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Full Length Research Paper

A study of heavy metal tolerant bacteria and their potential for bioremediation

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Panteka stream is a flowing stream polluted with wastes from the activities of mechanics. Water samples collected at different points of the stream were analysed in order to determine the level of heavy metal contamination and bacteria diversity with the view to elucidating the bioremediating potentials of the bacteria isolates. Four bacteria, tolerant to heavy metals, were isolated from Panteka stream. These were identified by morphological and biochemical techniques as *Staphylococcus epidermidis, Serratia marcescens, Proteus mirabilis* and *Escherichia coli.* The 16S rRNA gene sequencing and Basic Local Alignment search tool (BLAST) result confirmed *E. coli* and *Staphylococcus* spp. as heavy metal tolerant bacteria. Heavy metal tolerance analysis of the isolates exposed to nickel, zinc, lead, cadmium and iron showed that the isolates had maximum tolerance to the four heavy metal. Studies on bioremediation potential of the isolates to heavy metals in the stream revealed that mixed bacteria culture completely removed lead, nickel, zinc and cadmium. Analysis of pure isolates revealed *S. epidermidis* to be the most effective in removing lead (100%), nickel (100%), cadmium (90.29%), zinc (84.95%) and iron (54.82%). The results obtained from this study show that all four bacteria species isolated from Panteka stream have potential for bioremediation of heavy metals in contaminated water.

Key words: Panteka stream, mechanic workshop, heavy metals, bacteria isolates, bioremediation.

INTRODUCTION

The recent expansion of human industrial activity, including mining, smelting, and synthetic compound creation, has led to an exponential increase in the amounts of heavy metals released into the atmosphere, water, and soil (McConnell and Edwards, 2008). Many countries have regulatory guidelines for heavy-metal presence and exposure as well as remediation and treatment options. Screening of soil and water sources is conducted frequently to prevent overconsumption, but many of these programs and technologies are not readily available in developing nations (Li et al., 2006).

The term toxic heavy metal have particular application to cadmium (Cd), mercury (Hg), lead (Pb) and arsenic (As), all of which appear in the World Health Organization's

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list of 10 chemicals of major public concern. Other examples include manganese, chromium (Cr), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), selenium (Se), silver (Ag), antimony (Sb) and thallium (Ti). These heavy metals are non-degradable and must be reduced to acceptable limits before discharging into environment to avoid threats to living organisms (Alam et al., 2012).

The challenge of ensuring usable water in sufficient quantities to meet the needs of human and ecosystems emerged as one of the primary issues of the 21st century (Lawford et al., 2003). For example, inadequate water supply and poor water quality give rise to health and other societal issues, limit agricultural productivity and economic prosperity, and pose national security risk in some countries (Nwidu et al., 2008). The effect is damaging not only to individual species and populations but also to the natural biological communities and it accounts for the deaths of more than 14,000 people daily (WHO, 2007).

The interaction of bacterial species with metals and their use to remove metals from contaminated sites represent a unique process. Heavy metals are natural elements and in the most basic level are just atoms; degradation and metabolism are not possible. Instead, microorganisms have evolved coping strategies to either transform the element to a less-harmful form or bind the metal intra- or extracellular, thereby preventing any harmful interactions in the host cell. Plus, they are able to actively transport the metal out of the cell cytosol (Hamlett et al., 1992; White and Gadd, 1998).

Microbes deal with poisonous chemicals by applying enzymes to convert one chemical into another form and taking energy or utilizable matter from this process. The chemical transformations generally involve breaking of large molecules into several small molecules in simpler form. Microbial activity plays a key role in detoxification of metals in water. In view of the interest in water and wastewater treatment, the response of microorganisms towards toxic heavy metals is of importance. In some cases the by-products of microbial remediation are not only harmless but may prove useful (Gupta et al., 2003). There is therefore, need to search for such metal tolerant, metal absorbent as well as moderate thermophilic acidophilic organisms for bioremediation applications (Martin-Gonzalez et al., 2006; Umrania, 2006*)*.

Metal toxicity results from alterations in the conformational structure of nucleic acids, proteins or by interference with oxidative phosphorylation and osmotic balance (Yaoa et al., 2008). Use of bio-adsorbents such as bacteria, fungi, algae and some agricultural wastes that emerged as an eco-friendly, effective and low cost material option could offer potential inexpensive alternatives to the conventional adsorbents (Valls and Lorenzo, 2002).

No previous work on bioremediation has been done on Panteka stream. The contaminated stream may find its way into domestic sources of water located around the communities which can be hazardous to human health, hence the need to carry out research work for the presence of bacteria species with the potential for removal of heavy metals and providing a template for the decontamination of Panteka stream as well as other contaminated streams.

MATERIALS AND METHODS

Sample collection

Panteka is a place in Kaduna where all kinds of cars and motorcycle spare parts are sold, and their maintenance are carried out. The stream in Panteka is located at the Northern part of Kaduna, Nigeria. There were four sampling points in the course of the study. Point A, the entry point of Panteka stream which is at Rafin Guza (upstream), Point B, C and D are the part of the stream that flows from Panteka through National Eye centre (Downstream). Samples were collected with caution into sterile bottles. The lid of the bottle was removed, held by its base and was completely submerged below the surface of the water. The bottle was filled by holding it upstream in the flowing water and in sweeping motion, to prevent any water which has come in contact with the hand from entering the bottle. Samples were taken to NDA (Nigeria Defence Academy) Medical Centre Laboratory for culture and analysis.

Isolation of heavy metal tolerant bacteria

Using the pour plate method, water samples were inoculated into nutrient agar plates containing different concentrations (0.5 and 3 mg ml^{-1}) of the heavy metals in their salt form such as cadmium nitrate (Cd NO_3), nickel chloride ($NiCl_2$) zinc sulphate ($ZnSO_4$), ferric chloride ($FeCl_3$), and lead sulphate ($PbSO_4$). The plates were incubated at room temperature for 3 days. The bacteria were isolated and sub cultured to obtain their pure cultures.

Identification of isolated bacteria

Pure cultures of the isolates were identified based on colony characteristics like shape, colour, texture, form, elevation, gram staining and biochemical tests. Molecular methods were used to confirm the identities of the isolates.

Isolation of genomic DNA

200 µl of the bacteria cells was added in a 1.5 ml Eppendorf tube. 400 µl of lysis buffer and 25 µl proteinase k was added to the sample in 1.5 ml Eppendorf tube. The tube was placed on heat block at 60°C for minimum of 1 h. 400 µl of phenol chloroform (1:1) was added to the lysate and vortexed briefly. This was spun at 10000 rpm for 10 min to separate the phases. The upper layer was carefully removed with a micropipette into a new 1.5 ml Eppendorf tube.

Equal volumes of 100% ethanol was added with 20 µl of 3 M sodium acetate and mixed by inverting the tube several times which was incubated at -20°C overnight. The tube was spun at a maximum speed for 30 min. 70% ethanol was added and spun at a maximum speed for 5 min at 4°C. All traces of ethanol were removed by spinning for another 30 s. The DNA was dried by leaving the tube open for 10 min. The pellet was resuspended in 20 to 50 µl sterile water and the DNA quality was determined using a

Table 1. Morphological characterization of the heavy metal tolerant bacteria from Panteka stream.

Parameters	P3m1	P3m2	P2m3	P2m4
Form	Grape	Circular	Swarming	Circular
Colour	Cream	Cream with red pigment	Cream	White
Elevation	Raised	Umbonate	Raised	Convex
Shape	Cocci	Rod	Rod	Rod
Gram staining	+	-	-	-
Likely bacteria	*Staphylococcus epidermidis*	*Serratia marcescens*	*Proteus mirabilis*	*Escherichia Coli*

spectrophotometer (Jyothi et al., 2012)

Amplification of 16S rDNA genes by polymerase chain reaction (PCR)

The isolated DNA was amplified using 16S rDNA universal primers, (Forward, GGACTACAGGGTATCTAAT) and (Reverse, AGAGTTTGATCCTGG) (Kenneth et al., 1990). The PCR conditions were Pre- Denaturation: 5min at 94°C, Denaturation: 1min at 94°C, Annealing: 1 min at 52°C, Extension: 1 min at 72°C, Final extension: 5 min at 72°C for 35 cycles. The PCR products were first analysed by electrophoresis in 1.5% agarose gel which was stained with ethidium bromide and visualized under short wavelength UV light.

Nucleotide sequencing and alignment

16S rDNA sequencing of the isolated strain was carried out by Dye terminator cycle sequencing (Quick start kit). The gene sequences of each isolate obtained in this study was compared with known nucleotide database at the National Center for Biotechnology Information (NCBI) by using their world wide website, and the BLAST (Basic Local Alignment Search Tool) algorithm (Jyothi et al., 2012).

Maximum tolerance concentration (MTC)

The four bacteria isolates each were inoculated into a nutrient broth containing different concentrations of heavy metals. The concentrations of heavy metals used were determined based on the maximum amount each bacteria isolate could tolerate. The concentrations for nickel, cadmium, zinc and lead ranged from 0.5 to 4 mg ml^{-1}. The concentrations for Iron were 0.5, 0.7, 0.8, 0.9 and 1 mg ml^{-1}. The growth rate was monitored with a spectrophotometer at an absorbance of 600 nm against a nutrient broth (blank) containing the same amount of heavy metals (Pandit et al., 2013).

Bioremediation potentials of the heavy metals

100 ml of the water sample was poured into six 250 ml conical flasks containing 100 ml of nutrient broth each. The Stream water and nutrient broth was sterilized in an autoclave at 120°C for 15 min. Flask A was not inoculated with bacterium (Control), flask B, C, D and E were each inoculated with, *Staphylococcus epidermidis, Serratia marcescens, Proteus mirabilis* and *Escherichia coli,* respectively. Flask F was inoculated with a mixed Bacterial culture (MBC) of the four organisms. The samples were kept at room temperature. The concentration of cadmium, iron, lead, nickel and zinc was measured after 14 days of treatment with the isolates using atomic absorption spectrophotometer (AAS). Comparison was done on the values obtained before (control) and after treatment.

Statistical analysis

Data obtained for mixed bacteria culture and the most efficient pure isolate (*S. epidermidis*) in the removal of heavy metals from Panteka stream was compared using student t-test.

RESULTS

Identification of Bacteria by gram staining, morphological and biochemical characterization of Isolates P3M1, P3M2, P2M3, and P2M4 (Tables 1 and 2) identified *S. epidermidis, S. marcescens, P. mirabillis* and *E. coli* as heavy metal tolerant bacteria.

PCR amplification of 16S rRNA gene produced fragments of approximately 800 base pairs in size for the four bacteria isolates (Plate 1). Full length sequence of 16S rDNA gene was deduced for two isolates showing tolerance to high concentrations of the metals. Sequences were aligned and the closest match was detected using BLAST. Identification of strains by biochemical tests and 16S rRNA gene sequence analysis revealed that strain P2M4 showed 99% similarity to *E. coli* and strain P3M1 showed 100% similarity to *Staphylococcus* species.

Heavy metal tolerance of isolated bacteria

S. epidermidis and *E. coli* had MTC of 1.5 mg ml^{-1} for nickel, 3 mg ml^{-1} for zinc, 3 mg ml^{-1} for lead, 1.5 mg ml^{-1} for cadmium and 0.9 mg ml^{-1} for iron (Figure 1 to 5). *S. marcescens* and *P. mirabilis* had MTC of 1.5 mg ml^{-1} for nickel, 3 mg ml^{-1} for zinc, 4 mg/ml for lead, 1.5 mg ml^{-1} for cadmium, and 0.9 mg ml^{-1} for iron (Figure 1 to 5). The bacteria isolates showed similar pattern of heavy metal tolerance and this could be attributed to the fact that isolates are closely related genetically as the gram staining results showed that, most of the isolates were gram negative and belonged to the enterobacteriaceae family.

Table 2. Biochemical Characterization of heavy metal tolerant bacteria from Panteka stream.

Biochemical test	P3m1	P3m2	P2m3	P2m4
Mannitol	-	+	+	+
Orthinine decarboxylate	-	+	+	-
Nitrate reduction	-	+	+	+
Urease	+	-	+	-
Citrate	-	+	+	-
Methyl red	-	-	+	+
Catalase	+	+	+	+
Coagulase	-	-		-
Indole	-	-	-	+
Lactose	+	-	-	+
Hydrogen sulphide	+	-	+	-
Oxidase	-	-	-	-
Motility	-	+	+	+
Likely bacteria	*Staphylococcus epidermidis*	*Serratia marcescens*	*Proteus mirabilis*	*Escherichia coli*

+, Positive; -, negative.

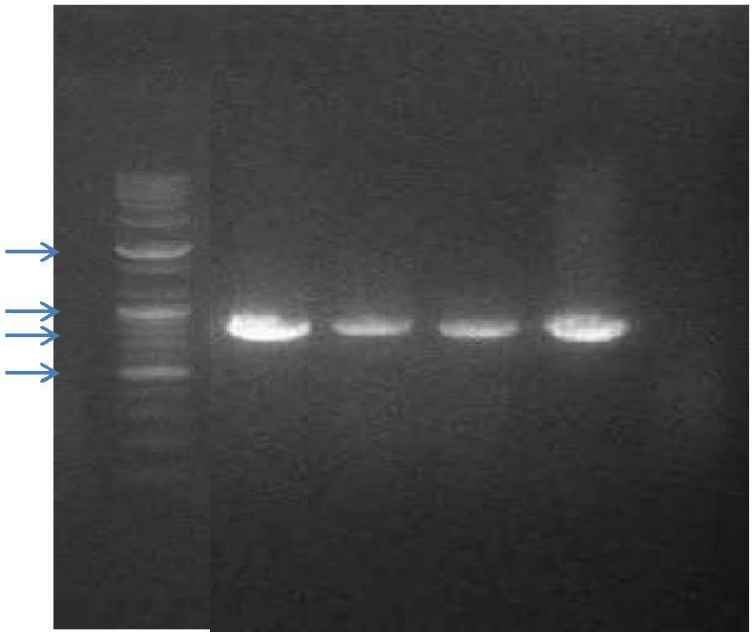

Plate 1. PCR amplicons of rRNA genes in four bacteria isolates from Panteka stream. Lane A, Ladder; Lane 1, P3M1; lane 2, P3M2; lane 3, P2M3; lane 4, P2M4; lane B, negative control.

Bioremediation potentials of the isolates to nickel, lead, zinc, cadmium and iron

Nickel and lead concentration was completely removed (100% reduction) by four bacteria isolates and mixed bacteria culture (MBC). Zinc concentration was reduced by *S. epidermidis* (84.95%), *S. marcescens* (58.73%), *P. mirabillis* (34.0%), *E. coli* (52.23%), and mixed bacteria culture (100%) (Figure 7). Cadmium concentration was reduced by *S. epidermidis* (90.29%), *S. marcescens* (66.82%), *P. mirabillis* (25.40%), *E. coli* (74.91%), and mixed bacteria culture (100%) (Figure 8). Iron concentration was reduced by *S. epidermidis* (54.82%), *S. marcescens* (52.01%), *P. mirabillis* (53.51%), *E. coli*

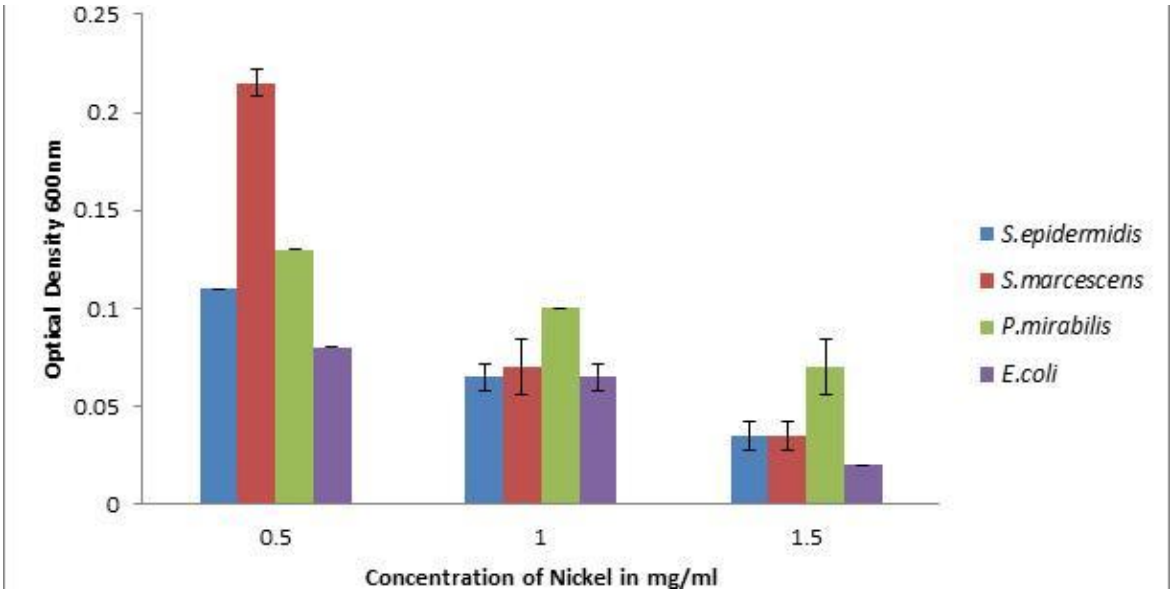

Figure 1. Effect of different nickel concentrations on the growth rate of bacteria isolates from Panteka stream. Data used are from two biological repeats. Error bars indicate ±SEM.

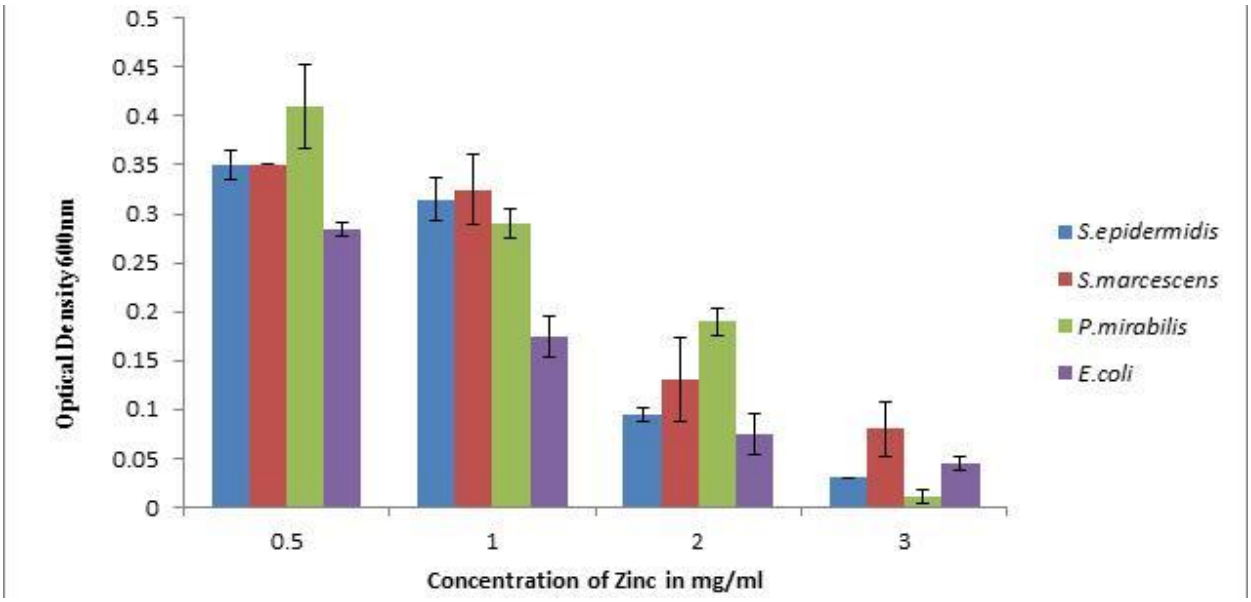

Figure 2. Effect of different zinc concentrations on the growth rate of bacteria isolates from Panteka stream. Data used are from two biological repeats Error bars indicate ±SEM.

(54.10%), and mixed bacteria culture (73.88%) (Figure 6). The best isolates for heavy metal reduction in Panteka stream in increasing order were *P. mirabilis, E. coli, S. marcescens,* and *S. epidermidis*. Mixed bacteria cultures had a highest percentages reduction of each heavy metal analysed in Panteka stream.

T-test data showed a significant difference between MBC and *S. epidermidis* in the removal of heavy metal; lead, nickel, cadmium, iron and zinc ($P < 0.05$).

DISCUSSION

The four heavy metal tolerant bacteria isolated were *S.*

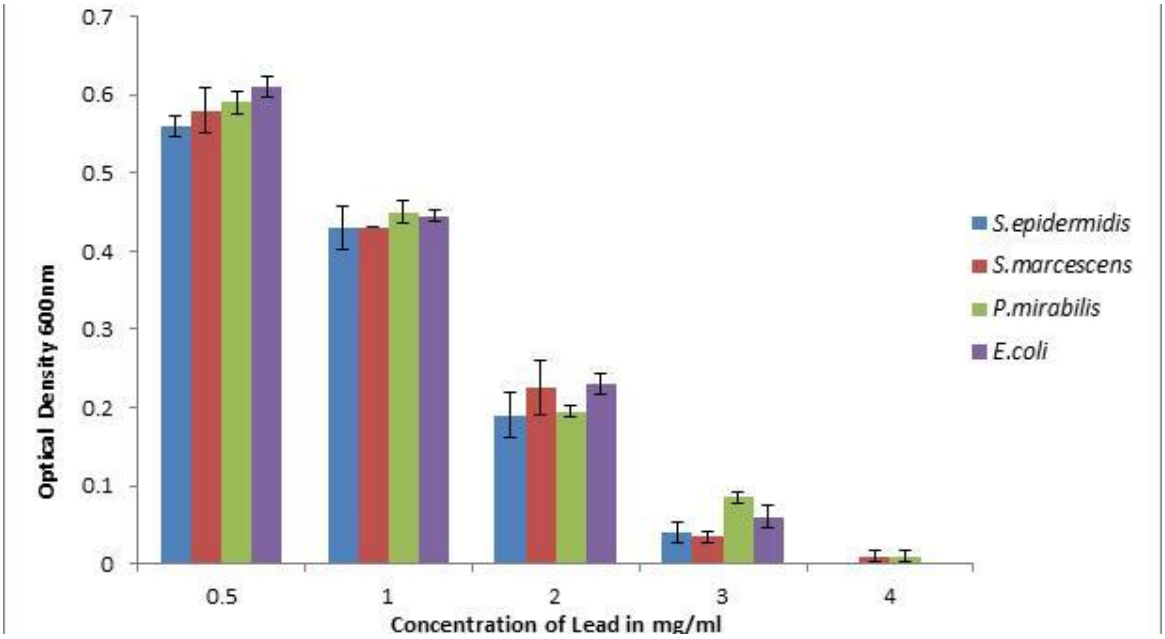

Figure 3. Effect of different lead concentrations on the growth rate of bacteria isolates from Panteka stream. Data from two biological repeats. Error bars indicate ±SEM.

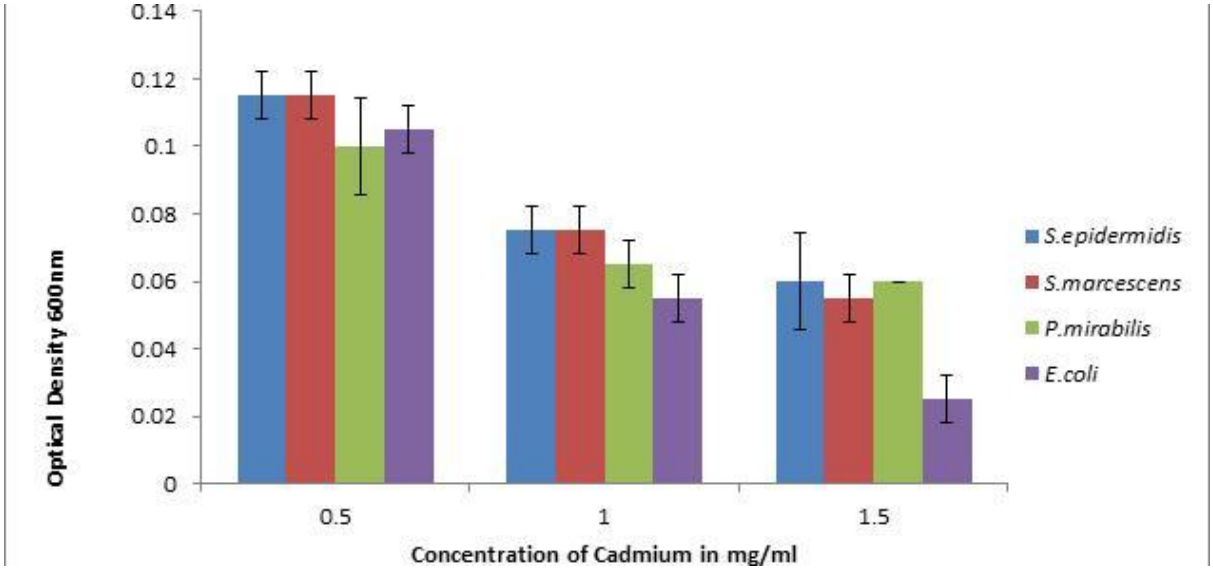

Figure 4. Effect of different cadmium concentrations on the growth rate of bacteria isolates from Panteka stream. Data used are from two biological repeats. Error bars indicate ±SEM.

epidermidis, S. marcescens, P. mirabilis, and *E. coli.* This result is similar to the report of Kolawole and Obueh (2015) that isolated *Staphylococcus spp., E. coli, Klebsiella, Salmonella* and *Pseudomonas* spp. as heavy metals tolerant bacteria. *Serratia marcescens* was reported to have been isolated as heavy metal tolerant bacteria (Nageswaran et al., 2012)

S. marcescens and *P. mirabilis* were the only isolates that showed maximum tolerance to lead at 4 mg/l. This compared favourably to the report of Owolabi and Hekeu (2015) who indicated in their works the high tolerance ability of *S. marcescens* and *Proteus* spp. to lead in a bioremediation study. The growth of the four heavy metals tolerant bacteria as reflected in their $O.D_{600}$ readings in increasing order was iron, cadmium, nickel, zinc, lead. Figure 3 shows that heavy metal tolerant

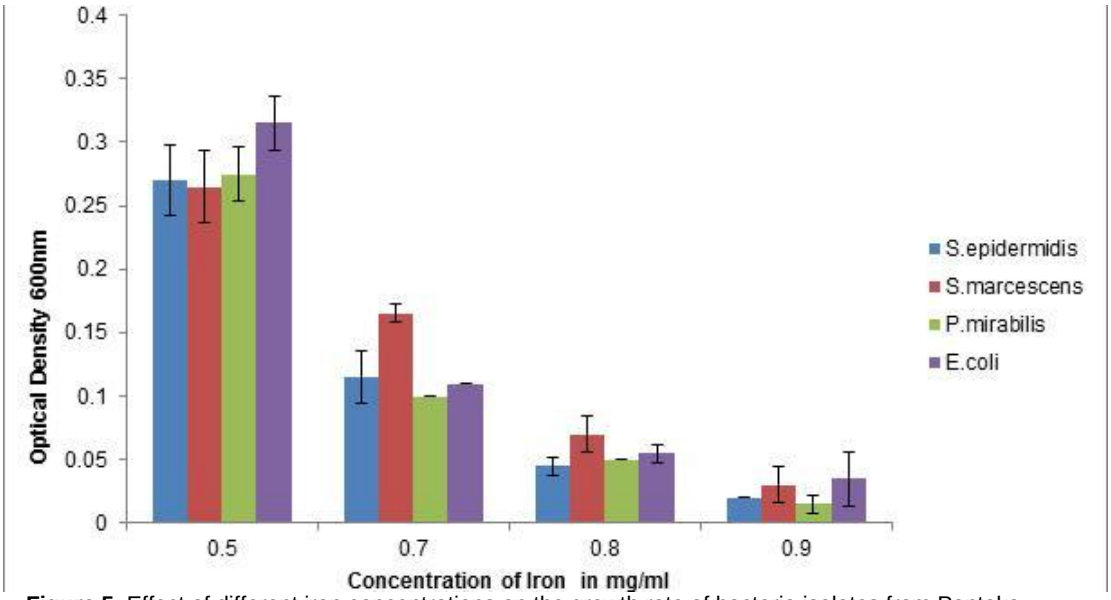

Figure 5. Effect of different iron concentrations on the growth rate of bacteria isolates from Panteka stream. Data used are from two biological repeats. Error bars indicate ±SEM.

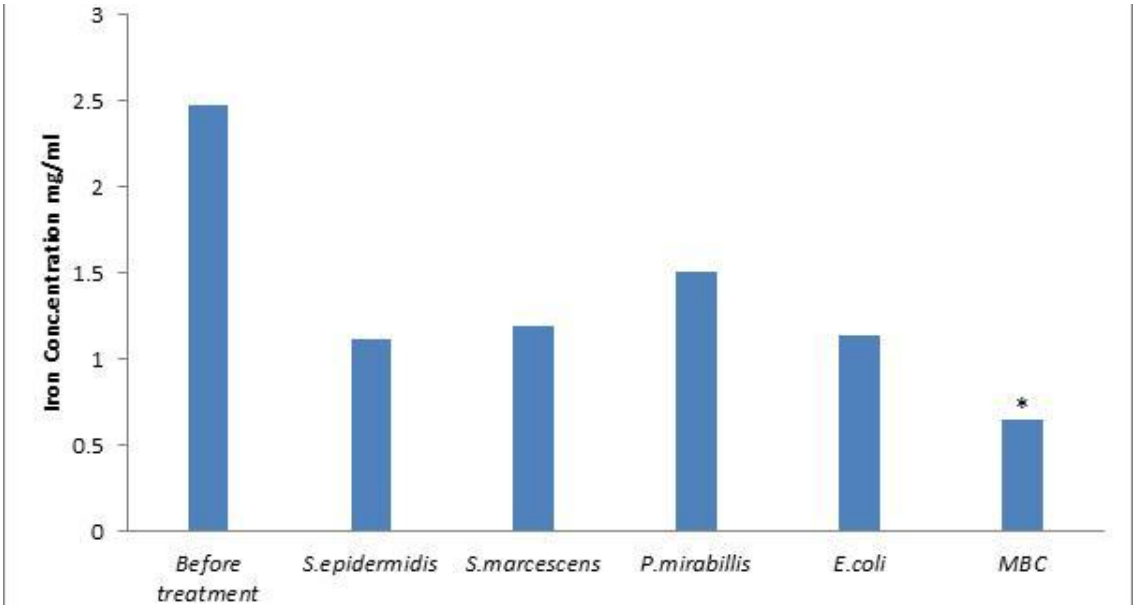

Figure 6. Iron reduction by bacteria isolates in Panteka Stream (* denotes significance difference from the most efficient single isolate, *S. epidermidis,* $p<0.05$).

isolates had high $O.D_{600}$ in the presence of lead as compared to other metals. The high tolerance to zinc could be attributed to being one of the essential trace metal ions for living organisms. Grass et al. (2001) stated that *E. coli* contains at least four zinc transport pumps, two zinc transporters (importers), ZnuABC and ZupT, and two zinc exporters, ZntA and ZitB. Fosmire (1990) however, reported that excess zinc can be harmful. Excessive absorption of zinc suppresses copper and iron absorption. Iron was poorly tolerated by the isolates. Simon (1998) stated that, iron is an essential nutrient for living agents due to its noticeable activity in electron transport reactions in biological systems, but its insolubility and reactivity lead to problems of poor availability and toxicity, respectively.

The bioremediation potential of the four heavy metal

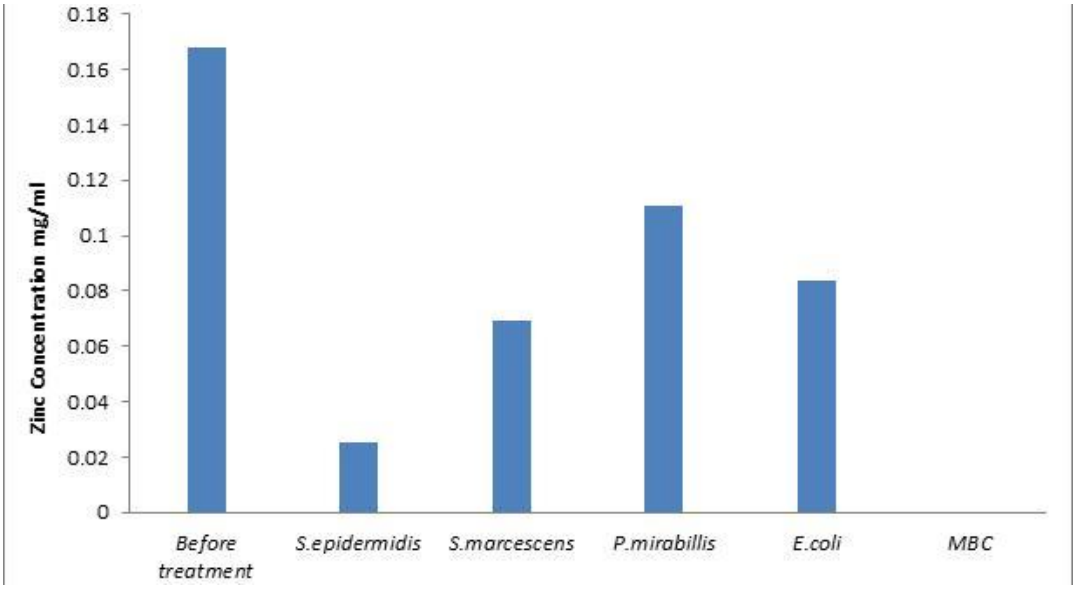

Figure 7. Zinc reduction by bacteria isolates in Panteka Stream.

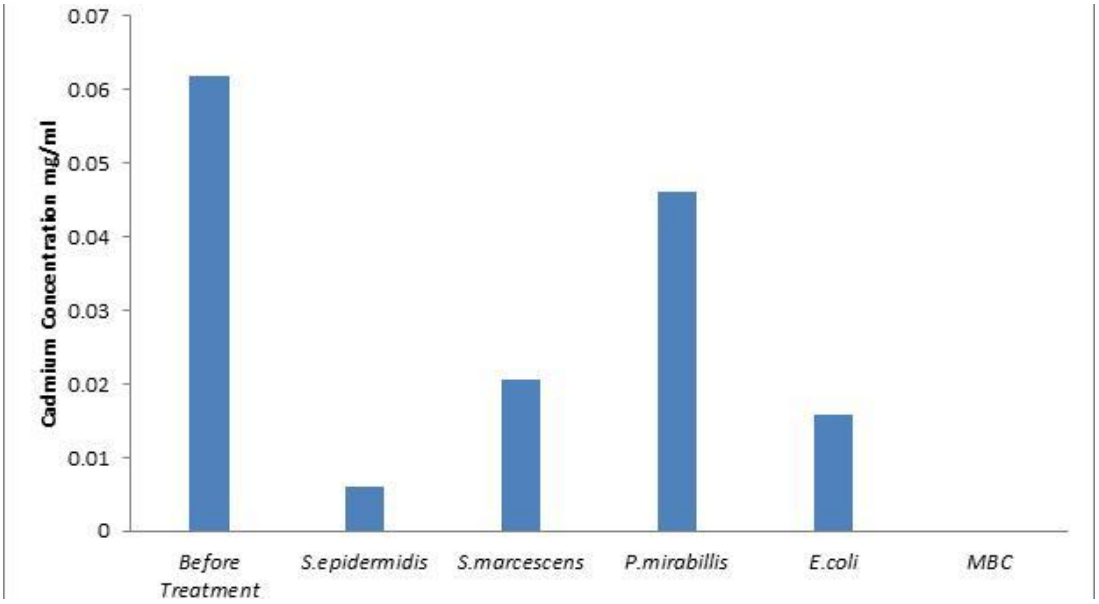

Figure 8. Cadmium reduction by bacteria isolates in Panteka Stream.

tolerant bacteria revealed that, *S. epidermidis* and *S. marcescens* show high potential for the removal of heavy metals in Panteka stream for pure isolates but mixed bacteria culture as being more effective than single cultures. The mixed bacteria culture completely removed lead, nickel, cadmium and zinc. This result compares favourably with the report of Oaikhena et al. (2016), who indicated in a bioremediation study of a petroleum refinery effluent that, mixed culture consortium was more efficient in the removal of heavy metals than pure isolates. Although lead is known for its toxicity as reported by Manton et al. (2000), it was observed that, the four heavy metal tolerant bacteria isolated from Panteka stream thrived well in the presence of lead.

Conclusion

The presence of bacteria capable of tolerating heavy metals from contaminated Panteka stream was

investigated. Bacteria that are capable of tolerating heavy metals were isolated in pure cultures, where three isolates were identified to belong to the enterobacteriaceae.

In summary, the results presented in this work revealed that the four isolates, characterized with remarkable tolerance to heavy metals could be potential agents for the development of inoculants applicable in bio augmentation of heavy metals polluted industrial sites. The genetic capacity of the isolates can also be exploited for the remediation of heavy metal polluted sites. The result obtained from the research proved that, mixed bacteria culture was more effective for bioremediation than pure culture bacteria isolates.

Conflict of Interests

The authors have not declared any conflict of interests.

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