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The effects of Aluminium waterbone exposure to mixtures of Cadmium and Lead on growth and mortality of mosquito fish *Gambusia affinis* H. (Poescillidae, Pisces)

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ABSTRACT: Combined effects of three metals were assessed through toxicological tests in binary and one tertiary mixtures. Death and changes in body weight were used as biological responses to the toxicity. Test solutions were prepared with metal salts grade Merck Analar at 65%. Concentrations were derived from the Congolese official standards from Mining Code and Regulations. These were 500 ppb fof Cd, 5000 ppb for Pb and 5000 ppb for Al. Acute tests were 96-h exposures of the Mosquito fish *Gambusia affinis* and chronic tests were based on the 35-d exposures. During the chronic exposure, fish were daily fed with the powder of the earthworm *Eisenia fetida.*
Results allowed to conclude that:
- Legal standards from the Congolese code were toxic to fish. They should be reduced or lowered.
- Mixtures of Cd and Pb develop synergism and remain very toxic to aquatic organisms.
- Mixtures of both metals with the Al in binary or tertiary combinations showed antagonism.
This information provides useful tools to water quality managers.

KEYWORDS: Heavy metals, toxic, Acute, chronic, mixtures.

1 INTRODUCTION

Metal pollution is one of the main environment threats in the D R Congo (Musibono, 1999; UNEP, 2011). Indeed, in this mineral-rich country, mining activities remain the main source of pollution with Cd, Pb and Al (UNEP, 2010). This study focuses on the combined effects of these three metals under acute and chronic conditions, as they are existing in combination in natural environment, especially in aquatic ecosystem within the mining and industrial areas. There is no evidence that Cadmium, lead and Aluminium are essential metals for biological processes (Eisler, 2000). However, Cd toxicity in freshwater biota is well documented (Eisler, 2000). For example, the ambient water concentrations exceeding 10µg/L are associated with the high mortality, reduced growth, inhibit reproduction and other adverse effects. For the drinking water for humans, the criterion is 10µg/L (Eisler, 2000). Proposed Cd criteria for the freshwater aquatic life protection at the water hardness of 50 mg/l $cac0_3$ is < 1.5 µg/L; < 3.0 µg/L at 100 mg/L $caco_3$ and < 6.3 µg/L at 200 mg/L $caco_3$ (USEPA, 1980). For tested species of fish, the criterion is < 0.9 µg/L for acute toxicity and < 1.8 µg/L for chronic toxicity (Hall et al., 1998). Of course, the D R Congo legal standard value of 500 µg/L of Cadmium in mining discharges remains higher (D R Congo, 2002).

The proposed lead criteria for the protection of natural resources and human health come to the same conclusion as for Cadmium. Indeed, Pb criterion for aquatic life for water bodies with 50 mg/L $caco_3$ hardness is < 1.3 µg/L; <3.2 µg/L for 100 mg/L $caco_3$ water hardness, and < 7.7 µg/L for 200 mg/L $caco_3$ water hardness (Demayo et al., 1982). For fish, the criterion is < 300 µg/kg of food (fresh weight). These are lower than the D R Congo legal value of 5000 µg/L Pb (DR Congo, 2003).

2 MATERIAL AND METHODS

Analytical reagent used to prepare test solutions with 65% of purity (chemicals) were the salts of Cadmium ($Cd\ Cl_2$), Pb $(NO_3)_2$ and $Al_2\ (SO4)_{3.5}$00ml plastic containers were used to support different test solutions. Three binary and one tertiary metal mixtures were made. These are: Cd + Pb; Pb+Al; Cd+Al and Al+Cd+Pb.

Concentrations were derived from legal standard values in the D R Congo Mining Code (2003). These were for Cd, 500 ppb; Pb, 5000 ppb and 5000 ppb for the Al. Test solutions were made by dissolving 530 mg of cdcl2 in 1L of distilled de-ionized water; 5197 mg of Pb (NO_3) 2 in 1L of distilled and de-ionized water and 20 583 mg $Al_2\ (SO4)_3$ in 1L of distilled and de-ionized water. The aim was to determine the acute and chronic effects of these metal salt mixtures on aquatic organisms targeting therefore Cd, Pb and Al, respectively. The choice of these metals was due to the fact that these metals are present in the DR Congo environments, especially in the urban, mining and industrial region of Kinshasa and Katanga. As the toxicity is commonly assessed through single element acute toxicity tests, chronic effects of mixtures are generally ignored, despite the fact that in nature, toxic elements occur in combinations or mixtures.

We also want to check to which extent the Congolese standards are harmless to aquatic organisms.

Mosquito fish 8 day-Old offspring, *Gambusia affinis,* were used as test organisms, as follows:

Two series of biotests were performed, both for acute and chronic toxicity. Acute effect was determined through the mortality-survival models after 96-h exposures while chronic effect was determined through body weight changes after 35 days exposures.

All test concentrations were in three replicates, and each test container received 5 offspring, thus meaning 15 individuals per concentration. A total of 75 individuals was exposed to pollutants for 96-hour acute tests and 75 individuals for chronic tests as well, as shown in the following Table1.

Table 1. Test solution preparations and arrangements

Mixtures	Test solutions			Total per concentration
	Replicate 1	Replicate2	Replicate3	
Cd + Pb LC_{50}-Cd +LC_{50}-Pb (500ppb Cd+ 5000 ppb Pb).	5	5	5	15
Cd+Al LC_{50}-Cd+LC_{50}-Al (500 ppb Cd + 5000 ppb Al)	5	5	5	15
Pb+Al LC_{50}-Pb+LC_{50}-Al (5000 ppb Pb + 5000 ppb Al)	5	5	5	15
Al+Cd+Pb LC_{50}-Al+LC_{50}-Cd+ CLC_{50}-Pb (500ppb of Cd+5000pb of Pb+Pb+5000ppb of +Al)	5	5	5	15
Control: No metal added	5	5	5	15

3 RESULTS AND DISCUSSION

All results are summarized in both Table 2 and 3 below.

Table 2. Summary of observed acute effect (mortality) after 96-h exposure to metal concentrations

Exposure	Survival trends during the 96-h exposures				% of survival after 96-h exposure	Comments
	Day1	Day2	Day3	Day4		
Cd alone	15	10	6	0	0	Toxic to freshwater fish
Pb alone	15	12	12	9	60	Not toxic
Al alone	15	14	14	10	67	Not toxic
Cd+Pb	15	0	0	0	0	Toxic
Cd+Al	15	11	10	9	60	Not toxic
Pb+Al	15	15	13	11	73	Not toxic
Al+Cd+Al	15	12	11	9	60	Not toxic
Control	15	15	15	15	100	Not toxic at all

Table 3. Chronic effect assessment as change in body weight after 35- day exposures to metal solutions

Test solution	Initial body weight (in mg/15 individuals)	Final body after 90- day exposures (in mg/15 exposed individuals)	Total Body weight change for 15 individuals	Average body weight change per individual
Cd (500ppb)	156	205	47	3.13
Pb (5000 ppb)	156.3	278	121.7	8.11
Al (5000 ppb)	156.2	325.4	169.2	11.28
Cd +Pb (5000ppb Cd + 500 ppb Pb)	156.2	189.3	33.1	2.21
Cd + Al (500ppb Cd + 5000 ppb Al)	156.3	253.6	97.3	6.49
Pb+Al (5000ppb Pb + 5000 ppb Al)	156.1	289.4	133.3	8.89
Cd+Pb+Al (500ppb Cd +5000ppb Pb+5000ppb Al)	156.3	278.2	121.9	8.13
Control =No metal added	156.2	437.4	281.2	18.75

Using the Null hypothesis test, above results showed significant difference in toxicity between Cadmium and lead single solutions, and those with Al added ($p < 0.00001$); and finally, all solutions showed significant differences with the control in both (acute and chronic) tests.

Toxicity can be ranged as followed, from the least to the most toxic:

Control < Al < Pb+Al < Cd+Pb+Al < Pb < Cd+Al < Cd < Cd+Pb.

These results allowed reveal that:

Mixtures of Cd+Pb exert **sygernism** effects in aquatic environment at normal ph values (ph= 6-9);

Cd+Al and Pb+Al mixtures showed **antagonistic** effects, and the Al reduced the toxicity in the mixtures of Cd+Pb. This could be explained by the fact that Al develops diaspores with other metals through adsorption processes (Musibono and Day, 2000; Musibono and Day, 1999; Musibono, 1998).

This paper suggests that even at legal standard values as defined by the DRC government (R D Congo, 2003), Cd and Pb exert toxic effects in chronic exposures at all concentrations tested. The combined toxicity of metals in aquatic environments is well documented (Deniseger et al., 1990; Arambasic *et al*., 1995; Jak et al., 1996).

Therefore, legal values for Cd and Pb mining are higher and Cd exerts acute toxic effects in exposed fish.

4 CONCLUSION AND SUGGESTION

The effect of Al to mixtures of Cd and Pb in aquatic environment (ph 6-9) is antagonistic and therefore useful since Al reduces toxicities. The survival of fish *Gambusia affinis* is an objective indicator due to the fact that survival is strongly depending on the toxic effect of exposures.

The same trends appeared regarding the chronic exposure. Additional tests are being performed to drinking water for chicks.

Actual effluent standards for mining industry in D R Congo should be revised and values lowered. Indeed, they are harmful to aquatic life.

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