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Predicting the Toxicities of Ternary Mixtures of two Metals and Sodium Dodecyl Sulfate to *Serratia marcescens* (SerEW01) from Otamiri River Water

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Authors' contributions

This work was carried out in collaboration among all authors. Author EIC conceptualized, designed and supervised the research work. Author RNO carried out the experiments in the laboratory and wrote the draft copy of the manuscript. Author CON carried out curve fitting and other statistical analyses. All the authors were involved in the revision of the draft manuscript and approved the final version for submission.

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ABSTRACT

Background: Otamiri river server as a source of water for domestic activities, urban farming, recreation, aquatic foods in Owerri and environs. It also receives untreated domestic, industrial and agricultural waste water and run offs from the municipality. Seepages from solid wastes dumps at the river banks and sand mining activity going on in the river could also constitute environmental hazards

Aims: This study aims at evaluating the interactive effects of the ternary mixtures of sodium dodecyl sulfate (SDS) and some divalent metals on preponderant bacterium (*Serratia marcescens* (SerEW01)) from the river.

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Study Design: Fixed ratio ray design was used for the study, with inhibition of dehydrogenase activity as end point.
Place and Duration of Study: Owerri, Imo State, Nigeria, June – December, 2019.
Methodology: The bacterium was earlier isolated as the preponderant bacterium isolate from the river water. Fixed ratio ternary mixtures (Equieffect concentration (EEC50) and arbitrary concentration (ABCR) ratios), SDS + Pb + Zn, SDS + Cd +Zn, SDS + Pb +Ni, SDS + Ni + Cd, SDS + Co + Pb and SDS + Co + Cd were designed to evaluate the combined toxicities of these toxicants. Toxicities predicted by concentration addition (CA) and independent action (IA) models were compared with the experimentally observed toxicities.
Results: The EC_{50S} observed ranged from 0.046 ± 0.003 mM (Zn) to 2.329 ± 0.092 mM (SDS). The EC_{50S} of the toxicants were statistically different from each other ($P<0.05$). The order of increasing toxicities were SDS >Pb >Ni > Co > Cd(II) >Zn. Concentration-dependent toxicities with progressive inhibition of the dehydrogenase activity as the concentration increased were observed.. In all ternary mixtures, both the experimentally derived, CA and IA-predicted EC_{50S} were statistically different from each other. Both models predicted lower toxicities compared to the experimental data. The Toxic Index and Model Deviation Ratio indicated synergistic interaction of SDS and metal ions against *S. marcescens* (SerEW01)
Conclusion: This study could constitute base line information towards assessing the possible environmental hazards associated with co-contamination of the environment by SDS and divalent heavy metals, more so when both pollutants are common aquatic pollutants.

Keywords: Toxicities; divalent metal ions; dehydrogenase assay; concentration addition; independent action; ternary mixtures.

1. INTRODUCTION

Surface water pollution naturally results from the presence of various substances whose harmful effects results from a complex of interactions in which environmental and physico-chemical parameters exert an essential modulating effect [1-3]. According to Kumar et al. [4], sodium dodecyl sulfate (SDS) is an alcohol detergent, derivative of alcohol sulfates, with molecular formula and weight as $C_{12}H_{25}NaO_4S$ or $CH_3{=}(CH_2)_{11}\text{-O-}SO_3\text{-}Na^+$ and 288.38 g/ mol respectively. SDS has several applications, ranging from molecular to biochemical researches involving electrophoresis. It is equally part of household, kitchen and laundry detergents and may be harmful to living organisms. Though hitherto regarded to be environmental friendly owing to its ease of biodegradation and low bioaccumulative tendency [5], SDS has however been reported to be toxic in acute exposure (e.g., 19.040 μg/l for *Utterbackiaimbecillis*). Currently, SDS is neither listed as a ground water contaminant nor checked in water systems, unlike other surfactants with similar uses [6-8]. Due to its rapid acting, broad based and reliable toxic effect, SDS is usually used as a reference toxicant in toxicity assays [9]. According to Fergusson [10], heavy metals are the inorganic metals with five times the specific gravity of water. They are wide spread pollutants of much concern owing to their non-biodegradability and persistent nature [11]. Though heavy metals occur naturally, many environmental contaminations however are due to human activities like mining and smelting activities, industrial productions, as well as domestic and agricultural purposes [12].

On a daily basis, living organisms are seldom exposed to single stressors, rather to a mixture of diverse stressors; either simultaneously, consecutively, or both [13-15]. The toxicity of chemical compounds on aquatic organisms depends on concentration in both sediments and the water, as well as in processes related to their dissociation. Bioaccumulation, biodegradation, desorption and solubilization processes that occur in these environmental compartments determine the amount of dissociated compounds that will attain toxic levels in the organs of aquatic organisms [16]. Otamiri River is one of the two major rivers in Owerri urban and environs. It serves as a source of water for drinking, domestic activities, urban farming, recreation, aquatic foods among others. It also receives untreated domestic, industrial and agricultural waste water and run offs from the municipality. Seepages from solid wastes dumps at the banks of the river and sand mining activity going on in the river could also constitute environmental hazards. This study therefore aims at evaluating the interactive effects of the ternary mixtures of SDS and some divalent metals on preponderant bacterium from the river, using fixed ratio ray design.

2. MATERIALS AND METHODS

2.1 Reagents and Test Bacterium

Salts of heavy metals including $CdSO_4.8H_2O$, $Pb(NO_3)_2$, $ZnNO_3.6H_2O$, $CoCl_2$, and $NiSO_4.6H_2O$ were used as sources of the heavy metal ions, Cd, Pb, Zn, Co and Ni ions respectively. These salts, SDS and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (Germany). The deionized distilled water used in the preparation of reagents was sterilized by autoclaving and the stock reagents by membrane filtration. The more numerous *Serratia marcescens* isolated from Otamiri river water [17], the identity of which was confirmed using 16S rRNA partial gene sequencing, was used as test organism.

2.2 Preparation of Inoculum

S. marcescens cells were cultured in nutrient broth (Lab M) on a rotary incubator (150 rpm) at room temperature (28 ± 2°C) for 16 hours. The cells were harvested from the culture by centrifugation (3000 rpm, 15 minutes, Newlife Centrifuge, NL80-2). The harvested cell pellet was washed in sterile deionized water by repeated centrifugation (x3) and suspended in sterile deionized water [12]. The optical density of the cell suspension was diluted to contain 1.1×10^8 cell/ml by making reference to McFarland turbidity standards.

2.3 Toxicity Testing of Metal Ions and SDS

Inhibition of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT)-dehydrogenase activity was used to assess the toxicity of metal ions and SDS to *S. marcescens*. In a 2-ml total volume in 15-ml screw-capped culture tubes, the reaction mixture contained nutrient broth (Lab M), MTT, SDS or metal ion and *S. marcescens* inoculum (pH 7.0). Each concentration of metal ion or SDS was prepared in triplicate screw-cap culture tubes. A 0.5 ml of nutrient broth (0.8% w/v), calculated volumes of SDS (50 mM) or heavy metal ion (10 mM) working stock solutions and sterile deionized water (to make up) were added into each tube. Subsequently, 0.1 ml each of aqueous solutions of MTT (0.1% w/v) and *S. marcescens* suspension were added. The final SDS and metal ion concentrations varied from 0.002 mM to 1.5 mM and 1 mM to 10 mM respectively. Control tubes which consisted of the medium without SDS or heavy metals were also set up. The cultures were incubated at ambient temperature (28 ± 2^oC) for 24 hours [12]. The purple-colored MTT-formazan (MTTF) was then extracted with 4 ml n-butanol. The light absorption of the extracts was measured with spectrophotometer at 590 nm.

2.4 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).

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

Where, C_A is the 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 ion. Subsequently, the EC_{50} was calculated by fitting the concentration-responses into 2-parameter logistic function (Eq. 2) using least square non-linear regression technique.

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

Where x is the SDS or metal ion concentration, EC_{50} is SDS or metal ion concentration that elicited 50% inhibition of dehydrogenase activity and b is the slope at EC_{50}.

2.5 Design of Ternary Mixture Ratios

The ternary mixtures (SDS+Pb+Zn, SDS+Cd+Zn, SDS+Pb+Ni, SDS+Ni+Cd, SDS+Co+Pb and SDS+Co+Cd) were designed to contain SDS and metals ions in fixed ratios. In each ternary combination, four mixture ratios including one EC_{50} equieffect concentration ratio which were combined on the basis of the EC_{50} of the components (EECR50) and three mixture ratios that were chosen arbitrarily (ABCR) were investigated. The relative proportions of SDS and heavy metal ions in each ternary combination are shown in Table 1. Each combination was prepared as 10 mM stock solution by mixing requisite volumes of 10 mM solutions of each metal ion and SDS in separate 100-ml Erlenmeyer flasks and then used as a composite mixture during toxicity testing.

2.6 Toxicity Testing of the Ternary Mixtures

The toxicity assay procedure as described for the individual toxicants was adopted. In triplicate 15-ml screw-capped culture tubes, 2-ml reaction mixture containing nutrient broth, MTT, bacterial inoculum and the three toxicants (SDS and two metal ions) were prepared (pH 7). Into each tube, 0.5 ml of 0.8 % w/v nutrient broth, required volume of the composite mixture and sterile deionized distilled water (to make up) were added. Then, 0.1 ml each of 0.1% MTT solution and *S. marcescens* suspension were added to obtain graded total concentrations of the ternary mixtures. The final concentrations of the ternary mixtures ranged from 0.02 mM to 3.0 mM. The controls consisted of the medium without SDS and heavy metals. Incubation of the cultures, extraction of MTT-formazan (MTTF) and the measurement of the light absorption was done as described previously.

2.7 Determination of EC_{50} of the Ternary Mixtures

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

2.8 Prediction of Mixture Toxicities

The toxicities of the mixture were predicted from the toxicities of the individual toxicants using concentration addition (CA) and independent action (IA) models. The CA model assumes that the components of the mixture acts similarly against the test organism [18]. It is expressed as shown in Eq. 3, where $EC_{x(\mathrm{mix})}$ represents the total concentration of the ternary mixture that caused *x*% inhibition of dehydrogenase activity, EC_{xi} is the concentration of *i*th component that caused *x%* inhibition of dehydrogenase activity when tested as a single toxicant, *n* is the number of toxicants in the mixture, π_i is the relative proportion of *i*th component in the mixture.

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

On the basis of Eq. 3, concentrations of the mixture that caused 1-99% inhibitions were predicted as described [12]. The EC_{50} of the mixtures based on CA model were determined using Eq. 3 based on the relative proportion and EC_{50} of the individual component.

Table 1. Ternary mixtures of two divalent metals and SDS

Mixture	Mixture ratios (%)								
	SDS +Pb(II) +Zn(II)			SDS +Cd(II) +Zn(II)			SDS +Pb(II) +Ni(II)		
	SDS	Pb	Zn	SDS	Cd	Zn	SDS	Pb	NI
EECR50	94.60	4.16	1.24	96.50	2.20	1.30	89.80	3.95	6.25
ABCR1	95	4	1	96	2	2	90	4	6
ABCR2	93	5	2	94	2	4	88	5	7
ABCR3	94	2	4	93	3	4	87	2	11
Mixture	**Mixture ratios (%)**								
	SDS +Ni(II) +Cd(II)			**SDS +Co(II) +Pb(II)**			**SDS +Co(II) +Cd(II)**		
	SDS	**Ni**	**Cd**	**SDS**	**Co**	**Pb**	**SDS**	**Co**	**Cd**
EECR50	91.53	6.37	2.10	94.02	1.84	4.14	95.92	1.88	2.20
ABCR1	92	6	2	94	4	2	94	3	3
ABCR2	90	7	3	93	4	3	95	2	3
ABCR3	93	5	2	95	3	2	96	2	2

The independent action (IA) model (Eq. 4) assumed that the components of a given mixture act dissimilarly [19].

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

Where $E(c_{mix})$ is the predicted total 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 is the concentration of the *i*th component and $E(c_i)$ is the inhibition by c_i concentration of the individual component. The concentration-response relationships of the individual component were used to determine their response $E(c_i)$, by substituting Eq 2 (scaled 0 to 1) into Eq. 4 for each toxicant to yield Eq 5:

$$E(c_i) = \frac{1}{1 + \left(\frac{\pi_i x}{EC_{50i}}\right)^{bi}} \qquad (5)$$

Thus, the IA model is simplified as shown in Eq. 6 [12].

$$E(C_{mix}) = \left[1 - \prod_{i=1}^{n}\left\{1 - \frac{1}{1 + \left(\frac{\pi_i x}{EC_{50i}}\right)^{bi}}\right\}\right] \times 100 \qquad (6)$$

Where, *x* is the total concentration of the mixture, $\pi_i x$ is the concentration of *i*th component in the mixture. The EC_{50i} and b_i as calculated from Eq. 2 for SDS and each metal ion are substituted into Eq. 6. The predicted inhibitions [$E(C_{mix})$] by the mixture for a total concentration (C_{mix}) ranging from 0.02 to 4 mM were calculated from Eq. 6 using Microsoft Excel 2003. The resulting concentration-inhibition data were plotted as a line graph to give a visualization of the concentration-response curve predicted from the IA model [12].

Eq. 6 which was simulated in Microsoft Excel 2003 was used 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%.

The experimentally-observed EC_{50}s for individual toxicants and for the various mixtures ratios in each mixture were compared. Similarly, within each mixture ratio, the experimentally-observed EC_{50}s were also compared with EC_{50}s predicted from CA and IA models using Duncan post-hoc tests in SPSS Statistics 21.

2.9 The Toxic Index

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

$$TI = \sum_{i=1}^{n}\frac{C_i}{EC_{50i}} = \sum_{i=1}^{n}\frac{\pi_i EC_{50mix}}{EC_{50i}} \qquad (7)$$

Where C_i is the concentration of the *i*th component 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 π*i* is the proportion of *i*th component in the mixture. The effect of the mixture is interpreted as antagonism or synergism if TI is greater than 1 or less than 1 respectively. The effect is described as additive if TI equals 1 [20].

2.10 Model Deviation Ratios (MDR)

The model deviation ratios (MDR) were calculated as the ratio of the predicted EC_{50} to the experimentally-derived EC_{50} (Eq. 8). The effect of the mixture is interpreted as antagonism if MDR is less than 1 while MDR greater than 1 indicated that the mixture is synergistic. The effect is described as additive if MDR equals 1.

$$\mathrm{MDR} = \frac{\text{Predicted } EC_{50}}{\text{Experimental } EC_{50}} \qquad (8)$$

3. RESULTS

3.1 Toxicity of Individual Toxicants and Ternary Mixtures

Fig. 1 showed the effects of metal ions and SDS on *S. marcescens* dehydrogenase activity. The toxicities of the stressors were concentration-dependent, increasing progressively as the concentration increases to produce sigmoidal relationships. Inhibitions greater than 95% occurred at 1.0 mM Zn and Ni, 0.5 mM Pb, Cd and Co and 8 mM SDS. The experimental EC_{50} values indicated that the toxicities of divalent metal ions and SDS differed significantly from each other (Table 2). The experimental concentration-response relationships of the ternary mixtures as well as the predictions made from CA and IA models for *S. marcescens* (SerEW01) are shown in Figs. 2-7. The toxicities were dependent on the total concentrations of the stressors giving rise to sigmoidal concentration-response relationships. In all the ternary mixtures, CA and IA models greatly predicted lower toxicities than the experiment would suggest.

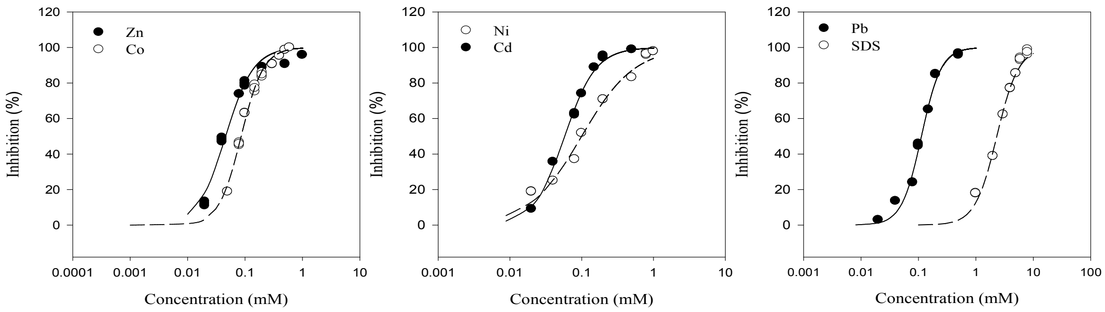

Fig. 1. Inhibition of dehydrogenase activity of *S. marcescens* (SerEW01) by the individual toxicant. The solid and dash lines are respective predicted toxicities

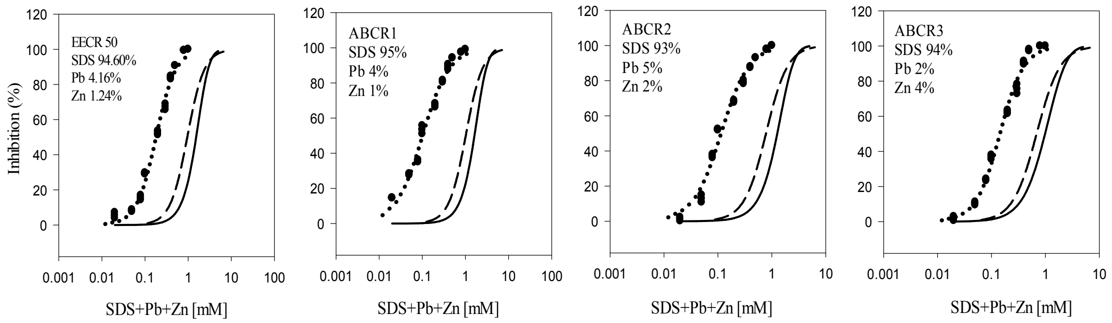

Fig. 2. Experimental and predicted inhibitory effects of ternary mixtures of SDS, lead and zinc ions on *S. marcescens* (SerEW01) dehydrogenase activity. The data points represent experimental dose-response data while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted based on the CA and IA model respectively

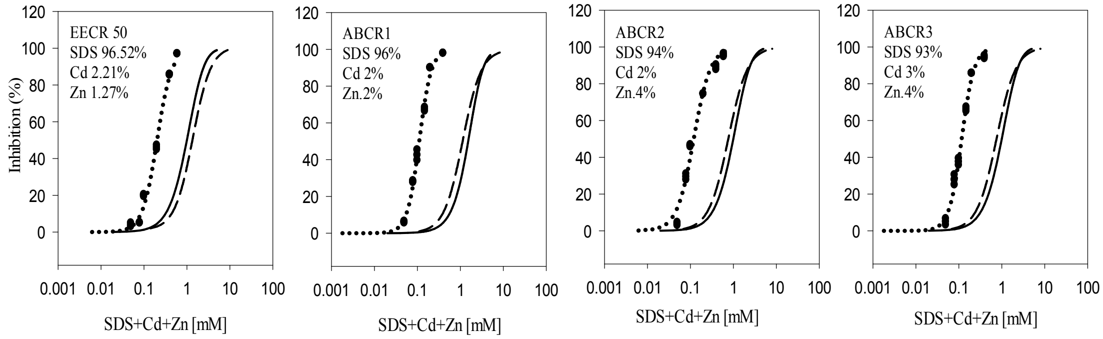

Fig. 3. Experimental and predicted inhibitory effects of ternary mixtures of SDS, cadmium and zinc ions on *S. marcescens* (SerEW01) dehydrogenase activity. The data points represent experimental dose-response data while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted based on the CA and IA model respectively

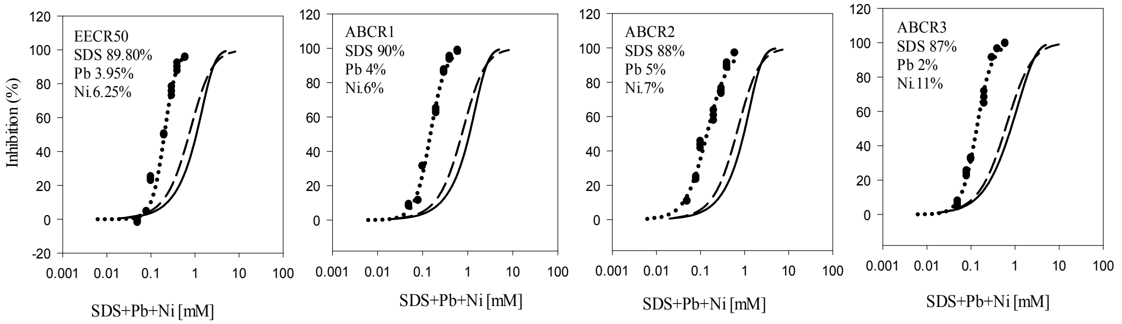

Fig. 4. Experimental and predicted inhibitions of ternary mixtures of SDS, lead and nickel ions on *S. marcescens* (SerEW01) dehydrogenase activity. The data points represent experimental dose-response data while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted based on the CA and IA model respectively.

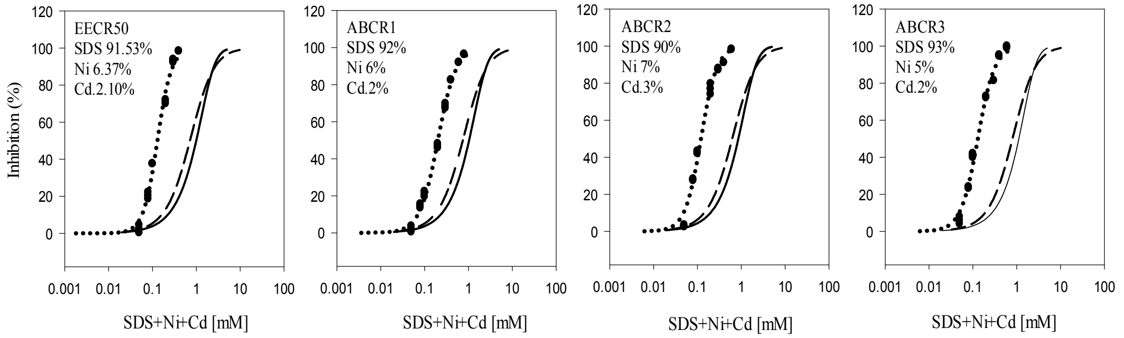

Fig. 5. Experimental and predicted inhibitory effects of ternary mixtures of SDS, nickel and cadmium ions on *S. marcescens* (SerEW01) dehydrogenase activity. The data points represent experimental dose-response data while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted based on the CA and IA model respectively.

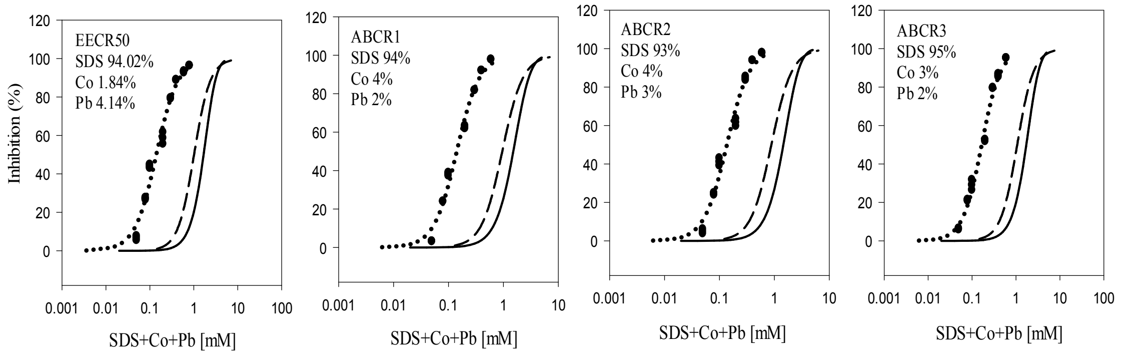

Fig. 6. Experimental and predicted inhibitory effects of ternary mixtures of SDS, cobalt and lead ions on *S. marcescens* (SerEW01) dehydrogenase activity. The data points represent experimental dose-response data while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted based on the CA and IA model respectively.

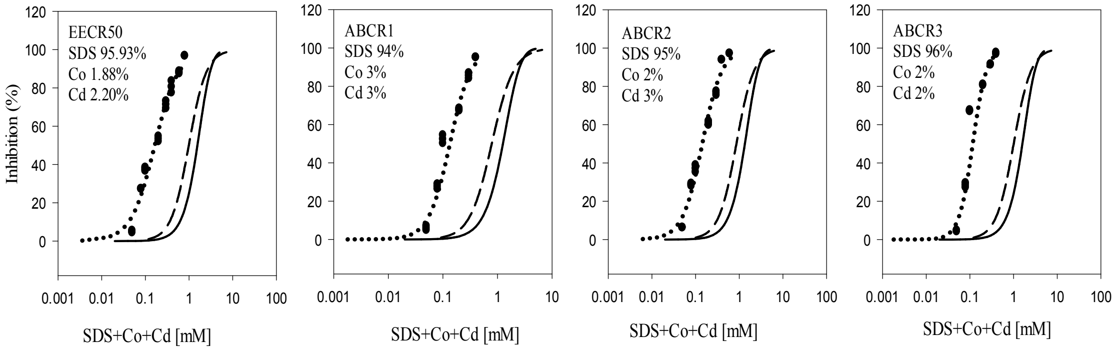

Fig. 7. Experimental and predicted inhibitory effects of ternary mixtures of SDS, cobalt and cadmium ions on *S. marcescens* (SerEW01) dehydrogenase activity. The data points represent experimental dose-response data while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted based on the CA and IA model respectively

Table 2 shows the median inhibitory concentrations (EC_{50}) of the ternary mixtures as derived from the experiments and as predicted from CA and IA models on the basis toxicity of individual metal ion and SDS. Also shown are the statistical relationships among the observed and predicted EC_{50} values. Generally, the EC_{50}s varied from 0.102 ± 0.006 mM in SDS 95% + Pb 4% + Zn 1% mixture to 0.203 ± 0.009 mM in SDS 96.52% + Cd 2.21% + Zn 1.27% mixture. In all ternary mixtures other than SDS + Ni + Cd and SDS + Co + Pb, the EC_{50} equieffect mixture ratio was less toxic to *S. marcescens*. As indicated by the EC_{50} values, the toxicities of arbitrarily-chosen concentration ratios were not significantly different from each other in SDS + Cd + Zn ternary mixture. However, the EC_{50} of equieffect mixture was significantly ($P<0.05$) different from the arbitrary mixture ratios. Similar trend was observed in SDS + Pb + Ni mixture. Also, in SDS + Pb + Ni mixture, toxicity increases as the concentration of nickel increased. In all the ternary mixtures except SDS + Co + Pb mixture, the toxicity (EC_{50}) of equieffect mixtures differed significantly from the toxicity of arbitrary mixture ratios. The observed EC_{50}, CA-predicted EC_{50} and IA-predicted EC_{50} values were significantly different from each other ($P < 0.05$), in all mixture ratios.

The TI, MDR and the interpreted effect of the SDS + metal ions ternary mixtures are shown in Table 3. The TI values ranged from 0.086 ± 0.023 to 0.276 ± 0.010, while model deviation ratio (MDR) ranged from 3.642 ± 0.134 to 10.219 ± 0.353 for CA and from 5.118 ± 0.145 to 15.853 ± 1.281 for IA. In all mixture ratios tested, the metals and SDS ternary mixtures were synergistic in their action against *S. marcescens*.

4. DISCUSSION

In the last few decades, water pollution has become a contemporary issue in both developed and developing countries around the globe. Pollutants such as divalent heavy metals and surfactants of various forms have been reported to contaminate both surface and ground water sources. Divalent metals like nickel, copper, manganese, zinc, cobalt are important elements required in minute quantity for metabolic and redox activities, others such as lead, cadmium, mercury, silver and aluminum don't have biological role and are therefore toxic to microbes [21,22].

Cadmium and lead have been reported to inhibit microbial population and enzymes activity in both aquatic and soil environment. For instance, increasing the concentrations of cadmium and lead in soil were reported to gradually decrease the microbial community and enzymes [23,24]. Similarly, lead was reported to inhibit β-galactosidase activity in *Providencia stuartii, Aeromonas dhakensis and Pantoea dispersa*, with IC_{50S} 0.0007 ± 0.002 mg/l (≈ 3.38 $\times 10^{-6}$ mM), 0.0016 ± 0.030 mg/l (≈ 7.72 $\times 10^{-6}$ mM) and 0.0009 ± 0.012 mg/l (≈ 4.34 $\times 10^{-6}$ mM) respectively [25]. In addition, an IC_{50} range of 0.199 mM to 0.239 mM Cd inhibited bioluminescence in photobacterium Q67 [26]. Furthermore, EC_{50S} of 0.023 ± 0.003 mM Cd and 0.135 ± 0.007 mM Pb were reported to inhibit dehydrogenase activity in *Pseudomonas* *fluorescence* from soil [27]. In the present study however, dehydrogenase activity of S. marcescens (SerEW01) from the river was inhibited by cadmium and lead at the thresholds of 0.058 ± 0.002 mM and 0.113 ± 0.005 mM respectively. The differences observed in the inhibitory thresholds could be attributed to differences in the test bacteria and the responses monitored.

Nickel, cobalt and zinc are required for metabolic activities of microorganisms; they nevertheless can be toxic at high concentrations. Zinc for instance was reported to inhibit *Vibrio fischeri* at

Table 2. Experimentally-observed and predicted toxicity thresholds (EC_{50}) of individual toxicants and the ternary mixtures of divalent metals and SDS on *S. marcescens* (SerEW01)

	EC_{50} (mM)‡ $^+$		
Toxicants and Mixtures	**Experimental†**	**CA-Predicted**	**IA-Predicted**
Ni	0.100 ± 0.008a	-	-
Cd	0.058 ± 0.002b	-	-
Pb	0.113 ± 0.005c	-	-
Zn	0.046 ± 0.003d	-	-
Co	0.086 ± 0.002e	-	-
SDS	2.329 ± 0.092f	-	-
SDS + Pb + Zn Mixtures			
EECR~~50~~	0.181 ± 0.010a*	0.960 ± 0.048**	1.530 ± 0.030***
ABCR1	0.102 ± 0.006b*	1.023 ± 0.050**	1.617 ± 0.030***
ABCR2	0.115 ± 0.007b*	0.785 ± 0.042**	1.261 ± 0.022***
ABCR3	0.144 ± 0.007c*	0.692 ± 0.043**	0.987 ± 0.026***
SDS + Cd +Zn Mixtures			
EECR50	0.203 ± 0.009a*	1.376 ± 0.073**	1.833 ± 0.008***
ABCR1	0.111 ± 0.020b*	1.138 ± 0.065**	1.526 ± 0.014***
ABCR2	0.120 ± 0.009b*	0.768 ± 0.049**	0.999 ± 0.033***
ABCR3	0.117 ± 0.004b*	0.761 ± 0.048**	1.000 ± 0.032***
SDS + Pb +Ni Mixtures			
EECR50	0.203 ± 0.005a*	0.736 ± 0.045**	1.072 ± 0.006***
ABCR1	0.150 ± 0.006b*	0.747 ± 0.046**	1.090 ± 0.007***
ABCR2	0.141 ± 0.010b*	0.659 ± 0.041**	0.961 ± 0.006***
ABCR3	0.135 ± 0.005b*	0.697 ± 0.043**	0.803 ± 0.032***
SDS + Ni + Cd Mixtures			
EECR50	0.130 ± 0.006a*	0.719 ± 0.042**	0.993 ± 0.004***
ABCR1	0.202 ± 0.006b*	0.747 ± 0.044**	1.033 ± 0.004***
ABCR2	0.121 ± 0.006a*	0.624 ± 0.036**	0.856 ± 0.003***
ABCR3	0.130 ± 0.007a*	0.806 ± 0.045**	1.116 ± 0.005***
SDS + Co + Pb Mixtures			
EECR50	0.141 ± 0.010a*	1.017 ± 0.040**	1.679 ± 0040***
ABCR1	0.142 ± 0.007a*	0.958 ± 0.034**	1.517 ± 0.001***
ABCR2	0.137 ± 0.008a*	0.886 ± 0.039**	1.460 ± 0.018***
ABCR3	0.167 ± 0.008b*	1.073 ± 0.039**	1.721 ± 0.012***
SDS + Co + Cd Mixtures			
EECR50	0.165 ± 0.012a*	0.991 ± 0.035**	1.556 ± 0.007***
ABCR1	0.135 ± 0.007b*	0.788 ± 0.027**	1.260 ± 0.011***
ABCR2	0.143 ± 0.009b*	0.864 ± 0.030**	1.346 ± 0.005***
ABCR3)	0.118 ± 0.005c*	1.011 ± 0.036**	1.597 ± 0.009***

Within column, among the individual toxicants, EC_{50} values with different letters are significantly different from each other

†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 ($P < 0.05$).

$^+$ Values are reported as Mean ± 1SD

Table 3. Toxic Index, MDR and Effect of Metals ions +SDS Ternary Mixtures on *S. marcescens* (SerEW01)

		MDR$^+$		Effect
Metal-SDS Mixtures	**Toxic Index (TI)**	**CA**	**IA**	
SDS +Pb +Zn				
EECR50	0.188 ± 0.001	5.312 ± 0.021	8.492 ± 0.608	Synergistic
ABCR1	0.086 ± 0.023	10.000±0.144	15.853±1.287	Synergistic
ABCR2	0.131 ± 0.026	6.811 ± 0.080	10.973±0.898	Synergistic
ABCR3	0.208 ± 0.003	4.807 ± 0.064	6.859 ± 0.156	Synergistic
SDS +Cd +Zn Mixtures				
EECR50	0.218 ± 0.005	6.766 ± 0.041	9.027 ± 0.387	Synergistic
ABCR1	0.133 ± 0.004	10.219±0.353	13.709±0.188	Synergistic
ABCR2	0.194 ± 0.010	6.422 ± 0.068	8.366 ± 0.327	Synergistic
ABCR3	0.208 ± 0.006	6.516 ± 0.218	8.574 ± 0.031	Synergistic
SDS +Pb +Ni Mixtures				
EECR 50	0.276 ± 0.010	3.624 ± 0.134	5.285 ± 0.149	Synergistic
ABCR1	0.201 ± 0.004	4.966 ± 0.089	7.261 ± 0.348	Synergistic
ABCR2	0.214 ± 0.002	4.673 ± 0.044	6.840 ± 0.510	Synergistic
ABCR3	0.223 ± 0.007	4.493 ± 0.149	5.948 ± 0.024	Synergistic
SDS +Ni +Cd Mixtures				
EECR 50	0.181 ± 0.002	5.516 ± 0.051	7.634 ± 0.362	Synergistic
ABCR1	0.271 ± 0.008	3.697 ± 0.106	5.118 ± 0.145	Synergistic
ABCR2	0.194 ± 0.002	5.158 ± 0.042	7.083 ± 0.343	Synergistic
ABCR3	0.161 ± 0.001	6.204 ± 0.042	8.620 ± 0.460	Synergistic
SDS +Co +Pb Mixtures				
EECR 50	0.139 ± 0.004	7.224 ± 0.232	11.952±0.876	Synergistic
ABCR1	0.149 ± 0.003	6.734 ± 0.115	10.683±0.629	Synergistic
ABCR2	0.155 ± 0.004	6.461 ± 0.165	10.667±0.790	Synergistic
ABCR3	0.156 ± 0.002	6.425 ± 0.074	10.323±0.563	Synergistic
SDS +Co +Cd Mixtures				
EECR 50	0.166 ± 0.006	6.029 ± 0.211	9.482 ± 0.704	Synergistic
ABCR1	0.172 ± 0.004	5.844 ± 0.102	9.350 ± 0.563	Synergistic
ABCR2	0.165 ± 0.005	6.052 ± 0.169	9.439 ± 0.626	Synergistic
ABCR3	0.116 ± 0.001	8.597 ± 0.039	13.585±0.589	Synergistic

$^+$ *Values are reported as Mean ± 1SD*

an EC_{50} of 0.86 ± 0.11 mg/l (≈ 0.002 mM) [28]. In a study on marine bacteria: *Providencia stuartii, Aeromonas dhakensis and Pantoea dispersa*, zinc inhibition of β-galactosidase biosynthesis in the three bacteria were at IC_{50S} of 0.0010 ± 0.004 mg/l (≈ 1.53 x10^{-5} mM), 0.0022 ± 0.032 mg/l (≈ 3.37 x10^{-5} mM) and 0.0010 ± 0.014 mg/l (≈ 1.53 x10^{-5} mM) respectively was reported [26]. In the present study, an EC_{50} of 0.046 ± 0.003 mM was recorded for zinc against the preponderant bacterium. A higher EC_{50} of 0.180 mM Zn against *Pseudomonas fluorescens* has been reported [12]. Similarly, Nweke and Orji [29] reported an EC_{50} of 0.91 mM Zn for microbial community of New Calabar River. However, tolerance of *Serratia* to zinc and other heavy metals has been reported elsewhere [30,31].

Cobalt and nickel were equally toxic to the bacterium even at low concentration in this study, with effective concentrations of 0.086 ± 0.002 mM and 0.100 ± 0.008 mM, respectively. A study by Hashida and Inouye [32] showed that increasing cobalt concentrations to 2 mM increased thermolysin activity in *Bacillus thermoproteolyticus* 3 to 4 times. This enhanced enzyme activity however decreased at a higher concentration range of 2-18 mM. Similarly, toxicity thresholds (EC_{50S}) of 0.099 ± 0,006 mM Co and 0.080 ± 0.006 mM Ni were reported against *Pseudomonas fluorescens* [27]. In the present study, *S. marcescens* (SerEW01) was however more sensitive to the effects of cobalt than nickel. Similar observations were made against *Pseudomonas* species [12,33]. To date, there is no information in literature on the toxicity of SDS on bacterial dehydrogenase activity. However, effects of SDS to aquatic biota, using other responses have been reported. For instance, inhibition of *Daphnia magna* acetyl cholinesterase by SDS at equieffect concentration (*EC_{50})* of 51.5 mg/l (≈ 0.18 mM) has been reported [34]. Similarly, an EC_{50} value of 2.6 mg/l SDS (9.02×10^{-3} mM SDS), was reported for *Vibrio fischeri* in a study that involved many taxa [35]. Sodium Dodecyl sulfate (SDS) recorded an EC_{50} of 2.329 ± 0.092 mM in the present study, indicating that *S. marcescens* (SerEW01) was more sensitive to the toxic effect of SDS, though the end points monitored, media composition and the test organisms were different. These could partly explain the observed variations in toxicity thresholds. The order of toxicity ranking for the toxicants was Zn > Cd > Co > Ni > Pb > SDS. This bacterium higher sensitivity to zinc as against cadmium and its relative tolerance to lead could not be explained. High tolerance of *S. marcescens* to lead and cadmium has been reported by [36].

Naturally, aquatic organisms are exposed simultaneously to several organic and inorganic compounds resulting from human agricultural, industrial and domestic activities [37]. In this study, SDS modulated the toxicity of the heavy metals and vice versa, giving EC_{50S} higher (lower toxicities) than those of the individual heavy metals but lower than that of SDS, in all the ternary mixtures tested. This modulation seems to be dependent on the relative proportions of the most toxic and least toxic components present, especially in SDS+Pb+Ni mixture. Similar assertion was made by [38], in a study on the effects of surfactants on the combined toxicity of TiO_2 nanoparticles and cadmium to *Escherichia coli*. According to the study, the toxicities of all the mixtures of nano TiO_2 and Cd with surfactant were generally lower than the toxicity of Cd as single species. In addition, the ternary mixtures were more toxic (lower EC_{50S}) than the binary mixtures of the toxicants (data not shown). Such differences in toxicities between binary and ternary mixtures of same toxicants have been reported [39].

The model deviation ratios (MDR) and the toxic index model (TI) used to analyse the ternary mixture toxicity indicated similar result, with regards to the toxicity of SDS and metal mixtures against the dehydrogenase activity of *S. marcescens* (SerEW01). The TI values obtained for all the ternary mixtures are less than 1, thus describing synergistic interactions [20]. Similarly, the MDR values for all the ternary mixtures of SDS and metal ions in this study are above 2.0 and thus indicate synergistic interactions [40]. Some authors have reported both synergistic and antagonistic interactions in studies with ternary mixtures of different heavy metals to bacteria and algae [12,41,42]. Although the mixtures in those studies had similar components (heavy metals) as the present study, none however had SDS as a component. Similarly, Xu *et al*. [43] reported synergistic interactions in a bioassay study that evaluated the effects of ternary mixtures of Cu+Zn+Pb and Cu+Zn+Cd on sea urchin embryo-larvae. However, additive effect was reported for the ternary mixture of Cd + As + Pb against water flea [44]. It has been reported that the types of interactions exhibited by mixture components is largely dependent on the proportion of each toxicants in the mixtures [45].

Concentration addition and independent action models have been used to predict toxicity of chemical mixtures based on the concentration-response relationship of the components of the mixture elsewhere [12]. In this study, both models were adopted to predict the joint effect of the ternary mixtures. In all mixture ratios tested, the CA and IA models grossly under estimated the toxic interactions of the toxicants against *S. marcescens* (SerEW01). Similarly, both underestimation and overestimation of ternary mixtures toxicity of heavy metal ions to *P. fluorescens* has been reported [12]. In addition, the values of EC_{50} predicted for SDS + Ni + Cd, SDS + Co + Pb and SDS + Co + Cd ternary mixtures by CA model are not far from those predicted by IA model. The ratio of CA-EC_{50} to IA-EC_{50} varied from 0.877 ± 0.047 to 0.915 ± 0.021, 0.728 ± 0.361 to 0.813 ± 0.177 and 0.844 ± 0.088 to 0.906 ± 0.026, with average of 0.889, 0.785 and 0.887, respectively for those ternary mixtures. This indicates that both models may have similar capacity in predicting the toxicity of SDS and metal mixtures.

5. CONCLUSION

Inhibition of dehydrogenase activity was used in assessing the toxic effects of ternary mixtures of SDS and divalent metal ions to *Serratia marcescens* (SerEW01). The results of this work indicated that the SDS and heavy metal ternary mixtures exhibited synergistic interactive effect on the organism. Both CA and IA models predicted lower toxicities compared to the experimental data. This study could constitute base line information towards assessing the possible environmental hazards associated with co-contamination of the environment with sodium dodecyl sulfate and divalent heavy metals, more so when both pollutants are common aquatic pollutants. To gain more insight into the toxic effects of the mixture of these toxicants, it is recommended that this study should be extended to microbial community of soil and aquatic environments.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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