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Assessing the toxicities of the binary mixtures of sodium dodecyl sulfate and heavy metals to *Serratia marcescens* (SerEW01) from Otamiri River

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Abstract

Toxicities of sodium dodecyl sulfate (SDS) + Pb(II), SDS + Cd(II), SDS + Ni(II), SDS + Zn(II), and SDS + Co(II) binary mixtures to *Serratia marcescens* (SerEW01) isolated from Otamiri river water, Owerri, Imo State, Nigeria were undertaken, using dehydrogenase activity as a response. Inhibitions of dehydrogenase activity by the individual toxicants were concentration-dependent, increasing steadily as the concentration increases. The observed EC_{50S} ranged from 0.046 ± 0.003 mM for Zn(II) to 2.329 ± 0.092 mM for SDS. Duncan tests indicated that the EC_{50S} of the individual toxicants differed significantly from each other. The order of decreasing toxicities was Zn(II) > Cd(II) > Co(II) > Ni(II) > Pb(II) > SDS. Fixed ratio mixtures [Arbitrary concentration ratio (ABCR) and EC_{50} equieffect concentration ratio (EECR 50)] were used to study the joint action of the binary mixtures. The mixtures progressively inhibited dehydrogenase activity in *S. marcescens* as the concentration increases. However, SDS 98.08% + Co(II) 1.92% mixture ratio was biphasic. The effects of the mixtures on the dehydrogenase activity were assessed using Toxic Index, Model Deviation Ratio and Isobolographic analyses. In addition, the toxicities of the mixtures were predicted with concentration addition (CA) and independent action (IA) models. In SDS+Ni(II) binary mixture, both models predicted similar toxicities. In all binary mixtures, both models greatly underestimated the mixture toxicities compared to the experimentally-observed data. Similarly, both the experimentally-observed, CA and IA-predicted EC_{50S} were statistically different from each another. Furthermore, the binary mixtures were generally synergistic against *S. marcescens* (SerEW01). This demonstrates the potential danger of co-contamination of the aquatic system by SDS and heavy metals

Keywords: Heavy metals, surfactants, mixture, toxicity, synergism, predictive models

INTRODUCTION

Environmental pollution by chemicals such as surfactants and heavy metals are as a result of increasing industrial human activities. The penetration of large amounts of these pollutants into surface water reservoirs could result in foaming, reduction in diffusion of atmospheric oxygen that dissolve in water and consequently lead to the death of many aquatic organisms due to deficiency of oxygen (Seifert & Domka, (2005). Sodium dodecyl sulfate (SDS) is an anionic surfactant commonly used in industrial, agricultural and domestic detergents and therefore, is a common contaminant of aquatic environments, through contaminated waters, sediments, or soils, thus threatening portable water sources, aquatic organisms or both (Singer & Tjeerdema, 1993; Cowan-Ellsberry *et al*., 2014). Toxic metals from various sources could also contaminate surface and ground water sources. Apart from natural sources, anthropogenic activities that have contributed to heavy metals environmental

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contamination include but not limited to metal mining, battery industry, burning of organic fuels, electroplating, electronics production, oil and petrochemicals spillage, as well as various agricultural practices (Chaturvedi & Tiwari 2013).

The toxicity of chemical compounds on aquatic organisms has been reported to be dependent on the concentrations of the chemicals in both sediments and the overlaying water, as well as in processes that relates to their bioavailability. Furthermore, such processes as bioaccumulation, biodegradation, desorption and solubilization which take place in these substrata determine the quantity of the bio-available compounds that will reach toxic levels in the organs of organisms living in aquatic environment (Flores *et al*., 2010). Microbes are indispensable for the normal functioning of the ecosystem; hence parameters that influence their metabolic activities and diversity should be of great interest. Microbes are known for their prompt responses to environmental perturbations, thus evaluation of microbial responses has been suggested as an immediate sign of ecosystem disturbances (Griffiths, 1983).

Otamiri River in Imo State, Nigeria is the major river serving Owerri and environs as a source of water for drinking, other domestic activities, fishing, urban agriculture, recreation and sand mining. It equally receives various types of man-made pollutants of industrial, agricultural and domestic origin, through run-offs and untreated sewage. Similarly, solid wastes from Owerri urban and environs are also dumped and incinerated along the river bank. These activities have resulted in the contamination of the river water and sediment by both anionic surfactants and some heavy metals (Okechi & Chukwura, 2020). So far, many studies have been conducted on the bacteriological quality as well as heavy metals contamination of the river and its sediment (Onyekuru *et al*., 2017; Oga *et al.,* 2018; Okechi & Chukwura, 2020). Sodium dodecyl sulfate (SDS) was reported to be the dominant anionic surfactant in Otamiri river water and sediment. *Serratia marcescens* has also been reported in Otamiri river by Ogbulie *et al*. (2008). Similarly, *Serratia marcescens* (SerEW01) was reported to be the preponderant bacterium among the bacterial population in the river water (Okechi & Chukwura, 2020). This high level of SDS contamination of Otamiri River may have led to possible selection of this bacterium in the river. Furthermore, SDS utilization as carbon and energy source by *Serratia marcescens* has been reported (Fedeila *et al*., 2018; Ahmad *et al*., 2019). The toxicological responses of the bacterial population of this aquatic system to SDS and heavy metals have not been studied. This study was therefore aimed at investigating the toxicities of SDS and some of the heavy metals found in the river. Dehydrogenase activity in *S. marcescens* was used as microbial response in assessing the toxicities of the SDS and the metals as individual toxicants and in binary mixtures with SDS.

MATERIALS AND METHODS

Reagents

The deionized water used in the preparation of chemicals was sterilized by autoclaving and the stock reagents by membrane filtration. The heavy metal ions: Cd^{2+}, Pb^{2+}, Zn^{2+}, Co^{2+} and Ni^{2+} were used as $CdSO_4.8H_2O$, $Pb(NO_3)_2$, $ZnNO_3.6H_2O$, $CoCl_2$, and $NiSO_4.6H_2O$, respectively. These metals recorded values higher than WHO recommended standards for drinking water in Otamiri river (Okechi & Chukwura, 2020). These metals, SDS and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were sourced from Sigma-Aldrich (Germany).

Samples collection

The sampled area has been described previously (Okechi & Chukwura, 2020). The river water samples for this study were collected from two sampling points along the river course. The first was adjacent the free zone, motor mechanic village, Nekede (5.465N, 7.035E), and the second was 100 meters downstream of the first sampling point (5.463N, 7.034E). These sampled points were down stream of the possible sources of the river contamination by heavy metals and SDS as used in this study. A 75-cL plastic container was used in collecting water sample from each of the two sampled points and thereafter pulled together in a 1.5 L plastic container. These containers were previously sterilized by soaking in 70% ethanol for 30 minutes before rinsing severally with sterile deionized water. The sample was taken to the laboratory for analysis, and was refrigerated at 4°C, until required.

Test organism and culture conditions

S. marcescens (SerEW01) used in this study was isolated from Otamiri river water as the preponderant bacterial isolate, with 33.33% occurrence and identified using 16S rRNA gene partial sequencing (Okechi & Chukwura, 2020). Culturing and preparation of test bacterium for toxicity assay was done as described by Nweke *et al.* (2018). *S. marcescens* (SerEW01) was grown in nutrient broth and incubated for 16 hours on rotary incubator (150 rpm), at 26 ± 2°C. Thereafter, the cells were harvested by centrifugation at 3000 rpm (Newlife Centrifuge, NL80-2) for 15 minutes. Harvested cells were washed thrice in sterile deionized water and then re-suspended in the sterile deionized water. The cell density was adjusted to $1.1x10^8$ cells mL^{-1} based on McFarland turbidity standards.

Binary ratios

The binary mixtures [SDS) + Pb(II), SDS + Cd(II), SDS + Ni(II), SDS + Zn(II), and SDS + Co(II)] were studied using fixed ratio design as a function of p(%) SDS and 100-p(%) metal ion weight to weight ratios (Table 1). An equieffect mixture (EECR 50) based on the EC_{50} of SDS and a metal ion and three arbitrary combinations (ABCR) were used to evaluate the toxicity of the binary mixtures. In each mixture, the mixture ratio was kept constant, while varying the total concentrations over a range that will enable a complete dose-response relationship to be obtained. Aqueous stock solutions (10 mM and 50 mM) of the binary mixtures were prepared by combining requisite volumes of the metal ion and SDS

stock solutions (10 mM or 50 mM respectively) to produce a particular concentration ratio.

Toxicity of SDS and individual toxic metal

The toxicity assay was based on inhibition of dehydrogenase activity in the test bacterium. Dehydrogenase activity measurement is a sensitive, rapid and cheap test to measure microbial growth and viability. Thus, the test has been used to assess chemical toxicity to microorganisms. A modified method of Nweke *et al*. (2018) was used to assay for dehydrogenase activity. The assay was done using MTT as the artificial electron acceptor. The reaction mixture contained nutrient broth (Lab M), MTT, *S. marcescens* suspension, SDS or metal ion to a total volume of 2 mL (pH 7.0) in 15 ml screw-capped culture tubes. A 0.5 mL of 0.8% w/v nutrient broth and requisite volumes of SDS (50 mM) or metal ion (10 mM) stock solution and sterile deionized water (to make up) were dispensed into triplicate tubes in order to obtain varying concentrations of SDS or metal ion. Thereafter, 0.1 mL each of 0.1% w/v aqueous solutions of MTT and *S. marcescens* suspension were also dispensed into every tube. The final concentrations of the metal ions ranged between 0.002 mM and 1.5 mM while that of SDS ranged between 1 mM and 10 mM. These concentration ranges were chosen based on the preliminary range-finding tests in order to achieve complete dose-response curve for the toxicants. Control cultures that consisted of inoculated medium without SDS or metal ions were also set up. The cultures were incubated at 26 ± 2°C for 24 hours. Thereafter, the purple-coloured MTT-formazan (MTTF) produced in each tube was extracted in 4 mL of n-butanol. Absorbance of the extract from each tube was measured in a spectrophotometer (VIS Spectrophotometer 721D) at 590 nm.

Toxicity of binary mixtures

The toxicity assay method was modified from Nweke *et al*. (2018). The study was carried out in 2-ml nutrient broth-MTT medium (pH 7) supplemented with varying concentrations of SDS and Cd(II), Pb(II), Zn(II), Co(II) or Ni(II) in triplicate 15 mL screw-capped culture tubes. Into each tube, 0.5 mL of 0.8 % w/v nutrient broth, requisite volumes of stock solutions of SDS + metal ion combinations and sterile deionized water (to make up) were dispensed. Thereafter, 0.1 mL each of 0.1% aqueous solutions of MTT and *S. marcescens* suspension were added. The final total concentrations of the binary mixtures ranged between 0.05 mM to 3.0 mM. The controls consisted of the medium without SDS and metal mixtures. The cultures were incubated at 26 ± 2°C for 24 hours, and subsequently extraction and quantification of MTTF produced were as stated above for individual toxicants.

Estimation of relative responses and EC_{50}

The relative inhibition of dehydrogenase activity (R) was calculated for each tube (Eq. 1).

$$R = \left[1 - \frac{T_A}{C_A}\right] \times 100 \quad (1)$$

Where, C_A is the mean absorbance of MTTF-extracted from the control tubes and T_A is absorbance of MTTF-extracted from the experiment with various concentrations of the SDS, metal or their mixtures. The concentration-response data of each toxicant and the binary mixtures were encoded into 2-parameter logistic model (Eq. 2) to estimate the EC_{50} as a parameter of the non-linear model.

$$R = \frac{100}{1 + \left(\frac{x}{EC_{50}}\right)^b} \quad (2)$$

Where x stands for toxicant concentration, EC_{50} represents the toxicant concentration that reduced the dehydrogenase activity by 50% inhibition while b is the slope at EC_{50}. In order to estimate, EC_{50} in biphasic concentration-responses, data was fitted into the hormetic model of Schabenberger *et al*. (1999) (Eq. 3).

$$R = 100 - \frac{100 - fx}{1 + \left[f + \left(\frac{2fEC_{50}}{100}\right)\right]\left(\frac{x}{EC_{50}}\right)^b} \quad (3)$$

Where f determines the extent of hormetic response, EC_{50} is as described in Eq. 2 while the quantity b no longer represents the slope at EC_{50} (Cedergreen *et al*., 2005).

Table 1. SDS and Metal Binary Mixtures

Mixture	Mixture Ratios (%)									
	SDS + Ni(II)		SDS + Cd(II)		SDS + Pb(II)		SDS + Zn(II)		SDS + Co(II)	
	SDS	Ni(II)	SDS	Cd(II)	SDS	Pb(II)	SDS	Zn(II)	SDS	Co(II)
EECR 50	93.49	6.51	97.76	2.24	95.79	4.21	98.70	1.30	98.08	1.92
ABCR1	94	6	98	2	96	4	99	1	98	2
ABCR2	92	8	96	4	94	6	98	2	96	4
ABCR3	91	9	94	6	93	7	96	4	90	10

EECR 50 = EC_{50} equi-effect concentration ratio, ABCR = Arbitrary concentration ratios

Prediction of mixture toxicities

The mixture toxicities were predicted based on the toxicities of the individual toxicants using concentration addition (CA) model (Eq. 4) (Berenbaum, 1985). The concentration addition model assumes that the components of the mixture share common mode of action on target the organism.

$$EC_{(xmix)} = \left[\sum_{i=1}^{n} \frac{\pi_i}{EC_{xi}} \right]^{-1} \quad (4)$$

In Eq. 4, $EC_{x(\mathrm{mix})}$ represents the total mixture concentration that gave *x*% inhibition of dehydrogenase activity. EC_{xi} represents the concentration of *i*th component that produced *x%* reduction in dehydrogenase activity when tested as a single toxicant, *n* is the number of toxicants, π_i is the relative proportion of *i*th toxicant in the mixture, On the basis of Eq. 4, the prediction of the mixture toxicities was as reported elsewhere (Nweke *et al*., 2018).

The independent action (IA) model (Eq. 5) assumed that the components of a given mixture have different mode of action (Faust *et al*., 2003).

$$E(C_{mix}) = 1 - \prod_{i=1}^{n} \left[1 - E(c_i) \right] \quad (5)$$

Where $E(c_{mix})$ is the total response (scaled from 0 to 1) of an *n*-component mixture, c_i is the concentration of the *i*th component and $E(c_i)$ is the response of each single toxicant. The concentration-response relationships of the individual toxicants were used to determine their response $E(c_i)$, by substituting Eq 2 (scaled 0 to 1) into Eq. 5 for each toxicant as Eq 6:

$$R = \frac{1}{1 + \left(\frac{\pi_i x}{EC_{50\,i}} \right)^{bi}} \quad (6)$$

The IA model was thus simplified as shown in Eq. 7.

$$E(c_{mix}) = \left[1 - \prod_{i=1}^{n} \left\{ 1 - \frac{1}{1 + \left(\frac{\pi_i x}{EC_{50i}} \right)^{bi}} \right\} \right] \times 100 \quad (7)$$

Where, $\pi_i x$ is the concentration of *i*th component in the mixture. The EC_{50} and *b* as calculated from equation 2 for each metal and SDS were used. The effect of the mixture for a total concentration (*x*) that ranged between 0.02 and 4 mM was computed using Microsoft Excel 2007. The resulting concentrations-effect data were plotted as a line graph to give a visualization of the dose-response curve predicted from the IA model (Nweke *et al*., 2018).

By using Eq. 7 which was fitted into Microsoft Excel 2007, the value of *x* in every mixture that gives $E(c_{mix})$ of 50% (predicted EC_{50} of mixture) was determined through trials. The EC_{50} of the mixtures based on CA model were determined using Eq. 5 based on the relative proportion and EC_{50} of each toxicant. The experimental $EC_{50\mathrm{S}}$ for individual toxicant as well as for the various mixtures ratios in each mixture were compared. Also, within each mixture ratio, the experimental, CA- and IA-models predicted $EC_{50\mathrm{S}}$ were equally compared using Duncan post-hoc tests implemented with SPSS Statistics 21.

Toxic index (TI)

TI was determined for each mixture by summing the toxic unit of every component of the mixture (Eq. 8).

$$TI = \sum_{i=1}^{n} \frac{C_i}{EC_{50i}} \quad (8)$$

Where C_i represents the concentration of the *i*th component in the mixture at the EC_{50} of the mixture (EC_{50mix}) and EC_{50i} is the concentration of the *i*th component that reduced dehydrogenase activity by 50%, when tested alone, TI = 1 denotes additivity, TI > 1 shows antagonism while TI < 1 denotes synergistic interaction (Nweke *et al*., 2018).

Model deviation ratios (MDR)

The model deviation ratios were computed using experimental EC_{50}, and predicted $EC_{50\mathrm{S}}$ (Eq. 9). MDR > 1 and MDR < 1 indicate underestimation and overestimation of toxicity respectively by the model (Li *et al*., 2014).

$$\mathrm{MDR} = \frac{\mathrm{Predicted}\,EC_{50}}{\mathrm{Experimental}\,EC_{50}} \quad (9)$$

Mixture isoboles

The observed EC_{50} of the mixtures were used to determine the isoboles of the binary mixtures as described by Nweke *et al*. (2018).

RESULTS

Toxicity of individual toxicants

The responses of *S. marcescens* to the toxicity of the toxicants were concentration-dependent. The toxicants increasingly reduced the dehydrogenase activity with increase in concentration, giving percentage inhibitions above 95% at 1 mM for Zn(II) and Ni(II), 0.5 mM for Pb(II), Cd(II) and Co(II) and 8 mM for SDS. The dose-response patterns are rather similar for SDS, Cd(II) and Co(II) (Figure 1). Table 2 shows the experimental and predicted toxicity thresholds (EC_{50}) of individual toxicants and their binary mixtures on *S. marcescens* (SerEW01). The $EC_{50\mathrm{S}}$ of the toxicants ranged from 0.046 ± 0.003 mM for Zn(II) to 2.329 ± 0.092 mM for SDS. The Duncan test shows that the $EC_{50\mathrm{S}}$ of the toxicants differed significantly from one another ($P < 0.05$) and the order of decrease in toxicity was Zn(II) > Cd(II) > Co(II) > Ni(II) > Pb(II) > SDS.

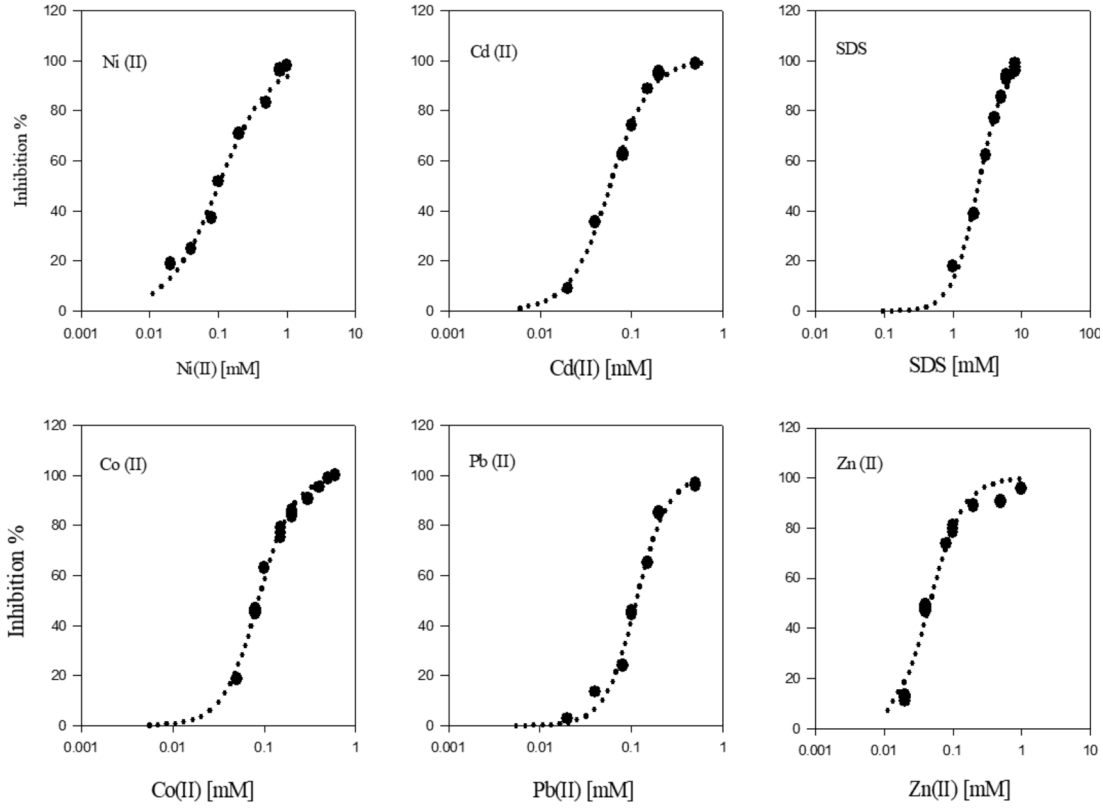

Figure 1. Inhibition of dehydrogenase activity of *S. marcescens* (SerEW01) by the individual toxicants

Toxicity of binary mixtures

The experimental concentration-response relationships of the binary mixtures and the predictions from CA and IA models are shown in Figures 2-6. In SDS 92% + Ni(II) 8% (ABCR2) and SDS 91% + Ni(II) 9% (ABCR3) mixture ratios, both CA and IA models slightly over-estimated the toxicities at low concentrations while underestimating at higher concentrations. In other SDS + Ni(II) mixture ratios, the models predicted slightly lower toxicities than the experimental data would suggest, even at lower concentrations (Figure 2). Also, similar mixture toxicities were predicted by both models, especially for SDS + Ni(II) mixtures, as their concentration-response curves were almost superimposed. In SDS + Cd(II) mixtures, inhibition of dehydrogenase activity took place even at low concentrations (Figure 3). Both CA and IA models predicted significantly lower toxicities than the observed. In SDS + Pb(II) mixtures, the models also grossly underestimated the toxicities relative to the experimental data (Figure 4). As shown in Figure 5, SDS + Zn(II) mixtures showed that the models slightly predicted lower toxicities, especially for SDS99% + Zn(II)1% (ABCR1) and SDS96% + Zn(II)4% (ABCR3) mixture ratios. In SDS + Co(II) mixtures, the equieffect mixture ratio (SDS98.08% + Co(II)1.92%) was found to be stimulatory to the dehydrogenase activity at low concentrations and inhibitory at higher concentration. Furthermore, other mixture ratios significantly underestimated the toxicities (Figure 6).

For the binary mixtures (Table 2), the experimental EC_{50S} in the SDS + Ni(II) binary mixture showed that ABCR2 mixture ratio had the highest EC_{50} (0.314 ± 0.013 mM) while ABCR1 mixture ratio had the least (0.239 ± 0.019 mM). Also, among the experimental EC_{50S}, the EECR50 and ABCR2 mixture ratios were statistically different from ABCR1 and ABCR3. In SDS + Cd(II) binary mixtures, the experimental EC_{50S} ranged from 0.115 ± 0.007 mM for ABCR2 to 0.207 ± 0.007 mM for EECR50.

In SDS + Pb(II) binary mixtures, there was no statistical difference between the experimental EC_{50} of EECR50 and ABCR1, as well as between ABCR2 and ABCR3. In SDS + Zn(II) binary mixtures, ABCR2 was the most toxic mixture ratio while ABCR3 was the least. In SDS and Co(II) binary mixtures, ABCR3 mixture ratio had the least EC_{50} (0.149 ± 0.009 mM) as against (0.303 ± 0.011 mM) recorded by EEC50 mixture ratio. In all SDS and metal ion binary mixtures, the experimental, CA- and IA-predicted EC_{50S} were statistically different from one another ($P < 0.05$). Similarly, in all but SDS + Ni(II) and SDS + Pb(II) binary mixtures, the experimental EC_{50S} for different mixture ratios were also statistically different from one another ($P < 0.05$).

The toxic index, model deviation ratio and effect of metals and SDS binary mixtures on *S. marcescens* (SerEW01) are shown in Table 3. The toxic index (TI) values ranged from 0.123 ± 0.002 to 0.543 ± 0.007, while model deviation ratio (MDR) ranged from 1.839 ± 0.028 to 10.771 ± 0.445. At all the tested mixture ratios, the metals and SDS binary mixtures were synergistic in their action against *S. marcescens*.

Table 2**:** Experimental and Predicted Toxicity Thresholds (EC_{50}) of Individual Toxicants and Their Binary Mixtures on *Serratia marcescens* (SerEW01)

Toxicants	EC_{50} (mM)‡ [+]		
	Experimental†	CA- Predicted	IA- Predicted
Na(II)	0.100 ± 0.008a	-	-
Cd(II)	0.058 ± 0.002b	-	-
Pb(II)	0.113 ± 0.005c	-	-
Zn(II)	0.046 ± 0.003d	-	-
Co(II)	0.086 ± 0.002e	-	-
SDS	2.329 ± 0.092	-	-
Binary Mixtures			
SDS + Ni(II)			
SDS 93.49% + Ni 6.51% (EECR50)	0.290 ± 0.016b*	0.952 ± 0.064 **	1.147 ± 0.027***
SDS 94% + Ni 6% (ABCR1)	0.239 ± 0.019a*	0.998 ± 0.066**	1.199 ± 0.026***
SDS 92% + Ni 8% (ABCR2)	0.314 ± 0.013b*	0.838 ± 0.059**	1.012 ± 0.032***
SDS 91% + Ni 9% (ABCR3)	0.243 ± 0.010a*	0.776 ± 0.055**	0.936 ± 0.035***
SDS + Cd(II)			
SDS 97.76% + Cd 2.24% (EECR50)	0.207 ± 0.007a*	1.241 ± 0.046**	1.640 ± 0.014***
SDS 98% + Cd 2% (ABCR1)	0.176 ± 0.009b*	1.306 ± 0.049**	1.720 ± 0.016***
SDS 96% + Cd 4% (ABCR2)	0.115 ± 0.007c*	0.907 ± 0.033**	1.188 ± 0.012***
SDS 94% + Cd 6% (ABCR3)	0.155 ± 0.008d*	0.695 ± 0.025**	0.880 ± 0.014***
SDS + Pb(II)			
SDS 95.79% + Pb 4.21% (EECR50)	0.251 ± 0.012a*	1.276 ± 0.053**	1.764 ± 0.009***
SDS 96% + Pb 4% (ABCR1)	0.249 ± 0.007a*	1.305 ± 0.055**	1.804 ± 0.009***
SDS 94% + Pb 6% (ABCR2)	0.193 ± 0.010b*	1.070 ± 0.045**	1.690 ± 0.206***
SDS 93% + Pb 7% (ABCR3)	0.201 ± 0.012b*	0.982 ± 0.042**	1.331 ± 0.015***
SDS + Zn (II)			
SDS 98.70% + Zn 1.30% (EECR50)	0.661 ± 0.015a*	1.419 ± 0.077**	1.799 ± 0.010***
SDS 99% + Zn 1% (ABCR1)	0.725 ± 0.017b*	1.560 ± 0.081**	1.941 ± 0.013***
SDS 98% + Zn 2% (ABCR2)	0.310 ± 0.011c*	1.173 ± 0.068**	1.518 ± 0.017***
SDS 96% + Zn 4% (ABCR3)	0.426 ± 0.021d*	0.784 ± 0.050**	0.995 ± 0.032***
SDS + Co(II)			
SDS 98.08% + Co 1.92% (EECR50)	0.303 ± 0.011a*	1.554 ± 0.056**	2.042 ± 0.026***
SDS 98% + Co 2% (ABCR1)	0.188 ± 0.010 b*	1.535 ± 0.058**	2.022 ± 0.024***
SDS 96% + Co 4% (ABCR2)	0.231 ± 0.010c*	1.142 ± 0.039**	1.547 ±0.006***
SDS 90% + Co 10% (ABCR3)	0.149 ± 0.009d*	0.649 ±0.024**	0.811 ± 0.012***

[+] Values compiled as Mean ± 1SD

†Within columns, in each toxicant mixture type, the experimental EC_{50}, values with the same letters are not significantly different from each other ($P < 0.05$).

‡ Within rows, in each mixture ratio, comparing between the experimental EC_{50}, CA-predicted EC_{50} and IA-predicted EC_{50}, values with the same number of asterisks are not significantly different from each other ($P < 0.05$).

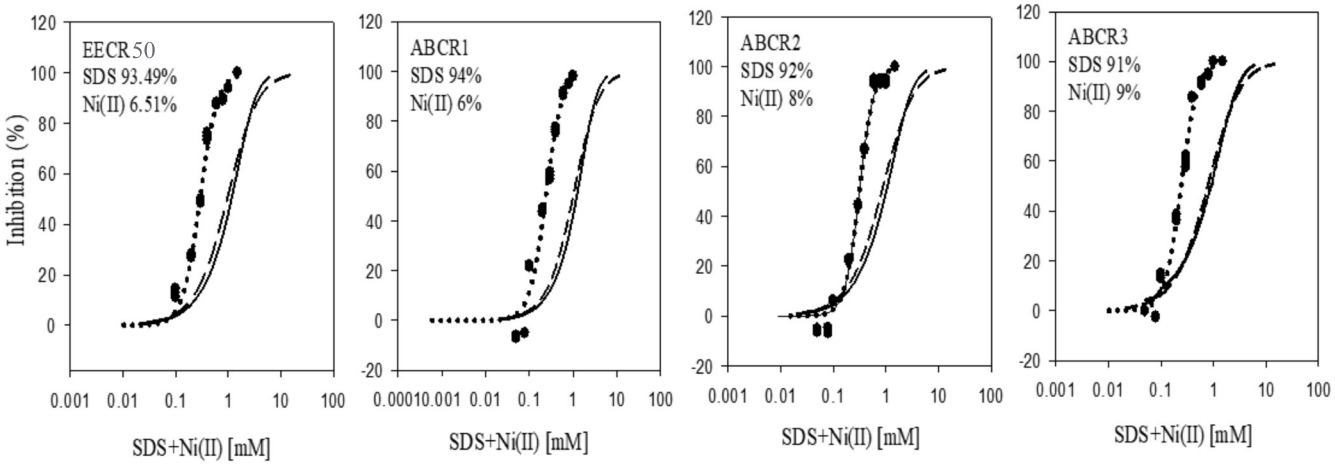

Figure 2. Experimental and predicted effects of binary mixtures of SDS and nickel ions on *S. marcescens* (SerEW01) dehydrogenase activity. The experimental dose-responses are represented by the data points. The toxicities estimated by fitting experimental data to logistic model (eq. 2) are presented as dotted line. The dashed and solid lines indicate the predicted toxicities from concentration addition and independent action models, respectively

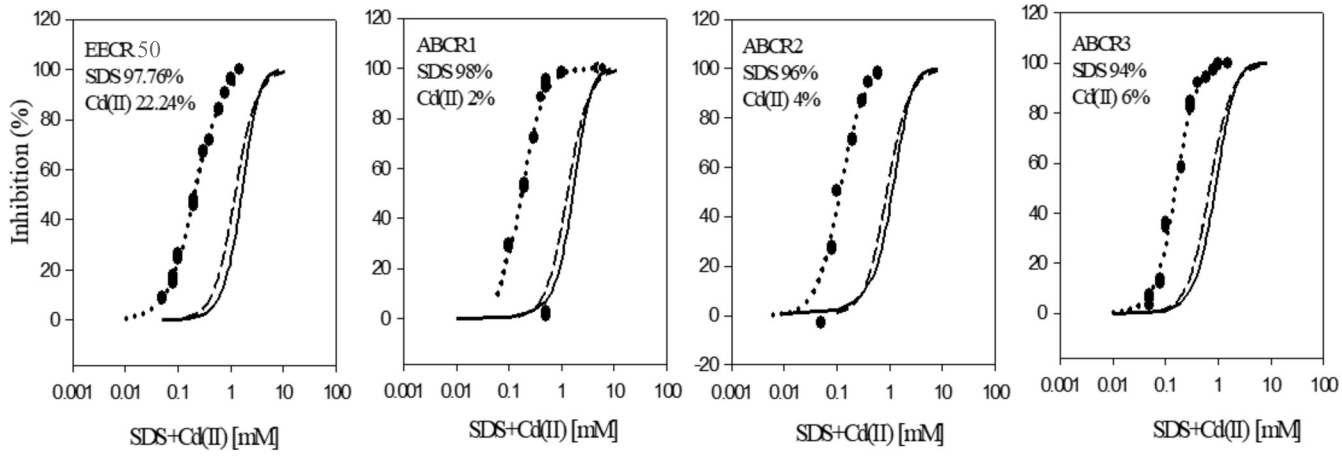

Figure 3. Experimental and predicted effects of binary mixtures of SDS and cadmium ions on *S. marcescens* (SerEW01) dehydrogenase activity. The experimental dose-responses are represented by the data points. The toxicities estimated by fitting experimental data to logistic model (eq. 2) are presented as dotted line. The dashed and solid lines indicate the predicted toxicities from concentration addition and independent action models, respectively

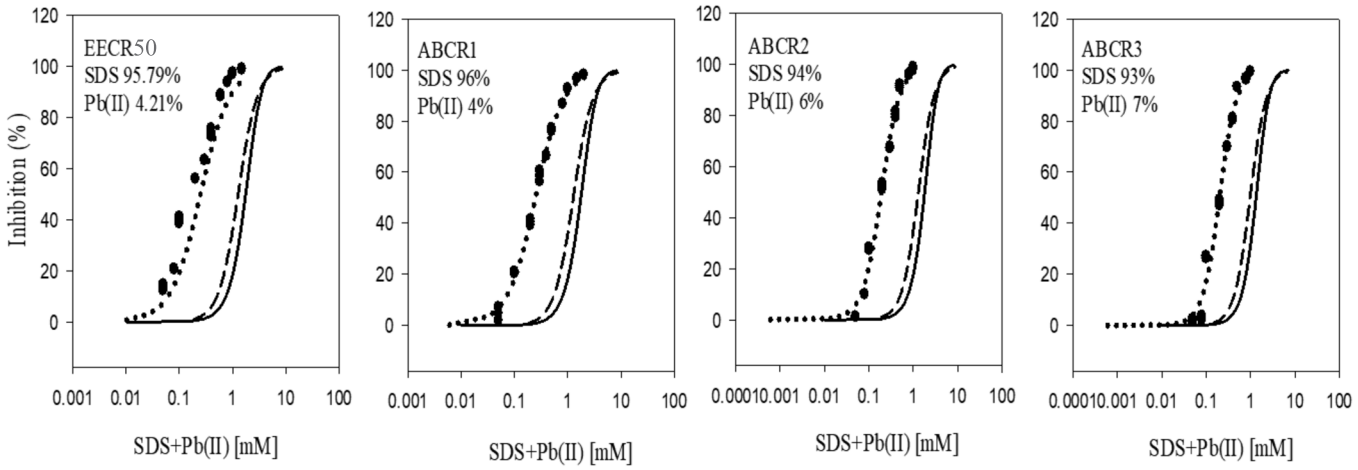

Figure 4. Experimental and predicted effects of binary mixtures of SDS and lead ions on *S. marcescens* (SerEW01) dehydrogenase activity. The experimental dose-responses are represented by the data points. The toxicities estimated by fitting experimental data to logistic model (eq. 2) are presented as dotted line. The dashed and solid lines indicate the predicted toxicities from concentration addition and independent action models, respectively

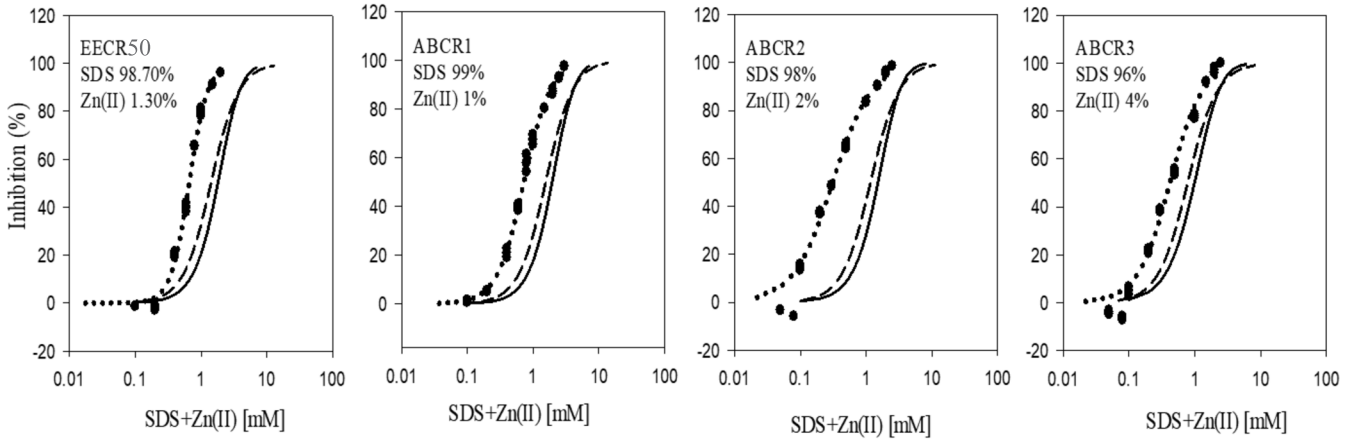

Figure 5. Experimental and predicted effects of binary mixtures of SDS and zinc ions on *S. marcescens* (SerEW01) dehydrogenase activity. The experimental dose-responses are represented by the data points. The toxicities estimated by fitting experimental data to logistic model (eq. 2) are presented as dotted line. The dashed and solid lines indicate the predicted toxicities from concentration addition and independent action models, respectively

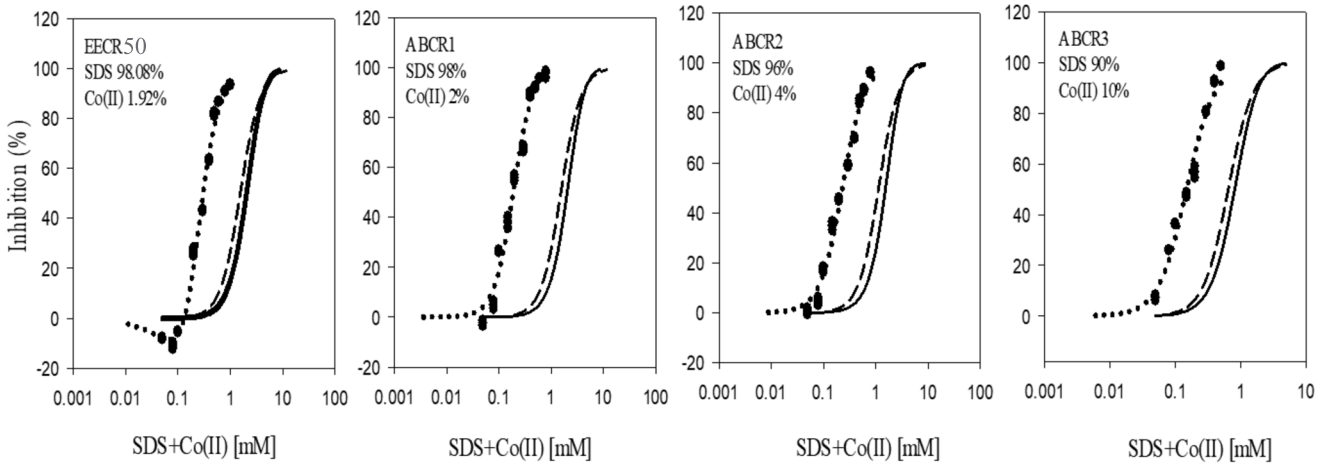

Figure 6. Experimental and predicted effects of binary mixtures of SDS and cobalt ions on *S. marcescens* (SerEW01) dehydrogenase activity. The experimental dose-responses are represented by the data points. The toxicities estimated by fitting experimental data to logistic model (eq. 2) are presented as dotted line. The dashed and solid lines indicate the predicted toxicities from concentration addition and independent action models, respectively.

The isobologram of the binary mixtures based on the EC_{50S} are shown in Figure 7. The analyses indicate synergistic effect for all metals and SDS binary mixtures ratios on the dehydrogenase activity. This observation was corroborated by the toxic index and model deviation ratios (Table 3).

DISCUSSION

Zinc, cobalt and nickel are among the trace elements, acting as enzyme co-factors during cell metabolisms. They can however be toxic to microorganisms at high concentrations. These heavy metal ions and SDS were inhibitory to *S. marcescens* (SerEW01) dehydrogenase activity at high concentrations. Heavy metals have been reported to inhibit dehydrogenase activity in pure bacterial cultures and microbial community in both soil and river water by previous authors (Nweke & Orji, 2009; Nweke *et al*., 2018). Lead and cadmium have no physiological function in living organisms and inhibit microbial metabolism even at low concentrations, thus they are considered as toxins (Mergeay *et al*., 1985). Cadmium was reported to compete with cellular zinc resulting in non-specific binding to DNA, inducing single strand breaks (Roane & Pepper, 2000). The toxic effects of lead include ion substitution or displacement of essential ions from cellular sites and blockade of essential functional groups in enzymes, polynucleotides, and essential nutrient transport systems (Gadd & Griffiths, 1977).

On the basis of bioluminescence inhibition in photobacterium Q67, median inhibitory concentration (IC_{50}) of 0.199 mM to 0.239 mM Cd(II) was reported by Ge *et al*. (2014). Similarly, IC_{50} of 0.022 mM Cd(II) against *Pseudomonas fluorescens* dehydrogenase activity was reported by Nweke *et al.* (2018). Furthermore, maximum tolerance concentration of 4 mg/mL Pb(II) ($\approx 2.0x10^{-5}$ mM) and 1.5mg/mL Cd(II) ($\approx 1.3x10^{-5}$ mM) were reported for *Serratia marcescens* by Nwagwu *et al.* (2017). However, in the present study, median inhibitory concentrations of 0.058 ± 0.002 mM and 0.113 ± 0.005 mM were recorded for cadmium and lead respectively. Tolerance of *Serratia* to zinc and other heavy metals has been reported (Nwagwu *et al*., 2017; Cider *et al*., 2017). Similarly, better tolerance to heavy metal toxicity by Gram negative bacteria has also been reported (Minz *et al*., 1996). A 50% effective concentration (EC_{50}) of 0.046 ± 0.003 mM for zinc was reported in this study, as against 0.180 mM recorded by Nweke *et al*. (2018) in their study with *Pseudomonas fluorescens*. Furthermore, an EC_{50} of 0.91 mM was recorded for Zn against microbial community of new Calabar River (Nweke & Orji, 2009). Like other heavy metals in the present study, cobalt inhibited dehydrogenase activity in *S. marcescens* (SerEW01) even at low concentrations. Cobalt was reported to stimulate thermolysin (from *Bacillus thermoproteolyticus*) activity at low concentrations while inhibiting it at high concentrations (Hashida & Inouye, 2007). Similarly, nickel stimulation of microbial growth at low concentrations (< 27 mg/L) (≈ 0.46 mM), has also been reported by Gikas, (2007). In the present study however, cobalt, with an EC_{50} of 0.086 ± 0.002 mM was more toxic to *Serratia marcescens* (SerEW01) than nickel (EC_{50} 0.100 ± 0.008 mM). Similar report has been recorded for *Pseudomonas fluorescens* (Nweke *et al*., 2018).

There is scarcity of published data on the toxicity of SDS using microbial dehydrogenase activity as a response. In a study that evaluated the effects of SDS on organisms that cut across different phyla, *Vibrio fischeri* was the most sensitive organism, with an EC_{50} of 2.6 mg/L ($9.02 x10^{-3}$ mM) SDS (Mariani *et a*l., 2006). Sodium Dodecyl sulfate (SDS) recorded an EC_{50} of 2.329 ± 0.092 mM in the present study, indicating that *S. marcescens* (SerEW01) was probably more tolerant to the effects of SDS than *Vibrio fischeri*. The variations in responses could be attributed to physiological differences in the organisms or the end point tested. Lopez-Roldan *et al*. (2012) reported the dependence of toxicity on the conditions in which the test was carried out.

Microorganisms show variations in their metal uptake systems as well as in their ways of accumulating metals

Table 3**:** Toxic Index, MDR and Effect of Metals and SDS Binary Mixtures on *Serratia marcescens* (SerEW01)

Metal+SDS Binary Mixtures	Toxic Index (TI)	MDR[+]		Effect
		CA	IA	
SDS + Ni(II)				
SDS 93.49% + Ni(II) 6.51% (EECR50)	0.305 ± 0.004	3.281± 0.040	3.959 ± 0.125	Synergistic
SDS 94% + Ni(II) 6% (ABCR1)	0.239 ± 0.003	4.179 ± 0.056	5.034 ± 0.294	Synergistic
SDS 92% + Ni(II) 8% (ABCR2)	0.375 ± 0.011	2.671 ± 0.080	3.228 ± 0.027	Synergistic
SDS 91% + Ni(II) 9% (ABCR3)	0.314 ± 0.009	3.188 ± 0.089	3.846 ± 0.027	Synergistic
SDS + Cd(II)				
SDS 97.76% + Cd(II) 2.24% (EECR50)	0.167 ± 0.001	5.994 ± 0.020	7.926 ± 0.200	Synergistic
SDS 98% + Cd(II) 2% (ABCR1)	0.135 ± 0.02	7.425 ± 0.103	9.785 ± 0.409	Synergistic
SDS 96% + Cd(II) 4%(ABCR2)	0.127 ± 0.003	7.900 ± 0.194	10.349±0.530	Synergistic
SDS 94% + Cd(II) 6%(ABCR3)	0.223 ± 0.004	4.489 ± 0.071	5.682 ± 0.203	Synergistic
SDS + Pb(II)				
SDS 95.79% + Pb(II) 4.21% (EECR50)	0.197 ± 0.002	5.077 ± 0.041	7.031 ± 0.320	Synergistic
SDS 96% + Pb(II) 4% (ABCR1)	0.191 ± 0.003	5.240 ± 0.071	7.247 ± 0.174	Synergistic
SDS 94% + Pb(II) 6% (ABCR2)	0.181 ± 0.001	5.536 ± 0.039	8.727 ± 0.757	Synergistic
SDS 93% + Pb(II) 7% (ABCR3)	0.205 ± 0.004	4.887 ± 0.085	6.633 ± 0.324	Synergistic
SDS + Zn(II)				
SDS 98.70% + Zn(II) 1.30% (EECR50)	0.466 ± 0,015	2.146 ± 0.067	2.723 ± 0.048	Synergistic
SDS 99% + Zn(II) 1% (ABCR1)	0.465 ± 0.013	2.152 ± 0.062	2.680 ± 0.045	Synergistic
SDS 98% + Zn(II) 2% (ABCR2)	0.264 ± 0.006	3.785 ± 0.091	4.905 ± 0.119	Synergistic
SDS 96% + Zn(II) 4% (ABCR3)	0.543 ± 0.007	1.839 ± 0.028	2.337 ± 0.040	Synergistic
SDS + Co(II)				
SDS 98.08% + Co(II) 1.92% (EECR50)	0.195 ± 0.000	5.129 ± 0.006	6.743 ± 0.159	Synergistic
SDS 98% + Co(II) 2% (ABCR1)	0.123 ± 0.002	8.168 ± 0.129	10.771 ± 0.445	Synergistic
SDS 96% + Co(II) 4% (ABCR2)	0.202 ± 0.002	4.939 ± 0.057	6.694 ± 0.278	Synergistic
SDS 90% + Co(II) 10% (ABCR3)	0.230 ± 0.006	4.371 ± 0.100	5.462 ± 0.238	Synergistic

[+] Values compiled as Mean ± 1SD

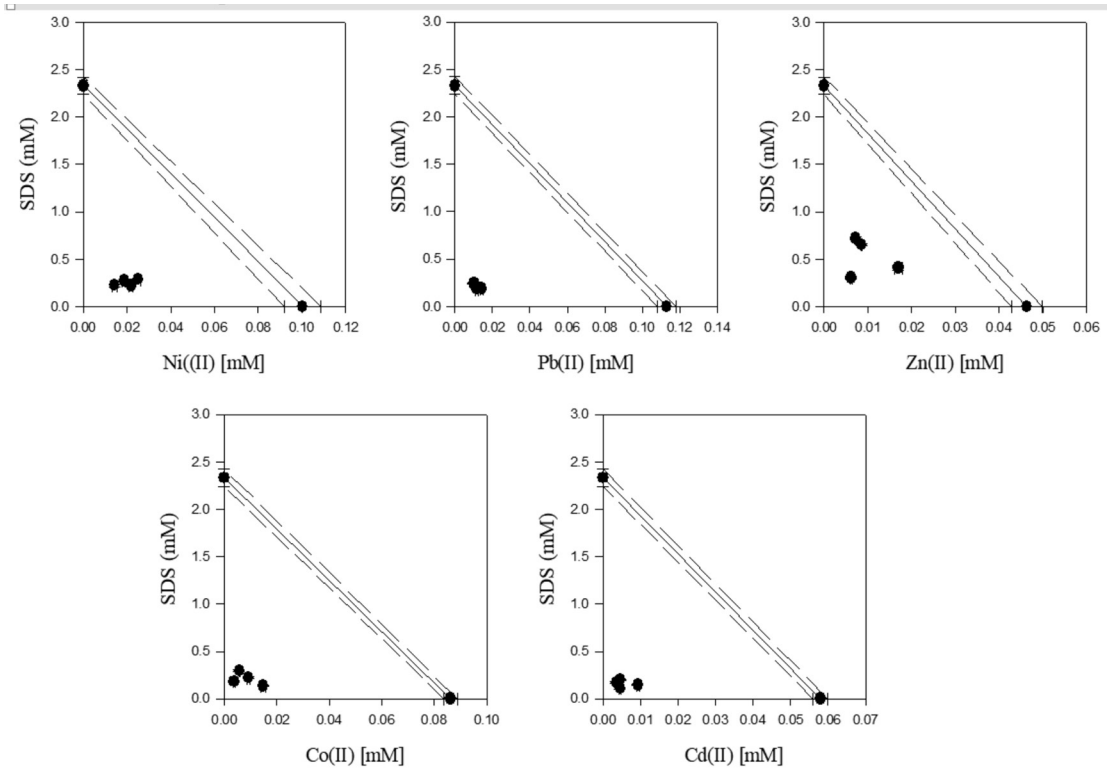

Figure 7. The EC_{50} isobole representation for SDS and metal ions as individual and mixtures tested on dehydrogenase activity of *S. marcescens* (SerEW01). The thick dots represent the standard deviation of the 95% confidence interval of the values. The solid and dashed lines are additivity lines and their 95% confidence belt

inside their cytoplasm. In microorganisms, increase in toxicity of metal ions is expected with proportional increase in concentration. Studies however show that in some cases, higher metal concentrations could trigger-off aggressive resistance mechanisms, thus increasing microbial tolerance to metals (Roane & Pepper, 2000). The order of toxicity of the toxicants as recorded in this study was Zn(II) > Cd(II) > Co(II) > Ni(II) > Pb(II) > SDS. The higher toxicity of zinc compared to cadmium, as well as relative tolerance to lead by this bacterium is not quite understood, although high tolerance of *Serratia marcescens* to lead and cadmium has been reported (Cristani *et al.,* 2011). Stimulatory effects have been recorded for metal ions against many microbial processes such as dehydrogenase activity, growth and bioluminescence (Roane & Pepper, 2000; Gikas, 2007; Rodea-Palomares, 2009) and for SDS (Dirilngen and Ince, 1995). However, the absence of stimulatory effects by the individual toxicants as observed in this study could be due to the sensitivity of *Serratia marcescens* (SerEW01) to the toxicants. The sensitivity of an organism to pollutants has been reported to vary considerably according on the type of pollutant (Wangberg *et al*., 1995). In addition, the similarity in the shapes of the dose-response curves for some of the toxicants, suggests possible similarity in the molecular mechanisms of action for those toxicants.

Naturally in the environments, microbes are exposed to mixtures of different chemicals whose toxicity often differs from the toxicities of their individual components. Sometimes, interactions may occur between the components of the mixture, resulting in modulation of toxicities of the mixture components. Such phenomenon was observed in this study with SDS and the five heavy metals. SDS modulated the toxicity of the heavy metals and vice versa, giving EC_{50S} higher than those of the individual heavy metals but lower than that of SDS in all the binary mixtures tested. As was observed by Nweke *et al*. (2018), this modulation seems to be dependent on the relative proportions of the most toxic (heavy metals) and least toxic components (SDS) present in the mixture.

The isobolographic analysis, model deviation ratios and the toxic index model indicated similar effects with respect to the toxicity of SDS and metal mixtures against *S. marcescens* (SerEW01) dehydrogenase activity. The TI values for all the binary mixtures ranged between 0.123 ± 0.002 and 0.543 ± 0.007, which is less than 1, thus describing synergistic interaction (Nweke *et al*., 2018). Similarly, the MDR values for all the binary mixtures ranged from 1.839 ± 0.028 to 10.771 ± 0.445. Li *et al.* (2014) suggested that MDR values of less than 0.5 and greater than 2 indicates antagonism and synergism respectively while MDR values of $0.5 \leq MDR \geq 2$ indicated additivity. From the present study, all the SDS + metal ion binary mixtures showed synergistic interaction, except SDS 96% + Zn(II) 4% mixture ratio that recorded marginal synergistic interaction. This weak synergistic effect could be attributed to the masking effect of SDS on zinc ions in the mixture. Furthermore, weak synergistic effect was also reported on the analysis of the joint toxicity of copper and perfluorinated carboxylic acids (PFCAs) against *Arthrobacter* species (Cai *et al*., 2019). However, Flores *et al*. (2010) reported joint toxicity of LAS and anthracene to be antagonistic on the growth of a microbial consortium isolated from polluted sediment

CA and IA models were used in predicting the toxicity of the toxicant mixtures from the concentration-response relationship of the components of the mixture. In SDS 92% + Ni(II) 8% mixture ratio, both models over-estimated the toxicity at low concentrations while underestimating at high concentrations. This is contrary to the observation by other authors on the toxic effects of binary mixtures of heavy metals. For instance, Gikas (2007) while using isobolographic analyses, reported both synergism and antagonism (at the zone of decreasing stimulation) in the binary mixtures of Ni(II) and Co(II) against the growth of activated sludge microbial community. Similarly, Nweke *et al*. (2018) reported underestimation of toxicity at a particular mixture ratio at low concentrations and overestimation at high concentrations of binary mixture of metals to *Pseudomonas fluorescens* by both models. They therefore concluded that in general, the effect of metal mixtures could depend on the mixture ratio under consideration. Similar assertion could be made about this work, despite the variations in the test bacterium and the toxicants studied. In addition, in SDS 91% + Ni(II) 9% mixture ratio, both CA and IA models predicted identical toxicities of the binary mixture. Studies have shown that prediction of similar EC_{50S} by both models is possible under some conditions (Drescher and Boedeker, 1995; Zhang *et al*., 2008; Huang *et al*., 2011; Chen *et al*., 2013).

In SDS + Zn(II) mixtures, both models predicted slightly lower toxicities in various mixture ratios tested. This resulted in weak synergistic interactions between SDS and zinc ions in the mixture. Furthermore, SDS 98.08% + Co(II) 1.92% mixture ratio was stimulatory at low concentration and inhibitory at high concentration of the binary mixture. Gikas (2007) reported a more drastic stimulation in the growth of the activated sludge microbial community by the tested Ni(II) and Co(II) mixture ratios at relatively low doses, compared to the stimulation by equal concentrations of the individual components, whilst at the same time acting as more potential inhibitors at relatively high concentrations. However, in other SDS + metal ions binary mixtures, both CA and IA models grossly underestimated the toxicities of the mixtures to *Serratia marcescens* (SerEW01).

CONCLUSION

The toxicities of the binary mixtures of metal ions and SDS to *Serratia marcescens* (SerEW01) from a tropical river were determined, using the inhibition of dehydrogenase activity as end point. From the results of this study, both the toxic index (TI) and model deviation ratios (MDRs) analyses showed that the mixture effect was synergistic. Although the result of this study may not be generalizable for all aquatic organisms, it indicates possible synergistic effects of SDS + metal ion mixtures to bacteria. More studies are recommended

on the effects of the mixtures of these toxicants on the natural microbial community of aquatic ecosystems.

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