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Synergistic interactions of senary mixtures of an anionic surfactant and five divalent metals to planktonic and sediment bacteria

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Abstract

The synergistic toxicities of senary mixtures of an anionic surfactant, Sodium Dodecyl Sulfate (SDS) with five divalent metal ions, Pb^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+} and Co^{2+}, to *Serratia marcescens* (SerEW01) and *Acinetobacter seifertii* respectively isolated from water and sediments in Otamiri River, Owerri, Imo State, Nigeria were critically analyzed with dehydrogenase activity inhibition as the response. The EC_{50}s observed for the *individual toxicants for S. marcescens* (Ser EW01) was between 0.046 ± 0.003mm (Zn^{2+}) and 2.329 ± 0.092mm (SDS) and between 0.011 ± 0.00mm (Cd^{2+}) and 2.810 ± 0.140mm (SDS), for *A. seifertii*. At $p < 0.05$, the EC_{50}s for individual toxicant were significantly different for each organism. To analyze the senary mixtures effects against the bacteria, fixed ratio mixtures of arbitrary combined ratios (ABCR) and EC_{50} equi-effect concentration ($EECR_{50}$) were designed. Logical function was used to describe the dose-response relationships between the individual toxicants and the mixtures. Based on the independent actions (IA) and the concentration addition (CA) models, there was a significant difference between the predicted and experimental toxicities. However, there was an underestimation of the mixture toxicities in both organisms by the predicted models, at high concentration and slight overestimation against *A. seiferii* at low concentration. Furthermore, CA-model made a better prediction of the mixture toxicities than IA- model at low concentrations, especially in ABCR 2 and 3 mixture ratios for *A. seiferii*. The Toxic Index (TI) and Model Deviation Ratio (MDR) analyses indicate synergistic interaction of the mixtures against both bacteria. Thus, in natural environment, mixtures of metals and surfactant could potentially harm the aquatic microbial ecosystems.

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Introduction

In toxicology, all substances are potentially toxic to living organisms and the degree of toxicity depends on the applied dosage (Magalhaes *et al.*, 2015). Microorganisms such as bacteria, yeasts, micro-algae, diatoms have been used to evaluate the impact of mixtures of chemical pollutants in the environment. A change in the biota is an effective way of accessing the impact of environmental pollutants, as it reflects integrated effects of the chemical mixtures. Both heavy metals and anionic surfactants by their uses can co-contaminate aquatic ecosystems, thus exposing aquatic bacteria to the joint effects of such toxicants mixtures (Okechi and Chukwura, 2020; Okechi *et al.*, 2020). In freshwater ecosystems, metals can produce aggregates of different sizes (Magalhaes *et al.*, 2015). Nystrand *et al.* (2012) categorized metals into three forms, namely; dissolved, colloidal and particulate, with the dissolved form as the most toxic due to their mobility and bioavailability. Some of the dissolved metal ions may be adsorbed on sediments, undergo ion-exchange with clay particles or form complexes with oxyanions, thus reducing their bioavailability to aquatic organisms (Bjerregaard and Andersen, 2007). Metals with larger particles are usually unavailable to aquatic biota but are increasingly deposited in sediments, which in turn serves as temporal reservoirs (Vignati *et al.*, 2006). However, modifications in water chemistry, such as increase in salinity, reduced conditions, lower pH and presence of complex organics, can lead to their re-solubilization (Bjerregaard and Andersen, 2007; Butler *et al.*, 2008; Gunkel-Grillon *et al.*, 2014).

Based on volume, the world's most widely used synthetic surfactants are anionic surfactants; sodium dodecyl sulfate (SDS) and linear alkylbenxene sulfonate (LAS). They are used in the production of domestically used products such as detergents, shampoos, shower gels, cosmetics, tooth pastes and academic materials. It is also widely used industrially **(**Sirisattha *et al.*, 2004). Sediment is a key component of the ecosystem which provides habitat, nutrient and breeding sites for aquatic animals, therefore, proper protection of sediment quality helps to restore and monitor the biological quality of water and in protecting aquatic life (USEPA, 2007).

Most toxicity studies are carried out by monitoring the effects of a single toxicant on an organism or a process. Naturally, aquatic organisms are simultaneously exposed to multiple toxicant mixtures. Although anionic surfactants were previously reported to be safe, some studies have however shown that the effects of heavy metal toxicity on aquatic organisms can be altered in the presence of surfactants (Swedmark and Granmo, 1981; Masakorala *et al.*, 2008). Okechi *et al.*, (2021a, b) recently reported that SDS enhanced the toxicities of heavy metals to aquatic bacteria. Though many mixture toxicity studies involving heavy metals and organic compounds have been carried out, only few published data exist on combined heavy metal toxicities and SDS to microorganisms. These studies involved only one or few metals and none had compared the responses of bacteria from different aquatic environmental strata to the mixtures of these toxicants. This study evaluated the joint effects of senary mixtures of five metals (Co^{2+}, Ni^{2+}, Pb^{2+}, Cd^{2+} and Zn^{2+}) and SDS on planktonic and sediment bacteria isolated from Otamiri River in Owerri, Imo State, Nigeria.

Materials and methods

Reagents and test bacteria

The following heavy metals salts $NiSO_4.6H_2O$, $ZnNO_3.6H_2O$, $CdSO_4.8H_2O$, $CoCL_2$, $Pb(NO_3)_2$ were used as sources of Ni, Zn, Cd, Co and Pd ions respectively. These salts, thiazolyl blue tetrazolium bromide (MTT) and SDS were obtained from Sigma-Aldrich (Germany). *Serratia marcescens* (SerEW01) and *Acinetobacter seifertii* reported as the preponderant bacteria isolates from Otamiri river water and sediment by Okechi and Chukwura (2020) were used as test organisms.

Preparation of the inocula

In separate nutrient broth cultures, rotary incubator of 150 rpm was used to grow *S. marcescens* and *A. seifertii* cells for 24 hours, under the temperature of 28 ± 2°C.

Centrifugation (Newlife centrifuge, NL80-2) at 3000 rpm for 15 mins was used to harvest of cells separately. The harvested cells from each culture were washed by repeated centrifugation (x3) and re-suspended in sterile deionized water. The cell densities in the cell suspensions were adjusted to $1.1x10^8$ cells/ml in accordance to McFarland turbidity standards.

Toxicity assay for metal ions and SDS

Inhibition of MTT-dehydrogenase activity was applied as response to analyze the toxicity of metal ions and SDS to each bacterium. The assay was conducted according to Nweke *et al.* (2020) with little modifications. Heavy metals or SDS was used to amend and evaluate the reaction mixture in nutrient broth of 2mL final volume. Both stock solutions of SDS or the selected heavy metals and sterile water (deionized) were added to 15mL culture tube containing 0.5mL nutrient broth (NB) of pH 7.0. The reaction mixture has a total of 0.2% w/v of nutrient broth, 0.1mL of 0.1% aqueous MTT, and 0.1mL standardized inoculum for each tube, thus varying concentrations of SDS and heavy metals were obtained. This was prepared in triplicates, alongside the control (the above mixtures without SDS or heavy metals). Incubation of culture was done for 24 h in the dark at 28 ± 2°C. In each tube, 4mL n-butanol was used to extract the purple-coloured MTT-farmazam (MTTF), while spectrophotometer (VIS Spectrophotometer 721D) was used to measure the extract absorption, at 590 nm.

Determination of EC_{50} of metal ions and SDS

The response of the organism to each concentration of SDS or metal ion was calculated as percent inhibition of dehydrogenase activity (R) relative to the mean control (Eq. 1).

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

In Eq. (1), C_A stands for mean absorbance of MTTF-extract in the control tubes, T_A is absorbance of MTTF-extract in the experiment with a particular concentration of SDS or metal ions. EC_{50} was consequently calculated by fitting the concentration-response values into 2-parameter logistic function (Eq. 2), using least square non-linear regression technique.

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

In Eq. (2), x is the SDS or metal concentration, EC_{50} is SDS or metal concentration that elicited 50% reduction in dehydrogenase activity and b is the slope of the fitted curve at EC_{50}.

Design of senary mixture ratios

The senary mixtures (SDS+Pb+Zn+Cd+Co+Ni) were designed to contain fixed ratios of SDS and metals ions. In the senary combinations, four mixture ratios comprising one EC_{50} equi-effect concentration ratio, determined based on the EC_{50S} of the components (EECR50) and three arbitrarily chosen mixture ratios (ABCR) were studied. Table 1 shows the relative proportions of heavy metals and SDS in the senary combinations for the organisms. Each combination was prepared as 10mm stock solution by mixing requisite volumes of 10mm solutions of each metal and SDS in separate 100-ml Erlenmeyer flasks and then used as a composite mixture during toxicity testing.

Table 1. Eqiu-effect concentration ratio (EECR50) and arbitrary concentration ratio (ABCR) mixtures.

	Mixture ratio (%)											
	Planktonic bacterium (*S. marcescens* (SerEW01))						Sediment bacterium (*A. seifertii*)					
Mixture	Cd^{2+}	Zn^{2+}	Pb^{2+}	Co^{2+}	Ni^{2+}	SDS	Cd^{2+}	Zn^{2+}	Pb^{2+}	Co^{2+}	Ni^{2+}	SDS
EECR 50	1.96	1.12	3.76	1.68	5.95	85.53	1.82	1.81	3.64	0.83	3.64	88.8
ABCR1	2	4	2	3	3	86	1	2	2	3	3	89
ABCR2	2	4	2	3	4	85	2	3	2	2	3	88
ABCR3	3	3	3	5	2	84	3	3	2	3	2	87

Toxicity assay for the senary mixtures

The toxicity assay procedure as described for the individual toxicants was adopted for the mixtures. Requisite volumes of composite mixtures of the toxicant (heavy metals and SDS), sterile deionized water and NB (0.5mL of 0.8% w/v) were added to a 15mL culture tube. Then, 0.1mL each of 0.1% MTT solution and *S. marcescens* suspension were added to

attain graded total concentrations of the senary mixtures in 2-ml total volumes. The same procedure was equally adopted for *A. seifertii* suspension. Controls were prepared, but without the toxicant mixtures. Incubation of the cultures, extraction of MTT-formazan (MTTF) and the measurement of the light absorption were as described earlier.

Determination of EC_{50} of the senary mixtures

The responses (*R*) of the organisms to each concentration of the senary mixtures were calculated relative to the mean control using Eq. 1 as described earlier. Subsequently, the EC_{50}s of the mixtures were calculated by fitting the concentration-response values into 2-parameter logistic function (Eq. 2), as described earlier.

Prediction of mixture toxicities

Concentration addition (CA) and independent action (IA) models were used to predict the toxicities of the mixtures from that of individual toxicants. The CA model assumes that the components of the mixture acts similarly against the test organism, as expressed below (Liu *et al.*, 2017).

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

In this equation, $EC_{x(mix)}$ stands for the senary mixtures' total concentration that leads to *x*% reduction in dehydrogenase activity, EC_{xi} is the *i*th component concentration which engenders *x%* reduction in dehydrogenase activity, when assayed as individual toxicant, *n* represents toxicant number in the mixture, π_i is the relative proportion of *i*th component in the mixture.

Based on Eq. 3, concentrations of the mixture that caused 1-99% inhibitions were predicted according to Nweke *et al.* (2018). The equation was also applied in determining the mixtures' EC_{50} based on CA model, according to the EC_{50} of the individual components and their relative proportions.

On the contrary, IA model assumes dissimilarity in action by the components of a given mixture, as expressed in eq. 4 below (Backhaus *et al.*, 2010).

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

In Eq. (4), $E(C_{mix})$ represents the total predicted inhibition of dehydrogenase activity (scaled from 0 to 1) caused by the total concentration (C_{mix}), of the components in the mixture, *n* is the number of mixture components, c_i represents the *i*th component concentration and $E(c_i)$ is the inhibition by c_i concentration of the individual component. This $E(c_i)$ is determined by the concentration-response association of individual components, by the substitution of Eq. 2 into Eq. 4, hence simplified IA model is formed as expressed in Eq. 5 (Nweke *et al.*, 2018).

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

In Eq. (5), *x* represents the total concentration of the mixture while $\pi_i x$ stand for the *i*th components concentration in the mixture. The EC_{50i} and b_i as calculated from equation 2 for SDS and each metal ion are substituted into Eq. 5. The predicted inhibitions [$E(C_{mix})$] by the mixture for a total concentration (C_{mix}) ranging from 0.02 to 4.6mm were calculated from Eq. 5, using Microsoft Excel 2003. Line graph was used to represent the resulting concentration-inhibition which gives a visual of the concentration-response curve predicted from IA model (Nweke *et al.*, 2018). Eq. 5 was simulated in Microsoft Excel 2003 to interactively determine the predicted EC_{50} of each mixture which is the value of c_{mix} in every mixture that gives $E(C_{mix})$ of 50%.

Duncan post-hoc test (SPSS 21) was used to compare the EC_{50} (experimental) between the individual toxicants and for the mixture ratios in each mixture. The same test was also used to compare the EC_{50} (experimental) and EC_{50} (predicted) from IA and CA models within each mixture ratio ($P<0.05$).

The toxic index

The Toxic Index (TI) for each mixture was calculated as the sum of toxic units for all mixture components (Eq.7).

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

In Eq. (6), C_i stands for the *i*th component concentration in the mixture and EC_{50i} is the concentration of the *i*th component that elicited 50% decrease in dehydrogenase activity when tested as an individual, *n* is the number of components in the mixture and πi represents the *i*th component proportion in the mixture. The effect of the mixture is interpreted as antagonism when TI is above 1, synergism when TI is below 1 but additive when TI equals 1 (Boillot and Perrodin, 2008).

Model deviation ratios (MDR)

The ratio of EC_{50} (predicted) to EC_{50} (experimental) gives the model deviation ratio (MDR) and is as expressed in Eq. 7 below. The effect of the mixture is interpreted as antagonism or synergism if MDR < 1 or MDR > 1 respectively. The effect is described as additive if MDR = 1.

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

Results

The responses of *S. marcescens* (Ser EW01) and *A. seifertii* to the effects of the toxicants as individuals were concentrations-dependent (Figs. 1 and 2). The higher the concentration of the toxicants, the more the dehydrogenase activity is hindered; hence more than 95% inhibition was achieved at 1.0 mM Zn^{2+} and Ni^{2+}, 0.5 mM Pb^{2+}, Cd^{2+} and Co^{2+} and 8 mM SDS (*S. marcescens* (SerEW01)), as well as 0.4 mM for Pb^{2+}, 0.0 5 mM for Co^{2+}, 0.08 mM for Cd^{2+}, 1 mM for Zn^{2+} and 10 mM for SDS (*A. seifertii*). Figs. 3 and 4 show the dose-response association (experimental) of the senary mixture and the CA and IA predictions for both organisms respectively. The experimental data were highly underestimated by the models and the mixtures equally inhibited the dehydrogenase activity even at low concentrations in both bacteria (Fig. 3).

In Fig. 4, at low concentration, slightly higher toxicities and at higher concentration, low toxicities, were predicted by both models relative to the experimentally-derived data, in ABCR2 and ABCR3 mixture ratios, against the sediment bacterium, *A. seifertii*.

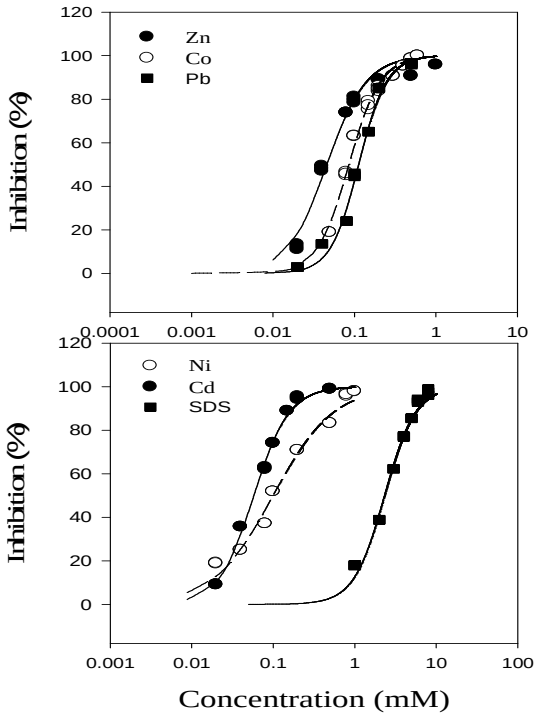

Fig. 1. Inhibition of dehydrogenase activity in *S. marcescens* (SerEW01) by the individual toxicants.

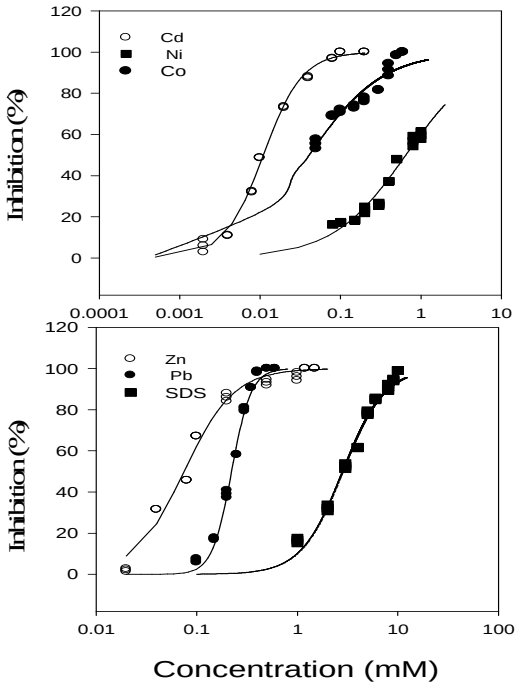

Fig. 2. Inhibition of dehydrogenase activity in *A. seifertii* by the individual toxicants.

Table 2 displays the toxicity threshold EC_{50} (experimental and predicted) for individual toxicants and the senary mixture for both organisms. In planktonic bacterium (*S. marcescens* (SerEW01)), Zn^{2+} was more toxic than other metal ions (0.046 ±0.003 mM) while Pb^{2+} was the least (0.113 ± 0.005 mM). In the case of sediment bacterium (*A. seifertii*), Cd^{2+} with EC_{50} of 0.011 ± 0.00 mM has the highest toxicity, while Ni^{2+} with EC_{50} of 0.649 ± 0.053 mM was the least toxic metal ion. SDS was more toxic to *S. marcescens* (SerEW01) (2.329 ± 0.092 mM) than *A. seifertii* (2.810 ± 0.140 mM).

In addition, *S. marcescens* (SerEW01) was generally more sensitive to the individual toxicants than *A. seifertii*, except for Cd^{2+} and Co^{2+}.

Table 2. Experimental and predicted toxicity thresholds (EC_{50}) of the individual and senary mixtures on the isolates.

Toxicant and mixtures	EC_{50} (mM)‡					
	Planktonic bacterium (*S. marcescens*)			Sediment bacterium (*A. seifertii*)		
	Experimental†	CA-Predicted	IA-Predicted	Experimental†	CA-Predicted	IA-Predicted
Singles						
Cd^{2+}	0.058 ± 0.002a	-	-	0.011 ± 0.000a	-	-
Zn^{2+}	0.046 ± 0.003b	-	-	0.075 ± 0.005b	-	-
Pb^{2+}	0.113 ± 0.005c	-	-	0.222 ± 0.005c	-	-
Co^{2+}	0.086 ± 0.002d	-	-	0.041 ± 0.008d	-	-
Ni^{2+}	0.100 ± 0.008e	-	-	0.649 ± 0.053e	-	-
SDS	2.329 ± 0.092	-	-	2.810 ± 0.140	-	-
SDS+5metals						
EECR 50	0.056±0.002a*	0.614±0.137**	0.902±0.034***	0.067±0.002a*	0.399±0.024**	0.500±0.056***
ABCR 1	0.910±0.003b*	0.416±0.023**	0.736±0.019***	0.142±0.008b*	0.421±0.041**	0.551±0.009***
ABCR 2	0.072±0.003a*	0.401±0.023**	0.729±0.053***	0.170±0.003c*	0.322±0.024**	0.397±0.059***
ABCR 3	0.053±0.003a*	0.389±0.019**	0.746±0.031***	0.163±0.004c*	0.237±0.018**	0.277±0.154***

Within column, among the individual toxicants, EC_{50}s with different letters differed significantly from one another

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

‡ Within rows, in each senary mixture ratio, comparing the experimental EC_{50}, CA-predicted *EC50* 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

The experimentally-derived EC_{50}s in senary mixture ranged from 0.053 ± 0.003mm for ABCR3 to 0.910 ± 0.003 mM for ABCR1 mixture ratios against *S. marcescens* (SerEW01). In addition, ABCR1 mixture ratio was statistically different from the other mixture ratios. Equi-effect (EECR 50) mixture ratio has the highest toxicity against *A. seifertii* (0.067 ± 0.002 mM), while ABCR 2 showed least toxicity (0.170 ± 0.003 mM). Furthermore, there is significant difference between the mixture ratio and the rest.

However, the predicted CA and IA EC_{50}s in all mixtures ratios were significantly different from each other in both organisms at $p < 0.05$.

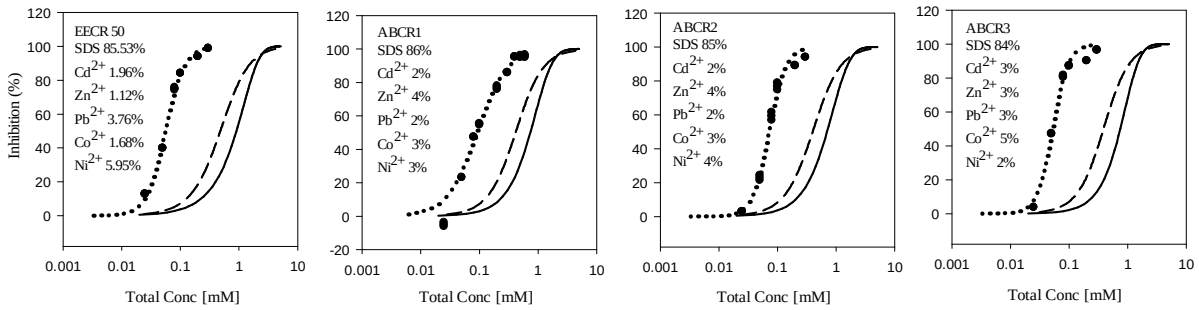

Fig. 3. Experimental and predicted toxicities of senary mixtures of SDS and five metals on *S. marcescens* (SerEW01) dehydrogenase activity. Data points represent experimental dose-response data while dotted lines represent toxicities derived by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from CA and IA models.

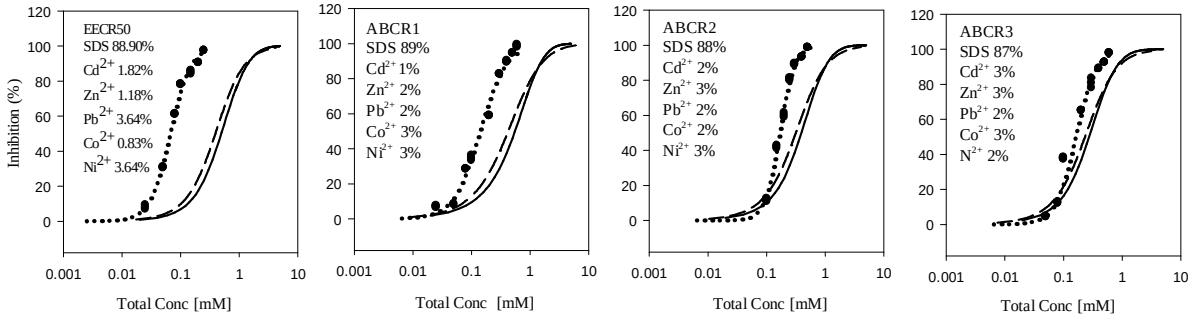

Fig. 4. Experimental and predicted toxicities of senary mixtures of SDS and five metals on *A. seifertii* dehydrogenase activity. Data points represent experimental dose-response data while dotted lines represent toxicities derived by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from CA and IA models.

The toxic index and model deviation ratios for the senary mixtures for both organisms are shown in Table 3. Against *S. marcescens* (SerEW01), the toxic index (TI) values ranged from 0.115 ± 0.003 to 0.219 ± 0.004, while MDR predicted on the basis of CA and IA models ranged from 4.551 ± 0.082 to 10.996 ± 2.198 and 8.068 ± 0.517 to16.216 ± 1.042 respectively. *A. seifertii* however recorded TI values that ranged from 0.168 ± 0.005 to 0.691 ± 0.033 and MDA ranged of 1.450 ± 0.070 to 5.955 ± 0.191 and 1.694 ± 0.074 to 7.467 ± 0.384 respectively, for CA and IA models' predictions. In all the mixture ratios evaluated, the action of heavy metals and SDS on the bacteria were synergistic.

Table 3. Toxic index and model deviation ratios based on EC_{50} thresholds.

	Planktonic bacterium (*S. marcescens*)			Sediment bacterium (*A. seifertii*)		
		MDR* Based on			MDR* Based on	
Mixture ratio	Toxic Index*	CA	IA	Toxic Index*	CA	IA
EECR 50	0.115 ± 0.003	10.996 ± 2.198	16.216 ± 1.042	0.168 ± 0.005	5.955 ± 0.191	7.467 ± 0.384
ABCR 1	0.219 ± 0.004	04.551 ± 0.082	08.068 ± 0.517	0.33 3± 0.015	3.011 ± 0.133	3.899 ± 0.278
ABCR 2	0.180 ± 0.003	05.560 ± 0.088	10.127 ± 0.332	0.531 ± 0.028	1.888 ± 0.101	2.329 ± 0.113
ABCR 3	0.135 ± 0.001	07.388 ± 0.058	14.211 ± 1.271	0.691 ± 0.033	1.450 ± 0.070	1.694 ± 0.074

+ Values are reported as Mean ± 1 SD

*All mixture ratios were synergistic to both bacteria according to TI and MDR values

Discussion

Studies on SDS toxicities on bacteria using dehydrogenase activity reduction as a response are limited in literature. However, its toxicities using other responses have been reported. For instance, an EC_{50} of 2.6mg/l SDS (≈ 9.02 x 10^{-3} mM SDS), was reported against *Vibrio fischeri* by Mariani *et al.* (2006), in a study involving organisms of different taxonomic levels. Similarly, EC_{50}s of 5.00 ± 2.95 x 10^{-6} mol l^{-1} (0.005 mM) and 3.33 ± 0.96 x 10^{-4} mol l^{-1} (0.33 mM) SDS were also reported to hinder cell growth and phosphorus absorption in *Acinetobacter junii* respectively (Hrenovic and Ivankovic, 2007). In the present study, however, the planktonic bacterium *S. marcescens* (SerEW01) was relatively more sensitive to SDS than the sediment bacterium *A. seifertii*. The differences in toxicities observed in SDS against these bacteria may be due to differences in their physiology and genetic makeups. A given surfactant can provoke multiple effects in an organism or at a given concentration, cause different levels of toxicities on different organisms (Li, 2008; Masakorala *et al.*, 2011). SDS is known to exert its toxicity primarily on membrane structures, it also induces lipid peroxidation, increase in production of glutathione and changes in carbon metabolism (Nickerson and Aspedon, 1992; Singer and Tjeerdema, 1993; Bindu and Babu, 2001). Heavy metals are known to render bacteria cells inactive by altering the proteins and nucleic acid structures and

forming complexes with the protein molecules (Bong *et al.*, 2010). In the present study, *S. marcescens* (SerEW01) was more sensitive to most of the heavy metals tested than *A. seifertii*, except for cadmium and cobalt. This observation however is in line with the numerous reports that planktonic bacteria are more sensitive to aquatic pollution than their sediment counterparts as reported elsewhere. The observed higher sensitivity of the sediment bacterium (*A. seifertii*) to Co^{2+} and Cd^{2+} ions compared to the planktonic bacterium (*S. marcescens* (SerEW01)) as recorded in this study is surprising. However, high toxicity of Cd^{2+} to sediment bacterial population of New Calabar River, with an EC_{50} < 0.2mm has been reported (Nweke and Orji, 2009). Furthermore, Nwagwu *et al.* (2017), also reported high heavy metal tolerance by *Serratia* species. High toxicity of Cd^{2+} to *Phosphobacterium phosphoreum* was reported by Zeb *et al.*, (2016), with an EC_{50} of 0.537mg/l (≈ 0.002 mM) as against 0.058 ± 0.002 mM and 0.011 ± 0.00 mM recorded in this study for the planktonic and sediment bacteria respectively.

At high concentration, trace elements such as Zn, Co and Ni can be very toxic and their adverse effects on microorganisms are widely recorded (Kelly *et al.*, 2003; Hashida and Inouye, 2007; Gikas, 2008). Co^{2+} ions were however more toxic to both bacteria than Ni^{2+} ions as recorded in this study. Similar results have been reported elsewhere (Gikas, 2007; Nweke *et al.*, 2018). Zn^{2+} ions have been reported to inhibit respiratory electron transport system in bacteria and eukaryotic organisms (Beard *et al.*, 1995; Nweke and Orji, 2009). In this study, planktonic and sediment bacterial dehydrogenase activity was inhibited by Zn^{2+} ions, with EC_{50}s of 0.046 ± 0.003 mM and 0.075 ± 0.005 mM respectively. *S. marcescens* (SerEW01) was highly sensitive to the effects of Zn^{2+} ions. The high toxicity of Zn^{2+} to this bacterium compared to Cd^{2+}, as well as its relative tolerance to Pb^{2+} as recorded in this study is not quite understood. Nevertheless, *S. marcescens* high tolerance to Cd and Pb has been reported by Cristani *et al.* (2011). In addition, there is a 50% inhibition of dehydrogenase activity by Zn^{2+} ions in *Bacillus* and *Micrococcus* species (sediment bacteria) from New Calabar River at 0.166 and 0.873 mM, respectively (Nweke *et al.*, 2007). Similarly, EC_{50} range of 0.236 ± 0.044 to 0.864 ± 0.138 mM for Zn^{2+} was reported for planktonic bacteria of New Calabar River by Nweke *et al.* (2006). In senary mixtures, the consistency in the higher tolerance of sediment bacterium against the planktonic bacterium to the toxic effects of mixtures of the toxicants was still observed. Similarly, the toxicities of the senary mixtures against both planktonic and sediment bacteria were higher than those of the quinary mixtures of the toxicants. This is reflected in their observed toxicity thresholds (EC_{50}s) (data not shown). This observation seemly contradicts the reported decreases in mixture toxicity with the increasing complexity of the mixtures. The toxic index and model deviation ratios in the senary mixtures showed strong synergistic interactions against both organisms, except in ABCR3 mixture ratio for *A. seifertii* that showed minimal synergy. In a study on the synergistic toxicity of the multiple chemical mixtures, Chen *et al.* (2015), reported all the six-component mixtures of toxicants to be strongly synergistic on earthworm. Similarly, synergistic interactions were reported in senary mixtures of heavy metals and 2-chlorophenols as well as heavy metals and phenol against *Pseudomonas fluorescens* isolated from the soil (Nweke *et al.*, 2020).

The above finding gives support to the declaration that the more complex the mixture, the more significant the synergistic effects (Chen *et al.*, 2015). However, the 96-hour acute toxicity of equi-toxic mixture of Pb+Cd+Hg+Cu+Zn+Ni was reported to be antagonistic against estuarine mysid (Verslycke *et al.*, 2003). It is important to note that this mixture had similar but not exactly the same mixture components as our study. Thus these variations in mixture components and organisms could partly account for the different effects observed.

The joint action of the senary mixtures of SDS and metal ions to both organisms were predicted using CA and IA models. The CA model predicted significantly lower toxicities in the mixture than the IA model

against the planktonic bacterium. This observation corroborated the report by Nweke *et al.* (2018) but contradicts the report by Nweke *et al.* (2020). Similarly, in all senary mixtures, the joint effects were greatly underestimated by both CA and IA-models for *S. marcescens* (SerEW01). However, for *A. seifertii,* in ABCR2 and ABCR3 mixture ratios, toxicity was over estimated at low concentration but underestimated at high concentration, by both models. Over estimation of the effects of mixtures toxicities at high concentrations against bacteria has however been reported by Nweke *et al.* (2020). Furthermore, the CA model correctly estimated the toxicities of the mixture at some points slightly above the underestimation region against the sediment bacterium in ABCR1 and ABCR2 mixture ratios. Thus, the CA model made a better prediction of the senary mixtures of metal ions and SDS against *A. seifertii,* at low concentration. This could be attributed to the similarities in the mode of actions of some of the components in the mixture.

Conclusion

The synergistic toxicities of the senary mixtures of SDS and metal ions (Cd^{2+}, Zn^{2+}, Pb^{2+}, Co^{2+} and Ni^{2+}) against *S. marcescens* (SerEW01) and *A. seifertii* isolated from Otamiri river and sediment were compared with the hindrance of dehydrogenase activity as the end point. Fixed ratio design involving EC_{50} equi-effect concentration ratio (EECR 50) and arbitrary concentration ratios (ABCR) were employed for the study. The result showed that the CA model made a better prediction of the toxicities of the senary mixtures against the organisms and that the mixtures were synergistic against both planktonic and sediment bacteria. These indicate the potential hazard to aquatic bacteria resulting from co-contamination of aquatic ecosystems by these toxicants.

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