



## Single and Joint Action Toxicity Studies of Trace Elements in Binary Mixtures against *Clarias gariepinus* and *Sarotherodon melanotheron*

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**ABSTRACT:** This study investigated the interactions of essential and non-essential trace elements when present in binary mixtures and resultant effects on acute toxicity in fish. The effects of four essential trace elements; Zn, Ni, Co and Cr respectively on the acute toxicities of three non-essential trace elements; Cd, Hg and Pb against *Clarias gariepinus* and *Sarotherodon melanotheron* were assessed using laboratory bioassays. The patterns of interaction of the elements affecting toxicity via antagonistic, synergistic or additive reactions were determined using the Synergistic Ratio (RA) and Concentration Addition (CA) joint action toxicity models. Single action toxicity indices showed that Hg was the most toxic element with a 96hr LC<sub>50</sub> value of 0.0004 and 0.0003 mmol/l and Co the least toxic element with a 96hr LC<sub>50</sub> value of 0.86 and 1.00 mmol/l, against the two fish species respectively. The SR and CA toxicity models showed that the essential trace elements (Zn, Ni, Cr and Co) reduced the toxicity of Pb against *C.gariepinus* as indicated by SR and RTU values < 1. Both models also showed that Zn reduced the toxicity of Pb and Hg respectively against *S.melanotheron*. However, the SR model showed that only Co reduced the toxicity of Cd against *C.gariepinus*. This study has established the possible beneficial interactions among essential trace elements and hazardous non-essential trace elements. Factors influencing such beneficial interactions including physiological processes in fish species, trace element concentrations and physicochemical parameters of exposure medium should be explored in future studies.

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Trace elements are a group of inorganic pollutants that are commonly detected in polluted aquatic ecosystems. Trace element pollution in aquatic ecosystems is a recurrent global environmental problem because they are not degradable by natural processes and are toxic to biological systems at low concentrations (Jiang *et al.*, 2012). They occur naturally in rocks and soils, however their use as raw materials in many industrial processes have resulted in elevated concentrations in the environment especially in aquatic ecosystems. They seldom exist in isolation in ecosystems, rather in mixtures with other elements and/or other organic pollutants. The toxicological effects of a trace element in an exposed organism may be aggravated or reduced by the interaction with other elements when two or more are present simultaneously in the surrounding media of the organism. Trace elements present in an ecosystem may interact with each other, competing for binding sites in an exposed organism and forming complexes which may or may not be easily excreted from the system (Otitolaju, 2002). The pattern of toxic interaction among trace elements when present in mixtures may be antagonistic, synergistic or additive (Otitolaju, 2002). These interactions may affect the uptake or excretion rates in exposed organisms (Franklin *et al.*, 2002) and

subsequently manifestation of toxic effects in the organism, final consumers and ecosystem at large. It is justifiable, based on their toxic properties and function or otherwise in biological systems, to investigate the influence of the essential trace elements on the toxicity of the non-essential trace elements when present in mixtures, as is the case in polluted ecosystems. The objective of this study was to evaluate the single and joint action toxicity of selected essential and non-essential trace elements in binary mixtures against *Clarias gariepinus* and *Sarotherodon melanotheron* as well as to assess the influence of the essential trace elements on the toxicity of non-essential trace elements by defining patterns of interaction via antagonistic, synergistic or additive actions using two mixture toxicity models.

### MATERIALS AND METHOD

*Test Organisms: description, source and acclimatization:* *Clarias gariepinus* and *Sarotherodon melanotheron* fingerlings (2-3 weeks old; mean total length, 4.0 ± 0.4 cm; mean total weight, 4.0 ± 0.2 g) were purchased from a fish farm in Lagos State, Nigeria and transported to the Ecotoxicology Laboratory, Department of Zoology, University of Lagos in plastic bags half filled with pond water. The

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fish species were acclimatized to laboratory conditions (Temperature;  $28 \pm 2^\circ\text{C}$  and Relative Humidity  $70 \pm 2\%$ ) in plastic tanks holding 35.0 L of dechlorinated tap water for a period of seven days. The water was changed once every 48 hours and was continuously aerated with an air pump (Bazgdon air pump, double type 1200). Photoperiod was maintained at a constant 14 hour light 10 hour dark cycle and the fishes were fed twice daily with fish food (Coppens) at 2% body weight.

*Test Compounds:* The non-essential elements used in this study were  $\text{Pb}(\text{NO}_3)_2$ ,  $\text{HgCl}_2$  and  $\text{CdCl}_2$  manufactured by J.T Baker (New Jersey, USA) purchased from Labio Scientific Center, Lagos, Nigeria. The essential elements were  $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$  and  $\text{ZnCl}_2$  manufactured by J. T Baker,  $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$  manufactured by L.N.L Laboratories and  $\text{CoCl}_2$  manufactured by Sigma-Aldrich (UK), purchased from Labio Scientific Center, Lagos, Nigeria. All elements were of analytical grade with 96-99.5% purity.

*Preparation of test compounds:* Stock solutions of trace elements for single action toxicity studies were prepared by taking computed amount of elements, which were made up to a desired volume using distilled water, to achieve solutions of known strength ( $\text{HgCl}_2$  and  $\text{CdCl}_2$  - 1 g/l;  $\text{Pb}(\text{NO}_3)_2$ ,  $\text{CoCl}_2$ ,  $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$  and  $\text{ZnCl}_2$  - 10 g/l). For binary mixture studies, the weight of each element salt in the mixture based on proportion in pre-determined ratios were computed and weighed out into a flask. This was made up to desired volume with distilled water and stirred with a glass rod to ensure proper mixing of constituents and to achieve stock solution of known strength. Pre-determined working concentrations for toxicity studies were made by serially diluting stock solutions of the trace elements or trace element mixtures. These were made up to required volume using de-chlorinated tap water in any series of toxicity studies. Actual concentration of trace element in each solution of known strength was computed based on molecular weight of test compound.

*Single action acute toxicity studies:* Test species (*Clarias gariepinus* and *Sarotherodon melanotheron*) were exposed in separate experiments, to different concentrations (pre-determined from range finding studies) of seven trace elements ( $\text{Pb}(\text{NO}_3)_2$  20-100 mg/l,  $\text{CdCl}_2$  10-45 mg/l,  $\text{HgCl}_2$  0.05-0.60 mg/l,  $\text{ZnCl}_2$  10-55 mg/l,  $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$  20-180 mg/l,  $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$  10-85 mg/l and  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  100-600 mg/l) and untreated controls respectively. Four active fingerlings of each species were taken from plastic holding tanks,

using a sieve and randomly assigned to bioassay aquaria holding media with test compound or untreated control respectively. Each treatment was replicated thrice, to give a total of 12 fingerlings exposed per concentration (APHA-AWWA-WPCF, 1995) and untreated control for each species. Mortality was assessed once every 24 hours for a period of 4 days (96 hours).

*Joint action acute toxicity studies of trace elements in binary mixtures:* Test species were exposed in separate experiments, to different concentrations of trace elements in binary mixtures of pre-determined ratios (w/w) of the essential and non-essential trace elements and untreated controls respectively. Exposure concentrations were determined after range finding studies and ranged within those used for single action studies for each element respectively. Each essential trace element (Zn, Ni, Co and Cr) was combined with the non-essential trace elements (Pb, Cd and Hg) in ratios 1:1, 1:4, 2:3 (w/w) respectively, with the weight of the essential trace element always being equal to or smaller than the non-essential trace element.

*Data analysis:* The Statistical Package for Social Sciences (SPSS version 16) was used to carry out Probit analysis (Finney, 1971) to extrapolate 96hr  $\text{LC}_{50}$  values from dose-response data obtained from acute toxicity studies.

*Binary mixtures toxicity assessment:* The Synergistic Ratio (SR) model after Hewlett and Plackett (1969) and the Concentration Addition (CA) model after Anderson and Weber (1975) were employed to assess the pattern of joint action interaction of the trace elements in binary mixtures.

*Synergistic Ratio (SR) Model:* This model assesses the contribution to mixture toxicity of each trace element in a mixture.

$\text{SR} = \text{LC}_{50}$  of trace element acting singly  $\div$   $\text{LC}_{50}$  of mixture

*Concentration Addition Model:* This model is generally based on the assumption that similarly acting toxicants when combined in a mixture will contribute equally to give an additive toxic effect.

Concentration Addition (CA) model was calculated as:

Relative Toxic Unit (RTU) = Predicted  $\text{LC}_{50}$   $\div$  Observed  $\text{LC}_{50}$

Where: Predicted  $\text{LC}_{50}$  is the  $\text{LC}_{50}$  value predicted for the mixture, calculated by summing up the  $\text{LC}_{50}$  of the

trace elements in the mixture when acting singly based on their proportions in the mixture. Observed LC<sub>50</sub> is the LC<sub>50</sub> of the mixture after the experiment.

SR/RTU = 1 describes additive action between trace elements in mixture; SR/RTU > 1 describes synergistic action between trace elements in mixture; SR/RTU < 1 describes antagonistic action between trace elements in mixture

## RESULTS AND DISCUSSION

### Single action toxicity of trace elements against *Clarias gariepinus* and *Sarotherodon melanotheron*:

Mercury was the most toxic element against the two fish species with the lowest 96hr LC<sub>50</sub> value (0.0004 and 0.0003 mmol/l for *C.gariepinus* and *S.melanotheron* respectively) while Co was the least toxic element (0.86 and 1.00 mmol/l for *C.gariepinus* and *S.melanotheron* respectively). Ramakritinan *et al.* (2012) have also reported Hg to be the most toxic element when compared to other elements they tested against aquatic species. The non-essential trace elements were generally more toxic to *C.gariepinus* than essential trace elements (Table 1). Non-essential trace elements have no known biological functions in living systems and have been reported to be highly toxic to living organisms (Guedenon *et al.*, 2012).

**Table 1:** 96hr LC<sub>50</sub> values of trace elements against the fish species

Trace Element	LC <sub>50</sub> 95% C.L (mmol/l)	Slope ± S. E	D.F	Probit line eqn	T. F
<i>Clarias gariepinus</i>					
Mercury	0.0004 (0.0001 - 0.0006)	2.664 ± 0.880	3	Y=2.615+2.664X	1
Lead	0.12 (0.09 - 0.15)	4.573 ± 1.173	3	Y=-7.296+4.573X	300
Cadmium	0.09 (0.07 - 0.12)	4.153 ± 1.141	3	Y=-5.161+4.153X	225
Zinc	0.16 (0.02 - 0.31)	0.859 ± 0.704	3	Y=-1.160+0.859X	400
Nickel	0.37 (0.22 - 0.78)	4.219 ± 1.694	3	Y=-8.381+4.219X	925
Cobalt	0.86 (0.67 - 1.03)	4.846 ± 1.147	3	Y=-11.196+4.846X	2,150
Chromium	0.21 (0.18 - 0.24)	7.330 ± 1.978	3	Y=-12.854+7.330X	525
<i>Sarotherodon melanotheron</i>					
Mercury	0.0003 (0.000 - 0.0006)	1.128 ± 0.493	3	Y=1.276+1.128X	1
Lead	0.14 (0.12 - 0.16)	6.272 ± 1.197	3	Y=-10.489+6.272X	466
Cadmium	0.17 (0.15 - 0.18)	13.333± 2.957	3	Y=-19.775+13.333X	566
Zinc	0.12 (0.07 - 0.16)	2.766 ± 0.780	3	Y=-3.335+2.766X	400
Nickel	0.11 (0.07 - 0.25)	2.522 ± 0.779	3	Y=-3.614+2.522X	366
Cobalt	1.00 (0.59 - 1.23)	4.231 ± 1.227	3	Y=-10.041+4.231X	3,333
Chromium	0.18 (0.13 - 0.37)	2.396 ± 0.738	3	Y=-4.015+2.396X	600

### Effects of the essential elements on the toxicity of the non-essential elements against *Clarias gariepinus* and *Sarotherodon melanotheron*:

**Zinc:** The SR and CA models showed that the interactions of Zn and Pb at all binary mixture ratios against the two fish species were antagonistic (SR and RTU values were < 1) resulting in reduced toxicity of Pb against the species. Zinc with Hg mixtures were less toxic against *C.gariepinus* at mixture ratios 1:1 and 1:4 and less toxic against *S.melanotheron* at all mixture ratios indicated by SR values < 1 (Table 2).

The ability of Zn to reduce the toxicity of Hg and Pb may be attributed to competition between the elements at the site of uptake, with Zn being an essential element preferentially absorbed by the organism than Hg or Pb, which are non-essential elements respectively.

There is a dearth of knowledge on the specific transport pathway of most non-essential trace elements including Hg and Pb in aquatic organisms, they have been reported to behave adventitiously

following existing pathways for essential trace elements (Deb and Fukushima, 1999).

The interaction of Zn with Cd against the two species was synergistic (enhanced toxicity of Cd) at all mixture ratios with SR and RTU values > 1, indicating that Zn and Cd mixtures were more toxic to the fish species than Cd alone (Table 2). Zinc is taken up through Ca ion channels in aquatic organisms, however Zn and Cd are chemically similar elements and are usually taken up through similar pathways (Rainbow and Blackmore, 2001). The increased toxicity of Cd in the presence of Zn can be attributed to displacement of Zn by Cd during uptake through the same channel/pathway.

**Nickel:** The toxicity models showed that acute toxicity of Pb against *C.gariepinus* was reduced in the presence of Ni (antagonism) at binary mixture ratios 1:1 and 2:3 as indicated by SR and RTU values < 1 (Table 3). However, Ni synergized the toxicities of Cd and Hg against *C.gariepinus* as well as the toxicities

of Pb, Cd and Hg against *S.melanotheron* as indicated by SR and RTU values > 1 (data not shown).

**Table 2:** Effects of Zinc on acute toxicity of Lead, Cadmium and Mercury against *Clarias gariepinus* and *Sarotherodon melanotheron*

Binary Mixtures	LC <sub>50</sub> 95% C.L (mmol/l)	SR	RTU
<i>Clarias gariepinus</i>			
Zinc + Lead (1:1)	0.16 (0.13 - 0.19)	0.75*	0.88*
Zinc + Lead (1:4)	0.20 (0.16 - 0.24)	0.60*	0.64*
Zinc + Lead (2:3)	0.18 (0.11 - 0.21)	0.67*	0.78*
Zinc + Cadmium (1:1)	0.06 (0.003 - 0.08)	1.50	2.08
Zinc + Cadmium (1:4)	0.08 (0.06 - 0.13)	1.13	1.30
Zinc + Cadmium (2:3)	0.07 (0.05 - 0.09)	1.29	1.69
Zinc + Mercury (1:1)	0.0006 (0.0005 - 0.0008)	0.67*	133.67
Zinc + Mercury (1:4)	0.0006 (0.0004 - 0.0008)	0.67*	53.87
Zinc + Mercury (2:3)	0.0004 (0.00009 - 0.0006)	1.00	160.60
<i>Sarotherodon melanotheron</i>			
Zinc + Lead (1:1)	0.16 (0.13 - 0.18)	0.88*	0.81*
Zinc + Lead (1:4)	0.15 (0.08 - 0.23)	0.93*	0.91*
Zinc + Lead (2:3)	0.20 (0.16 - 0.28)	0.70*	0.66*
Zinc + Cadmium (1:1)	0.03 (0.01 - 0.05)	5.67	4.83
Zinc + Cadmium (1:4)	0.01 (0.00 - 0.02)	17.00	16.00
Zinc + Cadmium (2:3)	0.04 (0.02 - 0.19)	4.25	3.75
Zinc + Mercury (1:1)	0.0005 (0.00005 - 0.0009)	0.60*	120.30
Zinc + Mercury (1:4)	0.0004 (0.0000 - 0.0006)	0.75*	60.60
Zinc + Mercury (2:3)	0.0007 (0.0005 - 0.001)	0.43*	68.82

LC: Lethal concentration; C.L: Confidence limits; SR: Synergistic Ratio; RTU: Relative Toxic Unit; \*: SR/RTU values < 1 indicating antagonistic reactions

**Table 3:** Effects of Nickel on acute toxicity of Lead against *Clarias gariepinus*

Binary Mixtures	LC <sub>50</sub> 95% C.L (mmol/L)	SR	RTU
<i>Clarias gariepinus</i>			
Nickel + Lead (1:1)	0.45 (0.31 - 0.76)	0.27*	0.55*
Nickel + Lead (1:4)	0.02 (0.01 - 0.11)	6.00	8.50
Nickel + Lead (2:3)	0.55 (0.47 - 0.75)	0.24*	0.44*

LC: Lethal concentration; C.L: Confidence limits; SR: Synergistic Ratio; RTU: Relative Toxic Unit; \*: SR/RTU values < 1 indicating antagonistic reactions

**Table 4:** Effects of Cobalt on acute toxicity of Lead, Cadmium and Mercury against *Clarias gariepinus* and *Sarotherodon melanotheron*

Binary Mixtures	LC <sub>50</sub> 95% C.L (mmol/L)	SR	RTU
<i>Clarias gariepinus</i>			
Cobalt + Lead (1:1)	0.14 (0.10 - 0.18)	0.86*	3.50
Cobalt + Lead (1:4)	0.11 (0.08 - 0.25)	1.09	2.44
Cobalt + Lead (2:3)	0.14 (0.10 - 2.21)	0.86*	2.97
Cobalt + Cadmium (1:1)	0.26 (0.18 - 2.43)	0.35*	1.83
Cobalt + Cadmium (1:4)	0.13 (0.10 - 0.16)	0.69*	1.88
Cobalt + Cadmium (2:3)	0.19 (0.14 - 0.29)	0.45*	2.09
<i>Sarotherodon melanotheron</i>			
Cobalt + Mercury (1:1)	0.0005 (0.0001 - 0.001)	0.60*	1000.30
Cobalt + Mercury (1:4)	0.0005 (0.0002 - 0.0008)	0.60*	400.48
Cobalt + Mercury (2:3)	0.0002 (0.00004 - 0.0004)	1.50	2000.90

LC: Lethal concentration; C.L: Confidence limits; SR: Synergistic Ratio; RTU: Relative Toxic Unit; \*: SR/RTU values < 1 indicating antagonistic reactions

**Table 5:** Effects of Chromium on acute toxicity of Lead against *Clarias gariepinus*

Binary Mixtures	LC <sub>50</sub> 95% C.L (mmol/L)	SR	RTU
<i>Clarias gariepinus</i>			
Chromium + Lead (1:1)	0.16 (0.0005 - 0.18)	0.75*	1.03
Chromium + Lead (1:4)	0.17 (0.07 - 0.20)	0.71*	0.81*
Chromium + Lead (2:3)	0.19 (0.09 - 0.22)	0.63*	0.82*

LC: Lethal concentration; C.L: Confidence limits; SR: Synergistic Ratio; RTU: Relative Toxic Unit; \*: SR/RTU values < 1 indicating antagonistic reactions

**Cobalt:** The SR model showed that interactions between Co and Pb as well as Co and Cd in binary mixtures against *C.gariepinus* were antagonistic indicated by SR values < 1 resulting in the reduced toxicities of Pb and Cd against the species. The model also showed that the interactions between Co and Hg at binary mixture ratios 1:1 and 1:4 against *S.melanotheron* were antagonistic (Table 4).

**Chromium:** The two models showed that interactions between Cr and Pb in binary mixtures against *C.gariepinus* were antagonistic as indicated by SR values < 1 resulting in reduced toxicity of Pb against the species (Table 5). Both models also showed that the interactions between Cr and the three non-essential elements (Pb, Cd and Hg) in binary mixtures against *S.melanotheron* were synergistic as indicated by SR and RTU values > 1 indicating the toxicities of the elements against the fish species were enhanced in the presence of Cr (data not shown).

**Conclusion:** This study showed beneficial antagonistic interactions among essential trace elements and some non-essential trace elements. These beneficial interactions should be investigated as a possible tool to develop remediation methods for organisms in polluted ecosystems in future studies. The pattern of interaction amongst trace elements should also be incorporated in setting safe discharge limits rather than relying on toxicity data for single elements as they are seldom present in isolation in polluted ecosystems rather in mixtures with other elements and/or other pollutants.

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