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Application of metal-tolerant bacteria isolated from aquaculture pond wastewater in the bioremediation of metal-containing effluent

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Abstract

The use of heavy metal tolerant bacteria for treatment of heavy metal wastes mostly from anthropogenic sources have gained prominence in recent years due to their efficiency and cheapness. This study was designed to investigate the ability of metal tolerating bacteria isolated from fish-pond wastewater to bioremediate paint manufacturing effluent. The physicochemical parameters of the fish pond wastewater, the metal concentration of both fish pond and paint-manufacturing were determined. Molecular characterization of the metal-tolerating bacteria was done by 16S rRNA sequencing. Single and consortium cultures of the metal-tolerating bacteria were used in the bioremediation setup for a period of 96 h. Three bacteria strains identified as *Bacillus thuringiensis* FPCO2 (BT), *Serratia liquefaciens* FPCO4 (SL) and *Bacillus anthracis* FPZN2 (BA), with minimum inhibitory concentration of ≥1000 µg/mL for all the tested metals were selected from the pool of isolates obtained and used for the bioremediation set-up. The metal removal rate significantly increased with time ($p<0.05$) with the consortium generally exhibiting higher metal removal than the single culture. The highest reduction for Cu (81%) was observed after treatment with BA+BT, while the highest reduction in the concentration of Fe (78%), Pb (84%) and Cr (75%) was observed in treatment with the consortium (BA+ SL + BT). Also, the highest reduction in Zn (97%) was recorded after treatment with BA+BT. The three metal-tolerant isolates obtained in this study have shown potential for the remediation of metal-containing wastewater with capacity to reduce the ecotoxicity of the effluent if released into the environment.

Keywords: Heavy metals, Fish pond, Paint-manufacturing effluent, Metal-tolerant bacteria, Consortium, Biosorption

1. Introduction

One of the major challenges faced globally is the presence of pollutants in the environment. The presence of these compounds, which could be naturally occurring or anthropogenically created, has severe adverse effects on humans, plants, and the microbiota. Among such compounds responsible for environmental challenges are heavy metals. Heavy metals are naturally occurring in the environment and have a density that is about five times greater than that of water [1]. The unguided and excessive use of metals in several industrial, agricultural, technological, and domestic applications have exposed humans to these compounds and this has led to increased concerns about the ecological and public health risk associated with the contamination of the environment by metal species [2]. Metals, unlike organic compounds, are non-biodegradable and persist in the environment for a very long period of time with deleterious effects on the microflora and fauna, especially when present at a toxic concentration [3].

Though there are several assumptions regarding the inter-relationship between heaviness and toxicity, heavy metals also include metalloids such as arsenic, that can induce profound toxicity even at a very low level of exposure [4] and mercury, that is a liquid at room temperature, yet relatively highly toxic. The toxicity of heavy metals results from changes in the conformational structure of nucleic acids, proteins or by interference with oxidative phosphorylation and osmotic balance in organisms [5]. The toxicity exerted by these

compounds are dependent on several factors which include, the dosage, medium of exposure, the relative availability of the metal and its form. Based on the toxicity of these compounds, metals such as arsenic, chromium, cadmium and mercury are among the metals that are constantly receiving attention from public health and environmental professionals [1].

He et al. [6] in their study on trace elements in agricultural systems reported that pollution of the environment with metals is more noticeable in point sources areas which include operations involving mining, smelting and other metal-based activities in certain industries. The major sources of these metals in the environment include atmospheric sources, pharmaceuticals, agricultural, industrial and the geogenic sources. This notwithstanding, weathering, and volcanic eruptions, which are natural phenomena, have contributed significantly to the challenge of metal pollution in the environment [2].

Paint manufacturing effluent often contains all the components of the manufacturing process such as residual acids, plating metals and toxic chemicals which are responsible for the high levels of organic compounds, suspended solids, coloured materials and heavy metals that are found in the resultant wastewater [7,10]. These components are known to be hazardous and if left untreated prior to discharge, pollute the surface, and sometimes leach into the subsurface environment and subsequently settle in the soil and sediment of water bodies [8].

Untreated or allegedly treated paint industrial effluents contain variable amounts of heavy metals such as arsenic, lead, nickel, cadmium, copper, mercury, zinc, and chromium [9] which have the potential to contaminate crops growing under such irrigation. These heavy metals have a marked effect on the aquatic flora and fauna which through biomagnification, enter the food chain and ultimately affect human beings as well. Heavy metal pollution is an ever-increasing problem of our oceans, lakes and rivers [10].

Several methods have been used in times past for the remediation of metal-containing wastewater and metal-polluted environment, and they include chemical precipitation (carbonate, hydroxide and sulphide precipitation), chemical oxidation and reduction, solvent extraction, reverse osmosis ion exchange, electrodialysis and adsorption [11]. However, these methods have been marred by several disadvantages, including generation of toxic secondary pollutants and lack of cost-effectiveness [12]. Several species of microbes have been proposed to be highly effective in the recovery and removal of heavy metals from soil and water compartments [13]. This has led research into the use of microbes especially bacteria for the clean-up of such environment owing to the cost-effective and ecofriendly nature of the approach, which is achieved via natural processes. Microorganisms engage different mechanisms such as biotransformation, extrusion, use of enzymes, production of exopolysaccharide and synthesis of metallothioneins to interact and survive in the presence of heavy metals [3].

Several bacteria strains such as *Bacillus cereus* MG257494.1, *Alcaligenes faecalis* MG9664410.1, *A. faecalis* Mg257493.1, *Bacillus safensis* have been reported to possess heavy metal biosorption properties and are thus useful for heavy metal containing waste water treatment [14,15]. Several investigations into paint manufacturing effluent treatment had focused on using adsorbent [16,17]. However, little investigation has been carried out on heavy metal removal from paint waste water using microorganisms. This study therefore was aimed at isolating metal-resistant bacteria from fish pond wastewater and utilizing the strains in the bioremediation of selected metals in a metal-containing paint-manufacturing effluent.

2. Materials and methods

2.1 Study sites and collection of samples

The study was carried out in Ado-Ekiti, Ekiti state, and Akure, Ondo state, Nigeria. The two states are located in the South-western part of Nigeria. Wastewater samples for the isolation of the metal-resistant bacteria were collected from selected aquaculture ponds in Ado-Ekiti (7° 37' 23.84" N 5° 13' 15.13" E), in sterile sample bottles for a period of one month. Samples were transported on ice to the Microbiology laboratory. Isolation of the bacteria was carried out within four hours of collection. The waste effluent which was used for the biosorption experiment was obtained from a paint manufacturing plant located in Akure, Ondo State (7° 15' 27.36" N 5° 10' 49.66" E).

2.2 Physicochemical and metal analysis of the wastewater

The physicochemical analysis of the wastewater was carried out using standard methods. The temperature of the samples was taken on site using a laboratory thermometer, while the pH and electrical conductivity were also measured on site using a pH meter and conductivity meter respectively. Other parameters such as chemical oxygen demand (COD), total suspended solids (TSS), total solids (TS), total dissolved solids (TDS), dissolved oxygen (DO) and biochemical oxygen demand (BOD) were determined using standard analytical methods for the examination of water and wastewater [18]. The concentration of metals in the samples was determined using the flame atomic absorption spectrophotometer according to the method of Hseu [19] after the samples have been digested using Nitric acid (HNO₃).

2.3 Preparation of metal solution

The stock solutions of the respective metals were prepared following the procedure of Narasimhulu et al. [20]. Appropriate quantity of the analytical grade, water soluble salts of the metals were dissolved in the required volume of water to make 1000 mg/L of each metal in solution. The solution was filter-sterilised using 0.22 μm membrane filter before the addition of the required volume to the cooled molten nutrient agar for the isolation procedure.

 2.4 Isolation and screening of bacteria on increasing metal concentration

Isolation of bacteria from wastewater samples was done on nutrient agar (Oxoid, UK) using the pour plate method, after the samples have been serially diluted. Aliquot (1000 μL) of the selected dilution was plated out on the culture medium and the set-up was incubated at 35±2°C for 24-48 h. Colonies developing on the medium were enumerated for the total heterotrophic bacterial count and recorded. Distinct colonies growing on the plates were selected and repeatedly sub-cultured until pure cultures were obtained. These were stored in nutrient agar slant for temporary storage. The isolates obtained were screened on increasing concentration (10-250 mg/L) of selected metals (chromium, zinc, lead, copper, iron, aluminum, and cobalt) for the determination of the minimum inhibitory concentration of the metals on the organisms. The medium was prepared by the addition of filter sterilized solution of the metals into already sterilized and cooled nutrient agar medium. The culture growing on a particular concentration was transferred to the next higher concentration until the isolates failed to grow on the meta-incorporated medium. This was done using the method of Narasimhulu et al. [20]. The set-up was incubated at 35±2°C for 24-48 h and observed for visible growth.

2.5 Molecular characterization of selected metal-resistant bacteria

Three bacterial isolates with codes; *Bacillus thuringiensis* FPCO2 (BT), *Serratia liquefaciens* FPCO4 (SL) and *Bacillus anthracis* FPZN2 (BA) with consistent growth and greater ability to tolerate increasing concentrations of all the metals were selected for the bioremediation studies. They were subsequently identified using the 16S rRNA sequencing.

2.6 DNA Extraction and Polymerase chain reaction (PCR) amplification

DNA of the selected isolates were extracted using the Jena Bioscience Bacteria DNA Preparation Kit. PCR was carried out in a GeneAmp 9700 PCR System Thermo Cycler (Applied Biosystem Inc., USA) using universal primers: Forward - 27F: 5'- AGAGTTTGATCMTGGCTCAG - 3' and reverse - 1492R: 5'- CGGTTACCTTGTTACGACTT- 3' PCR sequencing preparation cocktail consisted of 10 μL of 5× GoTaq colourless reaction, 3 μL of 25mM MgCl$_2$, 1 μL of 10 mM of dNTPs mix, 1 μL of 10 pmol each 27F 5'- AGAGTTTGATCMTGGCTCAG-3' and - 1525R, 5′-AAGGAGGTGATCCAGCC-3′ primers and 0.3 units of Taq DNA polymerase (Promega, USA) made up to 42 μl with sterile distilled water and 8μL DNA template. The PCR conditions were as follows: an initial denaturation step at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min and extension at 72°C for 1 min, and ending with a final extension at 72°C for 10 min [21]. The integrity of the amplified about 1.5Mb gene fragment was checked on a 1% Agarose gel. The amplified fragments were purified using ethanol to remove the PCR reagents. The purified fragment was checked on a 1.5% Agarose gel at a voltage of 110V for about 1hr, to confirm the presence of the purified product and quantified using a nanodrop of model 2000 (Thermo Scientific). The PCR products were stored at -80°C for further studies.

2.7 DNA sequencing and sequence analysis

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer (Applied Biosystems). The sequencing kit used was BigDye terminator v3.1 cycle kit. The sequences generated were compared to the national center for biotechnology information (NCBI) database by a basic local alignment search tool (BLAST) search to determine the identity of the isolates [22].

2.8 Bioremediation of paint-manufacturing effluent for the removal of metals

This experiment was carried out to test the ability of the three selected metal-tolerant bacteria in the removal of metals from a metal-polluted wastewater effluent samples from a paint manufacturing factory in Akure, Ondo State. The effluent was filter-sterilized using 0.25 μm Millipore membrane filters to remove microbial contaminants. Aliquot (10 mL) of 18h culture of the each of the three selected metal-tolerant bacteria (already

adjusted to 1.0 MacFarland standard was inoculated singly and in consortium into 1000 mL paint manufacturing effluent in sterilized Erlenmeyer flask. The set-up was incubated at 35±2°C for 96 h. The residual metal concentration was determined every 24 h using the flame atomic absorption spectrophotometer according to the method described by Syed and Chinthala [23]. Un-inoculated wastewater sample placed under the same condition served as the control. The heavy metal removal efficiency was calculated as percentage removal using the equation below:

$$\% \text{ HM removal } = \frac{\text{Initial HM concentration} - \text{Residual HM concentration}}{\text{Initial HM concentration}} \times 100 \qquad (1)$$

*HM denotes Heavy metal
The set up was as described below:
A: BA only (*Bacillus anthracis* FPZN2)
B: SL only (*Serratia liquefaciens* FPCO4)
C: BT only (*Bacillus thuringiensis* FPCO2)
D: BA + BT (*Bacillus anthracis* FPZN2 + *Bacillus thuringiensis* FPCO2)
E: BT + SL (*Bacillus thuringiensis* FPCO2 + *Serratia liquefaciens* FPCO4)
F: BA+ SL + BT (Consortium of the three selected isolates)

2.9 Scanning Electron Microscopy of cells exposed to and without metals

This was carried out following the method described by Akhtar et al. [24]. Prior to sample preparation 10 µL poly-L-lysine (0.1%) was deposited and spread on glass cover slips to form a basis for bacteria adherence on their surface. After the poly-L-lysine had dried, 20 µL of bacterial suspension was placed on the cover slips and left to dry again. The samples were prepared according to the following procedure: There was initial fixation in Karnosvsky solution for 36 h, followed by washing with cacodylate buffer (0.02 mol/L), with a post-fixation with osmium tetroxide for 1 h. This was followed by dehydration in acetone gradient and drying in a critical point apparatus. Gold metallization was performed to increase the electrical conductivity of the samples. Micrographs were taken with a Scanning Electron Microscope (LEO EVO 40 XPV).

3. Results and discussion

3.1 Physicochemical parameters of fish pond wastewater

Table 1 shows the mean physicochemical properties of the fish pond wastewater samples from which the metal-tolerating bacteria were isolated. The BOD, COD and DO were 152.3 mg/L, 185.30 mg/L and 7.5 mg/L respectively, while the TDS was 305.1 mg/L, TSS (38.7 mg/L), TS (328.36 mg/L), total alkalinity (20.25 mg/L), and total hardness was 62.00 mg/L. The mean temperature of the fish pond wastewater was 32.28°C, while the turbidity was 18.67 NTU, with conductivity measuring 108 mS/m. The mean pH of the fish pond water samples was 8.60. High levels of COD in the fish pond wastewater are indicative of poor water standards as it didn't comply with the set limits of 75 mg/L for COD [25]. Also, the high levels of BOD are an indication of possible contamination of the pond water by domestic sewage, crops, and animal waste.

Table 1 Mean physicochemical properties of fish pond wastewater samples.

Parameter	Fish pond wastewater
Temperature (°C)	32.28±0.04
Turbidity (NTU)	18.67 ±0.07
Conductivity (mS/m)	108±0.03
pH	8.60±0.02
TDS (mg/L)	305.1±0.05
TS (mg/L)	328.36±1.20
TSS (mg/L)	38.7±0.07
Total alkalinity (mg/L)	20.25±0.04
Total hardness (mg/L)	62.00±1.24
DO (mg/L)	7.5±0.04
BOD (mg/L)	152.3 ±0.06
COD (mg/L)	185.30 ±1.21

Note: Values are mean and standard error of three replicate samples.
DO: Dissolved Oxygen, BOD: Biochemical Oxygen Demand, TDS: Total Dissolved Solids, TSS: Total Suspended Solids, TS: Total Solids, ND: Not Detected.

The mean metal concentration in the fish pond wastewater is shown in Table 2. All the metals with the exception of cadmium were detected at varying concentration in the wastewater. The highest concentration of metal was with magnesium (9.50 mg/L), with the least concentration of metals apart from the one not detected was chromium (0.31 mg/L). The presence of lead in the pond wastewater could be as a result of lead pipes being used to supply water to the pond. Lead concentration at 0.35 mg/L in the water was found to be above the WHO standard limit of 0.01 mg/g. [26].

Ehiagbonare et al. [27] in their study of four fish ponds in Edo State observed that the values of the physicochemical parameters and heavy metals were not significantly different from the recommended acceptable standard for water and as such will not pose any health challenge as effluents. They however didn't detect cadmium and lead in the samples tested. Also, Saah et al. [28] also examined two fish ponds for heavy metal contamination as well as monitored the water quality. They detected Manganese and Zinc in all the selected fish ponds; with Mn levels in the ponds being significantly higher than the World Health Organization (WHO) recommended limit (< 0.500 mg/L). Also, Cr and Cd levels in two of the fish ponds exceeded the permissible limits with Pb and Cu levels detected were below the permissible limit. Metals such as Cr, Pb and Cd known to bioaccumulate in tissues and body of aquatic organisms in higher concentration than in the water [29]. This causes serious health problems in humans. The detection of Cd and Cr in some water samples pose a threat to both the aquatic organisms and humans.

Table 2 Mean metal concentration in the fish pond wastewater samples.

Metal	Concentration (mg/L)
Na	6.20±0.06
K	8.30±0.05
Ca	7.50±0.07
Mg	9.50±0.08
Zn	2.20±0.03
Fe	5.50±0.06
Cu	1.64±0.05
Pb	0.35±1.35
Cd	ND
Cr	0.31±0.04

Note: Values are mean and standard error of three replicate samples.
DO: Dissolved Oxygen, BOD: Biochemical Oxygen Demand, ND: Not Detected.

3.2 Molecular characterization of metal-tolerant isolates obtained

A total of thirty-five (35) metal-resistant bacteria were isolated from fish pond wastewater. All the isolates showed different responses on the media amended with different heavy metals. Some were resistant to only two heavy metals while some were susceptible to one or more heavy metals. Three bacterial isolates; BT, SL and BA with consistent growth and greater ability to tolerate increasing concentrations of all the metals on the metal-incorporated medium were selected for the bioremediation studies. The identity of the three selected metal-resistant bacteria obtained from the fish pond wastewater was confirmed after 16s rRNA sequencing and comparing to the NCBI database. BA and BT were *Bacillus anthracis* and *Bacillus thuringiensis* respectively with 99% similarity to the sequences already submitted to the GenBank. The third isolate was identified as *Serratia liquefaciens* SL with 65% similarity on the NCBI database. Piotrowksa-Seget et al. [30] reported that metal tolerance is often associated with members of the gram-positive genera such as *Bacillus, Arthrobacter, and Corynebacterium*. Ellis et al. [31] also reported that *Bacillus* sp. had a higher relative abundance in the heaviest metal-contaminated soil.

3.3 Metal concentration in the Paint-manufacturing effluent

Table 4 shows the initial metal concentration in the paint-manufacturing effluent before treatment with bacteria. Five metals namely Zinc (Zn), Iron (Fe), Copper (Cu), Lead (Pb) and Chromium (Cr) were the metal of interest selected for detection in the effluent. Of all the metals, the concentration of Cu was highest with a value of 8.22 mg/L, followed by Fe (5.20 mg/L) and Pb (5.08 mg/L). The remaining two metals, Cr and Zn, were present at a concentration of 3.76 mg/L and 3.40 mg/L respectively. Heavy metals are known to be toxic at high concentration. The oral route has been reported to be the main route through which heavy metals get into the human body system. People who fed on farm produce irrigated with untreated and partially treated wastewater have been found to bioaccumulate heavy metals with resultant health effects [32]. The level of Chromium, Copper and Lead detected in this study is higher than the permissible limits of 0.05 mg/L, 0.01 mg/L and <0.05 mg/L respectively thus suggesting the need for treatment before discharge into the environment. Tesfalem and Abdrie [33] investigated the heavy metal content of effluents from five paint industries and detected significantly high

levels of Lead, Chromium and Copper in the effluents which poses severe environmental and health risks if not properly treated. However, Aniyikaiye et al. [34] found out that the Copper, Lead and Chromium levels of waste discharge from 5 paint industries in Lagos, Nigeria were below detection limits.

Table 4 Initial concentration of metals in the paint-manufacturing effluent before bacterial treatment.

Metal	Concentration (mg/L)
Zn	3.40±0.05
Fe	5.20±0.02
Cu	8.22±1.16
Pb	5.08±1.24
Cr	3.67±0.07

Note: Values are mean and standard error of three replicate samples

3.4 Metal removal from the Paint-manufacturing effluent after bacterial treatment

Figure 1A shows the percentage of metals removed in the paint manufacturing effluent treated with BA. At the end of the 96-h experimental duration, the percentage removal of Cr was 70%, Pb (72%), Zn (87%), while for Cu and Fe, it was 43% and 96% respectively. The percentage removal of the five selected metals in the paint manufacturing effluent when treated with SL is shown in Figure 1B. The removal percentage for each of Cr and Fe was 64%, while for Cu, Zn, and Pb was 68%, 53% and 65% respectively.

Figure 1C shows the percentage removal of metals from the paint manufacturing effluent by BT. After the 96-h experimental duration, Cr had a percentage removal of 66% and Pb (68%). Zn, Cu and Fe had a percentage removal of 48%, 76%, and 59% respectively. The percentage removal of the five selected metals to the treated paint manufacturing effluent by a combination of BT and BA is shown in Figure 1D. The removal percentage of Cr and Fe after the 96-h experimental period was 69%, while Cu, Pb and Zn was 81%, 75%, and 97% respectively. Bacteria have been found to possess inherent or acquired ability to modify toxic metal ions into their respective non-toxic forms using ATPase dependent reduction pathways, thus ensuring their adaptation and survival in the environment [35]. Most microbes use the efflux mechanism located on the cell surface, and genes for tolerance have been discovered on both chromosomes and plasmids [36]. Some bacteria can combine detoxification and production of chelating agents that bound metals and reduce their toxicity [37].

Bhutada et al. [38] in their investigation observed that six bacteria isolates recovered from industrial effluent were able to remove up to 60% of the metals copper, zinc, cadmium up to a concentration of 2000 mg/L after 72 h of incubation and that mutated species could remove higher concentrations. Also, Kookhaee et al. [39] isolated *Lactococcus lactis* from tannery effluent that was able to remove 52.5% of Chromium (VI) with a minimum inhibitory concentration (MIC) value of 62.5 mg/L via biosorption. Orji et al. [37,26] investigated two bacteria strains *Paenibacillus* sp. and *Morganella* sp. recovered from a mining site and found their trend of heavy metals removal to be Pb (96.84% and 97.48%) Cu (86.36% and 100%), zinc (84.85% and 80.85%), cadmium (70% and 68.45%) respectively after 72 h of culturing in the heavy metal containing medium.

Figure 1E shows the removal percentage of the metals from the paint manufacturing effluent treated with BT and SL. There was a progressive reduction of the metals over the experimental duration. At 96 h, Zn and Pb had a percentage removal of 88% and 83% respectively, while Cu, Cr and Fe had 78%, 75% and 65% removal respectively. The percentage removal of metals from the treated paint manufacturing effluent by a consortium of BT, BA and SL is shown in Figure 1F. After the 96-h period, the percentage removal of Cu was 79%, Zn (96%), Pb (84%), while 75% and 78% of the initial concentration of Cr and Fe were removed respectively. Highest removal rate by the mixed cultures suggests synergy between the bacteria but prolonged incubation of the mixed culture could not improve the removal rate because of possibility of release of metabolites from one bacterium which can be inhibitory to the other, hence reduction in bioaccumulation rate. Bacteria are more stable and survive better when they are in mixed culture [3]. Several researchers have reported better metal removal results by bacterial consortium. In a study by Ge et al. [40], they observed that bacterial consortium had a better biosorption efficiency for removal of Cu (92.7%), Zn (90.3%) and Pb (86%) than single bacteria isolates. Kader et al. [41] also reported that, the effect of metal removal by consortia was comparatively better than the individual bacterial strains because of the combined efforts by the bacterial strains. Therefore, bacteria consortium is metabolically superior for biosorption of metals and are more appropriate for in situ bioremediation than single cultures.
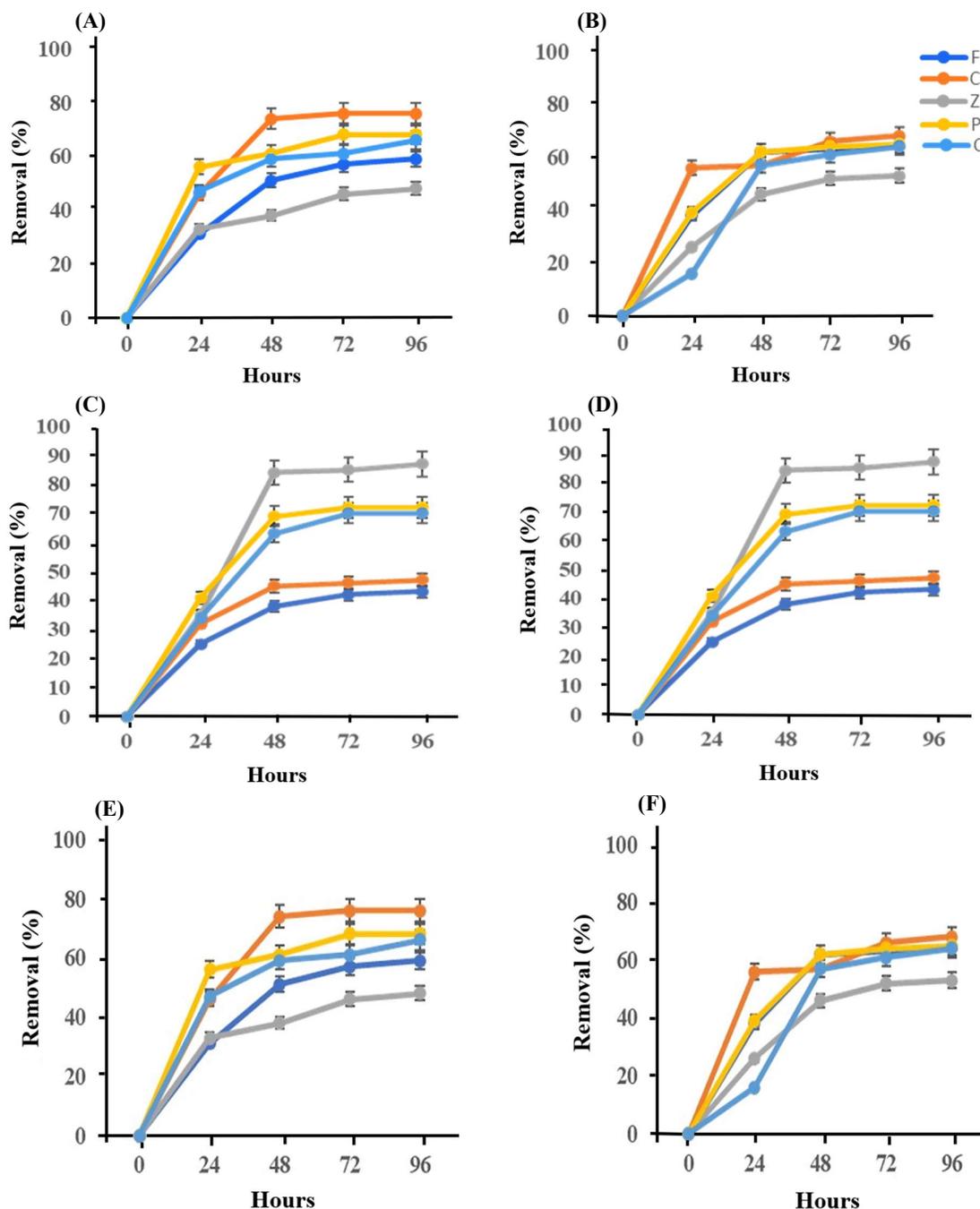
Figure 1 Percentage removal of metals from paint manufacturing effluent using (A) *Bacillus anthracis* FPZN2 *(B) Serratia liquefaciens FPCO4,* (C) *Bacillus thuringiensis FPCO2,* (D) combination of *Bacillus thuringiensis FPCO2 and Bacillus anthracis* FPZN2, (E) combination of *Bacillus thuringiensis* FPCO2 *and Serratia liquefaciens* FPCO4, and (F) the bacterial consortium.

3.5 Scanning Electron Micrograph of bacterial cells after treatment of paint-manufacturing effluent

Scanning electron microscopy (SEM) micrographs were used to analyse cell surface morphology before and after heavy metal biosorption [42]. This study showed that the shape of bacterium cells was modified after the adsorption of the heavy metals as shown in Figure 2(A-C) and (D-F). In a similar study by Chakravarty et al. [43], there was a clear change as depicted by the rough cell surface and membrane indentations observed in the outer surface of *Acidiphilium symbioticum* under metal stress condition. Also, Renu et al. [44] observed distortional changes in cell surface morphology of the bacterial isolate when observed under SEM after exposure to cadmium

with bulging of the cell surface which they suggested could be due to high exopolysaccharide production under Cd stress. Sinha and Mukherjee [45] reported that *Bacillus* sp. contains cell wall components which can be responsible for their ability for heavy metal bioremediation through the secretion of extracellular substances while Chang et al. [46] reported that in *Burkholderia cepacia* GYP1 cells were intact although there was distortion in the cell structure due to heavy metal toxicity.
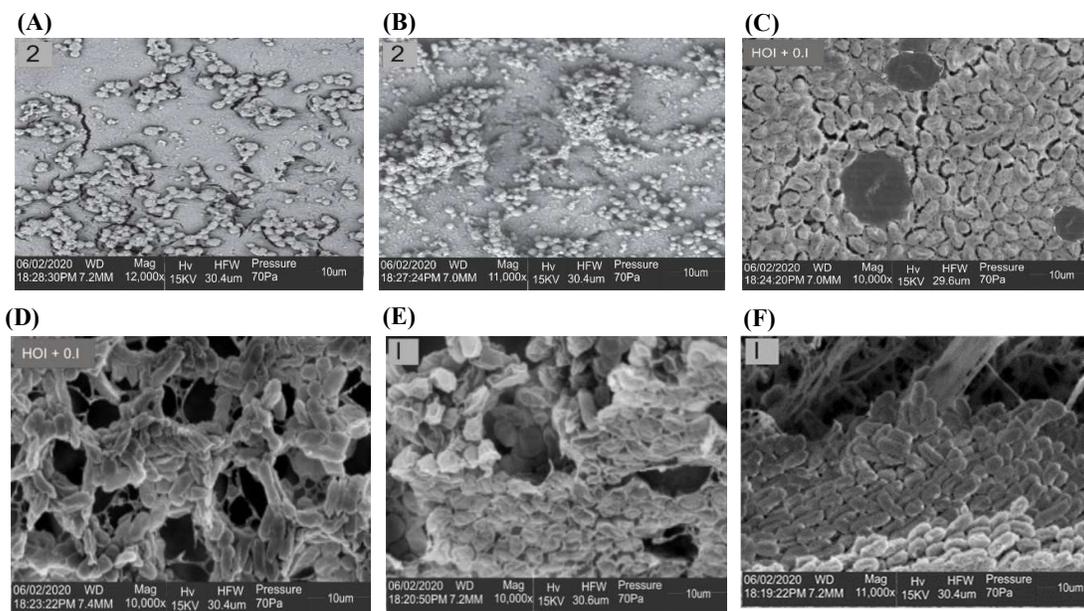

Figure 2 Scanning electron micrograph of (A) *Bacillus thuringiensis* FPCO2 (B) *Serratia liquefaciens* FPCO4 (C) *Bacillus anthracis* FPZN2 without metal stress, (D) *Bacillus thuringiensis* FPCO2, (E) *Serratia liquefaciens* FPCO4, and (F) *Bacillus anthracis* FPZN2 under metal stress.

4. Conclusion

The results from this study revealed the presence of some heavy metals in fish pond at concentrations which exceeded permissible limit. Bacterial isolates recovered from the fish pond exhibited growth patterns in metal amended medium with varied responses to these toxic compounds per time. Three of the isolates identified as BT, SL and BA based on 16S rDNA sequence displayed remarkable multiple resistance to some heavy metals. Although they singly removed significant amount of the heavy metals in paint effluent, the astounding removal rate of their mixed culture was remarkable. Changes in sizes and shapes also confirmed the adsorption of the metal ions in their cells. The benefit of using bacteria cells in clean-up was also highlighted in this study and established the role of these isolates in the adsorption, accumulation, degradation, and detoxification of heavy metal in paint wastewater. This study is among the very first to describe the potential of bacteria isolates in detoxification of paint manufacturing effluent which is rich in heavy metals. Consequently, the unique characteristics of these bacteria make them potential tools for the simultaneous removal of more than one heavy metal from a contaminated environment. They can also be harnessed in the removal of heavy metals from fish ponds to prevent bioaccumulation of heavy metals in fishes. Further studies towards the use of proteomics, genomics to discover genes, proteins, metabolites that could be involved in heavy metal tolerance by these bacteria is also necessary.

5. Acknowledgements

6. References

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