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Physiological stress response of *Macrobrachium vollenhoevenii* (Herklots 1857) to interacting effects of binary mixtures of industrial effluents

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The toxicities of effluents from paint and textile industrial establishments in Lagos State, Nigeria and their binary mixtures based on predetermined (1:1) and equitoxic (4:3) ratios were evaluated against the juvenile stage of prawn, *Macrobrachium vollenhoevenii* in laboratory bioassays. On the basis of derived toxicity indices, the paint effluent with 96 h LC_{50} = 54.73 ml/L was found to be 1.33 times less toxic than the textile effluent (96 h LC_{50} = 40.99 ml/L) when acting alone against *M. vollenhoevenii*. In the joint toxicity tests, the binary mixtures in a predetermined ratio (1:1) with 96 h LC_{50} = 49.45 ml/L was 1.16 times more toxic than the equitoxic mixtures (4:3) with 96 h LC_{50} = 57.7 ml/L. The interactions between binary mixtures showed significant departures from the action of individual constituent effluent when acting singly, and depended largely upon the proportions of additions of the mixture components. On the basis of synergistic (Synergistic Ratio, SR) and concentration-addition models (Relative Toxicity Unit, RTU), the relationship between binary mixtures (1:1) of paint and textile effluents against *M. vollenhoevenii* were in conformity with models of synergism and antagonism (SR = 1.11, 0.83; RTU = 0.97) while equitoxic mixtures (4:3) conformed to antagonism (S.R = 0.94, 0.71, RTU = 0.84) model. Furthermore, the isobologram model showed that both binary mixtures conformed to sub-additive action. Symptoms of toxicosis observed in the test organisms include loss of equilibrium, agitated swimming, spiral movement, followed by weakness, periods of quiescence and death. It is concluded that the incorporation of joint action toxicity evaluation with bio-monitoring tools are clearly relevant in setting effective and realistic environmentally safe limits of pollutants or verifying ecological significance of existing water criteria meant to protect aquatic biota.

Key words: Toxicity, effluent, synergism, antagonism, interactions, *M. vollenhoevenii*.

INTRODUCTION

Chemical characterization of industrial effluents from industries in Lagos metropolis has been shown to contain heavy metals and toxic constituents (Chukwu, 1991; Ajao, 1990; Oyewo, 1998). According to Oyewo (1998) in a survey carried out on industrial sources and distribution of heavy metals in Lagos lagoon, Nigeria, all industrial effluents were found to contain heavy metals at concentrations that varied within and between different categories of industries.

Although industrial effluents are not the sole source of

heavy metals that enter the environment, they are possibly the most important single source contaminating or polluting aquatic environments (Bryan and Langston, 1992). In Nigeria, over 85% of industries are situated in the Lagos metropolitan area (WES, 1997) and their effluents ultimately get into the Lagos lagoon complex directly or indirectly via drainages or streams. According to Singh et al. (1995), an estimated 10,000 m³ of industrial effluents are discharged into the Lagos lagoon per day. Estimates of the annual discharge rate of heavy metals from industrial effluents into the environment in Lagos metropolis indicated that iron and manganese were the most discharged yearly while mercury and cadmium were the least released (Oyewo and Don-Pedro,

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2003).

Several workers have employed short term bioassay techniques to assess levels of toxicity of industrial effluents against aquatic organisms. For example, Ajao (1985) reported on the acute toxicity of waste-water effluents from a textile mill and a detergent packing plant on hermit crab, *Clibanarius africanus*. Chukwu (2001) reported the acute toxicity of treated effluents from industrial establishments in Lagos metropolis on *Macrobrachium vollenhoevenii*. Furthermore, Chukwu and Ogunmodede (2005) evaluated the toxicological response and sensitivity of tropical estuarine benthic macro-invertebrates, *Clibanarius africanus* Aurivillus and *Tympanotonus fuscatus* var *Radula* under acute exposure to effluents from brewery, paint and textile industries and found that the brewery effluent was the most toxic to the test organisms.

Despite the fact that most human activities result in the introduction of multiple pollutants into the aquatic environment, most research on the biological action of the pollutants have concentrated on the action of single compounds against test organisms (Otitoloju, 2003). However, predicting the impact of pollutant chemicals and other environmental perturbations on bio indicator/sentinel animals and ecosystems requires us to take a different approach, particularly if we are to consider the possibility of complex synergistic interactions resulting in emergent and novel toxicities and pathologies from pollutant mixtures (Howard, 1997). For example, Otitoloju (2005) demonstrated that toxicity evaluations of the mixtures of crude oil and dispersant meant to stimulate environmental control settings of crude oil spillages in aquatic ecosystems revealed that the effects of the crude oil/dispersant mixtures varied, depending largely upon the proportion of addition of the mixture components. According to Moore et al. (2004), a major challenge in impact and risk assessment now facing ecotoxicologists in the new century is how to gain insight and real understanding of the joint action response of aquatic organisms to combinations of mixtures of chemical stressors.

The objective of this paper therefore is to evaluate the types of toxicological interactions existing between binary mixtures of effluents from paint and textile industrial establishments in Lagos State, Nigeria, against the juvenile stage of prawn, *M. vollenhoevenii* based on predetermined ratios and equitoxic ratios (that is, 96 h LC₅₀ values of the effluents) in laboratory bioassays. In Nigeria, *M. vollenhoevenii* are important commercial prawns in the Lagos lagoon, accounting possibly for 60% by weight of all prawn landings.

MATERIALS AND METHODS

Source and maintenance of test organisms

The test organisms were juvenile stages of *M. vollenhoevenii* (Arthropoda; Crustacean; Decapoda; Palaemonidae) of similar sizes (mean length 6.5 cm and mean weight, 240.5mg). They were collected at Makoko area of the Lagos Lagoon, Nigeria with the aid

of set nets.

Acclimatization of test organisms

The test animals were transported to the laboratory and kept in holding, glass tanks (72 × 28 × 28 cm) which contained aerated lagoon water. Mud was collected from the same site and placed in the holding tank as substrate. The test organisms were acclimatized to laboratory and experimental conditions (RH 70 ± 25%; temperature 28 ± 1°C; salinity, 16 ‰) for a minimum of 7 days before using them in laboratory bioassays. The water in the holding tanks was changed every 2 days to avoid accumulation of waste materials from the test organisms. Acclimatization of test organisms to laboratory conditions was in accordance with guidelines for bioassay techniques (APHA, 1985).

Test media

Industrial effluents used in this study were collected in 20 L plastic kegs from the main discharge point into the drainage system from two companies. Textile effluent was collected from Atlantic Textile Mill (ATM) located along Oshodi/Mile 2 expressway, Lagos. The paint effluent was collected from Fine Coat Paint Industries located at Ikeja, Lagos. Effluents were used immediately for bioassays in the laboratory to avoid decay.

General Bioassay Procedures

Bioassay containers: The bioassays were carried out in glass tanks (22 × 15 × 18 cm). These glass tanks were preferred to plastic containers as they minimize absorption of toxicants and prevent risk of corrosion and chemical reactions. Some plastics are known to react with some toxicant components (Don-Pedro, 1989).

Preparation of Substrate:

The soil substrate has been observed to increase the sensitivity of benthic organisms including hermit crabs and periwinkles to toxicants in laboratory bioassays (Otitoloju and Don-Pedro, 2002). The substrate used, however was collected from the site of collection of test animals and was subjected to its standardization procedure as described by Otitoloju (2002). A weighed mass of sieved soil (100 g) was used as substrate in each bioassay container.

Application of toxicants to test media:

Lagoon water was used as the medium for all the bioassay tests conducted. Pre-determined volumes of paint industrial effluent mixtures were measured using a measuring cylinder and introduced into the soil substrate and the volume made up to 100 ml/l by adding appropriate volumes of lagoon water. There was control in which test medium substrate was similar but no toxicant was introduced. Again a pre-determined volume of textile mill effluent was measured using a measuring cylinder and introduced into the substrate and the volume made up to 1000 ml by adding appropriate volumes of lagoon water. A glass rod was used to stir the mixture. This now served as the second toxicant for the experiment.

Assessment of quantal response:

The animal, *M. vollenhoevenii* is assumed dead when the appendages failed to respond on prodding with a blunt glass rod. Mortality

assessments were carried out at defined time intervals of 24, 48, 72 and 96 h.

Bioassays

Single action toxicity test:

Preliminary test was carried out to ascertain the range of activity of the test media before the final bioassays. Test solutions containing various concentrations of effluents were prepared using the dilution water (salinity 16‰). Active test animals were randomly assigned to a bioassay container already holding treated or untreated test media. The shrimps were not fed during the bioassays and the tanks were covered with wire gauze to prevent escape of irritated organisms.

A total of 60 test animals of *M. vollohoevenii* were exposed per treatment including untreated control in 3 replicates (15 animals per replicate) per treatment. The test animals were exposed to graded concentrations of test effluents; textile mill effluent against *M. vollohoevenii* at 10, 20, 40, 80, 160 ml/L and untreated control, and paint effluent against *M. vollohoevenii* at 10, 20, 40, 80, 160 ml/L and untreated control.

Joint action toxicity test:

Mixtures of textile and paint effluents were prepared in a predetermined ratio of 1:1 and used as a test compound. A procedure similar to that just described was carried out but in this instance the test medium contained equitoxic mixtures of the effluents, which was in ratio 4:3 for *M. vollohoevenii*. The test animals were exposed to graded concentrations of test effluents; *M. vollohoevenii* at the predetermined ratio were 10, 20, 40, 80, 160 ml/L and untreated control, and *M. vollohoevenii* at the equitoxic ratio were 20, 40, 80, 100, 120 ml/L and untreated control.

Measurement of the physico-chemical parameters of test effluents

A 2 L volume of fresh effluents from the discharge point of each industry was collected into a clean plastic keg and taken to the Environmental Analytical Laboratory in the Department of Chemistry, University of Lagos, for physico-chemical analysis. Procedures for physico-chemical analysis followed those described by APHA, (1985). The parameters analyzed include pH, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), total dissolved solids (TDS), conductivity, chloride, nitrate, and sulphate contents.

Statistical analyses

Toxicological dose-response data involving quantal response (mortality) for both single and joint action studies were analyzed by probit analysis (Finney, 1971). The indices of toxicity measurement derived from these analyses were LC₅₀ (median lethal concentration that causes 50% response (mortality) of exposed organisms), LC₉₅ (lethal concentration that causes 95% response (mortality) of exposed organisms), LC₀₅ (Lethal concentration that causes 5% response (mortality) of exposed organisms) and TF (toxicity factor for relative potency measurements e.g., ratio of 96 h LC₅₀ of a compound to LC₅₀ values at equivalent time intervals).

For the joint action toxicity of the industrial effluents mixtures, the three models employed for the analyses are as follows:

Model A: The synergistic ratio (SR) model after Hewlett and Plackett (1969). The synergistic ratio model is: $SR = LC_{50}$ of a che-

mical acting alone / LC₅₀ of chemical +additive (mixture), where SR= 1 describes additive action, SR<1 describes antagonism action and SR>1 describes synergistic action.

Model B: Concentration-addition model by Anderson and Weber (1975) with slight modification (relative toxic units (RTU) estimations) (Otilotoju, 2001). The concentration-addition model assumes that when similarly acting toxicants are mixed in any proportion, they will add together to give the observed response. In evaluating the joint action, a predicted response value (e.g. LC₅₀) is derived by summing up the LC₅₀ values of the separate toxicants according to the proportion of their contribution in the mixture. The predicted LC₅₀ values are then compared to the observed LC₅₀ value of the mixture so as to classify the type of interaction among the components of the mixture as:

- 1) Additive if the observed LC₅₀ value of the mixture is equal to the predicted LC₅₀ value of the mixture.
- 2) Synergistic if the observed LC₅₀ value of the mixture is less than the predicted LC₅₀ of the mixture.
- 3) Antagonistic if the observed LC₅₀ value of the mixture is greater than the predicted LC₅₀ of the mixture.

$$RTU = \text{Predicted LC}_{50} \text{ value} / \text{Observed LC}_{50} \text{ value}$$

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

Model C: The joint actions between the toxic compounds in binary mixtures are presented in form of isobolograms (Ariens et al., 1976) (Figure 1). Each isobole (I-IV) represents the amount of the toxicants in the formulations (employing multiple ratios with mixtures with same constituents but in varying ratios), which produces a given biological response (usually the 50% immobility response level, LC₅₀). In the theoretical isobole (Figure 1), points C and D represent the amounts of toxicant A and B which individually produced the biological response (LC₅₀ or median response levels in this work) which when connected gives the additive line. Isoboles (I-IV) where the two constituents of the test mixtures are separately active are described in the legend to Figure 1.

Data based on the derived 96 h LC₅₀ values of binary mixtures at predetermined and equitoxic ratios 1:1 and 4:3 respectively were fitted into isobolograms and compared to the theoretical model (Figure 1) in order to extrapolate the type of joint action depicted in this work, after Ariens et al. (1976).

Analysis of variance (ANOVA) at 5% significant level was used to test the significance of the effect of treatment. The comparison of replicate by means of Student Newman-Keuls (SNK) test was used to test for statistical differences in the results of 96 h toxicity tests.

RESULTS

Physico-chemical parameters of test media

The physico-chemical parameters of the effluent samples showed that the textile and paint effluents were dark blue and grey coloured, respectively. The temperature of the textile effluent ranged between 39.0 - 45 °C while the paint effluents were 26.0 - 29.1 °C. The dissolved oxygen content (>3.0 mg/l), biochemical oxygen demand (>15.0 mg/l) and chemical oxygen demand (>65.0 mg/l) of both effluents were low, but recorded high total suspended solids (<245.0 mg/l). The heavy metals calcium, nickel, copper and lead recorded levels as <65, <0.02, <0.075 and < 0.005 mg/l respectively for both effluents.

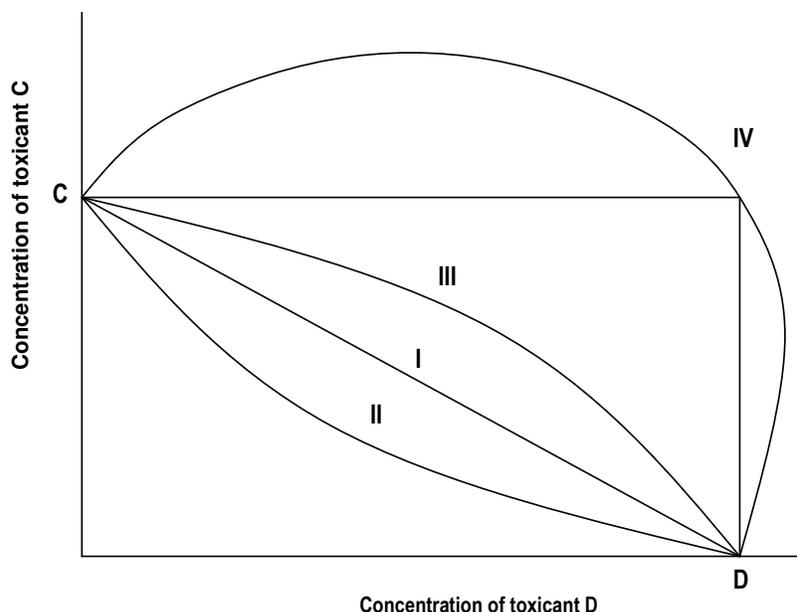


Figure 1. Isoboles depicting the types of interactions between two chemicals C and D (Arien et al., 1976). Isobole I depicts additive action; Isobole II depicts synergism; Isobole III depicts subadditive action; Isobole IV depicts antagonism.

Relative toxicity of paint and textile effluents against *M. vollenhoevenii*

The results of the dose mortality analysis of paint effluent against *M. vollenhoevenii* revealed that the derived toxicity indices (LC_{50} value) ranged from 54.73 ml/L (96 h LC_{50} value) to 138.84 ml/L (24 h LC_{50} value) while for the textile effluent, the derived toxicity indices (LC_{50} value) ranged from 40.99 to 94.5 ml/L (24 h LC_{50} value) (Table 1). On the basis of computed toxicity factor, the paint effluent with 96 h LC_{50} = 54.73 ml/L was found to be 1.33 times less toxic than the textile effluent with 96 h LC_{50} = 40.99 ml/L when acting alone against *M. vollenhoevenii*. The randomized analysis of variance (ANOVA) showed that there was significant difference ($P < 0.05$) between all the treatments (concentrations) at 24, 48, 72 and 96 h of exposure of *M. vollenhoevenii* to toxicants. Using Student Neumann keul (SNK) test at 5% level of significance, the mean mortality response of *M. vollenhoevenii* at 96 h of exposure to all concentrations of test media were significantly different from the control.

Joint action toxicity of binary mixtures (1:1) of paint and textile effluents against *Macrobrachium vollenhoevenii*.

The analysis of the dose-response data for the mixture of paint and textile effluents (1:1; 4:3) against *M. vollenhoevenii* revealed that the observed 96 h LC_{50} value of the binary mixture prepared based on predetermined ratio (1:1) with 96 h LC_{50} = 49.45 ml/L was 1.17 times more toxic than the equitoxic mixtures (4:3) with 96 h

LC_{50} = 57.7 ml/L (Table 2).

Based on the concentration – addition and synergistic ratio models, the binary mixture (1:1) of paint and textile effluents gave a synergistic ratio of 1.11 and 0.83 and Relative Toxic Unit (RTU) of 0.97 (Table 3). Both models show that the mixture of paint and textile effluents exhibited both antagonism and synergism with respect to individual toxicants, indicating that the toxicity of each constituent toxicant in the mixtures were either enhanced or reduced.

Similarly, the binary mixtures 4:3, of paint and textile effluents gave a synergistic ratio (SR) of 0.94 and 0.71 with respect to paint and textile effluents respectively and a relative toxic unit (RTU) of 0.84 (Table 3). Both models show that the binary mixture 4:3 of paint and textile effluents tends slightly towards antagonism ($SR < 1$; $RTU < 1$) indicating that toxicity of constituent toxicants in the mixtures was reduced.

Furthermore, based on the isobologram model, the binary mixtures of paint and textile effluents based on predetermined and equitoxic ratios 1:1 and 4:3 respectively conformed to sub-additive action when tested against *M. vollenhoevenii* (Figure 2). This is depicted by Isobole III in Figure 1. This is also an indication that the effluents were tending towards antagonism but the concentration were not significantly toxic to give a strong antagonistic effect.

Symptoms of effluent toxicity

It was observed that the test animal, *M. vollenhoevenii* showed basically the same behavioural response to both

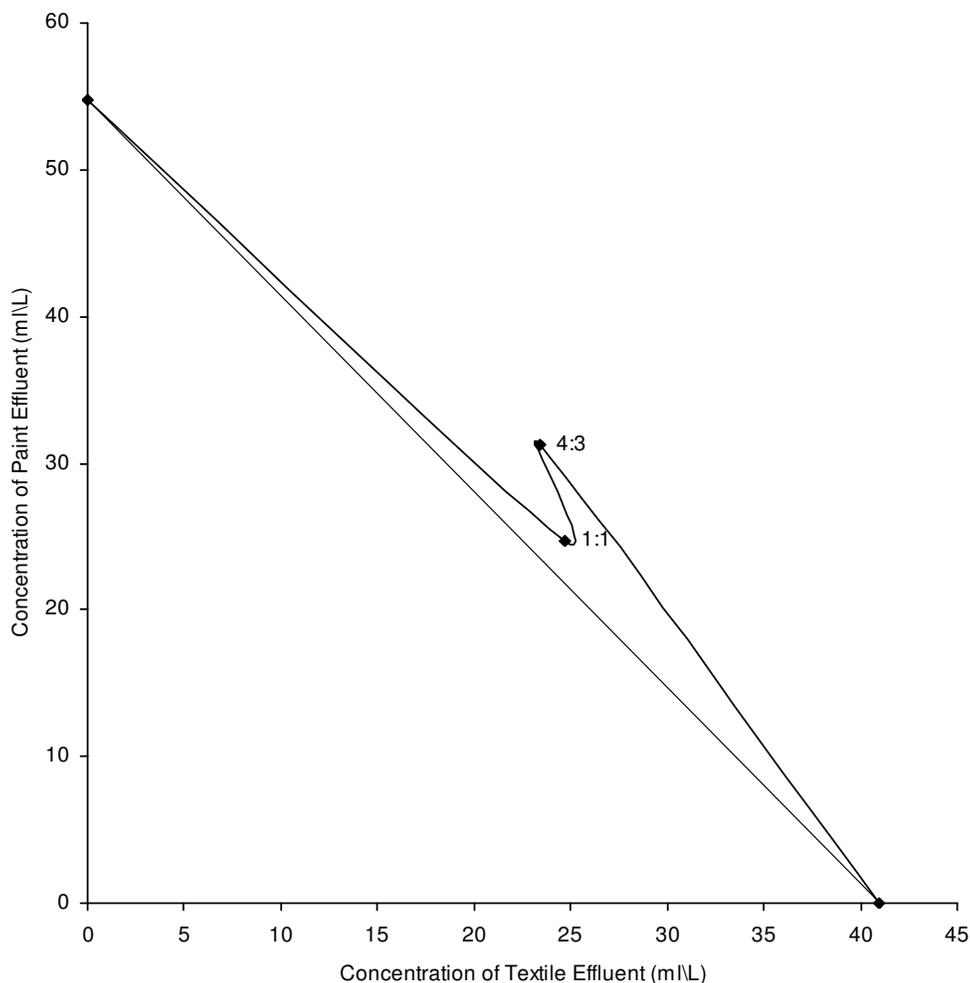


Figure 2. Isobole representation of the binary mixtures effect of paint and textile effluents at different proportions and tested against *M. vollehoevenii*.

the textile and paint effluents. They were observed to be active animals but after being treated with industrial effluents, their activity rate increased as seen in their agitated swimming and gradually reduced with loss of equilibrium, progressive weakness and periods of inactivity and quiescence. They eventually died and this was confirmed when there was no response to gentle prodding to a blunt pair of forceps. Following their death, the exoskeleton of the shrimps turned whitish from its original transparent colour. The intensity of these symptoms was directly related to the concentration of effluent as well as duration of exposure.

DISCUSSION

In the joint action toxicity studies, from their 96 h LC_{50} , it was revealed that the binary mixture 1:1 was 1.17 times more toxic than the binary mixture (4:3). Furthermore, comparison of the toxicity of the binary mixtures of paint

and textile effluents (1:1) and (4:3) using concentration-addition model also showed that both results conformed to antagonism ($RTU = 0.97$ and 0.84 , respectively) when tested with *M. vollehoevenii*. This result implied that this mixture of paint and textile effluents would be expected to cause more harm to the exposed animals.

The analysis of the joint-action results based on synergistic ratio model showed that the toxicity of the binary mixture 1:1 of paint and textile effluents against *M. vollehoevenii* conformed to synergism and antagonism ($SR1 = 1.11$ and $SR2 = 0.83$). The $SR1$ value is in relation to the paint effluent while the $SR2$ value is that of textile effluent. This is in agreement with the findings of Otitolaju (2005), who reported that the mixture of crude oil and dispersant, dissolved in ratio 9:1 against *M. vollehoevenii* conformed to the model of synergism while the mixture at ratio 6:1 conformed to the model of antagonism. It is therefore, probable that the same mixtures in different ratios could have caused a reduction (antagonism). Based on the isobologram model, binary

Table 1. Relative toxicity of paint and textile effluents against *M. vollenhoevenii*.

Exposure time (h)	LC ₅₀ (95% CL ml/L)	LC ₅₀ (95% CL ml/L)	LC ₉₅ (95% CL ml/L)	Scope + S.E.	DF	Probit line equation	TF	TF 2
Paint effluent								
24	14.89(5.56-24.25)	138.84(95.35-271.13)	1293.94(525.39-9437.58)	1.6968+0.33	3	Y=1.3647+16968x	1	
48	8.91(2.55-16.09)	114.44(77.75-223.61)	1469.96(547.65-13441.36)	1.4836+0.29	3	Y=1.9460+1.4836x	1.21	
72	9.056(3.69-14.81)	72.36(54.12-105.290)	577.23(301.17-1973.99)	1.8232+0.30	3	Y=1.6105+1.8232x	1.92	
96	7.49(3.21-12.13)	54.73(41.59-75.15)	399.77(226.70-1106.57)	1.9048+0.29	3	Y=1.6891+1.9048x	2.54	1
Textile effluent								
24	12.47(5.27-19.83)	94.50(70.05-145.48)	716.15(359.07-2772.94)	1.8701+0.32	3	Y=1.3058+1.8701x	1	
48	6.63(2.06-12.00)	74.08(53.37-116.45)	827.37(372.58-4199.48)	1.5695+0.29	3	Y=2.0657+1.5695x	1.27	
72	6.11(2.38-10.33)	48.50(36.44-66.22)	384.90(216.40-1094.98)	1.8283+0.29	3	Y=1.9180+1.8283x	1.95	
96	5.29(2.03-9.04)	40.999(30.68-55.01)	317.61(184.38-840.93)	1.8500+0.28	3	Y=2.0164+1.8500x	2.31	1.34

S.E. = Standard error; CL = confidence limit; D.F = degree of freedom; LC = lethal concentration.

TF1 = Toxicity Factor = LC₅₀ of test compound at 24 h / LC₅₀ of test compound at other hours (48, 72 and 96 h)

TF2 = Toxicity Factor = 96 h LC₅₀ of equitoxic mixture (4:3) / 96 h LC₅₀ of pre-determined mixture (1:1)

Table 2. Joint action toxicity of pre-determined (1:1) and equitoxic (4:3) binary mixtures of textile and paint effluents against *M. vollenhoevenii*.

Exposure time (h)	LC ₅₀ (95% CL ml/L)	LC ₅₀ (95% CL ml/L)	LC ₉₅ (95% CL ml/L)	Slope + S.E	DF	Probit line equation	TF	TF2
Binary mixture pre-determined ratio (1:1)								
24	15.83 (6.80-24.69)	116.84 (84.93-193.5)	862.32 (411.49-3911.56)	1.8949+0.3458	3	Y=1.0822+1.90x	1	
48	8.79 (2.28-15.39)	95.71 (67.83-163.23)	1042.05 (443.60-60.62)	1.586+0.2983	3	Y=1.857+1.586x	1.22	
72	7.49 (2.91-12.57)	62.87 (47.00-89.89)	527.76 (277.71-1760.69)	1.7861+0.2935	3	Y=1.798+1.780x	1.85	
96	6.61 (2.71-10.93)	49.45 (37.43-67.19)	369.75 (211-1009.38)	1.8825+0.2943	3	Y=1.810+1.882x	2.36	1
Binary mixture in equitoxic ratio (4:3)								
24	12.74 (2.45-24.03)	140.99 (103.58-259.04)	1560.36 (594.59-20574)	1.5755+0.3724	3	1.6139+1.5755x	1	
48	11.02 (2.61-20.48)	104.09 (79.90-152.19)	982.41 (453.60-608416)	1.6872+0.35744	3	1.597+1.6872x	1.25	
72	7.29 (1.88-14.49)	67.74 (50.16-88.79)	629.49 (330.79-2605.81)	1.6990+0.3394	3	1.8894+1.6996x	2.08	
96	7.17 (1.84-13.75)	57.70 (42.24-73.85)	464.23 (269.04-1431.64)	1.816+0.3393	3	1.8001+1.816x	2.44	1.17

S.E. = Standard error; CL = confidence limit; DF = degree of freedom; LC = lethal concentration.

TF1 = Toxicity Factor = LC₅₀ of test compound at 24 h / LC₅₀ of test compound at other hours (48, 72 and 96 h)

TF2 = Toxicity Factor = 96 h LC₅₀ of equitoxic mixture (4:3) / 96 h LC₅₀ of pre-determined mixture (1:1)

mixtures, 1:1 and 4:3 conformed to sub-additive action which is an antagonistic action that tends towards additive.

Several workers have reported antagonistic interactions between toxicants when tested against aquatic animals. Parrott and Sprague

(1993) reported that various combinations of mixtures of copper (0 - 150 m³/L) and Zn (0 - 150 mg/l) acted in an antagonistic pattern by causing

Table 3. Analysis (based on concentration – addition and synergistic ratio models) of the 96 h LC₅₀ values of textile effluent and paint effluent when acting jointly or singly against *M. vollehoevenii*.

Mixture of textile and paint effluents	Observed 96 h LC ₅₀ value	Predicted 96 h LC ₅₀ (95% CL)	Probit line equation	TF	RTU	Synergistic ratio	
						(SR ₁)	(SR ₂)
1:1	49.45 (37.43-67.19)	47.86	Y=1.805+1.883x	1.16	0.97	1.11	0.83
4:3	57.70 (42.24-73.65)	48.6	Y=1.801+1.816x	1.0	0.84	0.94	0.71
Textile alone	40.99 (30.68-35.01)		Y=2.016+1.851x	1.41	-	-	-
Paint alone	54.73 (4.95-75.15)		Y=1.689+1.905x	1.05	-	-	-

RTU = Relation toxic unit; SR₁ depicts paint; SR₂ depicts textile; TF, toxicity factor; SR, synergistic ratio.
 RTU = Predicted LC₅₀ value of binary mixtures / Observed LC₅₀ value binary mixtures
 T.F = 96 h LC₅₀ of least toxic compound / 96 h LC₅₀ of test compound
 SR = LC₅₀ of paint or textile effluent / LC₅₀ of paint + textile effluent

fewer changes in DNA, RNA and protein content of fat head minnows, *Pimephales promelas*, than when the fishes were exposed to the individual constituents. Otitoloju (2002) reported that the joint action toxicity evaluations of binary mixtures of heavy metal compounds (Zn-Cu, Zn-Cd and Cd-Cu) against the mangrove periwinkle *T. fuscatus var Radula* revealed that interactions between the constituent metals in the mixtures were largely antagonistic, except for a few mixtures where synergistic interactions between constituent metal compounds were observed. Antagonistic interactions in the mixture of pollutants where it exists could be an advantage in environmental management. This is because antagonism implies that interaction between the constituents results in the lowering of the toxicity of one or all the constituents of a mixture against the organism. Though most of the joint action toxicity results in this study depicted antagonistic interactions, there is always a possibility that when mixtures of heavy metals and other pollutants e.g. polychlorinated biphenyl, are present in mixtures in the ecosystem, the resultant interactions may be synergistic. Therefore, environmental protection should usually act more on the side of caution, in order to ensure reduction in the risk of damage caused by multiple pollutants as they occur in the ecosystem.

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