

Toxicity of Industrial Wastewater Acting Singly or in Joint-Ratios on *Clarias gariepinus*

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ABSTRACT

Freshwater ecosystems were the most vulnerable to pollution due to their easy accessibility for disposal of wastewaters leading to deterioration of water quality. Ona river, Ibadan, Nigeria is part of a dense network of inland watercourses emptying into the Lagos lagoon and receives industrial effluents from a food and beverage industry. The single and joint-action toxicity of effluents from these industries mixed in predetermined ratios of 75:25, 50:50 and 25:75% (v/v) (food:beverage); 100% (food) and 100% (beverage) respectively were tested against *Clarias gariepinus* fingerlings in a series of static bioassay to determine the pollution implications of interacting components of effluents and their effect on fish survival. Physico-chemical parameters of exposure concentrations and effluent samples were analyzed using standard methods. Effluent samples were further analyzed for heavy metals and interactions of composite mixtures were evaluated with models such as the Synergistic Ratio (SR), concentration-addition Relative Toxic Units (RTU) and isobologram (pictorial isobole) for joint-action evaluations. The 96 h LC₅₀ values were 23.00, 31.00, 71.79 and 33.50% (food) while no LC₅₀ value was derived for the 100% beverage effluent. Toxicity evaluation of effluent mixtures revealed decreasing toxicity in the order 75:25% (food: beverage); 50:50% (food: beverage); 100% (food) and 25:75% (food: beverage). Effluent samples and exposure concentrations were characterized by acidic pH and low DO (except for control exposures) while other effluent physico-chemical analysis revealed that Pb, Cr and Fe concentrations exceeded NESREA and WHO permissible limit for surface waters and protection of the aquatic ecosystem. The SR and isobologram models both depicted the food-beverage mixture of 75:25 and 50:50% as synergistic and the 25:75% mixture as antagonistic. The concentration-addition model depicted all mixture ratios as synergistic. The observed antagonistic interactions of 25:75% (food: beverage) ratio of synchronized effluent discharge by these industries towards setting safe limits for environmental discharge could be explored.

Keywords: Antagonistic interactions, composite mixtures, toxicity, synergism, joint-action

1. INTRODUCTION

Industrialization is considered to be vital to a nation's socioeconomic development, however the unregulated circumstances accompanying such development especially in developing countries have led to events of surface and ground water contamination by complex interacting chemicals and substances (Alumaa *et al.*, 2002; Clement *et al.*, 2006; Pandey, 2006; Casarett and Klaassen, 2007 Coors and Frische, 2011; Landrum *et al.*, 2012). This in turn has resulted in habitat loss and depletion of aquatic species diversity (MEA, 2005).

In order to have a clearer picture of interactions in natural environments, mixture toxicity protocols have

been given increasing attention in the last decade as a tool for predicting impacts of chemical pollutants on aquatic organisms (Silva *et al.*, 2002; Otitolaju, 2002; Backhaus *et al.*, 2003; Otitolaju and Okusada, 2003; Samuel *et al.*, 2008). This is as a result of the growing awareness that toxicity in natural ecosystems often times do not result from single substance exposure, but exposure to mixtures of toxicants (Altenburger *et al.*, 1996; Gardner *et al.*, 1998; Mu and LeBlanc, 2004).

Industrial effluents contain an array of components and when discharged into surface waters, give varying impacts depending on components, quantities of individual components and reaction potential in the face of other environmental factors (Adeogun and Chukwuka, 2011).

Investigations into possible joint effects between compounds have revealed that different types of interactions do occur when mixtures of metals, for example, jointly impact aquatic organisms. A number of authors have documented reports of synergistic interactions by mixtures of metals (Otitolaju, 2002; Otitolaju and Okusada, 2003; Khangarot *et al.*, 1982; Clark, 1992). Khangarot *et al.* (1982) reported that mixtures of compounds containing Zn^{2+} , Cu^{2+} and Ni^{2+} mixed in the ratio of 1:1 (wt/wt) and tested against the freshwater snail, *Lymanaea acuminata* had 48 h LC_{50} values of 6.59 mgL^{-1} for the trimetal mixture; 5.513 mgL^{-1} for Zn and Cu; and 2.68 mgL^{-1} for Cu and Ni bimetal mixtures. The observed LC_{50} values were higher in toxicity levels than the computed predicted 48 h LC_{50} values of 16.119 mgL^{-1} for the Zn, Cu, Ni mixture; 11.789 mgL^{-1} for Zn and Cu and 4.379 mgL^{-1} for Cu and Ni mixture revealing that interactions between the various combinations of heavy metals in triple and binary mixtures were synergistic.

Clark (1992) recorded a growth reduction of 67.8% in *Cristigera* species culture when equal concentrations of $HgCl_2$, $CuSO_4$ and $ZnCl_2$ were applied compared to 38.1% reduction when metal salts were applied individually thus suggesting a strong indication of synergistic interaction. Otitolaju (2002) observed that interactions between the test ratios of 1:1 and 2:3 (wt: wt) Zn-Cu tended to conform to the model of synergism when tested against the mangrove periwinkle *Tympanotomus fuscatus*. Binary mixture of spent carbide and spent engine oil (1:1 wt/wt) was also found to be more toxic than either test compound when acting singly indicating synergistic interaction (Otitolaju and Okusada, 2003). Some authors have however reported antagonistic reactions when mixtures of metals interact together. For example, Thorp and Lake (1974) observed that the 96 h LC_{50} value of a mixture of Zn and Cd (2:1 wt/wt) when tested against the fresh water shrimp *Paratya tasmaniensis* was higher (2.3 mgL^{-1}) and less toxic than the predicted 96 h LC_{50} value of 1.28 mgL^{-1} on the assumption that such interactions would be synergistic. Parrot and Sprague (1993) observed that various combinations of mixtures of Cu ($0-150\text{ mgL}^{-1}$) and Zn ($0-150\text{ mgL}^{-1}$) acted in an antagonistic manner, producing fewer changes in DNA, RNA and protein contents of the fathead minnow *Pimephales promelas* than when the fishes were exposed to the individual metal constituents. Antagonistic reactions, when they occur, are considered advantageous for environmental protection as the overall effect is a reduction in the toxicity of the individual components of such mixtures to exposed organisms (Otitolaju and Okusada, 2003).

Fagade *et al.* (1998) reported some degree of degradation in Ogunpa and Ona rivers and concluded that increased and uncontrolled urbanization along the river course was a major contributing factor to observed poor water quality. They also noted that pollution was mostly of organic origin as a result of low industrial activities in Ibadan during the period of study. Ten species of fish belonging to seven families were found in Ona river while three species of fish belonging to two families were found in Ogunpa river. This implies greater species diversity and reduced pollution for Ona river. With the recent increase in industrial activities along Ona river, it then became necessary to evaluate the effect of mixtures of effluents from these industries on *Clarias gariepinus* (a commercially important fish in Nigeria whose occurrence had been recorded in this river). The fingerling stage was chosen because of its relative importance in the survival and the recruitment pattern of organisms generally. The objectives of this study were to investigate the toxicity of effluents from a food and beverage industry acting singly and in joint ratios on *C. gariepinus* and to analyze effluent quality in order to ascertain likely pollution implication on water quality and species diversity.

2. MATERIALS AND METHODS

2.1. Collection of Test Organisms and Acclimation to Laboratory Conditions

Fingerlings of *C. gariepinus* with an average weight of 3.8g were collected from a private fish farm in Ibadan, transported to the laboratory in oxygenated waterproof bags and kept in holding tanks (120L capacity) filled with dechlorinated tap water for seven days to allow them acclimatize to laboratory conditions (temperature, $26.2\pm 1.6^\circ\text{C}$; pH, 7.0 ± 0.2 ; DO, $5.6\pm 0.3\text{ mgL}^{-1}$). Aeration was provided with an air pump and holding medium was changed every three days to prevent accumulation of metabolites. Fish were fed with 40% fish pellets (40% crude protein) during the acclimatization period.

2.2. General Bioassay Procedures

2.3. Collection of Effluents

Whole unfiltered effluents were collected fresh from the points of discharge of the food and the beverage industries in plastic kegs and transported to the laboratory for static bioassay tests as described by Reish and Oshida (1986) and OECD (2002).

2.4. Bioassay

2.5. Single Action Toxicity Test (Food and Beverage Industry Effluent)

Whole unfiltered effluents from a food and a beverage industry were taken as stock solutions. Required volumes were measured into graduated plastic containers and made up to the required concentration (19.1L) according to the methods of Reish and Oshida (1986) for toxicity evaluation (12g of fish:5L of water). Ten fingerlings of *C. gariepinus* (4.6g) were introduced into each concentration (10; 20; 30; 40%) including an untreated control (0%) in two replicates respectively.

2.6. Joint Action Toxicity Test (Food and Beverage Industry Effluent)

A predetermined volume of each effluent was mixed together in the ratio 1:1 (v/v) for the 50:50%; 3:1 (v/v) for the 75:25% (food: beverage) and 1:3 (v/v) for the 25:75% (food:beverage) concentrations. These were taken as stock solutions and serial dilutions with dechlorinated tap water gave concentrations of 10, 20, 30, 40 and 0% (control) in two replicates. Ten fingerlings of *C. gariepinus* (4.6g) were also introduced into each concentration in two replicates.

2.7. Assessment of Quantal Response (Mortality)

Fish were considered dead when there was a lack of both opercular and body movements when prodded by a glass rod (Reish and Oshida, 1986).

2.8. Physico-Chemical Analysis

Physico-chemical parameters (pH, dissolved oxygen and temperature) were measured at the beginning and end of the experiment for the test media and effluents with a digital CS-C933T Electrochemistry multimeter (Topac Instruments Inc).

Effluent samples were further analyzed for alkalinity, electrical conductivity, total solids, total suspended solids, sulphates, chlorides, phosphates and the presence of heavy metals. Alkalinity was determined by titrating a known volume of effluent sample with standardized 0.10M HCl. Sulphate (SO_4^{2-}) was determined by the turbidimetric method. Chloride (Cl^-) ion was analyzed by titration of a known volume of effluent with standardized 0.014N mercuric (II) nitrate solution while phosphates (PO_4^{3-}) was determined colorimetrically by ascorbic acid-molybdenum blue method (APHA-AWWA, 1995).

Heavy metals were determined by digesting a known volume of effluent sample with analytical grade HNO_3 . The digested sample was filtered into a 20 mL standard flask, made up to the mark with distilled-deionized water and stored in nitric acid prewashed polyethylene bottle in the refrigerator prior to analysis. The samples were then analyzed for metals- Pb, Cu, Cd, Mn, Cr, Ni, Ca, Zn and Fe with an Atomic Absorption Spectrophotometer (AAS).

2.9. Statistical Analysis

Toxicological dose response data for quantal (mortality) response were analyzed by the probit method (Finney, 1971).

To evaluate the joint toxicity of the industrial effluents, 3 models were used to quantitatively classify their mode of interaction. These were:

2.10. Model A: Synergistic Ratios (SRs)

Joint action toxicity data were analyzed based on the synergistic ratio model after Hewlett and Plackett (1969) where:

$$\text{S. R index} = \frac{\text{LC}_{50} \text{ of chemical acting alone}}{\text{LC}_{50} \text{ of chemical} + 2^{\text{nd}} \text{ compound}}$$

And S.R= 1: joint action is additive, S.R<1: joint action is antagonistic S.R>1: joint action is synergistic

2.11. Model B: Concentration-Addition Model

This model proposed by Anderson and Weber (1975) assumes that similarly acting toxicants mixed in any proportion, will add together to give the observed response. In evaluating the joint action, a predicted response value (e.g., LC_{50}) is derived by summing up the LC_{50} values of the separate toxicants according to the proportion of their contribution in the mixture. The predicted LC_{50} values are compared to the observed LC_{50} value of the mixture to give the Relative Toxicity Unit (RTU) which will be used to classify the type of interaction between the effluent mixtures as follows:

- Additive if the observed LC_{50} value of the mixture is equal to the predicted LC_{50} value
- Synergistic if the observed LC_{50} value of the mixture is less than the predicted LC_{50} value
- Antagonistic if the observed LC_{50} value of the mixture is greater than the predicted LC_{50} value

The relationship of the derived LC_{50} values to the predicted LC_{50} values (RTU) is estimated as:

$$\text{RTU} = \frac{\text{Predicted } \text{LC}_{50} \text{ value}}{\text{Experimentally derived } \text{LC}_{50} \text{ value}}$$

And RTU = 1 describes an additive action, RTU<1 describes antagonism and RTU >1 describes synergism.

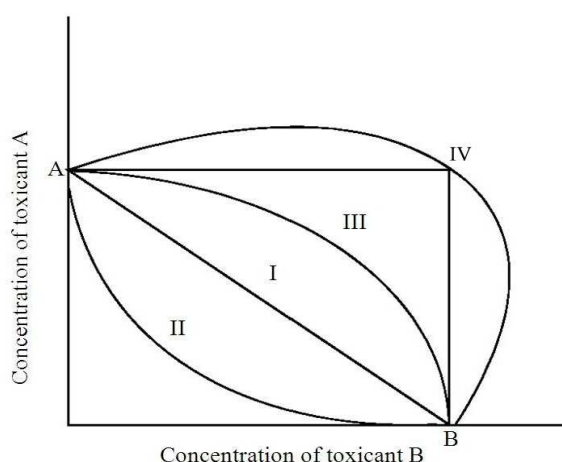


Fig. 1. Isoboles depicting the types of interactions between two chemicals A and B (Ariens et al, 1976). Isobole I depicts additive action; Isobole II depicts synergism; Isobole III depicts subadditive action; Isobole IV depicts antagonism.

2.12. Model C: Isobologram Model

Isobolograms (Ariens *et al.*, 1976) as depicted in **Fig. 1** involves the representation of the joint-actions between binary mixtures of test chemicals. Each isobole (I-IV) represents the amount of the toxicants in the formulations which produces a biological response. In the theoretical isobole (**Fig. 1**) points A and B represent the amounts of toxicant A and B which individually (singly) produced the biological response (LC_{50} in this study) which when connected gives the additive line. Isoboles (I-IV) represent the interactions of the test mixtures as described in the legend to **Fig. 1**. Three combinations of binary mixtures of effluents were tested at different ratios and individual effluents were also tested. The data accruing from the derived 96hr LC_{50} values were fitted into isobolograms and compared to the theoretical model (**Fig. 1**) to extrapolate the type of joint-action interactions in this study (Ariens *et al.*, 1976).

3. RESULTS

3.1. Physicochemical Properties

Table 1a and b show the mean variation in some physico-chemical parameters of exposure concentrations and effluents from the food and beverage industries. Average temperature for exposure concentrations were not significantly different (27.0-28.2°C) and were within

the range reported for tropical environment. Acidic pH (5.50-6.98) and low DO (0.4-3.6 mg L⁻¹) values were however recorded in exposure concentrations except the untreated control (**Table 1a**). The mean variation in physico-chemical parameters was also higher in food and beverage effluent (food was higher in values than beverage) than in the dilution water (**Table 1b**). Evident from the data obtained was that both wastes were acidic in nature, had very low DO and contained Fe, Cr and Pb in amounts that exceeded the permissible limits prescribed by NESREA {National Environmental Standards and Regulations and Enforcement Agency}, (NESREA, 2009) Nigeria for food and beverage industry effluents discharged into surface waters and WHO {World Health Organization} (WHO, 2004) guideline levels for drinking water and protection of aquatic ecosystems.

3.1. Relative Toxicity of Single and Joint Effluent Exposures on *C. gariepinus*

3.2. Single Action Toxicity (Food and Beverage Industry Effluent)

The analysis of the toxicity regime response data for food industry effluent on *C. gariepinus*, revealed a derived lethal concentration ranging from 34.00% (96 h LC_{50}) to 50.00% (48 h LC_{50}) (**Table 2**). The LC_{50} at 24 h was not detectable by the probit analysis giving values >100%. The 96 h LC_{50} values of the beverage industry effluent could not be determined as only 15.00% mortality was recorded in the highest concentration of 40.00% at the end of the exposure period compared with 70.00% mortality recorded in the food industry effluent using the same concentration range. This implies that the food industry effluent was more toxic than the beverage industry effluent:

3.3. Joint Action Toxicity (Food and Beverage Industry Effluents)

Results of the dose response data of *C. gariepinus* exposed to binary mixtures of food and beverage industry effluent in the ratios 3:1(v/v; 75:25%) showed a derived toxicity index ranging from 23.27% (96hr LC_{50}) to 30.77% (24 h LC_{50} values) (**Table 2**). Unlike the single toxicity of food industry effluent, binary mixtures gave Toxicity Factors (TF) with close and nearly overlapping ranges i.e., 1.00-1.30 between 24 and 96 h LC_{50} data. These results also depict that the addition of beverage industry effluent to food industry effluent in the ratio specified potentiated the ability of the food industry effluent to cause lethal effects to the fish in smaller amounts and within 24 h of exposure.

Analysis of exposure induced mortality of binary mixtures of food and beverage industry effluent in the

ratio 1:1 (v/v; 50:50%) showed that an increase in the ratio of beverage industry effluent to food industry effluent increased the amount of effluent required to cause detectable toxicity to 50% of the exposed *Clarias* population within 24h (Table 2). The increased toxicity of combinations of these effluents was apparent in lower LC₅₀ values derived (Table 2), where a smaller amount of the 75:25% (food: beverage) resulted in 50% mortality in exposed fish compared to the other mixture ratios and single acting effluent.

Based on the 96 h LC₅₀ values of the effluent mixtures and single exposures, the relative toxicity analyses showed that the 75:25% (food:beverage)

mixture was about 1.4 and the 50:50% (food:beverage) were 1.3 times more toxic than the food industry effluent used singly. The single acting food industry effluent was more toxic than the 25:75% (food:beverage) mixture by a factor of 2 (Fig. 2).

The synergistic evaluation of the food-beverage industry effluent mixtures on *C. gariepinus* based on the Synergistic Ratio (SR) model revealed that the interactions between the food-beverage effluent ratios of 75:25 and 50:50% conformed with the model of synergism i.e., S.R.>1 except for the 25:75% mixture which aligned with the antagonistic model (SR = 0.47) (Table 3).

Table 1a. Some physico chemical parameters of exposure concentrations

Parameter	Exposure concentrations (%)	Food (100%)	Beverage (100%)	Food:Beverage (50:50%)	Food:Beverage (75:25%)	Food:Beverage (25:75%)
Temperature (°C)	0.00	27.80	27.80	27.80	27.80	27.80
	10.00	27.70	27.20	27.90	27.80	27.90
	20.00	27.90	27.00	27.80	28.00	27.20
	30.00	28.00	27.30	28.00	27.90	27.70
	40.00	28.20	27.70	28.20	28.20	27.40
pH	0.00	7.02	7.02	7.02	7.02	7.02
	10.00	6.77	6.98	6.06	5.91	6.95
	20.00	6.58	6.95	5.91	5.68	6.85
	30.00	5.95	6.89	5.81	5.50	6.74
	40.00	5.68	6.79	5.67	5.48	6.61
Dissolved oxygen (mgL ⁻¹)	0.00	5.60	5.60	5.60	5.60	5.60
	10.00	2.80	3.60	2.40	2.20	3.20
	20.00	2.40	3.30	2.20	1.80	2.80
	30.00	1.80	3.20	0.80	0.80	2.40
	40.00	1.20	3.00	0.60	0.40	2.20

Table 1b. Physico-chemical parameters of Dilution water, Industrial effluent and NESREA/ WHO's standard for industrial effluent

Parameter	Dilution water	Food	Beverage	NESREA -2009	WHO -2011
pH	7.02	5.46	5.59	6.50-8.50	6.50-8.00
DO (mg/L)	5.60	0.10	0.20	6	4
EC	119	1380	880	-	500
TS(mg/L)	4.26	1600	670	2000	2000
TSS (mg/L)	-	130	38	0.25	-
Alkalinity (mg/L)	9.00	580	220	150	-
Cl ⁻	2.61	310	225	300	200-300
PO ₄ ³⁻	-	3.49	2.80	3.50	5
SO ₄ ²⁻	-	43.4	38.4	250	250
NO ₃ ²⁻	-	9.82	1.55	10	10
Pb (mg/L)	-	0.32	0.13	0.05	0.01
Cu (mg/L)	-	0.02	0.03	1	2
Cd (mg/L)	-	0.01	0.01	1	0.003
Ni (mg/L)	-	-	-	0.05	0.07
Cr (mg/L)	-	0.06	0.02	0.01	0.01
Mn (mg/L)	-	0.54	0.06	0.20	-
Zn (mg/L)	-	1.77	0.33	2	3.00-5.00
Fe (mg/L)	-	4.13	2.50	0.10	0.30

NESREA- National Environmental Standards Regulation and Enforcement Agency WHO-World Health Organisation

Table 2. Relative toxicities of single and binary effluent mixtures of food and beverage industries on *C. gariepinus*

Effluent type and ratio	Time (h)	LC ₉₀	LC ₅₀	LC ₁₀	Df	TF	Probit line Equation
Food (100%)	24	ND	ND	83.33	3	ND	Y= 0.15x-2.50
	48	79	50	20.37	3	1.47	Y= 1.35x-17.50
	72	60.29	42.65	19.12	3	1.25	Y= 1.7x-22.50
	96	50.00	34.00	16.96	3	1	Y= 2.35x-30.00
Food: Beverage (75%:25%)	24	43.08	30.77	18.46	3	1.3	Y= 3.25x-50.00
	48	41.05	29.11	17.16	3	1.25	Y= 3.35x-47.50
	72	40.44	28.68	16.91	3	1.23	Y= 3.40x-47.50
	96	34.24	23.27	12.31	3	1	Y= 3.65x-35.00
Food: Beverage (50%:50%)	24	ND	ND	50	3	ND	Y= 0.30x-5.00
	48	62.86	40	17.14	3	1.32	Y= 1.75x-20.00
	72	53.49	34.88	16.28	3	1.15	Y= 2.15x-25.00
	96	52.22	30.27	16.88	3	1	Y= 2.8x-32.50
Food: Beverage (25%:75%)	24	ND	ND	ND	-	-	-
	48	ND	ND	ND	-	-	-
	72	ND	ND	ND	-	-	-
	96	95.11	71.79	23.53	3	ND	Y= 0.85x-10.00

Df= degree of freedom; ND= not determinable; TF= Toxicity factor = 24hr, 48hr, 72hr LC₅₀ values/ 96hr LC₅₀ values

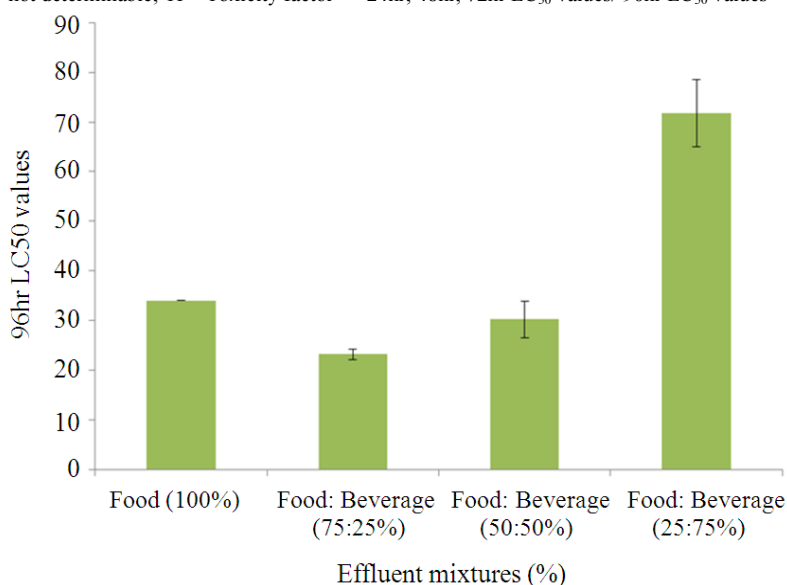


Fig. 2. Relative toxicity of food industry effluent, beverage industry effluent and their mixtures based on 96hr LC₅₀ values

The synergistic ratios derived from the 50:50% mixtures (1.08) were lower than that for 75:25% ratio (1.46) indicating lower potency (Table 3).

Analysis of the concentration mortality data using the concentration-addition model showed that the interactions between the food industry effluent and beverage industry effluent for all ratios were in conformity with the model of synergism (RTU>1) (Table 4). For the purpose of using the concentration-addition model, a 96 h LC₅₀ of 100% was assumed. This was as a result of the low toxicity of the beverage industry effluent and the consequent inability to derive the 96 h LC₅₀.

A further subsection of the joint-action toxicity data by fitting the derived 96 h LC₅₀ values of the tested mixtures and single compounds into the isobologram and comparing it to the theoretical pictorial isobole (Fig. 1) revealed that the resultant isobole from the binary mixtures of the effluents confirmed the results of the SR model which showed that the food-beverage ratio of 75:25% was synergistic. However contrary to the SR and RTU models, the isobologram model depicted that the food-beverage ratio 50:50% conformed to the criteria for sub-additive interaction while the food-beverage ratio 25:75% conformed to the criteria for antagonistic interaction (Fig. 3). This suggests that an increase in the concentration of the beverage effluent resulted in lower toxicity of the mixtures.

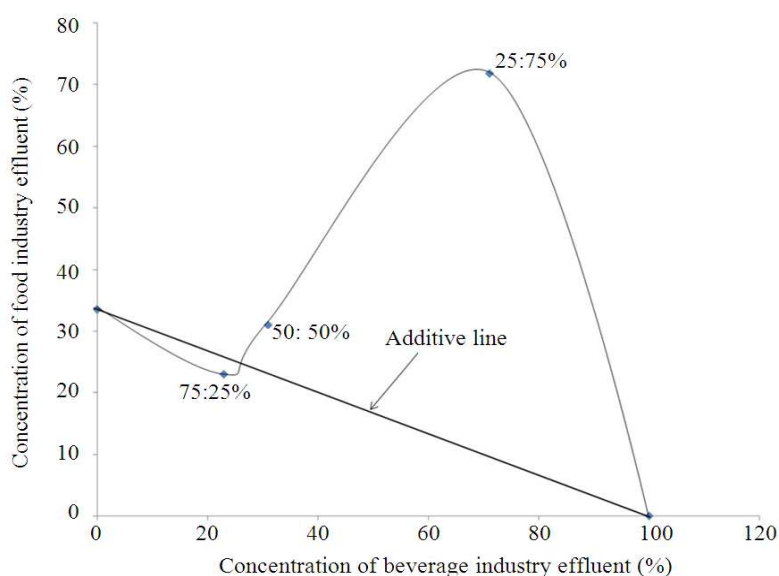


Fig. 3. Isobole representation of the joint-action of the food-beverage industry effluent when tested against *C. gariepinus*

Table 3. Summary of relative toxicity and synergistic ratios of binary mixtures of food and beverage industry effluent on *C. gariepinus* after 96h

Effluent ratios (v/v)	LC ₉₀ (95% C.L)	LC ₅₀ (95% C.L) (%)	LC ₁₀ (95% C.L) (%)	df (%)	Probit line equation	Synergistic ratio
Food-100%	50.00 (50.00-52.27)	34.07 (34.04-34.09)	16.96 (15.91-18.00)	3	Y=2.35x-30	-
Food/Beverage (75:25%)	34.24 (33.33-35.14)	23.27 (22.22-24.32)	12.31 (11.11-13.51)	3	Y=3.65x-35	1.46
Food/Beverage (50:50%)	52.22 (52.17-52.27)	30.27 (26.56-34.78)	16.88 (13.64-20.12)	3	Y=2.8x-32.5	1.08
Food/Beverage (25:75%)	95.11 (88.15-100.0)	71.79 (65.0-78.75)	23.53 (21.4-25.0)	3	Y=0.85x-10	0.47
Beverage (100%)	ND	ND	ND	-	-	-

Where ND= Not Determinable

Table 4: Synergistic analysis of effluent binary mixtures (based on concentration-addition model) tested against *C. gariepinus*

Test compound Mixtures	Experimentally observed 96 h LC ₅₀ (95% CL) (%)	Predicted 96h LC ₅₀ (95% CL) (%)	RTU
Food:Beverage (75:25%)	23.00	42.25	1.83
Food :Beverage (50:50%)	31.00	66.75	2.15
Food: Beverage (25:75%)	71.79	83.38	1.16

RTU= Relative Toxicity Unit

4. DISCUSSION

Acidic pH of the effluents used in this study resulted in lowering of the pH of exposure concentrations and this acidity could be attributed to mineral acids such as nitric, phosphoric and sulphuric acids which are essential raw materials for most industries. Low values of pH in exposure concentrations are a significant indication of the likely effects of effluents on the water quality of the receiving river as acidic pH affects metal solubility, alkalinity and water hardness. Low pH also has a negative effect on the metabolic activities of aquatic organisms (Wang *et al.*, 2002) and values reported were appreciably lower than

NESREA (2009) and WHO (2004) permissible limit for surface water quality.

The low levels of dissolved oxygen in the effluents and exposure concentrations (except control) was due to the high levels of nutrients, organic load and total solids content of the effluent and was lower than the NESREA (2009) and WHO (2004) guideline levels for surface waters and protection of aquatic ecosystem. DO is very crucial for the survival of aquatic organisms and the low levels recorded in exposure concentrations in spite of artificial respiration could be as a result of the high amount of oxygen required for chemical oxidation and decomposition of nutrients thereby depleting available

oxygen required for respiration of fish leading to asphyxiation in higher concentrations and more toxic mixtures. This observation is in agreement with the study of Fakayode (2005) who observed that industrial effluents discharged into Alaro river resulted in a depletion of DO downstream from the point of effluent discharge and could have dire consequences for aquatic organisms.

Effluents had a high EC, TS, TSS, Alkalinity, Cl^- , PO_4^{3-} and SO_4^{2-} compared with dilution water. The food industry effluent also had higher values indicating the presence of more toxic components than the beverage industry effluent. The activities of industries that require the use of phosphoric acid, hydrochloric acid, common salt (NaCl), sulphate salts and chloride containing compounds as raw materials particularly in the food and beverage industries and detergents for washing purposes in most industries (Fakayode, 2005) could be responsible for the high levels of the values recorded from effluent subsamples.

The levels of lead, iron and chromium recorded in effluent samples exceeded NESREA (2009) and WHO (2004) limit for food and beverage industry effluents discharged into surface waters. Some degree of accumulation is anticipated in the receiving Ona river. The anthropogenic contribution to the levels of metals in water, soil and food is well recognized (Backhaus *et al.*, 2003) and on a broad perspective, the implications of these findings on freshwater habitats are not likely to be favorable. Reports have it that freshwater organisms and their habitats have evolved with low metal levels thus small amount of metal pollutants introduced into their environment can augment existing concentrations resulting in adverse effects (Hill, 2010). The high concentration of iron (Fe) in effluent sample resulted in a corresponding decrease in dissolved oxygen concentrations in effluent sample and exposure concentrations. This observation is consistent with WHO (2004) report that depletion of DO in water supply increases the concentration of ferrous ion in solution.

These findings are particularly significant because preliminary evaluation of the fish and fisheries of Ona river revealed that only two species belonging to two families were occasionally encountered upstream from the point of effluent discharge and fish were no longer caught up to 2500 m downstream. The few species presently observed in catches compared with 10 species reported by Fagade *et al.* (1998) is a clear indication of the negative impact of pollutants on species diversity in river Ona. This coupled with the fact that some individuals resident around the industrial estate and

downstream of the receiving river use its water for drinking and other domestic purposes with little or no pretreatment under acute or chronic water shortage should be of serious health concern. Risks associated to humans on exposure of hexavalent chromium compounds through drinking water include enhanced UV- induced skin cancer (Costa and Klein, 2006) and stomach cancer (Sedman *et al.*, 2006). Lead-induced gametotoxic effects have been demonstrated in both male and female animals (Goyer, 1996). There is also evidence that lead may disrupt the hypothalamic-pituitary-gonadal axis. An increase in Maternal Blood Lead Levels (MBLL) may also contribute to premature birth and reduced birth weight in humans (ATSDR, 2005).

According to (Stenersen, 2004), if a non-toxic single acting substance enhances the toxicity of another, it is a synergistic interaction. If it reduces the toxicity of another it is an antagonistic interaction. A comparative evaluation of the single and joint toxicity of the food and beverage industry effluents revealed that most of the predetermined effluent ratios used in this study showed synergistic interactions as depicted by the Toxicity Factors (TF) and the synergistic evaluation models. In other words the addition of the beverage industry effluent potentiated the food industry effluent, but at increased ratios there was a deviation from potentiation towards sub-additive and antagonistic interactions. This trend is in line with the report of Otitolaju (2005) that the types of interactions exhibited by components of mixtures are largely dependent on the proportion of their occurrence in the mixture. Chukwu and Lawson (2009) also reported the possibility of synergistic reactions occurring when mixtures containing heavy metals interact with other pollutant mixtures present in the ecosystem.

From synergistic model analyses, the Synergistic Ratio (SR) model and isobologram model, showed complementary results, both indicating that the food-beverage ratio of 75:25 and 25:75% were synergistic and antagonistic respectively. The concentration-addition model classified all the effluent interactions as synergistic. This confirms reports (Chukwu and Lawson, 2009; Chapman, 2000) on the relative advantage of the SR model over other models in classifying joint-action binary and multiple mixtures and contrary to Samuel *et al.* (2008) each model may not be independently reliable for all varieties of mixtures. Furthermore it may be inferred that joint-action tests involving mixtures with components having far-apart toxicities and interactions bordering on synergistic (according to the SR model) and sub-additive (according to the isobologram model) may require the complementary role of the isobologram for accurate classification.

5. CONCLUSION

The higher mortalities recorded in composite mixtures compared with single concentrations imply that most of the mixture ratios were more toxic than each of the effluents acting singly thus indicating synergistic interaction. The deviation of effluent mixtures from potentiation to antagonism at certain ratios may be a promising tool for effluent discharge control. Industrial effluents of adjacent or proximal industries are most often discharged into common channels that end up as a point discharge into nearby water bodies, thus effluent discharge volumes could be adjusted and synchronized to ratios that can undoubtedly minimize toxicity. Furthermore, since the effluent discharge is not totally avoidable, poor water quality, fish mortality and the consequent reduction in species diversity due to industrial activities could be avoided if such management approach is considered. This, however, do not dispute the fact that the institutionalization and enforcement of stringent measures (i.e., policies and laws) to ensure compliance will go a long way to improve the quality of surface waters of the Nigerian environment.

The complementary role of the synergistic evaluation models should be further explored, so that predictive measures borne out of joint-action toxicity testing are developed with a high level of accuracy, especially for the purpose of establishing environmental safe limits for effluent discharges.

6. REFERENCES

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