Kinetics of zinc toxicity to environmental bacterial isolates
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ABSTRACT

Toxicity of zinc to *Pseudomonas*, *Escherichia*, *Proteus*, *Bacillus* and *Arthrobacter* species isolated from a tropical river and petroleum refinery effluent was assessed using TTC-dehydrogenase activity (DHA) inhibition test. At sufficient concentrations, zinc is toxic to these bacterial cells, and the exposure of the cells to zinc ion resulted in repression of dehydrogenase activity. The patterns of these toxic effects can be mathematically described with logistic dose-response models and in a manner similar to the non-competitive inhibition of enzymes. The threshold concentration above which toxic effect is observed ranged from 0.008 mM for *Pseudomonas* sp. DAF1 to 0.364 mM for *Proteus* sp. PLK2. The coefficients of inhibition K_i correlated with the IC_{50} and indicate that zinc toxicity is dependent on the organism. The K_i and toxicity threshold values predicted from the equations are comparable and are suitable indicators for kinetic analyses of zinc toxicity against bacteria.

Keywords: Zinc; dehydrogenase activity; toxicity thresholds; refinery effluent; inhibition coefficient.

Cinética da toxicidade de zinco para isolados ambientais de bactérias

RESUMO

Toxicidade de zinco para espécies dos gêneros *Pseudomonas, Escherichia, Proteus, Bacillus* e *Arthrobacter* isoladas de um rio tropical e de um efluente de refinaria de petróleo foi avaliada utilizando o teste de inibição de atividade de desidrogenase (TTC). Em concentrações suficientes, o zinco é tóxico para as células bacterianas e a exposição das células ao íon zinco resultou na repressão da atividade de desidrogenase. Os padrões desses efeitos tóxicos podem ser matematicamente descritos com base em modelos logísticos de dose-resposta de forma semelhante à não inibição competitiva de enzimas. A concentração limiar acima da qual o efeito tóxico foi observado variou de 0,008 mM para *Pseudomonas* sp. DAF1 até 0,364 mM para *Proteus* sp. PLK2. Os coeficientes de inibição K_i apresentaram correlação com o IC_{50} e indicaram que a toxicidade do zinco é dependente do organismo. Os limiares de K_i e de toxicidade previstos a partir das equações são comparáveis e são indicadores adequados para a análise cinética da toxicidade do zinco contra as bactérias.

Palavras-chave: zinco; atividade de desidrogenase; limiares de toxicidade de efluentes de refinarias; coeficiente de inibição.

1. INTRODUCTION

Zinc is an essential trace element required for optimal growth and metabolism of microorganisms (Borst-Pauwels, 1981; Ji and Silver, 1995). Although zinc is a component or activator of some enzymes, it becomes inhibitory to cellular activities at elevated concentrations (Babich and Stotzky, 1978; Gadd, 1993; Nweke et al., 2006). Zinc toxicity is caused by its interaction with sulfhydryl groups and other important enzyme radicals as well

as other proteins. Zinc is known to be a potent inhibitor of the respiratory electron transport systems of bacteria and eukaryotic organisms (Kasahara and Anraku, 1974; Beard et al., 1995; Choudhury and Srivastava, 2001). The intracellular concentrations of zinc must be finely adjusted to prevent either zinc deprivation or toxicity. Although microorganisms have mechanisms for homeostasis of toxic metals, there is a need to regulate the concentrations of metals in laboratory culture media, industrial bioprocess systems or natural ecosystems. To achieve this, adequate assessment of toxicity using microbial indicators is needed. Toxicity of zinc to microorganisms has been widely studied by quantifying observable metabolic changes such as, inhibition of growth, bioluminescence, dehydrogenase activity, nitrification etc. (Babich and Stotzky, 1978; Anderson et al., 1988; Kafka et al., 1997; Falih, 1998, Grunditz et al., 1998; Orji et al., 2008). However, kinetics of the toxic effects of metals has been studied to a lesser extent (Lewandowski et al., 1985; Volskay and Leslie Grady, 1988; Mazierski, 1994, Ren and Frymier, 2003). In this study, kinetics of the toxic effect of zinc to bacteria isolated from river water, river sediment and petroleum refinery wastewater were evaluated using dehydrogenase activity inhibition data.

2. MATERIALS AND METHODS

2.1. Bacterial strains

Bacterial strains were isolated from Port Harcourt petroleum refinery wastewater and New Calabar River, Port Harcourt, southeastern Nigeria. The method of isolation and identification of the bacterial strains was as described elsewhere (Nweke et al., 2006; 2007).

2.2. Dehydrogenase assay

Dehydrogenase activity was determined using 2,3,5-triphenyltetrazolium chloride as the artificial electron acceptor, which is reduced to red-coloured triphenylformazan (TPF). The assay was done in 3-ml volume of nutrient broth-glucose-TTC medium supplemented with varying concentrations of Zn^{2+} as $ZnSO_4$ in separate screw-capped test tubes. Portions (0.3 ml) of washed bacterial suspensions ($A_{420} = 0.5$) were inoculated into triplicate glass tubes containing 2.5 ml of phthalate-buffered (pH 6) nutrient broth glucose medium amended with Zn^{2+} and 0.2 ml of 0.4% (w/v) TTC in deionized distilled water was added to each tube to obtain final Zn^{2+} concentrations of 0.2 – 1.4 mM. The final concentrations of nutrient broth and glucose in the medium were 2 mg/ml each. The controls consisted of the isolates and the media without Zn^{2+}. The reaction mixtures were incubated under static conditions at room temperature (28 ± 2°C) for 24 h. The TPF produced was extracted in 4 ml of amyl alcohol and determined spectrophotometrically at 500 nm absorption maxima.

2.3. Data analysis

Inhibition of dehydrogenase activity by Zn^{2+} was calculated relative to the control as shown in Equation 1. The inhibition data generated are fitted into the kinetic models, Equation 2 (Kroiss et al. 1992; Juliastuti et al., 2003), Equation 3 (Ren and Frymier, 2003) and Equation 4 (Brains and Cousens, 1989; Abbondanzi et al., 2003). The kinetic parameters were estimated by iterative minimization of least squares using Levenberg-Marquardt algorithm of Table Curve 2D. All regression was done using the mean data and standard deviations. The Pearson product-moment correlation, linear regression analysis, t-test and analysis of variance (ANOVA) were done using Microsoft Excel 2003. The logistic dose-response functions, Equation 2 and Equation 4 were used to estimate the toxicity thresholds, IC_{20}, IC_{50} and IC_{80} which are the concentrations of Zn^{2+} that inhibited dehydrogenase activity by 20, 50 and 80% respectively. Pearson product-moment correlation was used to compare the inhibition constant

(K_i) estimated from Equation 2 and Equation 3 and the toxicity thresholds estimated from Equation 2 and Equation 4.

$$\text{Inhibition } (\%) = \left(\frac{C_A - T_A}{C_A} \right) \times 100 \qquad [1]$$

$$\text{Inhibition } (\%) = \left(1 - \frac{1}{1 + \dfrac{I^{KI}}{K_i}} \right) \times 100 \qquad [2]$$

$$\text{Inhibition}(\%) = \left(1 - \frac{1}{1 + \dfrac{I}{K_i} - \dfrac{a}{K_i}} \right) \times 100 \quad [3]$$

$$\text{Inhibition } (\%) = \frac{a_L}{1 + \left(\dfrac{I}{b} \right)^c} \qquad [4]$$

where: C_A = the absorbance of TPF produced in control test (without zinc); T_A= absorbance of TPF produced in the test with different concentrations of zinc; I = Inhibitor concentration (mM); K_i = inhibition constant (mM); KI= dimensionless inhibition parameter; a = threshold concentration above which toxic effect is observed (mM); a_L = maximum inhibition (%); b = slope parameter indicating the inhibition rate (mM); c = dimensionless inhibition parameter.

Equation 3 assumes that Zn^{2+} represses bacterial dehydrogenase activity by inhibiting the rate-determining step in the formation of triphenyl formazan in a manner similar to non-competitive inhibition of enzymes. In this case, the rate-determining step is the influx of electron to 2,3,5-triphenyltetrazolium chloride to form triphenyl formazan. This is catalyzed by a number of dehydrogenases, a battery of enzymes that transfer hydrogen and electron from substrate to 2,3,5-triphenyltetrazolium chloride (Rossel et al., 1997). Ren and Frymier (2003) have described the derivation of the model using bioluminescence inhibition data. A factor of 100 is added to the model to allow for the representation of repression of dehydrogenase activity in percentage.

3. RESULTS AND DISCUSSION

The New Calabar River is a short tidal coastal river situated in the Niger delta region of Nigeria. The river is impacted by effluent discharges from industries sited along its banks (Okpokwasili and Odokuma, 1993; Odokuma and Okpokwasili, 1997). Heavy metal resistant bacteria from this river have been reported (Odokuma and Ijeoma, 2003a, 2003b). More recently, the zinc content of the New Calabar River water and sediment were reported to be 5 mg/l and 65.8 mg/kg respectively (Nweke et al., 2007). The petroleum refinery effluent contains zinc at concentrations ranging from 0.02 mg/l in the rotary biodisk to 0.13 mg/l in the raw wastewater.

The selected bacterial strains used in this study were *Escherichia* sp. PLK1, *Proteus* sp. PLK2 and *Pseudomonas* sp. PLK5 isolated from the river water as well as *Bacillus* sp. SED1 and *Arthrobacter* sp. SED4 isolated from the river sediment. The petroleum refinery effluent

bacteria are *Pseudomonas* sp. DAF1, *Pseudomonas* sp. RWW2, *Bacillus* sp. DISK1 and *Escherichia* sp. DISK2. These bacteria are preponderant in their respective sources.
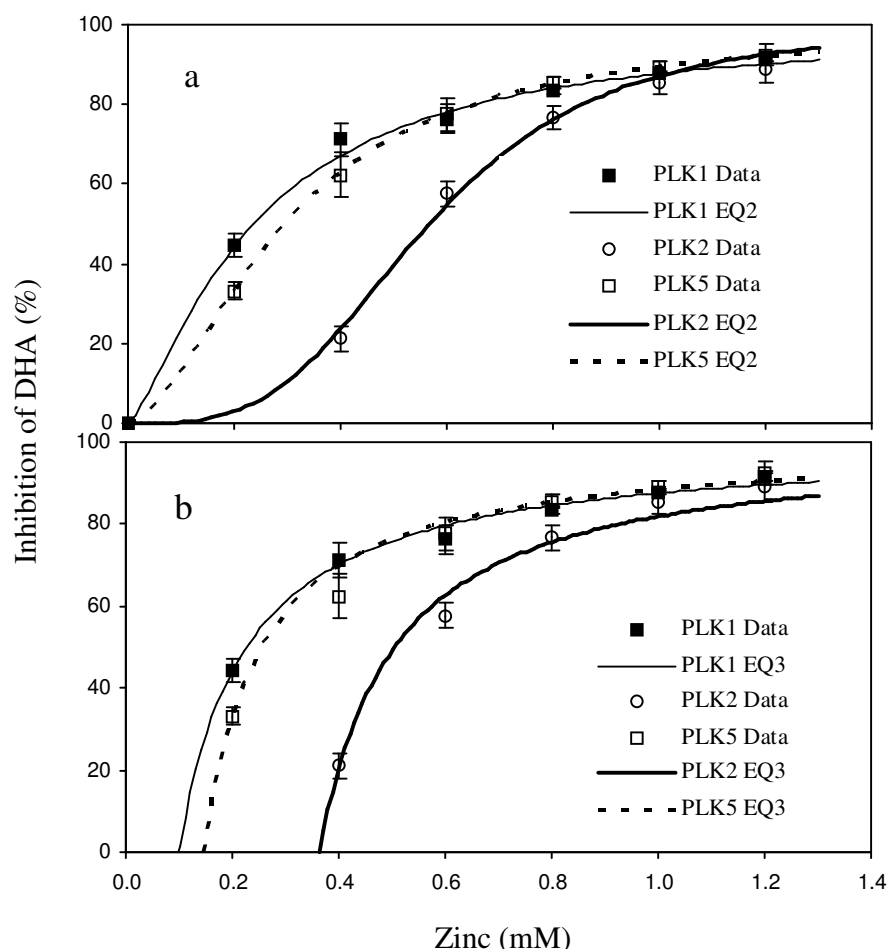

Figure 1. Effects of zinc on dehydrogenase activity (DHA) of *Escherichia* sp. PLK1, *Proteus* sp. PLK2 and *Pseudomonas* sp. PLK5 isolated from river water. The data points and bars represent experimental data mean ± standard deviation, and the dotted and continuous lines represent the data fit obtained with Equation 2 (a) and Equation 3 (b).

The effects of Zn^{2+} in terms of its relative inhibition of dehydrogenase activity in the bacterial strains are shown in Figures 1-3. The responses of the river water bacteria are dependent on the concentration of Zn^{2+}. In these bacterial strains, dehydrogenase activity was progressively inhibited with successive increase in zinc concentration. At 1.2 mM Zn^{2+}, dehydrogenase activities in *Escherichia* sp. PLK1, *Proteus* sp. PLK2 and *Pseudomonas* sp. PLK5 were inhibited by 91.702 ± 1.087%, 89.066 ± 3.584% and 92.384 ± 2.798%, respectively. Similarly, dose-dependent response was observed with sediment bacteria (Figure 2). In *Bacillus* sp. SED1, the dehydrogenase activity was progressively inhibited, and 89.182 ± 0.000% inhibition was observed at 1.0 mM. For *Arthrobacter* sp. SED4, total (100 %) inhibition of dehydrogenase activity occurred at 1.2 mM Zn^{2+}. The refinery effluent bacterial isolates were equally sensitive to toxicity of Zn^{2+} (Figure 3). The percentage inhibition of dehydrogenase activity increased with successive increase in the concentration of Zn^{2+}. This inhibition gradually increased in the case of *Pseudomonas* sp. RWW2 and *Pseudomonas* sp. DAF1. On the other hand, in the case of *Bacillus* sp. DISK1 and *Escherichia* DISK2, the inhibition increased sharply in the range of 0.05 to 0.2 mM Zn^{2+}. In *Bacillus* sp. DISK1, 0.1

mM resulted in 84.673 ± 3.199% inhibition of dehydrogenase activity, while 0.2 mM inhibited dehydrogenase activity by 63.333 ± 0.442% in *Escherichia* sp. DISK2. In both organisms, total inhibition of dehydrogenase activity occurred at 0.8 mM. Inhibition of dehydrogenase activity reported herein corroborates previously reported toxic effect of Zn^{2+} at high concentrations (Ji and Silver, 1995; Rensing et al., 1997). Although zinc is a trace element, it is toxic in excess of required concentration. Zinc is a potent inhibitor of respiratory activities in microorganisms (Kleiner, 1978; Kasahara and Anraku, 1974; Pérez-Garcia et al., 1993; Beard et al., 1995). Zinc ion concentrations of 0.0001 – 0.01 mM are required for optimal growth of most microorganisms *in vitro* (Sugarman, 1983).
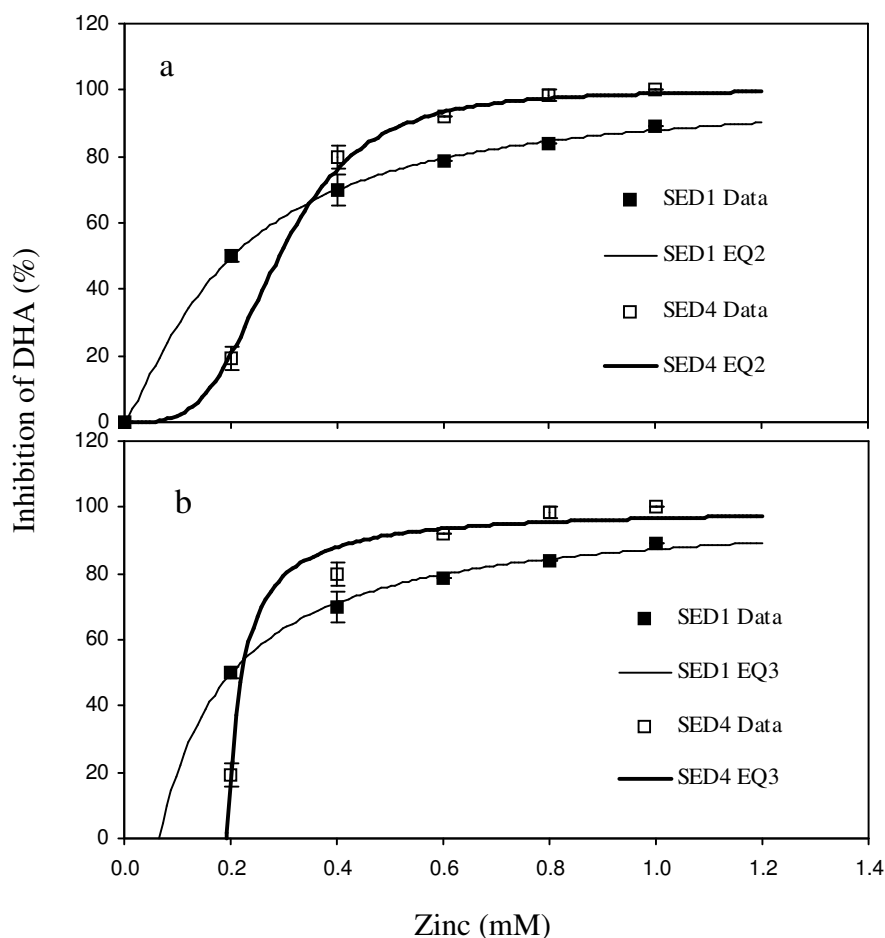

Figure 2. Effects of zinc on dehydrogenase activity (DHA) of *Bacillus* sp. SED1 and *Arthrobacter* sp. SED4 isolated from river sediment. The data points and bars represent experimental data mean ± standard deviation, and the continuous lines represent the data fit obtained with Equation 2 (a) and Equation 3 (b).
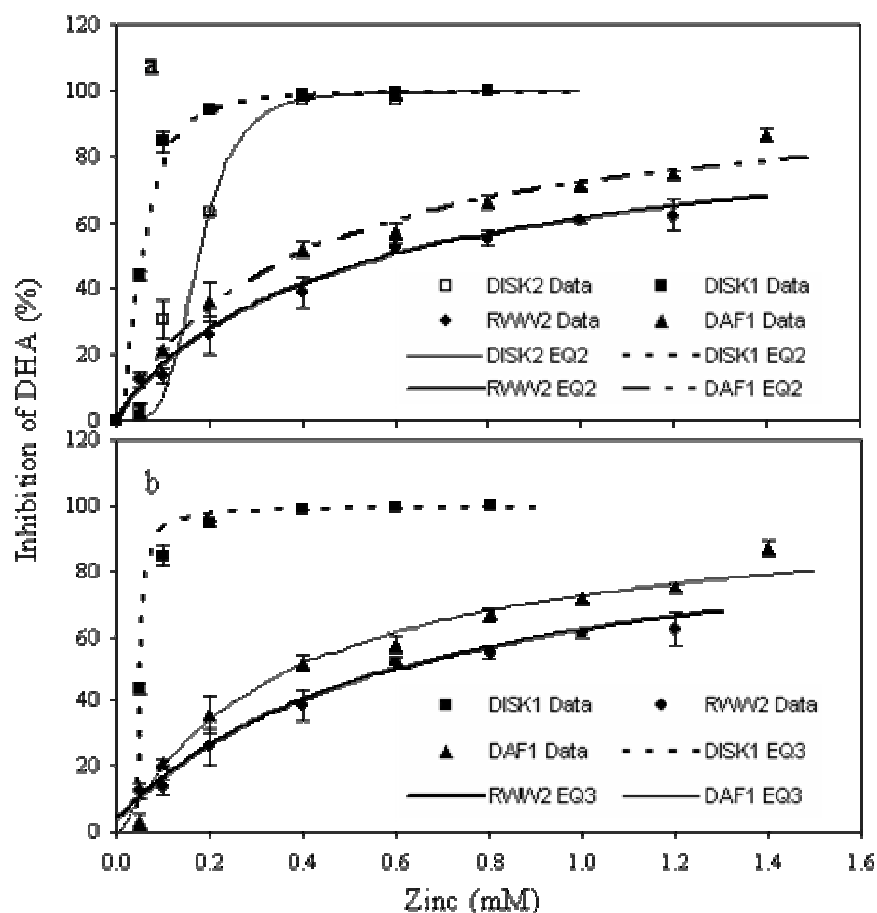
Figure 3. Effects of zinc on dehydrogenase activity (DHA) of *Pseudomonas* sp. DAF1, *Pseudomonas* sp. RWW2, *Bacillus* sp. DISK1 and *Escherichia* sp. DISK2 isolated from petroleum refinery wastewater. The data points and bars represent experimental data mean ± standard deviation. The continuous and broken lines represent the data fit obtained with Equation 2 (a) and Equation 3 (b).

The dose-response patterns of the bacterial strains are describable by kinetic models with high coefficient of regression ($R^2 > 0.98$). The inhibition coefficient (K_i) and toxicity threshold concentration (a) values are shown in Table 1. The results presented in Table 1 indicated that the inhibition of dehydrogenase activity varied and dependent on the bacteria. *Pseudomonas* sp. RWW2 had the highest K_i of 0.626 mM while *Escherichia* sp. DISK2 had the lowest K_i of 0.0005 mM. The inverse of the inhibition coefficient K_i represents the affinity of the enzyme to the inhibitor. Small K_i values imply that there is a strong affinity between the enzyme and the inhibitor and thus the enzyme will be more strongly inhibited for a given inhibitor concentration (Ren and Frymier, 2003). Likewise, higher K_i means lower toxicity and higher IC_{50}. Therefore, in terms of K_i, *Pseudomonas* sp. RWW2 is the most tolerant while *Escherichia* sp. DISK2 is the most sensitive bacteria. The linear regression of the IC_{50} and K_i predicted from Equation 2 gave high R^2 value of 0.8099, indicating good relationship between K_i and IC_{50} (Figure 4). Also, the association between the IC_{50} and K_i using the Pearson's product moment correlation coefficient ($r = 0.8999$) indicated that both parameters showed strong positive correlation. Ren and Frymier (2003) observed this kind of agreement between K_i and IC_{50} during toxicity assessment of metals against luminescent *Vibrio fischeri*. Although the mechanism of zinc inhibition was not investigated in this study, it is reasonable to assume

that the inhibition of dehydrogenase enzyme activity is similar to the non-competitive inhibition of enzymes. Zinc ion is not substrate analog and could not have bound competitively to the active site of dehydrogenase enzymes. According to inhibition coefficient (K_i), the order of sensitivity of the isolates to toxicity of zinc is *Escherichia* sp. DISK2 > *Bacillus* sp. DISK1 > *Arthrobacter* sp. SED4 > *Pseudomonas* sp. PLK5 > *Bacillus* sp. SED1 > *Escherichia* sp. PLK1 > *Proteus* sp. PLK2 > *Pseudomonas* sp. DAF1 > *Pseudomonas* sp. RWW2. Relatively, low concentration of zinc is required to inhibit dehydrogenase activity in refinery effluent bacteria. Similarly, *Bacillus* and *Escherichia* species of New Calabar River, having toxicity thresholds of 0.066 and 0.099 mM respectively would also be inhibited at low concentrations of zinc. Pearson product-moment correlation coefficient (r = -0.5098) indicated poor negative correlation between the toxicity threshold, a, and K_i. Although the minimum concentrations of Zn^{2+} that would inhibit dehydrogenase activity in the organisms were not determined experimentally, it would be some concentrations just above the respective toxicity threshold.

Table 1. Kinetic parameter estimates for zinc inhibition of dehydrogenase activity in bacterial isolates.

Bacteria/Source	Equation 2				Equation 3			
	KI	K_i (mM)	R^2	R^2Adj	a (mM)	K_i (mM)	R^2	R^2Adj
River water								
Escherichia sp. PLK1	1.365	0.140	0.9864	0.9773	0.099	0.128	0.9760	0.9600
Proteus sp. PLK2	3.366	0.148	0.9911	0.9822	0.364	0.141	0.9835	0.9669
Pseudomonas sp. PLK5	1.768	0.117	0.9998	0.9996	0.145	0.111	0.9950	0.9916
River sediment								
Bacillus sp. SED1	1.245	0.138	0.9939	0.9879	0.066	0.135	0.9882	0.9763
Arthrobacter sp. SED4	3.645	0.011	0.9963	0.9866	0.193	0.028	0.9616	0.9232
Petroleum refinery effluent								
Pseudomonas sp. DAF1	1.023	0.379	0.9899	0.9865	0.008	0.373	0.9903	0.9870
Pseudomonas sp. RWW2	0.887	0.626	0.9884	0.9838	-0.025	0.627	0.9869	0.9817
Bacillus sp. DISK1	2.119	0.002	0.9958	0.9930	0.047	0.004	0.9863	0.9772
Escherichia sp. DISK2	4.396	0.0005	0.9953	0.9905	-	-	-	-

The K_i values obtained from Equation 2 and Equation 3 were compared. This comparison is represented graphically in Figure 5a. The Pearson product-moment correlation coefficient (r) is 0.9991, indicating near perfect correlation. Linear regression analysis of the K_i values yielded the relationship, K_i (Equation 2) = 1.0079K_i (Equation 3) with high coefficient of regression of 0.9983. The result indicates that K_i (Equation 2) ≈ K_i (Equation 3). This is further elucidated by the plot of the function, K_i (Equation 2) = K_i (Equation 3) shown in Figure 5a. The linear regression line of K_i (Equation 2) versus K_i (Equation 3) coincides with the function K_i (Equation 2) = K_i (Equation 3). This indicates that the kinetic model (Equation 3) proposed by Kroiss et al. (1992) could be used not only as a suitable model to predict the inhibition coefficient K_i but also the concentration of a toxicant above which there is toxic effect. Although the model predicts negative inhibition of dehydrogenase activity for $I < a$, the model was not used to describe the effects of toxicant at concentrations below the threshold concentration. However, at concentrations below the threshold, *hormesis* or stimulation of dehydrogenase activity is expected. In *Proteus* sp. PLK2, Zn^{2+} stimulated dehydrogenase activity by 7.453 ± 3.00% at 0.2 mM (data not shown in the plots).

The inhibition thresholds, IC_{20}, IC_{50} and IC_{80} derived from the logistic models Equation 2 and Equation 4 are shown in Table 2. The median inhibitory concentrations (IC_{50}) of Zn^{2+} derived from Equation 4 ranged from 0.055 mM for *Bacillus* sp. DISK1 to 0.568 mM for

Pseudomonas sp. RWW2. According to IC_{50}, the order of sensitivity is *Bacillus* sp DISK1 > *Escherichia* sp. DISK2 > *Bacillus* sp. SED1 > *Escherichia* sp. PLK1 > *Arthrobacter* sp. SED4 > *Pseudomonas* sp. PLK5 > *Pseudomonas* sp. DAF1 > *Proteus* sp. PLK2 > *Pseudomonas* sp. RWW2. IC_{50} of zinc against *Pseudomonas fluorescens* in a TTC-dehydrogenase assay have been reported to vary from 0.64 to 1.27 mg/l (0.01 – 0.02 mM) (Abbondanzi et al., 2003). In a viability assay based on standard plate count, 0.25 mM of zinc killed 43% of soil bacteria (Keeling and Cater, 1998). A 100% lethal concentration of zinc ranging from 5 – 250 mg/l (0.077 – 0.385 mM) was reported for bacteria extracted from a pristine soil (Angle et al., 1993).

Table 2. Toxicity thresholds (mM) of zinc estimated from the kinetic models Equation 2 and Equation 4.

Bacteria/ Source	IC_{20}	IC_{50}	IC_{80}	R^2	R^2 adj	Fit Standard Error
				Equation 2		
River water						
Escherichia sp. PLK1	0.086	0.237	0.654	0.9864	0.9773	0.8259
Proteus sp. PLK2	0.375	0.567	0.856	0.9911	0.9822	3.0489
Pseudomonas sp. PLK5	0.135	0.297	0.650	0.9998	0.9997	0.3424
River sediment						
Bacillus sp. SED1	0.067	0.203	0.619	0.9939	0.9879	1.1091
Arthrobacter sp. SED4	0.199	0.291	0.426	0.9933	0.9866	1.5628
Petroleum refinery effluent						
Pseudomonas sp. DAF1	0.100	0.388	1.505	0.9899	0.9865	3.0614
Pseudomonas sp. RWW2	0.124	0.590	2.816	0.9884	0.9838	2.1157
Bacillus sp. DISK1	0.029	0.055	0.107	0.9958	0.9930	0.1787
Escherichia sp. DISK2	0.129	0.177	0.242	0.9953	0.9905	0.8093
				Equation 4		
River water						
Escherichia sp. PLK1	0.070	0.233	0.686	0.9916	0.9791	0.7472
Proteus sp. PLK2	0.392	0.556	0.845	0.9997	0.9987	0.6999
Pseudomonas sp. PLK5	0.136	0.296	0.647	0.9998	0.9997	0.3784
River sediment						
Bacillus sp. SED1	0.050	0.199	0.643	0.9990	0.9960	0.5547
Arthrobacter sp. SED4	0.197	0.292	0.432	0.9944	0.9776	1.7543
Petroleum refinery effluent						
Pseudomonas sp. DAF1	0.100	0.409	1.414	0.9902	0.9843	3.2562
Pseudomonas sp. RWW2	0.128	0.568	8.061	0.9904	0.9832	2.1100
Bacillus sp. DISK1	0.031	0.055	0.100	0.9977	0.9942	0.1532
Escherichia sp. DISK2	0.153	0.185	0.226	0.9990	0.9959	0.4626

The IC values obtained from Equation 2 and Equation 4 were compared. The Pearson product-moment coefficients (*r*) were 0.9944, 0.9981 and 0.9387 respectively for IC_{20}, IC_{50} and IC_{80} derived from Equation 2 and Equation 4. The relationships are shown in Figures 5b – d. Although Equation 4 described the experimental data better, as are indicated by the R^2 and fit standard error values, Equation 2 also proved to be suitable for predicting inhibition thresholds. In addition, the t-test (P < 0.01) indicated that there were no significant difference between the IC values obtained from Equation 2 and Equation 4. In all determinations, the linear regression coefficient (R^2) are greater than 0.98 and the regression line coincided with the function IC_x (Equation 2) = IC_x (Equation 4). It is noteworthy that Equation 2 is derived from the descending form of Equation 4, $y = a/(1+(x/b)^c)$ with a positive value of *c*. Equation 4 is ascending form with a negative value of *c*. The maximum value of *y* (% inhibition of
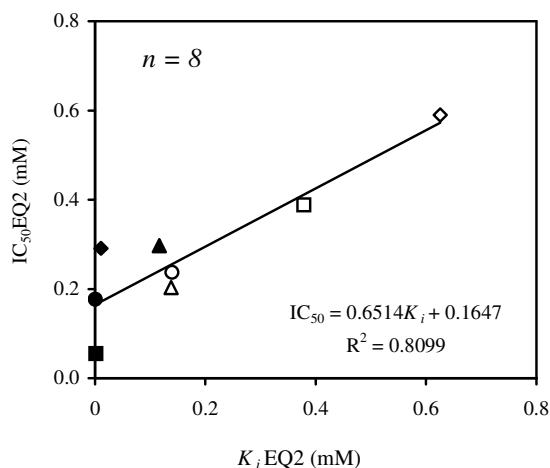
Figure 4. Comparison between K_i and IC_{50} values estimated from the kinetic model EQ2. Data represents: *Escherichia* sp. PLK1 (○); *Pseudomonas* PLK5 (▲); *Bacillus* sp. SED1 (Δ); *Pseudomonas* DAF1 (□); *Pseudomonas* sp. RWW2 (◊); *Arthrobacter* SED 4 (♦); *Bacillus* sp. DISK1 (■) and *Escherichia* sp. DISK2 (●) linear regression.
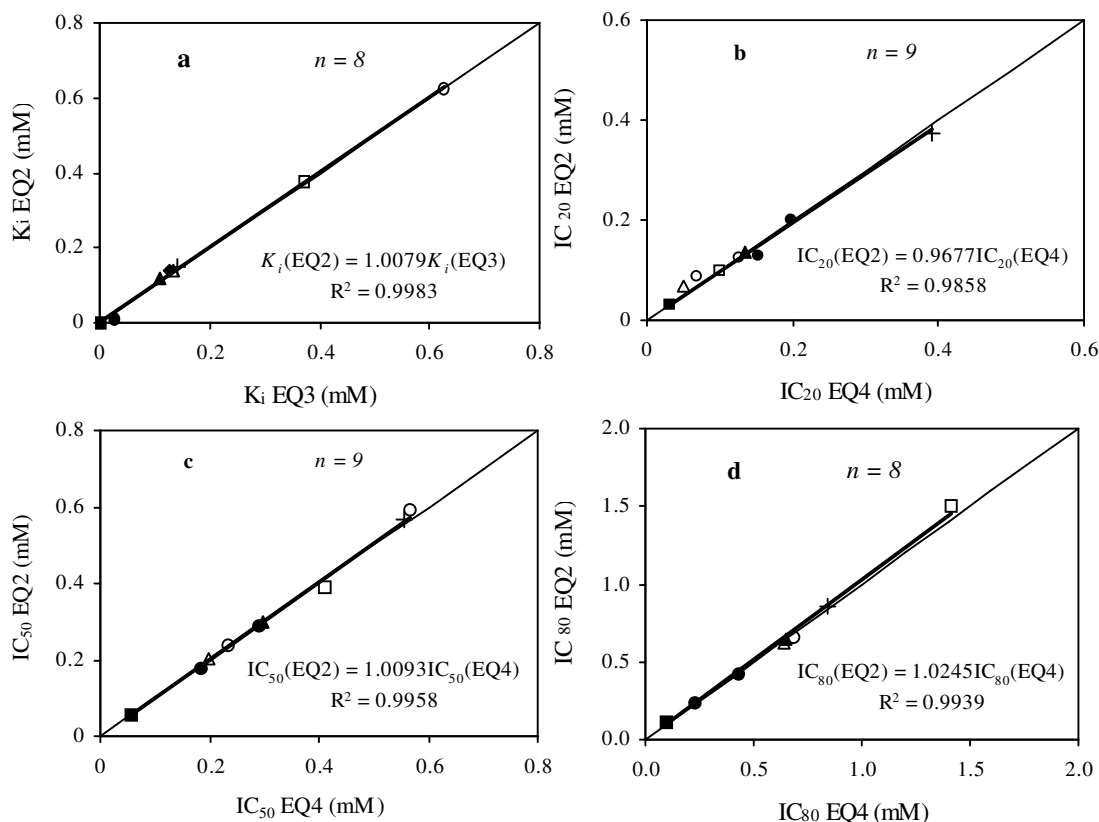

Figure 5. Comparisons between K_i values estimated from the kinetic models Equation 2 and Equation 3 (a) and between toxicity thresholds, IC_{20}, IC_{50} and IC_{80} (b-d) estimated from logistic dose-response models EQ2 and EQ4 . Data represents: *Escherichia* sp. PLK1 (○); *Proteus* sp. PLK2 (+)**;** *Pseudomonas* PLK5 (▲); *Bacillus* sp. SED1 (Δ); *Arthrobacter* SED 4 (♦); *Pseudomonas* DAF1 (□); *Pseudomonas* sp. RWW2 (◊); *Bacillus* sp. DISK1 (■) and *Escherichia* sp. DISK2 (●). The thin line represents the function $y = x$ and the thick line represents linear regression of data.

dehydrogenase activity) is set at 100% in equation 2 (Equation 2) and allowed as a parameter to be determined by successive iteration process in Equation 4. Thus the slight difference between the predicted values of equations 2 and 4 is brought about by the maximum value of the percentage inhibition used. Equation 2 is particularly applicable when there is no stimulation of enzyme activity at low concentrations of the test toxicant. The 2-way ANOVA showed that the dehydrogenase activity varied significantly ($p < 0.01$) with bacteria type and the concentrations of zinc.

4. CONCLUSION

The kinetics of zinc inhibition of dehydrogenase activity in bacterial strains can be mathematically described using logistic functions and as a non-competitive inhibition of rate-determining enzymatic reaction. There was agreement between K_i values obtained from the logistic function (Equation 2) and Equation 3, describing non-competitive inhibition of dehydrogenase activity. Also good agreement exists between the inhibition threshold concentrations (ICs) values predicted from Equation 2 and Equation 4. Additional information is provided by Equation 3 by predicting the respective threshold concentrations of zinc above which toxic effect is observed. These, represent the maximum concentration of zinc required for normal physiological activities of the bacteria. Thus, the information obtained from these kinetic models has bearing on the design and operation of bioprocess technologies, such as bioremediation, to prevent metal toxicity.

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