Toxicity of binary mixtures of sodium dodecyl sulfate and heavy metals to *Acinetobacter seifertii* from tropical river sediment

**Original Research Article**

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The toxicities of Sodium Dodecyl Sulfate (SDS) and heavy metal ions (Ni\(^{2+}\), Co\(^{2+}\), Cd\(^{2+}\), Zn\(^{2+}\) and Pb\(^{2+}\)), as individual and SDS + metal ion binary mixtures to *Acinetobacter seifertii* isolated from Otamiri river sediment, Owerri, in southeastern Nigeria were assessed, on the basis of dehydrogenase activity (DHA) inhibition. The responses of the bacterium to the toxic effects of the toxicants were concentration-dependent. The EC\(_{50}\)S observed ranged from 0.011 ± 0.000 mM for Cd\(^{2+}\) to 2.810 ± 0.140 mM for SDS. The EC\(_{50}\)S of the individual toxicants were statistically different from each other. The heavy metals were more toxic than SDS and the decreasing order of toxicity was Cd\(^{2+}\) > Co\(^{2+}\) > Zn\(^{2+}\) > Pb\(^{2+}\) > Ni\(^{2+}\) > SDS. Fixed ratio mixtures based on EC\(_{50}\) equi-effect concentration ratio (EECR50 mixtures) and arbitrary combinations (ABCR mixtures) were designed to assess the combined toxicities of SDS and metals. All the dose-response relationships of the ABCR and EECR50 mixtures and the individual toxicants could be described by logistic function. Toxicities predicted from concentration addition (CA) and independent action (IA) models were statistically different from the experimentally-derived toxicities. In SDS + Ni\(^{2+}\) binary mixture, CA and IA models overestimated the toxicity at low concentrations, while under estimating at high concentrations. Furthermore, both models predicted identical toxicities in SDS + Ni\(^{2+}\) and SDS + Cd\(^{2+}\) mixtures and there was also no statistical difference between CA and IA-predicted EC\(_{50}\)S for the two binary mixtures. In all but ABCR3 mixture ratio of SDS + Cd\(^{2+}\) mixture, both models significantly predicted lower toxicities. SDS + Zn\(^{2+}\) binary mixture was hormetic at low concentrations. The binary mixtures were generally synergistic to *A. seifertii*. This synergistic interaction of SDS and metals ions highlights the environmental implications of SDS and metal ions co-contamination.

**Keywords:** Inhibition, equi-effect concentration, dose-response, models, synergistic interaction, toxicant

**INTRODUCTION**

Sodium Dodecyl Sulfate (SDS), being an anionic surfactant, has been reported to be the most common synthetic organic compound contained in domestic detergents, shampoos, cosmetics, herbicides, as well as dispersants used to clean-up oil-spillage (Cowan-Ellsberry et al. 2014). Contaminated waters, sediments and soils remain the exposure route of SDS to aquatic environments, and this in-turn threatens domestic water supplies and/or organisms...
living in these environments (Singer and Tjeerdema, 1993). SDS is neither monitored in water supplies nor listed as a ground water contaminant presently, unlike other surfactants with similar uses (Singer and Tjeerdema, 1993; Rebello et al., 2014). Another group of contaminants that are discharged into the aquatic environments are the heavy metals. They are of great ecological importance due to their toxic effects and accumulative behavior. Unlike organic pollutants, heavy metals are naturally non-biodegradable and can undergo biogeochemical cycle, in which natural waters are the major pathways.

When heavy metals are discharged into aquatic environments, they associate instantly with particulate matters and are subsequently transferred to the bottom sediments (Hanson et al., 1993). The activity of heavy metals in aquatic ecosystems and their effect on aquatic organisms varies with the metal involved. However, of most significance in this regard is the capacity of trace elements to combine with other dissolved and suspended debris. Among these associations, the most outstanding is the interaction between heavy metals and organic compounds that originated from natural processes like vegetable decay or contamination by organic discharges of domestic and industrial origins with reasonable affinity and ability to associate with metals (Jones, 2010). Microorganisms are very important in ensuring effective and efficient working of all ecosystems; thus factors affecting microbial activities as well as their biodiversity are of great importance. Some researchers have therefore advocated monitoring microbial responses as an early indicator of ecological distress, as microbes are known to respond without delay to environmental stress (Odum, 1985). As a major component of the aquatic ecosystem, sediment provides food, spawning, habitat and breeding area for many aquatic organisms. Hence, protection of the quality of sediment aids in the restoration and monitoring the biological integrity of water (USEPA, 2007).

Otamiri river as the major river that transverses Owerri city and environs, in Imo State, Nigeria, serves for domestic, urban agriculture and industrial purposes. Solid wastes from the listed concerns are dumped and incinerated at the banks of the river. Similarly, leachates from the dumps, untreated sludge and run-offs from the city and environs gain unrestricted access into the river. Recreational and continuous sand dredging activities in the river, together with the afore mentioned may have contributed to the reported contamination of Otamiri river and sediment by heavy metals (Temitope et al., 2016). Detergent degrading bacterial flora has also been isolated from the river (Ogbulie et al., 2010). Recently, anionic surfactants, predominantly sodium dodecyl sulfate (SDS) was reported to be present in Otamiri river water and sediment (Okechi and Chukwura, 2020). Many researchers have established the toxicity of heavy metals and their mixtures to living organisms at low or high concentrations (Kouchou et al., 2017; Nweke et al., 2018). In addition, there were reports on the toxicity of some chemical surfactants by some authors (Yuan et al., 2014; Effendi et al., 2017). However, there is paucity of information on the toxicity of mixtures of SDS and heavy metal to bacteria inhabiting river sediments. Most importantly, the effects of metal and surfactant mixtures on the microbial population of Otamiri river water and sediment have not been studied. This study is therefore aimed at assessing the in vitro impacts of binary mixtures of SDS and some heavy metals detected in Otamiri river sediment, on the dehydrogenase activity (DHA) of preponderant bacterium isolated from the river sediment.

MATERIALS AND METHODS

Sample collection

Sediment samples were collected along the river course, adjacent Nekede motor mechanic village. Two locations were selected for study sampling. The first was 100 meters downstream of where Nworie River intersected Otamiri River (5.465N, 7.035E), while the second was approximately 100 meters downstream of the first location (5.463N, 7.034E). The samples were collected with Eckman grab sampler. They were later pooled together to form a composite sample in clean cellophane bag and immediately taken to Biotechnology department laboratories, Federal University of Technology, Owerri, for analysis.

Isolation of sediment bacterium and culture conditions

One gram (1g) of the sediment sample was placed in 9 ml of sterile water contained in a 100-ml Erlenmeyer flask and shaken for 1 minute. The sediment suspension was allowed to stand for 10 minutes as described by Fawole and Oso, (2004). Ten-fold serial dilution of sediment sample was then carried out. A 0.1 ml aliquot of $10^{-5}$ dilution of the sediment was aseptically inoculated onto duplicate sterile nutrient agar plates, spread with sterile glass rod and incubated at 37°C for 24 hours. The total heterotrophic bacterial counts of the sediment sample were determined by counting the colonies on the plates. The percentage occurrence of each isolates was determined. Discrete colonies were purified on Nutrient agar (NA) plates and stored on agar slants in the refrigerator at 4°C. The isolates obtained were subjected to identification through morphological and biochemical characterizations (Holt et al., 1994). The preponderant bacterial isolate with 42.10% occurrence was subjected to molecular characterization, using 16S rRNA gene partial sequencing. The identity was confirmed as Acinetobacter seifertii. To obtain cell suspension to be used as inoculum for the toxicity assay, the bacterium was grown in nutrient broth (NB) on a rotary incubator (agitation speed, 150 rpm) at a temperature of 28 ± 2°C for 24 h. The cells were harvested by centrifuging for 10 minutes at 3500 rpm (Newlife Centrifuge, NL80-2). The harvested cells were washed twice in sterile deionized water before re-suspension there-in. The cell density was adjusted to $1.1x10^8$ cells per ml according to McFarland turbidity standard.
Binary mixture ratios

The binary mixtures consisted of SDS and each of the five heavy metal (Ni\textsuperscript{2+}, Cd\textsuperscript{2+}, Zn\textsuperscript{2+}, Pb\textsuperscript{2+} and Co\textsuperscript{2+}), used as analytical grades of NiSO\textsubscript{4}·6H\textsubscript{2}O, CdSO\textsubscript{4}·8H\textsubscript{2}O, ZnNO\textsubscript{3}·6H\textsubscript{2}O, Pb(NO\textsubscript{3})\textsubscript{2} and CoCl\textsubscript{2} were ordered from Sigma/Aldrich Corporations, Germany. The binary mixtures were studied using fixed ratio design. In each mixture, at a constant mixture ratio, the total concentration was varied to obtain the complete concentration-response relationship. The mixtures were combined as p (%) SDS and 100-p (%) metal ion (Table 1). The SDS + metal binary mixtures were prepared as 10 mM and 50 mM working stock solutions by combining needed volumes of the heavy metal and SDS stock solutions to produce a particular concentration ratio. The mixtures were studied as if they are single toxicant solution during the toxicity assay.

Toxicity assay of individual heavy metal and SDS

The toxicity assay was carried out as modified from Nweke et al. (2018). The reaction mixtures were studied in 2-ml final volumes of low-strength nutrient broth (NB) amended with graded concentrations of SDS or heavy metals. To every 15-ml screw capped culture tube containing 0.5 ml NB (x 4-strength, pH 7.0), required volumes of sterile deionized water and stock solutions of each heavy metal or SDS were added. The total amount of NB in the reaction mixture was 0.2% w/v. Thereafter, 0.1 ml of 0.1% aqueous solution of MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-Tetrazolium Bromide) (Sigma chemicals) and 0.1ml of the standardized inoculum were added to each tube to give different concentrations of the heavy metal or SDS. Each concentration of SDS as well as the individual metal was prepared in duplicates. Duplicate control tubes without the toxicants were set up for SDS as well as for each heavy metal to give a total of 12 controls. The cultures were incubated at 28 ± 2°C for 24 h.

Toxicity assay of binary mixtures

The toxicity assay was carried out in 2-ml volumes of nutrient broth-MTT medium (pH 7) supplemented with varying concentrations of SDS and Ni\textsuperscript{2+}, Co\textsuperscript{2+}, Cd\textsuperscript{2+}, Zn\textsuperscript{2+} or Pb\textsuperscript{2+} in separate 15-ml screw-capped test tubes. In each duplicate tube, 0.5 ml of NB (x4-strength), required volumes of sterile deionized water and the respective SDS + heavy metal mixtures were added to obtain graded concentrations of binary mixtures of SDS + metal ratios. Then, 0.1 ml each of 0.1% aqueous solutions of MTT and the inoculum were also added into each tube. The controls consisted of the medium without SDS and heavy metals. The cultures were incubated at 28 ± 2°C for 24 h.

**Table 1: Binary mixtures of SDS and heavy metals**

<table>
<thead>
<tr>
<th>Mixture</th>
<th>SDS + Ni\textsuperscript{2+}</th>
<th>SDS + Cd\textsuperscript{2+}</th>
<th>SDS + Pb\textsuperscript{2+}</th>
<th>SDS + Zn\textsuperscript{2+}</th>
<th>SDS + Co\textsuperscript{2+}</th>
</tr>
</thead>
<tbody>
<tr>
<td>EECR 50</td>
<td>96.07</td>
<td>3.93</td>
<td>99.79</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>ABCR1</td>
<td>97</td>
<td>3</td>
<td>99</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ABCR2</td>
<td>95</td>
<td>5</td>
<td>98</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>ABCR3</td>
<td>90</td>
<td>10</td>
<td>96</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

**Extraction and quantification of MTT-formazan**

In each test, after the 24 h incubation, 4ml of n-butanol was added to stop the reaction and then shaken for about 10 min to extract the MTT-formazan produced into the n-butanol. The absorbance of the MTT-formazan extract was determined by spectrophotometry (VIS Spectrophotometer 721D) at 590 nm.

**Estimation of toxicity threshold (EC\textsubscript{50})**

Toxicity of the heavy metals, SDS and their mixture were expressed as percent inhibitions of DHA. The inhibitions were transformed relative to the mean control (equation 1).

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

Where R is the percentage inhibition of DHA, \( C_A \) is the mean absorbance of MTT-formazan extract in the control and \( T_A \) is the absorbance of MTT-formazan extract in test experiments with varying concentrations of SDS, heavy metals or their mixtures. The EC\textsubscript{50} values were calculated by fitting the dose-effect data of the individual toxicants and their mixtures with 2-parameter logistic function as shown in equation 2.

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

Where \( x \) is toxicant concentration, \( EC_{50} \) is the toxicant concentration that suppressed DHA by 50% and \( b \) is the slope at \( EC_{50} \). In the case of hormetic response, \( EC_{50} \) values were calculated by fitting the dose-effect data into the hormesis model of Schabenberger et al. (1999).

\[
R = 100 - \frac{100 + fx}{1 + \left[ 1 + \frac{fEC_{50}}{50} \right] \left( \frac{x}{EC_{50}} \right)^b}\]
Where \( f \) is the parameter showing the level of hormetic effect, \( EC_{50} \) is the concentration of the toxicant that gave 50% reduction in DHA. The parameter \( b \) lost its interpretation as the slope at \( EC_{50} \) (Cedergreen et al., 2005a).

**Prediction of mixture toxicities**

If the relative content of individual toxicant is known, the mixture toxicities could be predicted from toxicity of the respective component according to concentration addition (CA) model. The model assumes that the components of the mixture have similar mode of action against the test organism. CA model could be described as in equation 4: (Berenbaum, 1985).

\[
EC_{50}^{(mix)} = \left( \sum_{i=1}^{n} \frac{\pi_i}{EC_{50}^{(i)}} \right)^{-1}
\]  

(4)

Where \( EC_{50}^{(mix)} \) is the total dose of the mixture which produced \( x\% \) effect, \( EC_{50}^{(i)} \) represents the amount of individual (\( i \))th toxicant which resulted in \( x \) effect when evaluated individually, \( n \) is the number of components in the mixture, \( \pi_i \) is the relative amount of \( i \)th toxicant in the mixture. By applying Eq. 4, the mixture toxicities were predicted as described by Nweke et al. (2018). The independent action (IA) model is predicated on the assumption that the mixture constituents have varying modes of action against the organism. The IA model can be mathematically expressed as shown in equation 5 (Faust et al., 2003).

\[
E(C_{\text{mix}}) = 1 - \prod_{i=1}^{n} [1 - E(c_i)]
\]  

(5)

Where \( E(C_{\text{mix}}) \) represents the cumulative effect of a number of component \( (n) \) in a mixture, \( c_i \) is the amount of individual \( (i) \)th component; \( E(c_i) \) is the effect of \( i \)th component. By substituting logistic model (dose-response) (Eq. 2) with response scaled from 0 to 1, the IA model was simplified as shown in equation 6 (Nweke et al., 2018).

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

(6)

Where, \( x \) is the total amount of the mixture, \( \pi_i \) is the amount of individual \( (i) \)th component in the mixture. The \( EC_{50} \) and \( b_i \) as already defined in Eq. 2 for each component were applied. The effects of the mixture \( E(C_{\text{mix}}) \) at \( x \) ranging from 0 to 8 mM was calculated according to Eq. 6 encoded in Microsoft Excel 2003. The value of \( x \) in each mixture that gave \( E(C_{\text{mix}}) \) of 50% was estimated by trial and error. The mixture \( EC_{50} \) based on CA model was computed with Eq. 4 based on the relative proportion and the \( EC_{50} \) of each component. The experimentally-derived \( EC_{50} \) for the individual toxicants and the various mixtures ratios were compared. Similarly, for each mixture ratio, the experimentally-derived, CA- and IA- predicted \( EC_{50} \) were also compared for statistical difference using Duncan post-hoc tests carried out with SPSS Statistics 21.

**Toxic index (TI)**

The TI for every mixture was computed by summing the toxic units for all the mixture components (equation7).

\[
TI = \sum_{i=1}^{n} \frac{C_i}{EC_{50i}}
\]  

(7)

Where \( C_i \) represents the amount of individual \( (i) \)th component in the mixture at the mixture’s \( EC_{50i} \) (\( EC_{50\text{mix}} \)), \( EC_{50i} \) is the amount of the \( i \)th toxicant that gave 50% inhibition of DHA when evaluated individually; \( n \) represents the number of mixture components. According to Boillot and Perrodin, (2008) for a mixture, TI = 1, TI < 1 and TI > 1 indicate additive, synergistic and antagonistic interactions respectively.

**Model deviation ratios (MDR)**

The MDR represents the ratio of the predicted \( EC_{50} \) to the experimentally-derived \( EC_{50} \). MDR > 1 and MDR < 1 indicate higher and lower toxicities respectively by model predictions and thus deviated from additivity.

**Isobolographic analysis**

As noted by Nweke et al. (2018), isobolographic analysis of the binary mixture toxicity can be estimated with \( EC_{50} \) values. The interaction is termed synergistic or antagonistic respectively, when an isobole is below or above the additivity line.

**RESULTS**

**Toxicity of individual toxicants**

The responses of *A. seifertii* to the toxic effect of the individual toxicants were dependent on the concentration (Figure 1). The toxicants increasingly inhibited DHA as the concentration increases, resulting to inhibitions greater than 95% at 0.4 mM for Pb\(^{2+}\), 0.05 mM for Co\(^{2+}\), 0.08 mM for Cd\(^{2+}\), 1 mM for Zn\(^{2+}\) and 10 mM for SDS. Table 2 shows the experimental and predicted toxicity thresholds (\( EC_{50} \)) of individual heavy metal and SDS for *A. seifertii*. SDS with \( EC_{50} \) of 2.810 ± 0.14 mM had the least toxicity while cadmium with \( EC_{50} \) of 0.011 ± 0.00 mM had the highest toxicity. As shown in the results of this study, the \( EC_{50} \) of all the toxicants were statistically different (\( P < 0.05 \)) and the order of decreasing toxicity is Cd\(^{2+}\) > Co\(^{2+}\) > Zn\(^{2+}\) > Pb\(^{2+}\) > Ni\(^{2+}\) > SDS.

**Toxicity of binary mixtures of SDS and heavy metals**

The experimental dose-response relationships of the binary
mixtures as well as the predictions made from CA and IA models on *A. seifertii* are shown in Figures 2-6. As shown in Figure 2, the SDS 97% + Ni²⁺ 3% (ABCR1) binary mixture ratio had biphasic effect with stimulation of DHA at concentration range of 0.062 to 0.5 mM. There was insignificant stimulatory effect at 0.2 mM of equi effect (SDS 96.07% + Ni²⁺ 3.93%) and 0.05 mM to 0.1 mM of SDS 90% + Ni²⁺ 10% (ABCR3) binary mixtures. In all SDS + Ni²⁺ mixtures, the IA and CA models predicted lower toxicities than observed from the experimental assay. In the ABCR2 mixture ratio, both models slightly predicted higher toxicities at low concentrations, while underestimating toxicity at high concentrations. Furthermore, both IA and CA models predicted virtually identical toxicity pattern for most binary mixtures, especially in all SDS + Ni²⁺ and SDS + Co²⁺ mixture ratios, as well as ABCR3 (SDS 96% + Ni²⁺ 4%) of SDS + Cd²⁺ mixtures. In SDS + Cd²⁺ mixtures, inhibition of DHA took place even at low concentrations (Figure 3). In all its mixture ratios, both models predicted significantly lower toxicities than the experimental data, except for ABCR3 (SDS 96% + Cd²⁺ 4%) mixture ratio, where the models slightly overestimated the toxicity. In SDS + Pb²⁺ mixtures,
the models also grossly underestimated the toxicities relative to the experimental data and were toxic even at low concentrations (Figure 4). The SDS + Zn\textsuperscript{2+} mixtures had biphasic effect on the DHA of *A. seifertii* exhibiting hormesis at low concentrations up to 0.1 mM for ABCR1 and ABCR3 mixture ratios, 0.09 mM for ABCR2 and 0.3 mM for EECR50 (Figure 5). Above these hormetic concentration ranges, the mixture progressively inhibited the DHA of *A. seifertii*, reaching 95% at 0.8 mM for ABCR2, 96% at 2.5 mM for EECR50, 97% at 2.5 mM for ABCR1 and 98% at 1.5 mM for ABCR3 mixture ratios.

The inhibitory effects of SDS + Co\textsuperscript{2+} mixtures are shown in Figure 6. At low concentrations up to 0.12 mM, the EECR50 mixture ratio was hormetic. In ABCR1 mixture ratio, CA and IA models correctly predicted the toxicity at low concentrations while both models predicted slightly higher toxicities of EECR 50, ABCR2 and ABCR3 mixtures at low concentrations. In addition, in all SDS + Co\textsuperscript{2+} mixtures, as the concentrations increased, both models slightly underestimated toxicities.

Table 3 shows the experimental and predicted toxicity thresholds (*EC\textsubscript{50}*\textsuperscript{S}) of binary mixtures of metals and SDS on *A. seifertii*. The experimentally derived *EC\textsubscript{50}S* of SDS + Ni\textsuperscript{2+} binary mixtures ranged from 0.343 ± 0.014 mM (for ABCR3) to 1.243 ± 0.070 mM (for ABCR1) mixture ratios. All the experimentally derived *EC\textsubscript{50}S* were significantly different from each other. In all mixture ratios, the IA - and CA -predicted *EC\textsubscript{50}S* were not significantly different from each other but were however statistically different from the experimentally derived *EC\textsubscript{50}* (\(P < 0.05\)). In the binary
Figure 4: Experimental and predicted inhibitions of binary mixtures of SDS and Pb\(^{2+}\) on A. seifertii DHA. The experimental dose-responses are shown by the data points. The toxicities estimated by fitting experimental data to logistic model (eq. 2) were represented by the dotted line. The solid and dashed lines show the predicted toxicities from independent action and concentration addition models, respectively.

Figure 5: Experimental and predicted effects of binary mixtures of SDS and Zn\(^{2+}\) on A. seifertii DHA. The experimental dose-responses are shown by the data points. The toxicities estimated by fitting experimental data to logistic (eq. 2) or hormesis model (eq. 3) were represented by the dotted lines. The solid and dashed lines show the predicted toxicities from independent action and concentration addition models, respectively.

Figure 6: Experimental and predicted inhibitory effects of binary mixtures of SDS and Co\(^{2+}\) on A. seifertii DHA. The experimental dose-responses are shown by the data points. The toxicities estimated by fitting experimental data to logistic model (eq. 2) or hormetic model (Eq. 3) were represented by the dotted line. The solid and dashed lines show the predicted toxicities from independent action and concentration addition models, respectively.
mixtures of SDS + Cd\(^{2+}\), only EECR50 and ABCR2 mixture ratios were significantly different from each other for the experimentally derived \(\text{EC}_{50}\). Similarly, in ABCR3 mixture ratio, the experimentally-derived \(\text{EC}_{50}\) was statistically different from CA- and IA-predicted \(\text{EC}_{50}\). Other mixture ratios however showed statistical difference between the experimental and the predicted \(\text{EC}_{50}\) (\(P < 0.05\)). In the binary mixtures of SDS + Pb\(^{2+}\), the experimentally derived \(\text{EC}_{50}\) ranged from 0.202 ± 0.014 mM (for ABCR2) to 0.352 ± 0.060 mM for EECR50. Also, only ABCR2 mixture ratio had experimental \(\text{EC}_{50}\) significantly different from the other mixture ratios. In all SDS + Pb\(^{2+}\) mixture ratios, the predicted \(\text{EC}_{50}\) values as shown in Table 4. The TI, MDR and effect of binary mixtures of SDS and metals on \(A. seifertii\) are shown in Table 4. The TI values ranged from 0.114 ± 0.004 to 1.805 ± 1.22, while MDR was in the range of 0.556 ± 0.038 to 8.796 ± 0.293 for CA and 0.606 ± 0.045 to 13.275 ± 0.660 for IA. However, in all tested mixture ratios, the SDS + metal binary mixtures were synergistic in their actions, except ABCR3 (SDS 96%+Cd\(^{2+}\) 4%) that was antagonistic to the bacterium. The isoboles of the mixtures based on the \(\text{EC}_{50}\) are shown in Figure 7. There was synergistic effect in all SDS + heavy metal binary mixtures, except ABCR3 mixture ratio of SDS + Cd\(^{2+}\) mixture that was antagonistic as also shown by the isobologram. These observations corroborated the TI and MDR values as shown in Table 4.

### DISCUSSION

The contamination of aquatic ecosystems by heavy metal has become a serious concern due to their persistence and toxic nature. Apart from natural sources, human activities have been implicated in most environmental contamination.
Table 4: Toxic index, Model deviation ratio and effects of metals and SDS binary mixtures on *A. seifertii*

<table>
<thead>
<tr>
<th>Metal-SDS Mixtures</th>
<th>Toxic Index (TI)</th>
<th>CA</th>
<th>IA</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SDS + Ni(^{2+}) Binary Mixtures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDS 96.07% + Ni(^{2+}) 3.93% (EECR 50)</td>
<td>0.378 ± 0.003</td>
<td>2.646 ± 0.023</td>
<td>2.654 ± 0.076</td>
<td>Synergistic</td>
</tr>
<tr>
<td>SDS 97% + Ni(^{2+}) 3% (ABCR 1)</td>
<td>0.486 ± 0.003</td>
<td>2.056 ± 0.011</td>
<td>2.054 ± 0.079</td>
<td>Synergistic</td>
</tr>
<tr>
<td>SDS 95% + Ni(^{2+}) 5% (ABCR 2)</td>
<td>0.339 ± 0.006</td>
<td>2.951 ± 0.048</td>
<td>2.974 ± 0.076</td>
<td>Synergistic</td>
</tr>
<tr>
<td>SDS 90% + Ni(^{2+}) 10% (ABCR 3)</td>
<td>0.163 ± 0.003</td>
<td>6.136 ± 0.098</td>
<td>6.373 ± 0.239</td>
<td>Synergistic</td>
</tr>
<tr>
<td><strong>SDS + Cd(^{2+}) Binary Mixtures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDS 99.79% + Cd(^{2+}) 0.21% (EECR 50)</td>
<td>0.538 ± 0.005</td>
<td>1.882 ± 0.050</td>
<td>2.327 ± 0.047</td>
<td>Synergistic</td>
</tr>
<tr>
<td>SDS 99% + Cd(^{2+}) 1% (ABCR 1)</td>
<td>0.647 ± 0.021</td>
<td>1.547 ± 0.051</td>
<td>1.910 ± 0.112</td>
<td>Synergistic</td>
</tr>
<tr>
<td>SDS 98% + Cd(^{2+}) 2% (ABCR 2)</td>
<td>0.599 ± 0.018</td>
<td>1.671 ± 0.052</td>
<td>1.934 ± 0.048</td>
<td>Synergistic</td>
</tr>
<tr>
<td>SDS 96% + Cd(^{2+}) 4% (ABCR 3)</td>
<td>1.805 ± 0.122</td>
<td>0.556 ± 0.033</td>
<td>0.606 ± 0.045</td>
<td>Antagonistic</td>
</tr>
<tr>
<td><strong>SDS + Zn(^{2+}) Binary Mixtures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDS 96.07% + Zn(^{2+}) 3.93% (EECR 50)</td>
<td>0.182 ± 0.024</td>
<td>5.556 ± 0.752</td>
<td>8.022 ± 1.253</td>
<td>Synergistic</td>
</tr>
<tr>
<td>SDS 97% + Zn(^{2+}) 3% (ABCR 1)</td>
<td>0.141 ± 0.003</td>
<td>7.084 ± 0.156</td>
<td>9.725 ± 0.211</td>
<td>Synergistic</td>
</tr>
<tr>
<td>SDS 96% + Zn(^{2+}) 5% (ABCR 2)</td>
<td>0.114 ± 0.004</td>
<td>8.796 ± 0.293</td>
<td>13.275 ± 0.660</td>
<td>Synergistic</td>
</tr>
<tr>
<td>SDS 94% + Zn(^{2+}) 6% (ABCR 3)</td>
<td>0.178 ± 0.004</td>
<td>5.606 ± 0.128</td>
<td>8.913 ± 0.180</td>
<td>Synergistic</td>
</tr>
<tr>
<td><strong>SDS + Co(^{2+}) Binary Mixtures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDS 99.07% + Co(^{2+}) 0.93% (EECR 50)</td>
<td>0.218 ± 0.000</td>
<td>2.960 ± 0.147</td>
<td>2.928 ± 0.467</td>
<td>Synergistic</td>
</tr>
<tr>
<td>SDS 99% + Co(^{2+}) 1% (ABCR 1)</td>
<td>0.279 ± 0.012</td>
<td>3.595 ± 0.152</td>
<td>3.820 ± 0.178</td>
<td>Synergistic</td>
</tr>
<tr>
<td>SDS 97% + Co(^{2+}) 3% (ABCR 2)</td>
<td>0.492 ± 0.056</td>
<td>2.051 ± 0.228</td>
<td>2.310 ± 0.117</td>
<td>Synergistic</td>
</tr>
<tr>
<td>SDS 93% + Co(^{2+}) 7% (ABCR 3)</td>
<td>0.365 ± 0.054</td>
<td>2.780 ± 0.408</td>
<td>3.105 ± 0.398</td>
<td>Synergistic</td>
</tr>
</tbody>
</table>

* + values are reported as Mean ± 1SD

Figure 7: The EC₅₀ isobole representation for SDS and heavy metals as individual and mixtures tested against DHA of *A. seifertii*. The thick dots represent the standard deviation of the 95% confidence interval of the values. The solid and dashed lines represent additivity line and its 95% confidence belt.
by heavy metals. Cadmium, cobalt, nickel and zinc for example, have various industrial uses and as a result can jointly-contaminate terrestrial and aquatic ecosystems (Nies, 1992). The use of dehydrogenase activity to assess the toxicity of chemical compounds to pure cultures and microbial community has been reported elsewhere (Nweke et al., 2007; Wiatrowska et al., 2015). In the present study, cadmium was the most toxic heavy metal. This agrees with the observations on pure cultures of Pseudomonas species by Nweke et al. (2018). Cd has no known physiological function and has been reported to be toxic even at low concentrations (Nies, 1999). Cd²⁺ can replace Ca²⁺ and Zn²⁺ in proteins and can also induce oxidative stress. Furthermore, Cd²⁺ could distort the integrity of microbial plasma membrane and disrupt the proton movement across the membrane (Bitton et al., 1988). Like Cadmium, Pb²⁺ equally has no known biochemical function and is toxic to living organisms. A 50% effective concentration (EC₅₀) of lead against A. seifertii was 0.222 ± 0.005 mM in the present study. In soil, Pb²⁺ was reported to show noticeable toxicity against microbial community diversity even at 1 ppm (≈ 0.005 mM) (Sobolev and Begonia, 2008).

Although cobalt, nickel and zinc are micronutrients, they can be toxic at high concentrations. This is in line with their observed toxicities in this study. For instance, zinc is an important part of many microbial enzymes, and is therefore required for enzymatic activities and stability. However, at high concentrations Zn(II) could be toxic to microbial cells. Zinc for example is reported to inhibit the electron transport chain in all living organisms (Beard et al., 1995). Zinc inhibited dehydrogenase activity by 50% in sediment bacteria from New Calabar River at 0.166 and 0.873 mM for Bacillus and Micrococcus species respectively (Nweke et al., 2007). In a similar study, the median inhibitory concentration of 0.240 ± 0.012 mM was observed for zinc (Nweke et al., 2017). In the present study however, EC₅₀ of 0.075 ± 0.005 mM Zn(II) was recorded. Toxicity of nickel and cobalt to microorganisms has been widely reported and has also been critically reviewed by Gikas, (2008). Similarly, Co(II) has also been demonstrated to exhibit greater inhibitory potency to the growth of microorganisms than Ni(II) (Gikas, 2007). The same trend was observed in this study.

SDS has been reported to inhibit both cell multiplication and phosphorus uptake rate in Acinetobacter junii pure culture by 100% at concentrations of 10⁻³ mol L⁻¹ (1 mM) and higher, with an EC₅₀ 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) respectively (Hrenovic and Ivankovic, 2007). Similarly, Acinetobacter johnsonii and Oligotropha carboxidovorans isolated from sewage sludge showed nearly 50% and 20% loss of viability when treated with 0.2 and 2 mg/ml of SDS (0.694 mM and 6.94 mM), respectively (Malik et al, 2005). Toxicity of SDS to luminescent bacterium (Photobacterium phosphoreum), unicellular alga (Scenedesmus quadricauda), protozoan (Paramecium caudatum) and crustacean (Daphnia magna) has been reported by Esvyunina et al. (2016). The acute toxicity of alcohol sulfates to Daphnia magna increased with increasing alkyl chain length. In addition, at a concentration range of 0 - 15 mg/L (≈ 0 - 0.052 mM), morphological changes in the kidney and spleen of gilthead (Sparus aurata L.) induced by SDS resulted to substantial inhibitory effect on success of fertilization (Rosety et al., 2001). Furthermore, SDS has also been reported to inhibit both nitrogen-fixing capacity and growth of cyanobacterium, Gloeocapsa at 50 ppm (≈ 0.173 mM) (Cserhati et al., 2002). The EC₅₀ recorded in this study for SDS however was 2.810 ± 0.140 mM/L. The observed differences in the EC₅₀ threshold of SDS against the various organisms could be attributed to the differences in their genetic makeup, as organisms known to differ in their responses to even the same toxicant. In the present study also, A. seifertii generally appears to be more sensitive to heavy metals than SDS. These differences could be due to the differences in bacterial responses to different toxicants. This is in line with the report that microbial sensitivity or response to toxicants varies with the type of toxicant (Wangberg et al., 1995).

There are considerable pieces of information on the toxicity of heavy metals to bacteria, as well as SDS to aquatic animals, plants and algae (Nweke et al., 2018; Gibson et al., 2016; Masakorala et al., 2008b), but limited information exists on the toxicity of the mixtures of these toxicants to bacteria, particularly with the use of dehydrogenase enzyme activity as the endpoint. In the present study, the threshold concentrations observed in the binary mixtures of SDS and metal ions against A. seifertii were higher than those of the individual metals but lower than SDS. This shows interaction between the chemicals, resulting in modulation of the toxicity of each other in the mixture. SDS modulated the toxicities of the heavy metals and vice versa, as SDS has been reported to be less toxic than some metals and non-surfactant compounds (Wangberg and Blanck, 1988). This change in toxicity observed with mixtures compared with single components underscores the importance of studying mixture effects in evaluating pollution hazards to aquatic environment. Swedmark and Granmo, (1981) reported differences in toxicities to development of cod (Gadus morhua L.), between the combinations of metals and surfactant (LAS) and their single components. They also noted that generally, the surfactant decreased the toxicity of copper, while zinc decreased that of LAS.

The isobolographic analysis based on the EC₅₀ model deviation ratios (MDR) and toxic index model (TI) employed in the analysis of the binary mixture toxicity showed similarity in their result, to the toxicity of SDS and metal ions mixtures on the dehydrogenase activity of A. seifertii. According to Cedergreen, (2014), MDR values of 0.5 ± MDR ≤ 2 indicates that mixture effect is most likely to be additive. Thus, in the present study, marginal antagonistic as well as weak and strong synergistic interactions were observed. This weak synergistic effect could be attributed to the masking effect of SDS on cadmium and zinc ions in the mixture. Wang et al. (2012) reported clear and weak synergistic interactions at low
and higher concentrations respectively of Microcystin-LR and linear alkylenzene sulfonate against duckweed (Lemna minor). However, both synergistic and antagonistic interactions of anionic surfactants and heavy metal ions, as well as anionic surfactants and anthracene mixtures have been reported previously (Masakorala et al., 2008a; Cai et al., 2019).

The prediction of the combined actions of the binary mixtures was done using IA and CA models. In SDS 97% + Co2+ 3% and SDS 93% + Co2+ 7% mixture ratios of SDS + Co2+ binary mixture, as well as SDS 95% + Ni2+ 5% mixture ratio of SDS + Ni2+ mixture, both models overestimated the toxicity of the mixtures at low concentration, while underestimating at high concentration. On the contrary, both under- and overestimation of toxicity by IA and CA models for a particular mixture ratio, at low and high concentrations of metal mixtures against Pseudomonas fluorescens DHA was reported by Nweke et al. (2018). These differences could be attributed to differences in toxicants and physiology of the test organisms. The other mixture ratios of both binary mixtures however under estimated the toxicity, even at low concentration. SDS and Zn2+ binary mixtures showed biphasic effects upon exposure to A. seifertii. Similar hormetic effects were also observed in some SDS + Ni2+ mixtures, as well as EECR50 mixture ratio of SDS + Co2+ mixtures to the DHA of the bacterium. Biphasic response to chemicals is a phenomenon reported to be common among microbes as well as eukaryotic organisms. In this study, the observed stimulation of dehydrogenase enzyme activity at low concentrations (hormesis) and subsequent inhibition at higher concentrations is in agreement with the reported hormetic responses to zinc, nickel, cobalt and SDS (as individual toxicants) by microorganisms (Tozum-Calgan and Atay-Guneyman, 1994; Gikas, 2007; Nweke et al., 2007; Hashida and Inouye, 2007). However, in SDS + Pb2+ and SDS + Cd2+ binary mixtures, the IA and CA models underestimated the mixture toxicities, even at low concentrations, except in SDS 96% + Cd2+ 4% mixture ratio, where both models overestimated the toxic interactive effects of the mixture. Mixtures of Ni2+ and Co2+ synergistically inhibited the growth of activated sludge microbial community, but at the zone of decreasing stimulation, Ni2+ + Co2+ mixture was antagonistic (Gikas, 2007). Similar toxicities of the binary mixtures, specifically in SDS + Ni2+ and SDS + Co2+ binary mixtures, as well as SDS 96% + Cd2+ 4% mixture ratio was predicted by IA and CA models. Identical prediction of toxicity thresholds by both models has been reported under certain conditions (Nweke et al., 2019). Chen et al. (2013), observed that identical predictions by CA and IA models is possible if the concentration-effect relationship of each mixture component could be described by two-parameter Weibull equation, the curves are strictly parallel, with the slope parameter of 2.3. Furthermore, toxicity threshold predicted by both models for the binary mixtures of metals and pyrethroid insecticides against Daphnia magna was reported to be identical (Barata et al., 2006). According to Cedergreen and co-workers, binary mixtures of chemicals that have concentration-response curves with log-logistic slope of about 1 have similar IA and CA predictions (Cedergreen and Streibig, 2005b; Cedergreen et al., 2007; Cedergreen et al., 2008). This appears to be the case in the present study. The logistic function slope parameter for SDS, Co2+, Ni2+ and Cd2+ were 2.1, 0.96, 0.95 and 1.8 respectively. These values are not far from 1 and probably were the reasons for the similar CA and IA predictions observed in SDS + Ni2+, SDS + Co2+ and SDS + Cd2+ binary mixtures. Sodium dodecyl sulfate and heavy metal may act similarly against the bacteria, thus no significant difference existed between predicted threshold toxicities of the binary mixture on the basis of IA and CA for SDS + Ni2+ and SDS + Co2+ binary mixtures, and also SDS 96% + Cd2+ 4% mixture ratio. Huang et al. (2011) reported similar insignificant differences between mixture toxicities predicted on the basis of IA and CA models for phenols with similar and non-similar mechanisms of action. This shows that both models could be veritable instruments for predicting the toxicity of chemical mixtures (Faust et al., 2000).

CONCLUSION

At the end of this study, the results showed that though antagonism was observed, most of the binary mixtures however exhibited synergistic effects on A. seifertii. DHA inhibition was used as end point to study the toxicities of the binary mixtures of SDS with some heavy metals (Pb, Co, Cd, Ni and Zn) to A. seifertii isolated from Otamiri river sediment. The experiment was designed such that the bacterium was subjected to graded concentrations of the mixtures of SDS with each of the heavy metals, using fixed ratio design on the basis of EC20 equi-effect and arbitrary combinations. Although the result may not be readily extrapolated to other organisms, however it provided evidence of the possibility of synergistic effects of SDS + metal ion mixtures on bacteria. More study is advocated on the effects of the mixtures of these toxicants to the natural microbial community of sediment ecosystems.

Conflict of interests

The authors declare that there was no conflict of interests regarding the publication of this paper.

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