

*Full Length Research Paper*

# Inhibition of $\beta$ -galactosidase and $\alpha$ -glucosidase synthesis in petroleum refinery effluent bacteria by zinc and cadmium

C. O. Nweke<sup>1\*</sup> and G. C. Okpokwasili<sup>2</sup>

<sup>1</sup>Department of Microbiology, Federal University of Technology, P. M. B. 1526, Owerri, Nigeria.

<sup>2</sup>Department of Microbiology, University of Port Harcourt, P. M. B. 5323, Port Harcourt, Nigeria.

Accepted 7 February, 2011

**Inhibition of  $\alpha$ -glucosidase (EC 3.2.1.20) and  $\beta$ -galactosidase (EC 3.2.1.23) biosynthesis by zinc and cadmium in *Escherichia coli*, *Bacillus* and *Pseudomonas* species isolated from petroleum refinery wastewater was assessed. At sufficient concentrations, exposure of the cells to zinc and cadmium resulted in repression of  $\alpha$ -glucosidase and  $\beta$ -galactosidase induction. The extent of inhibition varied among the test bacteria, metal and enzyme. *Bacillus* with  $IC_{50}$  of  $0.125 \pm 0.004$  and  $0.120 \pm 0.007$  mM is most sensitive to cadmium inhibition of  $\alpha$ -glucosidase and  $\beta$ -galactosidase respectively. Biosynthesis of  $\beta$ -galactosidase in *Bacillus* species is most sensitive to toxicity of zinc. The patterns of these toxic effects can be mathematically described with logistic or sigmoid dose-response models. The coefficients of inhibition  $K_i$  correlated with the  $IC_{50}$  and indicate that inhibition coefficient can be used in addition to the  $EC_{50}$  values as a measure of toxicity. The information could have a bearing on the development of bacterial models for acute toxicity assays.**

**Key words:** Zinc, cadmium,  $\alpha$ -glucosidase,  $\beta$ -galactosidase, toxicity, inhibition coefficient.

## INTRODUCTION

Large volumes of wastewater are generated from petroleum refining activity. Wastewaters released by crude oil-processing and petrochemical industries are characterized by the presence of large quantities of crude oil products, polycyclic and aromatic hydrocarbons, phenols, metals and metal derivatives, surface-active substances, sulfides, naphthylenic acids and other chemicals (Suleimanov, 1995). Refineries also generate solid waste and sludges (ranging from 3 to 5 kg per ton of crude oil processed), 80% of which may be considered hazardous because of the presence of toxic organics and heavy metals (World Bank Group, 1998).

These chemicals can be toxic to aquatic life if the refinery effluent is discharged into the aquatic environment. There are a number of investigations on the biological impact of pollutants in refinery effluent (Onwumere and Oladimeji, 1990; Sherry et al., 1994; Krishnakumar et al., 2007; Nwanyaonu and Abu, 2010).

Characterization of Port Harcourt refinery (Nigeria) effluent indicates the presence of phenol, sulphates, phosphates, chlorides, nitrates, ammonium, zinc, iron and trace amounts of cadmium, cobalt, lead and copper (Otokunefor and Obiukwu, 2005; Nweke, 2010).

Although the concentrations of metals in the effluents are usually low, they may accumulate in the effluents and receiving water bodies due their non biodegradable nature. Some heavy metals like copper, nickel, zinc and cobalt are essential trace elements required for normal physiological function of microorganisms, while others like cadmium and mercury have no known physiological function and are toxic even at low concentrations. At high concentrations, both essential and non essential metal are toxic to organisms.

Metal ions inhibit metabolic activities of microorganisms involved in the degradation of organic compounds and thus reduce biodegradation rates (Sandrin and Maier, 2003). Due to its toxicity to microorganisms, metal ions often cause the breakdown of wastewater treatment systems and disturb ecological balance in natural environments. Thus, sensitive bioassays are required to

\*Corresponding author. E-mail: [xrisokey@yahoo.com](mailto:xrisokey@yahoo.com).

assess their toxicity.

Bioassays based on bacterial enzymes have become increasingly popular for assessment of the toxicity of environmental toxicants (Bitton and Koopman, 1992; Guven and Bashan, 1998; Odokuma and Okpokwasili, 2003; Guven et al., 2003; Nweke et al., 2007). Enzymes play pivotal roles in the physiology of microorganisms. They are key catalysts of metabolic reactions occurring in living cells. A wide range of enzymes have been used as indices of chemical toxicity with special emphasis on dehydrogenase activity (Christensen et al., 1982; Bitton and Koopman, 1986; Obst et al., 1988; Kim et al., 1994; Helliwell and Harden, 1996). Inhibition of  $\beta$ -galactosidase and  $\alpha$ -glucosidase activity has also been used to assess chemical toxicity to microorganisms (Barnhart and Vestal, 1983; Dutton et al., 1988).

$\beta$ -Galactosidase is an intracellular enzyme and lactose or its derivatives induce its biosynthesis. It catalyzes the breakdown of lactose to glucose and galactose. On the other hand,  $\alpha$ -glucosidase is an extracellular enzyme whose biosynthesis is induced by maltose and degrades maltose into glucose residues. Unlike inhibition of enzyme activity, inhibition of enzyme biosynthesis as a measure of chemical toxicity has been studied to a lesser extent. Reports on the inhibition of enzyme biosynthesis have been mostly the  $\beta$ -galactosidase of *Escherichia coli* and  $\alpha$ -glucosidase of *Bacillus licheniformis* (Dutton et al., 1988; Reinhartz et al., 1987; Dutton et al., 1990; Guven et al., 2003; Odokuma and Okpokwasili, 2003).

The objective of this study is to compare the inhibitory effect of zinc and cadmium on the biosynthesis of  $\beta$ -galactosidase and  $\alpha$ -glucosidase of *Pseudomonas*, *Bacillus* and *Escherichia* species isolated from petroleum refinery wastewater.

## MATERIALS AND METHODS

### Test chemicals and reagents

Deionized distilled water used in preparing chemical and reagent solutions was sterilized by autoclaving. The test metal ions,  $Zn^{2+}$  and  $Cd^{2+}$  were used as  $ZnSO_4$  and  $CdCl_2$  respectively. The enzyme substrates *p*-nitrophenyl- $\beta$ -D-galactopyranoside and *p*-nitrophenyl- $\alpha$ -D-glucopyranoside were obtained from Sigma (USA). The Z-buffer for the enzyme assays contained the following components:  $Na_2HPO_4 \cdot 7H_2O$ , 16 g/L;  $NaH_2PO_4$ , 5.6 g/L;  $MgSO_4$ , 0.12 g/L; KCl, 0.754 g/L and  $\beta$ -mercaptoethanol, 2.7 ml/L.

### Cell cultures and heavy metal stress treatment

Bacterial strains used were *Pseudomonas* sp. DAF1, *Pseudomonas* sp. RWW2, *Bacillus* sp. DISK1 and *E. coli* isolated from petroleum refinery wastewater (Nweke and Okpokwasili, 2010). They were maintained in nutrient agar slants at 4°C. The cells were grown by inoculating 50 ml of sterile nutrient broth media (HIMEDIA) in 100 ml Erlenmeyer flask. The cultures were incubated at room temperature ( $28 \pm 2^\circ C$ ) for 16 to 24 h on rotary shaker operated at 150 rpm. Cells were harvested by centrifugation at 4000 rpm for 10 min. Harvested cells were washed twice in sterile deionized distilled water and resuspended in the same water. The

cell suspensions were standardized in a spectrophotometer to an optical density of 0.6 at 420 nm.

The standardized cell suspensions were used as inocula in the enzyme assays. Portions (0.1 ml) of standardized cell suspensions were inoculated into sterile triplicate 20 ml screw-capped test tubes containing 1.9 ml of Z-buffered (pH 7.0) nutrient broth-lactose medium (consisting of 0.4 ml of Z-buffer, 0.4 ml of nutrient broth and 0.1 ml of 0.4 %w/v lactose and requisite volume of deionized distilled water to make up) supplemented with a particular concentration of heavy metal ion (0.05 to 1.4 mM). The controls consisted of inoculated medium without metal ions. The cultures were shake-incubated at room temperature for 1 h. In the case of  $\alpha$ -glucosidase activity assay, 0.4% w/v lactose was substituted with 0.4% w/v maltose as enzyme inducer.

### $\beta$ -Galactosidase activity assay

$\beta$ -Galactosidase activities were determined using *p*-nitrophenyl- $\beta$ -D-galactopyranoside, which is hydrolyzed to yellow-coloured *p*-nitrophenol. After 1 h incubation as previously described, 0.1 ml of 7% w/v sodium dodecyl sulphate (SDS) was added into each tube and shaken to solubilize the cells. Then, 0.1 ml of 0.4% w/v *p*-nitrophenyl- $\beta$ -D-galactopyranoside was added to the reaction mixture and incubated at room temperature for 24 h. The reactions were stopped with 1 ml of cold 1 M  $Na_2CO_3$  solution. The absorbances of *p*-nitrophenol solution produced were measured spectrophotometrically at 420 nm ( $\lambda_{max}$ ). The  $\beta$ -galactosidase activities were calculated relative to controls.

### $\alpha$ -Glucosidase activity assay

$\alpha$ -Glucosidase activities were determined using *p*-nitrophenyl- $\alpha$ -D-glucopyranoside that is hydrolyzed to yellow-coloured *p*-nitrophenol. After 1 h incubation as previously described, 0.1 ml of 0.4% w/v *p*-nitrophenyl- $\alpha$ -D-glucopyranoside was added to the reaction mixture and incubated at room temperature for 24 h. The reactions were stopped by adding 1 ml of cold 1 M  $Na_2CO_3$  solution. The absorbances of *p*-nitrophenol solution produced were measured spectrophotometrically at 420 nm ( $\lambda_{max}$ ). The  $\alpha$ -glucosidase activities were calculated relative to controls.

### Data analysis

The degree of inhibition was determined relative to control (100% enzyme activity) on the basis of measured absorbances as shown in Equation 1. Differences at enzyme activity levels between the controls and other samples were taken as metal ion effect 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 determine the toxicity thresholds of the toxicants ( $IC_{20}$ ,  $IC_{50}$  and  $IC_{80}$ ), the experimental data were fitted into non-linear logistic (Equation 2) or sigmoid (Equation 3) dose-response models by iterative minimization of sum of squares of the residuals based on Levenberg-Marquardt algorithm of Table Curve 2D. All regressions were done using the data mean and standard deviations at 95% confidence limit. The Pearson product-moment correlation and linear regression analysis were done using Microsoft Excell 2003:

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

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

$$\text{Enzyme activity (\% of control)} = \frac{a}{1 + e^{b(x-c)}} \quad (3)$$

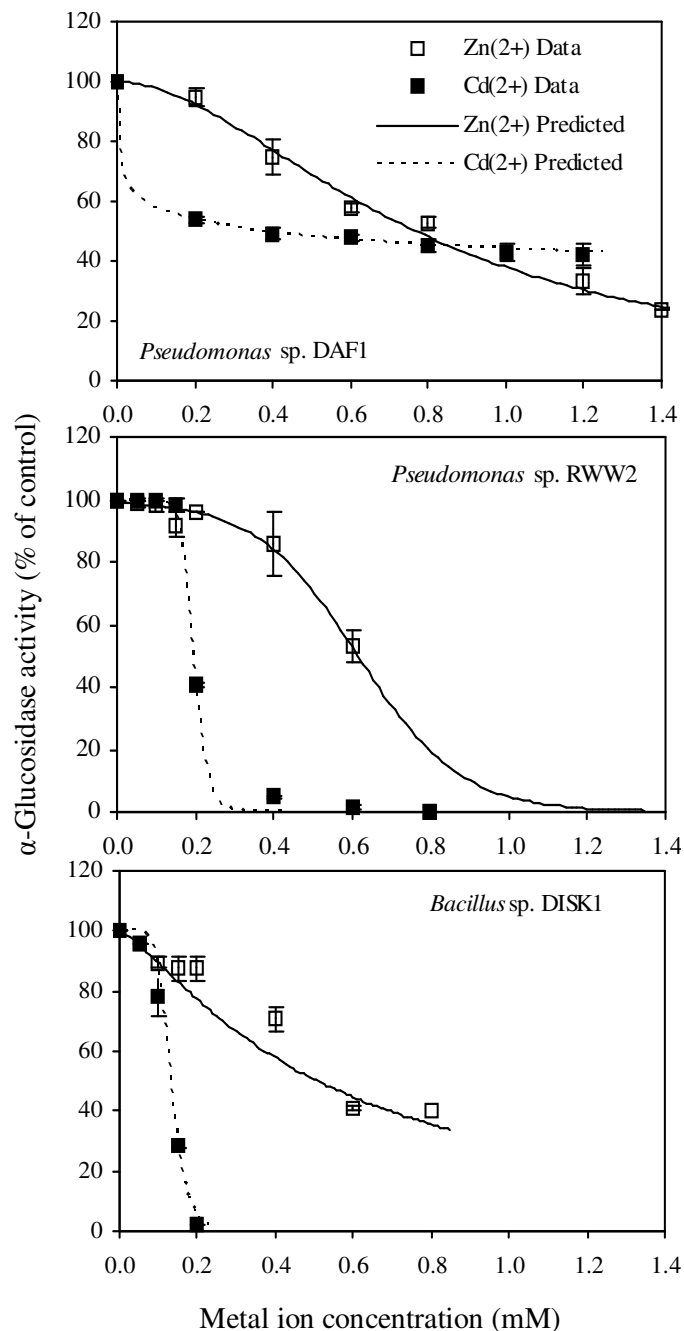
where  $C_A$  is the absorbance of *p*-nitrophenol in uninhibited control (without toxicant),  $T_A$  the absorbance of *p*-nitrophenol in inhibited test (with different concentrations of toxicant),  $x$  is the concentration of metal ion,  $a$  the uninhibited value of enzyme activity (100%),  $KI$  is dimensionless toxicity parameter,  $K_i$  is the coefficient of inhibition,  $b$  is slope parameter indicating the inhibition rate and  $c$  is  $IC_{50}$  (the value of  $x$  at  $y = 50\%$ ).

## RESULTS AND DISCUSSION

In the experiments, as was stated earlier, the differences at enzyme activity levels between the controls and other samples (containing metal ions) were taken as metal ion effect on enzyme biosynthesis. Given that the toxicant was added before enzyme induction, enzyme activity is a reflection of the amount of enzyme produced by the culture and thus the inhibition in the enzyme activity is taken to be inhibition of enzyme biosynthesis. The effects of  $Cd^{2+}$  and  $Zn^{2+}$  on the biosynthesis of  $\alpha$ -glucosidase by *Pseudomonas* sp. DAF1, *Pseudomonas* sp. RWW2, *Bacillus* sp. DISK1 and *E. coli* are shown in Figures 1 and 2. In all the bacterial strains,  $Cd^{2+}$  and  $Zn^{2+}$  repressed synthesis of  $\alpha$ -glucosidase as indicated in the repression of enzyme activity.

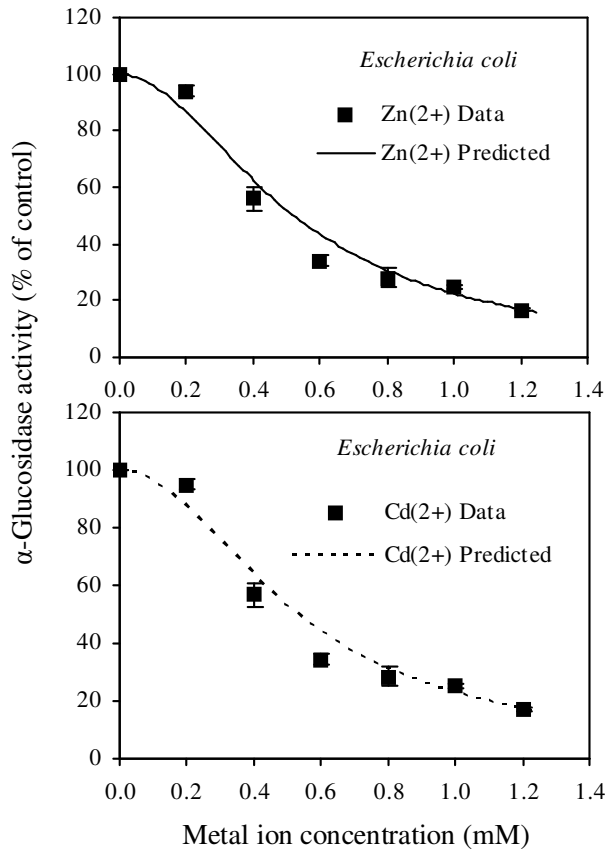
In *Pseudomonas* sp. DAF1, *Pseudomonas* sp. RWW2 and *Bacillus* sp. DISK1,  $Cd^{2+}$  was more inhibitory than  $Zn^{2+}$ . The responses of  $\alpha$ -glucosidase biosynthesis to zinc and cadmium appeared to be similar in *E. coli* (Figure 2). At 0.2 mM,  $Zn^{2+}$  and  $Cd^{2+}$  inhibited  $\alpha$ -glucosidase biosynthesis by  $6.00 \pm 2.00$  and  $4.858 \pm 2.024\%$  respectively. At 1.2 mM,  $Zn^{2+}$  and  $Cd^{2+}$  inhibited  $\alpha$ -glucosidase synthesis in *E. coli* by  $83.800 \pm 0.400$  and  $82.996 \pm 0.405\%$  respectively. The biosynthesis of  $\alpha$ -glucosidase in *Pseudomonas* sp. RWW2 and *Bacillus* sp. DISK1 were most sensitive to inhibitory effect of  $Cd^{2+}$ . At 0.2 mM  $Cd^{2+}$ ,  $\alpha$ -glucosidase synthesis was inhibited by 97.6% in *Bacillus* sp. DISK1. Also, in *Pseudomonas* sp. RWW2, 0.4 mM  $Cd^{2+}$  inhibited  $\alpha$ -glucosidase synthesis by  $95.208 \pm 0.625\%$ . Cadmium sharply inhibited  $\alpha$ -glucosidase synthesis in *Pseudomonas* sp. DAF1 at 0.2 mM and thereafter exhibited saturation effect at concentrations ranging from 0.4 to 1.2 mM. Such saturation effect of cadmium was observed with  $\beta$ -glucosidase activity in sediment bacteria (Montuelle et al., 1994).

The effects of zinc and cadmium ions on the biosynthesis of  $\beta$ -galactosidase are shown in Figure 3. In *E. coli* and *Pseudomonas* sp. DAF1,  $\beta$ -galactosidase synthesis was more sensitive to zinc than cadmium. However, in *E. coli*, zinc was more inhibitory at lower concentration ranging from 0 to 1.0 mM, and at 1.4 and



**Figure 1.** Effects of  $Zn^{2+}$  and  $Cd^{2+}$  on  $\alpha$ -glucosidase activity of *Pseudomonas* sp. DAF1, *Pseudomonas* sp. RWW2 and *Bacillus* sp. DISK1. The data points represent mean  $\pm$  standard deviation ( $n = 3$ ). The continuous and dotted lines are predicted values determined from dose response models. Differences at enzyme activity levels between the controls and other samples were taken as metal ion effect on enzyme biosynthesis.

1.8 mM,  $Cd^{2+}$  become more inhibitory than zinc. In this organism,  $Zn^{2+}$  appeared to exhibit saturation effect at higher concentration. Conversely, in *Bacillus* sp. DISK1,  $Cd^{2+}$  was more inhibitory to synthesis of  $\beta$ -galactosidase. The variations in the response could be attributed to

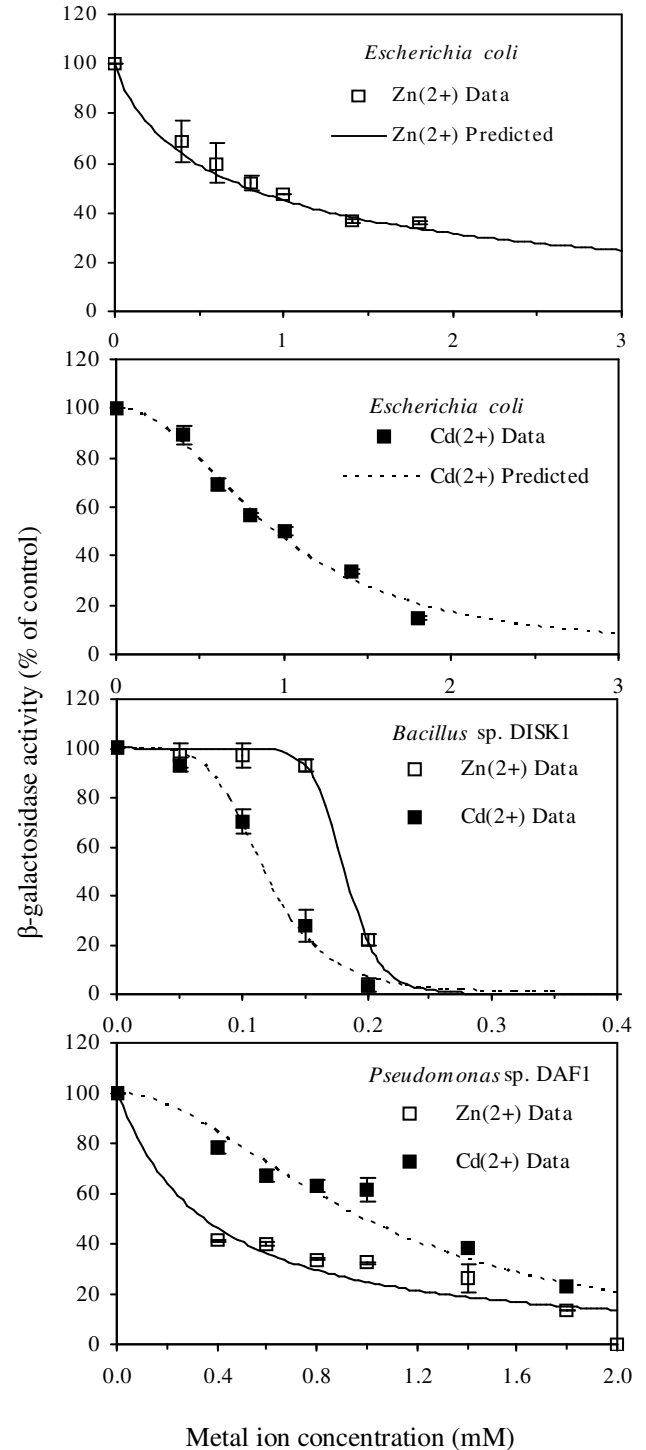


**Figure 2.** Effects of  $Zn^{2+}$  and  $Cd^{2+}$  on  $\alpha$ -glucosidase activity of *E. coli*. The data points represent mean  $\pm$  standard deviation ( $n = 3$ ). The continuous and dotted lines are predicted values determined from logistic dose response model. Differences at enzyme activity levels between the controls and other samples were taken as metal ion effect on enzyme biosynthesis

physiological differences in the organisms. Microorganisms have different metal uptake systems and vary in the way they accumulate metal in their cytoplasm. It is expected that toxicity of metal ions would increase proportionally with concentration. However, studies have found that in some cases, higher metal concentrations activate aggressive resistance mechanisms that increase microbial tolerance to metals (Roane and Pepper, 2000).

Comparatively, the  $\alpha$ -glucosidase biosynthesis is more sensitive than  $\beta$ -galactosidase biosynthesis to toxicity of zinc and cadmium ions in *E. coli*. In *Pseudomonas* sp. DAF1,  $\alpha$ -glucosidase is more sensitive to toxicity of cadmium while  $\beta$ -galactosidase synthesis is more sensitive to zinc. In *Bacillus* species,  $\beta$ -galactosidase biosynthesis was more sensitive to inhibitory effect of cadmium and zinc. The responses of the enzyme systems appeared to be dependent on the organism and the metal.

The responses of  $\alpha$ -glucosidase and  $\beta$ -galactosidase biosynthesis to toxicity of the heavy metals could be mathematically described with logistic and sigmoid dose-



**Figure 3.** Effects of  $Zn^{2+}$  and  $Cd^{2+}$  on  $\beta$ -Galactosidase activity of *Pseudomonas* sp. DAF1 *Bacillus* sp. DISK1 and *Escherichia coli*. The data points represent mean  $\pm$  standard deviation ( $n = 3$ ). The continuous and dotted lines are predicted values determined from logistic dose response model. Differences at enzyme activity levels between the controls and other samples were taken as metal ion effect on enzyme biosynthesis.

response models with high coefficient of regression ( $R^2 > 0.97$  for  $\alpha$ -glucosidase and  $R^2 > 0.91$  for

**Table 1.** Threshold concentrations of zinc and cadmium for inhibition of  $\alpha$ -glucosidase and  $\beta$ -galactosidase biosynthesis.

Bacteria	Metal	Threshold concentrations (mM)					
		$\alpha$ -Glucosidase			$\beta$ -Galactosidase		
		IC <sub>20</sub>	IC <sub>50</sub>	IC <sub>80</sub>	IC <sub>20</sub>	IC <sub>50</sub>	IC <sub>80</sub>
<i>Pseudomonas</i> sp. DAF1	Zn <sup>2+</sup>	0.356 ± 0.047	0.777 ± 0.056	1.700 ± 0.021	0.099 ± 0.004	0.368 ± 0.109	1.368 ± 0.109
	Cd <sup>2+</sup>	0.002 ± 0.001	0.385 ± 0.099	ND	0.448 ± 0.050	1.056 ± 0.062	2.492 ± 0.014
<i>Pseudomonas</i> sp. RWW2	Zn <sup>2+</sup>	0.474 ± 0.080 <sup>b</sup>	0.588 ± 0.031 <sup>b</sup>	0.702 ± 0.021 <sup>b</sup>	-	-	-
	Cd <sup>2+</sup>	0.172 ± 0.001	0.194 ± 0.001	0.219 ± 0.002	-	-	-
<i>Bacillus</i> sp. DISK1	Zn <sup>2+</sup>	0.247 ± 0.047	0.578 ± 0.023	1.377 ± 0.156	0.164 ± 0.004	0.182 ± 0.003	0.202 ± 0.002
	Cd <sup>2+</sup>	0.099 ± 0.006	0.125 ± 0.004	0.160 ± 0.000	0.090 ± 0.005	0.120 ± 0.007	0.160 ± 0.011
<i>E. coli</i>	Zn <sup>2+</sup>	0.240 ± 0.018	0.490 ± 0.030	0.974 ± 0.049	0.213 ± 0.117	0.873 ± 0.139	ND
	Cd <sup>2+</sup>	0.251 ± 0.019	0.496 ± 0.031	0.980 ± 0.048	0.497 ± 0.031	0.941 ± 0.032	1.781 ± 0.009

<sup>b</sup> threshold concentrations of zinc against  $\alpha$ -glucosidase synthesis in *Pseudomonas* sp. RWW2 was determined from sigmoid model (Equation 3). ND = not determined

**Table 2.** Coefficients of inhibition of  $\alpha$ -glucosidase and  $\beta$ -galactosidase biosynthesis by Zn<sup>2+</sup> and Cd<sup>2+</sup>.

Bacteria	Metal	$\alpha$ -glucosidase			$\beta$ -galactosidase		
		KI	K <sub>i</sub>	R <sup>2</sup> adj	KI	K <sub>i</sub>	R <sup>2</sup> adj
<i>Pseudomonas</i> sp. DAF1	Zn <sup>2+</sup>	1.8662	0.6123	0.9935	1.0479	0.3316	0.9112
	Cd <sup>2+</sup>	0.2428	0.7884	0.9994	1.8934	0.9728	0.9857
<i>Pseudomonas</i> sp. RWW2	Zn <sup>2+</sup>	-	-	0.9708 <sup>b</sup>	-	-	-
	Cd <sup>2+</sup>	11.5253	6.0935 × 10 <sup>-9</sup>	0.9951	-	-	-
<i>Bacillus</i> sp. DISK1	Zn <sup>2+</sup>	1.3089	0.4130	0.9790	13.3713	1.2877 × 10 <sup>-10</sup>	0.9985
	Cd <sup>2+</sup>	6.8210	9.4520 × 10 <sup>-7</sup>	0.9945	4.8662	2.9508 × 10 <sup>-5</sup>	0.9889
<i>Escherichia coli</i>	Zn <sup>2+</sup>	1.9109	0.2894	0.9895	0.8324	0.8180	0.9874
	Cd <sup>2+</sup>	1.9310	0.2944	0.9889	2.0841	0.8729	0.9778

<sup>b</sup> Coefficient of regression was determined from sigmoid model (Equation 3).

$\beta$ -galactosidase). The toxicity thresholds and the coefficients of inhibition generated from the models are shown in Tables 1 and 2. *Bacillus* sp. DISK1 is most sensitive to toxicity of cadmium, inhibiting  $\alpha$ -glucosidase and  $\beta$ -galactosidase biosynthesis by 50% at 0.125 ± 0.004 and 0.120 ± 0.007 mM Cd<sup>2+</sup> respectively. Other bacteria seem to have high resistance to cadmium. Cadmium as low as 0.01 mM was reported to be lethal to *E. coli* strain (Ferianc et al., 1998).

Cadmium concentrations of 0.05 and 0.1 mM barely inhibited the growth of *Rhodobacter capsulatus* B10 and at 0.15 mM, CdCl<sub>2</sub> inhibited cell proliferation, extended the lag phase and induced production and expression of cadmium-binding proteins. In the presence of 0.3 mM CdCl<sub>2</sub>, growth of *R. capsulatus* B10 was completely

inhibited (Mohamed et al., 2006). Cadmium and zinc were reported to inhibit the growth of *E. coli* by approximately 50% at 0.2 and 0.6 mM respectively (Isarankura-Na-Ayudhya et al., 2009). In *E. coli*, 25 ppm of CdCl<sub>2</sub> (0.136 mM Cd<sup>2+</sup>) inhibited  $\beta$ -galactosidase biosynthesis by 97.66 ± 7.9 % while 50 ppm of ZnCl<sub>2</sub> (0.367 mM Zn<sup>2+</sup>) inhibited  $\beta$ -galactosidase biosynthesis by 95.83 ± 3.1% (Guyen et al., 2003). By applying linear regression to the data of Guven et al. (2003), the median inhibitory concentrations (IC<sub>50</sub>) of ZnCl<sub>2</sub> and CdCl<sub>2</sub> were estimated at 22.698 ± 2.677 ppm (0.167 ± 0.02 mM) and 15.644 ± 1.279 ppm (0.085 ± 0.007 mM) respectively.

According to Dutton et al. (1990), 50% inhibition of  $\alpha$ -glucosidase and  $\beta$ -galactosidase biosynthesis by cadmium was achieved at 1.4 ± 0.05 mg/L (0.0076 ±

0.0003 mM) and  $1.3 \pm 0.18$  mg/L ( $0.0071 \pm 0.001$  mM) respectively. The reason for the increased tolerance of *E. coli* and *Pseudomonas* sp. DAF1 to cadmium inhibition of  $\alpha$ -glucosidase and  $\beta$ -galactosidase is not known. However, it can be attributed to cell envelope properties, inherent resistance factors, the mechanism of metal toxicity or the medium composition used to assess the response of bacteria to toxicity of heavy metals.

The reciprocal of the inhibition coefficient  $K_i$  represents the affinity of the enzyme operon system to the inhibitor. Small  $K_i$  value implies that there is strong affinity between the operon system and the inhibitor and consequently, the enzyme induction will be more strongly inhibited. Thus, higher  $K_i$  means lower toxicity and higher  $IC_{50}$ . The Pearson product-moment correlation coefficient ( $r = 0.9989$  and  $0.9843$  for  $Zn^{2+}$  inhibition of  $\alpha$ -glucosidase and  $\beta$ -galactosidase synthesis respectively;  $r = 0.6537$  and  $0.9998$  for  $Cd^{2+}$  inhibition of  $\alpha$ -glucosidase and  $\beta$ -galactosidase synthesis respectively) indicated good correlation between the  $K_i$  and  $IC_{50}$ .

In terms of  $IC_{50}$ , the order of sensitivity to  $Zn^{2+}$  is *E. coli* > *Bacillus* sp. DISK1 > *Pseudomonas* sp. RWW2 > *Pseudomonas* sp. DAF1 for  $\alpha$ -glucosidase and *Bacillus* sp. DISK1 > *Pseudomonas* sp. DAF1 > *E. coli* for  $\beta$ -galactosidase. Also, the order of sensitivity to  $Cd^{2+}$  is *Bacillus* sp. DISK1 > *Pseudomonas* sp. RWW2 > *Pseudomonas* sp. DAF1 > *E. coli* for  $\alpha$ -glucosidase and *Bacillus* sp. DISK1 > *E. coli* > *Pseudomonas* sp. DAF1 for  $\beta$ -galactosidase. These sequences are similar to the one based on inhibition coefficient ( $K_i$ ). The similarity 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.

Zinc is a trace element required for normal physiological function of microbial cells. However, at concentration higher than the physiologically required level, zinc becomes toxic and inhibits biochemical processes in living cells (Kleiner, 1978; Pérez-García et al., 1993; Gadd, 1993; Nweke et al., 2006). On the other hand, cadmium has no known biochemical function and thus is strong inhibitor of microbial metabolism even at low concentration (Ross, 1975; Gadd, 1993). Although cadmium has no physiological function in bacteria, it seems to be taken up by the  $Mn^{2+}$  uptake system and  $Mg^{2+}$  transport system (Perry and Silver, 1982; Nies and Silver, 1989). The inhibition of  $\alpha$ -glucosidase and  $\beta$ -galactosidase biosynthesis in this study is consistent with the reported toxic effects of  $Zn^{2+}$  and  $Cd^{2+}$ . In the cell cytoplasm,  $Cd^{2+}$  causes damage to cells by causing single-stranded DNA damage and disruption of synthesis of nucleic acid and proteins (Mitra and Bernstein, 1978; Mitra, 1984; Nyström and Kjelleberg, 1987). Cadmium (0.2 mM  $CdCl_2$ ) was reported to alter protein synthesis machinery, particularly elongation factor-TU (thermo unstable) and 30S ribosomal protein S1 of *E. coli* (Isarankura-Na-Ayudhya et al., 2009; Brocklehurst and Morby, 2000; Ferienc et al., 1998; Wang and Crowley, 2005). In addition to causing similar effect aforementioned, zinc causes reduction of

oligotrophic transport periplasmic binding proteins in *E. coli* (Isarankura-Na-Ayudhya et al., 2009; Easton et al., 2006). Although the mechanism of inhibition of  $\alpha$ -glucosidase and  $\beta$ -galactosidase biosynthesis in the test bacteria was not investigated in this study, the organisms may have responded to zinc and cadmium exposure via induction of proteins that are required for  $\alpha$ -glucosidase and  $\beta$ -galactosidase biosynthesis.

The result of this study further revealed that inhibition of  $\beta$ -galactosidase biosynthesis in *Bacillus* sp. DISK1 was most sensitive to zinc. Thus, the enzyme operon controlling the inducible  $\alpha$ -glucosidase and  $\beta$ -galactosidase synthesis in the *Bacillus* species was the most sensitive to  $Zn^{2+}$  and  $Cd^{2+}$ . This bacterium therefore can serve as a convenient model for assessment of heavy metal toxicity and good alternative to the widely used *E. coli* in enzyme induction assays.

## REFERENCES

- Barnhart CLH, Vestal JR (1983). Effects of environmental toxicants on metabolic activity of natural microbial communities. *Appl. Environ. Microbiol.*, 46: 970 – 977.
- Bitton G, Koopman B (1986). Biochemical tests for toxicity screening. In: Bitton G, Dutka BJ (Eds). *Toxicity Testing Using Microorganisms*. CRC Press, Boca Raton FL, pp. 27 – 55
- Bitton G, Koopman B (1992). Bacterial and enzymatic bioassays for toxicity testing in environment. *Rev. Environ. Contam. Toxicol.*, 123: 1 – 22.
- Brocklehurst KR, Morby AP (2000). Metal-ion tolerance in *Escherichia coli*: analysis of transcriptional profiles by gene-array technology. *Microbiol.*, 146: 2277 – 2282.
- Christensen GM, Olson D, Reidel B (1982). Chemical effects on the activity of eight enzymes: A review and a discussion relevant to environmental monitoring. *Environ. Res.*, 29: 247 – 255
- Dutton RJ, Bitton G, Koopman B (1988). Enzyme biosynthesis versus enzyme activity as a basis for microbial toxicity testing. *Toxicol. Assess.*, 3: 245 – 253.
- Dutton RJ, Bitton G, Koopman B, Agami O (1990). Effects of environmental toxicants on enzyme biosynthesis: a comparison of  $\beta$ -galactosidase,  $\alpha$ -glucosidase and tryptophanase. *Arch. Environ. Contam. Toxicol.*, 19: 395 – 398.
- Easton JA, Thompson P, Crowder MW (2006). Time-dependent translational response of *E. coli* to excess  $Zn(2+)$ . *J. Biomol. Tech.*, 17: 303 – 307.
- Ferianc P, Farewell A, Nyström T (1998). The cadmium stress stimulon of *Escherichia coli*-K12. *Microbiol.*, 144: 1045 – 1050.
- Gadd GM (1993). Interactions of fungi with toxic metals. *New Phytol.*, 124: 25-60.
- Güven K, Bashan M (1998). The effects of ionic and non-ionic detergents on *Escherichia coli*  $\beta$ -galactosidase activity. *Biochem Arch.*, 14: 275 – 286.
- Güven K, Togrul S, Uyar F, Ozant S, De Pomerai DI (2003). A comparative study of bioassays based on enzyme biosynthesis in *Escherichia coli* and *Bacillus subtilis* exposed to heavy metals and pesticides. *Enzyme Microbial Technol.*, 32: 658 – 664.
- Helliwell S, Harden TJ (1996). Bacterial toxicity assessment using resazurin reduction kinetics. *J. Chem. Edu.*, 73: 368 – 371.
- Isarankura-Na-Ayudhya P, Isarankura-Na-Ayudhya C, Treeratanapaiboon L, Kasikun K, Prachayasittikul V (2009). Proteomic profiling of *Escherichia coli* in response to heavy metal stress. *Eur. J. Sci. Res.*, 25(4): 679 – 688.
- Kim C-W, Koopman B, Bitton G (1994). INT-dehydrogenase activity test for assessing chloride and hydrogen peroxide inhibition of filamentous pure cultures and activated sludge. *Water Res.*, 28(5): 1117 – 1121.
- Kleiner D (1978). Inhibition of respiratory system in *Azotobacter*

- vinelandi* by divalent metal ions. FEBS Lett., 96(2): 366 – 368.
- Krishnakumar PK, Dineshbabu AP, Sasikumar G, Bhat GS (2007). Toxicity evaluation of treated refinery effluent using brine shrimp (*Artemia salina*) egg and larval bioassay. Fishery Technol., 44(1): 85 – 92.
- Mitra RS, Bernstein IA (1978). Single-strand breakage in DNA of *Escherichia coli* exposed to Cd<sup>2+</sup>. J. Bacteriol., 133: 75 – 80.
- Mitra RS (1984). Protein synthesis in *Escherichia coli* during recovery from exposure to low levels of Cd<sup>2+</sup>. Appl. Environ. Microbiol., 47: 1012 – 1016.
- Mohamed Fahmy Gad El-Rab S, Abdel-Fattah SA, Fukumori Y (2006). Effects of cadmium stress on growth, morphology and protein expression in *Rhodobacter capsulatus* B10. Biosci. Biotechnol. Biochem., 70(10): 2394 – 2402.
- Montuelle B, Latour X, Volat B, Gounot A-M (1994). Toxicity of heavy metals to bacteria in sediments. Bull. Environ. Contam. Toxicol. 53: 753 – 758.
- Nies DH, Silver S (1989). Metal ion uptake by plasmid-free metal-sensitive *Alcaligenes eutrophus*. J. Bacteriol., 150: 973 – 979.
- Nwanyanwu CE, Abu GO (2010). *In vitro* effects of petroleum refinery wastewater on dehydrogenase activity in marine bacterial strains. Ambi-Agua 5(2): 21 – 29.
- Nweke CO (2010). Kinetic modelling of toxicity, adsorption and biodegradation of phenolic compounds. A Ph.D. thesis of University of Port Harcourt, Nigeria. p. 90.
- Nweke CO, Okolo JC, Nwanyanwu CE, Alisi CS (2006). Response of planktonic bacteria of New Calabar River to zinc stress. Afr. J. Biotechnol., 5(8): 653 – 658.
- Nweke CO, Ntinugwa C, Obah IF, Ike SC, Eme GE, Opara EC, Okolo JC, Nwanyanwu CE (2007). *In vitro* effects of metals and pesticides on dehydrogenase activity in microbial community of cowpea (*Vigna unguiculata*) rhizosphere. Afr. J. Biotechnol., 6(3): 290 – 295.
- Nweke CO, Okpokwasili GC (2010) Influence of exposure time on phenol toxicity to refinery wastewater bacteria. J. Environ. Chem. Ecotoxicol., 2(2): 20 – 27.
- Nyström T, Kjelleberg S (1987). The effect of cadmium on starved heterotrophic bacteria isolated from marine waters. FEMS Microbiol. Ecol., 45: 143 – 153.
- Odokuma LO, Okpokwasili GC (2003). Bacterial enzyme biosynthesis: A tool for ecotoxicity assay. Global J. Pure Appl. Sci., 9(3): 311 – 318.
- Obst U, Holzapfel-Pschorn A, Wiegand-Rosinus M (1988). Application of enzyme assays for toxicological water testing. Toxic. Assess., 1: 81 – 91.
- Onwumere BG, Oladimeji AA (1990). Accumulation of metals and histopathology in *Oreochromis niloticus* exposed to treated NNPC Kaduna (Nigeria) petroleum refinery effluent. Ecotoxicol. Environ. Saf., 19: 123 – 134.
- Otokunefor TV, Obiukwu C (2005). Impact of refinery effluent on the physicochemical properties of a water body in the Niger Delta. Appl. Ecol. Environ. Res., 3(1): 61 – 72.
- Pérez-García A, Codina JC, Cazorla FM, de Vicente A (1993). Rapid respirometric toxicity test: sensitivity to metals. Bull. Environ. Contam. Toxicol., 50: 703 – 708.
- Perry RD, Silver S (1982). Cadmium and manganese transport in *Staphylococcus aureus* membrane vesicles. J. Bacteriol., 50: 973 – 979.
- Reinhartz A, Lampert I, Herzberg M, Fish F (1987). A new short term sensitive bacterial assay kit for the detection of toxicants. Toxic. Assess., 2: 193 – 206.
- Roane TM, Pepper IL (2000). Microorganisms and metal pollutants. In: Environmental Microbiology. Maier RM, Pepper IL, Gerba CP (Eds.). Academic press. New York, p. 404.
- Ross IS (1975). Some effects of heavy metals on fungal cells. Tans. Br. Mycol. Soc., 64: 175 – 193.
- Sandrin TR, Maier RM (2003). Impacts of metals on the biodegradation of organic pollutants. *Environ. Health Pers.* 111(8): 1093 – 1101.
- Sherry JP, Scott BP, Nagy E, Dutka BJ (1994). Investigation of the sub-lethal effects of some petroleum refinery effluents. J. Aquatic Ecosys. Health, 3: 129 – 137.
- Suleimanov RA (1995). Conditions of waste fluid accumulation at petrochemical and processing enterprises and prevention of their harm to water bodies. Meditsina Trudai Promyshlennaia Ekologiya, 12: 31 – 36.
- Wang A, Crowley DE (2005). Global gene expression responses to cadmium toxicity in *Escherichia coli*. J. Bacteriol., 187: 3259 – 3266.
- World Bank Group (1998). Petroleum refining. In: Pollution Prevention and Abatement Handbook. World Bank Group. pp 377 – 381.