Impact Of The Combined Toxicity Of Lead, Mercury, Cadmium And Aluminum On The Ecosystem Of The Matete River In Kinshasa

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RESUME

This work presents the results obtained as part of a study on the Contribution to the study of the combined toxicity of Lead, Mercury, Cadmium and Aluminum on the Matete River ecosystem in Kinshasa. The general objective pursued in this study is to evaluate the combined toxicity of lead, mercury, cadmium and aluminum on fish species populations. It was conducted in situ in the Matete River (measurements of physico-chemical pH and temperature parameters) and ex situ (ecotoxicological tests and elementary analyzes by X-ray spectrometry or X-ray fluorescence) respectively at the Ecotoxicology, ecosystem health, environmental health laboratory. Department of Environmental Sciences, Faculty of Science, University of Kinshasa and Central Analysis Laboratory (LCA) of CGEA / CREN-K. These experiments were conducted during the period from 01 August 2016 to 30 June 2017.

Water samples from the MATETE River were taken during the dry season and the rainy season to assess their influence of climate on the results. Results from in situ analyzes show that pH values in this aquatic ecosystem are close to neutral (ie 6.7 and 7.8 after rainfall, 6.6 and 7.5 without rain); the temperature values are respectively around 25.9 and 27.5ºC without rain and 25.8 to 27.5ºC after the rain. The X-ray fluorescence results obtained from the different samples of the Matete River reveal that the Mercury concentration varies between 0.00013 and <1.0 mg / l, Aluminum varies around 0.0020 and 2865 mg / l, Cadmium are in the range of 0.00020 and 2.4 mg / l and Lead, between 0.00008 and <2.0 mg / l. As for the ecotoxicological tests on the populations of *Gambusia affinis*, the results obtained in each of the solutions of these metals prepared in the laboratory have shown that the lethal concentrations (or effective) which kill at least 50% of the tested individuals (LC_{50} or EC_{50}) are of order: 0.000059 mg / ml for mercury solutions [Mercury Sulphate ($HgSO_4$)]; 0.00006mg / ml for Lead solutions (Lead Acetate [(CH_3COO) 4Pb)]; 0.0006mg / ml for solutions of Cadmium [Cadmium chloride ($CdCl_2$)] and 0.0064mg / ml for those of Aluminum

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[Aluminum trichloride ($AlCl_3$)]. The tests of the combined toxicity of the solutions of these four metals, namely Aluminum Trichloride ($AlCl_3$) + Lead Acetate (($CH_3COO)_4Pb$) + Cadmium Chloride ($CdCl_2$) + Mercury Sulfate ($HgSO_4$); reveal that the lethal concentration obtained is much lower than that of LC_{50} observed for each of the solutions prepared; which implies an increase of the toxicity towards the population of Gambusia affinis. This high toxicity would be related to the effects of synergy from each of four metals in the Matete River. These results would contribute to the management of "industrial" effluents discharges into aquatic ecosystems with a view to protecting mainly fish species (fish).

Key words: combined toxicity, heavy metals, Gambusia affinis, Matete River.

GENERAL INTRODUCTION

For more than fifty years, pollution has been one of the major problems facing our modern world. Pollution refers to the presence in the environment of dangerous, generally man-made chemicals and biological products, the harmful effects of which can be felt for long periods of time all over the planet. This pollution can affect water and land. It can be visible (oil slicks floating on the sea) just as much less visible (heavy metals dissolved in aquatic systems). The dumping of waste into rivers is nowadays of alarming proportions. For the sake of economy, factories and urban areas discharge their waste water directly into the natural environment, without having previously treated it. There are also toxic products that end up in rivers, killing many life forms. These pollutants include mercury (Hg), lead (Pb), cadmium (Cd) and aluminum (Al) from pulp mills, cosmetics, metallurgical plants, hospital facilities, etc. The flora and fauna living in this water can in this case become considerably poorer, not only quantitatively but also qualitatively (15).

The operation of an industry has often favored sites near watercourses for their facilities for three reasons: the transportation of raw materials, the water supply, which allows the cooling of the installations, and the possibilities of rejection. industrial effluents. For decades, rivers have inherited industrial effluents and wastewater, liquid wastes resulting from the extraction or processing of raw materials, and from all forms of production activity (6).

In the wild, terrestrial animals are generally exposed to heavy metals through their diet or by the air they breathe, while aquatic animals, in addition to their diet, are exposed to dissolved and particulate metals in animals. environments where they evolve. As absorption surfaces in aquatic environments are larger, therefore, the introduction of metals is much easier and the quantities of metals accumulated are likely to be greater (16).

Human activities have been impacting ecosystems for several decades, posing real environmental problems with regard to biodiversity and resources, especially in the marine environment, the final receptacle for chemical pollutants. Among these contaminants, certain so-called emerging substances, such as human and other

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cosmetic compounds and body care products, become a source of environmental concern (8).

The society is dependent on unpolluted rivers and rivers to provide for its needs: drinking water, fishing, agriculture, recreation, cultural and aesthetic amenity. These activities are closely linked to the quality of water and the good ecological functioning of rivers. Aquatic species are key elements in the functioning of the watercourse. They inhabit the aquatic environment and are co-adapted to them because of the evolutionary phenomena and many of them are sensitive to the alterations of their habitats.

It is for this reason that we have chosen to study the combined toxicity of Lead, Mercury, Cadmium and Aluminum on the Matete River ecosystem in Kinshasa, which crosses a large part of the industrial zone of the city.

2. HYPOTHESIS

The city of Kinshasa is full of a number of chemical industries whose effluents are generally discharged into watercourses, without any pretreatment (upstream). These effluents are loaded with chemical elements that combine or interact with each other and produce a toxic effect on the life of fish resources in various rivers, particularly in the Matete River. To these industrial effluents are added the rainwater that leaches the highly polluting wild dumps without neglecting domestic wastewater.

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3. OBJECTIVES OF THE STUDY

The general objective pursued in this study is to evaluate the combined toxicity of Lead, Mercury, Cadmium and Aluminum on the populations of fish species in order to contribute to the fight against food insecurity. To achieve this general goal, we have set ourselves the following specific objectives:

- Evaluate the quality of the Matete River by conducting physicochemical analyzes;
- Perform acute toxicity tests of lead, mercury, cadmium and aluminum solutions to determine their LC_{50};
- Determine the combined toxicity of these four metals;
- Produce a decision support tool to contribute to the sustainable management of industrial effluents.

STUDY ENVIRONMENT, MATERIAL AND METHODS

The Matete River in Kinshasa has its source in the municipality of Kisenso. It is one of the main major tributaries of the N'djili River, it crosses the following communes: Matete, Lemba and Limete Residential. It runs 10600 meters (length of the primary network of the river) on a very densely populated watershed of about 1276 hectares (area) before flowing into the N'djili River (Outlet) with a total flow of $110m^3$. is counted among the main rivers of the city of Kinshasa (Lubudi, Funa, N'djili, Yolo ...) and therefore it is an integral part of the hydrographic network of the city of Kinshasa.

It should be pointed out that its watershed is currently confronted with often delicate situations, in particular a strong anthropic pressure characterized by anarchic constructions thus causing repetitive erosions and / or land, but also an unchecked rampant population.

The climatic situation of the Matete River is almost identical with that of the city of Kinshasa. The city province of Kinshasa is located in the climate of low altitude. It is situated at an average altitude of 628m, and lies between 4º 19 'and 4º 25' S latitude and between 15º 18 'and 15º 22' east longitude, and covers an area of 9,965 km^2 (7). It is characterized by a humid tropical climate. The climate of Kinshasa is according to KÔPPEN, of the AW_4 type, that is to say a humid tropical climate with 4 months of dry season. It starts from mid-May to mid-September and the rest of the months is the rainy season. The average annual rainfall is about 1400 mm. The average annual temperature is 24 ° C. The absolute monthly absolute maxima of temperature exceed 35 ° C.
As for the dry season, it is characterized by the near absence of rains and it extends from mid-May to mid-September. The lowest temperatures of the year are observed in the dry season in the month of July in the range of 17.1°C to 17.5°C. March is the hottest month of the year.

Throughout the day, most of the year, the relative humidity is above 70%. Its annual average calculated over 24 hours is 81%: it oscillates from 76% during the day to 86% during the night.

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EXPERIMENTAL APPROACH

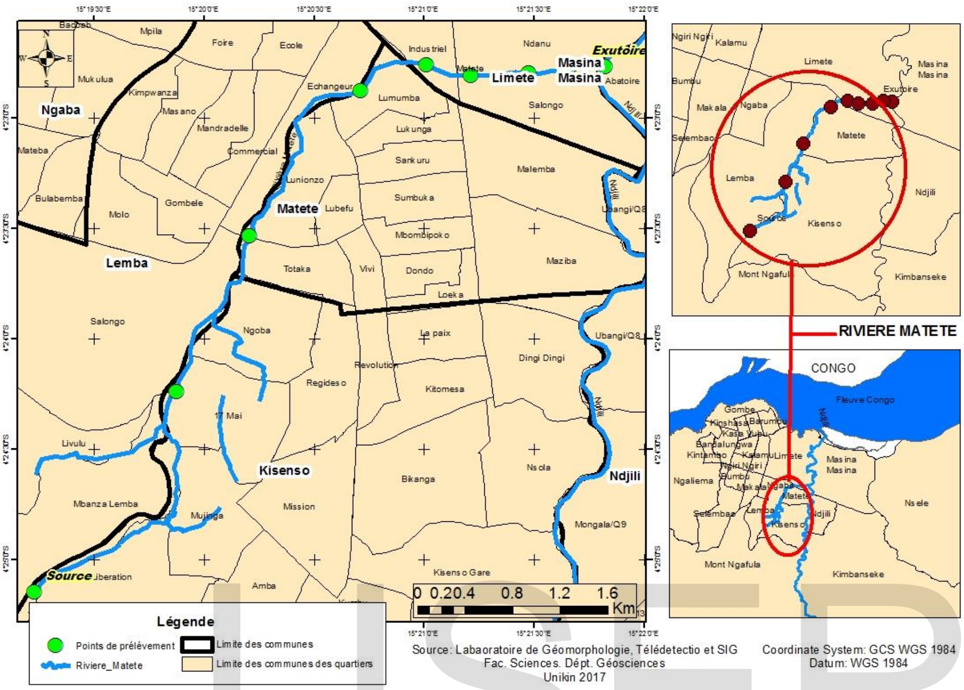
Water samples from the MATETE River were collected during the period from 01 August 2016 to 30 May 2017 (dry and rainy session). After each sampling, the water samples were placed in 1.5-liter plastic bottles previously washed, rinsed with tap water and then with distilled water and the water of the river to be analyzed. These were labeled and put in a cooler at a temperature of about $4^{O}C$ and transported to the laboratory.

II.2.1.1. PHYSICO-CHEMICAL PARAMETERS
II.2.1.1.1. Sampling and labeling

Sample samples consisted of Matete River water and algae living there.
Samples were collected every morning from two seasons (rainy and dry season) at different locations on the Matete River (from source to outlet) (see Map 1).
The 1.5-liter plastic bottles, cleaned and thoroughly rinsed with tap water and then sampled water (at least 3 times with river water from different locations) were used as flasks.

In situ, pH and temperature were measured at each sampling. Other physicochemical parameters, including the identification and content of metal trace elements, were analyzed at the CGEA / CREN-K central analysis laboratory (LCA).

SAMPLE SAMPLE SITES



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II.2.1.1.1.1. IN SITU ANALYSIS
II.2.1.1.1.1.1. pH

The Hydrogen potential measures the H + ion concentration of the water. It thus reflects the balance between acid and base on a scale of 0 to 14.7 being the pH of neutrality.
The multi-parameter brand "OAKTON, 35 Series" was used for this measurement. Once it is energized, rinse the electrode with distilled water and wipe it with a disposable tissue, then immerse the electrode in the solution to a minimum depth of two centimeters, wait until the value is stabilized before reading and finally, after reading, rinse the electrode again with distilled water and wipe it with a clean disposable tissue to make the next one.

II.2.1.1.1.1.2. Temperature

The temperature of the water is a parameter of comfort for the users. It corrects the analysis parameters whose values are related to the temperature (in particular the conductivity, dissolved oxygen, pH). In addition, by highlighting water temperature contrasts on a medium, it is possible to obtain indications on the origin and flow of water. The temperature must be measured in situ.

In this regard, we used the multi-parameter brand "OAKTON, 35 series" whose technique was to press the ON / OFF button of the multi-parameter device brand "OAKTON, 35 series" to put this last under tension, then, rinse the electrode with distilled water and wipe it with a disposable tissue, then put a little water in a 25ml beaker; immerse the instrument in water; until the measurement stabilizes; play while the unit is still in the water.

II.2.1.1.1.2. EX SITU ANALYZES

II.2.1.1.1.2.1. Measurement of physico-chemical parameters by X-ray fluorescence

Physico-chemical X-ray fluorescence analyzes were performed at the central CGEA / CREN-K laboratory to determine the concentration of aluminum, cadmium, mercury and lead in the different water samples. However, the X-spectrometer used has the advantage of giving the results of all other chemical elements whose atomic number Z ranges from 13 to 19 (see results in the appendix).

II.2.1.1.1.2.1.1. Principles of X-ray Fluorescence

The analyzes of the different chemical parameters are made using the X-ray fluorescence spectrometer, energy dispersive version (ED-XRF), XEPOS III, a multi-elementary method.
The samples were thus measured by an X-ray fluorescence spectrometer, using the four secondary targets, namely successively Molybdenum (39.76KV of voltage and 0.88mA of current), Aluminum oxide (49.15KV of voltage and 0.7mA current), Cobalt (35.79KV current) and finally HOPG Crystal Bragg (17.4KV voltage and 1.99mA current) of the anode palladium.

II.2.1.1.1.2.1.2. Operating mode

The sample is taken and placed to be analyzed under an X-ray beam. Under the effect of X-rays, the sample "resonates" and re-emits X-rays of its own; fluorescence. If we look at the energy spectrum of fluorescent X-rays, we see peaks characteristic of the elements present, we know what element we have, and the height of the peaks can determine in what quantity and concentration of the sample. From the results obtained from all these analyzes, we realized that these samples had to be subjected to ecotoxicological analyzes, in particular the toxicity tests.

II.2.1.2. Toxicity tests

These are short-term tests, the effects of which should be within a short time (from a few hours to a few days depending on the animal's life cycle) after administration of a single dose of the substance. We tested the toxicity of samples prepared in the laboratory.

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It was a question of testing their toxicity at different doses by the method of successive dilutions of the static type, that is to say without renewal of the toxic solutions.

These tests make it possible to establish a relationship between the concentration of exposure and the intensity of the effect. To do this, Gambusia affinis was taken as bioindicator, the biological material par excellence. Five times 3 Gambusia affinis were placed in each solution (concentration), ie 15 fish per concentration; which allowed us to observe them for 4 days according to the acute toxicity test. The technique consisted in observing the lethality in Gambusia affinis in the different solutions. Those who died were quickly removed or removed from the solution.

The results obtained made it possible to draw the median survival curves or LC_{50}.

The selection of young females of Gambusia affinis ranging in size from 2.5 to 3 cm (2; 3; 4 and 5) as biological indicators follows a series of criteria including:

- simplicity of handling and measurements;
- very high specific sensitivities to certain pollutants (Sensitivity to a very wide range of pollutants);
- reproducibility of the results;
- ease of obtaining and preserving biological organisms to be tested;
- representativeness of biological organisms and parameters to be tested.

Fished fish were acclimatized for two days (or 48 hours) under laboratory conditions in two plastic basins of 30 liters of dechlorinated tap water (dechlorinated tap water, that is, water collected from the tap and kept open for a day or twenty-four hours under open-air laboratory conditions).

The salts of the four metals chosen as chemical reagents (Cadmium Chloride $CdCl_2$, Lead Acetate (CH_3COO) 4Pb, Mercury Sulfate $HgSO_4$, and $AlCl_3$ Aluminum Trichloride) were weighed using a branded precision scale. KERN.
Starting from four different salts weighed separately, we prepared in turn the different solutions in four graduated glass feet of 2000ml capacity, the technique was to pour 500 ml of distilled water in a graduated foot and mix with 0.1 gram of salt stir until dissolution of the crystals (a homogeneous solution), then add distilled water until a solution of 1000ml (1 liter) of stock solution is obtained, that is to say the concentration 100; and keep in a 1.5 liter plastic bottle, then, using a 60ml syringe, we removed 100ml of the 10^0 concentration, then add 900ml of dechlorinated tap water to get the concentration of 10^{-1}; continue until you reach the 10^{-5} concentration.

The preparation technique of the different samples consists in preparing the different concentrations of the six-test solutions, the concentration of A1, B1, C1, D1, E1 and F1, that is to say that each 550ml plastic cup contained a given concentration. First 100ml of the concentration of 10^0 of the same solution, 100ml of the concentration of 10^{-1}, 100ml of the concentration of 10^{-2}, 100ml of the concentration of 10^{-3}, 100ml of the concentration of 10^{-4}, 100ml of the concentration of 10^{-5} and finally, 100ml sample solution of dechlorinated tap water in the laboratory and this is repeated five times.

For lead for example, six different concentrations and the control which makes a total of seven solutions. We did five repetitions: 7x5 = 35 cups use for each prepared solution and 140 550ml plastic cups used as experimental cells for the acute toxicity tests for the four 7cup metal solutions.

II.2.1.3. Combined toxicity tests

To perform our combined toxicity tests, we performed the first series of Acute Toxicity Tests for Mercury, Lead, Cadmium and Aluminum Salts separately, then we determined the LC_{50} of these four salts tested and finally from each LC_{50} found, we prepared a solution with the concentration corresponding to ½ of the LC_{50} value of each tested salt obtained and the mixture of these four ½ LC_{50} is the combined solution. The principle of this test is as follows: - if the mortality in the stock solutions of ½ of LC_{50} is 100%, the four salts tested are synergistic; - if it is high, the effects are additive; if it is zero, the salts are antagonistic.

II.2.1.4. Statistical approach

Statistical tests (ANOVA) were used to compare the toxicity of the different concentrations of Lead, Mercury, Cadmium and Aluminum solutions against test individuals (*Gambusia affinis*). The ANOVA test also allows you to know, in a multivariate study, which has the greatest impact on the final means (lethal concentration).

The average values obtained for different samplings were compared 2 to 2 by means of comparison tests of means (Student's t-test).

III. RESULTS AND DISCUSSION

This chapter has two parts, the first of which presents the results obtained from the various analyzes and the second discusses the results obtained.

III.1. RESULTS OBTAINED

III.1.1. RESULTS OF THE EXPERIMENTAL APPROACH

Globally, the different physicochemical parameters vary very clearly according to the seasonal periods (Before and after the rain).

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Table 3: Characterization of Matete River Water Samples

withdrawals / Parameters	Units	Standards	1	2	3	4	5	6	7	8	9
pH		6	7,4 7,4	7,5 7,5	7,4 7,5	6,6 6,8	6,6 6,7	7,5 7,8	7,5 7,7	7,2 7,8	7,1 7,5
Temperature	°C	25	25,9 25,8	26,6 26,6	27,1 27,2	26,3 26,5	26,5 27,5	27,5 26,7	27,1 27,1	27,2 27,5	27,1 27,5
Mercury	mg/l	0,0000	<1,0	<1,0	<1,0	<1,0	<0,3	<1,0	0,00013	0,00021	<1,0
Aluminum	mg/l	10,0067	<20	<20	0,0020	0,0020	39,3	474	1957	1474	2865
Cadmium	mg/l	5,9625	0,00020	<2,0	<2,0	<2,0	1,9	2,4	0,2	0,00062	1,8
Lead	mg/l	1,2939	<2,0	<0,4	<0,4	<0,1	0,8	0,6	0,00008	0,00059	1,2

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The results obtained on the characterization of the different samples of the Matete River go against the international standard because each metallic trace element does not meet the standards for surface water.

- Mercury values vary between 0.00013 and <1.0 mg / l. This situation would be due to the consideration of the river (from the source to the outlet) as a public trash can;
- Aluminum results vary around 0.0020 and 2865 mg / l. These values are believed to be due to the Matete River's (sandy) soil texture and some of the runoff sewage and leachate inputs from the wild dumps;
- Cadmium values are in the range of 0.00020 and 2.4 mg / l. These values are below the international standard of 5.9625 mg / l;

- Lead results vary between 0.00008 and <2.0 mg / l. These values would be due to the consideration of the Matete River as a landfill. Waste dumps that receive used batteries produce a metal-rich leachate of which the Matete River is the terminus.

Heavy rains can also affect aluminum levels in the aquatic environment through the leaching of large quantities of these metals from the watershed soils, the leaching of wild dumps that spill the banks of the river, and In the short term, trace metal concentrations caused by these events can have very significant effects in the aquatic environment.

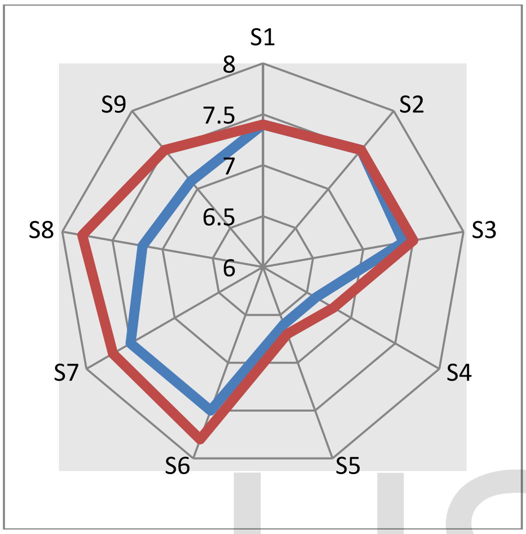

Figure 2, pH change of the Matete River before and after the rain

Based on the results obtained from the different samples of the Matete River, we find that the pH values are around 6.6 and 7.5 before the rain and 6.7 and 7.8 after the rain. They are almost neutral and are in the range recommended by (13). In this condition, aquatic life is in its ecological pre-referendum vis-à-vis the pH of their ecological niche.

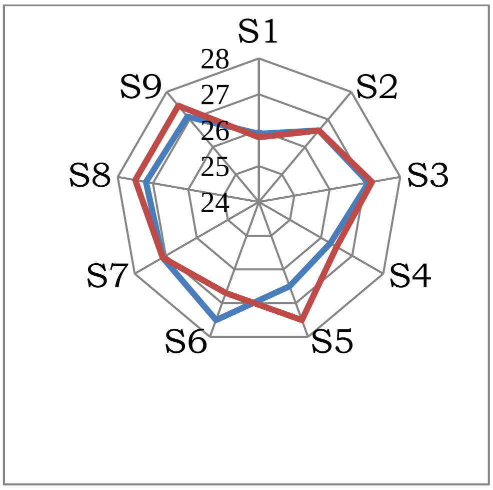

Figure 3, Temperature variation of the Matete River before and after the rain

The temperature values of our different samples vary between 25.9 and 27.5 before rain and 25.8 and 27.5 after rain. These values are above the norm of 25oC and reflect the influence of climatic conditions of R & D. Congo, one of the tropical countries in the aquatic environment of the Matete River.

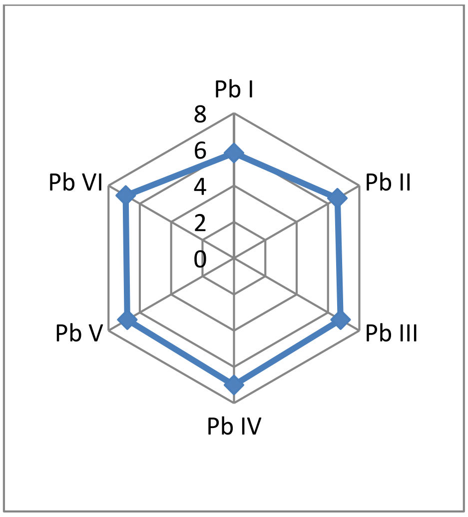

Figure 4, pH variation of the lead solutions of the different concentrations of prepared in the laboratory

The pH values of different lead solutions prepared in the laboratory are around 5.8 and 7. These values are close to the pH of the Matete River.

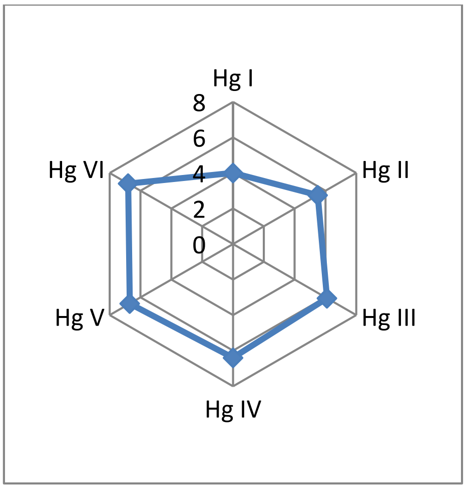

Figure 5, pH variation of Mercury solutions of different concentrations prepared in the laboratory

The pH values of the various solutions of Mercury prepared are seen to be between 4.0 and 6.8. These solutions are prepared under conditions close to those of the Matete River.

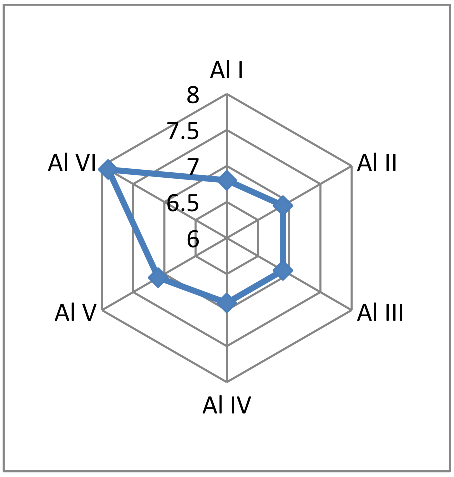

Figure 6, pH variation of aluminum solutions of different concentrations prepared in the laboratory

The pH results of different solutions of Aluminum prepared under the conditions convey the reality of the aquatic environment of the Matete River is a pH that we see that they are around 6.8 and 7.9.

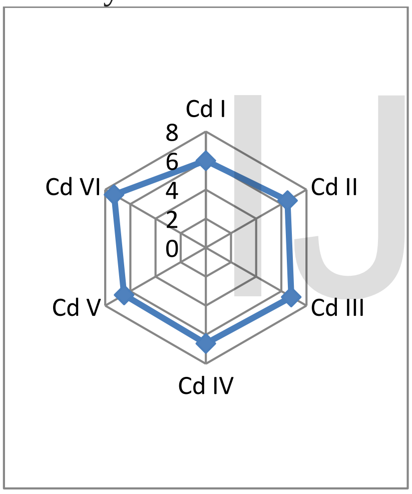

Figure 7, pH variation of cadmium solutions of different concentrations prepared in the laboratory

Based on the results obtained, the pH values of all the solutions prepared in the laboratory are between 6.0 and 7.3. These values reflect the conditions of the Matete River.

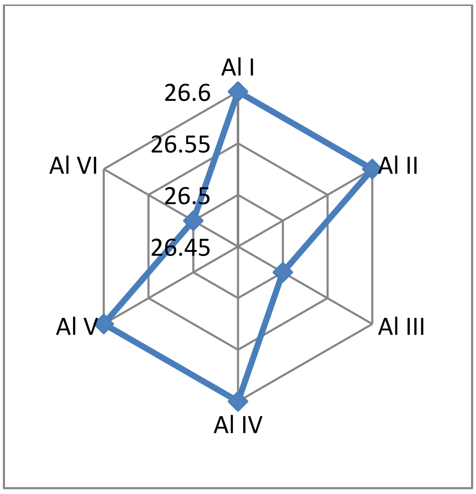

Figure 8, Temperature Variation of Aluminum Solutions of Different Concentrations Prepared in the Laboratory

The temperature of our different solutions of Aluminum we see that they are between 26,5 and 26,6°C. These values would be due to the influence of the heat of the preparation medium.

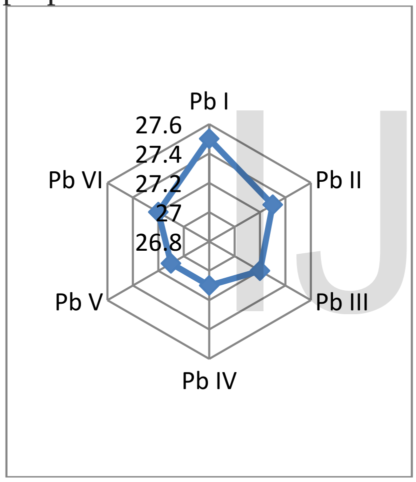

Figure 9, Temperature variation of the lead solutions of the different concentrations prepared in the laboratory

The temperature values of our lead solutions prepared at the laboratory are between 27.1 and 27.5°C. These values would logically be due to the influence of laboratory heat.

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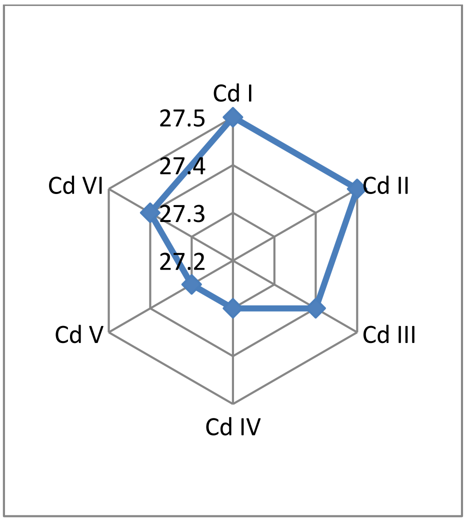

Figure 10, Temperature Variation of Cadmium Solutions of Different Concentrations Prepared in the Laboratory

In view of the results obtained, the temperature values of all the solutions of Cadmium prepared are between 27.3 and 27.5°C. This variation is due to the influence of the environment.

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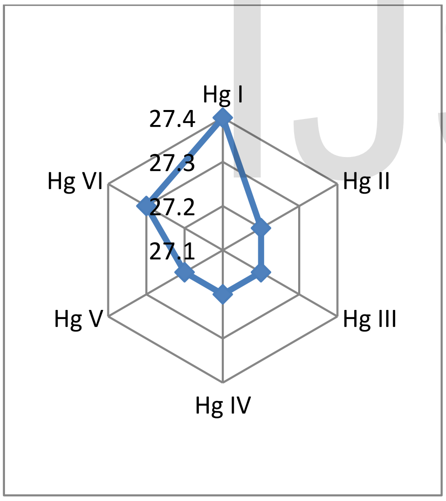

Figure 11, Temperature variation of Mercury solutions of different concentrations prepared in the laboratory

The temperature values of our various prepared Mercury solutions are between 27.2 and 27.4°C. These values would be due to the influence of ambient laboratory heat.

III.1.2. BIOTEST

In this part of this chapter, we present the results of the acute toxicity tests of solutions prepared in the ecotoxicology laboratory of the Department of Environmental Sciences of the Faculty of Science.

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Table 4: Number and percentage of *Gambusia affinis* survivors in lead solution

Concentrations	Number of deaths				Number of live	% of Survivors
	1st Day	2nd Day	3rd Day	4th Day		
10^0	3	-	-	-	0	0
10^{-1}	2	1	-	-	0	0
10^{-2}	1	0	1	0	1	33,33
10^{-3}	0	0	1	1	1	33,33
10^{-4}	0	0	1	0	2	66,66
10^{-5}	0	0	0		3	100
T	0	0	0	0	3	100

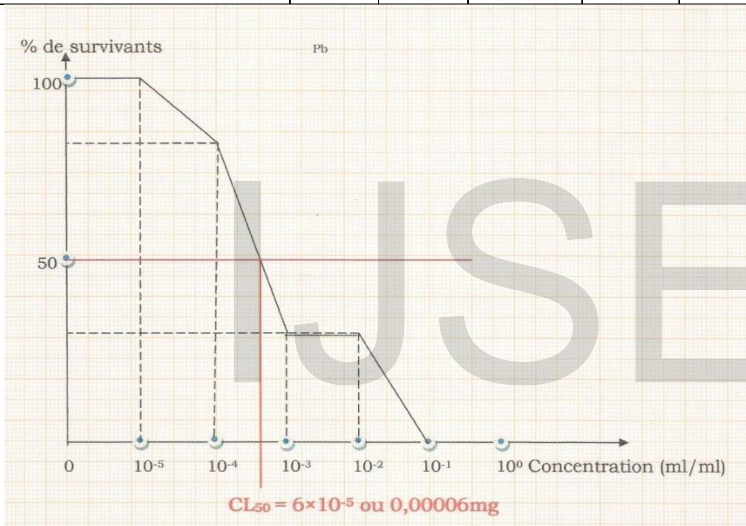

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Figure 1: Curve of *Gambusia affinis* Survivors in lead solutions

Table 4 and Chart 1 show that lead solution is very toxic to *Gambusia affinis*. Indeed, in the concentrations of 10^0 and 10^{-1}, no survivor was observed, ie 0% of survivors; monitoring concentrations of 10^{-2} and 10^{-3}, 33.3% of survivors were present in each concentration; in the 10^{-4} concentration, we observed 66.7% survivors and 100% survivors in the 10^{-5} concentration and in the control. The LC_{50} of the lead solution was around 6×10^{-5} or 0.00006 mg / ml, indicating its toxicity to *Gambusia affinis* populations.

VARIANCE ANALYSIS						
Source of variations	Sum of squares	Degree of liberty	Average squares	*F*	Probability	Critical value for F

Between Groups	9,51879699	6	1,58646617	3,90740741	0,00129676	2,171308818
Inside groups	51,1578947	126	0,40601504			
Total	60,6766917	132				

This table indicates that the F compute is greater than F tabular that is to say that there is a significant difference, in this respect, we reject the null hypothesis and confirm the alternative hypothesis. We can conclude that the lead solution is toxic to *Gambusia affinis.*

Table 5: Number and percentage of Gambusia affinis survivors in Cadmium solutions

Concentrations	Number of deaths				Number of live	% of Survivors
	1st Day	2nd Day	3rd Day	4th Day		
10^{0}	3	-	-	-	0	0
10^{-1}	2	1	-	-	0	0
10^{-2}	2	0	0	0	1	33,33
10^{-3}	1	0	0	0	2	66,66
10^{-4}	0	0	1	0	2	66,66
10^{-5}	0	0	0		3	100
T	0	0	0	0	3	100

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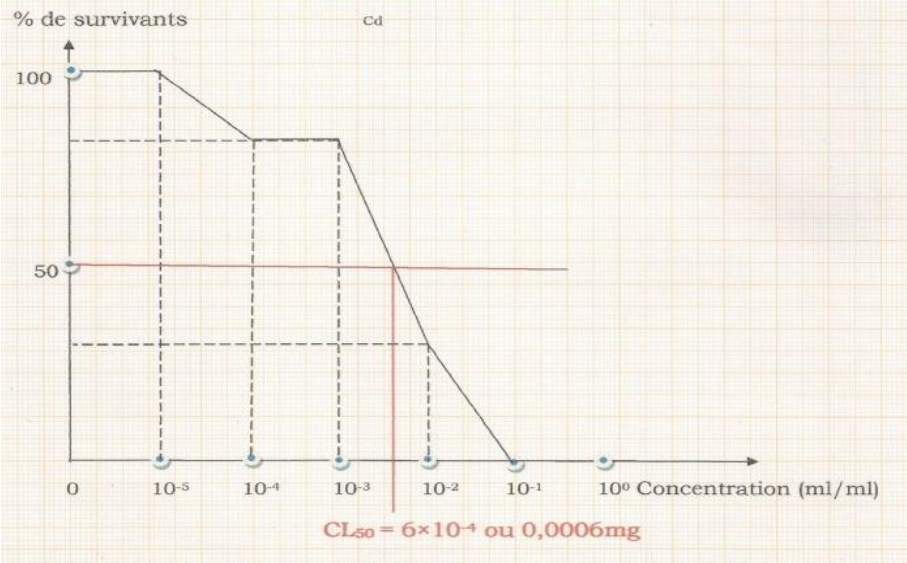

Figure 2: Curve of *Gambusia affinis* Survivors in Cadmium solutions

Table 5 and Chart 2 show that Cadmium solution is very toxic to *Gambusia affinis*. Indeed, in the mother (or 10^0) and 10^{-1} concentration, no survivor was observed in each of these concentrations, ie 0% of survivors; monitoring the concentration of 10^{-2}, with 33.3% of survivors. In the 10^{-3} and 10^{-4} concentrations, we observed 66.7% for each of the concentrations and 100% survivors in the 10^{-5} and control concentrations. The LC_{50} of the Cadmium solution was around the 6×10^{-4} or 0.0006mg / ml concentration, indicating its toxicity to *Gambusia affinis* populations.

VARIANCE ANALYSIS

Source of variations	Sum of squares	Degree of liberty	Average squares	*F*	Probability	Critical value for F
Between Groups	8,5112782	6	1,41854637	3,12132353	0,006935803	2,171308818
Inside groups	57,2631579	126	0,45446951			
Total	65,7744361	132				

This table indicates that the F compute is greater than F tabular that is to say that there is a significant difference, in this respect, we reject the null hypothesis and confirm the alternative hypothesis. We can conclude that the Cadmium solution is toxic to Gambusia affinis.

Table 6: Number and percentage of Gambusia affinis survivors in the Aluminum solution

Concentrations	Number of deaths				Number of live	% of Survivors
	1st Day	2nd Day	3rd Day	4th Day		
10^0	2	0	0	0	1	33,33
10^{-1}	1	1	0	0	1	33,33
10^{-2}	1	0	0	0	2	66,66
10^{-3}	0	0	0	1	2	66,66
10^{-4}	0	0	0	0	3	100
10^{-5}	0	0	0	0	3	100
T	0	0	0	0	3	100

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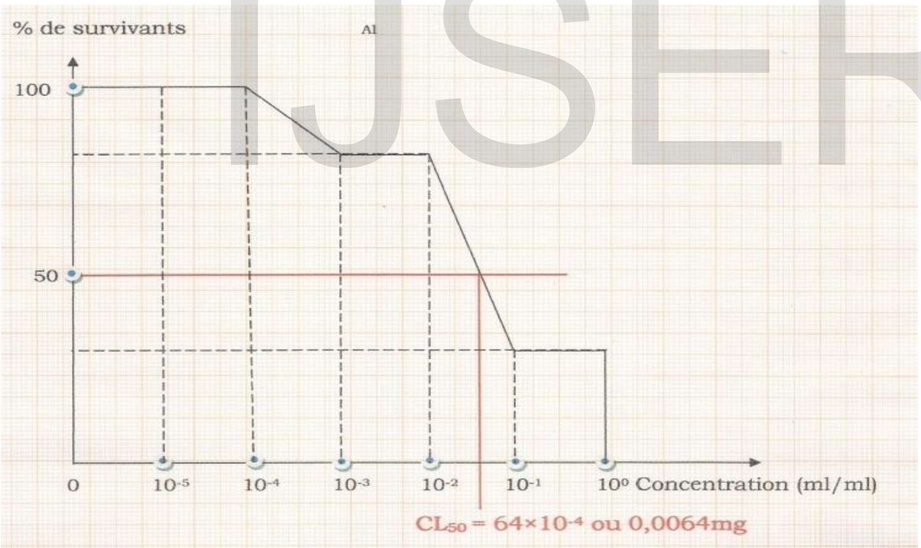

Figure 3: Survivors Curve of Gambusia affinis in Aluminum Solutions

Table 6 and Chart 3 show that the aluminum solution is very toxic to *Gambusia affinis*. Indeed, in its maternal concentration and 10^{-1}, 33.33% survived, followed by 10^{-2} and 10^{-3} concentrations, or we observed 66.7% for each of the concentrations and finally, 100% of survivors were observed in the concentrations of 10^{-4}.10^{-5} and in the control solution. The LC_{50} of the aluminum solution was around 64×10^{-4} or 0.0064 mg / ml, indicating its toxicity to *Gambusia affinis* populations.

VARIANCE ANALYSIS

Source of variations	Sum of squares	Degree of liberty	Average squares	*F*	Probability	Critical value for F
Between Groups	5,83458647	6	0,97243108	4,93220339	0,000145204	2,171308818
Inside groups	24,8421053	126	0,19715957			
Total	30,6766917	132				

This table indicates that the F compute is greater than F tabular that is to say that there is a significant difference, in this respect, we reject the null hypothesis and confirm the alternative hypothesis. We can conclude that the aluminum solution is toxic to *Gambusia affinis*.

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Table 7: Number and percentage of *Gambusia affinis* survivors in the Mercury solution

Concentrations	Number of deaths				Number of live	% of Survivors
	1st Day	2nd Day	3rd Day	4th Day		
10^{0}	3	-	-	-	0	0
10^{-1}	3	-	-	-	0	0
10^{-2}	3	-	-	-	0	0
10^{-3}	2	0	0	0	1	33,33
10^{-4}	0	0	1	0	2	66,66
10^{-5}	0	0	0	0	3	100
T	0	0	0	0	3	100

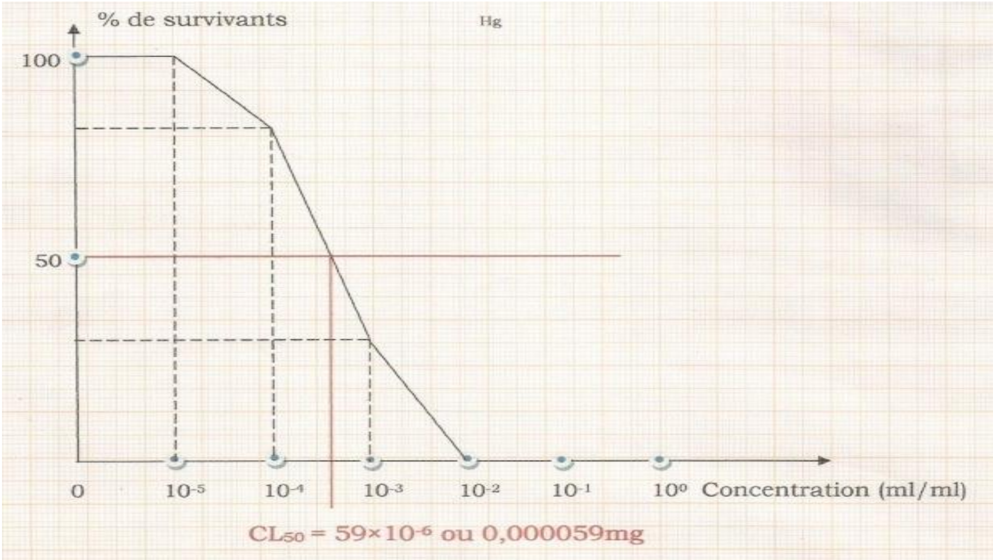

Figure 4: Curve of *Gambusia affinis* Survivors in Mercury Solutions

Table 7 and Chart 4 show that the mercury solution is very toxic to *Gambusia affinis*. Indeed, in the crude concentration (mother solution) or 10^0,10^{-1} and 10^{-2}, no survivor was observed in each of the concentrations, ie 0% of survivors, followed by the concentration of 10^{-3}. had 33.3% of survivors, in the 10^{-4} concentration, we observed 66.7% and 100% of survivors in the 10^{-5} concentration and in the control. The LC_{50} of the mercury solution was around 59×10^{-6} or 0.000059mg / ml, indicating its toxicity to *Gambusia affinis* populations.

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VARIANCE ANALYSIS

Source of variations	Sum of squares	Degree of liberty	Average squares	*F*	Probability	Critical value for F
Between Groups	8,466165 41	6	1,41102757	3,781645 57	0,10798977 1	2,1713088 18
Inside groups	99,78947 37	126	0,79197995			
Total	108,2556 39	132				

This table indicates that the F compute is greater than F tabular that is to say that there is a significant difference, in this respect, we reject the null hypothesis and confirm the alternative hypothesis. We can conclude that the Mercury solution is toxic to Gambusia affinis.

III.1.3. COMBINED TOXICITY

The technique consisted in preparing a combined concentration solution from half the LC_{50} of each of the solutions, ie we took half the lethal concentration of Lead (3×10^{-5} or 0.00003mg), Mercury (295×10^{-7} or 0.0000295mg), Aluminum (32×10^{-4} or 0.0032mg) and Cadmium (3×10^{-4} or 0.0003mg). In a cup (experimental pond), we obtained a combined concentration of four salts.

Three *Gambusia affinis* were put in the resulting solution, which allowed us to observe them for 4 days according to the acute toxicity test.

At the end of acute biological test, we found three cases of lethality of which one on the second day, one on the third day, and another on the third day with 0% of survivors, justifying the combined toxicity of Lead, Mercury, Aluminum and Cadmium for populations of *Gambusia affinis*. This indicates a synergy between the four salts in solution.

III.2. DISCUSSION

Under the conditions of the Matete River, this aquatic ecosystem has pH values between 6.7 and 7.8 after rainfall and 6.6 and 7.5 before rain. These values are higher than the norm of (13) fixed at 6 and this variation would be due to the way in which residents and industrialists consider the river as a landfill or a public garbage can. The observed pH variations influence the behavior of different metals (bioavailable or the ionic form which is the very toxic form for living organisms).

The temperature results are respectively around 25.9 and 27.5°C before the rain and 25.8 at 27.5°C after the rain. This is not surprising because the Matete River is in a tropical area. The solubility of aluminum and certain metallic trace elements is increased in the presence of complexing ligands in acidic or alkaline medium (pH <6 or> 8). Ambient temperature also influences the speciation of aluminum and, consequently, its solubility in the environment. Species should remain in their most toxic form at a higher pH if the temperature is low (2°C) compared to the higher temperature of 20°C (11). Therefore, at 2oC and a pH value <5.7, aluminum is mostly in the form of Al^{+3}. The dissolution of minerals generally increases with temperature but not the solubility of aluminum, which decreases. Climate change could explain up to 10% of the decline in aluminum concentrations in Czech lakes (14).

Under laboratory conditions, most of the metal is in dissolved or ionic form (the free form). Currently, it is considered that all of the metal introduced into the test medium is bioavailable.

After our various analyzes, the results of the biological acute toxicity tests of solutions of Aluminum Trichloride ($AlCl_3$), Lead Acetate ($(CH_3COO)_4Pb$), Cadmium Chloride ($CdCl_2$) and Mercury Sulfate ($HgSO_4$)
prepared in the laboratory revealed high toxicity in different concentrations to exposed *Gambusia affinis* populations.

Metal pollution is a particular problem because metals are not biodegradable. In addition, throughout the food chain, some are concentrated in living organisms. They can thus reach very high levels in the species tested.
Beyond a certain concentration (sometimes very low) in the medium, metals have harmful effects. The assessment of these effects must take into account the peculiarities associated with metals.

The lethal (or effective) concentration that kills at least 50% of the test individuals (LC_{50} or EC_{50}), such as the populations of *Gambusia affinis*, makes it possible to declare and classify the solutions in the order of toxicity. The latter is expressed in Charts 1, 2, 3 and 4.
Chart 4 shows the toxicity of the Mercury solution in its concentrations of $10^0.10^{-1}$ and 10^{-2} which are very toxic with a survival curve of the lethal concentration 50 around the concentration 59×10^{-6} or 0,000059mg / ml.
Chart 1 states that the 100 and 10-1 concentrations of the lead solution show high toxicity to the mosquito population with a lethal concentration around the 6×10^{-5} or 0.00006 mg / ml concentration that reveals its toxicity. vis-à-vis the receiving biophysical environment.
Graph 2 explains that cadmium solution is very toxic to the Gambusia affinis population in concentrations of 10^0 and 10^{-1} with a lethal concentration around 6×10^{-4} or 0.0006 mg / ml, justifying its toxicity to the health of the ecosystem.
Graph 3 shows that the concentrations 10^0 and 10^{-1} of the Aluminum solution are toxic for the indicator species with an effective concentration around the 64×10^{-4} concentration or 0.0064 mg / ml indicating its toxicity. These results confirm those of (9) who exposed rainbow trout (Oncorhynchus mykiss) to acute and subacute toxic combinations of Al, dissolved organic carbon (humic acids) and pH. The fish were exposed in a system with continuous renewal. For the acute toxicity test, mortality (25%) was noted at pH 8.03, humic acid concentration 6.17 mg • L^{-1}, and total aluminum concentration of 2 , 1 mg • L^{-1}. The 96-h LC50 were 3.75, 5.43, 4.60 and 5.22 mg • L^{-1} for respective concentrations of 1.4, 2.6, 6.6 and 10 of the humic acids, 1 mg • L^{-1}. Acute biological test results for the combined toxicity of Aluminum Trichloride ($AlCl_3$), Lead Acetate ($(CH_3COO)_4Pb$), Cadmium Chloride ($CdCl_2$) and Mercury Sulfate ($HgSO_4$) solutions reveal that the toxicity for the population of the test animals, which is comparable to the 10^0 and 10^{-1} concentrations of the lead solution, the 10^0 and 10^{-1} concentration of the cadmium solution, at the concentrations 10^0 and 10^{-1} of the solution of Aluminum and at concentrations 10^0, 10^{-1} and 10^{-2} of the Mercury solution. These results are due to the synergistic interactions of these four substances. On the other hand (12) demonstrated that the metals Copper and Manganese act in antagonism in an aquatic ecosystem while Aluminum and Copper are synergistic and as (1), which also found the existence of moderate interactions between these metals which, in the majority of cases, are synergistic with the test organism (Daphnia magna) and recommend that it is now necessary to continue this study, working on other metals commonly detected in industrial effluents (Pb, ...), by testing mixtures containing more than two pollutants, but also by working on pollutants present in particulate form. The effects on other aquatic organisms (algae, micro-organisms, molluscs, fish, etc.) and chronic effects are also to be studied, with a

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view to thoroughly evaluating the impact of industrial and urban effluents on the different organisms that live in the receiving environments concerned.

The fish-fishing scenario along the Matete River would better explain the manifestations of the combined effect of metals in this aquatic ecosystem and also the exposure of the health of the consuming population. Aquatic ecosystems are vulnerable to metals because some non-essential metals can accumulate in sediments, be released into pore water and increase the concentration of soluble or ionic metals, with real danger to aquatic life. as well as for the man. In view of the consumption of these contaminated fish, these levels present risks to the health of the populations (10).

CONCLUSION AND SUGGESTIONS

The purpose of this work was to evaluate the combined toxicity of Lead, Mercury, Cadmium and Aluminum for the Gambusia affinis population. At the end of this work entitled: "Contribution to the study of the combined toxicity of Lead, Mercury, Cadmium and Aluminum on the Matete River ecosystem in Kinshasa".
The Matete River is treated today as a natural trash can and also as a public dump of waste and very toxic effluents. These aquatic environments are mixing points of four pollutants and their effects are very toxic on populations of Gambusia affinis, because of their synergistic interactions.

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The biological tests carried out at the Ecotoxicology, Ecosystem Health and Environmental Biotechnology Laboratory of the Department of Environmental Sciences of the University of Kinshasa, reveal that mercury solutions [Mercury Sulfate ($HgSO_4$)] were more toxic than those of Lead (Lead Acetate [$(CH_3COO)_4Pb$)], Cadmium [Cadmium Chloride ($CdCl_2$)] and Aluminum [Aluminum Trichloride ($AlCl_3$)].
In this regard, we can conclude, by asserting the hypothesis that these effluents are loaded into chemical elements that combine with each other and produce a synergistic and highly toxic effect for individuals of Gambusia affinis in particular and the aquatic ecosystem of the Matete River, in general. This would justify the dead fish that are collected after the washing of industrial machinery.
It is therefore to be feared that this pollution will increase if appropriate measures are not taken and applied rigorously in the field.
In the field of environmental protection, the precautionary and preventive principle is essential in order to reduce the ecotoxicological risks associated with pollutants discharged into the receiving ecosystems. In this regard, we suggest to the authorities the following measures:

- control effluents discharged by the chemical industries;
- to recycle industrial effluents before their discharges into the receiving environment;
- use the bio-monitoring system for all effluents;
- apply the polluter pays charges;

- continue research on combined toxicity in the wider natural environment by applying binary solutions.

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