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Research Article

Bioremediation of some Heavy metals from Wupa River using *Bacillus subtilis* and *Pseudomonas putida*

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ABSTRACT

Pollution of water by heavy metals is a serious threat to the environment and the health of living organisms. This study was carried out to investigate the bioremediation potential of *Bacillus subtilis* and *Pseudomonas putida* on some heavy metals (Lead, copper and zinc) from water samples collected from Wupa River in Abuja. Water samples were collected and analyzed using standard procedures. The result shows that all the measured physicochemical parameters observed in the water sample from Wupa River are within the World Health Organization's maximum permissible limit for freshwater. In heavy metal mean concentration, lead records the highest (11.09 ± 0.84 mg/L) while zinc records the lowest in the sampled water. The absorbance of *Bacillus subtilis* and *Pseudomonas* increases from day 1 in all the treated heavy metals, except in the control sample, which records a decrease in absorption as sampling day increases. The application of *Bacillus subtilis* only (BS), *Pseudomonas putida* (P), and the combination of *Bacillus subtilis* and *Pseudomonas putida* in water showed a good remediation potential of heavy metals, as there was significant reduction in both physicochemical and heavy metals of the water samples. This study highlights the potential of fungal bioremediation as a sustainable and eco-friendly method for heavy metal removal from contaminated water bodies.

Keywords: *Bacillus subtilis;* Bioremediation; Heavy metals; *Pseudomonas putida*; Wupa River

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INTRODUCTION

Worldwide, water is an important component of living beings as it performs unique and indispensable activities (Mohammed *et al.,* 2020a). However, due to many anthropogenic activities, freshwater resources are deteriorating at a faster rate (Adam *et al.,* 2020). Increasing urban population growth, industrialization, and intensive farming have led to severe disturbance of drinking water across the world, resulting in limited access to clean and safe drinking water (Adamu *et al.,* 2022). The rise of urbanization and industrialization has left the environment exposed to numerous pollutants which are toxic to living things. Pollutants arising from different industrial processes are major sources of pollution to the soil and aquatic environment (Mohammed *et al.,* 2020a; Mohammed *et al.,* 2020b). Different types and quantities of heavy metals are released into the environment during different industrial process. For instance, wastewater from dye-producing companies are associated with antimony, chromium, and mercury (Methneni *et al.,* 2021). The application of fertilizers, pesticides, and herbicides in the agricultural sector generates pollutants that include aluminum, copper, zinc, nickel, lead, and arsenic to the environment (Ayilara *et al.,* 2020). Similarly, untreated pollutants from wastewater of the agri-food industries disposed into river canals and other waterbodies have harmful effects on the environment (Siric *et al.,* 2022 Mohammed *et al.,* 2020c).

Bioremediation refers to the use of microorganisms to eliminate or reduce the concentrations of hazardous wastes at a contaminated site (Yonghong *et al.,* 2014). One important characteristic of bioremediation is that it is carried out in non-sterile open environments comprising of a variety of microorganisms (Ibrahim *et al.,* 2023). Out of this diversified group of microorganisms, the central role towards degradation of contaminants is being accomplished by bacteria (Yonghong *et al.,* 2014). A biological treatment system comprising of these microorganisms has various applications such as the rehabilitation of contaminated sites, e.g., water, soils, sludges, and waste streams (Yonghong *et al.,* 2014; Ibrahim *et al.,* 2023a; Ibrahim *et al.,* 2023b).

Waste containing heavy metals are often improperly disposed into soil and water environments. When disposed into water bodies, they can lead to the death of fishes and other aquatic inhabitants, otherwise, they are biomagnified and cause chronic diseases in humans and animals (Mohammed *et al.,* 2020a; Mohammed *et al.,* 2020b; Mohammed *et al.,* 2020c). Therefore, there is a need for the remediation of these pollutants using physical, chemical, or biological methods. The physical and chemical methods have been used for years (Mohammed *et al.,* 2023; Mohammed *et al.,* 2024) but they come with their drawbacks which include the need for an expert and special equipment for the chemical bioremediation procedure while the physical bioremediation procedure is expensive (Mahmood *et al*., 2021). This has called for the need for a better alternative which is biological remediation (Bioremediation). Bioremediation is the most efficient, eco-friendly, and cost-effective technology for the transformation of contaminants (Ayilara and Babalola, 2023). Biological remediation can be carried out using both plants and microorganisms, nonetheless, plants take a longer time to grow and cannot be easily manipulated like the microbes which makes the microbes preferable (Hussain *et al*., 2022). In addition, microbes mitigate heavy metals and improve soil fertility and plant development (Chaudhary *et al*., 2023; Ayilara and Babalola, 2023). Thus, this study is aimed to ascertain the efficiency of *Bacillus subtilis* and *Pseudomonas putida* in bioremediation of heavy metals in water samples collected from Wupa River, Abuja, Nigeria.

MATERIALS AND METHODS

Study Area and Sample Collection

Wupa River lies between Longitude 7°017’00E and 7°022’12E and Latitude 8°056’48N and 9°001’48N. Wupa River is part of the Jabi River watershed in Abuja. The reach of the river under study covers a total length of 16 km. The river is narrow with maximum dry weather flow width varying from 10 m to 20 m. The river channel bed outcrops indicate medium roughness and the degree of sinusoidal is low to moderate with few sharp bends (Maishanu *et al.,* 2022). The temperature in the area varies from 27℃ to 36°C with an average value of 29℃. Rainfall varied from a monthly depth of 10 mm to 68 mm for the year 2021.

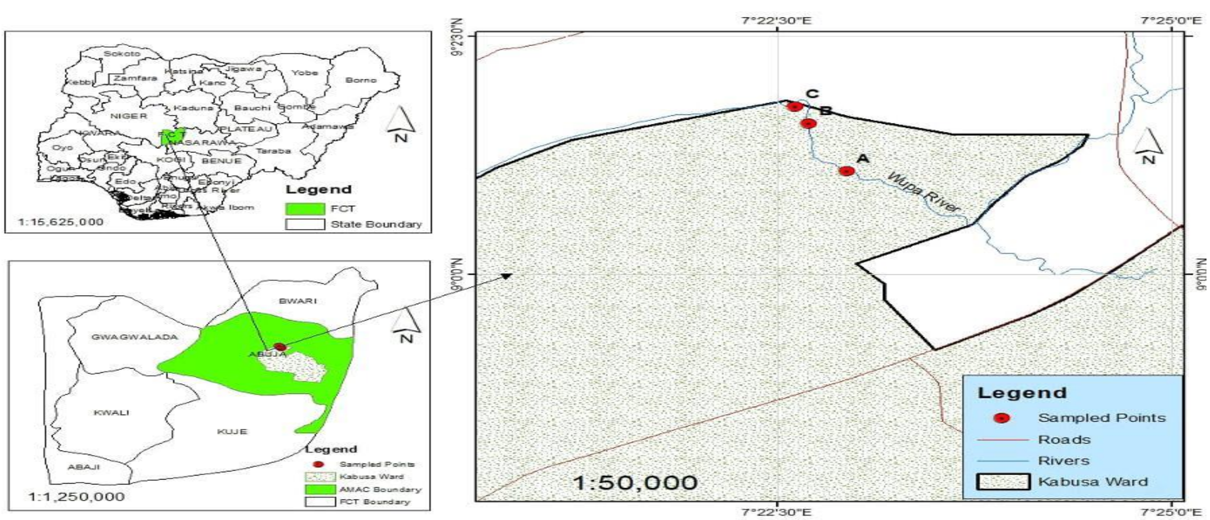

Figure 1: Administrative map of FCT showing Wupa River Sample collection

Prior to sample collection with 1L plastic bottles, sample bottles were washed with distilled water and rinsed with sampling water before collecting water for the study. The samples collected were labeled with the date, time, and location before taking to the laboratory for analysis. Also, pure isolates of *Bacillus subtilis* and *Pseudomonas putida* were obtained from National Institute of Pharmaceutical Research Development (NIPRD) Abuja Nigeria.

Determination of physicochemical parameters

The following physico-chemical parameters were determined using standard methods of American Public Health Association (APHA, 1998): Temperature, turbidity, conductivity, pH, biochemical oxygen demand (BOD), and dissolved oxygen (DO).

Determination of Heavy metals analysis of water samples from Wupa River Abuja Nigeria

The concentration of heavy metals such as Lead (Pb), Copper (Cu) and Zinc (Zn) in the digested water samples was determined using an Atomic Absorption Spectrophotometer equipped with a digital read-out system. Working standards were prepared after serial dilution of 1000 ppm metal stock solution in each case. Calibration curves were constructed by plotting absorbance values versus concentrations.

Determination of Bioremediation potentials of *Bacillus subtilis* and *Pseudomonas putida* on some heavy metals in Wupa River Abuja

Stock solution of zinc, lead, and copper was prepared in a sterile conical flask containing 500ml of distilled water for each. The flask was warmed on a hot plate simultaneously with gentle shaking and sterilized at 121ºC for 15 minutes in an autoclave. The solution was stored in a refrigerator at 4ºC until needed (Kumar *et al.,* 2014). Exactly 1.95g of Nutrient broth was prepared according manufacturer's instruction; the broth was dissolved in 150ml of distilled water in a conical flask and sterilized in the autoclave at 121°C for 15 min. After cooling to room temperature, 10ml of the nutrient broth media and 5ml of normal saline solution was measured into a bijou bottle. The pure culture of the *Bacillus subtilis* and *Pseudomonas putida* were inoculated into a different bijou bottle. The inoculum was placed inside an incubator shaker set for 7 days for proper synergetic growth of the organism (Khosro *et al.,* 2011).

Screening of *Bacillus subtilis* and *Pseudomonas putida* with Potential to remediate heavy metal in water sample

The isolation of bacteria was achieved by amending nutrient broth with the following quantities of salts before sterilization: CuSO4 5H20 (0.83 mg), Zn (NO3)2 (5.9 mg), Pb (NO3)2 (0.32 mg). After making the mineral salt medium (MSM) in a beaker, 0.1% of water sample was added to it and sterilized using an autoclave at 121 degrees Celsius for 15 minutes. 10ml of MSM was added into a test tube, 0.2ml of nutrient broth grown isolate was added into the sterile MSM, and it was then mixed properly and incubated for 6 days at 30 degrees Celsius. It was then checked using UV spectrophotometer. It was done in triplicate (Ibrahim *et al.,* 2023a; Ibrahim *et al.,* 2023b).

Bioremediation of wastewater samples using *Bacillus subtilis* and *Pseudomonas putida*

Degradation of heavy metals experiments by *Bacillus subtilis* and *Pseudomonas putida* was carried out in 250 ml of separate flasks containing 100 ml of effluents collected from study sites and 15ml of inoculum was added into the water sample and used for biodegradation (Khosro *et al.,* 2011). The pH was adjusted to 70.2 using Nacl and H_2SO_4. Then, the flasks were autoclaved at 121°C for 15 minutes. The autoclaved flasks were inoculated with 5ml of bacteria inoculum of each isolate. The flasks were kept in the mechanical shaker and incubated at 37 °C for 8 days. The heavy metal content was assessed with the help of Atomic Absorption spectroscopy.

RESULTS

Physicochemical parameters of water samples collected from Wupa River.

The mean physicochemical parameters of water samples collected from Wupa River are presented in Table 1. All the measured physicochemical parameters observed in a water sample from Wupa River are within the World Health Organization's maximum permissible limit for freshwater.

Heavy metal concentration of water samples collected from Wupa River

The heavy metal concentration of water samples collected from Wupa River was presented in Table 2. Lead was the record highest (11.09 ± 0.84 mg/L) in terms of mean concentration while zinc was the record lowest. All the heavy metals observed in water sample from Wupa River are within the World Health Organization maximum permissible limit for freshwater except lead which exceed the maximum permissible limit.

Screening of *Bacillus subtilis* and *Pseudomonas* sp for heavy metal removal in water samples collected from Wupa River

The potentials of heavy metal removal of *Bacillus subtilis* and *Pseudomonas* sp in water samples collected from Wupa River are shown in figures 2 and 3. The absorbance of *Bacillus subtilis* increased from day 1 in all the treated heavy metals except in the control sample which recorded decrease in absorption as the sampling day increased (Fig 2). The absorbance of *Pseudomonas* increased from day 1 in all the treated heavy metals except in the control sample which recorded a decrease in absorption as the sampling day increased (Fig 3).

Table 1. Physicochemical parameters of water samples collected from Wupa River

Parameters	Mean ± S. E	WHO permissible limit
Temperature	28.6 ± 0.11	40
pH	7.21 ± 0.07	7.0 – 8.5
Conductivity	0.25 ± 0.00	600
BOD	4.45 ± 0.59	5
DO	5.09 ± 0.05	5
Turbidity	4.71 ± 0.14	<40
Chloride	5.26 ± 1.12	
Sulphate	12.00 ± 1.90	250-400
Nitrate	20.53 ± 0.50	50
Total hardness	10.43 ± 0.26	

Table 2 Heavy metal concentration of water samples collected from Wupa River

Heavy metal (Mg/L)	Mean ± S. E	WHO permissible limit
Lead (Pb)	11.09 ± 0.84	0.02
Copper (CU)	5.49 ± 0.05	1.3
Zinc	0.10 ± 0.00	0.12

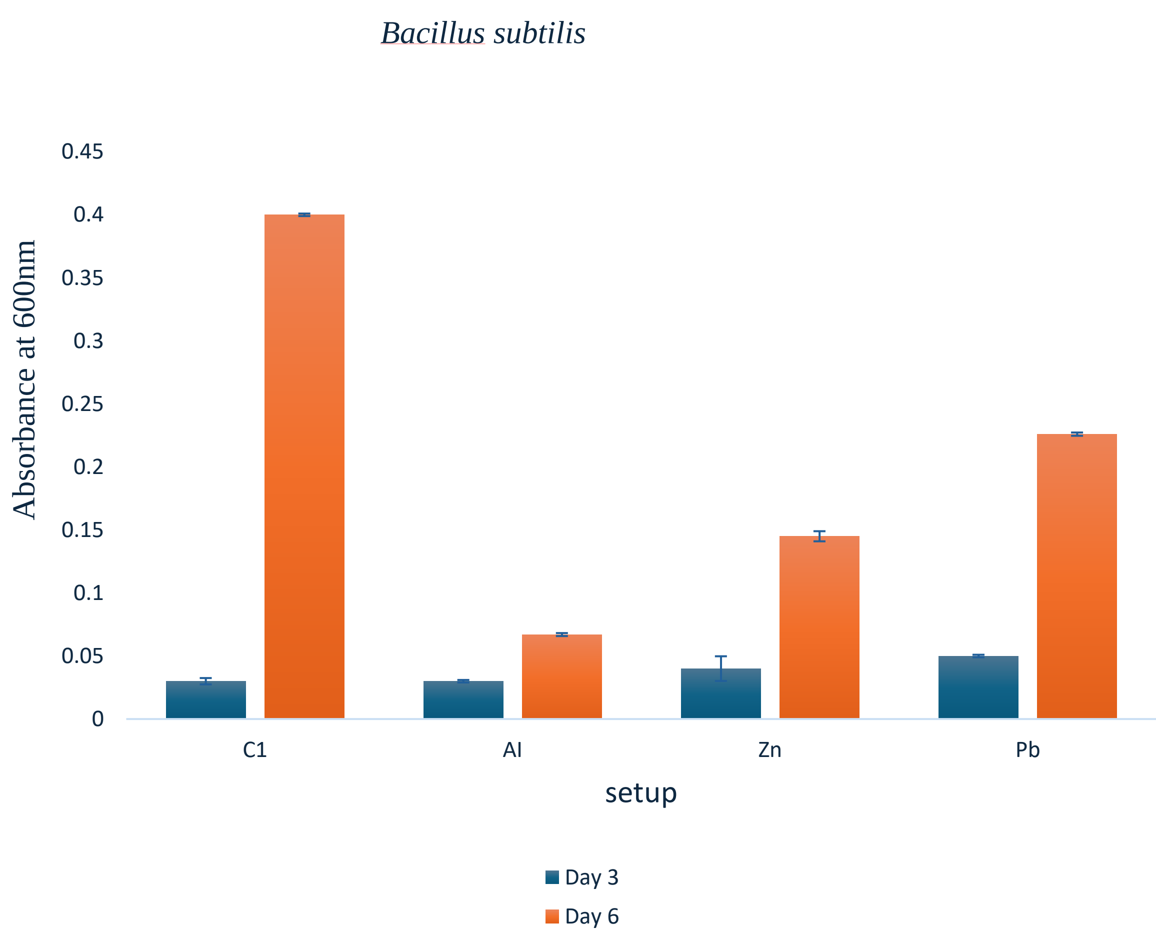

Figure 2. Biomass growth of *Bacillus subtilis* species in prepared heavy metal stock solutions

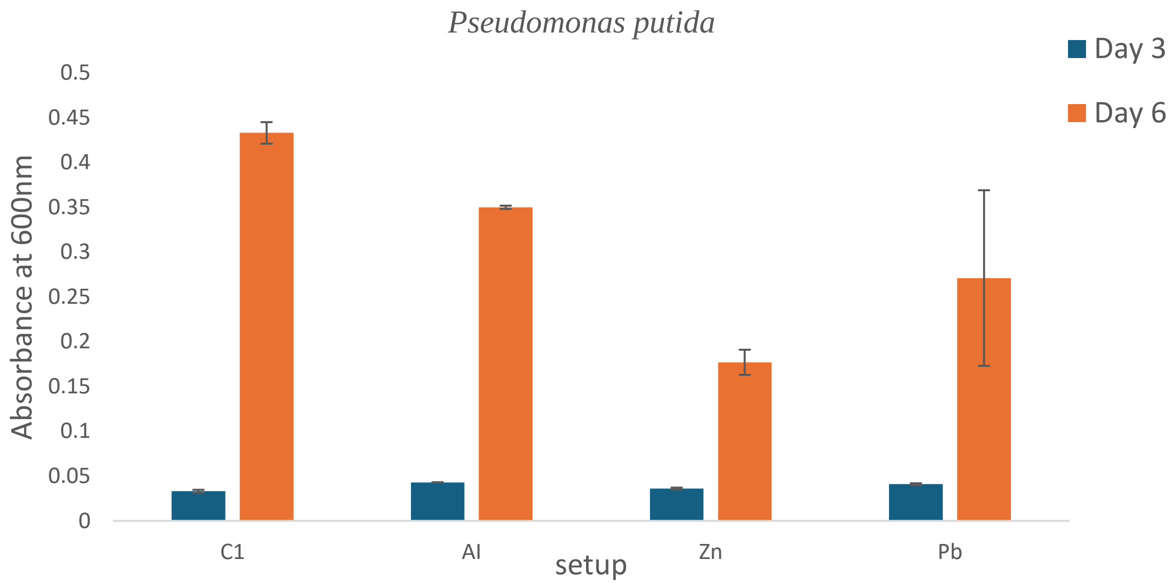

Figure 3. Biomass growth of *Pseudomonas putida* in prepared heavy metal stock solutions

Heavy metal removal efficacy of *Bacillus subtilis* and *Pseudomonas* sp in water samples collected from Wupa River

The potentials of heavy metal removal of *Bacillus subtilis* and *Pseudomonas putida* in water samples collected from Wupa River are shown in table 3. Water samples treated with *Bacillus subtilis* only (BS), *Pseudomonas putida* (P), and a combination of *Bacillus subtilis* and *Pseudomonas putida* showed a good remediation potential as there is a significant reduction in both physicochemical and heavy metals of the water samples. The bioremoval efficiency of *B. Subtilis* and *P. putida* for lead is shown in figure 4. All the isolates showed high removal efficiency for lead while combination of *B. Subtilis* and *P. putida* recorded the highest bioremoval efficiency (85.3%) in lead. The bioremoval efficiency of *B. Subtilis* and *P. putida* for copper is shown in Figure 5. All the isolates showed high removal efficiency for copper while combination of *B. Subtilis* and *P. putida* recorded the highest bioremoval efficiency (82.33%) in copper. The bioremoval efficiency of *B. Subtilis* and *P. putida* for zinc is shown in figure 6. All the isolates showed high removal efficiency for zinc while *P. putida* recorded the highest bioremoval efficiency (90%) in zinc.

Table 3. Heavy metal bioremediation removal of *Bacillus subtilis* and *Pseudomonas putida* in water samples collected from Wupa River

Parameters	Control	BS	PP	BSP
Temperature (°C)	28.6 ± 0.11	26.67 ±0.37	26.56 ± 0.32	26.76 ± 0.30
pH	7.21 ± 0.07	8.00 ± 0.01	7.91 ± 0.11	7.98 ± 0.10
Conductivity (µS/Cm)	0.25 ± 0.00	0.22 ± 0.03	0.20 ± 0.02	0.20 ± 0.03
BOD (mg/L)	4.45 ± 0.59	3.30 ± 0.54	2.93 ± 0.40	2.67 ± 0.80
DO(mg/L)	5.09 ± 0.05	4.06 ± 1.04	3.05 ± 0.80	2.64 ± 0.69
Turbidity (NTU)	4.71 ± 0.14	2.48 ± 0.01	2.67 ± 0.13	2.41 ± 0.51
Chloride (mg/L)	5.26 ± 1.12	4.10 ± 0.08	2.78 ± 0.54	3.43 ± 0.97
Sulphate (mg/L)	12.00 ± 1.90	6.32 ±1.72	6.30 ± 0.31	4.94 ± 1.59
Nitrate (mg/L)	20.53 ± 0.50	7.21 ± 1.53	9.26 ± 0.79	5.98 ± 1.82
Total hardness (mg/L)	10.43 ± 0.26	7.26 ± 1.42	7.36 ± 0.64	7.56 ± 0.34
Lead (mg/L)	11.09 ± 0.84	4.07 ± 1.12	3.05 ± 0.04	1.63 ± 0.44
Cupper (mg/L)	5.49 ± 0.05	2.13 ± 0.83	1.56 ± 0.75	0.97 ± 0.03
Zinc(mg/L)	0.10 ± 0.00	0.05 ± 0.03	0.01 ± 0.03	0.03 ± 0.00

Key; *Bacillus subtilis*(BS), *Pseudomonas putida*(PP*), Bacillus Subtilis+ Pseudomonas putida*(BSP)

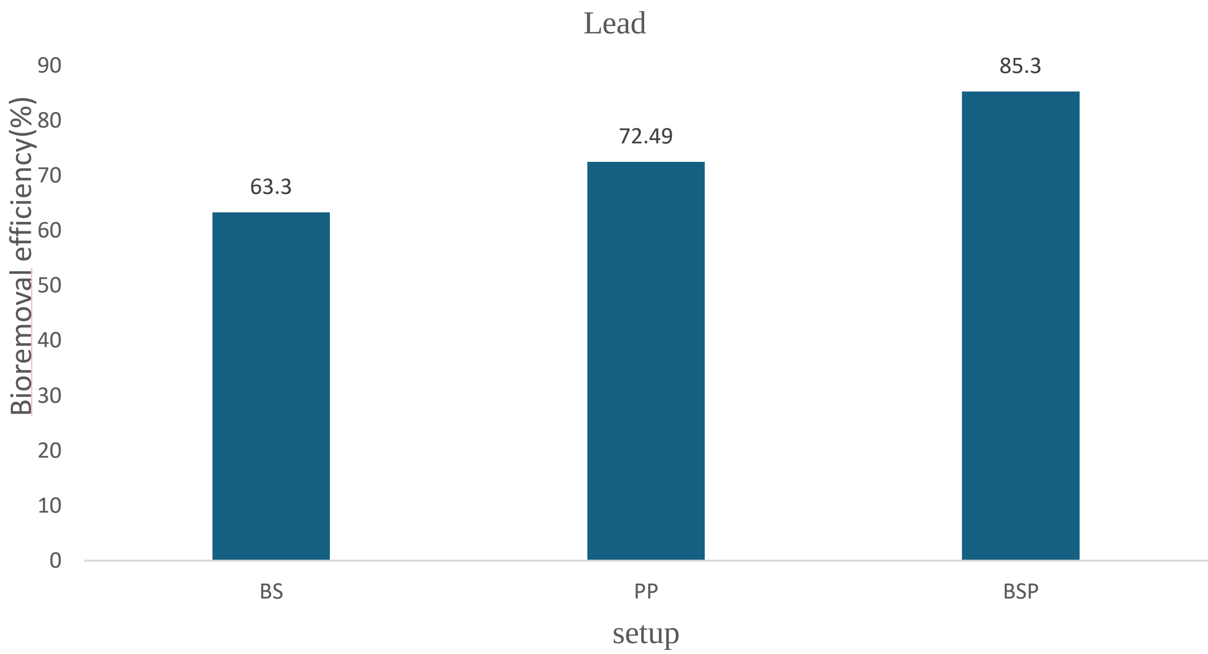

Figure 4: Bioremoval efficiency of *B. Subtilis* and *P. putida* for lead
Key: *Bacillus subtilis*(BS), *Pseudomonas putida*(PP*), Bacillus Subtilis+ Pseudomonas putida*(BSP).

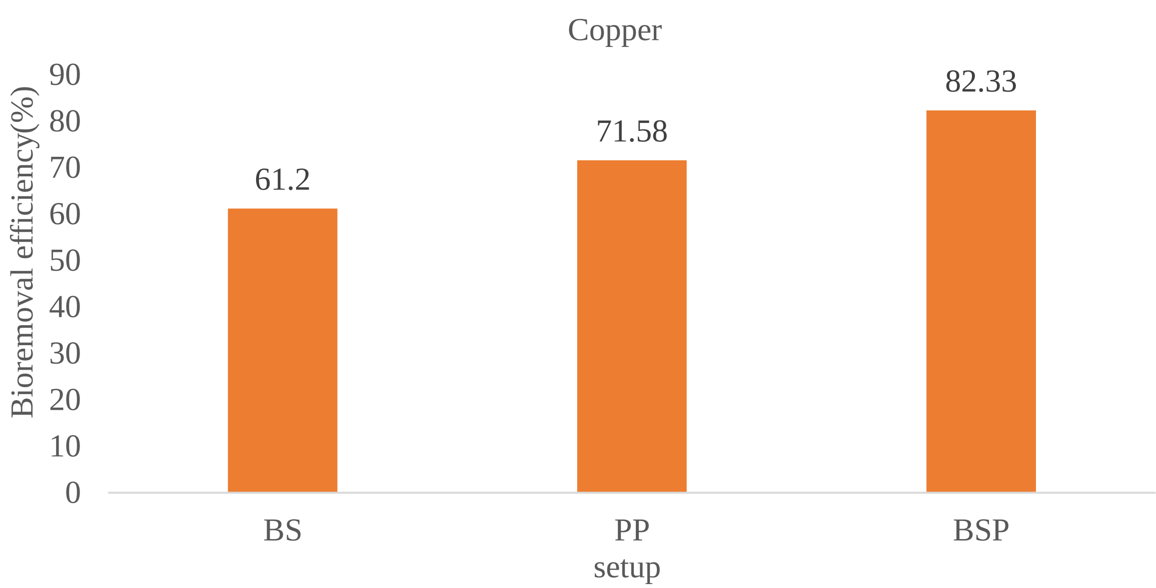

Figure 5: Bioremoval efficiency of *B.Subtilis* and *P. putida* for copper
Key: *Bacillus subtilis*(BS), *Pseudomonas putida*(PP*), Bacillus Subtilis+ Pseudomonas putida*(BSP).

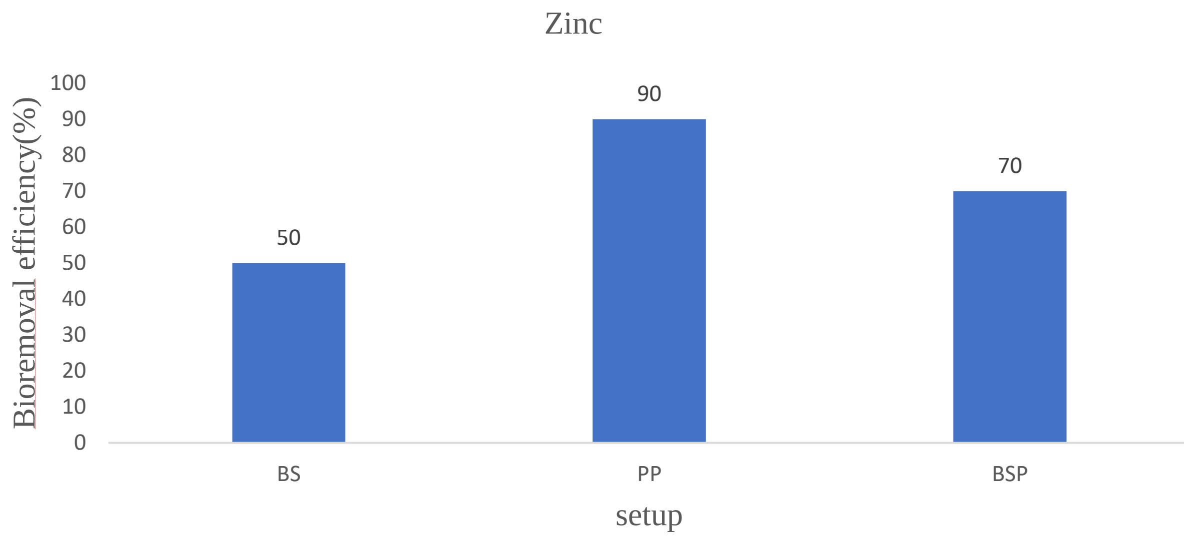

Figure 6: Bioremoval efficiency of *B.Subtilis* and *P. putida* for Zinc
Key: *Bacillus subtilis* (BS), *Pseudomonas putida* (PP*), Bacillus Subtilis+ Pseudomonas putida* (BSP)

DISCUSSION

Discharge of any kind of waste without proper treatment affects the physicochemical properties of water and soil which enters food chain and affects agriculture products, animal, and human health. Studies of water quality in various effluents revealed that anthropogenic activities have an important negative impact on water quality in the downstream sections of the major rivers (Kannel *et al.,* 2017). In this study, all the measured physicochemical parameters observed in water sample from Wupa River are within the World Health Organization maximum permissible limit for freshwater while all the heavy metal exceeds the maximum permissible limit for World Health Organization. The high values of heavy metals observed could be due to increasing human population in Wupa River and the discharge of waste water effluent from Wupa wastewater treatment plant into the Wupa River which serves as the source of water for the people of Wupa River.

Bioremediation is a process that uses living organisms to remove or neutralize contaminants in the environment. The results obtained in this study indicates that different bacterial strains exhibit varying bioremoval efficiencies for different heavy metals. For example, *B. Subtilis* showed the highest efficiency in removing lead, while *Pseudomonas putida* was most effective in removing zinc. However, when these two strains were combined (as BSP), they showed the highest efficiency in removing lead, indicating a synergistic effect. This study highlights the importance of using multiple bacterial strains in bioremediation processes to enhance removal efficiencies for different heavy metals. It also emphasizes the significance of comparing the efficiencies of different bacterial strains in removing specific heavy metals to optimize bioremediation strategies. Moreover, the results also suggest that the efficiency of bioremoval may vary depending on the type of wastewater being treated. For example, the bioremoval efficiency was found to be higher in wastewater treated with BSP compared to *B. Subtilis* alone. *Bacillus subtilis* and *Pseudomonas putida* shows the potential and efficacy of bioremediation of heavy metals like zinc, lead, and copper in water sample from Wupa River. *Bacillus subtilis* is a common bacterium that has shown the ability to absorb heavy metals from contaminated water. For instance, Acosta *et al,* (2005) reported that *Bacillus subtilis* have the ability to produces extracellular polymeric substances that can bind to heavy metals and also remediate heavy metals from the water. Additionally, *Bacillus subtilis* has been found to have the enzyme abilities necessary for the conversion of heavy metals into less toxic forms, which further aids in their removal from water sources (Ibrahim *et al.,* 2023a). *Bacillus* sp. has been reported to also have high remediation capacity. In this study *Bacillus subtilis* had high absorbance. The high absorbance rate at the sixth day6 the research reported in this study is consistent with related research carried out by Ibrahim *et al.,* (2024) who reported a remediation potential of *Bacillus subtilis* in Wupa WWTP, Abuja. The study also agrees with the findings of Kamika and Momba (2013) and Syed and Chinthala (2015) who reported high heavy metal remediation activity by *Bacillus sp*., as this could be attributed to the ability of *Bacillus subtilis* to exhibit robustness, adaptability, and diverse metabolic capabilities, including its potential for metal ion uptake (Syed and Chinthala, 2015; Ibrahim *et al.,* 2023b).

Pseudomonas putida is another bacterium known for its ability to remediate water contaminated with heavy metals. In this study, *Pseudomonas putida* had high absorbance. This high absorbance reported in this study is consistent with the findings of Ibrahim *et al.,* (2024) who reported a remediation potential of *Pseudomonas aeruginosa* and other bacteria species in Wupa WWTP, Abuja. This bacterium is capable of producing metal-binding proteins that can sequester heavy metals and prevent their uptake by other organisms. *Pseudomonas putida* also has the ability to metabolize heavy metals, converting them into less toxic compounds. Both *Bacillus subtilis* and Pseudomonas *putida* have been successfully used in bioremediation of heavy metals from wastewater sample of Wupa wastewater treatment plant and they show good efficiency in the removal of the wastewater (Ibrahim *et al.,* 2023a; Ibrahim *et al.,* 2023b). These bacteria can be applied in bioremediation systems such as bioreactors or in-situ bioremediation techniques to effectively remove heavy metals from water sources.

CONCLUSION

Human activities around water bodies pose a threat to the environment. All the heavy metals observed in this study exceeds the maximum permissible limit for World Health Organization although the physicochemical parameters were within the acceptable limit. *Bacillus subtilis* and *Pseudomonas putida* showed good remediation activities in the reduction of heavy metals like zinc, lead, and copper in water samples from Wupa River. There is a need to minimize the increase of anthropogenic activities around Wupa River and also stop the channeling of drainage or effluent into the water body. There is also need for further research and development of bioremediation techniques involving these bacteria which could lead to more sustainable and cost-effective solutions for heavy metal contamination in water.

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