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Inhibitory Effects of Ternary Mixtures of Sodium Dodecyl Sulfate and Heavy Metals to *Acinetobacter seifertii* from Otamiri River Sediment in Southeastern Nigeria

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Abstract

Toxicities of sodium dodecyl sulfate (SDS) and heavy metals, Pb(II), Cd(II), Ni(II), Zn(II) and Co(II), as individuals and ternary mixtures of two heavy metals and SDS to *Acinetobacter seifertii* isolated as preponderant bacterium from Otamiri river sediment, were assessed, using inhibition of dehydrogenase activity as end point. Among the individual toxicants, the EC_{50S} observed ranged from 0.011 ± 0.000 mM for Cd(II) to 2.810 ± 0.140 mM for SDS. The EC_{50S} of the toxicants were statistically different from one another and the order of increasing toxicities were SDS > Ni(II) > Pb(II) > Zn(II) > Co(II) >Cd(II). The responses of the bacterium were concentration -dependent. Arbitrary (ABCR) and EC_{50} equieffect (EECR) fixed ratio mixtures were used to evaluate the combined toxicities of the toxicants. The concentration-response relationships of all mixtures and individual toxicants were sigmoidal and fitted with logistic function. The observed toxicities (EC_{50S}) were compared with toxicities predicted from concentration addition (CA) and independent action (IA) models. In ABCR1 and ABCR3 mixture ratios of SDS+Ni(II)+Cd(II) and SDS+Co(II)+Cd(II) ternary mixtures, both CA- and IA-predicted EC_{50S} were not statistically different from each other. Furthermore, in all ternary mixtures, both models underestimated the mixture toxicities to *A. seifertii,* except in ABCR1 of SDS+Ni(II)+Cd(II) mixture, where both models almost correctly predicted the toxicities. Basically, synergistic interaction of the mixture components observed against *A. seifertii,* indicates their possible toxicological effects on the bacterial population of the aquatic ecosystems.

Keywords: Ternary mixtures, dehydrogenase activity, heavy metals, SDS, toxicants, synergy.

INTRODUCTION

Bacteria densely colonize freshwater and marine sediments where they constitute the primary agents of biogeochemical cycling of elements and also serve as food source for higher organisms (Nweke *et al*., 2007a). Heavy metal contamination of aquatic environment has been a serious problem because of their persistence and toxicity to most aquatic biota, including microorganisms (Lee *et al.,* 2005). As noted by Ince *et al*. (1999), heavy metals have received much interest amongst other chemicals in toxicological studies, due to their exceptionally adverse effects on aquatic organisms as a result of their natural and anthropogenic discharge into aquatic ecosystems. Synthetic surfactants also have a wide range of applications at homes, industries and agriculture, as well as in remediation processes and are thus key contaminants of numerous aquatic environments (Masakorala *et al.,* 2011). Although some researchers have reported anionic surfactants to be safe, investigations however showed that surfactants could change the adverse effect of heavy metals to fish and other aquatic lives (Karbe, 1975; Bianucci & Legnani, 1974; Swedimark *et al*., 1978). In aquatic ecosystems, sediments are the sinks for most of the discharged chemicals and play a major role

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in ecosystem processes (Burton *et al*., 2001)**.** Metals attach to organic and inorganic particles that are finally deposited beneath the water bodies. Agitations can redistribute these sediment-associated contaminants in the water phase and impair the activities of suspended microorganisms (Hanson *et al.,* 1993). The microbial communities in sediments process detrital organic matter and also serve as food source for higher organisms. *Acinetobacter* species has been widely reported in contaminated river and lake sediments by various authors and therefore could be a model bacterium for ecotoxicological studies involving chemical mixtures (Sheng *et al*., 2016; Huang *et al*., 2019)**.** Co-contamination of aquatic ecosystems by chemical mixtures could lead to possible interactions among the mixture components, with varied effects on aquatic biota, especially the microbial community. Such interactions may result in mixture effects greater or less than the sum of the effects of the individual chemicals. These situations are referred to as synergism and antagonism respectively. However, when the resultant effect is equal to the sum of the mixture effects of the individual chemicals (no interaction), it is termed additivity (Price *et al*., 2002). Many researchers have reported such interactive effects of chemical mixtures against aquatic microbiota, using different microbial responses (Xu *et al*., 2011; Nweke *et al*., 2016; 2017; Regenmortel *et al*., 2017; Yoo *et al*., 2020).

Chemical pollution of Otamiri River is as a result of anthropogenic activities in Owerri and environs (Okoro *et al*., 2016; Ogah *et al.,* 2018). Recently, Otamiri river water and sediment in Owerri, Imo State, Nigeria were reported to contain anionic surfactants, including sodium dodecyl sulfate (SDS) and heavy metals such as lead, cadmium, nickel, mercury, cobalt and zinc (Okechi & Chukwura, 2020). These heavy metals and surfactants have toxicological implications for the resident aquatic organisms. The toxicity of these pollutants to the microbial community of the river sediment has not been investigated. Furthermore, there is a lack of information on the toxicity of mixtures of organic pollutants and heavy metals to the bacterial population of the river and its sediment. This study was therefore aimed at investigating the combined toxicity of heavy metals and SDS to *Acinetobacter seifertii* isolated from the river sediment.

MATERIALS AND METHODS

Reagents

The deionized water used in reconstituting the reagents 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, SDS and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were all of analytical grades**.**

Test bacterium and cultural conditions

The test bacterium, *A. seifertii* was the most numerous bacterial isolate from Otamiri river sediment (Okechi & Chukwura, 2020). *A. seifertii* cells were prepared for bioassay by culturing in nutrient broth (Lab M) shaken at 150 rpm in a shaker incubator and incubated at 28 ± 2°C for 16 h. The cells were harvested and washed in sterile deionized water by repeated centrifugation (3000 rpm, 15 min, Newlife Centrifuge, NL80-2). Thereafter, the cells were suspended in sterile deionized water and the optical density adjusted to 0.1 at 540 nm in spectrophotometer (VIS Spectrophotometer 72 1D) (Nweke *et al*., 2014). This cell suspension was equivalent to $1.1x10^8$ cells/ml based on McFarland turbidity standards.

Ternary mixture ratios

The ternary mixtures consisted of SDS and two of the five heavy metals (Cd, Pb, Zn, Co and Ni), combined in fixed ratios. The ternary mixtures were SDS+Pb(II)+Zn(II), SDS+Cd(II)+Zn(II), SDS+Pb(II)+Ni(II), SDS+Ni(II)+Cd(II), SDS+Co(II)+Pb(II), and SDS+Co(II)+Cd(II). In each ternary combination of SDS and heavy metals, four mixture ratios including one EC_{50} equieffect concentration ratio (EECR50) and the three arbitrarily chosen mixture ratios (ABCR) were investigated. The relative proportion of SDS and heavy metals in each ternary combination are shown in Table 1. Each combination was prepared by mixing requisite volumes of the stock solutions of each component.

Table 1: Ternary mixtures of SDS and two heavy metals

	Mixture ratios (%)																	
	SDS +Pb(ii) +Zn(II)			SDS +Cd(II) +Zn(II)			SDS +Pb(II) +Ni(II)			SDS +Ni(II) +Cd(II)			SDS +Co(II) +Pb(II)			SDS +Co(II) +Cd(II)		
Mixture	SDS	Pb	Zn	SDS	Cd	Zn	SDS	Pb	Ni	SDS	Ni	Cd	SDS	Co	Pb	SDS	Co	Cd
EECR50	94.87	3.88	1.25	98.5	0.2	1.30	92.44	3.78	3.78	94.21	3.85	1.93	95.22	0.89	3.89	97.1	0.91	0.2
ABCR1	95	4	1	96	1	3	93	3	4	93	5	2	94	3	3	98	1	1
ABCR2	93	5	2	98	1	1	94	3	3	94	4	2	95	3	2	96	2	2
ABCR3	90	2	8	5	2	3	91	4	5	91	6	3	96	2	2	95	3	2

Toxicity assay

The cell viability assay with 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-Tetrazolium Bromide (MTT) was done in 2-ml volumes of nutrient broth-MTT medium containing graded concentrations of SDS, Cd(II), Pb(II), Zn(II), Co(II) or Ni(II) (for individual) or the mixtures in separate 15 ml screw-capped culture tubes (pH 7). In each tube, 0.5 ml portion of 0.8% nutrient broth and requisite volumes of sterile deionized water and stock solutions of the respective heavy metals, SDS or the ternary mixtures were added. Then, 0.1 ml each of 0.1% aqueous solutions of MTT and cell suspension were added to obtain final concentrations of 0.002 mM to 1.5 mM (individual heavy metal) and 1 mM to 10 mM (SDS). The final concentration of ternary mixtures varied between 0.02 mM and 2.0 mM. The control tubes contained the medium, MTT and bacterial inoculum but not SDS or heavy metals. The cultures were incubated at 28 ± 2°C for 24 h. After incubation, the purple coloured MTT-formazan (MTTF) produced in each tube was extracted in 4 ml of n-butanol. Absorbance of the extract was determined spectrophotometrically at 590 nm (VIS Spectrophotometer 72 1D) (Nweke *et al*., 2014).

Estimation of EC_{50S}

The relative inhibitions of dehydrogenase activity at each concentration of individual toxicant or the ternary mixtures were computed as shown in Eq 1.

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

Where R is the inhibition (%) of dehydrogenase activity, C_A is the mean absorbance of MTTF extract in the control experiment and T_A is absorbance of MTTF extract in the test experiment containing different concentrations of SDS and heavy metal or their mixture.

The concentration-inhibition data for the individual toxicants and the mixtures were fitted with a logistic function of 2 parameters (Eq. 2).

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

Where x is the concentration of toxicant, EC_{50} is the concentration of toxicant that inhibited dehydrogenase activity by 50% and b is the slope at EC_{50}.

Predicting mixture toxicities

The mixture toxicities were predicted from the toxicity of the individual component based on concentration addition (CA) model, Eq. 3. (Berenbaum, 1985).

$$EC_{x(mix)} = \left[\sum_{i=1}^{n} \frac{\pi_i}{EC_{xi}} \right]^{-1} \qquad (3)$$

Where ECx(mix) is the total concentration of the mixture that caused x% effect, ECx_i is the concentration of ith component that gave x effect as an individual, n is the number of components in the mixture, πi is the relative proportion of ith component in the mixture. Using Eq. 3, the concentration-inhibition relationships were determined as described elsewhere (Nweke *et al*., 2018).

The independent action (IA) model (Eq. 4) assumes that the components of any mixture have different mechanisms of action (Altenburger *et al*., 2000; Faust *et al*., 2003):

$$E(C_{mix}) = 1 - \prod_{i=1}^{n} \left[1 - E(c_i) \right] \qquad (4)$$

Where $E(c_{mix})$ represents the total effect (scaled from 0 to 1) of the mixture, c_i is the concentration of the ith component and $E(c_i)$ is the effect of the ith component. By applying the logistic model (Eq. 2) for a response scaled to 1 as maximum response, IA model can be simplified as shown in Eq. 5 (Nweke *et al*., 2018).

$$E(c_{mix}) = \left[1 - \prod_{i=1}^{n} \left[1 - \frac{1}{1 + \left(\frac{\pi_i x}{EC_{50i}} \right)^{bi}} \right] \right] \times 100 \qquad (5)$$

Where, $\pi_i x$ is the concentration of ith component in the mixture. EC_{50i} and b_i are the EC_{50} and the corresponding slope for each (ith) component. The concentration-inhibition relationships of the mixtures were calculated from Eq.5 encoded in Microsoft Excel 2007. The observed EC_{50} were compared with the predicted EC_{50S} by Duncan post-hoc test implemented in SPSS statistics 21.

Toxic index (TI)

The Toxic Index (TI) of each mixture was calculated as sum of toxic units for all the mixture components (Eq. 6).

$$TI = \sum_{i=1}^{n} \frac{C_i}{EC_{50i}} = \sum_{i=1}^{n} \frac{\pi_i EC_{50mix}}{EC_{50i}} \qquad (6)$$

Where C_i is the concentration of the ith component in the mixture at the EC_{50} of the mixture (EC_{50mix}) and EC_{50i} is the concentration of the ith component that caused 50% reduction in dehydrogenase activity when tested alone, n is the number of mixture components and π_i is the relative proportion of ith component in the mixture. The mixture is described as additive if TI equals 1. When TI is less than or greater than 1, the mixture is described as synergistic or antagonistic respectively (Boilot & Perrodin, 2008).

Model deviation ratios (MDR)

Model deviation ratios were calculated as shown in Eq. 7. MDR greater than 1 indicated synergism, while a value of less than 1 indicated antagonism. MDR of 1 indicated additivity.

$$\mathrm{MDR} = \frac{\text{Predicted } EC_{50}}{\text{Observed } EC_{50}} \qquad (7)$$

RESULTS

Toxicity of individual toxicant to A. seifertii

The observed and predicted $EC_{50\mathrm{S}}$ of metals, SDS and the ternary mixtures on *A. seifertii* are shown in Table 2. SDS with EC_{50} of 2.810 ± 0.140 mM had the least toxicity while cadmium with EC_{50} of 0.011 ± 0.000 mM was the most toxic. $EC_{50\mathrm{S}}$ of the individual toxicants were statistically different from one another (P < 0.05). The decrease in toxicities of the individual toxicants are as follow: Cd(II) > Co(II) > Zn(II) > Pb(II) > Ni(II) > SDS. The responses of the organism to the toxicity of the toxicants were concentration-dependent (Fig. 1). The toxicants increasingly inhibited dehydrogenase activity with increased concentrations, resulting in inhibitions of more than 95% at 0.4 mM for Pb(II), 0.05 mM for Co(II), 0.08 mM for Cd(II), 1 mM for Zn(II) and 10 mM for SDS. The concentration-response pattern for SDS and Ni(II) as well as for Cd(II) and Pb(II) were similar.

Toxicity of ternary mixtures of SDS and metals to A. seifertii

In Table 2, the observed $EC_{50\mathrm{S}}$ in SDS + Pb(II) +Zn(II)

Table 2: Observed and predicted $EC_{50\mathrm{S}}$ of metals, SDS and the ternary mixtures on *A. seifertii*

Toxicants and mixtures	EC_{50}(mM)‡ ⁺		
	Experimental†	CA-Predicted	IA- Predicted
Ni(II)	0.649 ± 0.053a	-	-
Cd(II)	0.011 ± 0.000b	-	-
Pb(II)	0.222 ± 0.005c	-	-
Zn(II)	0.075 ± 0.005d	-	-
Co(II)	0.041 ± 0.008e	-	-
SDS	2.810 ± 0.140f	-	-
SDS + Pb(II) + Zn(II)			
SDS 94.87% + Pb(II) 3.88% + Zn(II) 1.25%(EECR50)	0.328 ± 0.018a*	1.473 ± 0.068**	2.384 ± 1.018***
SDS 95% + Pb(II) 4% + Zn(II) 1% (ABCR1)	0.302 ± 0.016a*	1.535 ± 0.069**	2.490 ± 0.006***
SDS 93% + Pb(II) 5% + Zn(II) 2% (ABCR2)	0.368 ± 0.008b*	1.217 ± 0.058**	2.004 ± 0.197***
SDS 90% + Pb(II) 2% + Zn(II) 8% (ABCR3)	0.349 ± 0.023b*	0.679 ± 0.044**	0.868 ± 0.927***
SDS + Cd(II) +Zn(II)			
SDS 98.50% + Cd(II) 0.20% + Zn(II) 1.30%(EECR50)	0.713 ± 0.028a*	1.630 ± 0.082**	2.335 ± 0.831***
SDS 96% + Cd(II) 1% + Zn(II) 3% (ABCR1)	0.242 ± 0.020b*	1.274 ± 0.075**	1.707 ± 0.007***
SDS 98% + Cd(II) 1% + Zn(II) 1% (ABCR2)	0.639 ± 0.023c*	1.899 ± 0.097**	2.491 ± 0.093***
SDS 95% + Cd(II) 2% + Zn(II) 3% (ABCR3)	0.270 ± 0.030b*	1.210 ± 0.068**	1.715 ± 0.005***
SDS + Pb(II) +Ni(II)			
SDS 92.44% + Pb(II) 3.78% + Ni(II) 3.78%(EECR50)	0.538 ± 0.017a*	1.793 ± 0.076**	2.527 ± 0.467***
SDS 93% + Pb(II) 3% + Ni(II) 4% (ABCR1)	0.443 ± 0.018b*	1.894 ± 0.083**	2.532 ± 0.006***
SDS 94% + Pb(II) 3% + Ni(II) 3% (ABCR2)	0.270 ± 0.006b*	1.937 ± 0.083**	2.597 ± 0.047***
SDS 91% + Pb(II) 4% + Ni(II) 5% (ABCR3)	0.421 ± 0.012a*	1.720 ± 0.074**	2.448 ± 0.057***
SDS + Ni(II) + Cd(II)			
SDS 94.21% +Ni(II) 3.86% + Cd(II) 1.93%(EECR50)	0.116 ± 0.004a*	0.477 ± 0.024**	0.544 ± 1.100***
SDS 93% +Ni(II) 5% + Cd(II) 2% (ABCR1)	0.423 ±0.018b*	0.460 ± 0.023*	0.521 ± 0.019**
SDS 94% +Ni(II) 4% + Cd(II) 2% (ABCR2)	0.267 ± 0.005c*	0.463 ± 0.023**	0.526 ± 0.023***
SDS 91% +Ni(II) 6% + Cd(II) 3% (ABCR3)	0.184 ± 0.012d*	0.326 ± 0.017**	0.357 ± 0.103***
SDS + Co(II) + Pb(II)			
SDS 95.22% + Co(II)0.89% + Pb(II) 3.89%(EECR50)	0.277 ± 0.005a*	1.362 ± 0.117**	1.873 ± 0.879***
SDS 94% + Co(II) 3% + Pb(II) 3% (ABCR1)	0.334 ± 0.016b*	0.829 ± 0.112**	1.056 ± 0.045***
SDS 95% + Co(II) 3% + Pb(II) 2% (ABCR2)	0.197 ± 0.017c*	0.859 ± 0.120**	1.052 ± 0.455***
SDS 96% + Co(II) 2% + Pb(II) 2% (ABCR3)	0.261 ± 0.008a*	1.093 ± 0.134**	1.333 ± 0.138***
SDS + Co(II) + Cd(II)			
SDS 97.10% + Co(II) 0.91% + Cd(II).2%(EECR50)	0.198 ± 0.016a*	0.428 ± 0.027**	0.480 ± 0.530***
SDS 98% + Co(II) 1% + Cd(II) 1% (ABCR1)	0.216 ± 0.008a*	0.676 ± 0.049**	0.801 ± 0.001***
SDS 96% + Co(II) 2% + Cd(II) 2% (ABCR2)	0.248 ± 0.011b*	0.385 ± 0.029**	0.425 ± 0.035***
SDS 95% + Co(II) 3% + Cd(II) 2% (ABCR3)	0.169 ± 0.008c*	0.352 ± 0.030**	0.388 ± 0.235**

Within column, among the individual toxicants, EC_{50} values with different letters differed significantly from each other
†Within columns, in each individual toxicant or 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).
⁺ Values are reported as Mean ± 1SD

mixture showed that ABCR2 mixture ratio was the least toxic (0.368 ± 0.008 mM) while ABCR1 mixture ratio was the most toxic (0.302 ± 0.016 mM). In addition, among the observed EC_{50S}, the EECR50 and ABCR1 mixture ratios were statistically different from ABCR2 and ABCR3. In SDS + Cd(II) + Zn(II) mixtures, the observed EC_{50} values ranged from 0.242 ± 0.020 mM for ABCR1 to 0.713 ± 0.028 mM for EECR50. The observed EC_{50S} showed that EECR50 and ABCR2 mixture ratios were statistically different from ABCR1 and ABCR3 mixture ratios.

In SDS + Pb(II) + Ni(II) mixtures, for the observed EC_{50S}, EECR50 and ABCR2 mixture ratios were statistically different from ABCR1 and ABCR3 mixture ratios. In SDS + Ni(II) + Cd(II) mixtures, the observed EC_{50S} showed significant difference among mixture ratios. Similarly, the EC_{50} predicted by independent action model was statistically different from both the observed and concentration addition model-predicted EC_{50S} in ABCR1 mixture ratio ($P < 0.05$). In SDS + Co(II) + Pb(II) mixtures, among the observed EC_{50S}, ABCR1 and ABCR2 mixture ratios were significantly different from each other. In SDS + Co(II) + Cd(II) mixtures, the observed EC_{50S} of ABCR2 and ABCR3 mixture ratios were statistically different from each other. In ABCR3 mixture ratio, the observed EC_{50} was significantly lower than those predicted from CA and IA models ($P < 0.05$). However, in most mixture ratios, the observed EC_{50S} as well as those predicted from CA and IA models were statistically different from one another ($P < 0.05$).

Toxic index, model deviation ratio and effect of ternary mixtures of metals and SDS on *A. seifertii* are shown in Table 3. The TI values ranged from 0.139 ± 0.003 to 0.919 ± 0.019, while MDR values ranged from 1.088 ± 0.023 to 7.173 ± 0.148 for CA and 1.233 ± 0.041 to 9.621 ± 0.090 for IA. At all the tested mixture ratios, the ternary mixtures were synergistic in their actions against the bacterium, except for ABCR1 mixture ratio of SDS + Ni(II) + Cd(II) mixture, whose effect was rather additive. The observed concentration-response relationships of the ternary mixtures and the predictions made from CA and IA models for *A. seifertii* are shown in Figures 2-7. In the SDS + Pb(II) + Zn(II) mixture, both models greatly underestimated the toxicities except for ABCR3, where they slightly underestimated the toxicity (Figure 2).

In SDS + Cd(II) + Zn(II) and SDS + Pb(II) + Ni(II) mixtures, both CA and IA models also underestimated the toxicities relative to the observed data as shown in Figures 3 and 4 respectively. In

Table 3: Toxic index, MDR and effect of ternary mixtures of metals and SDS on *A. seifertii*

Metal-SDS Mixtures	Toxic Index (TI)	MDR+		Effect
		CA	IA	
SDS +Pb (II)+Zn(II)				
SDS 94.87% +Pb(II) 3.88%+Zn(II) 1.25% (EECR 50)	0.223 ± 0.002	4.493 ± 0.039	7.282 ± 0.384	Synergistic
SDS 95% +Pb(II) 4%+Zn(II)1% (ABCR1)	0.196 ± 0.001	5.090 ± 0.035	8.267 ± 0.395	Synergistic
SDS 93% +Pb(II) 5%+Zn(II) 2% (ABCR2)	0.303 ± 0.008	3.304 ± 0.087	5.558 ± 0.136	Synergistic
SDS 90% +Pb(II) 2%+Zn(II) 8% (ABCR3)	0.514 ± 0.002	1.946 ± 0.006	2.491 ± 0.553	Synergistic
SDS +Cd (II)+Zn(II)				
SDS 98.50% +Cd(II) 0.20%+Zn(II)1.30% (EECR 50)	0.499 ± 0.007	2.286 ± 0.025	3.278 ± 0.104	Synergistic
SDS 96% +Cd(II) 1%+Zn(II)3% (ABCR1)	0.393 ± 0.014	5.264 ± 0.139	7.074 ± 0.526	Synergistic
SDS 98% +Cd(II) 1%+Zn(II) 1% (ABCR2)	0.365 ± 0.006	2.969 ± 0.042	3.898 ± 0.097	Synergistic
SDS 95% +Cd(II) 2%+Zn(II) 3% (ABCR3)	0.246 ± 0.013	4.503 ± 0.243	6.406 ± 0.639	Synergistic
SDS +Pb (II)+Ni(II)				
SDS 92.44% +Pb(II) 3.78%+Ni(II) 3.78% (EECR 50)	0.300 ± 0.003	3.329 ± 0.033	4.696 ± 0.122	Synergistic
SDS 93% +Pb(II) 3%+Ni(II) 4% (ABCR1)	0.234 ± 0.001	4.277 ± 0.019	5.724 ± 0.168	Synergistic
SDS 94% +Pb(II) 3%+Ni(II) 3% (ABCR2)	0.139 ± 0.003	7.173 ± 0.148	9.621 ± 0.090	Synergistic
SDS 91% +Pb(II) 4%+Ni(II) 5% (ABCR3)	0.245 ± 0.004	4.084 ± 0.060	5.818 ± 0.152	Synergistic
SDS +Ni (II)+Cd(II)				
SDS 94.21% +Ni(II) 3.86%+Cd(II) 1.93% (EECR 50)	0.243 ± 0.007	4.120 ± 0.113	4.702 ± 0.122	Synergistic
SDS 93% +Ni(II) 5%+Cd(II) 2% (ABCR1)	0.919 ± 0.019	1.088 ± 0.023	1.233 ± 0.041	Additivity
SDS 94% +Ni(II) 4%+Cd(II) 2% (ABCR2)	0.578 ± 0.019	1.733 ± 0.058	1.970 ± 0.039	Synergistic
SDS 91% +Ni(II) 6%+Cd(II) 3% (ABCR3)	0.563 ± 0.017	1.779 ±0.054	1.945 ± 0.090	Synergistic
SDS +Co (II)+Pb(II)				
SDS 95.22% +Co(II) 0.89%+Pb(II) 3.89% (EECR 50)	0.204 ± 0.014	4.914 ± 0.332	6.761 ± 0.079	Synergistic
SDS 94% +Co(II) 3%+Pb(II) 3% (ABCR1)	0.406 ± 0.036	2.475 ± 0.218	3.157 ± 0.137	Synergistic
SDS 95% +Co(II) 3%+Pb(II) 2% (ABCR2)	0.231 ± 0.013	4.346 ± 0.235	5.340 ± 0.084	Synergistic
SDS 96% +Co(II) 2%+Pb(II) 2% (ABCR3)	0.243 ± 0.023	4.136 ± 0.380	5.097 ± 0.143	Synergistic
SDS +Co (II)+Cd(II)				
SDS 97.10% +Co(II) 0.91%+Cd(II) 2% (EECR 50)	0.463 ± 0.014	2.162 ± 0.066	2.430 ± 0.210	Synergistic
SDS 98% +Co(II) 1%+Cd(II) 1% (ABCR1)	0.320 ± 0.011	3.124 ± 0.104	3.708 ± 0.195	Synergistic
SDS 96% +Co(II) 2%+Cd(II) 2% (ABCR2)	0.647 ± 0.020	1.547 ± 0.048	1.715 ± 0.086	Synergistic
SDS 95% +Co(II) 3%+Cd(II) 2% (ABCR3)	0.483 ± 0.018	2.071 ± 0.078	2.291 ± 0.113	Synergistic

+ Values are reported as Mean ± 1SD

SDS + Ni(II) + Cd(II) mixture, the models slightly underestimated the toxicities and were toxic even at low concentrations, except in ABCR1 mixture ratio, where both models almost correctly predicted the experimentally-observed data at low concentrations, and underestimated the mixture toxicity at high concentrations. Similarly, in both SDS + Ni(II) + Cd(II) and SDS +Co(II) + Cd(II) mixtures, CA and IA models predicted similar toxicities, as their dose-response curves were almost superimposed (Figures 5 and 7). In ABCR1 mixture ratio of SDS + Co(II) + Pb(ll) and all SDS + Co(II) + Cd(II) mixtures, both models slightly predicted lower toxicities than the experimentally-observed data and were toxic even at low concentrations. In other SDS + Co(II) + Pb(ll) mixture ratios, both CA and IA models however grossly under-estimated the mixture toxicities (Figures 6).

DISCUSSION

Sodium dodecyl sulfate (SDS) was the least toxic to the sediment bacterium, among the toxicants evaluated in this study. This result is in agreement with the report that SDS was less toxic than a variety of metals and non surfactants compounds by previous authors (Whitton, 1967; Wangberg & Blanck, 1988). Sodium dodecyl sulfate has been reported to inhibit certain processes in different organisms at various concentrations. For example, inhibition in cell multiplication and rate of phosphate assimilation in pure cultures of *Acinetobacter junii* has been reported at EC_{50S} of 5.00 ± 2.95 x 10^{-6} mol/l (0.005 mM) and 3.33 ± 0.96 x 10^{-4} mol/l (0.33 mM) respectively (Hrenovic & Ivankovic, 2007). In a similar report, growth and nitrogen fixing ability in cyanobacterium *Gloeocapsa* were inhibited by SDS at 50 ppm (≈ 0.173 mM) (Cserhati *et al*., 2002). Furthermore, inhibion of overall up take of metals without altering cell membrane permeability in marine macroalga, *Ulva lactuca* by SDS was reported by Masakorala *et al*. (2008). However, SDS was not particularly toxic to *Ulva lactuca* at a concentration range of 0-10 mg/l (≈ 0.04 mM) (Masakorala *et al*., 2011) and towards algae, and invertebrates at environmentally realistic concentrations (Sandback *et al*., 2000). In the present study, SDS inhibited dehydrogenase activity in *A. seifertii* by 50% at 2.810 ± 0.140 mM after 24 hours.

Due to their persistence and toxicity, environmental pollution by heavy metals, particularly in aquatic ecosystems,

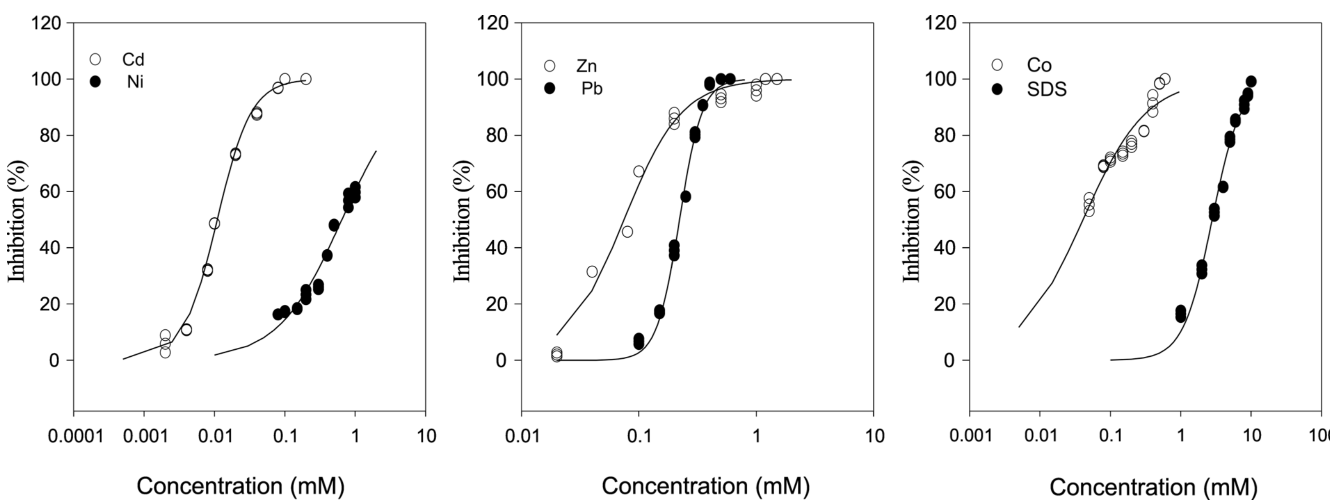

Figure 1: Individual toxicants inhibition of dehydrogenase activity in *A. seifertii*. The solid and dash lines represents predicted toxicities respectively.

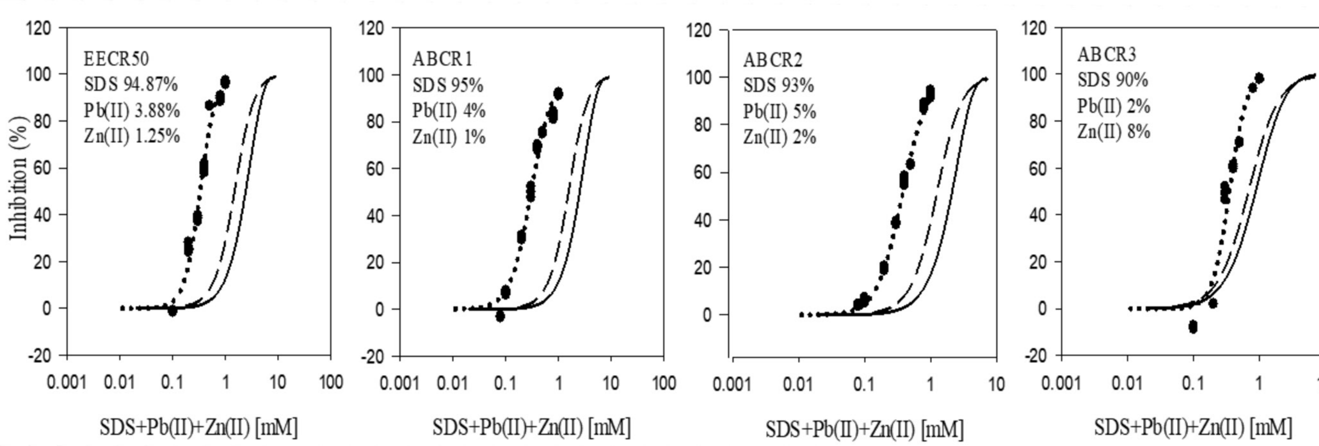

Figure 2: Observed (data points) and predicted (lines) inhibitory effects of ternary mixtures of SDS, lead and zinc ions on *A. seifertii* dehydrogenase activity. Dotted lines represent toxicities determined by nonlinear regression of observed data with logistic model (Eq. 2). Dashed and solid lines are toxicities predicted from the CA and IA models respectively.

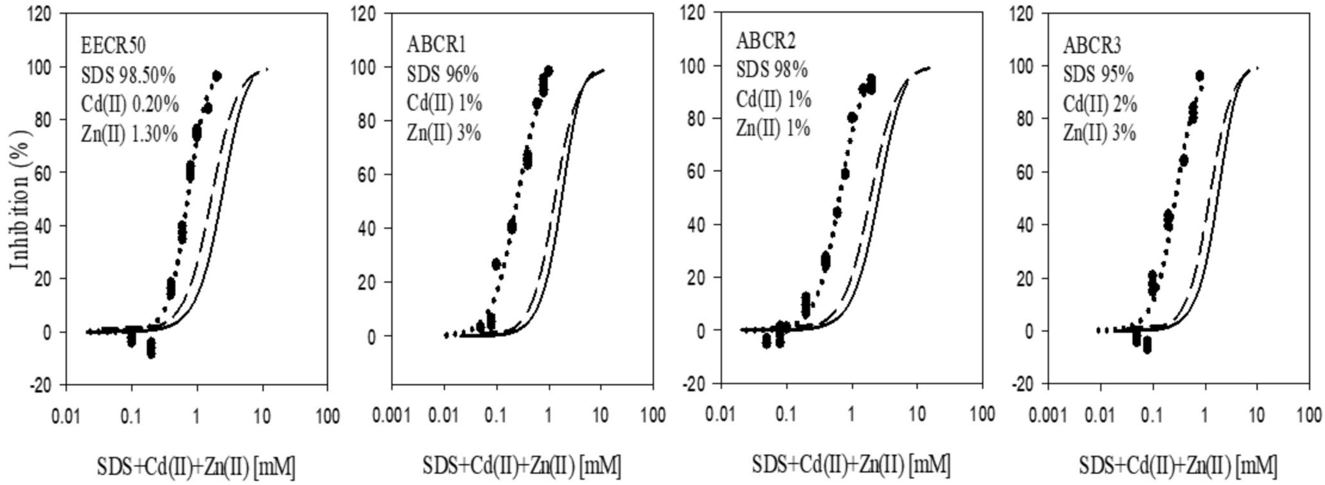

Figure 3: Observed (data points) and predicted (lines) inhibitions of *A. seifertii* dehydrogenase activity by ternary mixtures of SDS, cadmium and zinc ions. The data points represent observed concentration-response data. Dotted lines represent toxicities determined by nonlinear regression of observed data with logistic model (Eq. 2). Dashed and solid lines are toxicities predicted from the CA and IA models respectively.

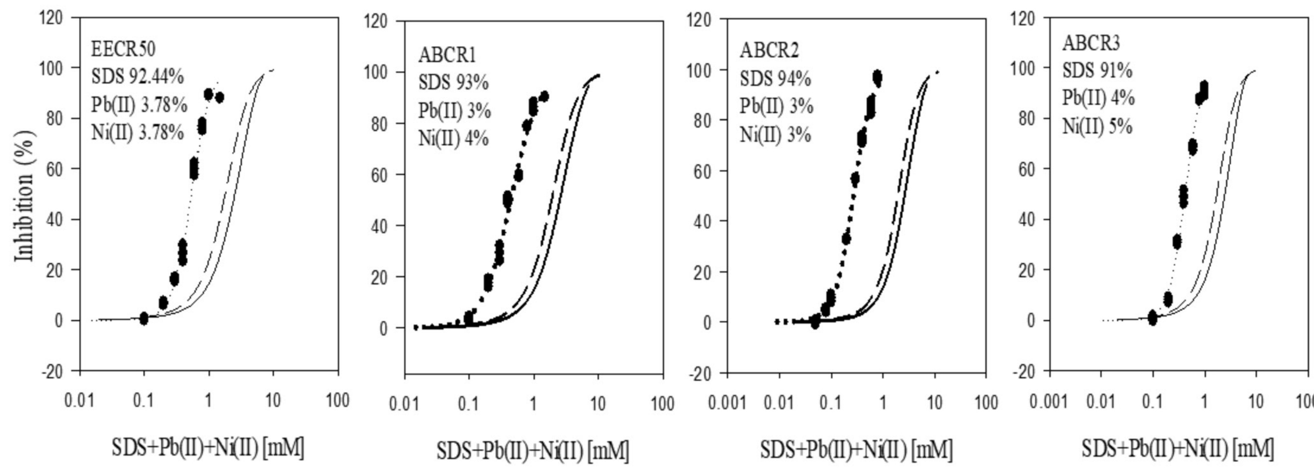

Figure 4: Observed (data points) and predicted (lines) inhibitions of *A. seifertii* dehydrogenase activity by ternary mixtures of SDS, lead and nickel ions. Dotted lines represent toxicities obtained by nonlinear regression of observed data with logistic model (Eq. 2). Dashed and solid lines are toxicities predicted from the CA and IA models respectively.

has been a serious concern. Heavy metals such as nickel, cobalt and zinc serve as micro nutrients to bacteria, required at trace concentrations. However, at higher concentrations, nickel, cobalt, zinc and "bad ions" (with no nutritional values) such as cadmium and lead are toxic to microorganisms (Nies, 1992). In the present study, the order of increasing toxicity among the trace metals were Ni > Zn > Co. Nickel was toxic to the bacterium, with an EC_{50} of 0.649 ± 0.053 mM. Possible mechanisms of nickel toxicity to microorganisms include: substitution of essential metal of metalloprotiens, binding to catalytic residues of non-metalloenzymes, binding outside the enzyme's catalytic site, resulting in allosteric inhibition and by indirectly causing oxidative stress (Macomber & Hausinger, 2011). Similarly, toxicity of cobalt to luminescent *Vibrio fischeri* has also been reported with a 15 min EC_{50} of 45.9 ± 4.59 mg/l (0.35 mM) (Fulladosa *et al*., 2005). In this study, cobalt inhibited dehydrogenase activity in *A, seifertii* with a 24 hours EC_{50} of 0.011 ± 0.000 mM. Furthermore, cobalt was more toxic than nickel. Such greater inhibition by cobalt to cell growth than nickel has been reported elsewhere (Gikas, 2007).

Zinc as component of many microbial enzymes, is needed for their catalytic activities and structural stability (Choudhury & Srivastava, 2001). However, Zn(II) may become toxic to cells at high concentrations. For example, a 50% decrease in dehydrogenase activity by zinc was reported in sediment bacteria from New Calabar river at 0.166 and 0.873 mM respectively, for *Bacillus* and *Micrococcus* species (Nweke *et al*., 2007a). Similarly, a 15 min EC_{50} of 0.86 ± 0.11 mgl^{-1} (≈ 0.003 mM) was reported for zinc against luminescent bacterium *Vibrio fischeri* by Fulladosa *et al*. (2005). Zinc inhibited dehydrogenase activity of the sediment bacterium *A, seifertii* in this study with 24 hours EC_{50} of 0.075 ± 0.005 mM. The variation in EC_{50S} observed could be attributed to the different bacterial species adopted for the studies.

Cadmium and lead have no physiological functions in living organisms and can be toxic at low concentrations. For instance, 15 min IC_{50S} of 0.537 mg/l (≈ 0.002 mM) and 1.231 mg/l (≈ 0.004 mM) were recorded for cadmium and lead respectively against *Phosphobacterium phosphoreum* T3S (Zeb *et al*., 2016). In a similar study, Mansour *et al.* (2015)

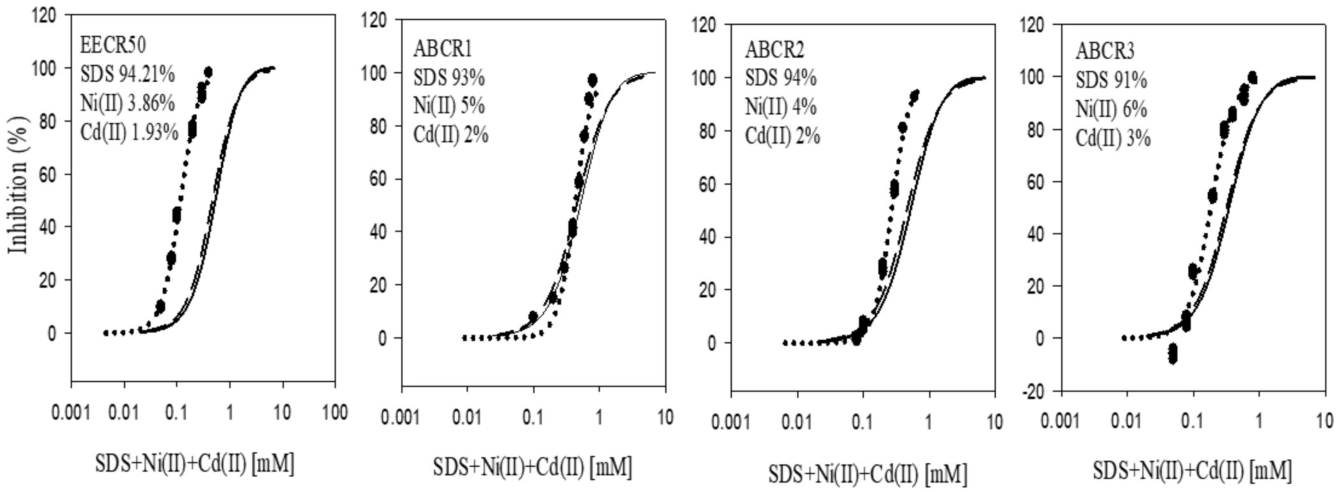

Figure 5: Observed (data points) and predicted (lines) inhibitory effects of ternary mixtures of SDS, nickel and cadmium ions on *A. seifertii* dehydrogenase activity. Dotted lines represent toxicities obtained by nonlinear regression of observed data with logistic model (Eq. 2). Dashed and solid lines are toxicities predicted from the CA and IA models respectively.

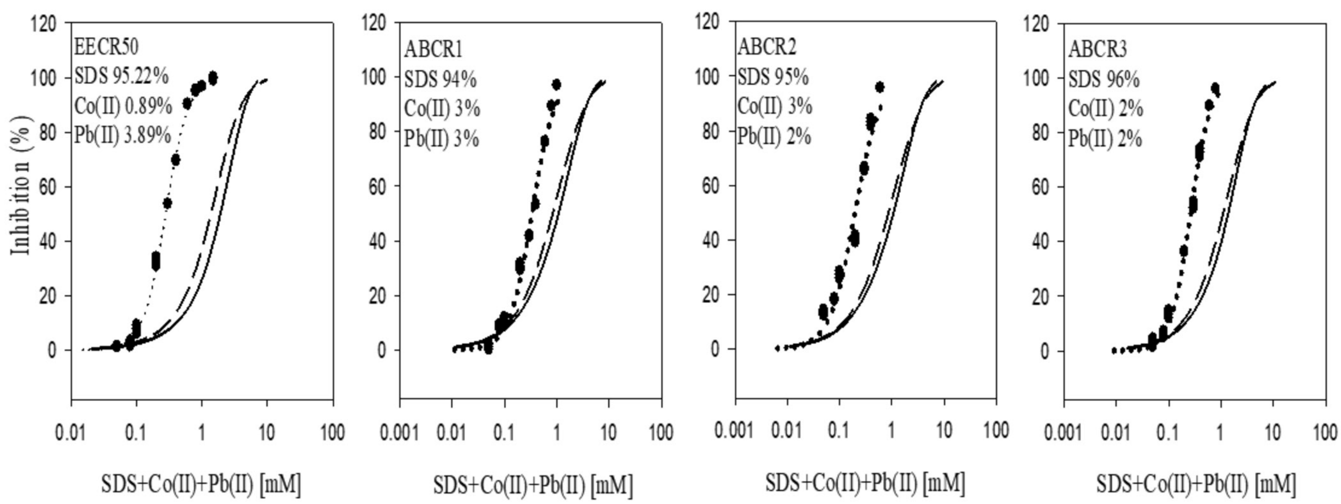

Figure 6: Observed (data points) and predicted (lines) inhibitory effects of ternary mixtures of SDS, cobalt and lead ions on *A. seifertii* dehydrogenase activity. Dotted lines represent toxicities obtained by nonlinear regression of observed data with logistic model (Eq. 2). Dashed and solid lines are toxicities predicted from the CA and IA models respectively.

reported 5 and 15 minutes EC_{50S} of 4.53 mg/l (≈ 0.018 mM) and 4.47 mg/l (≈ 0.014 mM), 6.60 mg/l (≈ 0.03 mM) and 5.83 mg/l (≈ 0.018 mM) for cadmium and lead respectively against inhibition of bioluminescence in *Vibrio fischeri*. In the present study however, cadmium and lead recorded 24 hours EC_{50S} of 0.011 ± 0.000 mM and 0.222 ± 0.005 mM against *A. seifertii.*

Though the concentrations of SDS and heavy metals recently reported in Otamiri river water and sediment by Okechi & Chukwura, (2020) were lower than the EC_{50S} of the toxicants employed in the present study, heavy metals are however known to be persistent and accumulative in the environment, and thus, could reach or even surpass these employed concentrations over time. Furthermore, progressive accumulation of heavy metals in Otamiri river sediment has been reported (Temitope *et al*., 2016). In addition, investigations have shown that surfactants can change the toxicity of heavy metals to aquatic organisms (Swedmark & Granmo, 1981; Masakorala *et al*., 2008). However, it is also possible that the bacterium may have evolved some tolerance mechanisms to these toxicants judging from their reported age long accumulation in the river, and as such, the response to the individual toxicants and their ternary mixtures may be different from that of *A. seifertii* isolated from unpolluted waterbodies. In addition, though metals have been reported to accumulate in Otamiri River, reasonable comparison cannot be made on the toxicities of heavy metals and surfactants on *A. seifertii* from the river. Not much work has been done on the ecotoxicological implications of these pollutants on the microbiological population of Otamiri River. Nweke *et al* (2017) assessed inhibition of INT-dehydrogenase activity in microbial community of Otamiri river water by Cd, Ni, Zn and Co). Although normal strength nutrient broth was used in the study, the microbial community with EC_{50} of 0.265 ± 0.015 mM Pb(II) was more sensitive to lead than *A. seifertii* in the present study.

There is scarcity of information on the toxic effects of ternary mixtures of SDS and heavy metals on bacteria. In the present study, SDS modulated the toxicity of the heavy

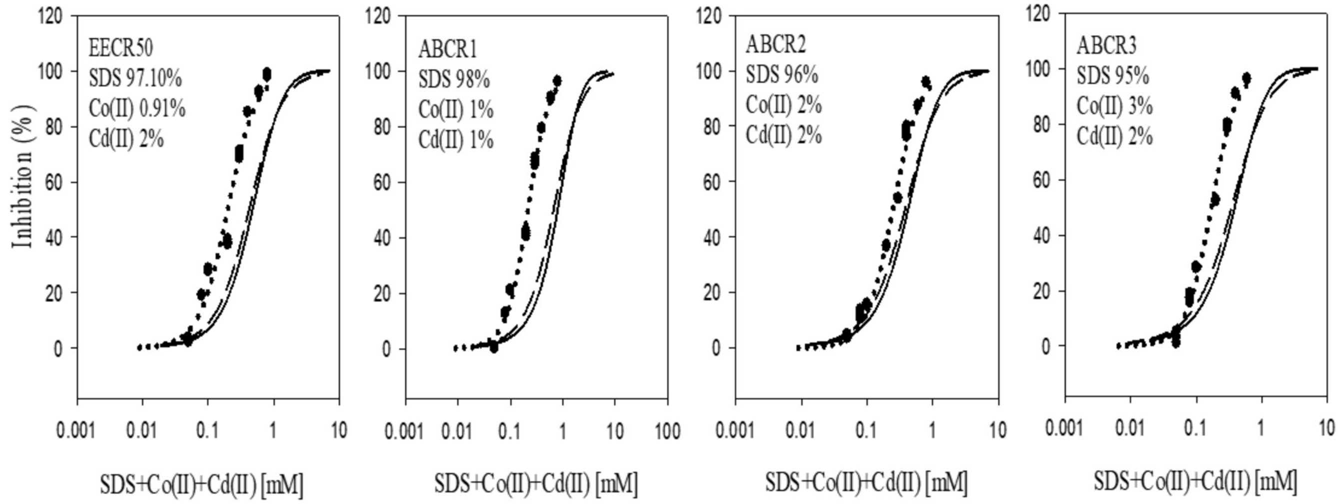

Figure 7: Observed (data points) and predicted (lines) inhibitory effects of ternary mixtures of SDS, cobalt and cadmium ions on *A. seifertii* dehydrogenase activity. Dotted lines represent toxicities obtained by nonlinear regression of observed data with logistic model (Eq. 2). Dashed and solid lines are toxicities predicted from the CA and IA models respectively.

metals and vice versa, thus giving EC_{50S} generally lower than SDS and in few cases lead as individual toxicants. Li *et al*. (2018), similarly noted that the toxicities of all the mixtures of nanoTiO_2 and Cd^{2+} with surfactant were lower than the single toxicity of Cd^{2+} to *Escherichia coli*. Although some researchers have claimed that anionic surfactants are healthy, adverse health effects have nevertheless been reported from exposure to multiple chemicals at low concentrations, which does not cause harm individually (Brain *et al*., 2007; Smith *et al*., 2013; Kortenkamp, 2014). Although it has been established that some anionic surfactants can enhance the toxicities of coexisting chemical species, such as metals, and anthracene (Swedmark & Granmo, 1981; Flores *et al*., 2010), this was not established in this study. Furthermore, though ternary mixtures of SDS+Pb(II)+Ni(II) and SDS+Ni(II)+Cd(II) were more toxic than their individual toxicities against the sediment bacterium, this increase seems to be more attributable to the effects of Pb and Cd ions than SDS, as such enhanced toxicities were not reflected in the binary mixture of SDS with nickel (data not shown).

The model deviation ratios (MDR) and the toxic index model (TI) used to analyse the ternary mixture toxicities indicated similar results, with regards to the toxicity of SDS and metal mixtures to *A. seifertii*. The TI values obtained for all the ternary mixtures were well below 1, except SDS93% + Ni(II)5% + Cd(II)2% mixture ratio that was almost 1, thus describing synergistic and additive interactions respectively. This ternary combination was especially important as an example of how the form of toxicological interaction in ternary mixtures would change in relation to their binaries (Boltes *et al*., 2012). The binary mixtures were synergistic and antagonistic in their interactions. Similarly, the MDR values for all the ternary mixtures of SDS and metal ions in this study also showed synergistic interactions, except the same mixture ratio that showed additivity. Some authors have reported both synergistic and antagonistic interactions in studies with ternary mixtures of different heavy metals to bacteria and liver cells (Xu *et al*., 2011; Lin *et al*., 2016; Nweke *et al*., 2018). Similarly, depending on metals concentrations, antagonism, additivity and synergism were reported on the acute toxicity of the ternary mixtures of metals to *Daphnia magna* (Traudt *et al.,* 2017). Reproduction in *Ceriodaphnia dubia* has also been reported to be significantly affected by the ternary mixtures of Cu+Pb+Zn, with more than additive effect (Cooper *et al*., 2009). It is important to note the variations in the mixtures components in these studies compared with the present study, as none had SDS as a component. It has been reported that the types of interactions exhibited by the components of mixtures largely depend on their relative proportions in the mixtures (Otitoloju, 2005).

Predictive models; CA and IA, have been used to predict the toxicity of chemical mixtures on the basis of concentration-response relationship of the mixture components. The CA model is based on the assumption that the mixture components act similarly while the IA model assumes that the mixture components act differently. In this study, CA and IA models either slightly or greatly underestimated the joint toxicity of the SDS and metals. Both models however made good predictions at low concentrations for ABCR1 mixture ratio of SDS + Ni(II) + Cd(II) mixture. Similar result was reported by Nweke *et al*. (2018). Furthermore, Regenmortel & De Schamphelaere, (2017) used both models to correctly predict Cu–Ni –Zn ternary mixtures on the growth of *Pseudokirchneriella subcapitata* in natural waters. It is important to note that there was no statistical difference between the observed EC_{50} and CA-predicted EC_{50}, in ABCR1 mixture ratio of SDS + Ni(II) + Cd(II) mixture, indicating additive effect of the mixture components. SDS and heavy metals may have similar modes of action against the bacterium, thus there was no substantial difference between predicted values of mixture toxicities on the bases of CA and IA-models in ABCR3 mixture ratio of SDS + Co(II) + CdII) mixture. Huang *et al*. (2011) reported similar insignificant differences in the toxicity of mixtures predicted from CA and IA models for phenol containing compounds with related and different mechanisms of action. In addition, similar toxicities for the ternary mixtures of SDS + Ni(II) + Cd(II) and SDS + Co(II) + Cd(II) were also predicted from CA and IA models in this study. Studies have shown that the EC_{50}s predicted from both models can be similar under certain conditions (Boedeker *et al*., 1993).

CONCLUSION

The toxicities of SDS and some heavy metals (Pb, Cd, Co, Zn, Ni) as individuals and in ternary mixtures to *A. seifertii*, isolated from Otamiri river sediment were examined, using inhibition of dehydrogenase activity as end point. The results of this study showed that both heavy metals and SDS exhibited varying toxicity levels to the sediment bacterium, both as individual toxicants and as ternary mixtures. The mixture interaction was generally synergistic, indicating the possibility of adverse effects of the mixture on the bacterial population of the river sediment.

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