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Effect of Heavy Metals on β-galactosidase Activity in Marine Bacteria

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Authors' contributions

This work was carried out in collaboration among all authors. Author CNA contributed in the course of conception, design, literature searches and manuscript writing. Author OJO contributed during design, literature searches, sample collection, laboratory analysis. Author AAI supervised the work and contributed during analysis and interpretation of results. All the authors read and approved the final manuscript.

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ABSTRACT

Background: Due to metal pollution and its toxicity in the coastal areas, the enzymatic activities of bacteria involved in the breakdown of organic compounds are repressed leading to decline in biodegradation rate.
Aim: The influence of heavy metals (copper, lead, zinc, manganese and iron) on β-galactosidase activity in three bacterial strains (*Providencia stuartii*, *Pantoea dispersa* and *Aeromonas dhakensis*) isolated from coastal marine sediment collected from coastal zone in Bonny Island, Nigeria was investigated.
Methodology: The strains were cultivated in Z- buffered medium having lactose as enzyme inducer. Beta galactosidase assay was done via 2-nitrophenol β-D-galactopyranoside as the substrate. The absorbances of p-nitrophenol solution formed were measured at 420 nm in a spectrophotometer. The β-galactosidase activities were calculated comparative to controls.
Results: Presence of the metals significantly affected β-galactosidase activities. Metal concentration of 0.001 mg/L triggered a decrease in enzyme activity. The sensitivity patterns of

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Pantoea dispersa and *Aeromonas dhakensis* were Cu>Pb>Zn>Mn>Fe while that of *Providencia stuartii* was Pb>Cu>Zn>Mn>Fe. The effect of metal stress to enzyme synthesis is reliant on the organism and the metal. This might be described with logistic dose-response model using elevated coefficient of inhibition (R > 0.81).

Conclusion: The results revealed that concentration of metal as low as 0.001 mg/L when deposited in the environment has detrimental effect on microbial activities and consequently on biogeochemical cycles. The isolated bacterial strains could serve as ideal organisms for heavy metal toxicity evaluation.

Keywords: Enzyme activity; β-galactosidase; heavy metals; pollution; sediment.

1. INTRODUCTION

Coastal regions are locations of release and buildup of a variety of ecological pollutants [1]. Heavy metals pollution of coastal sediment and water has been recognized to cause serious contamination as a result of industrial development. Anthropogenic sources of heavy metals are domestic waste water discharges, leaching of metals from piles of solid wastes, human and animal defecations, port activities (docking, vessel paints, antifouling, vessel repair amenities and anticorrosion chemicals) and activities of petroleum exploitation which introduce zinc (Zn), copper (Cu), lead (Pb) and other metals into the coastal waters [2,3,4].

Due to hydrophobic nature and low water solubility of heavy metals, they are intensely bound by sediments in aquatic environment [5]. As a result, extraordinary concentrations of heavy metals have been documented in coastal sediments close to industrialized towns [6]. The occurrence of these metals could lead to the killing of the existing organisms in the region leading to disruption of microbial activities that will adversely affect plants and animals in the area.

Sediments have diverse microbes which play important roles in aquatic ecosystem functions, such as ecological decontamination, bio-geochemical cycles and recycling of organic matter [7,8,9].

β -galactosidase which catalyses the breakdown of lactose to glucose and then to galactose is an intracellular enzyme and its biosynthesis is induced by lactose or its derivatives [10]. Factors that affect metabolic actions (e.g. β-galactosidase activity) of microbes are of great significance. Therefore, the aim of this research was to investigate the impact of heavy metals on β-galactosidase activity of bacteria in coastal sediment.

2. MATERIALS AND METHODS

2.1 Sample Collection

Sediment was collected from the coastal region of the Niger Delta in Bonny Island, Nigeria using Eckman grab sampler. The sample was collected in sterile glass container and was transported to the laboratory, immediately, for analysis.

2.2 Isolation of Culturable Bacteria

One gram of sediment was homogenized in 9 ml of sterile physiological saline from which tenfold serial dilution of the sample was carried out up to 10^{-6}. Then 0.1 ml portions of 10^{-5} and 10^{-6} dilutions were inoculated on sterile plates of Nutrient agar (Oxoid). The plates were incubated at 30ºC for 24-48 h. Colonies on the culture plates were macroscopically characterized. The three dominant isolates were purified by streaking onto fresh nutrient agar supplemented with sea water and identified using molecular methods.

2.3 Molecular Identification

2.3.1 DNA extraction (Boiling method and quantification)

Five milliliters of an overnight broth culture of the bacterial isolate in Luria Bertani (LB) was spun at 14000 rpm for 3 min. The cells were re-suspended in 500 µl of normal saline and heated at 95ºC for 20 min. The heated bacterial suspension was cooled on ice and spun for 3 min at 14000 rpm. The supernatant containing the DNA was transferred to a 1.5 ml micro centrifuge tube and stored at -20°C for other downstream reactions. The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer.

2.3.2 16S rRNA amplification and sequencing

The 16S region of the rRNA genes of the isolates were amplified using the 27F and 1492R primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 50 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, $MgCl_2$), the primers at a concentration of 0.4 M and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 min; denaturation, 95ºC for 30 seconds; annealing, 52ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 min. The product was resolved on a 1% agarose gel at 120V for 15 min and visualized on a UV trans illuminator. Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa.

2.3.3 Phylogenetic analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using Clustal X. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 [11]. The bootstrap consensus tree inferred from 500 replicates [12] is taken to represent the evolutionary history of the taxa analysed. The evolutionary distances were computed using the Jukes-Cantor method [13].

2.4 Source of Heavy Metal Ions

The metal ions Mn^{2+}, Zn^{2+}, Pb^{2+}, Fe^{2+} and Cu^{2+} were used as $MnSO_4.H_2O$, $ZnSO_4.7H_2O$, $Pb(NO_3)_2$, $Fe_2SO_4.3H_2O$ and $Cu(NO_3)_2.3H_2O$ respectively.

2.5 Preparation of Inocula

The three bacterial strains were cultivated to mid exponential stage using nutrient broth on a rotating incubator (150 rpm) at 30ºC. The cells were harvested by centrifugation at 3500 rpm for 15 min. Harvested cells were washed twice in deionized water and were standardized in a spectrophotometer to an optical density of 0.6 at 420 nm. The standardized cell suspensions were employed as inocula in the assay of the enzyme [10].

2.6 Culture Treatment with Heavy Metal and β-galactosidase Activity Assay

The method of Nweke and Okpokwasili [10] was employed with little modification. Small quantities of (0.1 ml) standardized cell suspensions were inoculated into sterile triplicate 20 ml screw-capped test tubes containing 1.9 ml Z- buffered (pH 7.0) nutrient broth- lactose medium (0.4 ml Z-buffer, 0.4 ml of nutrient broth and 0.1 ml of 0.4% w/v lactose and required volume of deionized water) supplemented with a specific concentration of heavy metal ion (0.001-200 mg/l). The control was made up of inoculated medium without metal ions. The cultures were incubated at 30ºC for 1 h. Then, 0.1 ml of 7% w/v sodium dodecyl sulphate (SDS) was added in each tube and shaken to solubilize the cells. Then, 0.1 ml of 0.4% w/v ρ-nitrophenyl-β-D-galactopyranoside solution was added to the reaction mixture and incubated at room temperature (28ºC-30ºC) for 24 hr. The reactions were stopped by adding 1 ml of cold 1 M Na_2CO_3 solution. The ρ-nitrophenyl-β-D-galactopyranoside was hydrolysed to yellow coloured ρ-nitrophenol. The absorbance of ρ-nitrophenol solution produced was measured spectrophotometrically at 420 nm (λ max). The β-galactosidase activity was calculated relative to the control [10].

2.7 Statistical Analysis

The degree of inhibition was determined relative to control (100% enzyme activity) on the basis of measured absorbance as shown in Equation 1. Dissimilarities at enzyme activity levels amid the control and other samples were considered as metal ion influence on enzyme biosynthesis. At least three replicate tests were carried out on each toxicant concentration. The data were plotted in terms of percent of enzyme activity in control test on *y*-axis versus metal concentration on *x*-axis with mean and standard deviation (n=3) shown as data points and bars respectively. To measure the toxicity thresholds of the toxicants (IC_{20}, IC_{50} and IC_{80}), the experimental data were fitted into non-linear logistic (Equation 2) dose-response models by iterative minimization of sum of squares of the residuals based on Levenberg Marquardt algorithm. All regressions were done using the data mean and standard deviations at 95% confidence limit using XLSTAT version 2015.4.01.21575.

$$\text{Enzyme activity (\% of control)} = \frac{T_A}{C_A} \times \frac{100}{1} \qquad (1)$$

$$\text{Enzyme activity (\% of control)} = \frac{a}{1 + \left(\frac{x}{K_i} \right)^{KI}} \qquad (2)$$

C_A is the absorbance of uninhibited control (without toxicant), T_A is the absorbance of inhibited test (with different concentrations of toxicant), x is the concentration of metal ion, a is the uninhibited value of enzyme activity (100%), *KI* is dimensionless toxicity parameter; K_i is the coefficient of inhibition [10].

3. RESULTS

3.1 Molecular Identification

The obtained 16S rDNA sequences from the 3 isolates produced precise matches during the mega blast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The 16S rDNA of B1, B2 and B3 exhibited a percentage similarity to *Providencia stuartii*, *Aeromonas dhakensis* and *Pantoea dispersa* at 99% as presented in Figs. 1, 2 and 3 respectively. The sequences are presented in Appendix 1, 2 and 3 respectively.

3.2 Inhibition of β-galactosidase Biosynthesis in *Providencia stuartii* by Heavy Metals

The effect of heavy metals on biosynthesis of β-galactosidase by *Providencia stuartii* is shown in Fig. 4. All the metals repressed synthesis of β-galactosidase in the bacterial strain as indicated in the repression of enzyme activity. Pb^{2+} was inhibitory to β-galactosidase more than the other metals. The toxicity thresholds and the coefficients of inhibition generated from the models for *Providencia stuartii* are shown in Table 1. *Providencia stuartii* is most sensitive to toxicity of Pb^{2+} inhibiting β-galactosidase biosynthesis by 50% at 0.0007 ± 0.002 mg/L Pb^{2+}. At 0.001 mg/L, Pb^{2+} and Cu^{2+} inhibited β-galactosidase activity by 73.2% and 64.6% respectively.

3.3 Inhibition of β-galactosidase Biosynthesis in *Pantoea dispersa* by Heavy Metals

The effect of heavy metals on biosynthesis of β-galactosidase by *Pantoea dispersa* is shown in Fig. 5. All the metals repressed synthesis of β-galactosidase in the bacterial strain as indicated in the repression of enzyme activity. Cu^{2+} was inhibitory to β-galactosidase more than the other metals. The toxicity thresholds and the coefficients of inhibition generated from the models for *Pantoea dispersa* are shown in Table 2. *Pantoea dispersa* is most sensitive to toxicity of Cu^{2+} inhibiting β-galactosidase biosynthesis by 50% at 0.0008 ± 0.012 mg/L Cu^{2+}. At 0.001 mg/L, Cu^{2+} and Pb^{2+} inhibited β-galactosidase activity by 65.2% and 60.6% respectively.

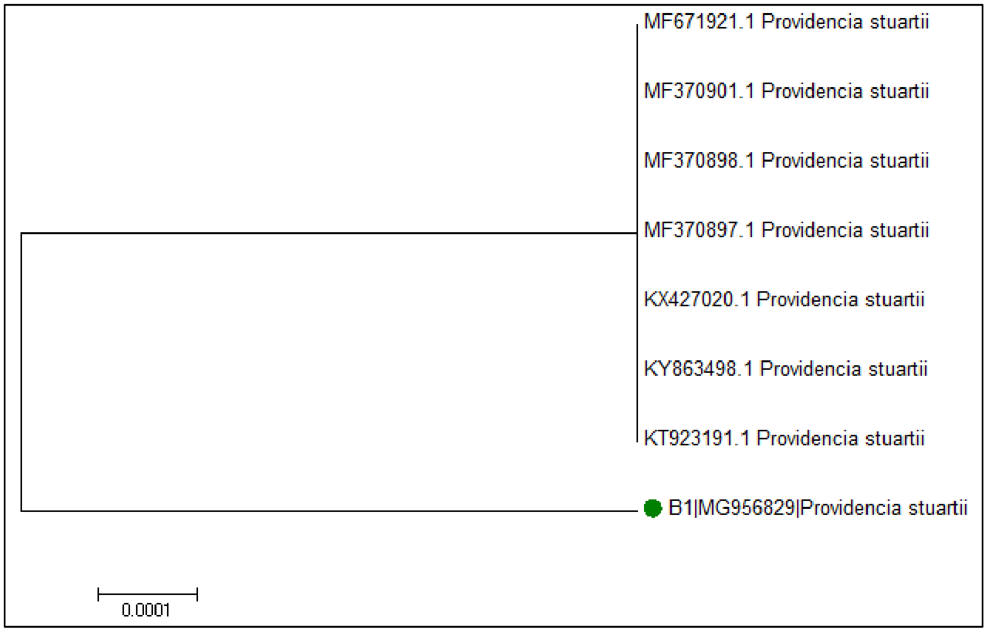

Fig. 1. Phylogenetic tree of B1

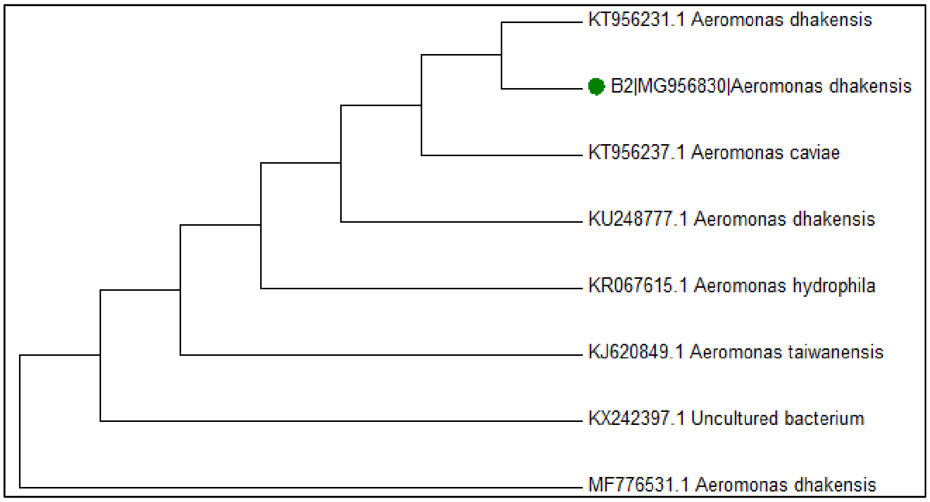

Fig. 2. Phylogenetic tree of B2

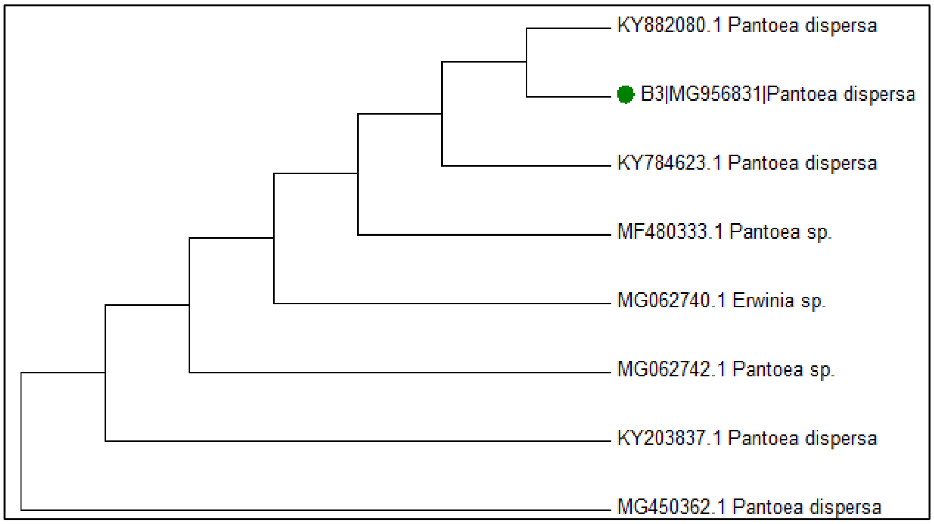

Fig. 3. Phylogenetic tree of B3

Table 1. Coefficient of inhibition by metals and threshold concentrations of metals for inhibition of β-galactosidase biosynthesis in *Providencia stuartii*

Metal	Coefficient of inhibition by metals			Threshold concentrations of metals (mg/L)		
	Ki	KI	R^2Adj	IC20	IC50	IC80
Cu	0.1190	0.4873	0.8529	0.0003 ± 0.004	0.0008 ± 0.003	0.0012 ±0.028
Zn	0.1200	0.2769	0.9655	0.0004 ± 0.001	0.0010 ± 0.004	0.0016 ± 0.021
Pb	0.1000	0.3972	0.8057	0.0002 ± 0.006	0.0007 ± 0.002	0.0011 ± 0.054
Mn	0.1209	0.3377	0.9650	0.0012 ± 0.001	0.0030 ± 0.008	0.0048 ± 0.029
Fe	0.1324	0.3000	0.9004	0.0418 ± 0.002	0.1045 ± 0.001	0.1672 ± 0.007

3.4 Inhibition of β-galactosidase Biosynthesis in *Aeromonas dhakensis* by Heavy Metals

The effect of heavy metals on biosynthesis of β-galactosidase by *Aeromonas dhakensis* is shown in Fig. 6. All the metals repressed synthesis of β-galactosidase in the bacterial strain as indicated in the repression of enzyme activity. Cu^{2+} was inhibitory to β-galactosidase more than the other metals. The toxicity thresholds and the coefficients of inhibition generated from the models for *Aeromonas dhakensis* are shown in Table 3. *Aeromonas dhakensis* is most sensitive to toxicity of Cu^{2+} inhibiting β-galactosidase biosynthesis by 50% at 0.0010 ± 0.006 mg/L Cu^{2+}. At 0.001 mg/L, Cu^{2+} and Pb^{2+} inhibited β-galactosidase activity by 52.9% and 31.71% respectively.

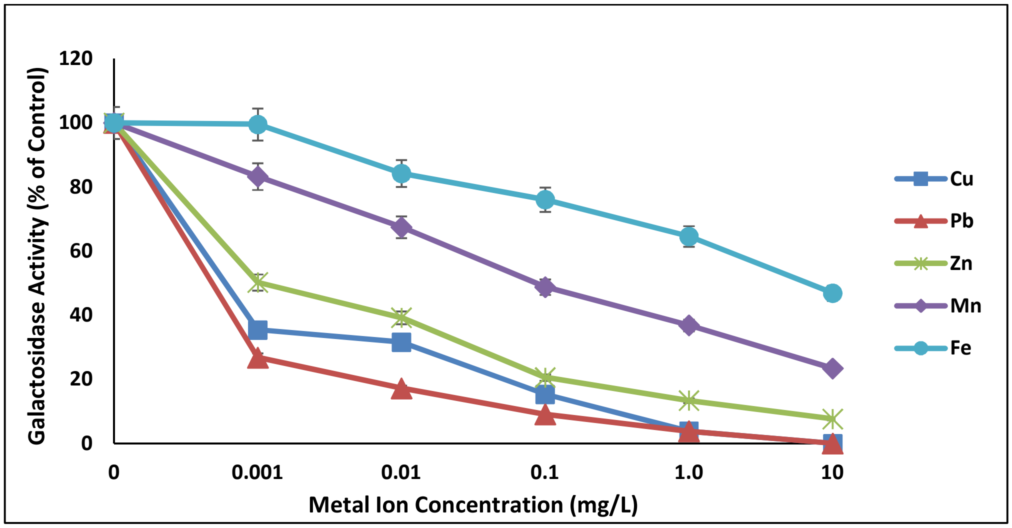

Fig. 4. Inhibition of β-galactosidase activity in *Providencia stuartii* by heavy metals

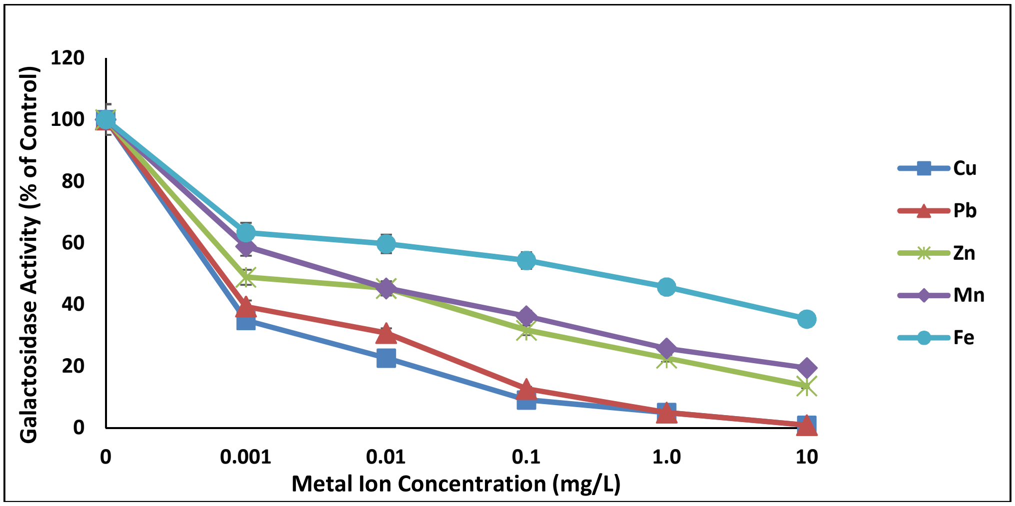

Fig. 5. Inhibition of β-galactosidase activity in *Pantoea dispersa* by heavy metals

Table 2. Coefficient of inhibition by metals and threshold concentrations of metals for inhibition of β-galactosidase biosynthesis in *Pantoea dispersa*

Metal	Coefficient of inhibition by metals			Threshold concentrations of metals (mg/L)		
	Ki	KI	R^2Adj	IC20	IC50	IC80
Cu	0.3400	1.0300	0.8187	0.0003 ± 0.054	0.0008 ± 0.012	0.0012 ± 0.020
Zn	0.3450	1.0100	0.9132	0.0004 ± 0.032	0.0010 ± 0.014	0.0016 ± 0.031
Pb	0.3410	1.0101	0.8793	0.0004 ± 0.051	0.0009 ± 0.012	0.0013 ± 0.021
Mn	0.3461	1.0110	0.8678	0.0005 ± 0.018	0.0012 ± 0.026	0.0020 ± 0.018
Fe	0.3522	0.3410	0.8953	0.0006 ± 0.009	0.0014 ± 0.008	0.0022 ± 0.021

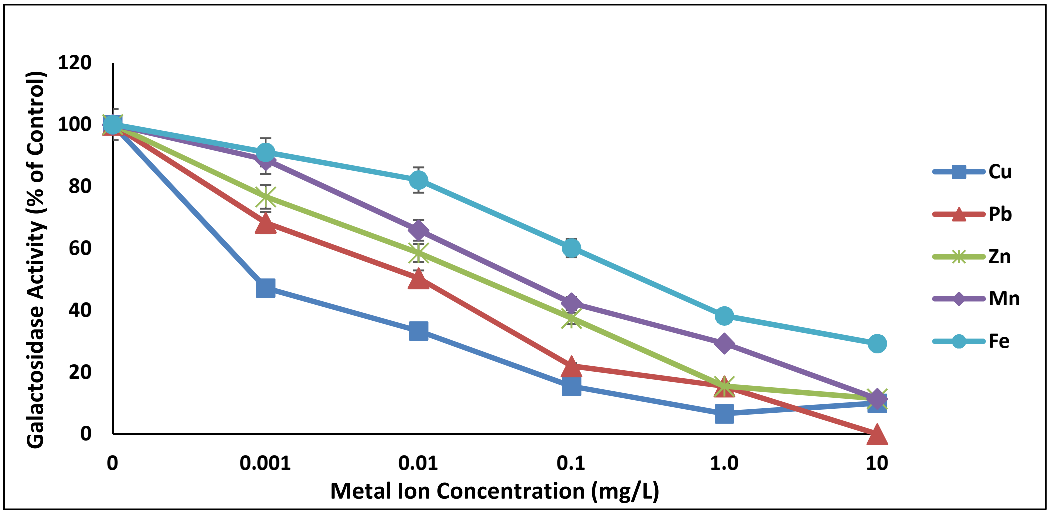

Fig. 6. Inhibition of β-galactosidase activity in *Aeromonas dhakensis* by heavy metals

Table 3. Coefficient of inhibition by metals and threshold concentrations of metals for inhibition of β-galactosidase biosynthesis in *Aeromonas dhakensis*

Metal	Coefficient of inhibition by metals			Threshold concentrations of metals (mg/L)		
	Ki	KI	R^2Adj	IC20	IC50	IC80
Cu	0.1074	0.5089	0.8525	0.0004 ± 0.027	0.0010 ± 0.006	0.0015 ± 0.051
Zn	0.1154	0.4536	0.9552	0.0009 ± 0.001	0.0022 ± 0.032	0.0035 ± 0.041
Pb	0.1084	0.4425	0.9124	0.0007 ± 0.001	0.0016 ± 0.031	0.0025 ± 0.034
Mn	0.1240	0.1712	0.9850	0.0018 ± 0.001	0.0044 ± 0.028	0.0070 ± 0.013
Fe	0.1320	0.3559	0.9497	0.0023 ± 0.002	0.0056 ± 0.041	0.0090 ± 0.012

4. DISCUSSION

Providencia stuartii, *Pantoea dispersa* and *Aeromonas dhakensis* isolated from sediment in this study are known to cause different human pathogenic diseases and are essential indicators in the environment when water quality is considered. *Providencia stuartii* is a Gram negative bacterium which is usually found in soil, sewage and water. It is a member of Enterobacteriaceae family and known to cause nosocomial infections [14]. *Pantoea dispersa* is a member of Enterobacteriaceae family that is found in soil, water and plants and seldom causes human infections but was in recent times associated with neonatal sepsis as a causal agent [15]. *Aeromonas dhakensis* is abundant in marine and other aquatic environments. It causes major skin and soft tissue infections and is regularly linked with floods and other water activities [16].

Different genera of bacteria are found in marine location with the utmost diversity in sediment [17]. The coastal marine location is recognized to habour diverse bacteria of antibacterial importance against human and fish pathogens [17]. The authors reported that the antagonistic bacteria are more in sediment than in any other samples. Other ecological roles the sediment bacteria carry out encompass decomposition of organic compounds and recycling of nutrients which are necessary for sustaining the biogeochemical stability of the environment.

Enzyme activities in soil (and sediment) are very sensitive to anthropogenic and natural instabilities and exhibit a rapid response to these variations [18]. A disturbance in the microbial activity of the microbes within the environment as a result of the effect of external influences will lead to an alteration in the community composition with potential negative consequences in the environment [19]. Methods that are centered on the enzymatic activities of microbes are clearly dependable ways for describing the ecological state of the environs [20].

The metals (Cu, Pb, Zn, Mn and Fe) used in this study inhibited the β-galactosidase activity in the marine bacteria (*Providencia stuartii*, *Pantoea dispersa* and *Aeromonas dhakensis*) isolated from sediment at concentration as low as 0.001 mg/L. Results obtained, therefore, suggest that the occurrence of these heavy metals in the environments will greatly reduce the population of these organisms and hence the microbial diversity of the affected ecosystem. Chemical toxicity of microorganisms has been evaluated through the inhibition of β-galactosidase activity [21,10]. Heavy metals can decrease enzyme activity by interacting with the enzyme-substrate complex, denaturing the enzyme protein or interrelating with the protein-active groups and can also disrupt the synthesis of microbial cells enzyme [22].

Lead as low as 1 mg/L was reported to be lethal to bacterial isolates [23]. The authors stated that the percentage survival of the isolates when exposed to 1 mg/L of Pb^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+} and Ni^{2+} were less than 30%.

Zinc is a trace element essential for normal physiological role of microbial cells. However, it is toxic to living cells and obstructs biochemical processes at concentration greater than the physiologically vital level [24]. Rensing and Grass [25] reported that copper is required for aerobic metabolism but when in excess is highly toxic to microorganisms. Copper ions inactivate proteins by damaging Fe-S clusters in cytoplasmic hydratases [26]. High concentration of iron and other trace elements could limit bacterial growth and alter their metabolic pattern [27]. The response of the enzyme system appeared to be dependent on the organism and the metal and the differences in the response to heavy metal toxicity can be ascribed to physiological dissimilarities in the organisms.

The responses of β-galactosidase synthesis to toxicity of the heavy metals can be mathematically defined with logistic dose-response models with high coefficient of regression ($R^2 > 0.80$). According to Nweke and Okpokwasili [10] small Ki value implies that there is strong affinity between the operon system and the inhibitor and as a result, the enzyme induction will be more intensely inhibited. They went further to deduce that higher Ki means lower toxicity and higher IC_{50} indicating good correlation between the Ki and IC_{50}. This relationship between Ki and IC_{50} was also observed in this study. In terms of IC_{50} the order of sensitivity of *Providencia stuartii* to metal ions is $Pb^{2+} > Cu^{2+} > Zn^{2+} > Mn^{2+} > Fe^{2+}$ and the order of sensitivity of *Pantoea dispersa* and *Aeromonas dhakensis* are $Cu^{2+} > Pb^{2+} > Zn^{2+} > Mn^{2+} > Fe^{2+}$. These sequences are like the one based on inhibition coefficient (Ki). The uniformity in the two sequences is an indication that the inhibition coefficient can be used in addition to the IC_{50} values as a measure of toxicity. Microbial activity affects the ecosystem balance and hence can be employed as an indicator of the ecological condition.

5. CONCLUSION

The study evaluated the effect of heavy metals (copper, lead, zinc, manganese and iron) on β-galactosidase activity in three bacterial strains (*Providencia stuartii*, *Pantoea dispersa* and *Aeromonas dhakensis*) isolated from coastal marine sediment. Metal concentration as low as 0.001 mg/L triggered a decrease in enzyme activity. The response of the enzyme system appeared to be dependent on the organism and the metal. The sensitivity patterns of *Pantoea dispersa* and *Aeromonas dhakensis* were Cu>Pb>Zn>Mn>Fe while that of *Providencia stuartii* was Pb>Cu>Zn>Mn>Fe. The isolated bacterial strains could serve as ideal organisms for heavy metal toxicity evaluation. Increase in the amount of metals deposited in the environment affects the microbial activities with negative effect on biogeochemical cycle.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

1) *Providencia stuatii* sequences

>MF370901.1 *Providencia stuartii* strain Y49W1 16S ribosomal RNA gene, partial sequence

GGTAACAGGGGAAGCTTGCTTCTCGCTGACGAGCGGCGGACGGGTGAGTAATGTATGGGGATC
TGCCCGATAGAGGGGGA
TAACTACTGGAAACGGTGGCTAATACCGCATAATCTCTTAGGAGCAAAGCAGGGGACCTTCGGG
CCTTGCGCTGTCGGAT
GAACCCATATGGGATTAGCTAGTAGGTAAGGTAATGGCTTACCTAGGCGACGATCCCTAGCTGG
TCTGAGAGGATGATCA
GCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCAC
AATGGGCGCAAGCCTGA
TGCAGCCATGCCGCGTGTATGAAGAAGGCCCTAGGGTTGTAAAGTACTTTCAGTCGGGAGGAAG
GCGTTGATGTTAATAC
CATCAACGATTGACGTTACCGACAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAA
TACGGAGGGTGCAAGC
GTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTAATTAAGTTAGATGTGAAATCCCC
GGGCTTAACCTGGGA
ATGGCATCTAAGACTGGTTAGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCATGTGTAGCGGTG
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2) *Aeromonas dhakensis* sequences

>CP023141.1 *Aeromonas dhakensis* strain KN-Mc-6U21, complete genome

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3) *Pantoea dispersa* sequences

>**MG450362.1 *Pantoea dispersa* strain PRPB12 16S ribosomal RNA gene, partial sequence**

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