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Original Research Article

Metalotolerance Capacity of Autochthonous Bacteria Isolated From Industrial Waste Effluent

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Abstract

Microbes play significant roles in remediation of heavy metal polluted industrial effluent using the mechanisms of biosorption and bioaccumulation. In the present study, six heavy metal resistant autochthonous bacteria species namely *Bacillus cereus, B. megaterium, B. subtilis, Flavobacterium aquatile, Pseudomonas flourescens and Pseudomonas putida* were isolated from effluent samples collected from Paper-mill industry (PMI), Paints and Chemicals Industry (PCI), and Steel-rolling Industry (SRI). The isolates were studied for their heavy metal tolerant capacities at different aqueous salt concentrations. Elemental analysis of the industrial effluent samples collected indicated the presence of heavy metals such as Copper (Cu^{2+}), Manganese (Mn^{2+}), Iron (Fe^{2+}) and Lead (Pb^{2+}) at varying concentrations in µg/ml. Generally, there were variations in the minimum inhibitory concentrations (MIC) of the heavy metal salt to each of the bacteria understudy. The MIC value of each of the bacterial isolates to aqueous solution of Cu_2SO_4 showed that *B. megaterium, B. subtilis, Pseudomonas flourescens* and *Pseudomonas putida* had the same MIC value of 20 ± 1.5 µg/mL while *Bacillus cereus* and *Flavobacterium aquatile* had MIC values of 13 ± 1.3 µg/mL and 25 ± 2.1 µg/mL respectively. This variation was also noticeable in aqueous salts of Mn_2SO_4, Fe_2SO_4 and Pb_2SO_4.The bacteria isolates showed sensitivity to heavy metals with increasing zone of inhibition as concentration increased with each isolate showing varying degree of metalotolerance. The effectiveness of the autochthonous bacteria as a means to bio-augment the remediation of heavy metal polluted industrial effluent was further proven and recommended.

Keywords: Industrial effluent; Autochthonous bacteria; Metalotolerance; Waste.

1.0 Introduction

Our environment faces careless increase in pollution level on account of uncontrolled increase in population, urbanization, industrialization and anthropogenic activities (Hanif *et al.,* 2005; Benson, 2006).Among the various pollutants, heavy metal contamination is one of the most critical environmental challenges in the twenty first century. Modern industries are majorly responsible for this problem of pollution as they continually and indiscriminately release pollutants into the ecosystem. Industrial effluents contain a large variety of toxic compounds; of which heavy metals with potentials to reach hazardous levels when accumulated in the environment is typically found (Nriagu and Pacyna, 1988). Heavy metals are elements with specific gravity equal to and greater than 5.0 and relative atomic weights ranging from 63.5 to 200.6, they are usually hazardous toxicants to humans, animals and the environment at a certain level.

Due to increase application of heavy metals in industrial processes, there is a steady rise in environmental pollution caused by heavy mental contamination from these processes.The major sources of heavy metals contaminants are indigenous waste waters and effluent from pharmaceutical industries, mines, metal processing industries, leather tanneries, pesticides, organic chemicals, rubber and plastics industries, lumber and wood production sites (Ansari *et al.*,2004; Vullo *et al.*, 2005).Heavy metals contamination spreads in the environment by run-offs which join other water bodies thereby contaminating these water sources (Downstream processes from industrial sites). Agricultural activities such as using agrochemicals and sewage sludge on farm land and cultivated plants also add substantial amount of these toxic metals to soils (Duxbury, 1994).

The problems of heavy metal as a pollutant cannot be overly emphasised because of its toxic nature to human and animal health and the general wellbeing of the ecosystem, this is because of their high occurrence as a toxicant, low solubility in the environment and, the classification of numerous members of these group as mutagens and carcinogens (Alloway, 1995). Heavy metals causing critical concern to human health and well-being are arsenic, mercury, lead, cadmium, chromium, copper and zinc (Lazrova *et al.*, 2005). It is estimated that more than a billion people are one way or the other subjected to elevated concentration of heavy metals and metalloids and millions of people all over the world may be suffering from sub-clinical metal poisoning (Nriagu, 1988; Nriagu and Pacyna 1988). Only recently about some few decades ago that increasing recognition of the health hazards and environmental consequences of heavy metals release into the environment has impelled the necessity for the decontamination of industrial effluent prior to release into natural water bodies.

Three types of metals are of more interest in the heavy metal pollution and these include:

- Toxicant such as Arsenite (As), Cadmium (Cd), Lead (Pb), Mercury (Hg), Chromium (Cr), Nickel (Ni),Copper (Cu), Cobalt (Co), Zinc (Zn), Tin (Sn) etc.
- Precious metals such asGold (Au), Silver (Ag), Palladium (Pd), Platinum (Pt), Ruthenium (Ru)etc.
- And radio nuclides such as Uranium (U),Radium (Ra),Thorium (Th), Americium, etc (Wang and Chen, 2006).

Physicochemical methods for removal of heavy metals from waste waters such as lime coagulation ion exchange, reverse osmosis, solvent extraction, and chemical precipitation cost a lot and these unfortunately do not remove heavy metals from waste water to the desired level (Kadirvelu *et al.*, 2001). It was only recent times that a new scientific era developed a technique that could help decontaminate and recover heavy metals from industrial wastes, this is the process of adopting and adapting natural biological processes to remediate the problems of heavy metals in industrial wastes before releasing the wastes into the natural environment. Biological method is an innovative technology that has been ever present for heavy metal polluted wastewaters remediation but was recognised only recently. Since microorganism have adapted survival tactics in habitats polluted with heavy metals, their different heavy metal degrading/detoxifying mechanisms such as bio-sorption, bioaccumulation, bio-mineralization or biotransformation can be applied either *in situ* or *ex situ* to design bioremediation processes that is economically viable (Crawford and Crawford, 1995;Lin and Lin,2005; Munoz, *et al.*, 2006; Umrania, 2006).The application of Bio-sorption in heavy metal remediation of waste water makes the subsequent recovery of these metals easier and economically feasible. The application of bio-sorption exploits the characteristics of microbes to adsorb metals, this can be applied commercially.

Since heavy metal tolerant microorganism can be found in heavy metal contaminated sites and industrial effluent, samples from the effluent and sites can be taken and analysed to identify the metals and the microbial population present in them. Hence, this paper investigates autochthonous microbes isolated from effluent samples and their capacity for the removal of heavy metals *in vitro* using aqueous heavy metal salts solutions at different concentrations.

2.0 Materials and Methods

2.1 Samples Collection

Collections of samples were carried out in three locations in South-Western Nigeria; this is as follows: Paper-mill industry (PMI), Ikeja Industrial Estate, Ikeja, Lagos State, Paints and Chemicals Industry (PCI), Agege, Lagos State and Steel-rolling Industry (SRI) at Osogbo, Osun State. The samples were collected into sterile 50ml bottles, taken to the laboratory and maintained at 4 ^{o}C to stop further biological activity. The pHs of the effluent samples was determined using pH meter.

2.2 Isolation and identification of Metalotolerant autochthonous bacteria from industrial effluent

In isolating bacteria from the industrial effluents, samples were cultured on nutrient agar media by serial dilution. 1mL of each sample of effluent was diluted serially with 9 mL of distilled water to a concentration of 10^{-1} to 10^{-5} in separate test tubes. Pour plate method was used by introducing 100 µL of the serial diluents effluent of concentration 10^{-3}, 10^{-4} and 10^{-5} respectively into separate plates before introducing the nutrient agar media, then incubated at 35 ^oC for 24 to 72 h. Isolates were sub-cultured onto fresh nutrient agar media and were characterized and identified based on their morphology, cultural, macroscopic andmicroscopic appearanceandtheir biochemical metabolic reactions.

2.3 Heavy metal determination by Atomic Absorption Spectroscopy

The concentrations heavy metals in each of the effluent samples were analysed using Atomic Absorption Spectroscopy (AAA) (Saxena, 1998; APHA, 1998). In doing this, samples were digested, and ultra-pure distilled water was added to make up to 30 ml of each sample that was dispensed into vials for the analyses. The elements determined include: Copper, Lead, Manganese, Iron, and Chromium.

2.4 Determination of heavy metal-resistant bacteria

This experiment was carried out using the method of Hassen *et al* (1998). Aqueous solutions of the determined heavy metals salts ($CuSO_4$, $PbNO_3$, $FeSO_4$ and $MnCl_2$) were prepared. Wells were aseptically dug into freshly inoculated Nutrient Agar plates and to each well, 100 µL of each prepared metal salt solution were introduced, the plates were incubated for 24 h at a temperature of 37 °C. The minimum inhibitory concentration (MIC) of each of the metal salts against each of the tested isolates were observed and recorded.

3.0 Results and Discussion

The adverse environmental impact of heavy metal pollution on human, animals and plant health and the general well-being of the environment should be swiftly and seriously dealt with by applying bioremediation techniques to abate the lethal levels of heavy metals in sites that are contaminated and on industrial wastes before they are released into the environment (Pandi, *et al., 2009*). From our work, elemental analysis of the industrial effluent samples collected indicated the presence of heavy metals such as Cu^{2+}, Mn^{2+}, Fe^{2+} and Pb^{2+}at varying concentrations in µg/ml as shown in Table 1. In PCI and PMI effluent samples, Fe^{2+} had the highest heavy metal concentration of 0.4 µg/mL and 0.5 µg/mL and least is Mn^{2+} (0.02 µg/mL) and Pb^{2+} (0.05 µg/mL) respectively. SRI also showed a similar trend of result with Fe^{2+} having the highest concentration (0.4 µg/mL) and least is Pb^{2+} (0.02 µg/mL). This indicates that Fe^{2+} is a prominent heavy metal present in PCI and SRI during industrial production processes.

Table 1: Heavy metal concentration. present in industrial effluent

Heavy metal	Source of effluent		
	PCI (µg/ml)	PMI (µg/ml)	SRI (µg/ml)
Mn^{2+}	0.02	0.40	0.03
Fe^{2+}	0.40	0.50	0.40
Pb^{2+}	0.03	0.05	0.02
Cu^{2+}	0.20	0.40	0.20

Paint & Chemical Industry PMI: Paper mill Industry SRI: Steel-rolling Industry.

Heavy metal concentrations in the environment have been reported not only to be hazardous, but as threat to the biotic component of the ecosystem. Adverse effects of heavy metals comprise, bio-accumulation in the tissue of many organisms, this leads to bio-magnification of toxic metal in the tissue of living organisms (Panikar *et al*., 2003) from which other adverse effects such as immunosuppression, carcinogenicity (Peakall, 1992), neurotoxicity which has high frequency among children (Cohen, 2005), enzyme inhibition, especially inhibition of some very important enzymes associated with synthesis of vital bio-molecules develops. In this study, six bacteria species were isolated from all the industrial effluents used in this study namely: *Bacillus cereus (Bc), B. megaterium (Bm), B. substilis (Bs), Pseudomonas flourescens (Pf), P. putida (Pp)* and *Flavobacterium aquatile (Fa).* Shukla *et al* (2007) in his work, reported to have isolated four chromate-tolerant bacteria strains which exhibited multiple metal and antibiotic resistances, Shukla's isolated bacterial strains in his paper shukla *et al* (2007) was reported to accumulated chromate to a high degree demonstrating that they have very promising potential in detoxification and recovery of chromate (Shukla, *et al.,* 2007). Of all the bacterial isolated for this research, only *Bacillus spp.* was dominant (Table 2).

Table 2: Microbial composition of effluent samples

Sources of effluent	Microbes present
PCI	*Pseudomonas fluorescence, P. putida*
PMI	*Bacillus cereus, B. megaterium*
SRI	*B. subtilis, Flavobacterium aquatile*

The bacteria were characterized and identified using colonial morphology, microscopy and biochemical tests. The microbial isolates showed varying degree of tolerance to aqueous heavy metal salts using plate diffusion method at different concentration as shown in figures 1-4. Kao et al (2008) carried out similar work when him and his team used a MerP expressing recombinant *Escherichia coli* which originated from Gram-positive (*B. cereus*) and Gram-negative (*Pseudomonas* sp.) were used to adsorb Nickel, Zinc and Chromate in aqueous solution (Kao *et al.,* 2008). Figure 1 showed the minimum inhibitory concentration (MIC) of each of the bacterial isolates to aqueous solution of Cu_2SO_4 with Bm, Bs, Pf and Pp having the same MIC value of 2 ± 1.5 µg/ml while Bc and Fa had MIC values of 13 ± 1.3 µg/mL and 25 ± 2.1 µg/mL respectively. MIC values of aqueous solution of Mn_2SO_4 to the microbial isolates showed that Bm, Bs, Pp had MIC values of 100 ± 6.7 µg/mL, Bc and Fa had MIC values of 50 ± 5.5 µg/mL while Pf had MIC value of 20 ± 2.6 µg/mL (Fig. 2).

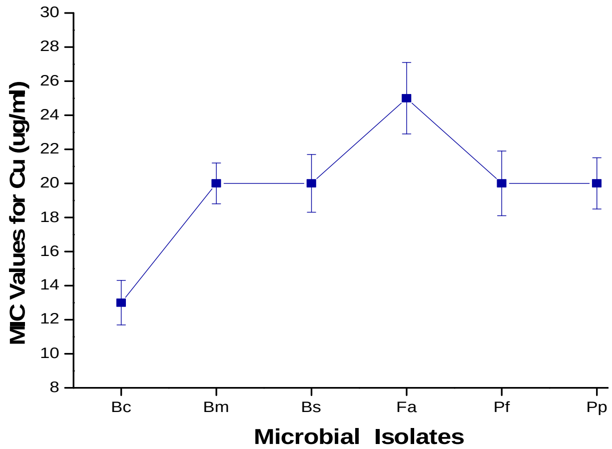

Fig. 1: MIC values of microbial isolates to aqueous salts of Cu_2SO_4.

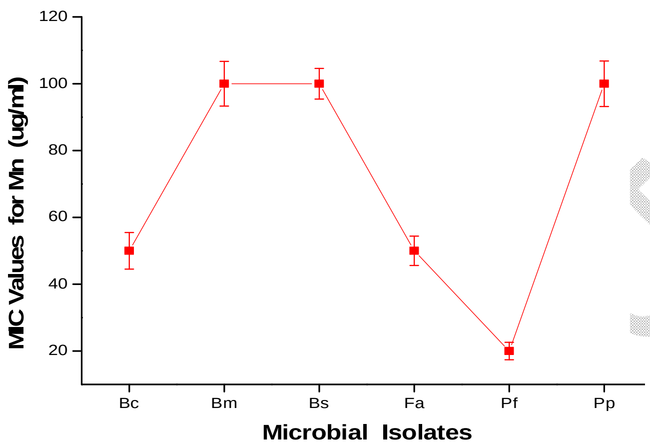

Fig.2: MIC values of microbial isolates to aqueous salts of Mn_2SO_4.

Figure 3 also shows the MIC values for Fe_2SO_4 in which Bm, and Pp had MIC values of 25 ± 2.5 µg/mL, Bs and Fa had MIC values of 50 ± 3.7 µg/mL, while Bc and Pf had MIC values of 100 ± 5.7 µg/mL and 25 ± 1.8 µg/mL respectively. The MIC values of the bacterial isolates to Pb_2SO_4 is as shown in Figure 4 in which Bc, Bm, Bs and Pp had MIC values of 20 ± 2.5 µg/mL while Fa and Pf had MIC values of 13 ± 1.5 µg/mL and 50 ± 4.2 µg/mL respectively.

Earlier work by Pardo (2003) also reported that inactive biomass of *Pseudomonas putida* has bio-sorption potentials for heavy metals. Similar studies involving the removal of Copper ions in effluent by *P. putida*, showed that *P. putida* have the capacity to remove over 80% of copper ions in copper contaminated wastewater. Results similar to this were also obtained from a study where *Trametes versicolor* were studied for their metal remediation properties, it was reported that copper ions, Lead ions, and Zinc ions were removal (Gulay,2003), *Bacillus* has been reported to effectively remove chromium (Shukla, 2007; Gulay, 2003), in another report, *Funaliatrogii* was found to have bio-sorption potential for Mercury, cadmium, and Zinc ion (Selvaraj, 2003), while *Bacillus firmus* can bio-absorb Lead, Copper, and Zinc (Salehizadeh, 2003).

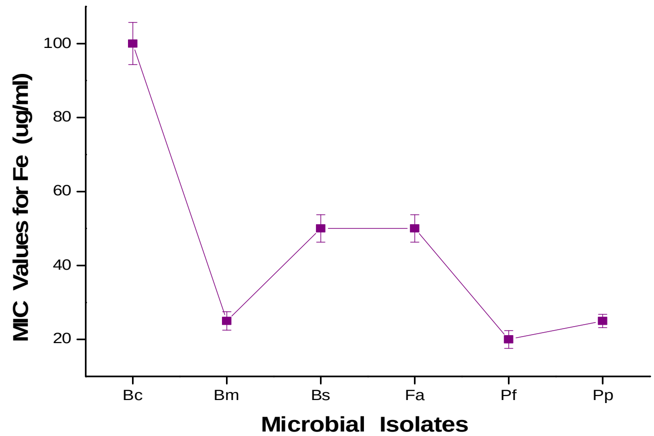

Fig. 3: MIC values of microbial isolates to aqueous salts of Fe_2SO_4

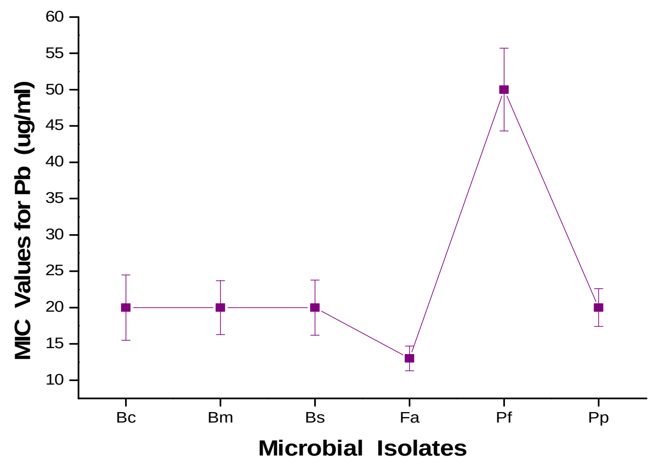

Fig. 4: MIC values of microbial isolates to aqueous salts of Pb_2SO_4

In general, micro-organisms uptake these metals either actively (bioaccumulation) or passively (bio-sorption). This is can be linked to bacteria surfaces affinity to metals ions resulting to their metal adsorbing and precipitating abilities. All these information discussed coupled with the result of this study clearly indicate that isolated bacteria like *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Flavobacterium aquatile*, *Pseudomonas flourescens*, *Pseudomonas putida* has proven to be a good development for efficient bio-sorbent for the removal and recovery of heavy metals.

4.0 Conclusion

Isolated microbes from this work such as; *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Flavobacterium aquatile*, *Pseudomonas flourescens*, and *P. putida* showed significant tendencies for use in metal-toxicity reduction and remediation processes.

5.0 Conflict Of Interest

All Authors have declared that there are no conflicts of interest.

Authors Contribution

ONM designed the experiment

EAA provided the data

AA and LE were involved in the discussion of results

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