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Interactive Effect of Quinary Mixtures of Phenols on *Pseudomonas fluorescens*

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Abstract
Background*: Chemicals do not exist in isolation in nature, but under certain conditions, may act jointly in a way that the overall level of toxicity is affected.Toxicity interaction of chemical mixtures have been widely reported and mathematically described to include a number of interactions such as synergism and antagonism; and in more complex models involving a mixture of homesis and synergism.Toxicity of 2,4-dichlorophenol (2,4-DCP), 4-chlorophenol (4-CP), 4-bromophenol (4-BP), 2-chlorophenol (2-CP) and phenol as single and quinary mixtures to Pseudomonas fluorescens using inhibition of dehydrogenase activity as toxicity response was assessed.*
Materials and Methods*: Quinary mixtures of the phenolic compounds were evaluated using arbitrary concentration ratios (ABCR) and equi-effects concentration ratios (EEC) to obtain mixtures of the compounds 2,4-D:4-CP:2-CP:4-BP:Phe. The ABCR ratios were (ABCR1;2%:6%:14%:4%:74%), (ABCR2;6%:2%:28%:2%:62%), (ABCR3; 1%:5%:10%:2%:82%) and (ABCR4;4.5%:3.4%:20%:3.1%:69%), while the EEC ratios evaluated were EE5, EE10, EE20, EE30, EE40 and EE50.*
Results*: Results obtained from the study showed that the phenolic compounds toxicity to dehydrogenase enzyme activity were logistic dose dependent. Assessment of the mixtures showed that among the ABCR mixtures, ABCR1 produced the highest toxicity with ecotoxic concentration 50% (EC_{50}) value of 1.632 ± 0.048mM, while EE10 mixture was the most toxic among the EEC ratios with EC_{50} value of 1.732 ± 0.028mM. The quinary mixtures of these phenolic compounds were found to be less toxic than the single compounds.Toxic index (TI) analysis of the mixtures indicated largely antagonistic interactions. Toxicity of the arbitrary concentration ratios mixtures was in the order ABCR1>ABCR2>ABCR3>ABCR4, while equi-effect concentration ratios was in the order EE10> EE50> EE40> EE20> EE30> EE5.* ***Conclusion:*** *The interactive effect of the mixtures of these compounds suggest that response of the isolate to their toxicity maybe largely dependent on the relative amount of these toxicants present in a mixture.*
Key words: *Toxicity,antagonism, phenolic compound, mixtures and Pseudomonas fluorescens,*

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I. Introduction

Phenols are important compounds widely applied in the modern chemical industries such as petroleum refining, drugs manufacture, paints, fertilizer etc. Phenol derivatives are abundant in the effluent component discharged into the environment by these industries (Whiteley and Bailey, 2000; Arutchelvan, *et al.,* 2005). Phenolic compounds maybe recalcitrant and toxic to most microorganisms; at higher concentrations, they can even inhibit the growth of microbial strains that are capable of assimilating them (Krastanov *et al.,* 2013). Chlorophenols are widespread pollutant of aquatic ecosystems, their toxicity may be genetic and influence the offspring of the affected organisms (Galindez-Mayer *et al.,* 2008). Microbial community are directly involved in biodegradation and maybe affected in frontline of a toxicity burst. Microbial cells are toxico-sensitive and can be applied as a biosensor in the detection of toxicity and environmental pollution (Chan *et al.,* 2013; Liu *et al.,* 2014; Vogrinc*et al.,* 2015; Zhou *et al.,* 2017). Phenol and its derivatives when degraded bya numbers of microorganisms may result to other products which pose environment risk to aquatic and terrestrial ecosystem (Krastanov*et al.,* 2013). In the Environment, chemicals do not act in an isolation but in the milieux of other equally potential toxicants. Toxicants may bioaccummulate in the environment; act independently or by interaction with other environmental toxicants. Toxicity interaction of chemical mixtures have been widely reported and mathematically described to include a number of interactions such as synergy and antagonism; and in more complex models involving a mixture of homesis and synergism (Cassee *et al.* 1998; Haddad *et al.,* 2000; Charles *et al.,* 2007; Kortenkamp *et al.,* 2009, SCHER, SCCS, SCENIHR, 2012; Nweke *et al.,* 2015; Nweke *et al.,* 2018). In the present study, *Pseudomonas fluorescens* dehydrogenase enzymes inhibition is applied as an index of cellular necrotic potential. The present study examines the invitro toxicity of phenols, 2-

Chlorophenol, 4-Chlorophenol, 2.4-dichlorophenol, 4-Bromophenol on *P. fluorescens* isolates, and the possible interactive effect of these compounds in a hypothetical quinary mixture using a randomized concentration ratio and equi-effect concentration ratio.

II. Materials and Methods

Test Chemical and Reagents

The phenolic compounds used in this study were Phenol (JHD, China), 2-Chlorophenol (Merck, Germany), 4-Chlorophenol (Merck, Germany), 2,4-dichlorophenol (JHD, China) and 4-bromophenol (Merck, Germany).The enzyme substrate 2,3,5-triphenyltetrazolium chloride was obtained from Sigma-Aldrich, Germany. All other reagents used were of analytical grade.

Isolation of Test Organism

The soil sample for the isolation of the test organism was collected from an arable soil in Owaelu Urata in Owerri-North LGA of Imo State, Nigeria.

The test bacterium *Pseudomonas fluorescens* was isolated from garden soil by inoculation of soil suspension on *Pseudomonas* agar for fluorescein (obtained from Sisco Research Laboratories (SRL), Mumbai, India) and incubated at 30^{o}C for 24 h (Collins *et al.,* 2004). The isolated organism was then characterized based on morphology, growth characteristics and biochemical test using oxidase production, oxidative fermentation, growth at 4 ^{o}C, no growth at 42 ^{o}C, nitrate reduction, phosphate solubilization (using Pikovskaya's broth, SRL, India), acid production from mannitol and maltose (Rhodes, 1959).

Preparation of Inoculum for Toxicity Assay

The *P. flourescens isolate* was grown in nutrient broth on a rotary shaker (150rpm) at room temperature (28 ± 2^{0}C) for 24 hrs. The cells were harvested by centrifugation at 4000 rpm for 10 min using centrifuge (Alpin medical England Model 90(1). Harvested cells were washed twice in sterile distilled water (Nweke *et al.,* 2014). The washed cells were suspended in the sterile distilled water and their optical density (OD) adjusted to 0.1 at wavelength of 540 nm using spectrophotometer (VIS Spectrophotometer 721D, Life Assisstance Scientific INST. CO).

Preparation of single chemicals

A 10mM stock solution of each of 2,4-dichlorophenol (2,4-DCP) and 4-chlorophenol (4-CP), and 50mM stock of 4-bromophenol (4-BP), 2-Chlorophenol (2-CP), and phenol were prepared in sterile deionized water and used for the study.

Preparation of mixtures of chemicals

The phenolic mixtures were prepared by diluting and mixing the stock solutions to achieve desired combination ratios in the mixtures as described in table 1. Arbitrary concentration ratios (ABCR) were based on random variations in the percentage composition of the mixtures. The equi-effect concentration ratios were achieved using the concentration of the single compounds with equal eco-toxic effect to create combination ratios (%). The mixtures were created in a final stock concentration of 10mM.

Design of Experiment

The quinary mixtures of all the phenolic compounds were studied through arbitrary concentration ratio (ABCR) and equi-effect concentration ratio (EE) and the various arbitrary concentration ratios (ABCR) evaluated were ABRR1, ABCR2, ABCR3 and ABCR4, while equi-effect concentration ratios evaluated were EE5, EE10, EE20, EE30, EE40 and EE50 as shown on table 1.

Table 1: Experimental design for the various Equi-effect and arbitrary concentration ratios of the phenols to the dehydrogenase activity of *Pseudomonas flourescens*

	Mixture ratios (%)				
Mixture	**2,4-DCP**	**4-CP**	**2-CP**	**4-BP**	**Phenol**
ABCR1	2.0	6.0	14.0	4.0	74.0
ABCR2	6.0	2.0	28.0	2.0	62.0
ABCR3	1.0	5.0	10.0	2.0	82.0
ABCR4	4.5	3.4	20.0	3.1	69.0
EE5	1.9	8.2	14.6	3.4	71.9
EE10	1.9	7.6	14.4	3.5	72.6
EE20	1.9	7.0	14.1	3.6	73.4
EE30	1.8	6.7	13.9	3.7	73.9
EE40	1.8	6.4	13.7	3.8	74.3
EE50	1.8	6.1	13.6	3.9	74.6

Toxicity Assay

Dehydrogenase activity inhibition was determined using 2,3,5-triphenyltetrazolium chloride (TTC) as the artificial electron acceptor which is reduced to the red-colored triphenylformazan (TPF) as described by Nweke *et al.,* 2014.The assay was carried out in 2ml volume of nutrient broth (pH 7) and TTC supplemented with varying concentrations of phenol, 2-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol, , 4-bromophenol. A 0.5ml portion of x4-strength of 0.1% nutrient broth and requisite volumes of sterile distilled water and stock solution (10mM for 2,4-DCP, 4-CP, 4-BP and 50mM for 2-CP and phenol) of the respective toxicants were added to each 20ml screw capped test tube to obtain different concentrations of the individual mixture ratios. Thereafter, 0.1 ml each of 0.1%w/v solution of TTC and bacterial suspension were added into each tube. The final concentrations of the toxicants ranged from 0 to 0.4 mM. The controls consisted of the medium without the toxicants. The cultures were incubated at room temperature (28 ± 2^0C) for 24 hrs. After incubation, 1ml of 1%v/v Triton X100 was added into each tube and allowed to stand for 10 min. the formazan produced in each tube was extracted in 4ml of butanol; and absorbances of the extracts were determined spectrophotometrically at 500nm.

Data Analysis

The inhibition of dehydrogenase activity at varying concentrations of the phenols (2,4-DCP, 4-CP, 2-CP, 4-BP and phenol) as well as mixtures were calculated as shown in equation below. The responses were generated as mean and standard deviation from triplicate determination as described by Nweke *et al.* (2014).

$$\%\ INH = \frac{C_A - T_A}{C_A} \times 100 \quad \text{.....} \quad \text{.....} \quad \text{.....} \quad \text{.....} \quad \text{.....} (1)$$

Where C_A is the absorbance of TPF extract in the control (without phenols); T_A is the absorbance of TPF extract in the test with different concentrations of the mixtures.

Determination of toxicity threshold

The dose-response data from the assessment of toxic effects of the toxicants (as individual and mixtures) to dehydrogenase activity of *Pseudomonas flourescen* were fitted into 2-parameter logistic model to obtain their respective ecotoxic threshold (EC_{50}) which is defined as the concentrations of the toxicants that inhibited the dehydrogenase activity of the isolate by 50%.

$$\%\ INH = \frac{100}{1 + \left(\frac{x}{IC_{50}}\right)^b} \quad \text{.....} \quad \text{.....} \quad \text{.....} \quad \text{.....} \quad \text{.....} \quad \text{.....} \quad \text{.....} (2)$$

Where x is the concentration of the chemical, EC_{50} is the concentration that caused 50% inhibition;b is parameter determining the relative slope at EC_{50}.

Analysis of combined effects using toxic index model

Toxic index (TI) model was also used to analyze the combined effect of the mixtures. The TI values were calculated using the expression:

$$TI = \sum_{i=1}^{n} TU_i \quad \text{.....} \quad \text{.....} \quad \text{.....} \quad \text{.....} \quad \text{.....} \quad \text{.....} \quad \text{.....} (3)$$

$$TU_i = \frac{Cmix_i}{IC_{50\,I}} \quad \text{.....} \quad \text{.....} \quad \text{.....} \quad \text{.....} \quad \text{.....} \quad \text{.....} \quad \text{.....} \quad \text{.....} (4)$$

Where $Cmix_i$is the concentration of the *i*th toxicant in the mixture and IC_{50i} is the IC_{50} of the same toxicant when tested as an individual.TI=1 signifies additive interaction, TI > 1 signifies antagonistic interaction and TI < 1 signifies synergistic interaction (Boillot and Perrodin, 2008).

III. Results

Figure 1.0 shows the fit curves for toxicity of the phenols, 2,4-dichlorophenol, 4-Chlorophenol, 4-Bromophenol, 2-Chlorophenol and phenol singly to dehydrogenase activity of *P. fluorescens.*

The phenols showed a logistic dose dependent inhibition of the dehydrogenase activity of the isolate; toxicity response of the isolates to the phenols ranked in the order 2,4-Dichlorophenol > 4-Bromophenol > Phenol > 4-Chlorophenol > 2-Chlorophenol. However, the response of the organism to phenol and 2-chlorophenol showed a narrow zone of low dose stimulation between 0-0.2mM.

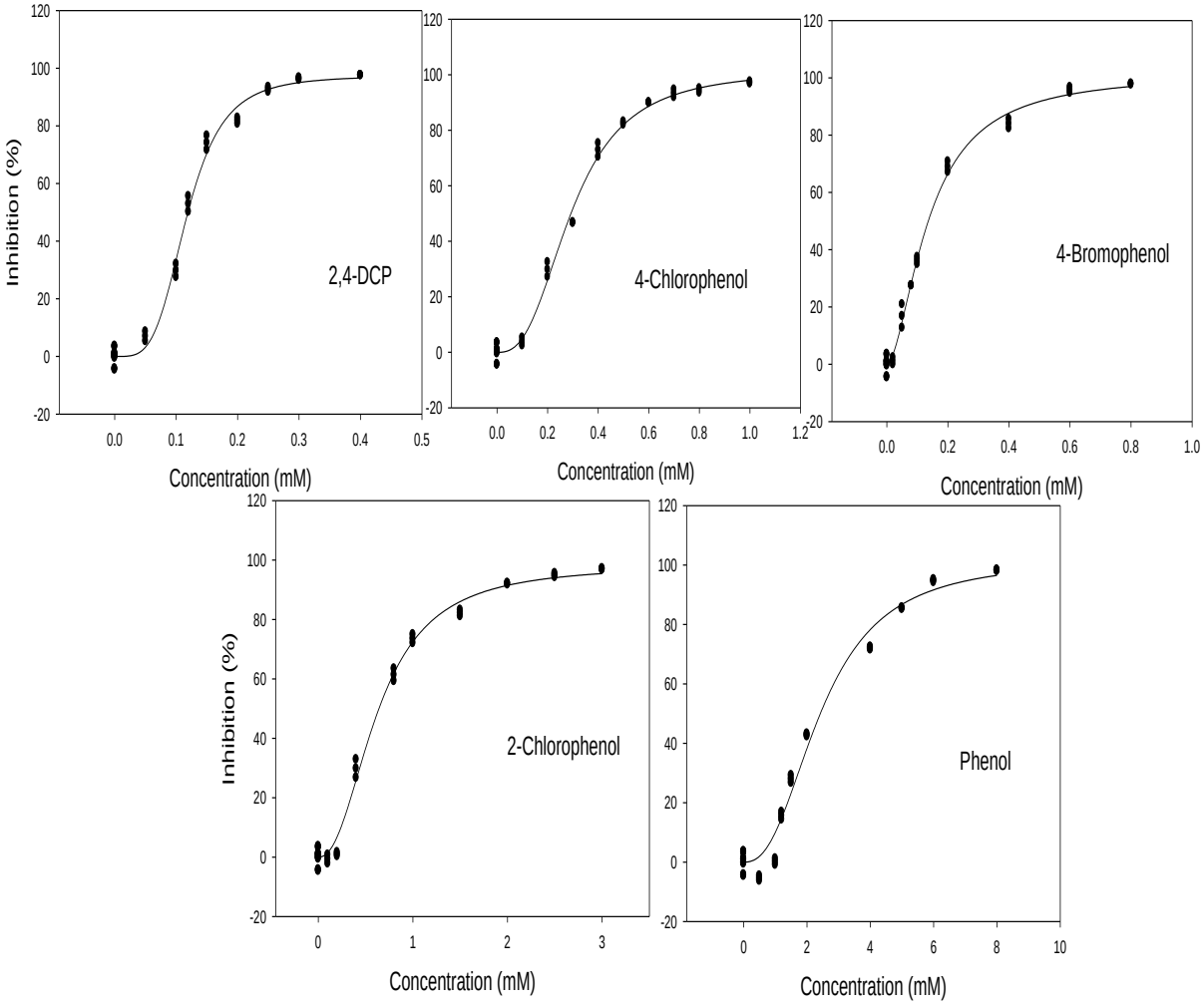

Figure 1: Toxicity of 2,4-dichlorophenol, 4-Chlorophenol, 4-Bromophenol, 2-Chlorophenol and phenol to *P. fluorescens*

Figure 2.0 shows the toxicity effect of quinary mixtures of 2,4-dichlorophenol, 4-Chlorophenol, 4-Bromophenol, 2-Chlorophenol and phenol to *P. fluorescens*using arbitrary concentration ratios.The quinary mixtures of phenols demonstrated a significantly increased inhibitory effect on the growth of *P. fluorescens.* Toxicity of the phenol mixtures progressively increased with increasing concentration in the four assessed ratios of phenol mixtures: ABRR1, ABCR2, ABCR3 and ABCR4 achieving a total inhibition of dehydrogenase activity. The mixtures ABRR1, ABCR2, ABCR3 and ABCR4 progressively inhibited dehydrogenase activity, reaching saturations at concentrations between 4.0-6.0mM of the mixtures.

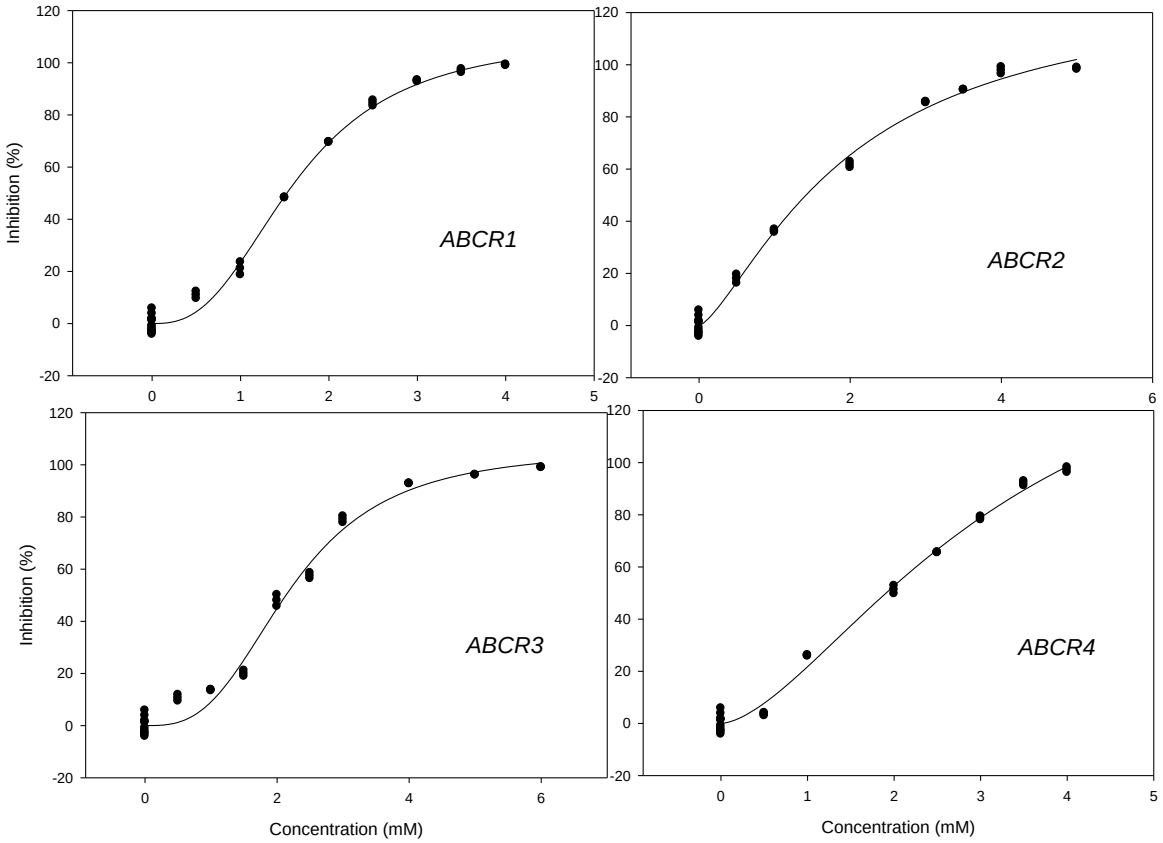

Figure 2: Toxicity of arbitrary concentration ratio quinary mixtures of 2,4-dichlorophenol, 4-Chlorophenol, 4-Bromophenol, 2-Chlorophenol and phenol to *P. fluorescens*

The fitted curves in figure 3 shows the response of *P. fluorescens*to toxicity of quinarymixtures of 2,4-dichlorophenol, 4-Chlorophenol, 4-Bromophenol, 2-Chlorophenol and phenol usingequi-effect concentration ratios. The equi-effect mixtures stimulated dehydrogenase activity at low doses, however, exhibited a steady increase in inhibition as concentration increased. At high concentration, the mixtures EE5, EE10, EE20, EE30, EE40 and EE50 demonstrated a saturation effect, with no significant changes in toxicity beyond 4.0mM.

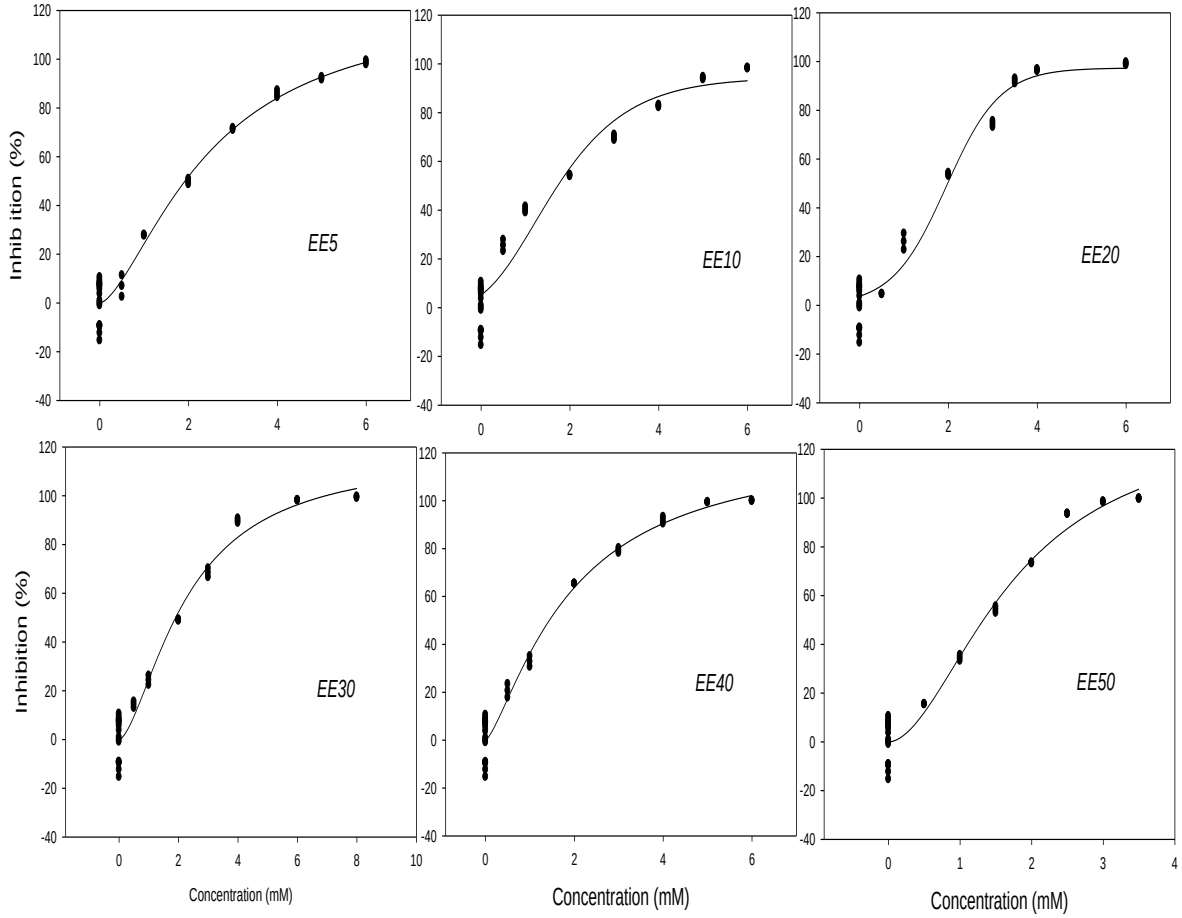

Figure 3: Toxicity of equi-effect quinarymixtures of 2,4-dichlorophenol, 4-Chlorophenol, 4-Bromophenol, 2-Chlorophenol and phenol to dehydrogenase activity of *P. fluorescens*

Table 2.0 shows thethresholdeco-toxic concentration (EC_{50})of individual and mixtures of phenols. The EC_{50} values obtained from the studies was 0.118 ± 0.002mM, 0.296 ± 0.006mM, 0.138 ± 0.005mM, 0.631 ± 0.019mM and 0.245 ± 0.012mM for the single compounds 2,4-Dichlorophenol, 4-Chlorophenol, 4-Bromophenol, 2-Chlorophenol and Phenol respectively. The EC_{50} of the arbitrary concentration ratio and equi-effect mixture are presented on table 2.0.

Table 2: Thresholdeco-toxic concentration (EC_{50}) of the singles and quinary mixtures of phenols to dehydrogenase activity of *P.fluorescens*

Toxicants/Mixtures	EC_{50} (mM)
2,4-Dichlorophenol	0.118 ± 0.002
4-Chlorophenol	0.296 ± 0.006
4-Bromophenol	0.138 ± 0.005
2-Chlorophenol	0.631 ± 0.019
Phenol	0.245 ± 0.012
Arbitrary concentration ratios	
ABCR1	1.632 ± 0.048
ABCR2	1.984 ± 0.014

ABCR3	2.216 ± 0.071
ABCR4	3.276 ± 0.056
Equi-effect concentration ratios	
EE5	2.443 ± 0.045
EE10	1.208 ± 0.013
EE20	1.947 ± 0.010
EE30	2.279 ± 0.092
EE40	1.922 ± 0.038
EE50	1.732 ± 0.028

Values are Mean ± standard deviation of 3 determinations

Table 3.0 shows the toxicity interactions of quinary mixtures of phenols to dehydrogenase activity of *P. fluorescens.*The TI values ranged from 6.31 ± 0.38 to 12.60 ± 0.41 for arbitrary concentration ratios, and 4.60 ± 0.23to 8.80 ± 0.70 for equi-effect concentration ratio mixtures. The interactions observed in the assessed mixtures were largely antagonism.

Table 3: Toxicity interactions of quinary mixtures of phenols to dehydrogenase activity of *P. fluorescens*

Mixtures	Toxic Index (TI)	Interactive Effect
Arbitrary concentration ratios		
ABCR1	6.31 ± 0.38	Antagonistic
ABCR2	7.30 ± 0.52	Antagonistic
ABCR3	8.63 ± 0.35	Antagonistic
ABCR4	12.60 ± 0.41	Antagonistic
Equi-effect concentration ratios		
EE5	7.15 ± 0.20	Antagonistic
EE10	4.60 ± 0.23	Antagonistic
EE20	7.54 ± 0.56	Antagonistic
EE30	8.80 ± 0.70	Antagonistic
EE40	7.42 ± 0.37	Antagonistic
EE50	6.71 ± 0.47	Antagonistic

Values are Mean ± standard deviation of 3 determinations

IV. Discussion

Toxicity interaction of chemical mixtures is paramount in assessment of environmental toxicity, industrial effluents are a complex mixture of ions, elements and compounds whose toxicity maybe modulated by others present in the environment.

In the present study, results obtained (Figure 1) shows the toxicity response of *P.flourescen* to the single phenolic compounds 2,4-DCP, 4-Chlorophenol, 4-Bromophenol, 2-Chlorophenol and phenol. These compounds demonstrated varying degree of inhibition on the dehydrogenase activity of *P. flourescen;* thetoxicity responses weretightly dose dependent and fitted into a logistic dose response model (Figure 1). Analysis of EC_{50} values of these single compounds (table 2) showed that the 50% eco-toxic concentration was 0.118 ± 0.002mM, 0.296 ± 0.006mM, 0.138 ± 0.005mM, 0.631 ± 0.019mM and 0.245 ± 0.012mM for 2,4-Dichlorophenol, 4-Chlorophenol, 4-Bromophenol, 2-Chlorophenol and Phenol respectively. Comparison of these EC_{50} of the single compound showed that toxicity ranked in the order 2,4-Dichlorophenol > 4-Bromophenol > Phenol > 4-Chlorophenol > 2-Chlorophenol.

Also, the mixtures of these phenolic derivatives with the parent compound presented interesting trends of toxicity responses. The arbitrary concentration and equi-effect ratio mixtures resulted in a marked increase in the EC_{50} values when compared to the single compounds. The EC_{50} values were 1.632 ± 0.048mM, 1.984 ± 0.014mM, 2.216 ± 0.071mM and 3.276 ± 0.056mM for the arbitrary concentration ratios ABCR1, ABCR2, ABCR3 and ABCR4 respectively. While for the equi-effect mixture ratios, EC_{50} were 2.443 ± 0.045mM, 1.208 ± 0.013mM,1.947 ± 0.010mM, 2.279 ± 0.092mM, 1.922 ± 0.038mM and 1.732 ± 0.028mM for EE5, EE10,

EE20, EE30, EE40 and EE50respectively. The highest toxicity response for the mixtures using arbitrary concentration and Equi-effect concentration ratio was recorded at ABCR1 with EC_{50} value of 1.632 ±0.048mM and EE10mixture with EC_{50} value of 1.208 ± 0.127 mM.These toxicity threshold were 10 times higher than that obtained for the most toxic single compound, 2, 4-dichlorophenol. The observed increases in the eco-toxic (EC_{50}) dose may be attributed to a possible shift in metabolism of the compound, towards a greater tolerance of the chemical mixtures.Also notable in the study,*P.flourescens* demonstrated a biphasic response to the equi-effect mixtures; low dose stimulation of growth of microorganisms by certain chemicals have been reported. Hormetic response observed in this studyis consisted with reported hormeticeffects of phenolic compounds on microorganisms (Calabrese and Blain, 2005, Nweke *et al.,* 2014, Christofi*et al.,* 2002, Nwekeand Okpokwasili, 2010).

Furthermore, the analysis of toxicity interaction of this mixtures using toxic index analysis (table 3) shows that both arbitrary concentration and equi-effects concentration ratios mixtures were largely antagonistic. The TI values ranged from 6.3 to 12.6 for arbitrary concentration ratios and 4.6 to 8.8 for equi-effect concentration ratios (Table 3). Toxic Index analysis is a widely applied method in ecotoxicological studies to explore the possible interactions of mixture of toxicants (Boillot and Perrodin, 2008; Nwanyanwu *et al.,* 2017; Asiwe *et al.,* 2018; Nweke *et al.,* 2018, Nzeh *et al.,* 2019). Boillot and Perrodin, (2008) described toxicity interaction of mixtures of chemicals, TI values =1 describes additive interaction, TI > 1 describes antagonistic interaction and TI < 1 describes synergistic interaction.

TI modeling of the resultscorroborated with the observed increase in EC_{50} of the phenolic mixtures against dehydrogenase activity of the *P. fluorescens* evaluated in this study. The TI values indicated largely antagonistic responses of the joint action of the quinary mixture. The TI values ranged from 6.30 to 12.6 for arbitrary concentration ratios, and 4.6 to 8.8 for equi-effect concentration ratio mixtures. These were far greater than the range 0.5 to 2.0 proposed by Deener (2000) as additive. The TI of the mixtures were largely greater than 1, indicating a strong antagonism of the phenolic derivatives to their individual toxicity. The antagonistic effect of chemical mixtures may be attributed to tolerance of the microorganisms to the mixtures or a possible chemical interaction of the mixture resulting in less toxic chelates (Nwanyanwu *et al.,* 2017). Similar observations were made by Boillot and Perrodin (2008) on seemingly antagonistic interaction between glutaraldehyde and surfactant against mobility of *Daphnia magma* and also by Nweke *et al*. (2014) on evaluation of toxicity of binary mixtures of formulated glyphosate and phenolic compounds on *Rhizobium* species. Mowat and Bundy (2002) predicted possibility of synergistic, additive and antagonistic interactions among binary and ternary mixtures of pollutants. Zhu and Chevion (2001) reported synergistic interaction of pentachlorophenol and copper mixture. Environmental contaminations are frequently encountered as mixtures and behaviors of chemicals in a mixture may not correspond to that from data on the pure compounds (Altenburger *et al.,* 2003). The chemicals may modulate the toxicity of each other in a mixture. Modulation of chemicals may have been established in this study with phenolic compounds evaluated. Environmental contaminants at increasing concentrations exert inhibitory effect on indigenous microorganisms of an ecosystem and thus disturb microbial balance of the ecosystem (Abdu *et al.,* 2017). The inhibition of dehydrogenase activity observed in this study at high concentrations of 4-chlorophenol, 2,4-dichlorophenol, 4-Bromophenol, 2-Chlorophenol and phenol is consistent with what has been widely reported (Agarry*et al.,* 2008; Agarry and Durojaiye, 2008; Heinaru*et al.,* 2001; Nweke *et al*. 2014; Nwanyanwu *et al.,* 2017). Phenols are membrane damaging biocides (Heipieper *et al.,* 1992; Okolo *et al.,* 2007), causing loss of cytoplasmic membrane integrity and thus disruption of membrane functions. Since dehydrogenases are membrane associated, loss of membrane integrity will ultimately affect their functions. In this study, the order of toxicity, 2,4-Dichlorophenol > 4-Bromophenol > 4-Chlorophenol > phenol > 2-Chlorophenol is in line with the report of Nweke *et al*. (2014) and Ren and Frymier (2002). Higher toxicity of 2,4-dichlorophenol when compared to 4-chlorophenol observed in this study corresponds to the findings of Rani *et al*. (2009) on degradation of mixture of phenolic compounds by activated sludge process using mixed consortia. This may be attributed to a higher number of substituent groups in structure and initial phenol concentration(Agarry*et al.,* 2008, Agarry and Durojaiye, 2008).

V. Conclusion

In the present study, the toxicity interaction of mixtures of fivephenolic compounds (2,4-Dichlorophenol, 4-Chlorophenol, 4-Bromophenol, 2-Chlorophenol and phenol) to *P. fluorescens* were assessed using inhibition of dehydrogenase activity as response. The single compounds exhibited varying level of toxicity to *P.fluorescens*. Toxicity ranked in the order 2,4-Dichlorophenol > 4-Bromophenol > Phenol > 4-Chlorophenol > 2-Chlorophenol.

However, EC_{50} analysis of the mixtures indicated that the mixture of the compounds using arbitrary concentration ratios and equi-effect concentration ratios were less toxic than the single compounds. Toxicity of the arbitrary concentration ratios mixtures was in the order ABCR1>ABCR2>ABCR3>ABCR4, while equi-effect concentration ratioswas in the order EE10> EE50 > EE40> EE20> EE30> EE5.Furthermore, toxic index

analysis of the phenolic compounds mixturesshowed that the mixture interaction werelargely antagonistic.This is indicative of a strong interaction of the phenolic derivatives in the mixture ratios studied resulting in less toxicity.

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