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Toxicity of quaternary mixtures of phenolic compounds and formulated glyphosate to microbial community of river water

Nweke, C.O.[1]*; Ike, C.C.[2] & Ibegbulem, C.O.[3]

1 Department of Microbiology, Federal University of Technology, P.M.B.1526, Owerri, Nigeria.

2 Department of Microbiology, Rhema University, P. M. B. 7021, Aba, Nigeria.

3 Department of Biochemistry, Federal University of Technology, P.M.B. 1526, Owerri, Nigeria.

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Abstract

The toxicity of mixtures of formulated glyphosate, phenol, 4-chlorophenol and 2,4-dichlorophenol to river water microbial community was investigated using non specific dehydrogenase activity as endpoint. The microbial community was exposed to individual toxicant and quaternary mixture ratios at concentrations ranging from 50 mg L^{-1} to 3000 mg L^{-1} over 24 h. The ecological doses (EC_{50}) as estimated from logistic or hormesis dose-effect models were 1317.946 ± 51.460 mg L^{-1}, 1046.414 ± 65.534 mg L^{-1}, 85.080 ± 4.468 mg L^{-1} and 60.897 ± 5.199 mg L^{-1} for formulated glyphosate, phenol, 4-chlorophenol and 2,4-dichlorophenol respectively. The toxicity and interaction of the mixtures were evaluated using concentration addition and toxic index models. The model deviation ratios (MDR) ranged from 0.553 ± 0.024 to 1.096 ± 0.021 while the toxic index (TI) varied between 0.912 ± 0.017 and 1.810 ± 0.078. However, the MDR and TI for all mixtures lie between 0.5 and 2.0. Thus, the joint action of the mixtures were considered additive.

Keywords: Dehydrogenase activity, concentration addition model, herbicide, EC_{50}.

INTRODUCTION

Glyphosate [N-(phosphonomethyl)glycine] is a post-emergence herbicide that inhibit the photosynthesis process of terrestrial and aquatic plants. It is the active ingredient of Roundup®, a widely used herbicide containing polyoxyethylene amime (POEA), a surfactant that enhances herbicidal action and to which toxicity of Roundup® is attributed (Tsui, 2003). In order to improve pesticide efficacy, glyphosate is usually applied as a mixture with other herbicides. Among the herbicides used in combination with glyphosate are phenoxy herbicides including 2,4-dichlorophenoxyacetic acid (2,4-D) (Lym, 2000; Sharma & Singh, 2001).

In soils and aquatic environments, the amine salts and esters of 2,4-D are degraded faster than glyphosate. The half-life of 2,4-D ranges from 1 to 14 days while the half life of glyphosate varied between 2 and 197 days (Giesey *et al*., 2000; US EPA, 2005). In the environment, 2,4-D is initially degraded to 2,4-dichlorophenol (2,4-DCP) (Daugherty and Karel, 1994) which can be degraded to 3,5-dichlorocatechol, 4-chlorophenol (4-CP) and phenol (Kohring *et al*., 1989; Zhang & Wiegel 1990; Fukumori & Hausinger, 1993) depending on the environmental conditions and the microorganisms involved. Therefore, these phenolic intermediates normally co-contaminate environmental media following application of glyphosate and 2,4-D mixture. Thus, it is imperative to evaluate the interactive toxicities of glyphosate and the phenolic intermediates of 2,4-D biodegradation

Although, as individual chemicals, toxicity of glyphosate and 2,4-D or its intermediates to microorganisms have been widely reported, not much work have been done on the toxicity of glyphosate and phenolic compounds as mixtures. Recently, Nweke *et al*. (2014, 2015) evaluated the joint action of binary mixtures of formulated glyphosate and phenolic intermediates (2,4-DCP, 4-CP and phenol) of 2,4-D biodegradation against dehydrogenase activity in pure culture of *Rhizobium* species isolated from the root nodule of a leguminous plant. The studies suggested additive interactions of formulated

*Corresponding author: Nweke. E-mail: xrisokey@yahoo.com

glyphosate with the phenolic compounds. There is need to extend such investigation to microbial communities of natural ecosystems. In natural environments, organisms are not usually subjected to toxicity of single pollutant but to mixture toxicity of a variety of pollutants. Combinations of pollutants even at no observed effect concentrations (NOEC) can produce significant toxic effects (Faust *et al*., 2003). Given the fact that microbes are vital for the efficient functioning of any ecosystem and are early warning indicator of ecosystem stress (Griffiths, 1983; Odum, 1985), it is important to investigate the mixture toxicity of pollutants on natural environmental microflora.

In this regard, toxicity assessment based on inhibition of dehydrogenase activity in natural microbial community using 2-(p-iodophenyl-3-(p-nitrophenyl)-5-phenytetrazolium chloride (INT) is widely used. Dehydrogenases are oxidoreductase enzymes that participate in respiration in microbial cells. Redox indicators serve as artificial electron acceptors in dehydrogenase activity assays to measure intracellular flux of electrons. INT is among the widely used tetrazolium salt in dehydrogenase activity assay. In this process, INT compete with the natural enzymes involved in microbial respiration, acting as electron acceptor and is reduced to red-coloured INT-formazan by a battery of microbial dehydrogenases that catalyze the movement of electrons from substrates to electron acceptors in respiratory chain (Trevors, 1984). Dehydrogenase activity is used as a measure of cell viability because dehydrogenase enzymes are intracellular and rapidly degraded following cell death (Rossel *et al*., 1997). Thus, INT could be used to assess ecotoxicological impacts of chemicals in an environment. INT and other tetrazolium salts have been used to measure dehydrogenase activity in many environments, including pure culture of microorganisms (Bitton *et al*., 1986; Baarschers *et al*., 1988), activated sludge (Kim *et al*., 1994; Caravalli *et al*., 2004), Biofilters (Fonseca *et al*., 2001), soil (Moreno *et al*., 2002) and aquatic systems (Aonofriesei, 2007; Schneider & Topalova, 2009).

In the present study, inhibition of INT-dehydrogenase activity was used to evaluate the toxicity of 2,4-DCP, 4-CP, phenol and formulaed glyphosate as well as their quaternary mixtures to microbial community of river water. Fixed ratio rays were employed to study the joint action of the mixtures at various concentrations and the effects of the mixtures on dehydrogenase enzyme activity in the microbial community was evaluated using concentration addition (CA) and toxic index (TI) models.

MATERIALS AND METHODS

Riverwater sample

River water was collected from Otamiri River at Ihiagwa, Imo State, south-eastern Nigeria. Water samples were collected midstream along the course of the river at three spots (5°24.25′0.32″ N, 7°0.36′0.036″ E; 5°24.28′0.55″ N, 7°0.38′0.36″ E and 5°23.55′0.20″ N, 6°59.46′0.39″ E) from a depth of 30 cm and pooled in 1-litre sterile plastic bottle. The pooled sample was stored in a cooler and taken to laboratory. The bacterial load of the sample was determined on nutrient agar plates within 6 h of collection using standard microbiological procedure. Within the period, the water sample was used as inoculum for the toxicity assay. The bacterial load of the water sample was estimated at 1.32 x 10^{10} CFU/ml.

Quaternary mixture ratios

The quaternary mixtures of glyphosate (as active ingredient in formulated glyphosate pesticide, Roundup), phenol, 4-CP and 2,4-DCP were studied along fixed ratio rays. The bioassays were aimed at determining the toxicity of each of the three phenolic compounds mentioned above in quaternary mixtures with glyphosate as a function of weight to weight ratios as shown in Table 1.

Table 1- Quaternary mixture ratios of glyphosate, phenol, 4-CP and 2.4-DCP

Mixture	Mixture ratio (%) Glyphosate: Phenol: 4-CP: 2,4-DCP
1	10:10:40:40
2	60:20:10:10
3	5:5:10:80
4	80:10:5:5
5	5:80:5:10
6	25:25:25:25

Dehydrogenase activity assay

A colorimetric assay with 2-(p-Iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) as an artificial electron acceptor was applied for the assay of dehydrogenase activity using the method of Nweke *et al*. (2014) with little modification. The reaction mixture consisted of 2-ml final volumes of phosphate-buffered (pH 7) nutrient broth supplemented with varying concentrations of 2,4-dichlorophenol, 4-chlorophenol, phenol and glyphosate. Into each tube containing 0.5 ml portion of X4-strength nutrient broth, requisite volumes of distilled water and stock solutions (800 or 8000 mg L^{-1}) of respective phenolic compound and glyphosate were added. Thereafter, 0.1ml of 0.2% aqueous solution of INT and 0.5 ml of riverwater sample were added into each tube to obtain varying concentrations of toxicants in each quaternary mixture ratio. The final concentrations of the toxicants ranged from 0 to 3000 mg L^{-1}. Each concentration of the quaternary mixture and the individual toxicants were prepared in triplicates. Controls were prepared without the toxicants. Triplicate control tubes were prepared for each test toxicant, giving a total of 12 controls. The cultures were incubated at room temperature (28 ± 2°C) for 24 h. After incubation, 1 ml of 1% v/v triton X-100 was added into each tube, shaken for 1 min and allowed to stand at room temperature for 10 min to permeabilize the cell membrane (Lee *et al*., 1988). The INT-formazan (INTF) produced in each tube was then extracted in 4 ml of butanol.

The formazan extract was cleared by centrifugation at 3000 rpm for 10 mins. Thereafter, absorbances of the extracts were determined spectrophotometrically at 500 nm using butanol as blank. There was no colour interference and non enzymatic reduction of INT by the toxicants, triton X-100, and butanol. Thus, no colour correction factor was applied in the assay.

Data analysis

The inhibition of dehydrogenase activity from each toxicity assessment was transformed relative to the mean of controls (with standard deviation < 5%) to a 0 to 100% scale as shown in Eq. 1. The normalized responses were generated as mean and their standard deviations from triplicate determinations.

$$R = \frac{C_A - T_A}{C_A} \times 100 \quad (1)$$

Where R is the inhibition (%) of dehydrogenase activity, C_A is the mean absorbance of INTF extract in the control experiments and T_A is absorbance of INTF extract in the test experiment with different concentrations of toxicants.

The dose-response data of the individual toxicants as well as the mixtures were then graphed and fitted with 2-parameter logistic function (Eq. 2).

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

Where x is the concentration of toxicant, EC_{50} is the concentration of toxicant that inhibited dehydrogenase activity by 50% and b is the slope at EC_{50}.

In order to predict stimulation of dehydrogenase activity at low doses of glyphosate or its mixtures with 2,4-DCP, 4-CP or phenol, the dose-response data were fitted to hormetic model (Eq. 3) of Schabenberger *et al*. (1999).

$$R = 100 - \left[\frac{100 + fx}{1 + \left[\frac{p}{100 - p} + \left\{ \left(\frac{100}{100 - p} \right) \frac{fEC_p}{100} \right\} \right] \cdot \left(\frac{x}{EC_p} \right)^b} \right] \quad (3)$$

Where f is the parameter describing the degree of hormetic response, p is the percentage decrease in the response, EC_p is the concentration of the toxicant at a given p. The parameter b is no longer the slope at EC_{50} (Cedergreen *et al*., 2005).

The toxic unit (TU) and toxic index (TI)

The toxicity of each mixture component expressed in TU was calculated as shown in Eq. 4.

$$TU = \frac{C_{mix}}{EC_{50}} \quad (4)$$

Where C_{mix} is the concentration of the component in the mixture at the EC_{50} of the mixture and EC_{50} is the concentration of the component that elicited 50% decrease in dehydrogenase activity when tested as an individual.

The Toxic Index (TI) of each mixture was calculated as sum of TU for all the components of the mixture (Eq. 5).

$$TI = \sum_{i=1}^{n} TU \quad (5)$$

Where n is the number of components in the mixture. TI = 1 describes additivity, TI > 1 describes antagonistic interaction and TI < 1 describes synergistic interaction (Boillot & Perrodin, 2008).

Prediction of mixture toxicities

Effective concentration of the mixture ($EC_{x(mix)}$) obeying concentration addition (CA) can be predicted from the equation:

$$EC_{x(mix)} = \left(\sum_{i=1}^{n} \frac{\pi_i}{EC_{xi}} \right)^{-1} \quad (6)$$

Where n is the number of components, π_i is the proportion of ith component in the mixture, such that the sum of $\pi_i = 1$, EC_{xi} is the concentration of ith component that gave x effect when tested as an individual. In an n-component mixture, Eq. 6 for an EC_{50i} can be substituted into Eqs. 2 and 3 to give Eqs. 7 and 8 respectively:

$$R = \frac{100}{1 + \left(\sum_{i=1}^{n} \frac{\pi_i x}{EC_{50\,i}} \right)^b} \quad (7)$$

$$R = 100 - \frac{100 + fx}{1 + \left[1 + \left(\frac{2f}{100 \left(\sum_{i=1}^{n} \frac{\pi_i}{EC_{50\,i}} \right)} \right) \right] \cdot \left(\sum_{i=1}^{n} \frac{\pi_i x}{EC_{50\,i}} \right)^b} \quad (8)$$

Where x is the total concentration of all the components in the mixture and b is the average slope for individual components (Rider & LeBlanc, 2005).

The CA models (Eq. 7 and 8) were used to predict the joint effect of the quaternary mixtures depending on whether there was hormesis or not (Nweke *et al*. 2015).

The size of hormetic response f was predicted from the relative proportion of each compound in the mixture as described by Belz *et al*. (2008). If two compounds A and B are mixed with a proportion p% of A and (100-p)% of component B, then the expected f at p (f_p) is given by:

$$f_p = f_A \frac{p}{100} + f_B \frac{100 - p}{100} \quad (9)$$

Thus,

$$f_p = f_A \pi_A + f_B \pi_B = \sum_{i=1}^{n} f_i \pi_i \quad (10)$$

Where n is the number of components, π_i is the proportion of ith component in the mixture. Using the predicted f (obtained

by fitting the dose-response relationships of individual components into hormesis model) and average *b*, the effect of the quaternary mixture on stimulation of dehydrogenase activity was predicted on the basis of concentration addition (CA) using the hormesis model. In each case, the predicted EC_{50} was compared with the experimental EC_{50}.

Curve fittings were implemented with TableCurve 2D v5.01. A 2-way Anova with replication to test for the influence of the different concentration ratios and the method of prediction on the EC_{50}s was implemented with Microsoft Excel 2003. The 2-way Anova was based on the different mixtures (as factor A on the columns) and the method of predicting the EC_{50}, experimentally derived or CA-predicted (as factor B in the rows). The Duncan post-hoc tests were done using IBM SPSS Statistics 21.

Model deviation ratios (MDR)

The model deviation ratios (MDR) were calculated as the ratio between the predicted effect concentration and the experimentally observed effect concentration at EC_{50}. A MDR of greater than 1 indicated that the model underestimated toxicity, while a value of less than 1 indicated that the model overestimated toxicity.

$$\text{MDR} = \frac{\text{Predicted } EC_{50}}{\text{Experimental } EC_{50}} \quad (11)$$

RESULTS AND DISCUSSION

The responses of the microbial community to the toxicity of glyphosate, phenol, 4-CP and 2,4-DCP as individuals are shown in Figure 1. Glyphosate had biphasic effect on the dehydrogenase activity of the microbial community. Hormesis occurred at concentrations up to 800 mg L^{-1} glyphosate. Above this hormetic concentration range, glyphosate progressively inhibited dehydrogenase activity of the community, reaching 96.92% inhibition at 3000 mg L^{-1}. Similarly, phenol, 4-CP and 2,4-DCP progressively inhibited dehydrogenase activity reaching 96.68%, 98.82% and 99.68% at 3000 mg L^{-1}, 600 mg L^{-1} and 1500 mg L^{-1} respectively. The median inhibitory concentrations (EC_{50}) of the toxicants are shown in Table 2. Glyphosate with the EC_{50} of 1317.946 ± 51.460 mg L^{-1} was the least toxic while 2,4-DCP with the EC_{50} of 60.897 ± 5.199 mg L^{-1} was the most toxic.

The toxicity of the quaternary mixtures of glyphosate, phenol, 4-CP and 2,4-DCP are shown in Figure 2. As was the case with glyphosate as individual, the 60:20:10:10 (mixture 2) and 80:10:5:5 (mixture 4) glyphosate: phenol: 4-CP: 2,4-DCP mixtures exhibited hormesis at low doses up to 200 mg L^{-1} and 600 mg L^{-1} respectively. Other mixtures inhibited dehydrogenase activity even at low doses. The responses of the community to the mixtures as predicted from the CA model are also shown in Figure 2. The experimentally-derived

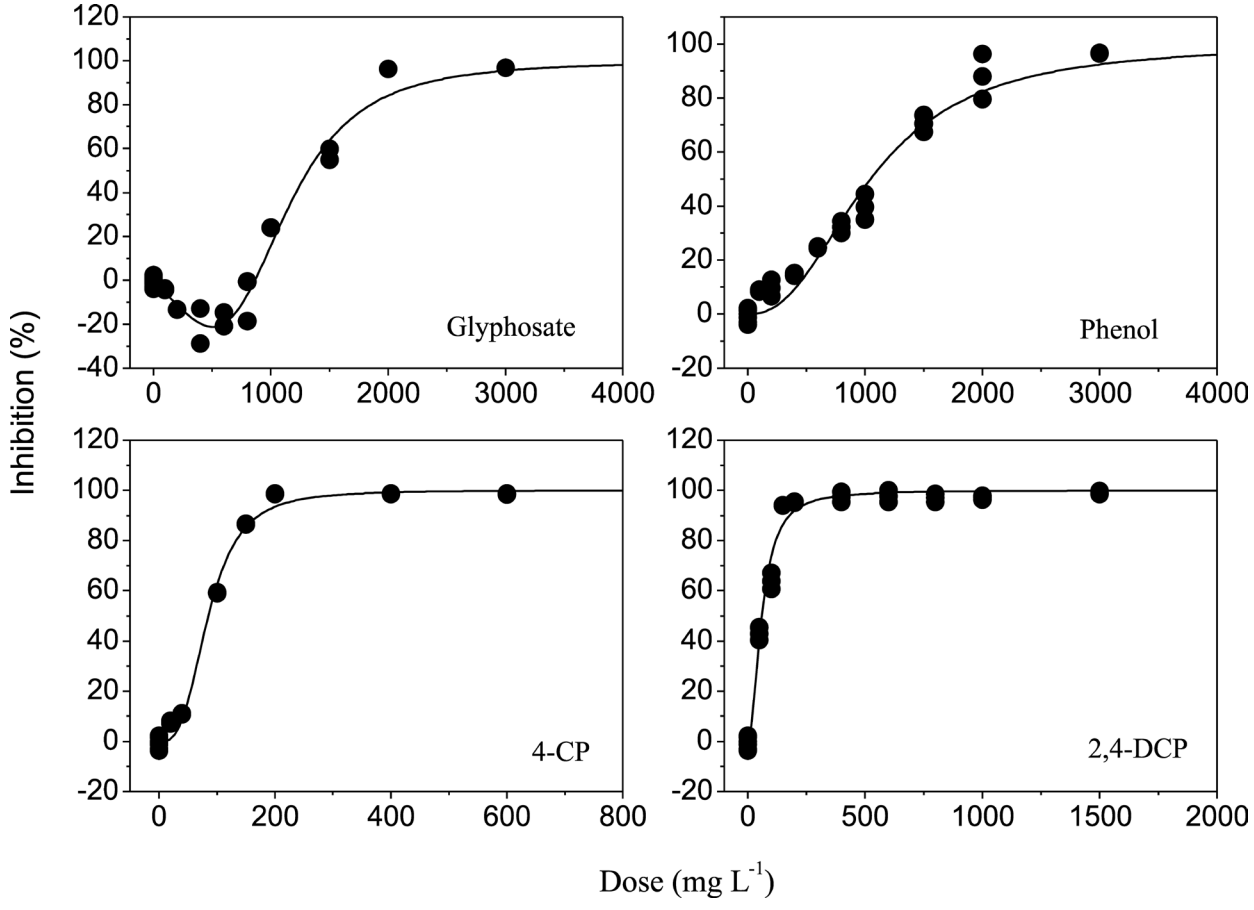

Figure 1– Inhibition of dehydrogenase activity in riverwater microbial community by the individual toxicants

Table 2– The EC_{50} of individual substances

Compound	EC_{50} (mgL^{-1})
Glyphosate	1317.946 ± 51.460
Phenol	1046.414 ± 65.534
4-CP	85.080 ± 4.468
2,4-DCP	60.897 ± 5.199

EC_{50}s and CA-derived EC_{50}s, the model deviation ratios and the toxic index for the various mixtures are shown in Table 3. The CA model predicted similar toxicity of 5:5:10:80 (mixture 3) as the experiment would suggest. Similarly, with 5:80:5:10 (mixture 5) mixture, the CA model predicted toxicities that were close to the experimentally-derived values. However, the model underestimated the toxicity of mixture 3 and overestimated the toxicity of mixture 5 at low doses (Fig. 2). In the other mixtures, CA model slightly overestimated

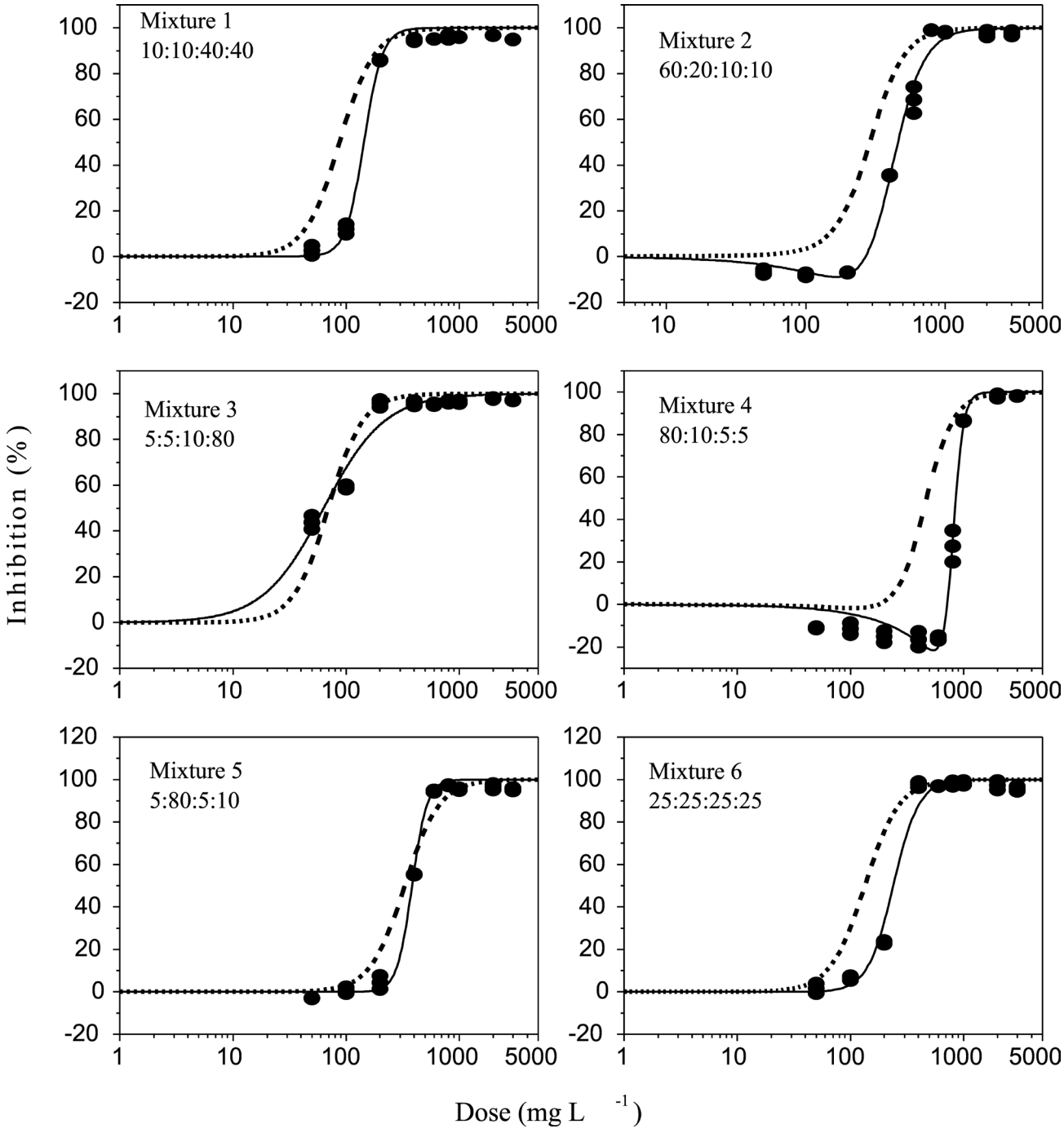

Figure 2– Experimental and predicted dose-response data for the inhibition of dehydrogenase activity in riverwater microbial community by mixtures of glyphosate and phenolic compounds (in the ratio; glyphosate: phenol: 4-CP: 2,4-DCP). Data points represent experimentally-derived values. The solid lines represent the logistic model (Eq. 2) or hormesis model (Eq. 3) fit to the experimental data and the dashed lines represent values predicted from CA models (Eq 7 (without hormesis) or Eq 8 (with hormesis)).

Table 3 – Toxicological parameters of the quaternary mixtures of glyphosate and phenols

Mixture	EC_{50} (mgL^{-1})		MDR	TI	Effect**
	Experimental †	Predicted ‡			
1	143.781 ± 6.868[a]	87.386 ± 6.240[a]	0.607 ± 0.014	1.647 ± 0.039	ANT
2	459.411 ± 16.648[b]	288.630 ± 19.315[b]	0.628 ± 0.019	1.594 ± 0.049	ANT
3	63.432 ± 6.438*[c]	69.450 ± 5.730*[,a]	1.096 ± 0.021	0.912 ± 0.017	ADD[a]
4	855.434 ± 16.153[d]	473.516 ± 29.322[c]	0.553 ± 0.024	1.810 ± 0.078	ANT
5	382.943 ± 10.245[e]	329.742 ± 23.985[d]	0.860 ± 0.040	1.164 ± 0.054	ADD[b]
6	239.454 ± 12.454[f]	133.803 ± 9.444[e]	0.558 ± 0.011	1.791 ± 0.036	ANT

† Within column, all experimental EC_{50} values are statistically different ($p < 0.05$) from each other

‡ Within column, predicted EC_{50} values with same letters are not statistically different ($p > 0.05$) from each other

*No significant difference ($p > 0.05$) between experimental and predicted EC_{50} for mixture 3. Other predicted EC_{50s} without asterisks are statistically different from the corresponding experimental values.

**Effects are shown as additive (ADD) and antagonistic (ANT); [a] the slightly synergistic effect had TI value close to 1 and was taken to be additive; [b] the slightly antagonistic effect had TI value close to 1 and was taken to be additive

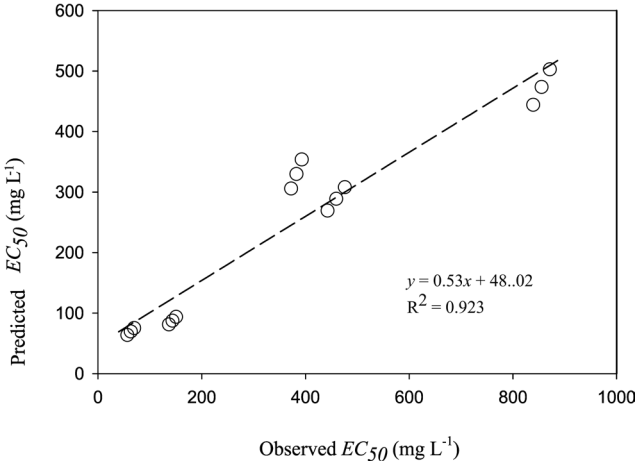

Figure 3– Correlation between the predicted and experimentally-observed EC_{50} of the quaternary mixtures.

the inhibition of dehydrogenase activity. The 2-way Anova comparisons among the different concentration ratios and between the experimentally observed and CA-predicted toxicity indicated strong statistical difference between the toxicity of the different metal mixtures (p = 6.4E-28) and that strong statistical difference existed between experimentally observed and predicted toxicities (p = 1.8E-18). The relationship between the predicted and observed EC_{50} is shown in Figure 3. The R^2 value of 0.923 indicated strong positive correlation between the predicted and observed toxicity.

Hormesis is a dose-effect phenomenon that is characterized by low-dose stimulation and high-dose inhibition (Calabrese & Baldwin, 2001). This biphasic response to chemicals occurs widely in microorganisms and higher forms of life (Calabrese & Blain, 2005). Nweke *et al*. (2014) reported hormetic effects of glyphosate at low doses on the dehydrogenase activity in pure culture of *Rhizobium* species. Stimulation of microbial activity following glyphosate application in soil has been reported (Araujo *et al*., 2003; Ratcliff *et al*., 2006; Partoazar *et al*., 2011; Allegrini *et al*., 2015). The stimulation of dehydrogenase activity at low doses of glyphosate could be attributed to possible use of glyphosate as carbon and energy source. Several bacterial species have been demonstrated to grow on glyphosate and its biodegradation intermediate (Dick & Quinn, 1995; Gimsing *et al*., 2004; Moneke *et al*., 2010). Thus, the observed hormesis could be due to increase in respiration response at low concentrations due to sarcosine, an intermediate of glyphosate biodegradation (Allegrini *et al*., 2015). In addition, it could be a stress response in glyphosate sensitive species at low concentrations due to the "energy drain" resulting from the ATP used in the accumulation of shikimate and hydroxybenzoic acids (Zabaloy *et al*., 2012).

The inhibition of dehydrogenase activity observed in this study at higher concentrations of glyphosate and phenols corroborated other reports. Toxicity of glyphosate to bacteria and other microorganisms have been reported (Botelho, *et al*., 2012; Shehata *et al*., 2013, Nweke *et al*., 2014). Glyphosate exhibits higher toxicity in soil-free media (Busse *et al*., 2001). The non-specific dehydrogenase activity appeared to be a less sensitive response for assessing toxicity of glyphosate to bacteria. An IC_{50} of 18.2 mg L^{-1} glyphosate for *Vibrio fischeri* bioluminescence was reported by Bonnet *et al*. (2007). Similarly, Tsui & Chu (2003) reported IC_{50} of 162.0 mg L^{-1} and 24.9 mg L^{-1} for glyphosate (isopropylamine salt) and Roundup® respectively. Based on inhibition of non-specific dehydrogenase activity, Nweke *et al*. (2014) reported an IC_{50} of 661.614 ± 33.234 mg L^{-1} glyphosate (in Roundup®) against pure culture of root nodule *Rhizobium* species..

Phenolic compounds are known to disrupt membrane functions by causing loss of cytoplasmic membrane integrity (Heipieper *et al*., 1991). Dehydrogenase enzymes are membrane- associated; thus loss of membrane integrity will eventually affect their activity. The order of toxicity is

2,4-dichlorophenol > 4-chlorophenol > phenol > glyphosate. This trend is similar to what was obtained with pure culture of *Rhizobium* species (Nweke *et al*., 2014). However, the microbial community was more tolerant to the formulated glyphosate than *Rhizobium* species. A lower IC_{50} of 177 and 210 mg L^{-1} phenol based on inhibition of dehydrogenase activity in *Pseudomonas fluorescens* ATCC 13525 was reported (Abbondanzi *et al*., 2003). In *Escherichia coli*, Cenci *et al*. (1987) estimated median inhibitory concentration (IC_{50}) of phenol and 4-chlorophenol against dehydrogenase activity at 6.76 mM (636.18 mg L^{-1}) and 1.6 mM (205.78 mg L^{-1}) respectively. More recently, 115.824 ± 11.05 mg L^{-1} 4-chlorophenol was reported to inhibit 50% of dehydrogenase activity in *Pseudomonas* species isolated from petroleum refinery wastewater (Nweke & Okpokwasili, 2010b). In a similar study, IC_{50} ranging from 471.419 to 1415.671 mg L^{-1} phenol against dehydrogenase activity in wastewater bacteria was reported (Nweke & Okpokwasili, 2010a)

The good correlation between the observed and predicted toxicity might be due to the toxicants exhibiting same mode of action against the microbial community. The phenols used in this study are known to be polar narcotics (Aptula *et al*., 2002) and as mentioned earlier, they disrupt the functions of the cell membrane. Although, glyphosate may not have narcotic effect, the polyoxyethylene amine (POEA) used in its formulation is a surfactant and may have membrane-damaging effect on microorganisms. Navarro & Martinez (2014) reported that (POEA) can cause toxic effects in fish, which are related to an imbalance in the redox state. Thus, CA model was expected to have good prediction of the mixture toxicity. As was mentioned earlier, CA model gave good prediction of the toxicities of mixtures 3 and 5, indicating that they are additive.

In our previous study, we reported the mutual modulation of toxicities in binary mixtures of formulated glyphosate with 2,4-dichlorophenol, 4-chlorophenol or phenol to produce synergistic and antagonistic effects. (Nweke *et al*., 2014; 2015). However, statistical analysis lead to the conclusion that the joint action of the mixtures on *Rhizobium* species dehydrogenase activity was additive. Similar observations were made in this study with microbial community of river water. The MDR and TI values indicated slight synergistic and antagonistic interactions among the components of the chemical mixtures. Nevertheless, the values are within 0.5 - 2.0 range, thus the mixtures are said to be additive (Petersen & Tollefsen, 2011; Li *et al*., 2014).

CONCLUSION

The *in vitro* toxicities of the quaternary mixtures of formulated glyphosate and phenols (2,4-DCP, 4-CP and phenol) were predicted using concentration addition (CA) and toxic index (TI) models. The models indicated possibility of synergistic and antagonistic actions. However, the MDR and TI values lie between 0.5 and 2.0. These values are within the expected inter-laboratory/inter-experiment deviation for most species. We therefore concluded that the joint actions of the mixtures on microbial community of the river water were additive.

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