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Article

Synergistic toxicities of binary and ternary mixtures of an anionic surfactant and divalent metals to *Lysinibacillus fusiformis* isolated from a vegetable farm

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Abstract: The toxicities of the heavy metals (Pb, Cd, Ni, Zn, and Co) and their ternary mixtures with Sodium Dodecyl Sulfate (SDS) to *Lysinibacillus fusiformis* isolated from *Talinum fruticosum* farms irrigated with Otamiri River water in Owerri, Imo State, Nigeria, were assessed using dehydrogenase activity (DHA) restriction as an endpoint. Fixed ratio mixtures (arbitrary concentration ratio (ABCR) and equi-effect concentration ratio (EECR) mixtures) were formulated to evaluate the combined toxicities of these toxicants. Toxicities were predicted with concentration addition (CA) and independent action (IA) models and compared with the experimentally observed toxicities. The response of the bacterium to the toxicants' toxicities was concentration-dependent and gradually inhibited the DHA as the concentration increased, with percentage inhibitions greater than 95% at 0.5 mM for Zn, 1 mM for Ni, 0.3 mM for Pb, 0.08 mM for Cd, 0.7 mM for Co, as well as 10 mM for SDS. The 50% effective concentrations (EC_{50S}) of the individual toxicants differed significantly from one another ($P < 0.05$). All the dose-response relationships of the ABCR and EECR mixtures and the individual toxicants could be described by a logistic function. In most binary mixtures, predicted toxicities from the CA and IA models were significantly different from the observed toxicities. In ABCR1 mixture ratio of SDS + Cd^{2+} mixtures, CA and IA models correctly predicted the experimental data at different points, while the IA model correctly predicted the experimental data in the EECR50 mixture ratio of SDS + Pb^{2+} mixture. In SDS + Co^{2+} mixtures, EC_{50S} predicted by both models were identical. The effects of the mixtures interactions showed both weak and strong synergism, as well as additive against the soil bacterium. Similarly, in all but ABCR1 and ABCR2 mixture ratios of SDS + Cd + Zn ternary mixtures, the experimentally observed EC_{50}, CA- and IA-predicted EC_{50S} were significantly different from one another ($P < 0.05$). Furthermore, both models greatly underestimated the mixture toxicity at all tested mixture ratios and were strongly synergistic against the soil bacterium. The use of such contaminated water for irrigation could negatively affect the soil bacterial community and, by extension, soil fertility, going by the possible interaction between heavy metals and SDS.

Keywords: SDS; toxicities; concentration addition; independent action; heavy metals

1. Introduction

Urban farming, which is gradually becoming popular worldwide because of its numerous benefits to the community and the environment. However, the availability of adequate water is seriously becoming a restraining factor, thus prompting a search for alternative resources [1]. The use of different sources of water for the irrigation

of crops has been reported [2,3]. Long-term application of such contaminated irrigation waters can result in the accumulation of metals in soil [4,5]. Heavy metals can also contaminate the soil through other human activities like land application of fertilizers, animal manures, sewage sludge, pesticides, among others [5,6]. Some heavy metals, such as zinc, copper, cobalt, and nickel, are trace elements, required in small quantities by microorganisms as coenzymes for their metabolism. Others like lead, cadmium, silver, mercury, and aluminum have no known physiological roles and are regarded as being toxic to living organisms. Thus, heavy metal contamination of soils is a serious concern, not only due to their persistence and accumulative nature in the environment but also because of the health risks posed to humans and the ecosystems [7]. These risks could be through direct ingestion or contact with contaminated soil, the food chain, drinking of contaminated groundwater, reduced food quality via phytotoxicity, reduction in use of land for farming, and land tenure problems [8].

Similarly, Sodium dodecyl sulfate (SDS) is an anionic surfactant that is common in aquatic environments. Co-contamination of the environment by heavy metals and SDS is expected due to the latter's widespread use in many domestic products. According to Fendinger et al. [9], the prevalence of SDS in the environment is mostly from its presence in domestic and industrial effluents as well as its direct discharge from some applications. SDS has also been reported to enhance the toxicity of some heavy metals in their mixtures. Zhu et al. [10] noted that even a low concentration of sodium dodecyl sulfate (SDS), a widely used bactericidal surfactant, can significantly change the species, quantity, and genetic functions of microorganism communities in natural waters.

Microorganisms are known to play vital roles in restoring soil fertility; 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 [11]. There is increasing evidence suggesting that microorganisms are by far more susceptible to heavy metal toxicity than soil animals or plants growing on the same soils [12]. The bioavailability of metals is dependent on their dissociation, and over time they undergo time-dependent chemical processes that may render them unavailable for uptake by plants [13,14].

Otamiri River watersheds are used for all-season farming. Crops such as maize, fluted pumpkin, and water leaves are planted at the riverbanks and irrigated with the river water. The river water and its sediment have been reported to be polluted by heavy metals, anionic and cationic surfactants from different sources [15,16]. In the same study, sodium dodecyl sulfate was also reported to be the predominant anionic surfactant in the river [15]. The toxic effects of heavy metals on soil microorganisms have been widely reported [17]. Similarly, detrimental effects of joint actions of heavy metals and some organic compounds to soil bacteria have also been reported [18,19]. To date, however, no study has been undertaken to assess the possible effects of irrigation with heavy metals and SDS-contaminated Otamiri River water on soil bacteria of the cultivated soils. Considering the roles of microbes in maintaining soil fertility, this study therefore aims at assessing the toxicities of SDS

and some heavy metals (identified in the river), as individuals and in their ternary mixtures, on the *Lysinibacillus fusiformis* isolated from the soil of the vegetable farm at the riverbank.

2. Materials and methods

2.1. Sample collection

Two vegetable (*Talinum triangulare*) farms located 100 m apart, at the Otamiri River bank, in Owerri, Imo State, Nigeria, were used for the study. The river water is used for all-season irrigation of the farms. A clean shovel (disinfected with 70% ethanol) was used to scrape off the topsoil to about 3 cm; thereafter, a soil auger was then used in collecting surface soil at the two farms located at 5.465°N, 7.035°E and 5.463°N, 7.034°E, respectively. These samples were pooled together in a cellophane bag and mixed thoroughly, then taken to Biotechnology Laboratory, Federal University of Technology, Owerri, and analyzed within 24.

2.2. Isolation of soil bacteria

One gram (1g) of the mixed soil sample was suspended in 9 mL of sterile water contained in a 100-mL flask and stirred for a minute with sterile glass before allowing it to stand for about 10 min. Ten-fold serial dilution of the soil suspension was then carried out sequentially to the 6th test tube [20]. Then 0.1 mL of the 10^{-5} dilution of the suspension was aseptically inoculated onto sterile nutrient agar plates in duplicates, with a sterile Pasteur pipette, and then spread with a sterile glass rod, then incubated at 37 °C for 24 h. Discrete colonies were further subcultured on Nutrient agar plates to obtain pure cultures, which were then stored on agar slants in the refrigerator at 4 °C. The isolates obtained were further tested for their ability to degrade SDS in an enriched culture medium as described by Adekanmbi and Usinola [21]. At the end of 96 h incubation, two of the seven isolates were able to utilize SDS as the only source of carbon. The isolates were identified as *Lysinibacillus fusiformis*, using morphological and biochemical tests [22]. The identity was further confirmed using 16S rRNA gene partial sequencing.

2.3. Reagents and test bacterium

The heavy metal salts ($CdSO_4.8H_2O$, Pb $(NO_3)_2$, $ZnNO_3.6H_2O$, $CoCl_2$, and $NiSO_4.6H_2O$) were used as sources of their respective metal ions. The salts, SDS and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were obtained from Sigma-Aldrich (Germany). The deionized distilled water used in reagents' preparation was sterilized by autoclaving and the stock reagents by membrane filtration. *L. fusiformis* identified as SDS degrading bacterium was used as test bacterium.

2.4. Inoculum preparation

Lysinibacillus fusiformis cells were cultured in nutrient broth (Lab M) on a rotary incubator (150 rpm) at 26 ± 2°C for 16 h. The cells were harvested from the culture by centrifugation (3000 rpm, 15 min, Newlife Centrifuge, NL80-2). The

harvested cell pellet was repeatedly washed in sterile deionized water by centrifugation and suspended there-in [23]. The optical density of the cell suspension was adjusted to contain 1.1×10^8 cell/mL in accordance with McFarland turbidity standards.

2.5. Design of the binary mixture ratios

The binary mixtures (SDS+Pb, SDS+Ni, SDS+Zn, SDS+Cd and SDS+Co) were designed to contain SDS and one of the five metals ions in fixed ratios. In each binary combination, 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 required volumes of the heavy metal and SDS stock solutions to produce a given concentration ratio. The mixtures were treated as if they were single toxicant solution all through the assay.

Table 1. Binary mixtures of SDS and metals.

Mixture	Mixture Ratios (%)									
	SDS + Ni^{2+}		SDS + Cd^{2+}		SDS + Pb^{2+}		SDS + Zn^{2+}		SDS + Co^{2+}	
	SDS	Ni^{2+}	SDS	Cd^{2+}	SDS	Pb^{2+}	SDS	Zn^{2+}	SDS	Co^{2+}
EECR50	97.89	2.11	99.77	0.23	96.01	3.99	99.31	0.69	99.41	0.59
ABCR1	98	2	99	1	97	3	98	2	98	2
ABCR2	96	4	98	2	95	5	97	3	94	6
ABCR3	95	5	97	3	94	6	96	4	96	4

2.6. Design of the ternary mixture ratios

Table 2. Ternary mixtures of two metals and SDS.

Mixture	Mixture ratios (%)								
	SDS + Pb^{2+} + Zn^{2+}			SDS + Cd^{2+} + Zn^{2+}			SDS + Pb^{2+} + Ni^{2+}		
	SDS	Pb	Zn	SDS	Cd	Zn	SDS	Pb	NI
EECR50	95.44	3.89	0.67	99.08	0.23	0.69	95.88	3.91	0.21
ABCR1	96	3	1	97	1	2	96	3	1
ABCR2	94	4	2	95	2	3	94	2	4
ABCR3	95	2	3	93	3	4	95	4	1
Mixture	SDS^{2+} + Ni^{2+} + Cd^{2+}			SDS + Co^{2+} + Pb^{2+}			SDS + Co^2 + Cd^{2+}		
	SDS	Ni	Cd	SDS	Co	Pb	SDS	Co	Cd
EECR50	97.66	2.11	0.23	90.90	5.4	3.70	94.20	5.6	0.20
ABCR1	98	1	1	91	6	3	94	5	1
ABCR2	96	3	1	89	7	4	93	4	3
ABCR3	97	2	1	92	6	2	95	3	2

The ternary mixtures comprising SDS and two of the following five metals (Cd, Pb, Zn, Co and Ni) in fixed ratios were used in the study. Three arbitrarily chosen

mixtures ratios (ABCR) and one EC_{50} equieffect concentration ratio (EECR50) were combined to assess the effect of the ternary mixtures. The percentage of the mixture components are as shown in **Table 2**. Every mixture was constituted by using varying volumes of 10 mM and 50 mM stock solutions of metal ions and SDS respectively and used as composite mixture in the DHA assay.

2.7. Toxicity assay for metal ions and SDS

Toxicity assay was done using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-Tetrazolium Bromide (MTT), with the inhibition of dehydrogenase activity (DHA) as the end point [24]. In a 2-mL total volume in 15-mL screw-capped culture tubes, the reaction mixture consisted of nutrient broth (NB), MTT, SDS or metal ion and *L. fusiformis* inoculum (pH 7.0). Each concentration of metal ion or SDS was prepared in duplicate screw-cap culture tubes. In each tube, a 0.5 mL of NB, calculated volumes of SDS (50 mM) or metal ions (10 mM) working stock solutions and sterile deionized water (to make up) were dispensed. Subsequently, 0.1 mL each of aqueous solutions of MTT and *L. fusiformis* suspension was added. The final concentrations of the toxicants ranged from 0.002 mM (metal ions) to 1.0 mM (SDS). The controls consisted of the medium without SDS or metal ions. The cultures were incubated in the dark at 26 ± 2 °C for 24 h. After incubation, 4 mL n-butanol was added into each tube and shaken for 10 min to extract the purple MTT-formazan produced by enzymatic reduction of MTT. The absorbance of each extract was determined in a spectrophotometer (VIS Spectrophotometer 721D) at 590 nm.

2.8. Toxicity testing for the mixtures

The toxicity assay procedure as described for the individual toxicants was adopted for the mixtures. In duplicate 15-mL screw-capped culture tubes, 2-mL reaction mixture containing NB, MTT, bacterial inoculum and the two toxicants (SDS and a metal ion) were prepared (pH 7) (binary mixtures). For the ternary mixtures, the reagents were the same but the assay medium was instead supplemented with varying concentrations of SDS and two of the metals (Cd^{2+}, Pb^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}), in different ternary combinations. Incubation of the cultures, extraction of MTT-formazan (MTTF) and the measurement of the absorption was done as described above.

3. Data analysis

3.1. Determination of EC_{50S} of the toxicants and their mixtures

The response of the organism to each concentration of SDS, metal ion and their mixtures were calculated as percent inhibition of DHA (R) relative to the mean control Equation (1).

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

Where, C_A is the mean absorbance of MTTF-extract in the control tubes, T_A represents absorbance of MTTF-extract in the experiment with a particular concentration of SDS, metal ion or their mixtures. Subsequently, the EC_{50} was

calculated by fitting the concentration-responses into 2-parameter logistic function (Equation (2)) using least square non-linear regression technique.

$$R = \frac{100}{1 + \left(\frac{x}{EC50}\right)^{b}} \tag{2}$$

Where x stands for the SDS or metal ion concentration, EC_{50} is SDS or metal ion concentration that produced 50% reduction in DHA and b is the slope at EC_{50}.

3.2. 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. It is expressed as shown in Equation (3) [25].

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

Where $EC_{x(\mathrm{mix})}$represents the total concentration of the binary mixture that elicited x% inhibition of DHA, EC_{xi} is the concentration of ith component that gave % inhibition in DHA when tested alone, n is the number of components in the mixture, π_i is the relative proportion of ith component in the mixture. Applying Eq. 3, the mixture toxicities were determined as described by Okechi et al. [24]. The independent action (IA) model is predicated on the assumption that the mixture constituents have independent modes of action against the bacterium. The IA model could be expressed mathematically as shown in Equation (4) [26].

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

where $E(cmix)$ represents the cumulative effect of a number of component (n) in a mixture, c_i stands for the amount of individual (i-th) component; $E(ci)$ is the effect of i-th component. By substituting logistic model (dose-response) (Equation (2)) with response scaled from 0 to 1, the IA model was simplified as shown in Equation (5) [18].

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

where, x is the total amount of the mixture, π_{ii} is the amount of individual (i-th) component in the mixture. The EC_{50i} and bi as already defined in Equation (2) for each component were applied. The effects of the mixture $E(cmix)$ at x ranging from 0 to 10 mM was calculated according to Equation (5) encoded in Microsoft Excel 2010. The value of x in each mixture that gave E(c_{mix}) of 50% was estimated by trial and error. The mixture EC_{50} based on CA model was computed with Equation (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_{50S} were also compared for statistical difference using Duncan post-hoc tests carried out with SPSS Statistics 21.

3.3. The toxic index

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

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

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 DHA when tested alone, *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 > 1 or < 1 respectively. The effect is described as additive if TI = 1 [27].

3.4. Model deviation ratios (MDR)

The model deviation ratios (MDR) were calculated as the ratio of the predicted EC_{50} to the experimental EC_{50} (Equation (7)). The effect of the mixture is interpreted as synergistic or antagonistic if MDR > 1 or < 1 respectively, while MDR = 1 shows additivity [28].

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

3.5. Isobole analysis

The isobolographic analysis of the binary mixture toxicity was estimated on the basis of the EC_{50} values as reported by Okechi et al. [29]. The interactive effect indicates antagonism or synergism respectively, when an isobole is above or below the additivity line.

4. Results

4.1. Toxicity of individual toxicants

The responses of the *L. fusiformis* to the toxicity of the toxicants were dose-dependent (**Figure 1**), with the toxicants gradually inhibiting the DHA as the dose increases, giving percentage inhibitions greater than 95% at 0.5 mM for Zn^{2+}, 1 mM for Ni^{2+}, 0.3 mM for Pb^{2+}, 0.08 mM for Cd^{2+}, 0.7 mM for Co^{2+} as well as 10 mM for SDS. The experimental and predicted toxicity thresholds (EC_{50}) of individual metal ions and SDS on *L. fusiformis* are shown in **Table 3**. The EC_{50S} of the toxicants ranged from 0.013 ± 0.001 mM for Cd^{2+} to 2.613 ± 0.173 mM for SDS. The Duncan test indicates that the $EC_{50}$of the toxicants were significantly different from one

another other ($P < 0.05$) and the order of toxicity ranking is Cd^{2+}> Co^{2+}> Zn^{2+}> Ni^{2+}> Pb^{2+}> SDS.

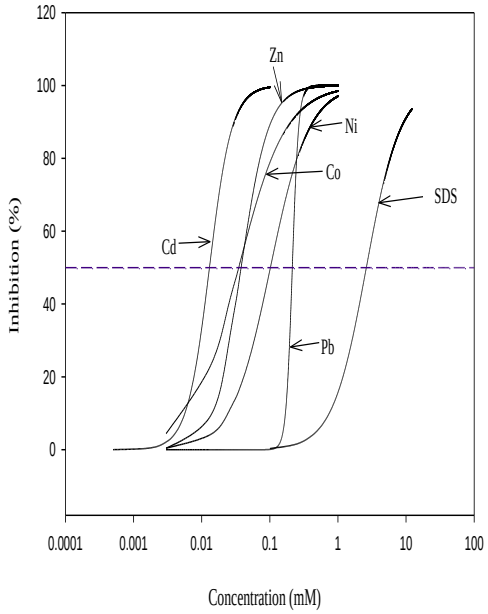

Figure 1. Inhibition of DHA in *L. fusiformis* by individual toxicants.
Predicted toxicities are represented as solid lines.

Table 3. Experimentally-observed and predicted toxicity thresholds (EC_{50}) of binary mixtures on *L. fusiformis*.

Toxicant Mixtures	***EC*$_{50}$ (mM)‡**		
	Experimental†	**CA-Predicted**	**IA-Predicted**
Ni^{2+}	0.102 ± 0.004a	-	-
Cd^{2+}	0.013 ± 0.001b	-	-
Pb^{2+}	0.214 ± 0.001c	-	-
Zn^{2+}	0.037 ± 0.002d	-	-
Co^{2+}	0.035 v 0.003e	-	-
SDS	2.613 ± 0.173f	-	-
SDS + Ni^{2+}			
SDS 97.89% + N^{2+} 2.11% (EECR50)	0.869 ± 0.052a*	1.981 ± 0.038**	1.981 ± 0.037**
SDS 98% + Ni^{2+} 2% (ABCR1)	1.218 ± 0.114b*	1.753 ± 0.103**	2.013 ± 0.041***
SDS 96% + Ni^{2+} 4% (ABCR2)	0.551 ±0.025c*	1.319 ± 0.072**	1.540 ± 0.013***
SDS 95% + Ni^{2+} 5% (ABCR3)	0.520 ± 0.028c*	1.173 ± 0.063**	1.371 ±0.001***
SDS + Cd^{2+}			
SDS 99.77% + Cd^{2+} 0.23% (EECR50)	1.211 ±0.087a*	1.790 ± 0.125**	2.318 ± 0.060***
SDS 99% + Cd^{2+} 1% (ABCR1)	0.818 ±0.013b*	0.871 ± 0.064*	1.076 ± 0.025***
SDS 98% + Cd^{2+} 2% (ABCR2)	0.268 ±0.027c*	0.523 ± 0.039 **	0.612 ± 0.033***
SDS 97% + Cd^{2+} 3% (ABCR3)	0.257 ±0.020c*	0.373 ± 0.028**	0.420 ± 0.026**

Table 3. (*Continued*).

Toxicant Mixtures	EC_{50} (mM)‡		
	Experimental†	CA-Predicted	IA-Predicted
SDS + Pb^{2+}			
SDS 96.09% + Pb^{2+} 3.99% (EECR50)	0.387 ± 0.040a*	1.816 ± 0.083**	0.420 ± 0.026*
SDS 97% + Pb^{2+} 3% (ABCR1)	0.162 ± 0.017b*	1.954 ± 0.097**	2.695 ±0.179***
SDS 95% + Pb^{2+} 5% (ABCR2)	0.137 ± 0.010b*	1.673 ± 0.071**	2.703 ± 0.147***
SDS 94% +Pb^{2+} 6% (ABCR3)	0.209 ± 0.013c*	1.561 ± 0.061**	2.606 ± 0.091***
SDS + Zn^{2+}			
SDS 99.31% + Zn^{2+} 0.69% (EECR50)	0.801 ± 0.038a*	1.775 ± 0.112**	2.221 ± 0.052***
SDS 98% + Zn^{2+} 2% (ABCR1)	0.274 ± 0.006b*	1.104 ± 0.073**	1.383 ± 0.026***
SDS 97% + Zn^{2+} 3% (ABCR2)	0.312 ± 0.016b*	0.856 ± 0.057**	1.045 ± 0.027***
SDS 96% + Zn^{2+} 4% (ABCR3)	0.273 ± 0.014b*	0.700 ± 0.047**	0.834 ± 0.028***
SDS + Co^{2+}			
SDS 99.41% + Co^{2+} 0.59% (EECR50)	0.523 ± 0.019a*	1.821 ± 0.132**	1.952 ± 0.032**
SDS 98% + Co^{2+} 2% (ABCR1)	0.226 ± 0.008b*	1.057 ± 0.082**	1.175 ± 0.020***
SDS 94% + Co^{2+} 6% (ABCR2)	0.184 ± 0.010c*	0.482 ± 0.040**	0.532 ± 0.369**
SDS 96% + Co^{2+} 4%(ABCR3)	0.218 ± 0.011b*	0.662 ± 0.054**	0.718 ± 0.270**

†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 ($P < 0.05$).
The asterisks (***) as used in the data in Table 3 are already explained in the 2nd statement of the footnote above: This stated thus "‡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 ($P < 0.05$)".

4.2. Toxicity of the mixtures

The experimental dose-response relationships of the binary mixtures and the predictions made from CA and IA models for *L. fusiformis*are shown in **Figures 2**–**6**. In SDS + Ni^{2+} mixture, both models slightly underestimated the mixture toxicities than the experimentally-derived data would suggest, even at lower concentrations especially for ABCR2 and EECR50 mixture ratios (**Figure 2**). In SDS + Cd^{2+} mixtures, in ABCR1 mixture ratio, between 0.78 mM to 0.85mM, CA-model correctly predicted the experimental data, as could also be seen in Table 3. However, below this concentration range, the model slightly overestimated the toxicities. Similarly, the IA model almost correctly predicted the experimental data from 0.84mM concentration. Both models however slightly underestimated the toxicities at low concentration. In other mixture ratios, inhibition of dehydrogenase activity took place even at low concentrations (**Figure 3**). In SDS+Pb^{2+}, SDS + Zn^{2+}, and SDS + Co^{2+} mixtures, both CA and IA models greatly predicted lower toxicities than the experimentally-derived data would suggest, even at low concentration (**Figures 4**–**6**), except in EECR50 mixture ratio of SDS+Pb^{2+} mixture, where IA model correctly predicted the experimental data (**Figure 4**). Similarly, in SDS + Zn^{2+} binary mixture, both models predicted similar toxicities, as their dose-response curves were almost superimposed (**Figure 6**).

The isobolographic analyses of the binary mixtures based on the EC_{50S} are shown in **Figure 7**. The isobologram indicated synergistic effect in most metals and SDS binary mixtures, except ABCR1 mixture ratio in SDS + Cd^{2+} mixture that was additive. This observation was corroborated by the toxic index and model deviation ratio values as shown in **Table 3**.

The experimental dose-response relationships of the ternary mixtures as well as the predictions made from concentration addition (CA) and independent action (IA) models for *L fusiformis* are shown in **Figures 8**–**13**. In most ternary mixtures both models greatly predicted lower toxicities even at low concentration, compared to the experimentally-observed data.

The experimental and predicted toxicity thresholds (EC_{50S}) of binary mixtures of metals and SDS against *L. fusiformis* are shown in **Table 3**. The experimentally-derived EC_{50S} in the binary mixture of SDS + Ni^{2+} ranged from 0.520 ± 0.028 mm to 1.218 ± 0.114 mM for ABCR3 and ABCR1 mixture ratios, respectively. Also, among the experimentally-derived EC_{50S}, there was no statistical difference betweenABCR2 and ABCR3 in SDS + Ni^{2+} and SDS+Cd^{2+} binary mixtures. In addition, in EECR50and ABCR3 mixture ratiosof SDS + Ni^{2+} and SDS+Cd^{2+} mixtures respectively, EC_{50}valuespredicted on the basis ofCA- and IA- models were not statistically different from each other ($P < 0.05$). Furthermore, no significant difference existed between the experimentally-derived *EC50* and that predicted on the basis of CA model ($P < 0.05$), in ABCR1 mixture ratio of SDS+Cd^{2+} binary mixtures.

In SDS + Pb^{2+} binary mixtures, there was no statistical difference between the experimentally- derived EC_{50} of ABCR1 and ABCR2 mixture ratios, as well as between experimentally-derived EC_{50} and that predicted on the basis of IA-model within EECR50 mixture ratio ($P < 0.05$). In SDS + Zn^{2+} binary mixtures, experimentally-derived EC_{50} in EECR50 mixture ratio was significantly higher than the EC_{50S} of the other mixture ratios. Experimental EC_{50S}, as well as EC_{50S} predicted from CA- and IA-models were statistically different from one another ($P < 0.05$), .in all mixture ratios. In SDS + Co^{2+} binary mixtures, ABCR2 mixture ratio was the most toxic (0.184 ± 0.011 mM) while EECR50 mixture ratio was the least (0.523 ± 0.019 mM). There was however no statistical difference between ABCR1 and ABCR3 mixture ratios in the experimentally-derived EC_{50S}. Similarly, only ABCR1 mixture ratio showed statistical difference between the experimentally-derived *EC50* and EC_{50S} predicted from CA- and IA-models ($P < 0.05$).

Experimentally-observed and predicted toxicity thresholds (EC_{50}) of ternary mixtures are also shown in **Table 4**. In general, the experimentally-observed EC_{50S} for the ternary mixtures ranges from 0.087 ± 0.004 mM for EECR50 mixture ratio of SDS + Pb + Zn mixture to 0.418 ± 0.018 mM for ABCR1 mixture ratio of SDS + Pb + Ni mixture. In SDS + Pb + Zn, SDS + Pb + Ni and SDS + Ni + Cd ternary mixtures, the experimentally-observed EC_{50S} for all mixture ratios differed statistically from one another ($P < 0.05$). Similarly, in SDS + Cd + Zn and SDS + Co + Pb, ABCR2 and ABCR3 mixture ratios differ statistically from the ABCR1 and EECR50 mixture ratios, while only ABCR3 mixture ratio of SDS + Ni + Cd mixtures differed from the other mixture ratios for experimentally-observed EC_{50S} ($P < 0.05$). In all but ABCR1 and ABCR2 mixture ratios of SDS + Cd + Zn ternary

mixtures, the experimentally-observed EC_{50}, CA- and IA-predicted EC_{50S} were significantly different from each other ($P < 0.05$).

Table 4. Experimentally-observed and predicted toxicity thresholds (EC_{50S}) of the ternary mixtures on *L. fusiformis*.

Toxicant Mixtures	EC_{50} (mM)[‡+]		
	Experimental[†]	CA-Predicted	IA-Predicted
SDS + Pb^{2+} + Zn^{2+}Mixture			
SDS 95.45% + Pb^{2+} 3.89% + Zn^{2+} 0.67% ($EECR_{50}$)	0.087 ± 0.004a*	1.379 ± 0.070**	2.304 ± 0.943***
SDS 95% + Pb^{2+} 2% + Zn^{2+} 3% ($ABCR_1$)	0.107 ± 0.009b*	1.293 ± 0.071**	2.007 ± 0.001***
SDS 94% + Pb^{2+} 4% + Zn^{2+} 2% ($ABCR_2$)	0.122 ± 0.007c*	0.928 ± 0.052**	1.403 ± 0.535***
SDS 96% + Pb^{2+} 3% + Zn^{2+} 1% ($ABCR_3$)	0.140 ± 0.011d*	0.798 ± 0.049**	1.050 ± 0.055***
SDS + Cd^{2+}+Zn^{2+}Mixtures			
SDS 99.08% + Cd^{2+} 0.23% + Zn^{2+} 0.69% ($EECR_{50}$)	0.346 ± 0.021a*	1.480 ± 0.233**	2.125 ± 0.634***
SDS 97% + Cd^{2+} 1% + Zn^{2+} 2% ($ABCR_1$)	0.205 ± 0.012b*	0.743 ± 0.249**	1.089 ± 0.333**
SDS 93% + Cd^{2+} 3% + Zn^{2+} 4% ($ABCR_2$)	0.124 ± 0.009c*	0.504 ± 0.228**	0.715 ± 0.775**
SDS 95% + Cd^{2+} 2% + Zn^{2+} 3% ($ABCR_3$)	0.135 ± 0.004c*	0.268 ± 0.020**	0.378 ± 0.314***
SDS + Pb^{2+} +Ni^{2+} Mixtures			
SDS 85.89% + Pb^{2+} 3.91% + Ni^{2+}0.20%($EECR_{50}$)	0.234 ± 0.022a*	1.753 ± 0.080**	2.687 ± 1.376***
SDS 95% + Pb^{2+} 4% + Ni^{2+} 1% ($ABCR_1$)	0.418 ± 0.019b*	1.651 ± 0.080**	2.401 ± 0.054***
SDS 96% + Pb^{2+} 3% + Ni^{2+} 1% ($ABCR_2$)	0.192 ± 0.012c*	1.184 ± 0.058**	0.556 ± 0.742***
SDS 94% + Pb^{2+} 2% + Ni^{2+} 4% ($ABCR_3$)	0.344 ± 0.011d*	1.542 ± 0.070**	2.421 ± 0.040***
SDS + Ni^{2+} + Cd^{2+} Mixtures			
SDS 97.67% +Ni^{2+} 2.11% + Cd^{2+} 0.23%($EECR_{50}$)	0.203 ± 0.015a*	1.871 ± 0.038**	1.871 ± 0.363***
SDS 98% +Ni^{2+} 1% + Cd^{2+} 1% ($ABCR_1$)	0.088 ±0.004b*	0.805 ± 0.057**	1.073 ± 0.016***
SDS 97% +Ni^{2+} 2% + Cd^{2+} 1% ($ABCR_2$)	0.132 ± 0.006c*	0.699 ± 0.047**	0.985 ± 0.508***
SDS 96% +Ni^{2+} 3% + Cd^{2+} 1% ($ABCR_3$)	0.179 ± 0.010d*	0.749 ± 0.052**	1.032 ± 0.034***
SDS + Co^{2+} + Pb^{2+} Mixtures			
SDS 90.89% + Co^{2+} 5.40% + Pb^{2+} 3.70%($EECR_{50}$)	0.142 ± 0.009a*	0.484 ± 0.037**	0.584 ± 0.261***
SDS 92% + Co^{2+} 6% + Pb^{2+} 2% ($ABCR_1$)	0.111 ± 0.007b*	0.454 ± 0.035**	0.534 ± 0.016***
SDS 91% + Co^{2+} 6% + Pb^{2+} 3% ($ABCR_2$)	0.089 ± 0.004c*	0.396 ± 0.031**	0.467 ± 0.076***
SDS 89% + Co^{2+} 7% + Pb^{2+} 4% ($ABCR_3$)	0.097 ± 0.005c*	0.463 ± 0.037**	0.533 ± 0.013***
SDS + Co^{2+} + Cd^{2+} Mixtures			
SDS 9.19% + Co^{2+} 5.60% + Cd^{2+} 0.20%($EECR_{50}$)	0.095 ± 0.004a*	0.473 ± 0.039**	0.561 ± 0.014***
SDS 94% + Co^{2+} 5% + Cd^{2+} 1% ($ABCR_1$)	0.123 ± 0.008b*	0.391 ± 0.031**	0.536 ± 0.000***
SDS 95% + Co^{2+} 3% + Cd^{2+} 2% ($ABCR_2$)	0.121 ± 0.007b*	0.263 ± 0.021**	0.328 ± 0.147***
SDS 93% + Co^{2+} 4% + Cd^{2+} 3% ($ABCR_3$)	0.129 ± 0.007b*	0.362 ± 0.028**	0.473 ± 0.037***

[†]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.

The asterisks (***) as used in the data in Table 4 are already explained in the 2nd statement of the footnote above: This stated thus "[‡]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 ($P < 0.05$)".

The toxic index, model deviation ratio and effect of metals and SDS binary mixtures on *L. fusiformis* are shown in **Table 5**. The toxic index (TI) values ranged from 0.082 ± 0.002 to 0.942 ± 0.054, while model deviation ratio (MDR) ranged from 1.070 ± 0.062 to 12.605 ± 0.100 and 1.090 ± 0.048 to 19.790 ± 0.304 for CA and IA respectively. At all the mixture ratios tested, the metals and SDS binary mixtures were synergistic in their action on the bacterium, except in ABCR1 of SDS + Cd^{2+} that was additive.

Table 5. Toxic index, MDR and effect of Metals + SDS binary mixtures on *L. fusiformis*.

Metal+SDS Binary Mixtures	Toxic Index (TI) [+]	Mean MDR[+]		Effect
		CA	IA	
SDS + Ni^{2+}				
SDS 97.89% + Ni^{2+} 2.11% (EEC50)	0.505 ± 0.001	2.332 ± 0.013	2.283 ± 0.094	synergistic
SDS 98% + Ni^{2+} 2% (ABCR1)	0.694 ± 0.024	1.492 ± 0.096	1.661 ± 0.123	weak syner
SDS 96% + Ni^{2+} 4% (ABCR2)	0.418 ± 0.004	2.430 ± 0.067	2.797 ± 0.104	synergistic
SDS 95% + Ni^{2+} 5% (ABCR3)	0.443 ± 0.001	2.300 ± 0.071	2.642 ± 0.125	synergistic
SDS + Cd^{2+}				
SDS 99.77% + Cd^{2+} 0.23% (EEC50)	0.677 ± 0.002	1.516 ± 0.066	1.918 ± 0.093	synergistic
SDS 99% + Cd^{2+} 1% (ABCR1)	0.942 ± 0.054	1.070 ± 0.062	1.315 ± 0.029	additivity
SDS 98% + Cd^{2+} 2% (ABCR2)	0.512 ± 0.013	2.026 ± 0.133	2.289 ± 0.106	synergistic
SDS 97% + Cd^{2+} 3% (ABCR3)	0.689 ± 0.003	1.492 ± 0.072	1.633 ± 0.028	weak syner
SDS + Pb^{2+}				
SDS 96.01 + Pb^{2+} 3.99% (EEC50)	0.213 ± 0.012	4.911 ± 0.421	1.090 ± 0.048	synergistic
SDS 97% + Pb^{2+} 3% (ABCR1)	0.083 ± 0.004	12.605±0.100	16.711±0.609	synergistic
SDS 95% + Pb^{2+} 5% (ABCR2)	0.082 ± 0.002	12.581±0.622	19.790±0.304	synergistic
SDS 94% + Pb^{2+} 6% (ABCR3)	0.134 ± 0.003	7.643 ± 0.332	12.485±0.346	synergistic
SDS + Zn^{2+}				
SDS 99.31 + Zn^{2+} 0.69% (EEC50)	0.452 ± 0,009	2.252 ± 0.082	2.777 ± 0.072	synergistic
SDS 98% + Zn^{2+} 2% (ABCR1)	0.249 ± 0.011	4.051 ± 0.188	5.041 ± 0.066	synergistic
SDS 97% + Zn^{2+} 3% (ABCR2)	0.365 ± 0.006	2.793 ± 0.110	3.354 ± 0.097	synergistic
SDS 96% + Zn^{2+} 4% (ABCR3)	0.390 ± 0.006	2.608 ± 0.104	3.056 ± 0.066	synergistic
SDS + Co^{2+}				
SDS 99.41 + Co^{2+} 0.59% (EEC50)	0.288 ± 0.010	3.523 ± 0.148	3.734 ± 0.081	synergistic
SDS 98% + Co^{2+} 2% (ABCR1)	0.215 ± 0.009	4.720 ± 0.218	5.196 ± 0.120	synergistic
SDS 94% + Co^{2+} 6% (ABCR2)	0.382 ± 0.011	2.668 ± 0.116	2.890 ± 0.024	synergistic
SDS 96% + Co^{2+} 4% (ABCR3)	0.330 ± 0.010	3.088 ± 0.134	3.300 ± 0.207	synergistic

[+] Values are reported as Mean ± 1SD.

The toxic index, model deviation ratio and effect of metals and SDS ternary mixtures on *L. fusiformis* are shown in **Table 6**. The toxic index values ranged from 0.063 ± 0.000 to 0.505 ± 0.022, while model deviation ratio (MDR) ranged from 2.007 ± 0.096 to 16.101 ± 0.487 for CA and 2.709 ± 0.112 to 26.502 ± 0.657 for IA.In all mixture ratios tested, the metals and SDS ternary mixtures were strongly synergistic in their action against the soil bacterium.

Table 6. Toxic index, MDR and effects of Metals+SDS ternary mixtures on *L. fusiformis*.

Metal-SDS Mixtures	Toxic Index (TI) [+]	Mean MDR[+]		Effect
		CA	IA	
SDS +Pb+ Zn Mixtures				
SDS 95.45% + Pb^{2+} 3.89% + Zn^{2+} 0.67% ($EECR_{50}$)	0.063 ± 0.000	16.101±0.487	26.502±0.657	synergistic
SDS 95% + Pb^{2+} 2% + Zn^{2+} 3% ($ABCR_1$)	0.083 ± 0.002	12.510±0.699	18.881±1.229	synergistic
SDS 94% + Pb^{2+} 4% + Zn^{2+} 2% ($ABCR_2$)	0.132 ± 0.001	7.759 ± 0.299	11.518±0.479	synergistic
SDS 96% + Pb^{2+} 3% + Zn^{2+} 1% ($ABCR_3$)	0.176 ± 0.002	5.878 ± 0.305	7.539 ± 0.382	synergistic
SDS + Cd^{2+}+Zn^{2+}Mixtures				
SDS 99.08% + Cd^{2+} 0.23% + Zn^{2+} 0.69% ($EECR_{50}$)	0.257 ± 0.002	4.382 ± 0.813	6.150 ± 0.454	synergistic
SDS 97% + Cd^{2+} 1% + Zn^{2+} 2% ($ABCR_1$)	0.343 ± 0.005	3.734 ± 1.374	5.328 ± 1.329	synergistic
SDS 93% + Cd^{2+} 3% + Zn^{2+} 4% ($ABCR_2$)	0.335 ± 0.002	4.236 ± 2.099	5.792 ± 2.373	synergistic
SDS 95% + Cd^{2+} 2% + Zn^{2+} 3% ($ABCR_3$)	0.505 ± 0.022	2.007 ± 0.096	2.797 ± 0.022	synergistic
SDS + Pb^{2+} +Ni^{2+} Mixtures				
SDS 85.89% + Pb^{2+} 3.91% + Ni^{2+}0.20%($EECR_{50}$)	0.133 ± 0.006	7.773 ±0.565	11.506±0.393	synergistic
SDS 95% + Pb^{2+} 4% + Ni^{2+} 1% ($ABCR_1$)	0.253 ± 0.001	4.013 ± 0.109	5.745 ± 0.054	synergistic
SDS 96% + Pb^{2+} 3% + Ni^{2+} 1% ($ABCR_2$)	0.162 ± 0.003	6.299 ± 0.256	8.107 ± 0.461	synergistic
SDS 94% + Pb^{2+} 2% + Ni^{2+} 4% ($ABCR_3$)	0.223 ± 0.003	4.535 ± 0.106	7.045 ± 0.040	synergistic
SDS + Ni^{2+} + Cd^{2+} Mixtures				
SDS 97.67% +Ni^{2+} 2.11% + Cd^{2+} 0.23%($EECR_{50}$)	0.153 ± 0.001	6.694 ± 0.289	9.264 ± 0.629	synergistic
SDS 98% +Ni^{2+} 1% + Cd^{2+} 1% ($ABCR_1$)	0.109 ± 0.003	9.287 ± 0.348	12.195±0.203	synergistic
SDS 97% +Ni^{2+} 2% + Cd^{2+} 1% ($ABCR_2$)	0.189 ± 0.004	5.379 ± 0.187	7.469 ± 0.235	synergistic
SDS 96% +Ni^{2+} 3% + Cd^{2+} 1% ($ABCR_3$)	0.240 ± 0.003	4.255 ± 0.156	5.761 ± 0.219	synergistic
SDS +Co + Pb Mixtures				
SDS 90.89% + Co^{2+} 5.40% + Pb^{2+} 3.70%($EECR_{50}$)	0.293 ± 0.005	3.494 ± 0.140	4.124 ± 0.051	synergistic
SDS 92% + Co^{2+} 6% + Pb^{2+} 2% ($ABCR_1$)	0.244 ± 0.005	4.190 ± 0.170	4.824 ± 0.041	synergistic
SDS 91% + Co^{2+} 6% + Pb^{2+} 3% ($ABCR_2$)	0.225 ± 0.007	4.510 ± 0.190	5.242 ± 0.067	synergistic
SDS 89% + Co^{2+} 7% + Pb^{2+} 4% ($ABCR_3$)	0.209 ± 0.007	4.870 ± 0.210	5.510 ± 0.027	synergistic
SDS +Co + Cd Mixtures				
SDS 9.19% + Co^{2+} 5.60% + Cd^{2+} 0.20%($EECR_{50}$)	0.201 ± 0.008	5.046 ± 0.237	5.908 ± 0.040	synergistic
SDS 94% + Co^{2+} 5% + Cd^{2+} 1% ($ABCR_1$)	0.314 ± 0.006	3.259 ± 0.137	4.378 ± 0.207	synergistic
SDS 95% + Co^{2+} 3% + Cd^{2+} 2% ($ABCR_2$)	0.462 ± 0.008	2.208 ± 0.091	2.709 ± 0.112	synergistic
SDS 93% + Co^{2+} 4% + Cd^{2+} 3% ($ABCR_3$)	0.357 ± 0.007	2.854 ± 0.116	3.664 ± 0.161	synergistic

[+] Values are reported as Mean ± 1SD.

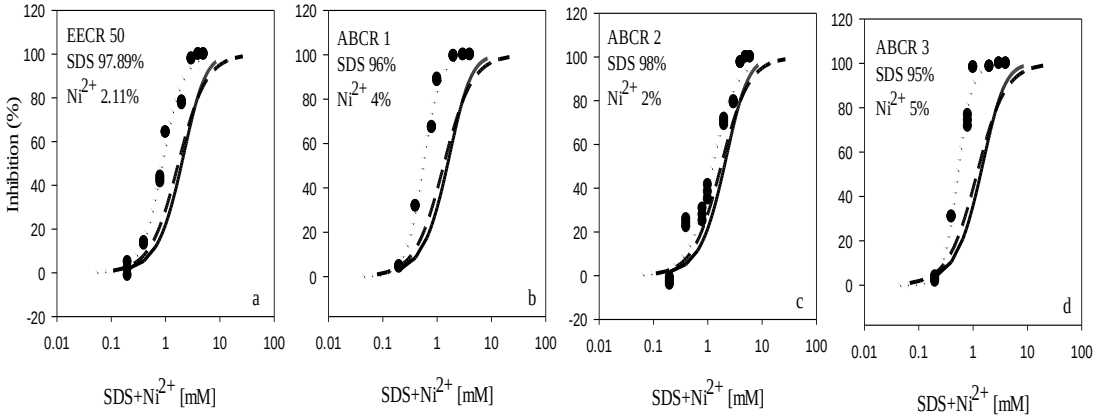

Figure 2. Experimental and predicted inhibitions of binary mixtures of SDS and nickel ions on *L. fusiformis* DHA.

Data points (●) are the experimental dose-response data, while the dotted lines are toxicities obtained by fitting experimental data to logistic model (Equation (2)). Dashed and solid lines are toxicities predicted from the CA and IA models respectively.

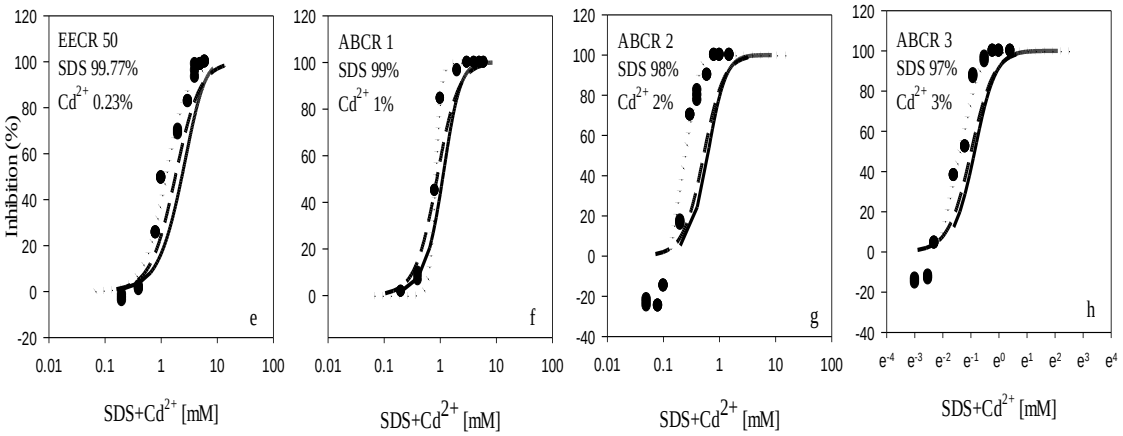

Figure 3. Experimental and predicted inhibitions of binary mixtures of SDS and cadmium ions on *L. fusiformis* DHA.

Data points (●) are the experimental dose-response data, while the dotted lines are toxicities obtained by fitting experimental data to logistic model (Equation (2)). Dashed and solid lines are toxicities predicted from the CA and IA models respectively.

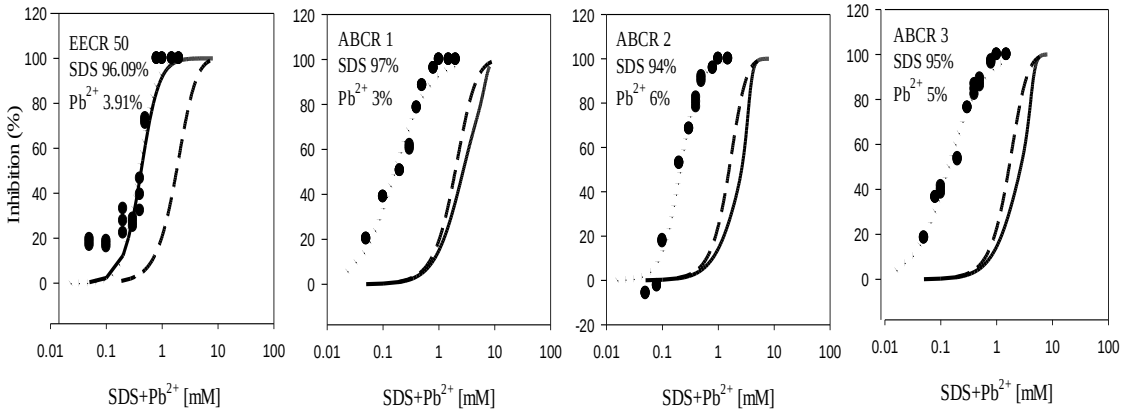

Figure 4. Experimental and predicted inhibitions of binary mixtures of SDS and lead ion on *L. fusiformis* DHA.

Data points (●) are the experimental dose-response data, while the dotted lines are toxicities obtained by fitting experimental data to logistic model (Equation (2)). Dashed and solid lines are toxicities predicted from the CA and IA models respectively.

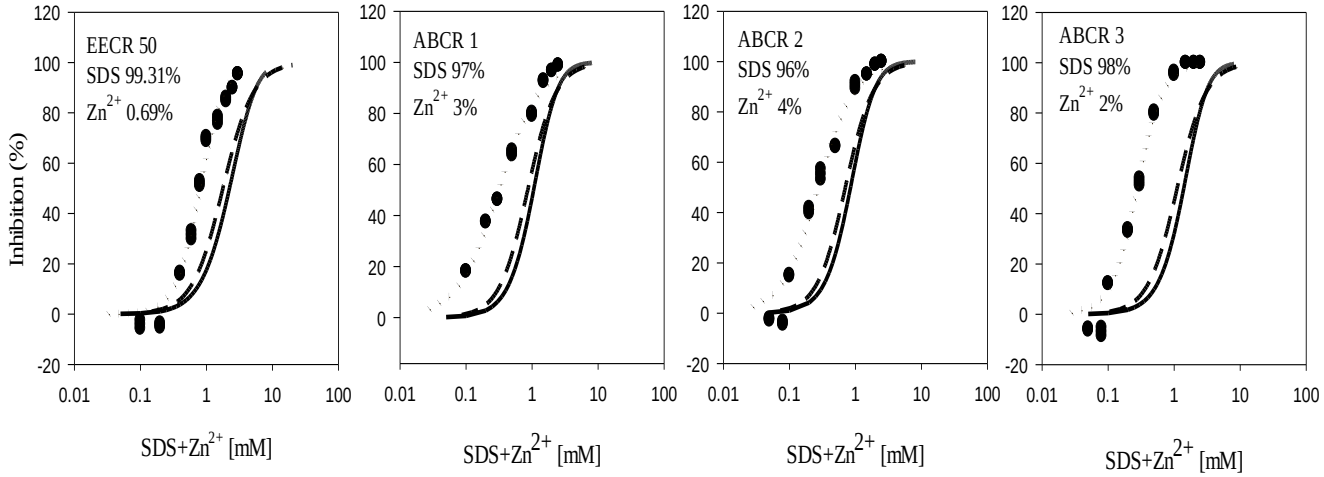

Figure 5. Experimental and predicted effects of binary mixtures of SDS and zinc ions on *L. fusiformis* DHA.

Data points (●) are the experimental dose-response data, while the dotted lines are toxicities obtained by fitting experimental data to logistic model (Equation (2)). Dashed and solid lines are toxicities predicted from the CA and IA models respectively.

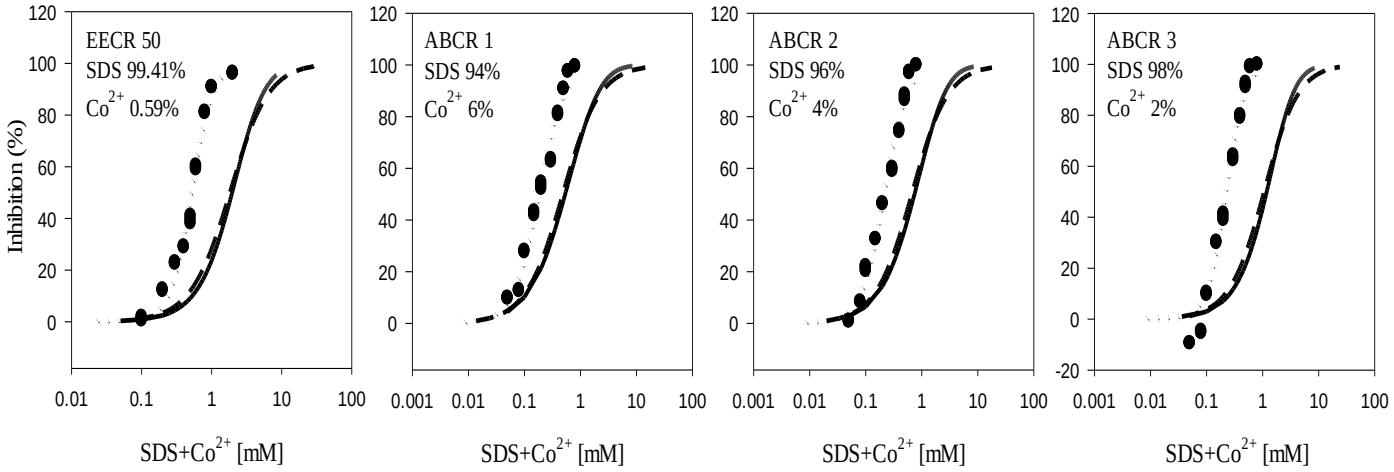

Figure 6. Experimental and predicted inhibitory effects of binary mixtures of SDS and cobalt ions on *L. fusiformis* DHA.

Data points (●) are the experimental dose-response data, while the dotted lines are toxicities obtained by fitting experimental data to logistic model (Equation (2)). Dashed and solid lines are toxicities predicted from the CA and IA models respectively.

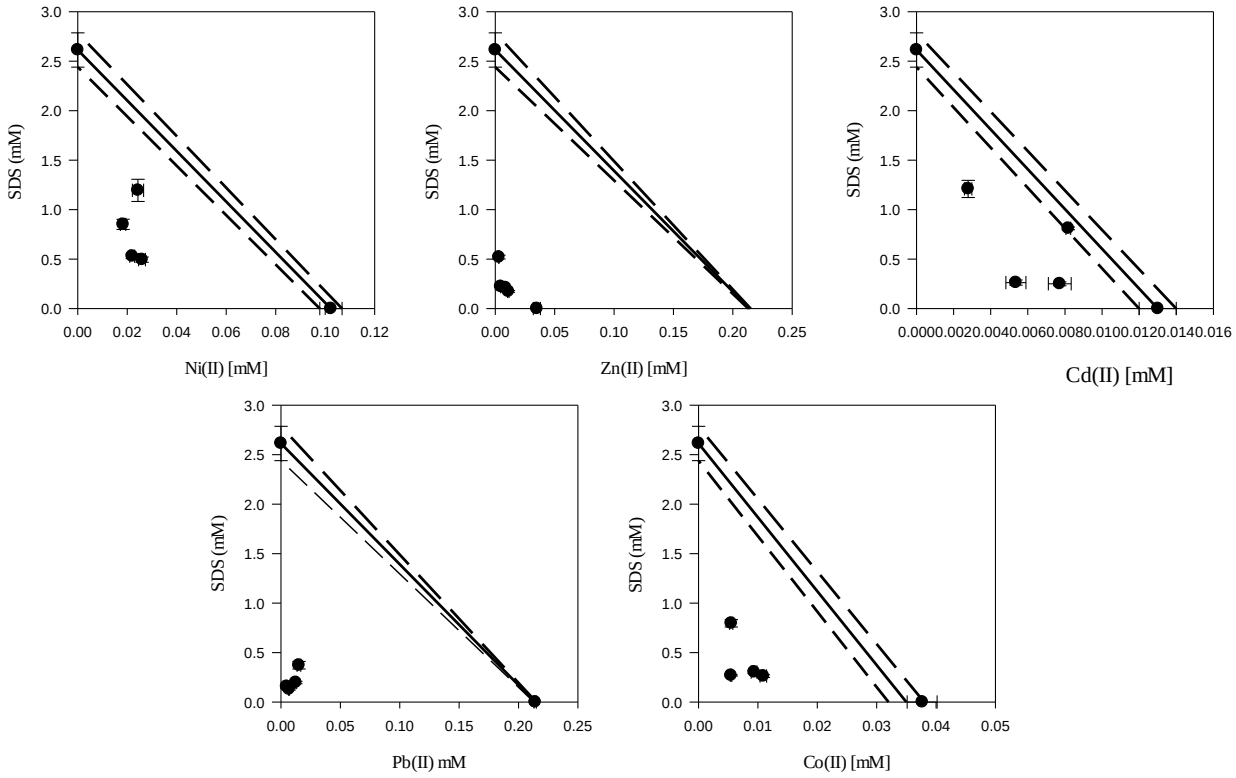

Figure 7. The EC_{50} isobole representation for SDS and metal ions as individual and mixtures tested against DHA of *L. fusiformis*.

The thick dots stand for the standard deviation of the 95% confidence interval of the values, while the solid and dashed lines stand for additivity line and its 95% confidence belt respectively.

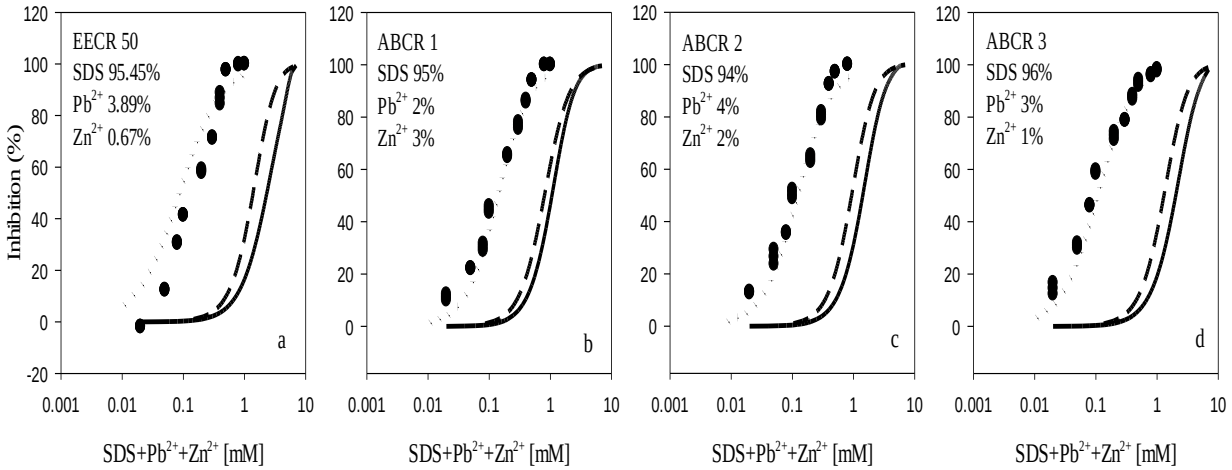

Figure 8. Experimental and predicted inhibitory effects of ternary mixtures of SDS, lead and zinc ions on *L. fusiformis* DHA.

Data points (●) are experimental dose-response data, while the dotted lines are toxicities obtained by fitting experimental data to logistic model (Equation (2)). Dashed and solid lines are toxicities predicted from the CA and IA-models respectively.

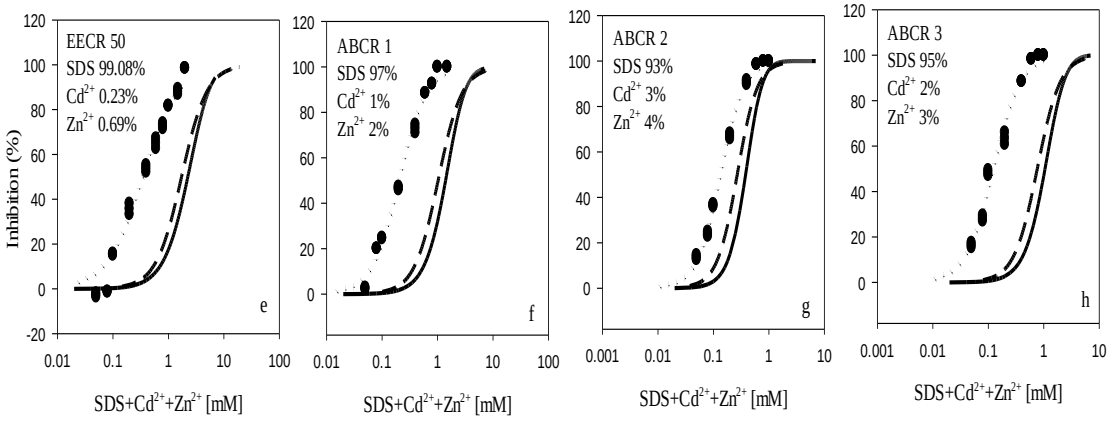

Figure 9. Experimental and predicted inhibitory effects of ternary mixtures of SDS, cadmium and zinc ions on *L. fusiformis* DHA.

Data points (●) are experimental dose-response data, while the dotted lines are toxicities obtained by fitting experimental data to logistic model (Equation (2)). Dashed and solid lines are toxicities predicted from the CA and IA-models respectively.

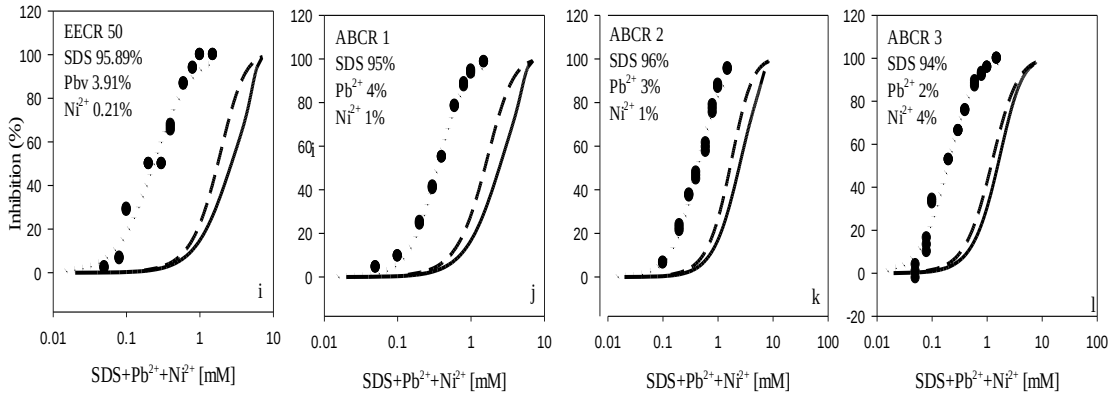

Figure 10. Experimental and predicted inhibitory effects of ternary mixtures of SDS, lead and nickel ions on *L. fusiformis* DHA.

Data points (●) are experimental dose-response data, while the dotted lines are toxicities obtained by fitting experimental data to logistic model (Equation (2)). Dashed and solid lines are toxicities predicted from the CA and IA-models respectively.

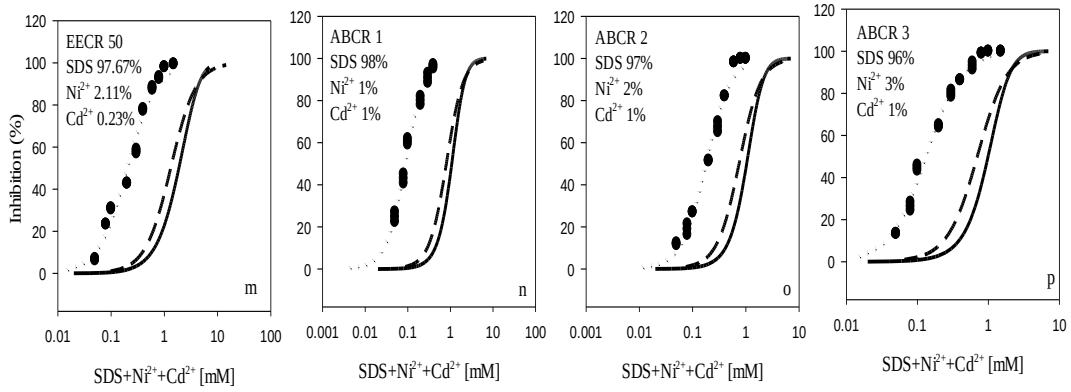

Figure 11. Experimental and predicted inhibitory effects of ternary mixtures of SDS, nickel and cadmium ions on *L. fusiformis* DHA.

Data points (●) are experimental dose-response data, while the dotted lines are toxicities obtained by fitting experimental data to logistic model (Equation (2)). Dashed and solid lines are toxicities predicted from the CA and IA-models respectively.

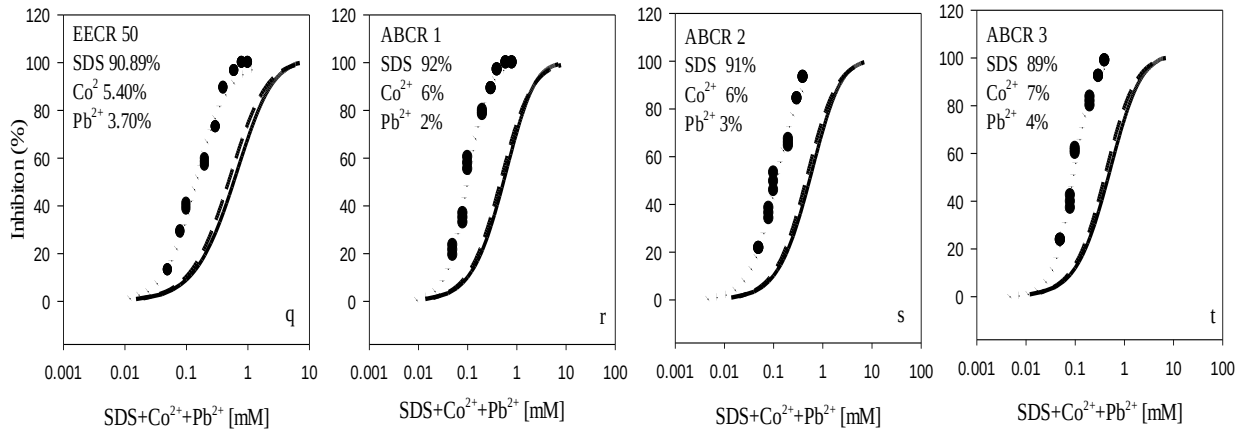

Figure 12. Experimental and predicted inhibitory effects of ternary mixtures of SDS, cobalt and lead ions on *L. fusiformis* DHA.

Data points (●) are experimental dose-response data, while the dotted lines are toxicities obtained by fitting experimental data to logistic model (Equation (2)). Dashed and solid lines are toxicities predicted from the CA and IA-models respectively.

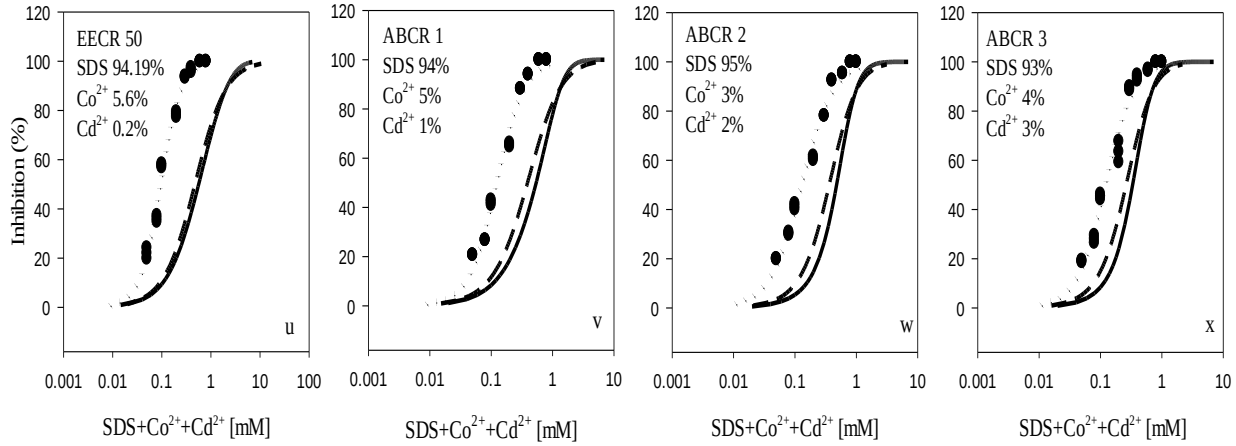

Figure 13. Experimental and predicted inhibitory effects of ternary mixtures of SDS, cobalt and cadmium ions on *L. fusiformis* DHA.

Data points (●) are experimental dose-response data, while the dotted lines are toxicities obtained by fitting experimental data to logistic model (Equation (2)). Dashed and solid lines are toxicities predicted from the CA and IA-models respectively.

5. Discussion

Environmental pollution by heavy metals has become a serious threat to living organisms in different ecosystems [3,30]. The physiological and biochemical properties of microorganisms can be altered by the presence of heavy metals. Heavy metals such as cadmium and lead have been reported to be toxic to microorganisms, even at low concentrations [31]. In the present study for example, cadmium was the most toxic heavy metal against the soil bacterium *L. fusiformis*. This agrees with the reports elsewhere [18–24]. Cadmium and lead inhibited DHA thresholds in *L. fusiformis* at 24-h EC_{50S} of 0.013 ± 0.001 mM and 0.214 ± 0.001 mM respectively. Cadmium and lead toxicities to microorganisms are mostly by denaturing proteins, destroying nucleic acids, inhibiting enzymes activity and hindering cell division and transcription [32]. A 24-h EC_{50} of 0.023 ± 0.003 mM was reported for cadmium

against *Pseudomonas fluorescens* by Nweke et al. [18]. In the same study, lead inhibited DHA in the bacterium at an EC_{50} of 0.135 0.007 mM, after 24 h. Similarly, in a study on ternary mixtures of two metals and SDS on aquatic bacterium (*Serratia marcescens* (SerEW01)), 24-h EC_{50} thresholds of 0.058 ± 0.002 mM Cd and 0.113 ± 0.005 mM Pb were reported to inhibit DHA [24]. *Lysinibacillus fusiformis* was more sensitive to the toxic effect of cadmium but more tolerant to the effect of lead than the other bacteria. The observed differences in sensitivity could be due to variations in genetic makeup of the test organisms. Although better tolerance to heavy metals by Gram negative bacteria compared to Gram positive bacteria has been reported [33], *L. fusiformis* relative tolerance to lead in this study is quite surprising. Nevertheless, heavy metal tolerance by soil bacteria has been reported [34,35].

Zinc, nickel and cobalt are trace elements, required in small quantities by bacteria for various physiological processes. They have however been reported to be toxic to soil bacteria at high concentrations. Zinc toxicity to microorganisms could lead to decrease in biomass, growth inhibition and even death [36]. In the present study, zinc inhibited the DHA in the soil bacterium by 50% at an EC_{50} of 0.037 ± 0.002 mM. In a study on combined effects of metals and chlorophenols on soil bacterial consortium, a 24-h EC_{50} toxicity threshold of 0.328 ± 0.015 mM Zn was reported by Nwanyanwu *et al.* [37]. Similarly, Nweke et al. [18] reported an EC_{50} threshold of 0.184 ± 0.017 mM Zn to inhibit DHA in *Pseudomonas fluorescens* isolated from soil.

Nickel and cobalt toxicities to soil bacteria at high concentrations have been reported. For instance, EC_{50}*s* of 0.649 ± 0.052 mM Ni and 0.041 ± 0.008 mM Co were reported to inhibit DHA by 50% in *Acinetobacter seifertii* isolated from Otamiri river sediment by Okechi et al. [38]. In the present study however, EC_{50} thresholds of 0.102 ± 0.004 mM Ni and 0.035 ± 0.003 mM Co were reported to inhibit DHA in *L. fusiformis*. Similarly, cobalt was a more potent DHA inhibitor than nickel against the soil bacterium. Similar assertion has been made previously [18–24].

Although sodium dodecyl sulfate was previously regarded as being safe due to its ease of biodegradation, acute toxicity of SDS to some organisms and microbial community structure have however been reported. For instance, study has shown that 2–6 mg/L SDS (≈ 0.69–2.08 mM SDS) significantly changed species and gene functions of the soil microorganisms in lake-terrestrial ecotone [39]. Similarly, equi-effect concentration values of 2.810 ± 0.140 mM and 2.329 ± 0.092 mM were reported to inhibit DHA by 50% in *A. seifertii* and *S. marcescens* (SerEW01) respectively [23]. In the present study, SDS inhibited DHA in *L. fusiformis* at EC_{50} threshold of 2.613 ±0.173 mM. Sodium dodecyl sulfate exerts its toxic effect principally on membrane structures; it can equally induce lipid peroxidation, as well as increase production of glutathione and alterations in carbon metabolism. The observed differences in EC_{50} thresholds could be due to differences in the bacterial genetic makeups and physiology, as organisms are known to react differently even to the same toxicant [40, 41]. The order of toxicity ranking of the individual toxicants in this study is Cd^{2+}> Co^{2+}> Zn^{2+}> Ni^{2+}> Pb^{2+}> SDS. This shows that the heavy metals were more toxic to the soil bacterium than the anionic surfactant SDS. Most metals have been reported to be more toxic to microorganisms than SDS [42,43].

Bacterial genera such as *Lysinibacillus, Staphylococcus, Bacillus* and *Paenibacillus* have been reported to biodegrade sodium dodecyl sulfate in detergent wastewater [21]. Similarly, *Lysinibacillus* was recently reported to be the second most abundant heterotrophic bacteria in Otamiri river [44].

The binary mixtures of SDS and heavy metal were more toxic than SDS alone but relatively less toxic than the individual heavy metals. These could be seen from their 24 h EC_{50} thresholds values. The interactive effects of the mixture components have resulted in SDS seemingly reducing the toxic effects of the metal ions in the mixtures compared to their metals toxicities. Similarly, the metals may have enhanced the toxicity of SDS in all the binary mixtures, giving rise to mixture toxicities much lower than that of SDS as an individual toxicant. These results agreed with the reports of modulation in toxicities between heavy metals and anionic surfactants [38–45]. Similarly, some anionic surfactants have also been reported to enhance the toxicities of co-existing chemical species, such as metals [38]. This seems to be the case with lead in this study, as the toxicities of most SDS + Pb^{2+} binary mixture ratios are lower than that of lead as an individual toxicant.

The model deviation ratio, toxic index and isobologram analysis determined on the basis of EC_{50} thresholds**,** showed that the interactive effect of the binary mixtures were both weakly and strongly synergistic against *L. fusiformis* DHA. The effect of ABCR1 mixture ratio of SDS+Cd^{2+} binary mixture was however additive to the soil bacterium. The weak synergy reported in some of the mixture ratios of SDS+Ni^{2+} and SDS+Cd^{2+} binary mixtures could be due to the modulation effects of SDS on the metal ions as noted earlier. Marginal antagonistic, as well as weak and strong synergistic interactions were reported in binary mixtures of SDS and metal ions against *Acinetobacter seifertii* [29]. Similarly, both synergism and all effect levels were reported in a study on the binary mixtures of heavy metals and phenols as well as metals and chlorophenols against DHA in *Pseudomonas fluorescens* isolated from soil and bacterial consortium respectively [18–37].

In SDS + Cd^{2+} mixtures, both models correctly predicted the experimentally-derived data at different points or concentrations for ABCR1 mixture ratio while slightly underestimating the experimental data in the other mixture ratios against the soil bacterium. Similarly, CA and IA models greatly predicted lower toxicity in other binary mixtures, except in EECR50 mixture ratio of SDS+Pb^{2+} mixture where IA model correctly predicted the experimental data. Such good predictions by both CA and IA models on the binary mixtures of Zn + Cd and Ni + Co against *Pseudomonas fluorescens* has been reported elsewhere [46]. Furthermore, accurate prediction of metal mixture toxicities by IA model at low effect levels was reported against *Daphnia magna, Ceriodaphnia dubia*, and *Hordeum vulgare* by Nys et al. [47]. However, both underestimation and overestimation of the binary mixture of SDS and Cd by both models was reported against *A. seifertii* [29]. These observed differences could be due to differences in both mixture components and the test bacteria.

In addition, both models predicted similar EC_{50} equi-effect toxicities in SDS + Zn^{2+} binary mixture against the soil bacterium. According to [48], both models can show similar predictions 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. A 50% effective concentration

(EC_{50}) predicted by both models for the binary mixtures of SDS and some metals against *A. seifertii* were reported to be identical [29]. Sodium dodecyl sulfate and heavy metals may have similar mode of action against *L. fusiformis*, thus there was no significant difference between toxicities determined from CA and IA models for most of the binary mixtures of the toxicants. Similar assertion was made by previous authors [49].

The toxicity indices and the models deviation ratios employed in analyzing the effect of the ternary mixtures on the DHA in *L. fusiformis* indicated similar results. In all the ternary mixtures, the TI values were much less than 1 and MDR greater than 2. These results indicate that the interactive effects of the mixtures against the soil bacterium were strongly synergistic [27,28]. Synergistic interaction was reported in ternary mixtures of SDS and heavy metals against *Serratia marcescens* (SerEW01) by Okechi et al. [24]. Similarly, a synergistic effect was also reported in ternary mixtures of Cu + Zn + Pb and Cu + Zn + Cd against sea urchin embryo-larvae. In the same study, Pb + Cd + Cu and Pb + Cd + Zn ternary mixtures, however, exhibited an additive effect on the embryo-larvae [50]. Though the components of the ternary mixtures in our study, the end point monitored, as well as the test organism, differ from those studied by Xu and co-workers, nevertheless, the types of interactions shown by the components of any mixtures are largely dependent on their relative proportions in the mixtures [51].

Concentration addition (CA) and independent action (IA) models were used in predicting the effect of the mixtures on the soil bacterium in the present study. In the present study, CA and IA models predicted lower toxicities for the ternary mixtures of SDS and heavy metals compared to the experimental data. Similar predictions of lower toxicities by both models against planktonic bacteria were reported by Okechi et al. [24]. Furthermore, Nweke et al. [46] also reported underestimation of the ternary mixtures toxicities of heavy metals by both models against *Pseudomonas fluorescens.* SDS and metal ions may exhibit identical mode of action on *L. fusiformis*; thus, no statistical differences were observed in the toxicity thresholds determined on the basis of CA and IA models for ABCR1 and ABCR2 mixture ratios of SDS + Cd^{2+} + Zn^{2+} ternary mixtures, as observed earlier. Similar observations have been reported elsewhere [47–50].

6. Conclusion

The effects of the binary and ternary mixtures of SDS and some heavy metals against *Lysinibacillus fusiformis* isolated from vegetable farms (irrigated with the toxicants contaminated river water) at the bank of the Otamiri River, Owerri, Imo State, Nigeria, were evaluated, with the inhibition of DHA as the end point. The interactive effects of the mixtures were strongly synergistic, and the CA and IA models greatly underestimated the mixture toxicities against the soil bacterium. Thus, the continued use of such water to irrigate the farms could impact negatively on the soil bacterial community and, by extension, the soil fertility.

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