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Bioremediation Potentials of *Bacillus subtillis* and *Aspergillus niger* on Selected Heavy Metals from Wupa Wastewater Treatment Plant, Abuja

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Abstract

Bioremediation is the process of cleaning up the environment with microorganisms, such as fungi and bacteria. These microorganisms employ processes including biosorption, biodegradation, and bioaccumulation to remediate the waste materials. Potential for bioremediation of heavy metals from wastewater collected from the Wupa wastewater treatment plant (WWTP) in Abuja was examined for *Aspergillus niger* and *Bacillus subtilis*. Samples were analyzed using standard methods and procedure. The bioremediation potentials of isolates were assessed on a heavy metal solution, monitored for growth and absorbance. The remediation potential was evaluated using ultraviolet-visible spectrophotometer and microplate reader. Between the influent and effluent, every heavy metal evaluated in the treated and untreated samples demonstrates a significant difference ($p<0.05$). The Wupa wastewater treatment plant's mean value for the heavy metals under study varied from 0.31±0.10 mg/L in treated wastewater to 0.37±0.20 mg/L in untreated wastewater. This indicates a substantial ($p<0.05$) difference in manganese levels between the influent and effluent. There was a substantial ($p<0.05$) difference in the mean iron value between the influent and effluent, ranging from 0.91±0.3 mg/L in the influent to 1.58±0.2 mg/L in the effluent. The treated and untreated wastewater exhibit a significant difference ($p<0.05$) in mean value of zinc ranging effluent wastewater. The findings indicate that *Aspergillus niger* grew to its maximum with an absorbance of 0.612 ± 0.62 and Bacillus subtilis to its maximum with an absorbance of 0.729 ± 0.01.The results of this investigation allow us to draw the conclusion that *Aspergillus niger* and *Bacillus subtillis* have the ability to withstand and grow on heavy metals, which helps to remove the metals from wastewater by absorbing them and increasing the biomass of the microorganisms.

Keywords: Bioremediation, Aquatic pollution, Heavy metals, Wastewater
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Introduction

As the population of the earth is increasing, the rate of waste release into the environment is increasing. Certain pollutants are discharged into water bodies, affecting the chemical and physical characteristics of the water. These wastes include organic waste and inorganic wastes (hydrocarbons and heavy metals). A diverse range of compounds, including chemicals, heavy metals, pathogens, medications, and minerals like phosphorus and nitrogen, are included in this category of contaminants (Adamu et al., 2022). As untreated or poorly treated wastewater infiltrates rivers, lakes, oceans, and groundwater, it disrupts the delicate balance of aquatic ecosystems and poses significant risks to human health (Vijayaraghavan and Yun, 2008). This pervasive issue poses multifaceted challenges that warrant immediate attention and proactive measures by authorities. At the beginning of the 20th century, household sewage from each house in rural and suburban areas was first treated using septic tanks. Later, a few towns along with companies realized that dumping sewage straight into streams may have negative health effects. Consequently, wastewater treatment plants, sometimes referred to as sewage treatment facilities, were constructed. (Chukwu and Oranu 2018).

The biological, physicochemical, and biochemical processes that are present in wastewater treatment plants make them intricate systems. (Chukwu and Oranu 2018). For such reason, the Federal Capital Territory (FCT) enacted the Wupa wastewater treatment plant (WWTP), To treat the wastewater, the microbial population in mixed suspension is essential to the facility's activated sludge process. The facility was built in response to the rising concern over wastewater treatment as Abuja developed into a worldwide metropolis. (Saminu et al., 2017). However, according to the world population review, this population has grown by almost 140% since 2010. It occupies an area of 297,000m^2 and receives primarily domestic waste from households and schools, including medical wastewater from hospitals in the metropolis. The population was expected to be 776,298 in the most recent census, which was conducted in 2006. However, between 2000 and 2020 estimates show that the population now exceeds 2.4 million, and this population is projected to grow to over 6 million by the year 2035 (Sadigov, 2022). This poses questions on whether the current WWTP has the capacity to clean properly, and the increasing volumes of influence it will continue to receive presently and in the coming years. For this reason, there is a need to explore other eco-friendly wastewater treatment techniques. One practical and long-term method for treating wastewater polluted with different types of contaminants is bioremediation. Its reliance on natural biological processes, coupled with continuous innovation, holds immense potential in addressing environmental challenges and ensuring the preservation of water resources for future generations (Ibrahim et al., 2024).

The main source of heavy metal contamination in rivers and streams is industrialization (Ibrahim et al., 2024). Heavy metals are stored in river sediments, and this might provide a secondary risk of metal contamination to the interconnected aquatic systems. (Wang, 2017). Because heavy metals are harmful and non-degradable by nature, heavy metal contamination in rivers has been the focus of several research and has attracted attention from all around the world. (Shafie et al., 2014).

Most Nigerian towns and cities have been plagued by the indiscriminate discharge of liquid garbage. Urban residents frequently release liquid waste into the environment with little thought to the consequences for social welfare or the ecology. Wastewater in the WUPA treatment plant frequently isn't treated before being released into the environment because of staff incompetence, equipment failure, power supply outages, high maintenance costs, and a lack of chemicals available for treatment (Ibrahim et al., 2023). Therefore, this research will contribute to the assessment of the introduced organisms' (*Aspergillus niger* and *Bacillus subtilis)* bioremediation capacity in the bioremediation of heavy metals present in wastewater.

Materials and Methods

Study Area

The study was conducted from December 2022 to August 2023 at the Wupa Wastewater Treatment Plant in Abuja, Nigeria. The Wupa WWTP is situated at coordinates 7°23"N, 9°01E in the Idu district of Abuja Municipal Area Council (AMAC). The effluent-receiving river, Wupa River, lies behind the facility. It was built to handle effluent from the Abuja Metropolis's Phases I, II, and III. (Chukwu & Oranu, 2018).

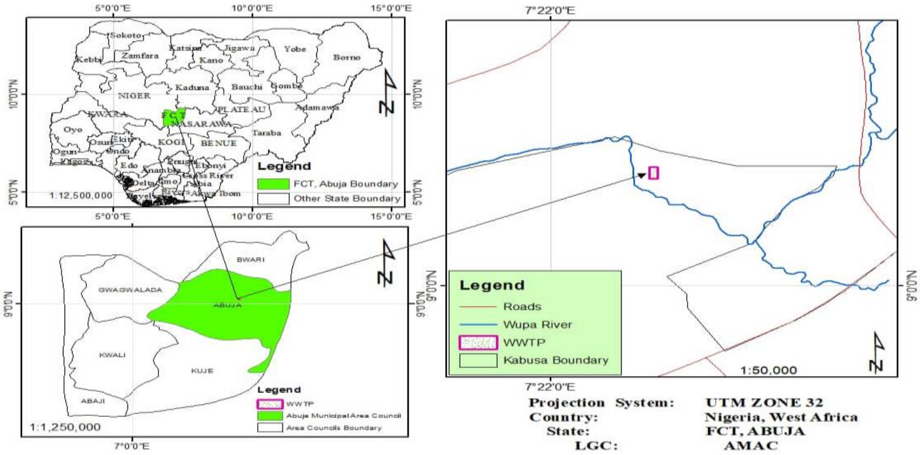

Fig 1: The Wupa Treatment Plant site is indicated on a map of the Federal Capital Territory.

Collection of Samples

The research involved the selection of two (2) distinct sample locations at the Wupa wastewater treatment plant: one for processed wastewater (effluent) and one for untreated wastewater (influent). After opening the sampling sites and letting the wastewater drain for 30 seconds, the wastewater was collected aseptically using a sterile 250 ml sample vial, which was promptly corked to prevent additional contamination. When the samples were collected, the temperature of the effluent was recorded. To analyse physicochemical parameters, microbiological characteristics, and heavy metals, water samples were taken in triplicate (Ezra et al*.,* 2021).

Determination of Heavy Metals Concentration in the wastewater

Using a triple acid solution in 10 milliliters (HNO_3, H_2SO_4 and $HClO_4$ in a 9:2:1 ratio, respectively), two (2) milliliters of wastewater (influent and effluent) were collected and digested until the effluent became colorless. Using two rounds of Whattman number 1 filter paper, the digested sample was put through Atomic Absorption Spectroscopy (SHIMADZU AA-7000 model) for heavy metal assay, in accordance with the standard protocol that the American Public Health Association (2014) suggested.

Reconfirmation of Aspergillus niger and Bacillus subtilis Isolates

Inactive isolates of *Aspergillus niger* and *Bacillus subtilis* were collected in a nutrient agar slant from the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. The isolates were made active by subculturing them into potato dextrose agar and nutrient agar that had been produced in accordance with the manufacturer's instructions. Following preparation and inoculation, the nutrient plates carrying *Bacillus subtillis* were incubated for 48 hours at 37 °C, and *Aspergillus niger* was subcultured on potato dextrose agar, which was incubated for 5 days at 25 °C. Molecular identification of the isolates was done using standard methods which include involved Genomic DNA extraction, DNA amplification region using Polymerase Chain Reaction (PCR) and Gel electrophoresis of amplicons.

Screening Aspergillus niger and Bacillus subtilis for Heavy Metal bioremediation potentials.

Stock solution of iron, zinc and manganese was prepared by dissolving 0.1g of iron, 0.1g of zinc and 50ml of manganese solution in a sterile conical flask containing 500ml of distilled water. The flask was concurrently heated to 121ºC while being gently shaken, and it was then sterilized for 15 minutes at that temperature. Until it was needed, the solution was kept in a refrigerator at 4ºC (Kumar et al*.,* 2017).

Heavy metal resistance screening was carried out for bacteria and fungi isolates by modification of the method reported by Amalesh et al*.* (2012). The ability of isolated bacteria and fungus to use wastewater was examined**.** The produced stock solution of heavy metals was added to sterile nutrients agar (NA) and potato dextrose agar (PDA), which were then put into sterile petri plates and solidified.

Agars enriched with heavy metals were infected with pure isolates. The isolates of bacteria were incubated at 35ºC for 48 hours, while the isolates of fungus were cultured for

5 days at room temperature (25±2 ^{0}C). After the period of incubation, the plates were monitored for growth for seven days, and absorbance was measured using Microplate reader (EMP M201). All the results were properly recorded. (Idowu and Ijah, 2017).

Preparation of Inoculum

Precisely 1.95g of nutrient broth was prepared in accordance with the manufacturer's guidelines. The broth was dissolved in 150 milliliters of distilled water and then autoclaved for 15 minutes at 121°C. Five milliliters of normal saline solution and ten milliliters of the nutrient broth were measured into a bijou bottle. Afterwards, pure *Bacillus subtilis* was inoculated into the bottle and placed in an incubator shaker for seven days to ensure that the organism grew in a suitable synergistic manner (Khosro *et al.,* 2011).

Flakes of potato dextrose broth (PDB) were collected and inoculated with two discs (1 mm in diameter) of *Aspergillus niger* colonies. The flasks were placed in an incubator shaker for 14 days at 25 ±2°C for synergetic growth. The broth was filtered using filter paper (Whatman No. 1) to extract the fungal mycelia that had grown in, and a Millipore filter (0.45 µm) was used for further filiteration. The filtrates were separated and stored in sterile flasks under sterile conditions (Cheesebrough, 2010).

Bioremediation potential of Aspergillus niger and Bacillus subtilis to utilize Wastewater from Wupa WWTP

Twelve sterile containers were used for the remediation procedure, each holding 1500 ml of wastewater inoculated with *Bacillus subtilis*, *Aspergillus niger*, both *Bacillus subtilis* and *Aspergillus niger,* and a control container (Khosro et al., 2011).

To aid in the biodegradation of the wastewater, 15 millilitres of inoculum were introduced. The growth and remediation potential were monitored every two days for eight days. Using an ultraviolet (UV) spectrophotometer calibrated at 600 nm to observe biomass development, the remediation ability of the selected species was assessed (Khosro et al., 2011).

Statistical Analysis of Data

Descriptive statistics were used to analyse the data using Microsoft Excel package 365. The standard error of mean (SEM) for the experimental data was shown as mean ±. Statistical Package for Social Science (SPSS) 23.0 was utilised to do one-way analyses of variance (ANOVA) to ascertain whether there was a significant difference ($p<0.05$) between the variables.

Results

Heavy metal analysis of wastewater collected from Wupa WWTP.

The wastewater (influent and effluent) from Wupa WWTP's mean values of the heavy metal's parameters, as shown in Table 1, revealed that Manganese, Iron and Zinc differs significantly ($p<0.05$) as the influent recorded high value than the effluent from the wastewater. All the measured heavy metal in both treated and untreated wastewater exceeds the WHO standard.

Table 1. Heavy metal characteristics of wastewater collected from Wupa WWTP

Heavy metals	Influent	Effluent	WHO standard
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	0.50±0.10	0.1

The gel electrophoresis of the two test organisms (*Aspergillus niger* and *Bacillus subtillis*) are represented in figure 2. Agarose gel electrophoresis of targeted ITS 1 and ITS 4 region for *Aspergillus niger had* amplified products of approximately 600 bp while *Bacillus subtilis* had amplified products of approximately 789 bp.

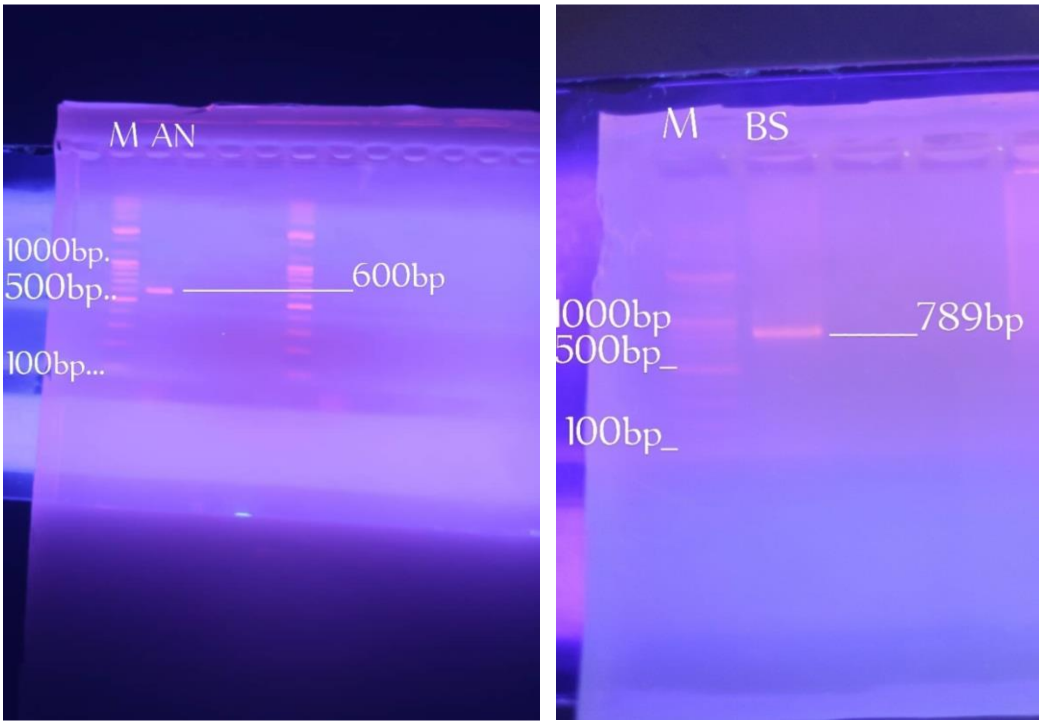

Plate 1: Agarose Gel electrophoresis of targeted ITS 1 and ITS 4 region for *Aspergillus niger* and *Bacillus subtillis* isolates using 100 bp Bioline Hyper Ladder

Table 2: Evaluation of *Aspergillus niger* and *Bacillus subtilis*'s capacity for heavy metal bioremediation in wastewater

Isolate	Growth observed after 7 days	Mean ± SD absorbance
Aspergillus niger	+++	0.612 ± 0.62
Bacillus subtilis	+++	0.729 ± 0.01

+++ Maxinium Growth; ++Moderate Growth; + Minimum Growth; - No Growth

The mean growth and absorbance of *Aspergillus niger and Bacillus subtilis isolates* were determined as presented in Table 2. *Bacillus subtillis* had the highest absorbance (0.729 ± 0.01) compared to *Aspergillus niger* (0.612 ± 0.62). The two test organisms demonstrated exuberant growth as indicated by (+++) in the seven days culture.

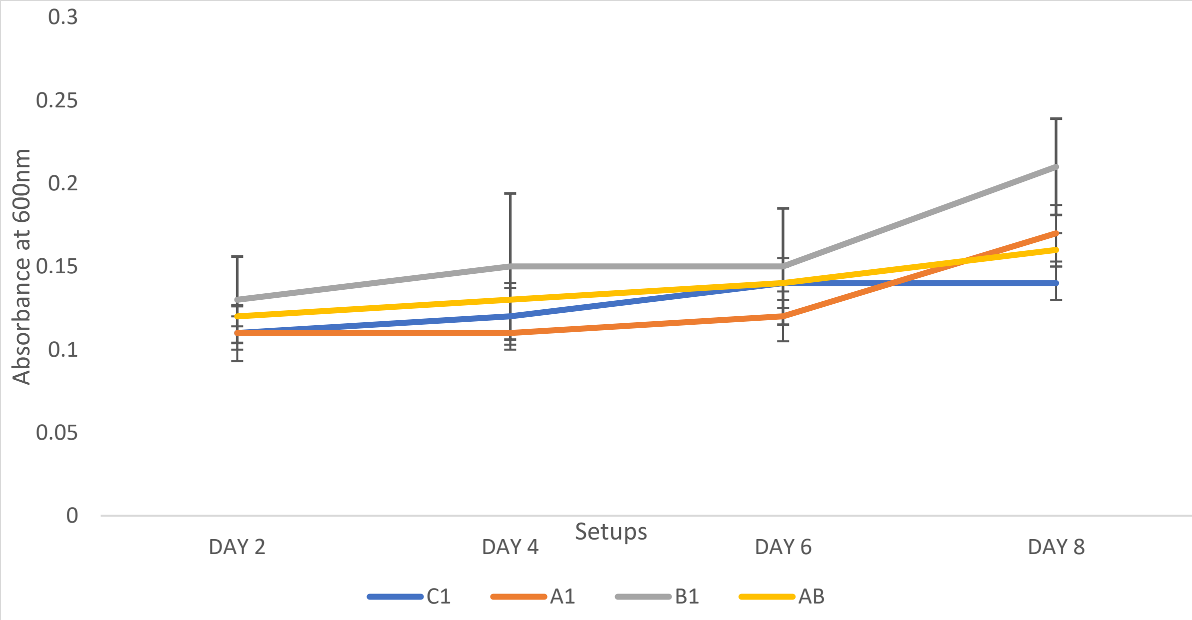

Fig 2: Mean Biomass growth of *Aspergillus niger* and *Bacillus subtilis* of Wupa WWTP influent.

Note. Key: C: Container for control; A: Treatment for *Aspergillus niger*; B: Treatment for *Bacillus subtillis*; AB: Treatment for *Bacillus subtillis* and *Aspergillus niger*

The mean biomass growth of the *Aspergillus niger and Bacillus subtilis* were measured in two days interval for eight days experimental setup, as presented in Fig 4.2. At day two (2), *Bacillus subtillis* (B1) had the highest growth with value of 0.13 followed by consortia of *Aspergillus niger* and *Bacillus subtillis* (AB) which recorded 0.12 while control (C1) and *Apergillus niger* (A1) were 0.11. At day four (4) B1 maintained the highest growth of 0.15 while AB increased to 0.13 and C1 increased to 0.12 and A1 remain unchanged with same value of 0.11 At day six (6) B1 maintained the highest but there was no increase (0.15) followed by an increase in C1 and AB which recorded value of 0.14 and B1 recorded lowest value of 0.12 At day eight (8) B1 recorded highest value of 0.21 followed by A1 with increase of 0.17, AB with increase of 0.16 while C1 maintained a value of 014.

Discussion

Heavy metals usually find their ways into freshwater bodies through human activities and some of the metals have tend to have effect on human and aquatic life causing serious problem to the environment (Chen et al., 2017; Du et al., 2020). The concentration of Zinc, Iron and Manganese recorded for the influent and effluent were seen to be above the WHO permissible value. so, in cases where the wastewater is not been treated and discharged to the environment, the high Iron content in water has been known to cause neuro degenerative conditions and contributes to soil acidification leading to loss of phosphorous (Abagale *et al.,* 2013). While, on the other hand, Manganese in water bodies has been known to cause oxidative stress and generally affects the soil structure.

The mean value of the studied heavy metal (manganese, iron and zinc) of Wupa wastewater treatment plant shows significant difference ($p<0.05$) between the influent and effluent as the effluent record low values after treatment. Manganese, iron, and zinc are commonly present in wastewater due to their occurrence in industrial discharges, storm water runoff, and domestic sewage (Sheoran et al., 2019). If not appropriately handled, their presence in wastewater can have a substantial negative impact on the operation of wastewater treatment facilities and provide environmental problems (Ibrahim et al., 2023). According to the data generated from this study, the concentrations of heavy metals are in decreasing order of magnitude. Fe > Mn > Zn. The findings of this investigation are consistent with those of Mustapha and Adeboye's (2014) study, which found elevated iron levels. The concentrations of Fe, Mn and Zn were all above the safe limit for WHO standards for effluent before discharge (WHO 2018).

The water treatment process which was an oxidative type (Saminu et al., 2017) was not sufficient in the reduction of heavy metals within WHO accepted limit of wastewater before discharge to environment. The result shows significant reduction of Iron (Fe), Manganese (Mn) and no reduction of Zinc (Zn) concentration in the effluent as against the high concentration reported for the influent.

Evaluation of Bacillus subtilis and Aspergillus niger's capacity for heavy metal bioremediation in wastewater

Bacillus sp. has been reported to also have high remediation capacity. In this study *Bacillus subtilis* had absorbance of 0.729 ± 0.01. This high absorbance 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 Momba and Kamika (2013) and Syed & Chinthala (2015) who reported high remediation activity in *Bacillus spp*. 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 & Chinthala, 2015). Many theories have been proposed to explain how microbes become resistant to metals (Syed & Chinthala, 2015). All of these techniques, meanwhile, generally work to either actively pump out the metal ions from the cell or to stop them from entering it (Roane and Pepper, 2000). The highest biosorption of heavy metals was shown by *Bacillus subtilis* in this investigation, with a value of 0.729 ± 0.01; these results are consistent with the findings of other studies (Chang and Chen 1998; Chang and Chen 1999; Syed & Chinthala, 2015).

Aspergillus niger is a fungus known for its various applications in biotechnology, food production, and industrial processes. In certain conditions, it can be involved in the absorption or bioaccumulation of heavy metals. In this study a maximum growth *Aspergillus niger* was observed on heavy metal stock solution with absorbance of 0.612 ± 0.62 within 7 days. This could be due to the mechanism of heavy metal absorption or bioaccumulation in *Aspergillus niger* involves several processes like adsorption, absorption, and complexation (Ali-Shtayeh, 1999). The cell wall of fungi typically contains various functional groups like carboxylic, amino, hydroxyl, and sulfhydryl groups that can bind and sequester heavy metals Ali-Shtayeh, 1999). Research into this area has shown promising results, suggesting that *Aspergillus niger* has the potential to be used in bioremediation processes to remove heavy metals from contaminated environments (Dhanushree & Hina Kousar, 2017). *Aspergillus niger* has been observed to accumulate zinc in varying concentrations, typically reported in the range of tens to hundreds of milligrams per kilogram of dry biomass (Dhanushree & Hina Kousar, 2017).

The biomass of isolates in different experimental setups of Influent wastewater shows a significant increase when compared to the control in all the three setups. The biomass of *Aspergillus niger* setup increased from 0.11- 0.14, *Bacillus subtilis* setup increased from 0.11-0.17 while the consortium of *Apergillus niger* and *Bacillus subtilis* recorded a biomass of 0.12-0.16. The increase in biomass of all the setup could be because of tolerance exhibited by the organism; these systems provide favorable environment for microbial growth and interaction with heavy metals (Faryal et al., 2006; Abdel-Raheem, & Shehata, 2020). Biomass can facilitate the process of bioaccumulation, where living organisms accumulate heavy metals within their tissues (Gadd, 1993). This accumulation helps in removing metals from the water, effectively reducing their concentration (Javaid et al., 2011; Singh et al., 2020). The biomass of *Aspergillus* species can effectively adsorb heavy metals onto its surface, thus reducing their concentration in the water (Gadd, 1993). The chelating and complexing abilities of *Aspergillus* biomass components contribute to its effectiveness in capturing and immobilizing heavy metals (Abdel-Raheem, & Shehata, 2020). Bioaccumulation of heavy metals in solution by living fungal cells offers better uptake of toxic metals than non-living biomass (More et al., 2010). Species of Aspergillus have been isolated from contaminated areas and their ability to withstand elevated levels of harmful metals has been evaluated (Pandey and Banerjee, 2012). Among fungi, their capacity for heavy metal bioaccumulation is unique (Al-Garni et al., 2009).

Conclusion

Assessment of the Wupa WWTP revealed crucial insights into its operational efficiency and environmental impact. The characterization of heavy metal contamination elucidated the prevalence of manganese, iron, and zinc within the wastewater, highlighting the need for targeted remediation strategies to mitigate potential ecological risks. The study's results indicate that the microorganisms in the wastewater exhibited the ability to withstand and proliferate on heavy metals, hence aiding in the elimination of such metals from the wastewater through absorption, however the removal by the introduction of *Bacillus subtillis* and *Aspergillus niger* yielded a better remediation of heavy metals. The results of the findings have reported the ability of the test organisms to remediate stock solution of heavy metals with the *Bacillus subtillis* having the highest absorbance of 0.729 ± 0.01. There was a significant increase when compared to the control in all the three setups. The biomass of *Aspergillus niger* setup increased from 0.11- 0.14, *Bacillus subtilis* setup increased from 0.11-0.17 while the consortium of *Apergillus niger* and *Bacillus subtilis* recorded a biomass of 0.12-0.16. The increase in biomass of all the setup could be as a result of tolerance exhibited by the organism; these systems provide a conducive environment for microbial growth and interaction with heavy metals and thus give aid to the organism in remediating heavy metals from wastewater.

Recommendation

1. Wastewater analyses should be conducted before discharging wastewater to the environment.
2. Explore the feasibility of scaling up the bioremediation process from laboratory experiments to pilot-scale studies, addressing practical challenges and optimizing the technology for real-world application.
3. Investigate the genetic and molecular mechanisms that underlie the organisms' heavy metal absorption and tolerance processes to pinpoint the genes or pathways that lead to effective bioremediation.

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