



Ternary Mixture Toxicity of 2,4-Dichlorophenoxyacetic Acid and Heavy Metals on Alkaline Phosphatase Activity in *Desmodesmus abundans*

Ugochukwu Romanus Onyeukwu^{1*}, Chikodili Gladys Anaukwu², MaryRose Ogechi Echeta¹,
Chizoba Precious Tony-Nze¹ and Stephen Nnaemeka Ezekwueche¹

¹Department of Microbiology, Faculty of Science and Computing, University of Agriculture and Environmental Science, Umuagwo, P.M.B. 1038, Imo State, Nigeria.

²Department of Applied Microbiology and Brewing, Nnamdi Azikiwe, University of Awka, P.M.B. 5025, Anambra State, Nigeria.

*Corresponding author: ugorom4@gmail.com

Abstract	Article History
This study investigated the toxic effects of unary and ternary mixtures of heavy metals with 2,4-dichlorophenoxyacetic acid (2,4-D) on alkaline phosphatase activity in the freshwater microalga <i>Desmodesmus abundans</i>. The heavy metals assessed include Copper (Cu^{2+}), Zinc (Zn^{2+}), Lead (Pb^{2+}), Chromium (Cr^{2+}), and Cadmium (Cd^{2+}). Toxicity assays were conducted using fixed ternary mixture ratios of 20:40:40 and 40:30:30 to evaluate the combinatory interactions. Results demonstrated that all individual metal ions exerted significant inhibitory effects on phosphatase activity in a concentration-dependent manner. Among the ternary mixtures at the 20:40:40 ratio, combinations containing 2,4-D/Pb^{2+}/Cr^{2+}, 2,4-D/Pb^{2+}/Cd^{2+}, and 2,4-D/Cr^{2+}/Cd^{2+} exhibited the highest toxicity, with other mixtures following a descending toxicity pattern. For the 40:30:30 ratio, the most toxic mixtures were 2,4-D/Cr^{2+}/Cd^{2+}, 2,4-D/Pb^{2+}/Cr^{2+}, 2,4-D/Cu^{2+}/Cr^{2+}, and 2,4-D/Cu^{2+}/Zn^{2+}. Toxicity rankings further reflected varying intensities among the mixtures, with some displaying synergistic effects (2,4-D/Pb^{2+}/Cr^{2+}, 2,4-D/Cr^{2+}/Cd^{2+}), others additive (2,4-D/Cu^{2+}/Cr^{2+}, 2,4-D/Cu^{2+}/Zn^{2+}), and the majority antagonistic interactions. The toxic index analysis revealed predominately antagonistic behaviour in 20:40:40 ratio mixtures, except for specified synergistic and additive exceptions. Overall, increasing proportions of heavy metals in the mixtures intensified the toxic impact of 2,4-D. These findings highlight that mixtures of heavy metal ions with relatively low-toxicity pesticides such as 2,4-D can pose substantial ecotoxicological risks to freshwater algae, demonstrating complex interaction patterns dependent on mixture composition. Keywords: <i>Desmodesmus abundans</i>, additive toxicity, antagonism, synergism, ecotoxicity, alkaline phosphatase, ternary mixtures	Received: 17 Oct 2025 Accepted: 27 Oct 2025 Published: 01 Nov 2025 Scan QR code to view*  License: CC BY 4.0  Open Access article.
How to cite this paper: Onyeukwu, U. R., Anaukwu, C. G., Echeta, M. O., Tony-Nze, C. P., & Ezekwueche, S. N. (2025). Ternary Mixture Toxicity of 2,4-Dichlorophenoxyacetic Acid and Heavy Metals on Alkaline Phosphatase Activity in <i>Desmodesmus abundans</i>. <i>IPS Interdisciplinary Journal of Biological Sciences</i>, 4(4), 166–176. https://doi.org/10.54117/ijbs.v4i4.95	

1. Introduction

The paradigm of chemical risk assessment has evolved from focusing on individual substances to addressing complex mixtures, alongside a shift from traditional animal testing to alternative methodologies mandated by chemical regulations (Jongwoon *et al.*, 2022). Environmental chemical risk assessments are designed to address both qualitative and quantitative ecological risk challenges involving individual chemical pollutants (O'Brien and Keough, 2014; Braglia *et al.*, 2021; Schuijt *et al.*, 2021; Onyegeme-Okerenta *et al.*, 2025a). Despite this, Carole *et al.* (2018) highlighted a significant knowledge gap in understanding mixture toxicity and its

impact on aquatic organisms. When chemicals are present in mixtures with comparable toxicity levels, their combined effects can manifest as acute, additive, synergistic, antagonistic, or reductive interactions, which differ from the effects of single compounds (Carole *et al.*, 2018; Onyeukwu *et al.*, 2023; Onyeukwu and Edna, 2022a,b). Agrochemicals employed for aquaculture disease management frequently exist in chemical combinations, necessitating an evaluation of their ecotoxicological interactions to improve toxic effect interpretations (Vanessa *et al.*, 2023). Agrochemical pesticides are largely introduced into the environment through agricultural and horticultural practices, often seeping into

subsoil layers via precipitation. The complexity of possible chemical cocktails presents challenges for toxicity testing, requiring expertise because chemicals at no observed effect concentrations (NOEC) may still contribute to toxicity in mixtures, regardless of the chemical types involved (Jongwoon *et al.*, 2022; Onyegeme-Okerenta *et al.*, 2025b).

In aquatic ecosystems, microorganisms such as microalgae; which are primary producers and sensitive to physicochemical changes are routinely exposed to mixtures of organic and inorganic pollutants rather than isolated substances (Aronzon, Peluso and Coll, 2020; Kovalakova *et al.*, 2020; Topaz *et al.*, 2020; Zhang *et al.*, 2021; Ahmadmahmoodi *et al.*, 2020). Microalgae serve as critical models for toxicity assessment due to their rapid and measurable responses, enabling exploitation of various endpoints (Martínez-Ruiz and Martínez-Jerónimo, 2015; Nguyen, Moon and Lee, 2020; Nunes *et al.*, 2018). Beyond phosphatase inhibition, pollutants may induce chronic effects including genotoxicity, mutagenicity, carcinogenicity, teratogenicity, immunosuppression, and endocrine disruption, especially within complex mixtures (Exposito *et al.*, 2021). Among emerging micropollutants, pesticides, heavy metals, and pharmaceutical residues are notable for their abundance and diverse biological impacts (Göbölös *et al.*, 2024). Biochemical markers like the inhibition of hydrolytic phosphatase activities provide crucial endpoints for assessing environmental toxicant effects (Onyeukwu and Edna, 2022a,b; Wenq *et al.*, 2021).

Given the impracticality of experimentally testing all possible mixture combinations, modeling is indispensable for predicting the toxicological properties of mixtures. Existing models, including Concentration Addition (CA) and Independent Action (IA), are commonly referenced for estimating combined chemical effects (Cedergreen *et al.*, 2008; Göbölös *et al.*, in Bliss, 1939; Wang *et al.*, 2020a; b). CA is applicable when chemicals share a similar mode of action (Loewe, 1926), while IA applies to chemicals with dissimilar modes (Bliss, 1939). However, these models primarily predict additive effects. For instance, Cedergreen *et al.* (2008) found that CA and IA only accounted for about 10% accuracy in 20% of 158 binary mixtures tested (Göbölös *et al.*, in Loewe, 1926). Synergistic interactions, notably between heavy metals, can be evaluated using the Combination Index (CI) method, also known as the Chou-Talalay method (Kahatagahawatte and Hara-Yamamura, 2020; Roell *et al.*, 2017). Although research on mixtures of heavy metals and pesticides has increased, mostly focusing on binary mixtures (Belden *et al.*, 2007; Baek, 2019) the nearly infinite potential chemical combinations complicate comprehensive empirical assessment.

Quantitative Structure-Activity Relationship (QSAR) models have been employed to predict activities of both single chemicals and mixture components but are often restricted to binary combinations and additive toxicities (Altenburger, Nendza, and Schüürmann, 2003). These models are limited by the quality and scope of experimental datasets, which frequently encompass only a few values, insufficiently representing complex biological interactions. Enhanced QSAR model accuracy requires broader, more reliable

experimental data to reflect complex systems effectively (Kolmar and Grulke, 2021; Belfield *et al.*, 2023).

This study aimed to evaluate the acute toxicity effects of single and ternary mixtures involving Copper (Cu^{2+}), Zinc (Zn^{2+}), Lead (Pb^{2+}), Chromium (Cr^{2+}), Cadmium (Cd^{2+}) ions, and 2,4-dichlorophenoxyacetic acid (2,4-D) on alkaline phosphatase activity in *Desmodesmus abundans*. We investigated the individual and combined effects of unary and ternary mixtures by assessing acute inhibition of alkaline phosphatase activity. Synergistic, additive, and antagonistic interactions at differing effective concentrations were quantified using the CI method. Additionally, the contribution of each chemical to mixture effects was analyzed using sigma statistical plots, based on relative response estimates and median inhibitory concentration (MIC_{50} or EC_{50}) parameters. Predictive modeling employed statistical tools and the mixtox model, with the interaction parameter 'a' indicating synergy ($a < 0$) or antagonism ($a > 0$) (Onyeukwu and Edna, 2022a,b; Greco *et al.*, 1992, 1995; Jonker *et al.*, 2005).

2. Materials and Methods

2.1 Study Area

The study was conducted in Nworie-Otamiri River in Ihiagwa, a town situated within Owerri West Local Government Area, Imo State, southeastern Nigeria, approximately 12 km south of Owerri city. The Otamiri River flowing through Ihiagwa is located at latitude $4^{\circ}54'14.00''$ N and longitude $7^{\circ}08'30.00''$ E, draining a watershed covering roughly 10,000 km^2 with annual rainfall ranging between 2250 and 2500 mm. The watershed is predominantly covered by depleted rainforest, with mean temperatures consistently around 27°C . The river faces pollution from organic waste and chemicals originating from intensive human activity, agriculture, poor drainage, industrial discharge, and is a drinking water source when public supplies fail. Inefficient waste management contributes to elevated pollutant levels, including phosphates, nitrates, metals, pesticides, and herbicides.

2.2 Sampling

2.2.1 Sampling Bottles

Water samples were collected using sterile glass bottles with a minimum volume of 200 mL, capped with ground glass or screw caps lined with silicon rubber capable of withstanding sterilization at 160°C . Bottles and caps were protected with sterilized aluminum foil to prevent contamination. Sampling intervals were scheduled every 10 minutes per site, controlled by a stopwatch operated by field assistants.

2.3 Sample Collection and Transport

Water was collected midstream at three selected points along the river; upper, middle, and lower courses at specified coordinates and ~ 30 cm depth. Samples were pooled into sterile 1-L plastic or 200 mL glass bottles, immediately stored in insulated coolers, and transported for laboratory analysis within six hours to preserve cell viability.

2.4 Isolation of Algae

Pooled samples were processed within six hours via washing or centrifugation, followed by agar plating. Pure cultures of algal isolates were obtained and maintained axenically for 2-3

weeks under controlled microbiological conditions until toxicity assays commenced. Algal load was quantified as colony-forming units per milliliter (CFU/mL) (Tomaselli, 2004; Parvin *et al.*, 2007).

2.5 Algological Analysis

2.5.1 Agar Plate Preparation

Agar plates with 2% (w/v) agar in Bold's Basal Medium (BBM) were autoclaved at 126°C for 15 minutes, cooled, and supplemented with 5 mL warm medium. Plates were inverted and dried for 72 hours before streaking with enriched Bold basal medium modified with trace metals and F/2 vitamin solutions (Stein, 1973; Nichols and Bold, 1965).

2.5.2 Culture and Purification

Washed algal samples (12 mL) were centrifuged at 3000 rpm for 15 minutes; supernatants discarded, and cells washed six times with sterile water to remove contaminants (Parvin and Habib, 2007). Isolates were streaked onto selective agar containing antibacterial and antifungal agents, incubated under fluorescent light at $28 \pm 2^\circ\text{C}$ for two weeks, and subsequently transferred to liquid Bold basal medium for sustained culture in exponential growth.

2.5.3 Subculture and Storage

Axenic cultures were maintained in inorganic liquid medium per OECD guidelines (1981). Stock cultures were incubated at $20 \pm 2^\circ\text{C}$ under white fluorescent light (3000–4000 lux) on a rotary shaker; fresh stocks were initiated every 40–60 days by inoculating 5×10^4 cells/mL (Jonsson and Aoyama, 2007).

2.5.4 Inoculum Standardization

A loopful of stored isolates was inoculated into 200 mL BBM broth and incubated with shaking (150 rpm) at $28 \pm 2^\circ\text{C}$ for 24 hours. Cells were harvested by centrifugation, washed twice, and standardized spectrophotometrically to an optical density of 1.8 at 600 nm.

2.6 Morphological Identification

Isolates were assessed morphologically by colony characteristics, green pigmentation, and chlorophyll a/b content. Colony features such as colour, form, transparency, and diameter were recorded; additional biochemical tests included catalase, phosphatase, starch, and lipase activities (Stein, 1973).

2.7 Molecular Identification

2.7.1 DNA Extraction

DNA was extracted from dense pure cultures using the ZR fungal/algal/bacterial DNA MiniPrep Kit (Inqaba, South Africa). Samples underwent mechanical lysis in bead beating tubes, followed by centrifugation and successive purification steps per manufacturer instructions. Purified DNA was eluted and stored at -20°C .

2.7.2 DNA Quantification

Extracted DNA was quantified using the NanoDrop 1000 spectrophotometer with sterile distilled water for blanking. Two microliters of DNA were measured to determine concentration.

2.7.3 18S rRNA Gene Amplification

The 18S rRNA gene was amplified using primers 18S C-2 b (5'-ATTGGAGGGCAAG TCTGGT-3') and 18S D-2 b (5'-ACTAAGAACGGCCATGCAC-3') on an ABI 9700 thermal cycler. PCR conditions consisted of an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds, 53°C for 30 seconds, and 72°C for 30 seconds, with a final extension at 72°C for 5 minutes. The amplified products were separated on 1% agarose gels and visualized under blue light to confirm successful amplification. This protocol ensured specific and reproducible amplification for downstream sequencing and phylogenetic analysis.

2.7.4 Sequencing

Sequencing of the 18S rRNA gene was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI 3510 Genetic Analyzer (Inqaba Biotechnological, Pretoria). The sequencing reactions consisted of purified PCR product, BigDye mix, sequencing buffer, and specific primers. Thermal cycling was set for 32 cycles with denaturation at 96°C for 10 seconds, annealing at 55°C for 5 seconds, and extension at 60°C for 4 minutes per cycle. The amplified products were purified to remove unincorporated dyes and analyzed by capillary electrophoresis. This approach ensures high-quality, accurate base calling suited for downstream phylogenetic analysis and strain identification of *Desmodesmus abundans*. Sequence quality was verified before alignment and comparison against reference databases, maintaining consistency with established microalgal molecular identification protocols.

2.7.5 Phylogenetic Analysis

Sequence editing was performed using Trace Edit software to ensure high-quality base calling and removal of ambiguous nucleotides. Reference sequences were retrieved from the National Center for Biotechnology Information (NCBI) database using BLASTN to identify homologous 18S rRNA gene sequences of *Desmodesmus abundans* and related taxa. Multiple sequence alignments were conducted using MAFFT with default parameters, ensuring accurate alignment of conserved and variable regions across sequences. Phylogenetic trees were constructed employing the Neighbor-Joining method implemented in MEGA 6.0, based on the Jukes-Cantor evolutionary model to calculate genetic distances. Bootstrap analysis with 500 replicates was performed to assess the robustness and statistical support of the inferred phylogenies. This methodological framework enabled a reliable taxonomic placement of the isolate within the genus *Desmodesmus*, addressing phenotypic variability and clarifying species relationships consistent with prior taxonomic reviews and molecular studies. These steps align with standard phylogenetic practices facilitating accurate identification and evolutionary contextualization of microalgal isolates (Burgel, *et al.*, 2022; Felsenstein, 1985).

2.7.6 Phosphatase Extraction

Following the protocol of Jonsson and Aoyama (2007), algal pellets of *Desmodesmus abundans* were flash-frozen using liquid nitrogen to preserve enzymatic activity and facilitate cell disruption. The frozen pellets were then macerated in ice-cold acetate buffer (pH typically 5.0–6.0) to stabilize

phosphatase enzymes. Cell lysis was achieved by applying multiple cycles of probe sonication on ice, ensuring efficient cell wall disruption while minimizing heat-induced denaturation. The resulting suspensions were centrifuged at 10,000 rpm for 20 minutes at 4°C to pellet cellular debris. The clarified supernatants containing soluble alkaline phosphatase enzymes were carefully collected and stored on ice or at -20°C for subsequent enzymatic activity assays. This extraction method maintains enzyme integrity and yields reliable extracts optimal for measuring phosphatase activity, which serves as a sensitive biochemical biomarker in toxicity studies involving heavy metals and herbicides in microalgae.

2.8 Toxicity Assessment of Unary and Ternary Mixtures on Alkaline Phosphatase Activity

Graded concentrations of individual metals (Cu^{2+} , Zn^{2+} , Cr^{2+} , Pb^{2+} , Cd^{2+}) and 2,4-dichlorophenoxyacetic acid (2,4-D) were tested for acute toxicity. Metal stock solutions were prepared at 10 mM. Concentration ranges were: Cu^{2+} , Zn^{2+} , Cr^{2+} at 0-0.5 mM; Pb^{2+} , Cd^{2+} at 0-7.0 mM; 2,4-D at 0-25 mM. Ternary mixtures were formulated at ratios of 20:40:40 and 40:30:30 for pesticide/metal mixtures, with toxicities evaluated from 0-9.0 mM concentrations. Alkaline phosphatase inhibition assays were performed in triplicate in 2 mL reaction mixtures containing toxicants, buffered enzyme, substrate (p-NPP), and distilled water, incubated at 37°C for 30-40 minutes. Reactions were terminated with sodium hydroxide, centrifuged, and absorbance measured at 410 nm.

2.9 Relative Response Estimation, Median Inhibitory Concentration, and Modeling

Relative responses were calculated as percentages relative to controls (Equation 1) and dose-response data from unary and ternary mixtures were fit to models (Equations 2-10) using SigmaPlot v10. EC_{50} values for individual chemicals and mixtures were extracted from fitted models, which incorporated hormesis parameters such as slope and maximum response [nonlinear regression models and median inhibitory concentrations (IC_{50}) were derived as the concentration causing 50% inhibition] (Cedergreen *et al.*, 2005).

Relative responses (Equation 1) in *Desmodesmus abundans* enzyme assays were calculated as percentages relative to controls using:

$$\text{Relative Response (\%)} = \left(\frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}} \right) \times 100 \quad (1)$$

where A_{sample} , A_{control} , and A_{blank} represent absorbance measurements of treated, untreated control, and blank samples, respectively.

Several models (such as in equations 2-7) is commonly used to estimate IC_{50} and EC_{50} include:

1. Linear Regression:

$$Y = aX + b, \text{IC}_{50} = \frac{0.5 - b}{a} \quad (2)$$

2. Log-Transformed Linear Model:

$$Y = a \log(X) + b \quad (3)$$

3. Four-Parameter Logistic (4PL) Model:

$$Y = D + \frac{A - D}{1 + \left(\frac{X}{C} \right)^B} \quad (4)$$

where, A and D are asymptotic minimum and maximum responses, B is the slope, and C is the IC_{50} .

4. Hill Equation:

$$Y = \frac{X^n}{\text{EC}_{50}^n + X^n} \quad (5)$$

where, n is the Hill slope.

5. Weibull Model:

$$Y = 1 - e^{-\left(\frac{X}{\lambda} \right)^k} \quad (6)$$

where, λ is scale and k shape parameters.

6. Gompertz Model:

$$Y = A \exp(-\exp(B - CX)) \quad (7)$$

Parameters A , B , and C modulate the curve shape.

The dose-response data were also analyzed incorporating hormesis where applicable, accounting for low-dose stimulation effects. To interpret mixture toxicity, the Combination Index (CI) method was applied, quantifying interactions as synergistic ($\text{CI} < 1$), additive ($\text{CI} = 1$), or antagonistic ($\text{CI} > 1$).

2.10 Toxicity Interaction Analysis Using Toxic Index Model

The toxicity interaction of the mixtures was assessed using the toxic index (TI), (Equations 8-10) which sum the components toxic unit (Boillot and Perrodin, 2008). The TU values were calculated using the expression:

$$\text{TU}_i = \frac{\text{Cmix}_i}{\text{IC}_{50i}} \quad (8)$$

While TI is the summation of TU for n toxicants in the mixture

$$\text{TI} = \sum_{i=1}^n \text{TU}_i \quad (9)$$

$$\text{Cmix}_i = \frac{\text{Ratio}}{100} \text{IC}_{50i \text{ mix}} \quad (10)$$

Where Cmix_i is the concentration of the i th toxicant in the mixture and EC_{50i} is the EC_{50} of the same toxicant when tested singly. $\text{TI}=1$ implies additive interaction, $\text{TI} > 1$ implies antagonistic interaction and $\text{TI} < 1$ implies a synergistic interaction. Toxic units (TU) and Toxic Index (TI) were calculated to assess mixture interactions based on component concentrations and EC_{50} values (Boillot and Perrodin, 2008).

2.11 Statistical Analysis

EC_{50} values and experimental data were analyzed using one-way ANOVA ($p < 0.05$) with IBM SPSS Statistics v22. SigmaPlot v10 was also employed for modeling and graphing analytical results.

3. Results

3.1 Morphological and Biochemical Characterization of Isolate R2-KJ676104

The isolate designated as R2-KJ676104 was examined under an electron microscope (Figure 1), revealing cell dimensions ranging from 11 to 15 μm ($\pm 0.48 \mu\text{m}$) in length and 4 to 6 μm ($\pm 0.5 \mu\text{m}$) in width. Morphological analysis showed that the isolate existed both as unicellular and colonial forms, the latter composed of 2-4 cells bearing spiny projections. The cells displayed an ellipsoidal or obovate shape with a heavily granulated surface, enclosed within a mucilaginous sheath. Cellular arrangements included tetrads, alongside single and double-cell forms, with colonial cells distinctly exhibiting spiny extensions. Based on these morphological features, the isolate was preliminarily classified as belonging to the genus *Desmodesmus*, consistent with descriptions by Charmaine *et al.*, (2021), Hegewald (2000), Perumal *et al.*, (2012), and Nichols and Bold (1965). The biochemical and morphological characteristics are summarized in Table 1.

Table 1. Morphological and biochemical screening assay of *Desmodesmus abundans*

Parameter	Result
Shape	Obovate / ellipsoidal
Form	Tetrad / mucilaginous
Chloroplast	Present
Colour	Green pigment
Type	Spiny projection
Cell diameter	11–15 $\mu\text{m} \pm 0.48 \mu\text{m}$
Phosphatase test	++
Nitrogenase test	++
Chlorophyll analysis	++
Nitriles test	++
Lipoxygenases	+
Amylase	+
Lipase test	+
Urease test	++
Thiolase / gelatinase test	+
Catalase	+
Hydrogenases	+
Carbonic anhydrase	+

Key: +: Low reactivity; ++: High reactivity

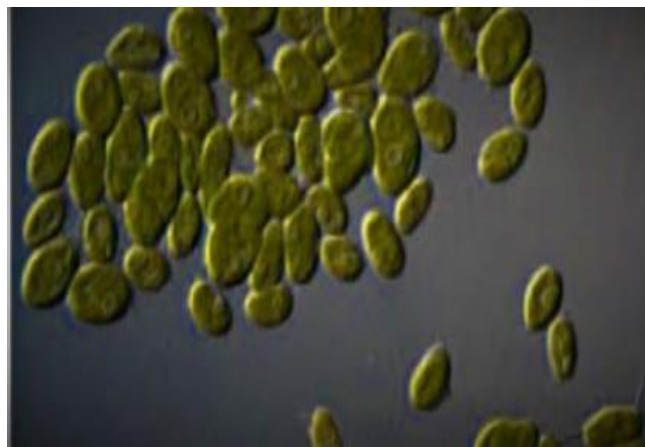


Figure 1. Electron microscopy image illustrating the cellular morphology of *Desmodesmus abundans* isolate.

3.2 Molecular Identification of Isolate R2-KJ676104

The molecular identification process, including DNA extraction, quantification, 18S rRNA gene amplification, sequencing, and phylogenetic analysis, confirmed the isolate's taxonomic placement. The 18S rRNA sequence of R2-KJ676104 matched with a 99–100% identity to reference sequences available in the NCBI non-redundant nucleotide database. Phylogenetic reconstruction using the Jukes-Cantor evolutionary model corroborated the isolate's affiliation with *Desmodesmus* species, closely related to *Desmodesmus abundans*.

3.3 Acute Toxicity of Individual Metals and 2,4-D on Phosphatase Activity of *Desmodesmus abundans*

Figure 2 presents the dose-response curves for unary toxic effects of copper (Cu^{2+}), lead (Pb^{2+}), chromium (Cr^{2+}), cadmium (Cd^{2+}), zinc (Zn^{2+}), and 2,4-dichlorophenoxyacetic acid (2,4-D) on phosphatase activity. The inhibition data fitted well to a three-parameter sigmoidal model with determination coefficients (R^2) ranging from 0.98 to 0.99. Median inhibitory concentrations (IC_{50}) for each toxicant are detailed in Table 2.

Table 2. Median inhibitory concentration (IC_{50}) of the effect of Unary toxicity of (metal/pesticide) to phosphatase enzyme activity from *Desmodesmus abundans*.

Toxicant	IC_{50} (mM)
Cu^{2+}	2.18 ± 0.47
Zn^{2+}	0.13 ± 0.00
Pb^{2+}	0.16 ± 0.01
Cr^{2+}	0.17 ± 0.01
Cd^{2+}	0.16 ± 0.01
2,4-D	0.26 ± 0.01

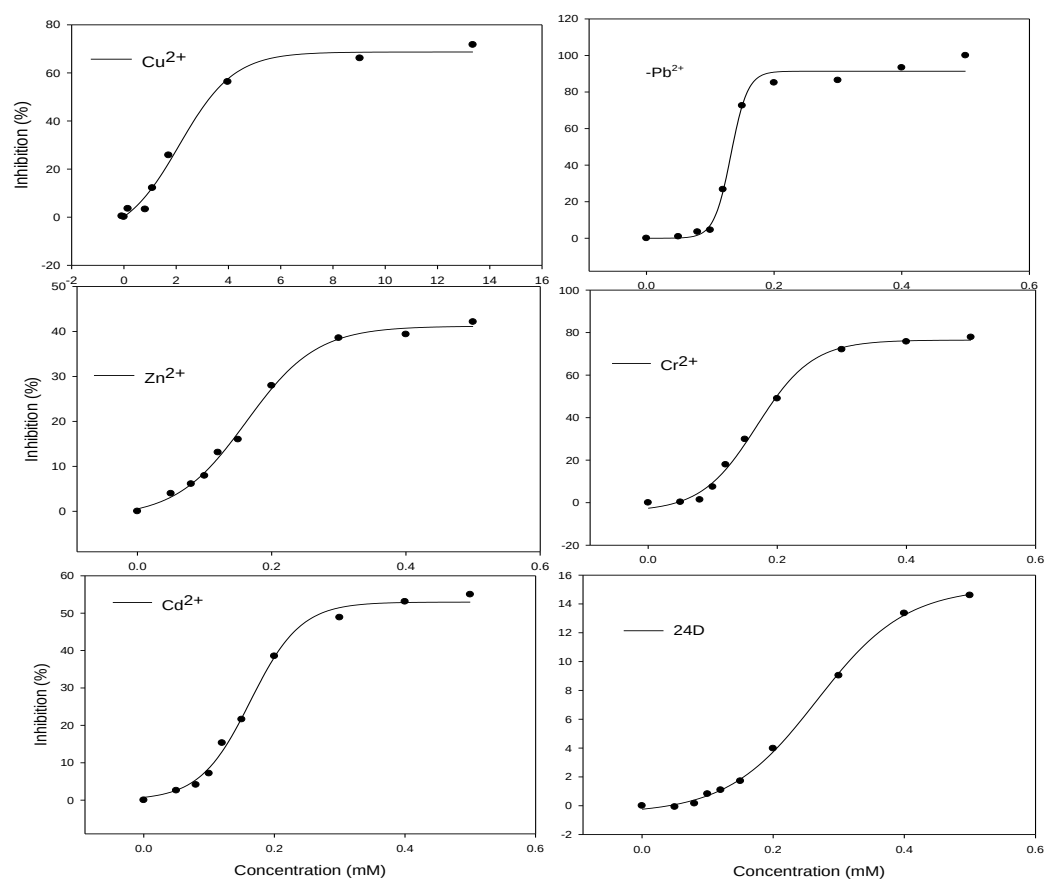


Figure 2: Experimental data points and model predictions of unary toxicity dose-response curves for the inhibition of phosphatase activity by Cu^{2+} , Zn^{2+} , Pb^{2+} , Cr^{2+} , Cd^{2+} , and 2,4-D in *Desmodemus abundans* using three- and four-parameter sigmoidal models.

3.4 Toxicity of Ternary Mixtures of Metals with 2,4-D on Phosphatase Activity

Figures 3 and 4 depict dose-response curves for ternary mixtures composed of heavy metals with 2,4-D at two fixed ratios (20:40:40 and 40:30:30). The inhibitory effects

conformed to a four-parameter sigmoidal model with R^2 values between 0.96 and 0.99. Table 3 lists the median inhibitory concentrations (IC_{50}), toxicity indices, and interaction types for each mixture.

Table 3. Median inhibitory concentration (IC_{50}) of the effect of Ternary toxicity of (metal/pesticide) to phosphatase enzyme activity from *Desmodemus abundans*.

Toxicant: Mixture	IC_{50} (mM)	Toxicity index	Interaction
Ternary mixture ratio (20:40:40)			
2,4-D/ Cu^{2+} / Pb^{2+}	5.68 ± 0.23^c	19.61 ± 0.90^c	Antagonism
2,4-D/ Cu^{2+} / Zn^{2+}	5.81 ± 0.19^c	23.41 ± 1.17^c	Antagonism
2,4-D/ Cu^{2+} / Cr^{2+}	4.37 ± 0.25^c	14.44 ± 0.72^c	Antagonism
2,4-D/ Cu^{2+} / Cd^{2+}	5.06 ± 0.17^c	17.47 ± 0.87^c	Antagonism
2,4-D/ Pb^{2+} / Zn^{2+}	5.29 ± 0.31^c	33.57 ± 1.67^c	Antagonism
2,4-D/ Pb^{2+} / Cr^{2+}	2.77 ± 0.03^c	15.57 ± 0.77^c	Antagonism
2,4-D/ Pb^{2+} / Cd^{2+}	0.37 ± 0.06^c	2.13 ± 0.10^c	Antagonism
2,4-D/ Zn^{2+} / Cr^{2+}	6.38 ± 0.09^c	39.55 ± 1.97^c	Antagonism
2,4-D/ Zn^{2+} / Cd^{2+}	4.22 ± 0.18^c	26.78 ± 1.33^c	Antagonism
2,4-D/ Cr^{2+} / Cd^{2+}	2.77 ± 0.03^c	15.57 ± 0.77^c	Antagonism
Ternary mixture ratio (40:30:30)			
2,4-D/ Cu^{2+} / Pb^{2+}	6.61 ± 0.43^c	23.47 ± 1.17^c	Antagonism
2,4-D/ Cu^{2+} / Zn^{2+}	3.92 ± 0.42^c	15.61 ± 0.78^c	Antagonism
2,4-D/ Cu^{2+} / Cr^{2+}	3.72 ± 0.35^c	12.79 ± 0.63^c	Antagonism
2,4-D/ Cu^{2+} / Cd^{2+}	4.42 ± 0.25^c	15.69 ± 0.78^c	Antagonism
2,4-D/ Pb^{2+} / Zn^{2+}	4.37 ± 0.25^c	25.00 ± 1.25^c	Antagonism
2,4-D/ Pb^{2+} / Cr^{2+}	2.77 ± 0.03^c	14.34 ± 0.71^c	Antagonism
2,4-D/ Pb^{2+} / Cd^{2+}	4.28 ± 0.20^c	22.63 ± 1.13^c	Antagonism
2,4-D/ Zn^{2+} / Cr^{2+}	4.28 ± 0.20^c	22.63 ± 1.13^c	Antagonism
2,4-D/ Zn^{2+} / Cd^{2+}	5.29 ± 0.31^c	30.26 ± 1.51^c	Antagonism
2,4-D/ Cr^{2+} / Cd^{2+}	2.77 ± 0.03^c	14.34 ± 0.71^c	Antagonism

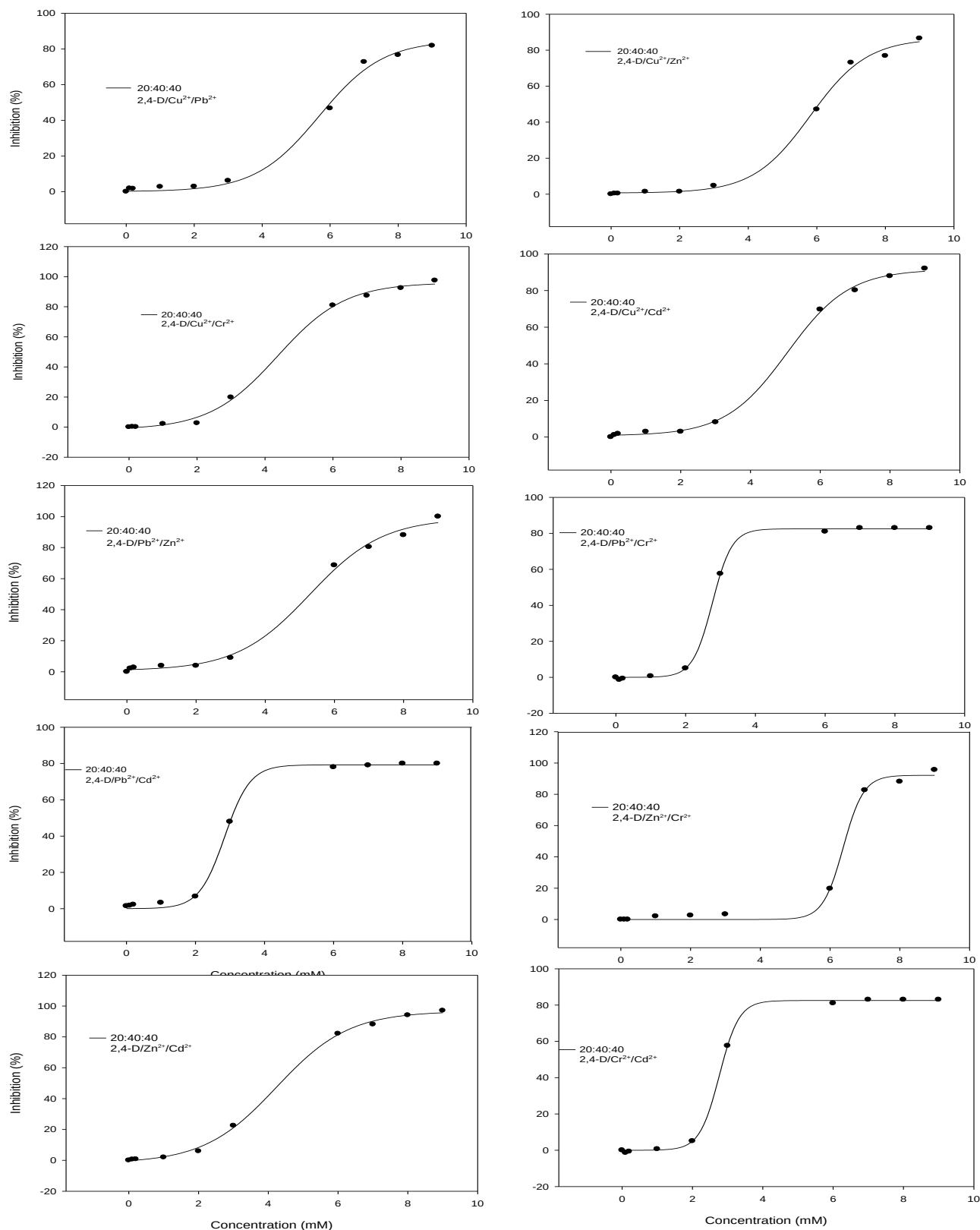


Figure 3. Dose-response curves for phosphatase activity inhibition by ternary mixtures (20:40:40 ratio) including 2,4-D/Pb²⁺/Cd²⁺, 2,4-D/Zn²⁺/Cr²⁺, 2,4-D/Zn²⁺/Cd²⁺, and 2,4-D/Cr²⁺/Cd²⁺.

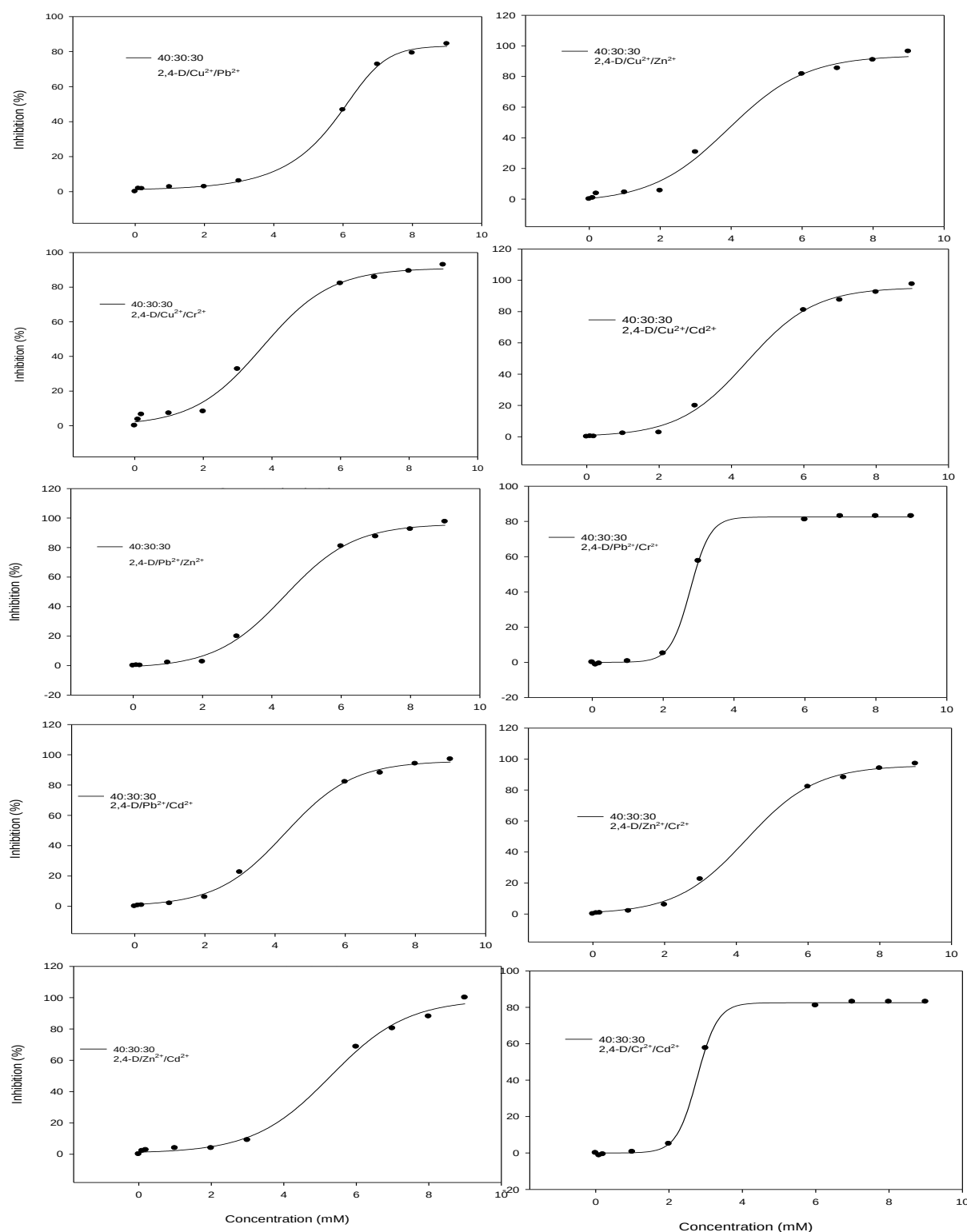


Figure 4. Dose-response curves for phosphatase activity inhibition by ternary mixtures (40:30:30 ratio) including 2,4-D/Cu²⁺/Pb²⁺, 2,4-D/Cu²⁺/Zn²⁺, 2,4-D/Cu²⁺/Cr²⁺, 2,4-D/Cu²⁺/Cd²⁺, 2,4-D/Pb²⁺/Zn²⁺, and 2,4-D/Pb²⁺/Cr²⁺.

4. Discussion

4.1 Morphological and Biochemical Identification

Microscopic analysis confirmed that isolate R2-KJ676104 exhibits cell dimensions and morphological features consistent with *Desmodesmus* species, exhibiting unicellular and colonial forms with characteristic spiny projections and mucilaginous

sheaths. These observations align with prior descriptions by Charmaine *et al.*, (2021), Hegewald (2000), and others. Biochemical assays (Table 1) corroborate this identification and concur with previous studies reporting similar profiles for *Desmodesmus abundans* (Yamamoto *et al.*, 2004; Ilman *et al.*, 2002; Champenois *et al.*, 2015; Borowitzka, 2018).

4.2 Molecular Identification

The sequencing and phylogenetic analyses placed the isolate firmly within *Desmodesmus abundans*, demonstrating a molecular similarity of 99–100% with related species. The evolutionary distance metrics further support this classification.

4.3 Toxicity of Single Metals and Ternary Mixtures

The median inhibitory concentration values indicate zinc ion as the most potent inhibitor of phosphatase activity in *Desmodesmus abundans* ($IC_{50} = 0.13 \pm 0.00$ mM), followed by lead, cadmium, chromium, and 2,4-D, with copper exhibiting the lowest toxicity ($IC_{50} = 2.18 \pm 0.47$ mM) (Table 2, Figure 2). These findings are consistent with reported toxicities of zinc and lead nanoparticles on freshwater algae (Saxena and Harish, 2019; Al-Hasawi *et al.*, 2020; Mohy El-Din and Abdel-Kareem, 2020). Cadmium's toxic effects align with previous literature although it is generally considered a lower ecological risk compared to other metals (Wang *et al.*, 2020). The relatively lower toxicity of 2,4-D supports its classification as agro-friendly; however, its toxicity may increase in mixtures with metals.

Ternary mixture evaluations revealed predominantly antagonistic interactions ($TI > 1$) across tested ratios (20:40:40 and 40:30:30), except for certain combinations such as 2,4-D/ Pb^{2+} / Cr^{2+} and 2,4-D/ Cr^{2+} / Cd^{2+} , which exhibited additive or synergistic effects ($TI \leq 1$) (Table 3). The ecotoxicity ranking for mixtures indicated variation in inhibitory potency, with particular mixtures demonstrating increased toxicity synergistically, consistent with previous reports on metal-nanoparticle mixtures (Adochite and Andronic, 2021).

4.4 Statistical Comparisons

One-way ANOVA analysis confirmed significant differences ($p < 0.05$) between the IC_{50} values of unary and ternary mixtures for phosphatase inhibition (Table 4). Superscript comparisons indicate distinct toxicological effects among tested compounds and their combinations.

5. Conclusion

The study demonstrated that ternary mixtures of 2,4-D with heavy metals at ratios of 20:40:40 and 40:30:30 exert substantial inhibitory effects on *Desmodesmus abundans* alkaline phosphatase activity. Although 2,4-D alone exhibited relatively low toxicity, its combination with metal ions enhanced toxicity, emphasizing that the agrochemical's ecotoxicological profile is significantly altered in mixed contaminant scenarios. These findings highlight the need for urgent control over pesticide-metal mixtures in urban-rural aquatic settlement.

Compliance with ethical standards

Acknowledgment

I acknowledge the staff of Silverpresh Laboratories, Owerri, Imo State, Nigeria.

Disclosure of conflict of interest

There was no conflict of interest.

Statement of Ethical approval

All procedures were conducted in accordance with ethical guidelines

Statement of informed consent

All participants were fully informed about the study's purpose, procedures, risks, and their rights before participation.

Table 3: One-Way ANOVA Comparison of Unary and Ternary IC_{50} Values for Metal/Pesticide Effects on Phosphatase Activity in *Desmodesmus abundans*

Toxicant	<i>Desmodesmus abundans</i> (mM)
Unary	
Cu^{2+}	2.18 ± 0.47^b
Zn^{2+}	0.13 ± 0.00^a
Pb^{2+}	0.16 ± 0.01^a
Cr^{2+}	0.17 ± 0.01^a
Cd^{2+}	0.16 ± 0.01^a
2,4-D	0.26 ± 0.01^a
Ternary Mixture (20:40:40)	<i>Desmodesmus abundans</i> (mM)
2,4-D/ Cu^{2+} / Pb^{2+}	5.68 ± 0.23^{ef}
2,4-D/ Cu^{2+} / Zn^{2+}	5.81 ± 0.19^{fg}
2,4-D/ Cu^{2+} / Cr^{2+}	4.37 ± 0.25^{cd}
2,4-D/ Cu^{2+} / Cd^{2+}	5.06 ± 0.17^{de}
2,4-D/ Pb^{2+} / Zn^{2+}	5.29 ± 0.31^{ef}
2,4-D/ Pb^{2+} / Cr^{2+}	2.77 ± 0.03^b
2,4-D/ Pb^{2+} / Cd^{2+}	0.37 ± 0.06^a
2,4-D/ Zn^{2+} / Cr^{2+}	6.38 ± 0.09^g
2,4-D/ Zn^{2+} / Cd^{2+}	4.22 ± 0.18^c
2,4-D/ Cr^{2+} / Cd^{2+}	2.77 ± 0.03^b
Ternary Mixture (40:30:30)	<i>Desmodesmus abundans</i> (mM)
2,4-D/ Cu^{2+} / Pb^{2+}	6.61 ± 0.43^g
2,4-D/ Cu^{2+} / Zn^{2+}	3.92 ± 0.42^{de}
2,4-D/ Cu^{2+} / Cr^{2+}	3.72 ± 0.35^d
2,4-D/ Cu^{2+} / Cd^{2+}	4.42 ± 0.25^e
2,4-D/ Pb^{2+} / Zn^{2+}	4.37 ± 0.25^{de}
2,4-D/ Pb^{2+} / Cr^{2+}	2.77 ± 0.03^c
2,4-D/ Pb^{2+} / Cd^{2+}	4.28 ± 0.20^{de}
2,4-D/ Zn^{2+} / Cr^{2+}	4.28 ± 0.20^{de}
2,4-D/ Zn^{2+} / Cd^{2+}	5.29 ± 0.31^f
2,4-D/ Cr^{2+} / Cd^{2+}	2.77 ± 0.03^c

Means with differing superscript letters indicate statistically significant differences at $p < 0.05$.

References

- Adochite, C and Andronic, L. (2021). Aquatic Toxicity of Photocatalyst Nanoparticles to Green Microalgae *Chlorella vulgaris*. *Water*, 13(1), 1-77.
- Ahmadmehmoodi, R. Z., Malekabadi, M. B., Rahimi, R and Johari, S. A. (2020). Aquatic pollution caused by mercury, lead, and cadmium affects cell growth and pigment content of marine microalga, *Nannochloropsis oculata*. *Environment Monitoring Assess*, 192:330.
- Al-Hasawi, Z. M., Mohammad, I., Hamid, A., Almutairi, A., W and Touliabah, E. H. (2020). Response of *Pseudokirchneriella subcapitata* in Free and Alginate Immobilized Cells to Heavy Metals Toxicity. *Molecules*, 1, 23-30.
- Altenburger, R., Nendza, M., and Schuurmann, G. (2003). Mixture toxicity and its modeling by quantitative structure-

- activity relationships. *Environmental Toxicology and Chemistry*, 22, 1900–1915. doi: 10.1897/01-386.
- Aronzon, C. M., Peluso, J and Coll, C. P. (2020). Mixture toxicity of copper and nonylphenol on the embryo-larval development of *Rhinella arenarum*. *Environmental Science and Pollution Research*, 27, 13985–13994.
- Baek, K., Lee, Y., Nam, O. and Park, S. (2019). Introducing *Dunaliella* *LIP* promoter containing light-inducible motifs improves transgenic expression in *Chlamydomonas reinhardtii*. *Biotechnology Journal*, 11:384–392.
- Belden, J. B., Gilliom, R. J., Lydy, M. J. (2007). How Well Can We Predict the Toxicity of Pesticide Mixtures to Aquatic Life? *Integrated Environmental Assessment and Management*, 3, 364–372.
- Belfield, S.J., James, W.F., Steven, J.E., Judith, C.M., Knut, E.T., Mark, T.D.C. (2023). A review of quantitative structure-activity relationship modelling approaches to predict the toxicity of mixtures. *Journal of Computational Toxicology*, 25:100251.
- Bliss, C.I. (1939). The Toxicity of Poisons Applied Jointly. *Annals of Applied biology*, 26, 585–615.
- Boillot, C., and Perrodin, Y., (2008). Joint-action ecotoxicity of binary mixtures of glutaraldehyde and surfactants used in hospitals: use of the Toxicity Index model and isobologram representation. *Ecotoxicology and Environmental Safety*, 71, 252–259.
- Borowitzka, M. A. (2018). Biology of Microalgae. In: A. Levin and J. Fleurence, (Eds.), *Microalgae in Health and Disease Prevention*. USA: Academic Press, pp.23–72.
- Braglia, R., Rugnini, L., Malizia, S., Scuderi, F., Redi, E.L., Canini, A and Bruno, L. (2021). Exploiting the Potential in Water Cleanup from Metals and Nutrients of *Desmodesmus* sp. and *Ampelodesmos mauritanicus*. *Plants*, 10, 1461.
- Burgel, G., Ribas, P.G., Ferreira, P.C., Passos, M.F., Santos, B., Savi, D.C., Ludwig, T.A.V., Vargas, J.V.C., Galliterasawa, L.V and Kava, V.M. (2022). Morphology, molecular phylogeny and biomass evaluation of *Desmodesmus abundans* (Scenedesmaceae-Chlorophyceae) from Brazil. *Braz J Biol*, 21; 82.
- Carole, D. P., Costil, K., Bouchart, V., Halm, L. and Marie-Pierre (2018). Toxicity assessment of five emerging pollutants, alone and in binary or ternary mixtures, towards three aquatic Organisms. *Environmental Science and Pollution Research*, (25), 7, 6122–6134.
- Cedergreen, N., Christensen, A.M., Kamper, A., Kudsk, P., Mathiasen, S.K., Streibig, J.C., Sørensen, H. A. (2008). Review of Independent Action Compared to Concentration Addition as Reference Models for Mixtures of Compounds with Different Molecular Target Sites. *Environmental Toxicology and Chemistry*, 27, 1621–1632.
- Cedergreen, N., RitZ, C and Streibig, J. C. (2005). Improved empirical model describing hormesis. *Environmental Toxicology Chemosphere*, 24:3166–3172.
- Champenois, J., Marfaing, H and Pierre, R. (2015). Review of the taxonomic revision of *Chlorella* and consequences for its food uses in Europe. *Journal of Applied Phycology*, 27, 1845–1851.
- Charmaine, L., Kai, H., Kar, L. L., ImalaGana, V., Sarah, M. F., Teng, R. C., Hui, M. M., Wei, X.S., Sarah, L., Oscelyn, J. N., Nazurah, S. B. N., Nurhazlyn, B. M., Zephyr, Y.W. E., Punithavathy, M. and Jen, Y. (2021). New Identification of microalgae cultured in Bold's Basal medium from freshwater samples, from a high-rise city. *Scientific Reports*, 11:4474.
- Expósito, N., Carafa, R., Kumar, V., Sierra, J., Schumacher, M and Papiol, G.G. (2021). Performance of *Chlorella vulgaris* Exposed to Heavy Metal Mixtures: Linking Measured Endpoints and Mechanisms. *International Journal Environmental Research and Public Health*, 18, 1037.
- Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39:783–791.
- Göbölös, B., Sebők, R. E., Szabó, G., Tóth, G., Szoboszlai, S., Kriszt, B., Kaszab, E., and Háhn, J. (2024). The Cocktail Effects on the Acute Cytotoxicity of Pesticides and Pharmaceuticals Frequently Detected in the Environment. *Toxics*, 12, 189.
- Greco, W. R., Bravo, G and Parsons, J. C. (1995). The search for synergy: A critical review from a response surface perspective. *Pharmacological Reviews*, 47, (2): 331–385.
- Greco, W., Unkelbach, H. D., Pösch, G., Sühnel, J., Kundi, M and Bödeker, W. (1992). Consensus on concepts and terminology for combined action assessment: The Saarisekä agreement. *Archives of Complex Environmental Studies*, 4, (3): 65–69.
- Hegewald, E.H. (2000). New combinations in the genus *Desmodesmus* (Chlorophyceae, Scenedesmaceae). *Algological Studies*, 96, 1–18.
- Illman, A.M., Scragg, A.H and Shales, S.W. (2002). Increase in *Chlorella* strains calorific values when grown in low nitrogen medium. *Enzyme of Microbial Technology*, 27, 631–635.
- Jongwong, K., Myungwon, S., Jiwon C. and Minju, N. (2022). MRA Toolbox v.1.0: a web-based toolbox for predicting mixture toxicity of chemical substances in chemical products. *Scientific Reports*, 12:8880.
- Jonker, M. J., Svendsen, C., Bedaux, J. J., Bongers, M and Kammenga, J. E. (2005). Significance testing of synergistic/antagonistic, dose level-dependent, or dose ratio-dependent effects in mixture dose-response analysis. *Environmental Toxicology and Chemistry*, 24, 2701–2713.
- Jonsson, C. M. and Aoyama, H. (2007). *In vitro* effect of agriculture pollutants and their joint action on *Pseudokirchneriella subcapitata* acid phosphatase. *Chemosphere*, 69:849–855.
- Jukes, T. H and Cantor, C.R. (1969). Evolution of protein molecules. In: H.N Munro, (Ed), *Mammalian Protein Metabolism*. New York, Academic Press, New York. 21–132.
- Kahatagahawatte, Y.B.P. and Hara-Yamamura, H. (2020). Review on Mixture Toxicity of Pharmaceuticals in Environmental Waters and Wastewater Effluents. In *Resilience, Response, and Risk in Water Systems*; Springer Transactions in Civil and Environmental Engineering; Springer: Singapore, 105–126.
- Kolmar, S. S and Gruke, C. (2021). Determining the predictive Limit of QSAR Models. The United States Environmental Protection Agency's Center for Computational Toxicology and Exposure. 15070.
- Kovalakova, P., Cizmas, L., McDonald, T. J., Marsalek, B., Feng, M and Sharma, V. K. (2020) Occurrence and toxicity of antibiotics in the aquatic environment: A review. *Chemosphere*, 251, 126351.
- Loewe, S.; Muischenk, H. Kombinationswirkungen. 1. Mitteilung: Hilfsmittel Der Fragestellung (1926). *Naunyn-Schmiedeberg's Archive Experimental Pathway Pharmacol.*, 24, 316–326.
- Martínez-Ruiz, E. B and Martínez-Jerónimo, F. (2015). Nickel has biochemical, physiological, and structural effects on the green microalga *Ankistrodesmus falcatus*: An integrative study. *Aquatic Toxicology*, 169, 27–36.
- Mohy El-Din, S. M and Abdel-Kareem, M. S. (2020). Effects of Copper and Cadmium on the Protein Profile and DNA Pattern of Marine Microalgae *Chlorella salina* and *Nannochloropsis salina*. *Environmental Process*, 7, 189–205.

- Nguyen, M. K., Moon, J.Y and Lee, Y. C. (2020). Microalgal ecotoxicity of nanoparticles: An updated review. *Ecotoxicology and Environmental Safety*, 201, 110781.
- Nichols, H and Bold, H. (1965). Growth media-fresh water. In: *Hand Book of Physiology*. Methods; Stein, J. R., Ed.; Cambridge University Press: Cambridge, UK, pp. 7-24.
- Nunes, B. W., Hedlund, K. F., Oliveira, M. A and Carissimi, E. (2018). Ecotoxicological analysis of acrylamide using a microalga as an Indicator organism. *Water Environment Research*, 90, 442–451.
- O'Brien, A. L and Keough, M. J. (2014). Ecological responses to contamination: A meta-analysis of experimental marine studies. *Environmental Pollution*, 195, 185–191.
- Onyegeme-Okerenta, B. M., Ezeji, M. M., and Akpoji, B. S. (2025a). Assessment of polycyclic aromatic hydrocarbons exposure to soil and vegetable near a gas-powered plant in Rivers State, Nigeria: Impact of industrially affected soil to public health. *IPS Journal of Toxicology*, 3(4), 76–99.
- Onyegeme-Okerenta, B. M., Godwin, I. J., Belonwu, D. C., and Anacletus, F. C. (2025b). Contamination and Ecotoxicological Health Risk Assessment of Potential Toxic Elements in Surface Water and Sediment from Two Communities along Bonny River. *IPS Journal of Toxicology*, 3(2), 35–48.
- Onyeukwu, R. U., Asiwe, E. S., Nweke, C. O., Vivian, K. G.M. (2023). Toxicity of single and ternary mixtures of heavy metals and 2,4-dichlorophenoxyacetic acid to *Chlorella vulgaris* alkaline phosphatase activity. *World Journal of Advanced Research and Reviews*, 17(03), 206–223.
- Onyeukwu, U. R and Edna, I. C. (2022a). Toxicity of binary mixture of heavy metals and 2,4-dichlorophenoxyacetic acid on phosphatase activity of *Chlorella vulgaris*. *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, 16, 24-36.
- Onyeukwu, U. R and Edna, I. C. (2022b). Influence of binary mixture of heavy metals and 2,4-dichlorophenoxyacetic acid on phosphatase activity of *Desmodesmus abundans*. *International Journal of Innovative Science and Research Technology*, 7, 76-87.
- Parvin, M., Zannat, M.N. and Habib, M.A.B. (2007). Two important techniques for isolation of microalgae. *Asian Fisheries Science* 20:117-124.
- Perumal, P., B. Balaji-Prasath., P. Santhanam, S. Ananth, A. Shenbaga- Devi2 and S. Dinesh-Kumar.(2012). Isolation and culture of microalgae. *Workshop on Advances in Aquaculture Technology*.
- Roell, K.R., Reif, D.M., Motsinger-Reif, A.A. (2017). An Introduction to Terminology and Methodology of Chemical Synergy-Perspectives from across Disciplines. *Frontiers in Pharmacology*, 8, 158.
- Saxena, P and Harish, (2019). Toxicity assessment of ZnO nanoparticles to freshwater microalgae *Coelastrella terrestris*. *Environmental Science and Pollution Research international*, 26, 26991-27001.
- Schuijt, L.M.; Peng, F.J.; Van den Berg, S.J.P.; Dingemans, M.M.L.; Van den Brink, P.J. (2021). Ecotoxicological tests for assessing impacts of chemical stress to aquatic ecosystems: Facts, challenges, and future. *Science of the Total Environment*, 795, 148776.
- Stein, J.R.(1973). Handbook of Phycological Methods.Culture methods and growth measurements. Cambridge University Press, Cambridge, 56-60.
- Tomaselli, L. (2004). The microalgal cell. In: A. Richmond (Ed.), *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*. Oxford, UK: Blackwell Science press Ltd.
- Topaz, T., Egozi, R., Suari, Y., Ben-Ari, J., Sade, T., Chefetz, B and Yahel, G. (2020) Environmental risk dynamics of pesticides toxicity in a Mediterranean micro-estuary. *Environmental Pollution*, 265, 114941.
- Vanessa A.M., Ariana G. W., Juliano S. G., Gabriel C. W., Cristiane V. T., Héla T., Jean-François F., Claudemir M. R., Cleder A. S and Sylvie C. (2023). An alternative approach to assess ecotoxicological effects of agrochemical combinations used in Brazilian aquaculture farms. *Environmental Science and Pollution Research*. 30:70713–70721.
- Wang, D., S. Wang, L. Bai, M.S. Nasir, S. Li, and W. Yan, (2020) Mathematical Modeling Approaches for Assessing the Joint Toxicity of Chemical Mixtures Based on Luminescent Bacteria: A Systematic Review. *Frontiers Microbiology*, 11:1651.
- Wang, Q., Yalin, D. Sun, F. Deng, X and Chang, H. (2020). Ranking Ecological Risk of Metals to Freshwater Organisms in Lake Taihu, China. *Hindawi Journal of Chemistry*, 6.
- Yamamoto, M., Kurihara, I. and Kawano, S. (2005). Late type of daughter cell wall synthesis in one of the Chlorellaceae, *Parachlorella kessleri* (Chlorophyta, Trebouxiophyceae). *Planta*, 221,766-75.
- Zhang, F., B. Meng, S. Gao, R. Hough, P. Hu, Z. Zhang, S. Yu, K. Li, Z. Liu, and S. Cui, (2021). Levels, Inventory, and Risk Assessment of Heavy Metals in Wetland Ecosystem, Northeast China: Implications for Snow Cover Monitoring. *Water*. 13, 2161.

IPS Intelligentsia Publishing Services

HOME ABOUT JOURNALS IPS BOOKS ARCHIVES SUBMISSION SERVICES CAREER CONTACT US

PUBLISH WITH US FOR WORLDWIDE VISIBILITY

Enter Search... CS



FEATURED PUBLICATIONS

Antioxidant and Dietary Fibre Content of Noodles Produced From Wheat and Banana Peel Flour

This study found that adding banana peel flour to wheat flour can improve the nutritional value of noodles, such as increasing dietary fiber and antioxidant content, while reducing glycemic index.

DOI: <https://doi.org/10.54117/ijbs.v2i2.24>

Cite as: Oguntuyinbo, O. O., Olumurewa, J. A. V., & Omosa, O. S. (2023). Antioxidant and Dietary Fibre Content of Noodles Produced From Wheat and Banana Peel Flour. *IPS Journal of Nutrition and Food Science*, 2(2), 46–51.

Impact of Pre-Sowing Physical Treatments on The Seed Germination Behaviour of Sorghum (*Sorghum bicolor*)

This study found that ultrasound and microwave treatments can improve the germination of sorghum grains by breaking down the seed coat and increasing water diffusion, leading to faster and more effective germination.

Submit your manuscript for publication: [Home - IPS Intelligentsia Publishing Services](https://www.ips-journals.com)

* Thank you for publishing with us.