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Joint toxicity of binary mixtures of surfactants and heavy metals on *Micractinum pusillum* isolated from Otamiri River

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Abstract

Toxicity of lead (Pb), cadmium (Cd), cobalt (Co), nickel (Ni), zinc (Zn), sodium dodecylsulphate (SDS), cetylpyridinium chloride (CPC) and their binary mixtures were evaluated based on the growth (OD_{610}) inhibition of *Micractinum pusillum*. The responses of the organism to the toxicity of Pb^{2+}, Cd^{2+}, Co^{2+} and Ni^{2+} showed a dose-dependent inhibition to the microbial growth while Zn^{2+} , SDS and CPC showed hormetic curves which were characterized by stimulation of growth at low concentrations and toxicity at high concentrations. Zinc showed hormetic effect up to 1.0 mM while SDS showed hormetic effect at concentrations up to 2.5 mM. Relatively, CPC among other toxicants exhibited greater stimulatory effect on the growth of the alga at concentration up to 8.0 mM. At doses above the stimulatory range, the toxicants progressively inhibited the growth of the organism. The EC_{50} values were evaluated using monotonic and hormetic dose-response models. The EC_{50} of the toxicants ranged from 0.12±0.05 to 11.88±1.60 mM. Statistically, the results obtained from the Tukey HSD POSTHOC test showed that the EC_{50} values of the toxicants were significantly different ($P<0.05$) and the order of decreasing toxicity is $Co^{2+}>Pb^{2+}>Ni^{2+}>Cd^{2+}>Zn^{2+}>SDS>CPC$. The results of the joint effect of the mixtures on the growth of the test organism indicated additive, antagonistic and synergistic interactions for the mixtures analyzed. The chemicals may accumulate in the biota and get bio-magnified in the food chain, ultimately affecting man. Therefore, the results have the potential to inform decision-making processes related to environmental protection, chemical management, and public health.

Keywords: Joint toxicity; Surfactant; Heavy metal; Microalgae; Effective concentration; Toxic index

1. Introduction

Surfactants are widely used in daily life and industry in significant quantities. After use, surfactant residues are dumped into sewage systems or surface waters (Invally and Ju, 2017). Surfactants introduced into sewage systems may be degraded by wastewater treatment facilities. The degree of degradation, however, depends on a variety of factors, including the chemical structure of the surfactant and environmental factors including pH, temperature, and dissolved oxygen concentration (Invally and Ju, 2017). By acting at the surfaces and interfaces of different substances, surface active chemicals lower surface tension. The polar, hydrophilic head group and the non-polar, hydrophobic tail of surfactants are responsible for their amphipathic properties (Bajpai, 2018). They are helpful in many cleaning applications, such as detergents, emulsifiers, wetting and foaming agents, and many more, due to their remarkable solubility and cleaning characteristics (Ivanković and Hrenović, 2010).

Sodium dodecyl sulphate (SDS) is an anionic synthetic amphiphile that is widely used in pharmaceuticals, household detergents, cosmetics products such as shower gels, toothpastes, and soaps, as well as industrial processes. It is one of

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the most difficult new toxins that wastewater frequently releases into the environment (Suaibu *et al*., 2021). This is why they are harmful to the environment when they enter it through agrochemicals, industrial goods, and daily activities' effluents (Li *et al*., 2018b). Regarding its biological activity, SDS has the ability to bind to a wide range of biomolecules, such as starch, proteins, enzymes, and nucleic acids, as well as intercalate into various cellular components, such as phospholipid membranes (Sirisattha *et al*., 2004). According to Boccafoschi *et al*. (2017), it has the ability to solubilize nuclear and cellular membranes, denature and change the ultrastructure of proteins. In particular, SDS binding affects the surface charge of proteins, which can lead to protein unfolding and loss of cell function (Sirisattha *et al*., 2004).

Another surfactant, a cationic quaternary ammonium that is frequently used as an antibacterial agent in pharmaceutical and personal care products (PPCPs) is cetylpyridinium chloride (CPC, $C_{21}H_{38}ClN$). It is particularly used in oral hygiene products like toothpaste and mouthwash (Pottel *et al*., 2020). Items containing CPC may be discharged directly into the environment or into wastewater treatment plants (WWTPs) due to their widespread use (Russo *et al*., 2019).

The CPC's hexadecane chain causes the lipid membrane to become disorganized, which causes microbial cell membrane to burst completely. This mode of action illustrates the broad-spectrum antibacterial activity of CPC as an antiseptic or disinfectant in a range of formulations, including mouthwashes, lozenges, tablets, and cleaning solutions for foodstuffs (Nasila *et al*., 2021).

Elements with atomic masses greater than 20 and densities greater than 5.0 g/cm3 are classified as heavy metals (Ali and Khan, 2017). Due to weathering of soils and rocks, mining, waste processing, fertilizer use, corrosion of pipes, wires, and sheets, burning of coal and wood, sand mining, oil spills, and agricultural operations, metals are introduced into aquatic systems (Nwankwoala and Ekpewerechi, 2007; Okechi and Chukwura, 2020; Kalu *et al*., 2023). Biological systems are used to classify heavy metals as essential or non-essential. The essential heavy metals like nickel, iron, and zinc are needed by living things and are necessary for biological processes while non-essential heavy metals, including lead, cadmium, and mercury, are poisons and are not necessary for the biological systems (Nweke *et al*., 2018). For distinct groups of life, such as plants, animals, and microbes, the necessity of heavy metals may differ. However, heavy metals are found naturally in the environment and are thought to be necessary for life because of the physiological and biochemical roles they play in biological systems. If they get beyond a certain threshold, they might potentially have detrimental effects on health. Even at low concentrations, aquatic creatures such as plankton, aquatic plants, invertebrates, and vertebrates are negatively impacted by toxic heavy metals (Atici *et al*., 2008). The main negative impacts of heavy metals on microalgae are lowered enzyme activity, impaired cell growth, and hindrances to photosynthesis and the production of organic molecules (Soad, 2016).

One type of freshwater unicellular chlorella that is commonly seen in rivers is *Micractinum pusillum*. According to Ming-Li and Siu-Wan (2018), they are the first species to be affected by pollution and the main producers at the base of the aquatic food chain. Even non-lethal effects of pollution on primary producers may have an impact on the flow of energy up the food chain because photosynthesis is the basic process that underpins food webs. Their high photosynthetic efficiency, simple structure, high sensitivity and specificity in detecting pollutants, and ability to grow well in harsh environmental conditions like the presence of heavy metals, high salinity, nutrient stress, and extreme temperature have made them popular as bio-indicators of environmental change (Krytian *et al*., 2015).

Otamiri river is a highly significant river for the economy of Imo state (Kalu *et al*., 2023) and drains a variety of geologies, soil types, and land uses. Among the sources of pollution are the following: fertilizer application, leachate from refuse dumps, chemical fishing, swimming, and mining activities; corrosion of sheeting, wires, and pipes; discharge from washings; run-off of oil and grease from rising fuel stations and auto mechanic workshops, as well as wastes from hospitals and institutions (Nwankwoala and Ekpewerechi, 2007; Okechi and Chukwura, 2020). These activities may have contributed to the reported contamination of river water and sediment by surfactants and heavy metals. As a result, the river's water quality indicators have been compromised (Kalu *et al*., 2023). Otamiri river water and sediment have recently been discovered to contain sodium dodecyl sulphate as well as other anionic and cationic surfactants (Kalu *et al*., 2023; Okechi and Chukwura, 2020). According to earlier studies (Soad, 2016; Batsalova *et al*., 2017; Ming-Li and Siu-Wan 2018; Nweke *et al*., 2018; Okechi *et al*., 2020b), heavy metals and their mixes are harmful to living things at different concentrations. As with surfactants, reports have been made regarding their toxicity to living things (Shrivas and Wu, 2007; Lima *et al*., 2011; Azizullah *et al*., 2012; Okechi *et al*., 2020b). The toxicity of surfactants and heavy metal combinations on microalga isolated from Otamiri river water, however, is not well understood. Hence, the study assessed the effects of their binary mixtures because of the possibility of toxicity from synergistic interactions on microalgae.

2. Materials and Methods

2.1. Sample Collection, Isolation and Cultivation

Sample collection and isolation of microalgae were done as described by Kalu *et al*. (2023). The micropipette method, as outlined in Kalu *et al*. (2023), was used for isolation. Using 18S rRNA gene partial sequencing, the microalgal isolate was subjected to molecular characterization. *Micractinum pusillum* was confirmed to be the identification. Stock culture of the isolate was inoculated into fresh sterilized Bristol medium in a ratio of 1/10. The medium contained ampicillin ($100\mu gL^{-1}$), chloramphenicol ($25\mu gL^{-1}$) and amphotericin B ($2.5\mu gL^{-1}$) in a 1-L flask to inhibit the growth of bacteria and fungi. The flask was incubated for 96 hours under natural low light (veranda reflection) (Oyewumi and Olukunle, 2018) and shaken periodically on a shaker (HY-4AKS) at a speed of 150 rpm. According to Abdelaziz *et al*. (2014), all cultures were exposed to ambient carbon dioxide (CO_2) by plugging the culture flask with sterile cotton wool. Harvesting and washing of algal cells was done as described in Kalu *et al*. (2023). Algae cells at exponential growth phase (96h) were harvested by centrifugation at 3000rpm for 15minutes. Harvested cells were washed twice in sterile deionized water and suspended in the same medium.

2.2. Standardization of Isolate

The maximum wavelength of the isolate was inspected by scanning washed sample cultures between 540-750nm using a spectrophotometer (721D). To obtain cell suspension to be used as inoculua for the toxicity assay, 96 h old microalgae were standardized at an optical density (OD) of 0.5 at 610nm by diluting the washed cells with sterile deionized water and measuring the OD using a UV-Visible (721D) (Ming-Li and Siu-Wan, 2018).

2.3. Toxicity assay of individual heavy metal and SDS

To evaluate the toxicity, growth inhibition (OD610) was employed. The reaction mixture was made up of 2-ml final volume. Sterile deionized water and stock solutions of the appropriate SDS/CPC or metal ions were added in the necessary quantities to each 15-ml culture tube that had 1.0 ml of Bristol medium (pH 7.0). Then, each tube received 0.1 ml of the standardized microalgal suspension. For every concentration of SDS, CPC and metal ions, triplicates of each were prepared, and control tubes were made without toxicants for each surfactant and metal ion. Incubation and culture conditions were as described above. The inhibition of growth (OD_{610}) was measured using a UV-VIS Spectrophotometer (721D) following 96 hours of incubation.

2.4. Binary mixture ratios

Using a fixed ratio design, the binary mixtures of SDS, CPC and each of the five heavy metals (Pb^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, and Zn^{2+}) were examined in relation to weight to weight ratios. German-based Sigma-Aldrich Corporations provided analytical grades of the salts utilized as $Pb(NO_3)_2$, $CdSO_4.8H_2O$, $NiSO_4.6H_2O$, $Co(NO_3)_2.6H_2O$, and $ZnNO_3.6H_2O$. The tests were prepared using arbitrary concentration ratios (ABCR). P (%) = SDS or CPC and 100-p (%) = metal ions were the ratios used to combine the mixtures. The combinations were made by mixing the necessary volumes of the heavy metal and SDS or CPC stock solutions to create a certain concentration ratio, starting with 10 mM and 100 mM stock concentrations (for the heavy metals and SDS, respectively). To establish the whole dose-response relationship, the component concentrations were changed for each mixture at a fixed mixture ratio. During the toxicity assay, the mixtures were examined as a single toxicant solution (Okechi *et al*., 2020b).

2.5. Toxicity assay of binary mixtures

The toxicity testing was conducted in separate 15-ml test tubes using 2-ml volumes of Bristol medium supplemented with different doses of SDS,CPC, Pb^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, or Zn^{2+}. To achieve graded concentrations of binary mixtures of SDS/CPC + metal ratios, 0.5 ml of Bristol medium, necessary amounts of sterile deionized water, and the corresponding SDS/CPC + heavy metal mixtures were added to each triplicate tube (test and control) to obtain graded concentrations of binary mixture ratios. The standardized microalgal suspension (0.5 ml) was then added into each tube. The tubes were incubated for ninety-six hours as previously mentioned under low natural light. After incubation, growth inhibition (OD_{610}) was measured with a UV-VIS Spectrophotometer (721D).

2.6. Estimation of toxicity threshold (EC_{50})

As demonstrated in Okechi *et al*. (2020b), the response of the organism to the SDS/CPC and metal ions as individuals and mixtures was determined as mean percent inhibition of growth (R) relative to the mean control (equ. 1). Three sets of results were used to calculate the mean and standard deviations.

$$R = \left(\frac{C_A - T_A}{C_A}\right) x100 \quad (1)$$

Where: R = %Inhibition, C_A is the mean absorbance in control, T_A is mean absorbance in test for growth with different concentrations of toxicants as single or binary mixtures.

The EC_{50s} were calculated by graphing and fitting the dose- response curve into logistic, 2-parameter model (equ.2). For hormesis, i.e. stimulation of growth at low concentration of individual toxicant or the mixtures, the EC_{50} was estimated using reparameterized Brain-Cousens model (equ. 3) (Schabenberger *et al*., 1999).

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

$$R = 100 - \left(\frac{100+fx}{1+\left[1+\left\{\frac{2fEC_{50}}{100}\right\}\right]\left(\frac{x}{EC_{50}}\right)^b}\right) \quad (3)$$

Where x is the surfactant or metal ion concentration, EC_{50} is the concentration of the toxicant that inhibited growth by 50%, b is the slope at EC_{50} and f is the parameter describing the degree of hormetic response.

Determination of Toxic Index (TI)

The Toxic index model was used to analyze the combined effect of the mixtures as described in equ. 4.

$$TI = \sum_{i=1}^{n} TU_i \quad (4)$$

TU_i is the toxic unit of the ith component in the mixture. Each toxic unit was calculated from the relationship:

$$TU_i = \frac{C_{mixi} x \pi i}{EC_{50i}} \quad (5)$$

Where C_{mixi} is the concentration of the ith toxicant in the mixture, πi is the proportion of the individual component and EC_{50i} is the EC_{50} of the toxicant when tested singly.

TI =1 connotes additive interaction, TI< 1 depicts synergistic interaction while TI >1 describes antagonistic interaction (Boillot and Perrodin, 2008).

2.7. Data Analysis

All analyses were performed in three replicates. Curve fittings were done using Sigma Plot 10.0. Analysis of variance (ANOVA) was performed using IBM SPSS Statistics 25 to test the differences between the means at various concentrations. When the ANOVA results showed that the treatments were significant at P<0.05, Tukey's multiple comparison test was used to compare the values of the mean EC_{50s}.

3. Results and Discussion

3.1. Toxicity of individual toxicants to *Micractinium pusillum*

The responses of the organism to the toxicity of Pb^{2+}, Cd^{2+}, Co^{2+} and Ni^{2+} showed a dose dependent inhibition to the microbial growth (OD_{610}) while Zn^{2+} , SDS and CPC showed hormetic curves which were characterized by stimulation of growth at low concentrations and toxicity at high concentrations (Figure 1). Zinc showed hormetic effect up to 1.0 mM while SDS showed hormetic effect at concentrations up to 2.5 mM. Relatively, CPC among other toxicants exhibited greater stimulatory effect on the growth of the alga at concentration up to 8.0 mM. At doses above the stimulatory range, the toxicants progressively inhibited the growth of the organism. Table 1 shows the experimental toxicity threshold (EC_{50}) of individual metal ions and surfactants on *Micractinium pusillum*. The EC_{50} of the toxicants ranged from 0.12±0.05 to 11.88±1.60 mM. Cobalt (Co^{2+}) with EC_{50} of 0.12±0.05mM recorded the highest toxicity while CPC with EC_{50} of 11.88±1.60had the least toxic effect on the organism. Statistically, the results obtained from the Tukey HSD POSTHOC test showed that the EC_{50s} of the toxicants were significantly different (P<0.05) and the order of decreasing toxicity is Co^{2+}>Pb^{2+}>Ni^{2+}>Cd^{2+}>Zn^{2+}>SDS>CPC.

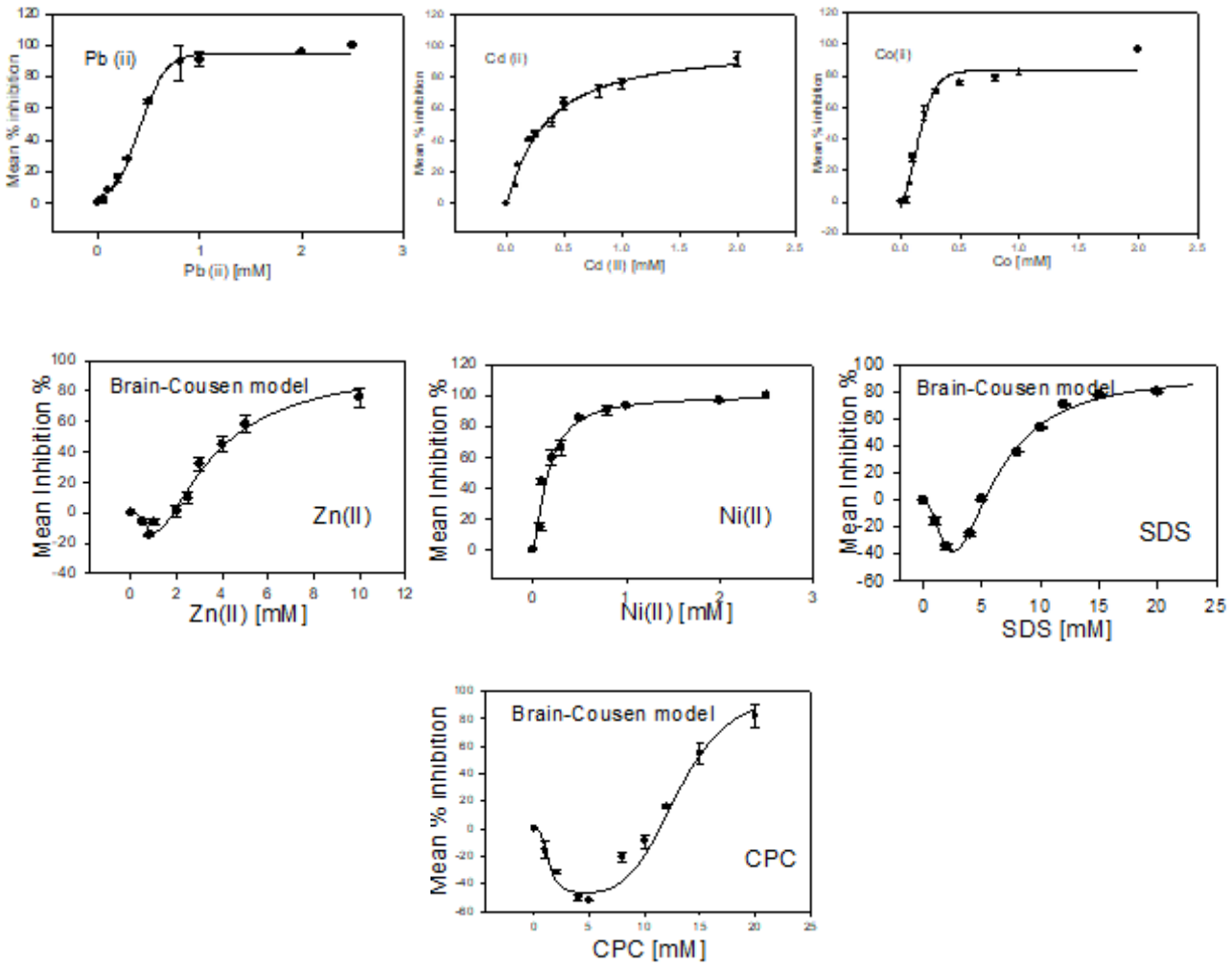


Figure 1 Growth inhibition of *M. pusillum* by single chemicals Pb (II), Cd(II),Co(II), Zn(II) Ni (II), SDS and CPC. The data points represent experimental dose-response data while the lines represent the toxicities obtained by fitting the data to logistic model or reparameterized Brain-Cousen model.

Table 1 Experimental Toxicity Thresholds (EC_{50}) of individual metal ions/surfactants on *M. pusillum*

Toxicant	**EC_{50} (mM)**
Pb^{2+}	0.41 ± 0.01^{b}
Cd^{2+}	1.33 ± 0.06^{d}
Co^{2+}	0.12 ± 0.05^{a}
Zn^{2+}	3.34 ± 0.27^{e}
Ni^{2+}	0.76 ± 0.02^{c}
SDS	7.89 ± 0.80^{f}
CPC	11.88 ± 1.60^{g}

The EC_{50} values of the toxicants are expressed as mean ± SD and they are significantly different at $P < 0.05$.

3.2. Toxicity of binary mixtures of SDS, CPC and heavy metals to *M. pusillum*

The dose-response relationships of the binary mixtures on *M. pusillum* are shown in Figure 2. The EC_{50} values obtained from the mixture reactions indicated a strong toxic effect of toxicants to the isolate as reflected in the lower toxic threshold concentration obtained. The EC_{50} values obtained in all the mixtures analyzed ranged from 0.10±0.05 to 3.21±1.10 mM. Statistically, they are different from each other ($P<0.05$) except ABCR- 3 in SDS+Ni mixture, ABCR-2 in SDS+Cd and ABCR-3 in CPC + Zn mixture which showed no significant difference.

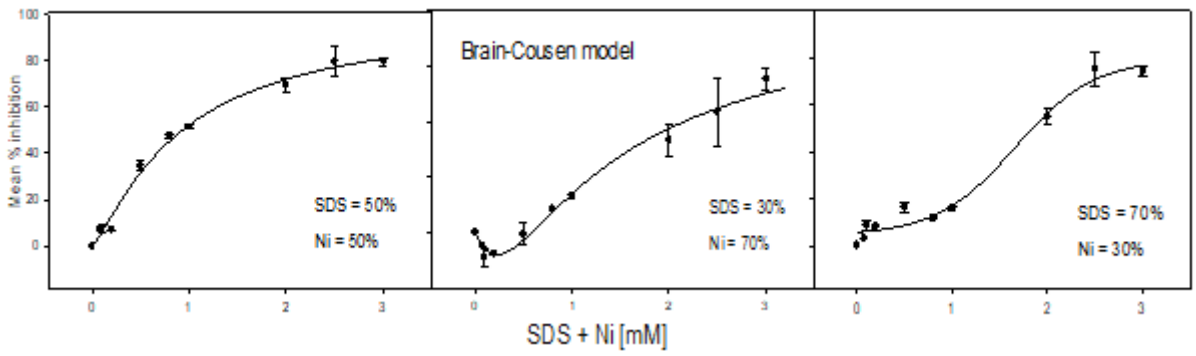
Figure 2 and Table 2 show the growth inhibition of SDS and metal mixtures. SDS 30%+ Ni^{2+} 70% (ABCR 1) binary mixture ratio had biphasic effect with stimulation of growth at concentration range of 0.0 to 0.4 mM. As binary mixtures, 96 h EC_{50} obtained showed that 50% SDS + 50% Ni among the various mixtures of sodium dodecyl sulphate and nickel had highest toxicity with EC_{50} value of 0.93 ± 0.21 mM while 30% SDS + 70% Ni with EC_{50} of 1.81±0.84 mM was least toxic. Their toxicity thresholds are significantly ($P<0.05$). In ABCR 3 mixture ratio, the EC_{50} is comparable to the EC_{50} of Ni used as a single toxicant. The mixtures of SDS and Ni (SDS+Ni) at the ratio of 70%SDS +30%Ni had TI value of 1.68±0.21 showing that the mixture had an antagonistic effect. On the other hand, ABCR 2 and ABCR 3 ratios synergistically exerted toxic effect on the test organism.

In the case of SDS + Cd binary mixtures, the mixtures inhibited growth in a dose-dependent manner. The concentration ratio 30% SDS + 70% Cd showed highest toxicity with EC_{50} of 0.93±0.01 mM. The three mixture ratios ABCR 1, ABCR 2 and ABCR 3 are significantly different ($P<0.05$). 30%SDS and 70%Cd mixture had comparable toxic effect with toxicity threshold related to that of cadmium as single chemical. The toxic effects derived from the toxic index show that ABCR 1, ABCR 2, ABCR 3 are antagonistic, synergistic and additive respectively.

Among the SDS+Pb concentration ratios, 70% SDS + 30% Pb with EC_{50} of 1.35 ± 0.68 mM and 50% SDS + 50% Pb with EC_{50} of 1.82 ± 0.34 mM had the highest and lowest toxicities respectively. Binary mixtures of sodium dodecyl sulphate and lead showed relatively low toxicity with EC_{50} values that were higher than the value observed for lead alone. The mixtures of SDS + Pb at the three different mixture ratios of 70%SDS +30%Pb, 30%SDS +70%Pb and 50%SDS+50%Pb were all antagonistic as their TI values were greater than one.

The EC_{50} obtained showed that 30% SDS + 70% Co among the mixtures of sodium dodecyl sulphate and cobalt had highest toxicity with EC_{50} value of 1.09 ± 0.58 mM while 70% SDS + 30% Co with EC_{50} of 2.29±0.62 mM was least toxic. Their EC_{50s} are significantly different ($P<0.05$). Relatively, the toxicity of cobalt as a single toxicant is higher than the toxicity of the mixture ratios base on the EC_{50} values. The mixture showed a biphasic interaction response at equilibrium concentration as stimulation and inhibition were both observed. All the mixture ratios exhibited antagonism. The mixture reactions and responses of SDS+ Zn were not determined.

In all the mixture ratios of cetylpyridinium chloride (CPC) with the heavy metals, the response of the test organism to the toxicants was dose-dependent (Figure 3, Table 3). The mean EC_{50} values are significantly different ($P<0.05$). ABCR 3 of CPC+Pb mixture was found to be most toxic with an EC_{50} of 0.10±0.05 mM while ABCR 2 of CPC+Zn mixture with EC_{50} of 3.21±1.10 mM showed the least toxicity to *M. pusillum*. ABCR 1 and ABCR 2 of CPC+Ni ratios produced antagonistic effect whereas ABCR 3 showed additive effect. The interactions of cetylpyridinium chloride and cadmium mixture ratios were antagonistic except for ABCR 2 which was synergistic. While the interaction effect of CPC+Zn were all synergistic, the response of *M. pusillum* to CPC+Pb mixture ratios produced synergistic, additive and antagonistic effects for ABCR 1, ABCR 2 and ABCR 3 respectively.



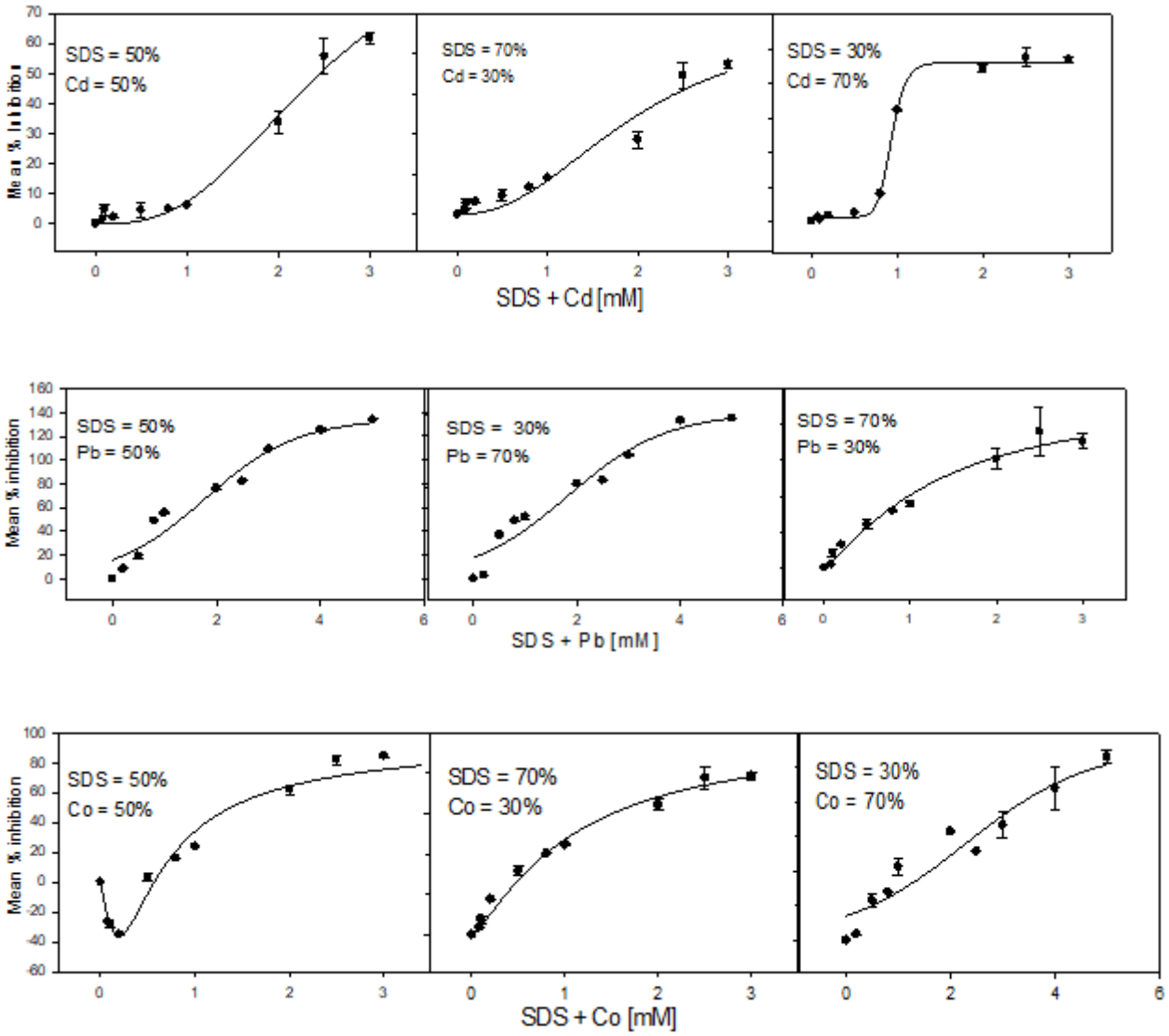


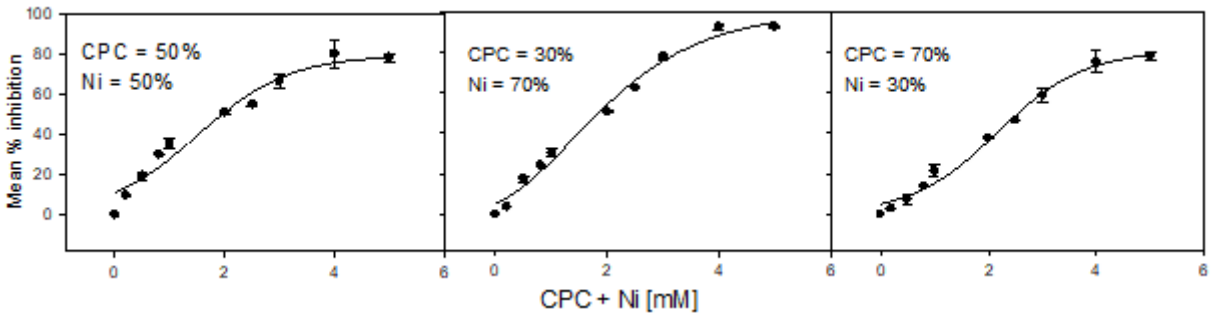
Figure 2 Growth (OD_{610}) inhibition of *M. pusillum* by binary mixtures of SDS+Ni, SDS + Cd, SDS + Pb, and SDS + Co.

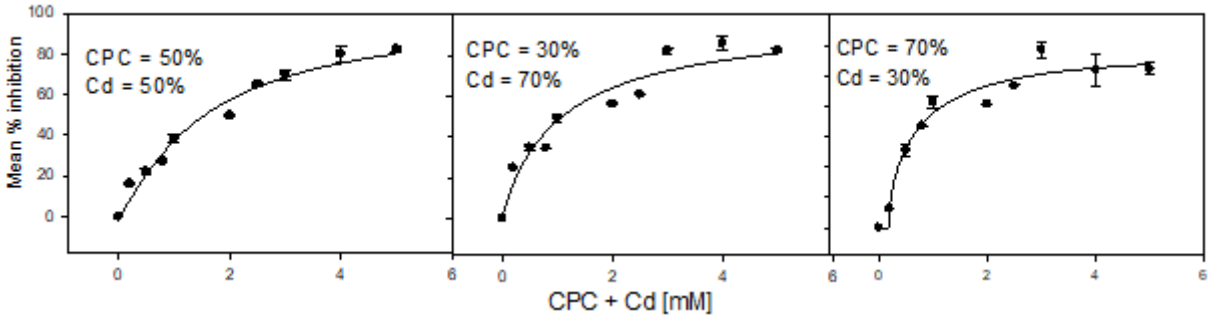
Table 2 The mean EC_{50}, Toxic Index and Toxic Effects of Binary mixtures of SDS and metal ions on *M. pusillum*

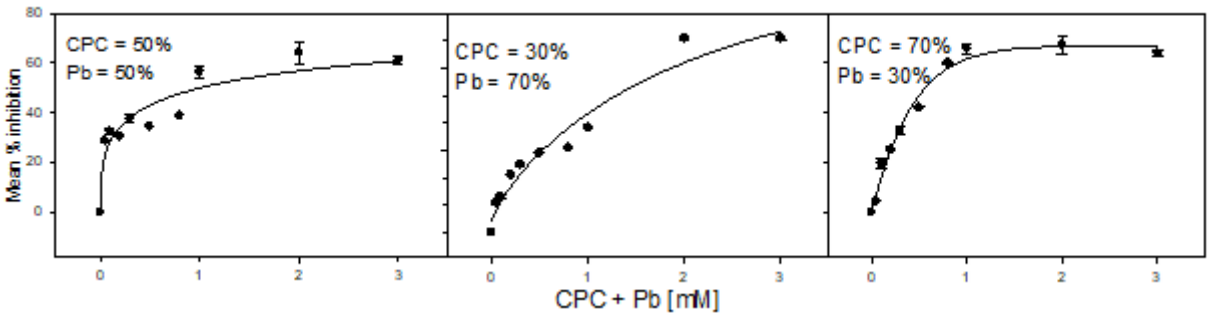
Toxicant mixture	R^2 value	EC_{50}(mM) ±SD	Toxic Index	Toxic effect
SDS + Ni				
70%SDS +30%Ni (ABCR 1)	0.9818	$1.66±0.15^{j}$	1.68±0.21	Antagonism
30%SDS +70%Ni (ABCR 2)	0.9811	$1.81±0.84^{k}$	0.78±0.03	Synergism
50%SDS +50%Ni (ABCR 3)	0.9919	$0.93±0.21^{d}$	0.67±0.32	Synergism
SDS + Cd				
70%SDS +30%Cd (ABCR 1)	0.9542	$2.02±1.02^{l}$	1.24±0.72	Antagonism
30%SDS +70%Cd (ABCR 2)	0.9979	$0.93±0.01^{d}$	0.25±0.11	Synergism
50%SDS +50%Cd (ABCR 3)	0.9859	$2.45±0.60^{m}$	1.08±0.41	Additive
SDS + Pb				

70%SDS +30%Pb (ABCR 1)	0.9796	1.35±0.68^{g}	2.42±0.54	Antagonism
30%SDS +70%Pb (ABCR 2)	0.9495	1.77±0.29^{k}	1.67±0.19	Antagonism
50%SDS+50%Pb (ABCR 3)	0.9412	1.82±0.34^{k}	2.16±0.29	Antagonism
SDS +Co				
70%SDS +30%Co (ABCR 1)	0.9160	2.29±0.62^{m}	14.21±0.70	Antagonism
30%SDS +70%Co (ABCR 2)	0.9670	1.09±0.08^{d}	3.32±0.60	Antagonism
50%SDS +50%Co (ABCR 3)	0.9468	1.31±0.73^{f}	5.54±0.68	Antagonism

Values are represented as mean ± SD. EC_{50} values with different superscript are significantly different (P<0.05).







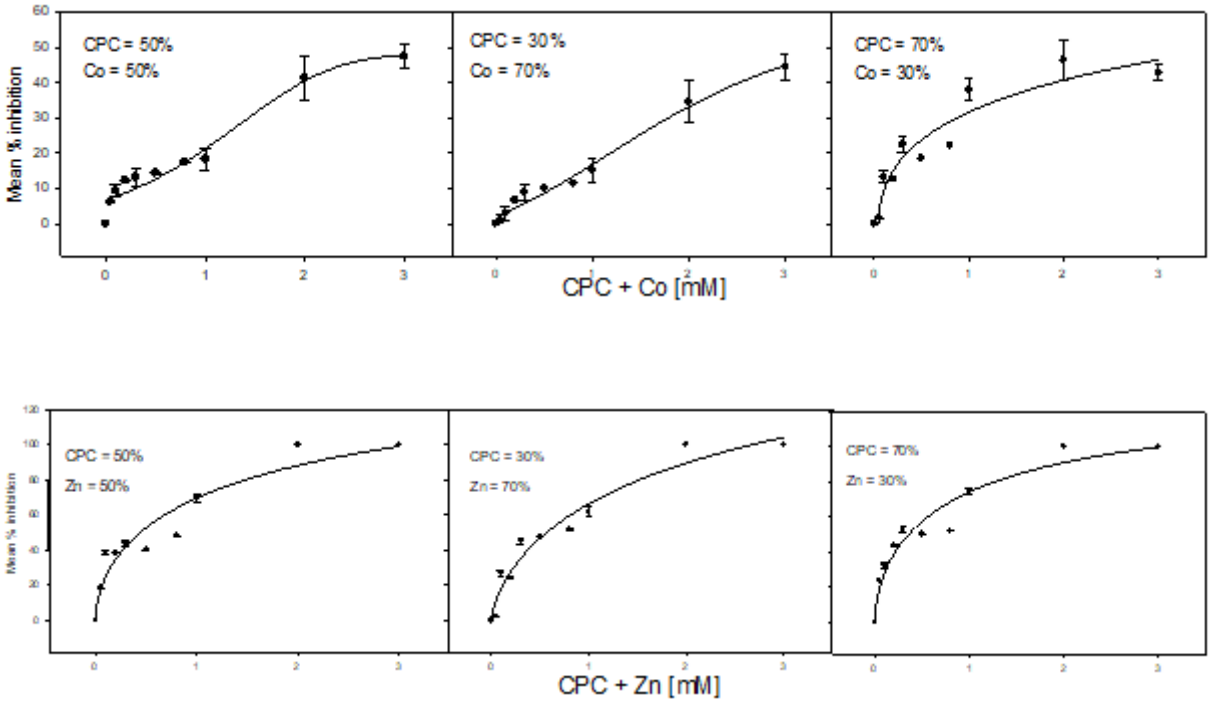


Figure 3 Growth (OD_{610}) inhibition of *M. pusillum* by binary mixtures of CPC+Ni, CPC+Cd, CPC+Pb, and CPC+ Co.

Table 3 The mean EC_{50}, Toxic Index and Toxic Effects of Binary mixtures of CPC and Metal ions on *M. pusillum*

Toxicant mixture	R^2 value	EC_{50}(mM)	Toxic Index	Toxic effect
CPC +Ni				
70%CPC+30%Ni (ABCR 1)	0.9949	1.67 ± 0.36^{j}	2.29±0.27	Antagonism
30%CPC+70%Ni (ABCR 2)	0.9868	1.37 ± 0.13^{g}	1.14±0.22	Antagonism
50%CPC+50%Ni (ABCR 3)	0.9589	1.51 ± 0.23^{h}	1.08±0.19	Additive
CPC +Cd				
70%CPC+30%Cd (ABCR 1)	0.9644	0.55 ± 0.14^{c}	1.25±0.23	Antagonism
30%CPC+70%Cd (ABCR 2)	0.9388	1.08 ± 0.04^{d}	0.37±0.67	Synergism
50%CPC+50%Cd (ABCR 3)	0.9765	1.55 ± 0.62^{i}	1.38±0.58	Antagonism
CPC+Pb				
70%CPC+30%Pb (ABCR 1)	0.9830	0.29 ± 0.03^{b}	0.28±0.04	Synergism
30%CPC+70%Pb (ABCR 2)	0.9567	1.36 ± 1.02^{g}	1.03±0.25	Additive
50%CPC+50%Pb (ABCR 3)	0.9020	0.10 ± 0.05^{a}	2.26±0.48	Antagonism
CPC+Co				
70%CPC+30%Co (ABCR 1)	0.9169	2.02 ± 0.42^{l}	1.89±0.32	Antagonism
30%CPC+70%Co (ABCR 2)	0.9824	1.64 ± 0.79^{j}	1.16±0.65	Antagonism
50%CPC+50%Co (ABCR 3)	0.9584	1.14 ± 0.18^{e}	1.60±0.22	Antagonism
CPC+Zn				
70%CPC+30%Zn (ABCR 1)	0.9431	0.55 ± 0.19^{c}	0.29±0.14	Synergism

30%CPC+70%Zn (ABCR 2)	0.9557	3.21 ± 1.10^{n}	0.12±0.01	Synergism
50%CPC+50%Zn (ABCR 3)	0.9590	0.92 ± 0.20^{d}	0.40±0.07	Synergism

Values are represented as mean ± SD. EC_{50} values with different superscript are significantly different (P<0.05).

4. Discussion

4.1. Toxicity of Single Metal ions (Pb^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Zn^{2+}) and Surfactants (SDS and CPC) to *M. pusillum*.

There is no doubt that microorganisms respond differently to different heavy metals under different conditions. Some can mobilize, transform, uptake, and immobilize heavy metals upon interaction (Verma and Kuila, 2019). And so can be efficiently used as bioaccumulators of these toxicants in aquatic ecosystems and effluents in bioremediation activities without generating toxic by-products (Sharma and Dietz, 2009) whereas, further increases beyond a particular threshold exerts deleterious effects that are mostly concentration-dependent (Abhinandan *et al*., 2019).

As seen in Table1, *M. pusillum* showed significantly different toxicities on exposure to Co^{2+}, Pb^{2+}, Ni^{2+}, Cd^{2+}, SDS and CPC as indicated by their EC_{50s}. Okechi *et al*. (2020b) similarly recorded statistically different EC_{50} values of all the toxicants they studied on *Acinetobacter seifertii.* However, their results indicated that Cd^{2+}exhibited the highest toxicity (0.011 ± 0.00 mM), while SDS showed the least (2.810 ± 0.14 mM). But in the present work, Co^{2+} is the most toxic of all the toxicants (0.12±0.05 mM) whereas CPC showed the least toxicity (11.88±1.60 mM). Further, the order of decreasing toxicity for *A. seifertii* in the study of Okechi *et al*. (2020b) was Cd^{2+}> Co^{2+}> Zn^{2+}> Pb^{2+}>Ni^{2+}> SDS, that of Su (2013) from high to low on the inhibition of the growth of *Chlorella autotrophica* was Hg^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+} and Cd^{2+}, while in the present study, the order of decreasing toxicity was Co^{2+}> Pb^{2+}> Ni^{2+}> Cd^{2+}> Zn^{2+}> SDS > CPC for *M. pusillum*, Another study evaluated the acute toxic effects of Cu^{2+}, Cd^{2+} and Zn^{2+} on microalgae *Karenia mikimotoi* and *Prorocentrum minimum* and recorded a gradual decrease in the relative growth rate of both populations of the two marine microalga with the increasing concentrations of the heavy metals, and the sensitivity was Cu^{2+}> Cd^{2+}> $Zn^{2+,}$ though *P. minimum* was more sensitive to the toxicant stress (Yu *et al*., 2012).The disparity could relate to their evolutionary differences, their isolation sites, minimum metal concentrations applied, and method of data analysis employed.

Furthermore, the growth inhibition of *M. pusillum* by the toxicants was dose- dependent while the responses to $Zn^{2+,}$ SDS and CPC had biphasic effect. This agrees with the findings of Okechi et al. (2020b) in the case of *S. marcenses* where the toxicants progressively inhibited the dehydrogenase activity at increased concentrations. Onyeukwu *et al*. (2023) reported that the inhibition of *Chlorella vulgaris* phosphatase activity by single metals –Cu^{2+}, Pb^{2+}, Cr^{2+}, Cd^{2+}, Zn^{2+} and 2,4-D - occurred in a decreasing order Cr > Cu > Zn >Pb> Cd > 2,4-D.

Abhinandan *et al*. (2019) cultivated two acid-tolerant microalgae namely *Heterochlorella* sp. MAS3, and *Desmodesmus* sp. MAS1 at pH 3.5 for mitigation of heavy metals such as Cu, Fe, Mn, and Zn and recorded that 10–20 mg L^{-1} concentration of Cu supported the growth of microbes which resulted to 40–60% of heavy metal removal. In the present study, *M. pusillum* was inhibited even at low concentrations, as reflected in the EC_{50} values of 0.12±0.05 and 3.34±0.27 for Co and Zn respectively showing that Co^{2+} exhibited the most toxicity.

When the dose-response data of Pb (II) and Cd(II) were fitted to logistic model, the EC_{50} values were 0.41±0.01(Pb^{2+}) and 1.33±0.06 (Cd^{2+}). A similar study by Zamni-Ahmadmahmoodi *et al*. (2020) which investigated the acute (72h) and sub-acute (14 days) toxicity of lead and cadmium to the green microalga, *Nannochloropsis oculata* (modified OECD guideline (No. 201) reported 72-h IC_{50} values of Pb, and Cd exposed to *N. oculata* as 1.81, and 4.97 mgL^{-1}, respectively which indicated that Lead is about 2.7 times more toxic than Cadmium. He *et al*. (2017) reported that Cd disturbs respiration in plants and algae while Cheng *et al*. (2016) reported its effect on the growth and antioxidant response for freshwater algae *Chlorella vulgaris*.

4.2. Toxicity of Binary mixtures of metal ions and surfactants to *M. pusillum*

The EC_{50} values obtained from all the mixtures analyzed indicated a strong toxic effect of toxicants to *M. pusillum* and ranged from 0.10±0.05 to 3.21±1.10 mM. Statistically, they are different from each other (P<0.05) except ABCR- 3 in SDS+Ni mixture, ABCR-2 in SDS+Cd and ABCR-3 in CPC + Zn mixture. Similarly, in their toxicological assessment of anionic and amphoteric surfactants individually and in binary mixtures (applied at 95% concentration of the metal/ surfactant) on marine microalgae, *Phaeodactylum tricornutum*, Ríos *et al*. (2023) reported EC_{50} values ranging from 4.27 mgL^{-1} to 93.05 mgL^{-1}. The current study however was applied at varying percentage mixture concentrations of 30:70, 70:30 and 50:50, of surfactant/metal mixtures. This could account for the differences in reported EC_{50} ranges. Koppel *et al*. (2019) investigated cellular accumulation following exposure to a mixture of cadmium, copper, nickel, lead, and

zinc on two Antarctic marine microalgae, *Phaeocystis antarctica* and *Cryothecomona sarmigera*. In both microalgae, cellular cadmium, copper, and lead concentrations increased with increasing exposures while cellular nickel and zinc did not. For both microalgae, copper in the metal mixture drives inhibition of growth rate with R^2 values >0.84 for all cellular fractions in both species.

The toxic index results of the study indicated additive, antagonistic and synergistic interactions similar to the reports of Nwanyanwu *et al*. (2017), Nlemolisa *et al*. (2020) and Okechi *et al*. 2020b. Overall, in the present study, the EC_{50} values and R^2 values provide valuable information about the differential toxicity of SDS/CPC and metal mixtures, shedding light on the nuanced effects of their combinations on *M. pusillum* and contributing to the understanding of environmental toxicology and microbial responses to complex chemical mixtures.

5. Conclusion

In conclusion, the results of the toxicity study showed that exposing the test organism to the toxicants either as single or mixture toxicant pose great danger in the ecosystem. Research reports point to the possibility that chemicals, even when mixed at levels where they individually offer little danger, might cause joint toxicity. The toxic effects of some of the combinations were synergistic. This is worrisome as the combinations will produce greater damage than the effect of sum of the individual toxicants. The chemicals may accumulate in the biota and bio-magnify in the food chain, ultimately affecting man.

Compliance with ethical standards

Disclosure of conflict of interest

There are no conflicts of interest in connection with this paper, and the material described is not under publication or consideration for publication elsewhere.

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