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Combined Effects of Binary Mixtures of Commonly Used Agrochemicals: Patterns of Toxicity in Fish

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Abstract: The toxic effects of the binary mixtures of NPK 12:12:17 fertilizer and NPK 20:10:10 fertilizer was evaluated against *Oreochromis niloticus* fingerlings, in order to determine the interactions existing between the two fertilizers when acting singly and in joint action by adopting predetermined ratio (1:1) and the equitoxic ratio (4:3) that depict their individual $96hLC^{50}$ values. Based on the derived toxicity indices NPK 12:12:17 (96hLC50, 2.856ml/L) was found to be 1.3 times less toxic than NPK 20:10:10 fertilizer ($96hLC^{50}$, 2.198ml/L) when acting singly against *O. niloticus*. In the joint toxicity tests carried out on the binary mixtures of N.P.K fertilizers (1:1 and 4:3) it was found that the predetermined ratio (1:1) with $96hLC^{50}$ = 5.458ml/L) was 1.54 times less toxic than the equitoxic mixture 4:3 $96hLC^{50}$ = 3.537ml/L. The analysis of variance (ANOVA) showed that there were significant differences ($P<0.05$) in the quantal response of *O. niloticus* to different concentrations of NPK 12:12:17 fertilizer and NPK 20:10:10 fertilizer during the exposure period. The concentration-addition model (relative toxicity unit, RTU) and synergistic models (synergistic ratio, SR) used to analyze the mixtures revealed that the two fertilizers at the predetermined and equitoxic ratios conformed to the model of antagonism (RTU = 0.46 and SR = 0.807, 0.621) and (RTU = 0.73 and SR = 0.523, 0.403) respectively. Furthermore, the isobologram model showed that the test ratio 1:1 was significantly antagonistic while the test ratio 4:3 was sub additive. The findings of the study suggest that the joint action toxicity evaluation of agrochemicals is important in risk assessment and impact prediction of diffuse aquatic pollutants.

Key words: Joint Action Toxicity, Binary Mixtures, Agrochemicals, Nile Tilapia, Fingerlings.

INTRODUCTION

Land use practices such as forestry, grazing, agriculture, urbanization, and mining disrupt aquatic ecosystems by altering watershed processes that ultimately influence the attributes of streams, lakes, and estuaries. The world is focused on increasing the agricultural productivity due to this fertilizer is being used in most countries where agriculture is now well developed. In Nigeria like many other third world countries, land for agricultural uses is fast diminishing hence resulting in increased attention being paid to the productivity. Farmers in the Northern part of the country, use more than 60% of the fertilizers in their farms [1,2]

The use of fertilizer in aquaculture is as important as it is in agriculture and it has been reflected in the increase in fish production and also in crop production in agriculture. In aquaculture, fertilizers have been used in various forms and quantities to enhance fish production for greater abundance of fish food organisms but excessive uses of fertilizer has been shown to have adverse effect on water quality [3,4] and also causes gill damage.

Agro-chemical pollutants have different effects on different organisms the intensity depends on the different organisms the intensity of which depends on the generic make up of species, its previous history as well as temperature, pH and salinity [5,6]. The most important effect of agro-chemical pollutants in the aquatic environment is ecological disruption which results in the imbalance created between organisms and their environment and between communities of organism of different species [7,8].

Organic and inorganic fertilizers used in agricultural processes are rich in nutrients like nitrates, phosphate and organic nutrient which could lead to excessive enrichment of water bodies[9,10] resulting in high biological oxygen demand, depletion of oxygen and ultimately death of aquatic organisms[11]. Excessive

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application of fertilizers encourage over abundance of plankton which may cause oxygen depletion in fishponds [12,13]. Fishes are repelled by low oxygen concentrations particularly at high temperature [14] and if prevented, they develop clear signs of distress that is observed as rate of gill movement increases as oxygen content falls [15]. The effect of poison on fish depends on a number of factors such as type of fish species exposed to the poison [16] exposure time and the concentration of poison used [17,18] type of toxicant used, life cycle stage of the fish exposed to the toxicant environmental factors such as dissolved oxygen concentration (DO) salinity, hardness of water, temperature [19,20].

Although fishes are extremely sensitive to aquatic pollution, a lot of work still needs to be done on joint action toxicity tests due to the variations in the resulting toxic effects. This is quite necessary because the current control and management of pollutant involves the setting of safe limits and standards aimed at restricting levels of hazardous chemicals below injurious thresholds [21,22]. Thus, the need to consider the joint action toxicity tests rather than the single action toxicity tests with the aim of checking for the biological effects of mixtures of pollutants to set up standards for environmental management protections need not be over emphasized.

Oreochromis niloticus is a member of the family Cichlidae that is common in brackish water and swamps adjoining the Lagos lagoon network in Nigeria [23,24]. This family consists of over 600 species distributed mostly in Africa and is cultured in over 25 countries. They grow well in both freshwater and brackish water. Some of them are omnivorous feeders, which can filter feed on plankton as well as accepting larger food particles. It is a native to West, Central, and east Africa and has also been introduced to many other countries throughout the tropics and beyond. They are found in the lagoon and in addition to this, they have the ability to withstand extreme water temperature and low levels of dissolved oxygen [25]. The possession of many desirable characteristics makes *O. niloticus* very suitable for culture.

In view of the foregoing the study set out to investigate the differential and joint-action toxicity of fertilizers NPK 20:10:10 and NPK 12:12:17 against *O. niloticus* fingerlings based on predetermined ratios and equitoxic ratios in laboratory bioassays.

MATERIALS AND METHODS

Test Animals: Live fingerlings of *Oreochromis niloticus* (mean weight: 1.93g, mean length: 3.57cm) used for this study were collected from Animashaun farms in Badagry, Lagos state. The fishes were transported to the laboratory in oxygenated polythene bags.

Acclimatization of Test Organisms: In the laboratory, the fishes were kept in glass holding tanks (50cm x 30cm x 30cm) and acclimatized for a period 7days. The water was continuously aerated with 220V air pumps and changed once in 2 days. The fishes were fed twice daily during the period of acclimatization with commercial fish feed pellets at 5% body weight. The fishes were fed every day until a day preceding the bioassay test. Stocking and experimentation was carried out under ambient laboratory conditions (temperature 27±3^{0}C, relative humidity 79±2%).

Test Compounds: The NPK fertilizers are composed of Nitrogen, Phosphorus, and Potassium respectively in different concentrations used for growing crops. NPK 20:10:10 fertilizer and NPK 12:12:17 fertilizer were obtained from Golden Fertilizer Co. Ltd. in Iganmu Lagos, Nigeria. 100grams of each fertilizer was dissolved in a 1litre of dechlorinated water and a brownish viscous liquid was obtained with sediments at the base. The aqueous solution was then kept in a glass bottle, under ambient laboratory conditions (temperature 27± 3^{0}C, relative humidity 79±2%) until the time of use.

General Bioassay Procedures:

Bioassays Containers: The bioassays were carried out in glass tanks (22cm x 15cm x 18cm). 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 crude oil components [26].

Application of Toxicants to Test Media: De chlorinated tap water was measured using a measuring cylinder into clean, dry bioassay containers, and a predetermined volume of the fertilizer was added into the water to make it up to 2000mL (total volume of test media) to achieve the desired test concentration.

Assessment of Quantal Response: The fish, *O. niloticus* were assumed to be dead if there was no movement of appendages, opercular and mouth or failed to respond to the touch of forceps /glass rod. The distress behaviour and general conditions of the fish were carefully observed during experimentation.

Bioassay Procedure:

Relative Acute Toxicity Tests Of NPK fertilizers against *Oreochromis niloticus* fingerlings: Ten fishes of similar sizes in three replicates were introduced randomly into the test media in bioassay containers. A total of 30 fish fingerlings were exposed per treatment including untreated control (de chlorinated tap water).The test animals were exposed to varying concentrations of NPK fertilizers as follows:

NPK 20:10:10 fertilizer- 1ml/L, 2ml/L, 4ml/L, 6ml/L and 8ml/L

NPK 12:12:17 fertilizer - 1ml/L, 2ml/L, 4ml/L, 6ml/L and 8ml/L

Mortality was assessed once every 24 hours for 4 days (96hours)

Joint Action Toxicity Tests Of NPK Fertilizers Against *Oreochromis niloticus* Fingerlings: Mixtures of NPK 20:10:10 and NPK 12:12:17 fertilizers were prepared in pre-determined 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 fertilizers, which was in ratio 4:3 for *O. niloticus* .The test animals were exposed to graded concentrations of fertilizers as follows:

O. niloticus at the predetermined ratio were 2ml/L, 4ml/L, 6ml/L, 8ml/L 10ml/L and untreated control

O. niloticus at the equitoxic ratio were 2ml/L, 4ml/L, 6ml/L, 8ml/L 10ml/L and untreated control

Statistical Analysis: Toxicological dose-response data involving quantal response (mortality) for both single and joint action studies were analyzed by probit analysis[27]. The indices of toxicity measurement derived from these analyses were LC_{50} (median lethal concentration that causes 50% response (mortality) of exposed organisms), LC_{95} (lethal concentration that causes 95% response (mortality) of exposed organisms), LC_{05} (Lethal concentration that causes 5% response (mortality) of exposed organisms) and T.F. (toxicity factor for relative potency measurements e.g., ratio of 96-h LC_{50} of a compound to LC_{50} values at equivalent time intervals). For the joint action toxicity of the fertilizers mixtures, the three models employed for the analyses are as follows:

Model A: The synergistic ratios (SR) model after [28]. The synergistic ratio model is;

$$SR = \frac{LC_{50} \text{ of a chemical acting alone}}{LC_{50} \text{ of chemical +additive (mixture)}}$$

Where SR=1 describes additive action, SR<1 describes antagonism action and SR>1 describes synergistic action.

Model B: concentration-addition model by[291] with slight modification (relative toxic units (RTU) estimations[21]. 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 values (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 is then compared to the observed LC_{50} value of the mixture so as to classify the type of interaction among the components of the mixture as:

Additive if the observed LC_{50} value of the mixture is equal to the predicted LC_{50} value of the mixture

Synergistic if the observed LC_{50} value of the mixture is less than the predicted LC_{50} of the mixture.

Antagonistic if the observed LC_{50} value of the mixture is greater than the predicted LC_{50} of the mixture.

$$RTU = \frac{\text{Predicted } LC_{50} \text{ value}}{\text{Observed } LC_{50} \text{ value}}$$

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

Model C: Isobolograms after[30]. The joint actions between the toxic compounds in binary mixtures are presented in form of isobologtams (Fig 1). Each isobole (I-IV) represent 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, LC_{50}). In the theoretical isobole (Fig. 1), points C and D represent the amounts of toxicant A and B which individually produced the biological response (LC_{50} 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 (Fig. 1).

Data based on the derived 96hr LC_{50} values of Binary mixtures at predetermined ratio 1:1 and equitoxic ratio 4:3 were fitted into isobolograms and compared to the theoretical model Fig 1 in order to extrapolate the type of joint action depicted in this work, after[31].

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 96hrs toxicity tests.

RESULTS AND DISCUSSION

Physico-Chemical Parameters: The results of the physico-chemical parameters of the test media obtained during the bioassay studies indicate that the values for dissolved oxygen, pH, and temperature were 5.2±0.3mg/L, 7.0; and 27±2^{o}C respectively.

Single-Action Toxicity Test of NPK Fertilizers Against *Oreochromis niloticus* Fingerlings: The toxicity of the NPK 12:12:17 fertilizer and NPK 20:10:10 fertilizer varied slightly based on their 96-h LC_{50} values was 2.856ml/L and 2.198ml/L respectively. The computed toxicity factors show that after 96hours exposure NPK 20:10:10 fertilizer was 1.30 times more toxic than NPK 12:12:17 fertilizer. When tested against *O. niloticus* fingerlings (Table 1).

Joint-action toxicity of binary mixtures of NPK 12:12:17 fertilizer and NPK 20:10:10 fertilizer Against *Oreochromis Niloticus* Fingerlings: The lethal effects of the bioassays test with the binary mixtures in the redetermined ratio of 1:1 and the equitoxic ratio of 4:3 against *O. niloticus* fingerlings were 5.458ml/L and 3.537ml/L respectively (Table 2). Also the computed toxicity factors reveal based on the 96h LC_{50} values of exposure. The equitoxic ratio was 1.5 times more toxic than the predetermined ratio when tested against the organisms.

Based on the 96-h LC_{50} values, it was observed that the mixtures were significantly less toxic against *O. niloticus* fingerlings than when NPK 12:12:17 and NPK 20:10:10 fertilizers acted singly (Table 2). When the same sets of 96-h LC_{50} values were subjected to additional analysis based on concentration-addition model ,the interaction between the NPK 12:12:17 and NPK 20:10:10 fertilizers in predetermined (1:1) and equitoxic ratios (4:3) were consistently below 1(RTU=0.73 and RTU=0.46) tending to agree with the antagonism model (Table 3).

Furthermore, analysis of the joint action results based on the synergistic ratios model with reference to NPK 12:12:17 shows that mixtures prepared according to the proportion of the level of occurrence (predetermined ratio 1:1) and equitoxic ratio(4:3) was found to be less toxic (SR^1=0.523 and SR^2=0.807) (antagonism) than the fertilizer when acting singly. Similarly, the interaction in fertilizer (1:1 and 4:3) mixtures with reference to NPK 20:10:10 conformed to the model of antagonism (SR^2=0.621 and SR^2=0.403) (Table 3).

Further analysis of the joint-action toxicity test by fitting the derived 96-h LC_{50} values of the tested mixtures and single compounds into the isobolgram, Model C and comparing it to the theoretical pictorial isobole (Fig. 1) revealed that the resultant isobole from the binary mixtures of fertilizers conformed with the model of antagonism in the two mixtures (Fig 2).

Behavioural Observations of *Oreochromis niloticus* Fingerlings in Test Media: *O. niloticus* fingerlings showed sensitivity to the test media when introduced into different concentrations of the test solutions by swimming rapidly and attacking the sides of the glass tanks. After about 10 – 15 minutes, the fish rested at the bottom of the tank. It was observed that the test animals exhibited agitated movements, and lost their balance at higher concentrations of test media. The test organisms began to show great signs of stress, their fins became stretched, swam briefly on their sides before eventually dying. This was observed more in the higher concentrations of the test media. The opercular movement also became faster in the test solutions when compared with control and the test organisms were often found at the surface gulping for air by opening the mouth and rapidly taking in the test solution.

Discussion: All the physico-chemical parameters show that the quality of the water was conducive for the growth of the test organisms over the 96-h LC_{50} values of acute toxicity tests. NPK fertilizers like most other agrochemical substances are toxic to organisms at increased concentrations but the results of the study revealed that NPK 12:12:17 fertilizer in 96-h LC_{50} values gave 2.856ml/L while that of NPK20:10:10 fertilizer in 96-h LC_{50} gave 2.198ml/L. This shows that NPK 20:20:10 fertilizer is 1.3 times more toxic than NPK 12:12:17 fertilizer. The differential toxicity observed between the test chemicals can be attributed to the fact that the physical characteristics and chemical composition of the test compounds are different[31,14].

The toxicity of NPK 12:12:17 fertilizer and NPK 20:10:10 fertilizer studied showed that the joint-action toxicity analysis agreed mainly with the model of antagonism which implies that the binary mixtures were less toxic compared to when they acted singly. The mechanism responsible for antagonistic interaction between components in a mixture can be attributed to the competition for binding sites in the biological interface reactions between the various components[32,33]. Antagonistic interactions in mixtures of pollutants where it occurs could be an advantage in environmental management, which was observed in this case study.

Furthermore, it is also important to note that the three models used in classifying the joint-action toxicity results in this case study were in agreement most of the time which shows that each of the models can provide its own classification that is reliable and accurate.

Table 1: Relative Toxicity of NPK Fertilizer 12:12:17 against *Orechromis niloticus* fingerlings

Exposure Time (Hrs)	LC_{50} (95% CL ml/L)	LC_5 (95% CL ml/L)	LC_{95} (95% CL ml/L)	Slope S.D	Probit Equation	D.F	T.F1	T.F2
NPK Fertilizer Ratio 12:12:17								
24	28.409 12.004 - 401.72	1.652 0.111 - 2.877	488.587 65.569 - 1108.821	1.33 ± 0.49	Y = 3.06 + 1.33x	3	1	
48	12.806 7.625 - 63.497	0.872 0.106 - 1.652	187.969 45.512 - 292.43	1.41 ± 0.41	Y = 3.49 + 1.41x	3	2.218	
72	5.788 3.493 - 23.766	0.103 0.076 - 0.449	326.823 49.464 - 608.37	0.94 ± 0.33	Y = 4.28 + 0.94x	3	4.908	
96	2.856 1.891 - 4.031	0.211 0.216 - 0.515	38.695 17.134 - 304.397	1.45 ± 0.33	Y = 4.34 + 1.45x	3	9.947	1
NPK Fertilizer Ratio 20:10:10								
24	30.808 11.912-229.021	1.170 0.0163 - 2.307	811.097 79.936 - 2471.906	1.16 ± 0.45	Y = 3.28 + 1.16x	3	1	
48	26.777 10.017-172.122	0.421 0.088- 1.239	1703.11 105.829-9895.325	0.91 ± 0.38	Y = 3.69 + 0.91x	3	1.151	
72	5.321 3.017 - 30.757	0.587 0.089 - 0.354	482.554 54.897 - 2204.601	0.84 ± 0.32	Y = 4.39 + 0.84x	3	5.789	
96	2.198 1.489 - 2.907	0.264 0.566 - 0.539	18.314 10.572 - 58.124	1.79 ± 0.35	Y = 4.39 + 1.79x	3	14.016	1.30

$$TF1 = \text{Toxicity Factor} = \frac{LC_{50} \text{ of Test Compound at 24 Hours}}{LC_{50} \text{ of Test Compound at other hours (48, 72, 96 hours)}}$$

$$TF2 = \text{Toxicity Factor} = \frac{96hLC_{50} \text{ of NPK Fertilizer 12:12:17}}{96hrLC_{50} \text{ of Fertilizer 20:10:10}}$$

SD = Standard Deviation D.F = Degree of Freedom
CL = Confidence Limit LC = Lethal Concentration

Table 2: Joint Action Toxicity of Pre-determined (1:1) and Equitoxic (4:3) Binary Mixture of NPK 12:12:17 Fertilizer and NPK 20:10:10 Fertilizer against *Orechromis niloticus* fingerlings

Exposure Time(Hrs)	LC_{50} (95% CL ml/L)	LC_5 (95% CL ml/L)	LC_{95} (95% CL ml/L)	Slope ± S.E	Probit Equation	D.F	T.F1	T.F2
Pre-determined Ratio (1:1)								
24	20.669 11.597 -- 413.298	1.802 0.094 -- 3.193	237.019 50.830 - 1508.545	1.55 ± 0.56	Y = 2.958 + 1.55x		3	1
48	12.521 8.565 -- 40.658	1.285 0.145 - 2.364	121.965 38.649 - 917.059	1.66 ± 0.50	Y = 3.17 + 1.66x	3	1.651	
72	8.164 6.432-- 12.408	1.377 0.402 -- 2.243	48.425 24.241 --291.634	2.13 ± 0.49	Y = 3.06 + 2.13x	3	2.532	
96	5.458 4.418 -- 6.743	1.068 0.527--2.088	27.886 14.268--56.508	2.33 ± 0.47	Y = 3.28 + 2.33x	3	3.787	1
Equitoxic Ratio (4:3)								
24	24.515 11.745 -- 38791.76	1.042	576.513 0.068 -- 2.429	1.20 ± 0.51 69.297 -- 716.253	Y = 3.33 + 1.20x	3	1	
48	12.130 8.193 -- 45.166	1.033 0.0623 -- 2.087	142.381 40.694 - 258.879	1.54 ± 0.48	Y = 3.33 + 1.54x	3	2.02	
72	6.283 4.861-- 8.780	0.876 0.170 -- 1.627	45.049 22.299 -- 295.585	1.92 ± 0.46	Y = 3.47 + 1.92x	3	3.90	
96	3.537 2.663 -- 4.311	1.224 0.318 -- 1.368	14.820 10.551-- 28.698	2.64 ± 0.48	Y = 3.55 + 2.64x	3	6.93	1.54

TF1 = Toxicity Factor = $\frac{LC_{50} \text{ of Test Compound at 24 Hours}}{LC_{50} \text{ of Test Compound at other hours (48, 72, 96 hours)}}$

TF2 = Toxicity Factor = $\frac{96hLC_{50} \text{ of pre-determined ratio}}{96hrLC_{50} \text{ of equitoxic ratio}}$

SD = Standard Deviation D.F = Degree of Freedom
CL = Confidence Limit LC = Lethal Concentration

Table 3: Analysis (Based on Concentration-Addition and Synergistic Ratio Models) of the 96h LC_{50} values of NPK 12:12:17 Fertilizer and NPK 20:10:10 Fertilizer) when Acting Jointly of Singly Against *Orechromis niloticus* fingerlings

Fertilizer Mixtures	Experimentally Observed 96h Lc_{50}(95% Cl) ml/L	Predicted 96h Lc_{50} (95% Cl) ml/L	Probit Line Equation	RTU	Synergistic SR1	Ratio SR2
Equitoxic Mixtures 4:3	3.537 (2.663 - 4.311)	2.574(1.719-3.549)	Y = 3.55 + 2.64x	0.73	0.807	0.621
Predetermined Ratio 1:1	5.458 (4.418 - 6.743)	2.527(1.690-3.469)	Y = 3.28 + 2.33x	0.46	0.523	0.403
NPK 12:12:17 Fertilizer Alone	2.856 (4.031 - 1.891)		Y = 4.34 + 1.45x			
NPK 20:10:10 Fertilizer Alone	2.198 (2.907 - 1.489)		Y = 4.39 + 1.79x			

KEY:
RTU = Relation Toxic Unit
RTU = $\frac{\text{Predicted } LC_{50} \text{ Value Binary Mixture}}{\text{Observed } LC_{50} \text{ Value Binary Mixture}}$
SR = Synergistic Ratio
CL = Confidence limits
SR^1/SR^2 = Synergistic ratio of the 1st / 2nd fertilizer

SR = $\frac{\text{(LC50 value of the fertilizer acting alone)}}{\text{(LC50 value of the mixture)}}$

SR^1/SR^2 = 1 indicates additive action
SR^1/SR^2 > 1 indicates synergism
SR^1/SR^2 < 1 indicates antagonism

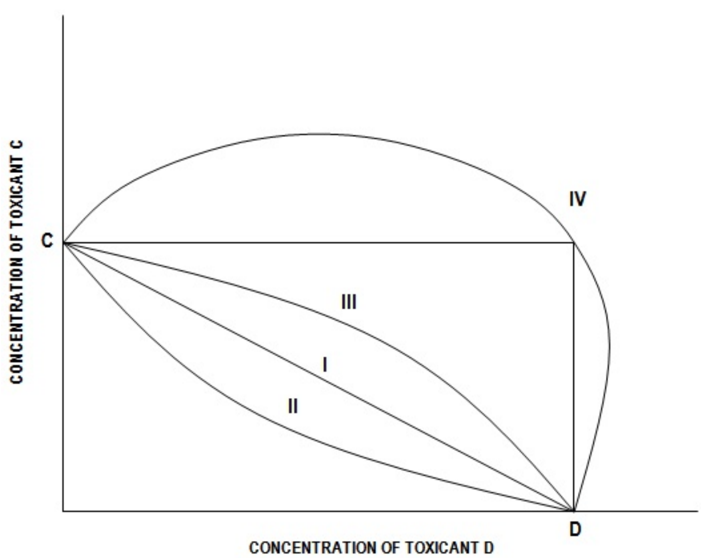

Fig. 1: Isoboles depicting the types of interactions between two chemicals C and D (after Arien's et al., 1976). Isobole I depicts additive action; Isobole II depicts synergism; Isobole III depicts subadditive action; Isobole IV depicts antagonism

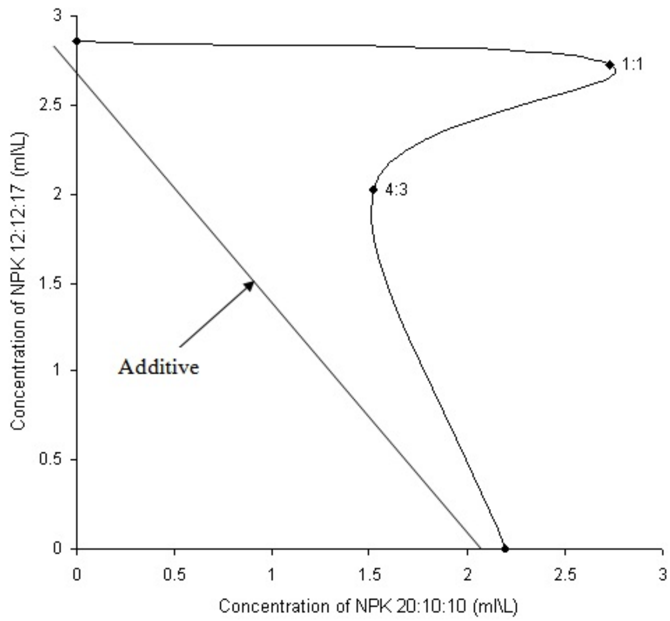

Fig. 2: Isobole Representation of the Binary Mixtures Effect of NPK 20:10:10 and NPK 12:12:17 at Different Proportions and Tested against *O. niloticus*

The SR model of [28] has the advantage of classifying the type of joint-action for each of the individual components in a mixture whether binary or multiple due to its ability to classify the type of strength of interactions between each of the components in the mixture. This shows that this model would be more useful when joint-action evaluations are carried out with a view of setting environmental safe limits of pollutants as reported by[34]. In addition, the pictorial isobole model of[31], has the advantage of giving an overall display of the types of interactions existing among the mixtures, but it is also important to always take into consideration the joint-effect of each of the different proportions of the mixtures when evaluating the type of joint-effect of binary mixtures of toxicants[34,21].

Edokpayi (1993) reported that Calcium Ammonium a farm fertilizer is a feasible short-term test to assess the stressful levels of *Tilapia zilli*. A lot of work needs to be done on the joint-action on fertilizers and other pollutants due to its increasing discharge into our aquatic bodies of water which could result to reduction in the number of deaths, detrimental in increasing the mortality or additive leading to equal rates of single-action and joint-action toxicity.

The development of predictive measures is an aim of joint-action toxicity testing. This information can be used to regulate discharges and enforce compliance. It is therefore easy to imagine that a safe time whose starting point is the toxicity of a single chemical acting against sensitive species under conditions where such a chemical occurs in a mixture with one or more other chemicals that significantly antagonise action of the initial compounds. This was confirmed by the experimental 96h LC_{50} bioassay with toxicity with toxicity of NPK 12:12:17 fertilizer and NPK 20:10:10 fertilizer in the predetermined and equitoxic ratios. This information can be used to regulate discharged and enforce compliance. Further work should also be done on other ratios of fertilizers to ascertain whether the pollutant could be effective in reducing the effect of mortality. Also bioaccumulation and histopathology studies should be carried out to investigate the secondary effects of these pollutants on *O. niloticus*. Hence, the critical part of these pollutants should be determined.

In conclusion this study revealed that NPK 20:10:10 fertilizer is more toxic than NPK 12:12:17 fertilizer and the joint action revealed that the binary mixtures lead to a decrease in the number of deaths which could be an advantage in the biomonitoring and management of the aquatic environment. In view of this work, it is therefore suggested that these two fertilizers be used together as against been used singly so as to reduce the concentrations of their toxicity hence leading to better management of our aquatic bodies of water.

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