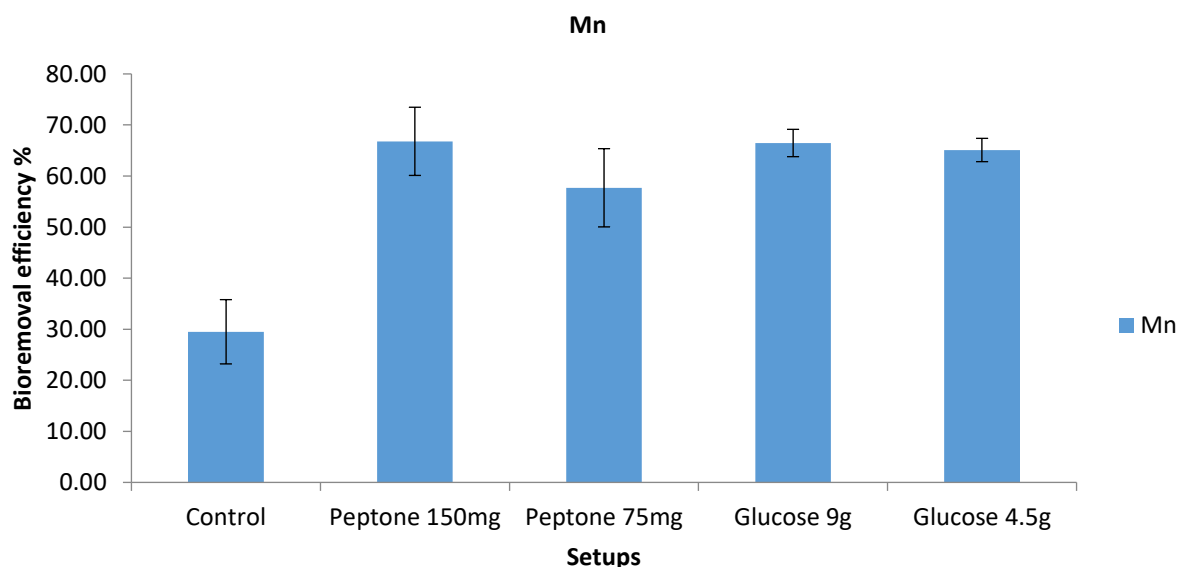
and 29.50% for Glucose (9g), Glucose (4.5g), Peptone (75mg) and Control respectively.

Bioremoval efficiency of Peptone and Glucose biostimulation for Fe was presented in Figure 5. The highest value was recorded in Glucose (9g) for treatment water with bioremoval efficiency of

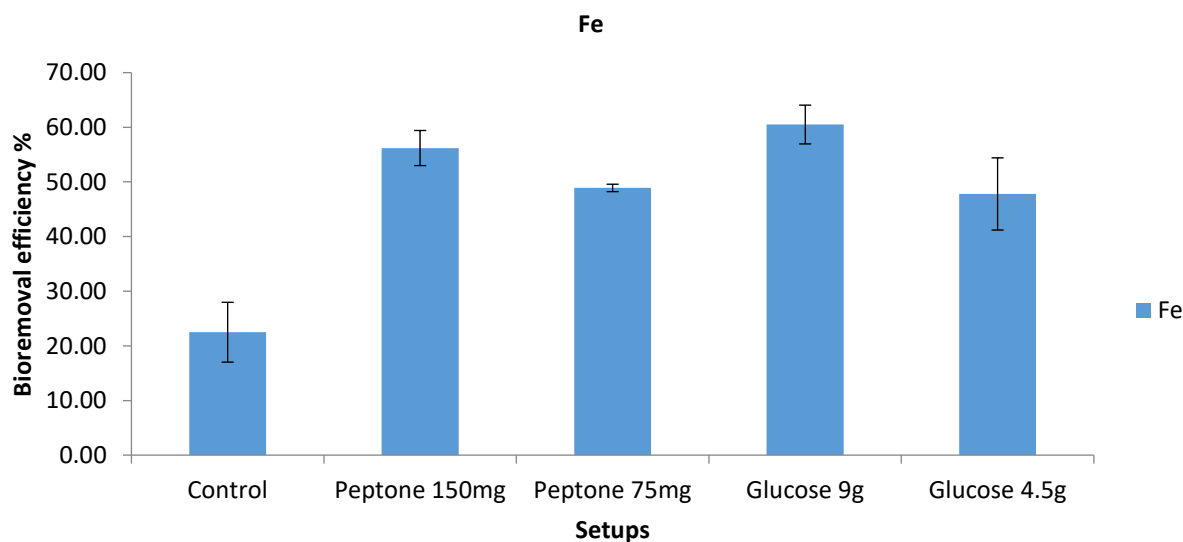
60.50%. This is followed by 56.20, 48.90, 47.80 and 22.50% for Peptone (150mg), Peptone (75mg), Glucose (4.5g) and the Control respectively.

Bioremoval efficiency of Peptone and Glucose biostimulation for Zn was presented in Figure 6. Treatment with Glucose showed higher Fe removal

with efficiency of 70.20 and 63.60% at 9g and 4.5g respectively. This is followed by Peptone with efficiencies of 62.40 and 55.29% at 150mg and 75mg respectively. The least efficiency is seen in the Control with 28.90%.



**Figure 4: Bioremoval efficiency of Peptone and Glucose for Mn in Wupa wastewater**



**Figure 5: Bioremoval efficiency of Peptone and Glucose for Fe in Wupa wastewater**



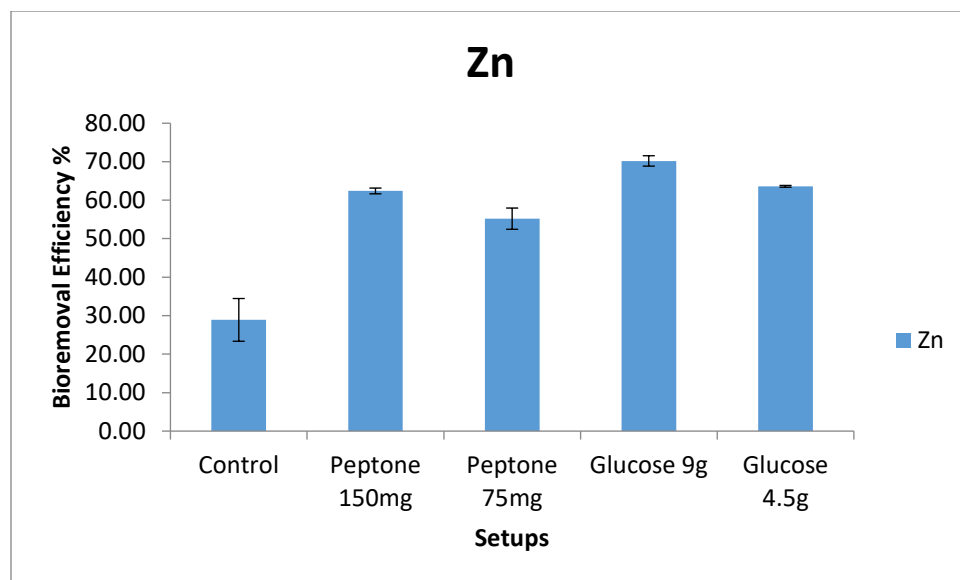


Figure 6: Bioremoval efficiency of Peptone and Glucose for Zn in Wupa wastewater

## DISCUSSION

The results on almost all measured physicochemical parameters showed significant differences ( $p < 0.05$ ) between the influent and effluent, except for pH and temperature. This indicates that the treatment process had a notable effect on most quality parameters of the wastewater.

The pH values of both influent ( $7.14 \pm 0.02$ ) and effluent ( $7.25 \pm 0.03$ ) fall within the World Health Organization (WHO) permissible limit (6.5–8.5), suggesting that the treatment process maintained near-neutral conditions suitable for microbial activities that drive biodegradation and biostimulation processes. Similar findings were reported by Adelegan (2020) and Eze et al. (2021), who observed that stable pH conditions between 6.5 and 8.0 are optimal for microbial enzymatic activity involved in the biodegradation of organic matter in wastewater.

The temperature values ( $27.3 \pm 0.01$  °C in influent and  $29.4 \pm 0.02$  °C in effluent) also remained within acceptable discharge standards ( $< 40$  °C), indicating that thermal changes during treatment were minimal. This temperature range is typical for tropical wastewater systems and supports mesophilic microbial populations responsible for organic and metal transformation (Oyetibo *et al.*, 2020).

A significant reduction was observed in conductivity, turbidity, total dissolved solids (TDS), total suspended solids (TSS), biochemical oxygen demand (BOD), and chemical oxygen demand (COD) after treatment. The

reduction in conductivity (from 53.4  $\mu\text{S}/\text{cm}$  to 35.5  $\mu\text{S}/\text{cm}$ ) suggests a decrease in ionic concentration due to sedimentation, adsorption, and microbial utilization of nutrients. Similar conductivity reduction patterns were noted by Adeleye and Okoh (2022) in their assessment of municipal wastewater treatment efficiency in Nigeria.

The turbidity reduction from 181.0 NTU in the influent to 24.0 NTU in the effluent indicates efficient removal of suspended particles and colloidal matter, likely facilitated by sedimentation and microbial flocculation. The observed TSS reduction (149 mg/L to 22 mg/L) further supports this, showing a substantial decrease in particulate load beyond the WHO discharge limit of 30 mg/L. Comparable findings were reported by Ojo et al. (2020) in Lagos State, who documented an 80–85% reduction in suspended solids following biological treatment processes.

BOD and COD are important indicators of organic pollution and treatment efficiency. The marked decline in BOD (from 41.0 mg/L to 3.0 mg/L) and COD (from 77.9 mg/L to 5.7 mg/L) reflects high treatment efficiency, indicating that the majority of biodegradable and oxidizable organic matter had been metabolized or removed during treatment. This aligns with findings by Singh et al. (2021) and Adesina et al. (2022), who reported similar reductions in BOD and COD during biological wastewater treatment processes driven by activated sludge and microbial consortia.

Regarding heavy metal concentrations, the results indicate significant reductions in Mn, Fe, and Zn levels after treatment. The concentration of Mn decreased from  $0.37 \pm 0.10$  mg/L in the influent to  $0.31 \pm 0.02$  mg/L in the effluent, while Fe dropped from  $1.58 \pm 0.20$  mg/L to  $0.91 \pm 0.30$  mg/L. Zn concentration remained relatively stable at  $0.50 \pm 0.10$  mg/L but still exhibited a statistically significant difference ( $p < 0.05$ ) between influent and effluent, indicating partial removal. These reductions suggest that the treatment system exhibits some level of heavy metal removal capacity, possibly through microbial biosorption, sedimentation, and co-precipitation mechanisms.

However, despite these reductions, the residual concentrations of all three metals in the effluent exceeded WHO discharge standards (Mn = 0.05 mg/L, Fe = 0.3 mg/L, Zn = 0.1 mg/L). This highlights the limited capacity of conventional treatment processes to eliminate heavy metals and underscores the need for enhanced biostimulation strategies or tertiary treatment stages. Similar limitations were documented by Osuolale and Okoh (2017) and Ude and Eze (2020), who reported that most wastewater treatment plants in Nigeria are primarily designed for organic matter removal rather than metal detoxification.

The persistence of elevated metal concentrations poses potential ecological risks if effluents are discharged into receiving water bodies. Metals like Fe, Zn, and Mn can accumulate in sediments and aquatic organisms, leading to bioaccumulation and possible trophic transfer, as emphasized by Nwankwoala et al. (2022).

Overall, the observed physicochemical patterns indicate that the Wupa Wastewater Treatment Plant is effective in reducing organic pollution and suspended solids but less efficient in removing heavy metals to WHO-compliant levels. This finding justifies the investigation into bacterial biostimulation as an alternative or complementary strategy for heavy metal remediation, since specific microbial species can enhance metal uptake, transformation, and immobilization.

The bacterial load of wastewater from the Wupa Wastewater Treatment Plant (WWTP) revealed marked differences between the influent and effluent samples, indicating the effectiveness of the treatment process in microbial load reduction. The influent

samples showed bacterial concentrations that were too numerous to count (TNTC), reflecting the high microbial content typically associated with raw domestic and industrial wastewater (Adewumi et al., 2020). In contrast, the treated effluent recorded a mean bacterial count of  $7.14 \times 10^6$  CFU/ml, suggesting substantial microbial removal efficiency through the plant's treatment processes. Similar findings were reported by Egbueri (2018), who observed significant reductions in microbial counts between influent and effluent samples in a comparable wastewater treatment system in Lagos, Nigeria. However, the persistence of bacterial colonies even after treatment emphasizes the possibility of resistant microbial species surviving the disinfection process (Choudhary et al., 2021).

Molecular identification of isolates from the WWTP revealed the presence of five bacterial genera—*Stenotrophomonas*, *Bacillus fusiformis*, *Lysinibacillus* sp., *Enterococcus casseliflavus*, and *Duganella callida*. Among these, *Stenotrophomonas* was the most frequently detected. This genus has been widely associated with wastewater environments and is known for its metabolic versatility and tolerance to heavy metals (Ryan et al., 2009; Alka et al., 2021). *Stenotrophomonas maltophilia* has been reported to produce extracellular enzymes and biosurfactants that enhance heavy metal mobilization and uptake during bioremediation (Wu et al., 2020). The detection of *Bacillus* and *Lysinibacillus* species further supports the bioremediation potential of the microbial consortium identified in Wupa WWTP, as both genera are known for producing chelating compounds that bind metals such as Zn, Fe, and Mn, facilitating their removal from contaminated media (Gadd, 2010). The presence of *Enterococcus casseliflavus*—a species shown to tolerate and remove heavy metals—and *Duganella callida*, from a genus implicated in biogeochemical cycling and transformation of toxic selenium species, suggests potential contributions to organic matter processing and resilience to metal(loid) stress in the system (Maia et al., 2020; Raths & Bücking, 2021).

The biomass growth pattern of the isolates across nine days indicated that the type and concentration of carbon and nitrogen supplements influenced microbial proliferation. Treatments with glucose (9 g and 4.5 g) exhibited the highest optical densities (0.33 and 0.18 respectively), indicating enhanced bacterial

growth compared to treatments with peptone (150 mg and 75 mg) and the control. This suggests that glucose served as a more readily available carbon source, promoting faster metabolic activity and cell replication (Kumar *et al.*, 2020). These findings are consistent with those of Bhattacharya *et al.* (2019), who reported that glucose supplementation increased bacterial biomass and enzymatic activity during the biostimulation of contaminated water systems. Conversely, reduced growth in peptone treatments could be attributed to the slower nitrogen assimilation rate or suboptimal carbon-to-nitrogen (C:N) ratio, which can limit bacterial energy production (Li *et al.*, 2018). The slight decrease in absorbance at day six across treatments could be due to nutrient depletion or metabolic adaptation, which later stabilized as cells reached stationary growth phases.

The bacterial species identified play crucial roles in enhancing heavy metal bioremediation through biostimulation. The glucose and peptone amendments likely acted as metabolic enhancers, stimulating indigenous microorganisms to produce metabolites such as siderophores, organic acids, and biosurfactants that bind, mobilize, or precipitate heavy metals (Kumar *et al.*, 2020). *Stenotrophomonas maltophilia*, for instance, is known to secrete metal-binding proteins that facilitate bioaccumulation of Fe, Mn, and Zn (Singh *et al.*, 2021). Similarly, *Bacillus fusiformis* produces exopolysaccharides that immobilize metals, thus reducing their bioavailability and toxicity (Ayangbenro & Babalola, 2017). The effectiveness of biostimulation observed in this study aligns with the report of Rahman *et al.* (2019), who demonstrated significant enhancement of bioremediation efficiency when glucose and peptone were used as organic stimulants in metal-polluted wastewater.

The results revealed that biostimulation had a notable impact on the physicochemical properties of wastewater, leading to significant reductions in several pollution indicators. The pH values across all treatment setups remained relatively stable, varying only slightly within the range of 0.27–1.25%, and stayed within the WHO acceptable limits (6.5–8.5). This near-neutral pH provided a favorable environment for microbial metabolism and enzymatic activity, which is essential for biodegradation processes. Similarly, the temperature remained below the WHO

threshold (<40°C), indicating that the microbial activities were maintained under suitable environmental conditions. These findings agree with Singh *et al.* (2020b) and Chaudhary *et al.* (2019), who reported that stable pH and moderate temperature promote optimal microbial growth and degradation efficiency in wastewater systems.

The conductivity, total dissolved solids (TDS), and total suspended solids (TSS) showed remarkable reductions, suggesting microbial utilization and removal of ionic and particulate matter efficiently. Conductivity decreased by 0.6–22.1%, reflecting a reduction in the concentration of dissolved ions. TSS reduced substantially by 40.4–82.8%, while TDS decreased by 1.6–22.8%, both indicating active microbial metabolism and sedimentation of organic materials. Similar patterns were observed by Adekunle *et al.* (2021) and Okoh *et al.* (2019), who attributed such reductions to microbial metabolism, assimilation and degradation of organic and inorganic particles in effluents. The observed decreases also indicated that bacterial activity improved the clarity and quality of the treated wastewater.

The biological oxygen demand (BOD) and chemical oxygen demand (COD) recorded the most significant decreases, ranging from 19.0–94.9% and 63.0–97.3%, respectively. These reductions show that microorganisms effectively oxidized organic pollutants, thereby improving effluent quality. The substantial decline in oxygen demand aligns with the findings of Akunna *et al.* (2022) and Gadd (2020), who explained that bacterial enzymes such as oxidoreductases play a crucial role in degrading complex organic compounds. The enhanced reduction observed in glucose and peptone treatments also supports the assertion of Abatenh *et al.* (2017) that carbon and nitrogen supplementation enhance microbial metabolism and co-metabolism during bioremediation.

The overall improvements in water quality parameters recorded in this study are consistent with previous findings by Eze *et al.* (2020) and Das and Dash (2021), who demonstrated that nutrient-induced bacterial stimulation significantly enhances biodegradation efficiency in wastewater systems. The observed results further suggest that indigenous microorganisms at the Wupa WWTP have the natural capacity to adapt and utilize available nutrients for

pollutant degradation, as supported by Obinna and Nworie (2018).

The biostimulation of indigenous bacteria for the removal of heavy metals in wastewater from the Wupa Wastewater Treatment Plant, Abuja, demonstrated that nutrient amendment using carbon and nitrogen sources (glucose and peptone) significantly enhanced the bioremoval efficiency of Mn (Mn), Fe (Fe), and Zn (Zn). The observed trends in Figures 4–6 indicated that both glucose and peptone supplementation improved bacterial metabolic activity and consequently increased metal uptake compared to the control.

For Mn, the highest bioremoval efficiency (66.80%) was achieved with peptone at 150 mg, followed closely by glucose treatments (66.47% and 65.10% at 9 g and 4.5 g, respectively). This suggests that nitrogen-rich media (such as peptone) can stimulate bacterial growth and enzymatic activities required for metal complexation and reduction. Similar findings were reported by Abioye et al. (2018), who observed that nitrogen sources enhanced Mn removal efficiency in microbial consortia due to increased synthesis of metallothionein-like proteins. The moderate removal observed in glucose treatments also indicates that carbon sources can serve as energy substrates, driving bacterial growth and bioaccumulation processes (Anand et al., 2021).

In the case of Fe, glucose supplementation produced the highest bioremoval efficiency (60.50%) at 9 g concentration, indicating that carbon-enriched environments promote bacterial metabolism and facilitate the reduction of ferric ions to ferrous forms that can be immobilized or sequestered. This aligns with the observations of Liu et al. (2020), who reported enhanced Fe (III) reduction in glucose-supplemented systems due to the stimulation of heterotrophic bacterial respiration. The relatively lower performance of peptone in Fe removal may be attributed to slower utilization rates compared to glucose, as carbon source availability is a key factor influencing microbial metal transformation and reduction processes (Brahmacharimayum, Mohanty, & Ghosh, 2019; Sarkodie et al., 2022).

For Zn removal, glucose also demonstrated superior performance, with efficiencies of 70.20% and 63.60% for 9 g and 4.5 g treatments, respectively. Peptone treatments (150 mg and 75 mg) followed with 62.40% and 55.29% removal, while the control showed the

lowest value (28.90%). The higher Zn removal in glucose-supplemented systems suggests that carbon-induced bacterial proliferation enhances biosorption capacity, as more biomass and extracellular polymeric substances (EPS) are produced. These EPS provide binding sites for Zn ions through functional groups such as hydroxyl, carboxyl, and amine (Yin et al., 2020; Khan et al., 2023). This result corroborates the work of Ekhaie and Anyasi (2012), who reported improved Zn bioremediation in nutrient-enriched wastewater due to enhanced bacterial growth and biofilm formation.

The control setups consistently showed the lowest bioremoval efficiencies, emphasizing the importance of nutrient supplementation in promoting bacterial growth and enzymatic activity for effective bioremediation. The high removal efficiencies observed (ranging from 55–70%) confirm the potential of biostimulation as an eco-friendly and cost-effective strategy for treating metal-contaminated wastewater, in line with findings from Olowo et al. (2021) and Alrumman et al. (2018), who both reported significant metal reductions through microbial biostimulation approaches.

## CONCLUSION

This study demonstrated that while the conventional treatment process at the Wupa Wastewater Treatment Plant is effective at reducing organic and particulate pollution, it is inadequate for remediating heavy metals to safe levels. The identification of a resilient, metal-tolerant indigenous microbiome, predominantly *Stenotrophomonas* and *Bacillus* species, revealed a significant, untapped bioremediation potential. Crucially, the application of a simple biostimulation strategy using carbon (glucose) and nitrogen (peptone) sources significantly enhanced this innate microbial activity, leading to a dramatic increase in the removal efficiency of Mn, Fe, and Zn—more than doubling the performance of the unamended control. Glucose, as a readily available carbon source, proved particularly effective, especially for the removal of Zn and Fe. These findings affirm that nutrient limitation is a key constraint on the natural bioremediation capacity within the plant and that biostimulation presents a viable, eco-friendly strategy to augment the existing treatment process.

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