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Evaluation of Acute Chromium (III) Toxicity in Relation to *Daphnia similis*

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Abstract

Acute chromium (III) toxicity in relation to *Daphnia similis*, most appropriate for Brazilian environment has been evaluated. The preliminary toxicity test showed that the median effective concentration was 10 mg/l. After having performed the final test, the immobility percentage was calculated for each concentration in relation to the total number of the used organisms within the chosen pH range. It was shown that the proposed methodology rendered realistic results and the 48-h CE50 value under the test conditions is 3.24 mg/l. The minimum concentration that did not show any toxicity to *Daphnia similis* was 2.5 mg of chromium (III) per liter. The obtained level is superior to the maximum limit recommended by the Brazilian norms, 0.5 mg/l.

Keywords: Water Pollution, Chromium (III), *Daphnia similis*, Acute Toxicity Test

1. Introduction

In the last decades, the raising water pollution has led to the increase in research concerning the toxic and non-toxic effects of metal contamination. As a result, the wastewater of industrial origin can play a negative role as to the utilization of a receptor water body. The bioactive cations, especially present in aquatic environments, can lead to undesirable physical, chemical and biological alterations of the water. In particular, the aquatic beings of economic interest do not resist the presence of rejected materials, the effluent becoming a direct carrier of the elements harmful to human health. Moreover, the accumulation of certain elements in the food chain can reach dangerous trophic levels [1]. The presence of heavy metals in these industrial residues is an accomplished fact [2].

The metals reach the organisms through different ways. The transference of a chemical contaminant from one trophic level to another varies from 50% to 100%. Due to the high affinity of heavy metals to the sulfur-containing aminoacids, metal ions are captured by the organisms and remain enclosed in proteins, accumulating during the consumer's life. The higher the trophic level, the higher will be the amount of residues in the organisms under consideration. This fixation is a part of the whole bio-accumulation process [3].

Due to its extensive use in various industrial sectors chromium is considered an important element in world's economy. However, it may considerably contaminate soil and freshwater. The metal itself and its compounds are toxic to vertebrates and invertebrates. In general, the hexavalent form is known as the most aggressive to man, as, other than poisonous, it may be carcinogenic. The toxicity depends on its capacity of being easily transported through cellular membrane with the posterior reduction to chromium (III). The latter is capable of producing intracellular free radicals that damage DNA ribbons. On the contrary, when in the extracellular pool, the trivalent chromium is relatively non-toxic to man, as it does not pass into the cell [4]. The osteoblasts seem to be more sensitive to chromium (III) presence [5].

The tests for acute chemical toxicity with aquatic organisms constitute an effective tool for the detection, prediction and evaluation of the pollutants negative influence on living organisms. Their usage in toxicity tests was oficialized in 1985 [6].

In these tests representative aquatic organisms are exposed to varied concentrations of a certain compound during given periods. The acute toxicity is a quick adverse response, normally lethal, to such stimuli, which is ob-

served, as a rule, during the first 48 hs. The unity employed for the measurement is the median effective concentration (CE50), which, according to the Brazilian Association of Technical Norms [7], attests to the acute effect with the immobility of 50% of the exposed individuals.

As to the toxic agents present in liquid effluents, their evaluation can be carried out using two techniques: studying a separate effect of a specific hazardous substance and testing the effluent as a whole. In most cases, the former approach is more appropriate [8].

The *Daphnia* is a freshwater *Cladoceran* used worldwide as test organism in aquatic toxicity assays. As to the different *Daphnia* species, *Daphnia magna* is suitable for the environments with freshwater hardness between 175 and 225 mg/l of $CaCO_3$. Most tests for chromium (III) toxicity that have been performed in the USA and Europe employed this micro-crustaceous and $CrCl_3$ as chromium-containing standard [9,10]. Nevertheless, the natural properties of Southern hemisphere freshwater, and those of Mato Grosso do Sul, Brazil, in particular, are characterized by a much lower hardness: from 40 to 48 mg/l of $CaCO_3$. That means that some other *Daphnia* species, better accommodated to these conditions would be preferable in order to obtain more demonstrative and realistic data.

Given the predominant presence in the ambient of Mato Grosso do Sul, *D. similis* are the most appropriate for the present study. The algae are their main nourishment, and, on the other hand, *Daphnia* themselves represent food supply to the secondary consumers. It is to be noticed that the process of their cultivation *in vivo* involves little or no difficulties and they are especially sensitive to the presence of heavy metals. The purpose of this investigation is the evaluation of acute chromium (III) toxicity in relation to *Daphnia similis.*

2. Methodology

The method used to evaluate the toxicity was a standard technique established and published in the Brazilian Norm "Aquatic Ecotoxicology Acute Toxicity Experimental Test with *Daphnia* spp (*Cladocera*, *Crustacean*)" by the ABNT [7]. Below follow some important experimental details of the tests performed.

Test organisms:The *Daphnia similis*'s samples used to start the initial culture were obtained from the laboratory belonging in the Sector of Water Toxicology and Biological Assays of CETESB (Ambiental Sanitation Technology Inc.), São Paulo.

2.1. Stock Solution

The stock solution was prepared by dissolving chromium (III) chloride (Merck) in water. Its concentration in chromium (III), as in the previously quoted tests, was 1000 mg/l. The freshwater used for *Daphnia similis* cultivation and for diluting the stock solution was MS medium [7].

2.2. Freshwater for Cultivation and Dilution

In order to check its admissibility for application, the above freshwater was previously tested without chromium content. Twenty young organisms of *Daphnia similis* ageing between 6 and 24 hs were incubated for 48 hs in the dark at 20℃ without feeding. At the end of the period the number of mobile and immobile organisms was counted. According to ABNT [7], the freshwater is considered acceptable if the counting renders less than 10% of immobile organisms. In our experiment there were no immobile *Daphnia*s.

2.3. Cultivation and Maintenance of *Daphnia similis*

The culture was kept at 20°C, photo period being 16 hs of light with the intensity of 1000 lux and 8 hs of darkness. A culture vessel made of clear glass and provided with stopper was used for all operations. The culture was daily fed with suspension of algae containing of 5×10^6 cells/ml. Total water hardness and pH of the nutrient system were 40-48 mg/l of $CaCO_3$ and pH 7.0-7.6 respectfully. It is to be noted that the pH values used in this test were somewhat different than those recommended for ordinary *Cladocerans*, that is 5.0-7.0 [1]. Twenty- four hours before the start of the test, in the evening, all young organisms were withdrawn from the incubation containers with Pasteur pipette and used for testing.

2.4. Characteristics of the Preliminary Test

The initial solution of 100 mg/l was prepared diluting the stock solution with deionized water. The test solutions, had the following chromiun contents: 10 mg/l, 1.0 mg/l, 0.1 mg/l, 0.01 mg/l, 0.001 mg/l and 0.0001 mg/l.

The necessary condition for chromium (III) to penetrate into citosol is its presence in a dissociated form and not in a form of suspension or precipitate. Therefore it was important to adjust the pH of the solutions in order to maintain the element in its soluble basic form $Cr(OH)^{+2}$ of considerable availability and viable to the microorganisms [11]. So, the pH values were adjusted to the interval 6.0–6.5 in accordance to the range of existence of the monohydroxy ion.

Test-vessels contained about 20 ml of each solution, in duplicate. Five organisms were placed in each test-vessel

with the minimum of water possible. The incubation was carried out for 24-h at 20℃ in the dark, without feeding. The result of the preliminary toxicity test showed 24-h CE50 value of 10.0 mg of chromium (III) per liter.

2.5. Characteristics of the Final Test

The concentrations to be used in the final test were chosen according to the results of the preliminary test. The test-solutions were prepared with intermediary concentrations growing geometric progression with ratio no higher than 2. Experimental conditions are summarized in **Table 1**.

3. Results and Discussion

All forms of chromium are potentially toxic, but chromium (III) in the form of chromate is less dangerous for the environment than chromium (VI). Consequently, the total chromium content and its components are of great importance, since its excess may be detrimental to health, provoking a series of pathological conditions. In the Brazilian state of Mato Grosso do Sul the largest source of water pollution by chromium is the tanning process. As it was shown earlier, chromium (III) is the only species present in the effluents of the tanneries selected to study metal emissions.

The usage of *Daphnia* for the evaluation of its levels is worldwide known, but the choice of a concrete organism largely depends on the local water properties. So *Daphnia magnus*, employed in the USA and Europe is not the most appropriate organism for the trials performed in the Southern hemisphere and in Brazil, in particular, where water hardness is much lower. The present investigation deals with the usage of *Daphnia similis* because of its affinity to the waters with the hardness 40-48 mg/l of $CaCO_3$ and because of its predominant presence in the environment of Mato Grosso do Sul.

A study carried out so far on a large amount of aquatic species, showed that chromium (III) did not exhibit any toxicity to bacteria, marine algae or fish. An obvious reason to this fact should be the practical absence of chromium (III) ions in milieu with neutral and weak alkaline pH values, as chromium would be sequestered in the form of insoluble $Cr(OH)_3$. So it is rather difficult to explain that even in these conditions *Daphnia* species were sensible to chromium concentrations around 6-9 mg/l [12]. Most probably it is due to the unaccountable variation in pH with consequent hydroxide dissolution.

Therefore it is extremely important to point out that the pH values should be always maintained in the range 6.0-6.5. Actually, if we consider the solubility product of chromium (III) hydroxide $[Cr^{+3}][OH^-]^3 = 6.3 \times 10^{-31}$ [13], so at neutral pH, when $[OH^-] = 10^{-7}$ mol/L, free chromium (III) concentration would be 6.3×10^{-10} mol/L or less than 10^{-3} mg/l. Thus there would be practically no chromium dissolved to test against *Daphnia*. Nevertheless, yet at lower pH chromium will be available in the form of the aforementioned basic ions $[Cr(OH)_2]^+$ and consequently toxicity tests will become reliable.

The preliminary toxicity test showed that the median effective concentration was 10 mg/l. After having performed the final test, the immobility percentage was calculated for each concentration in relation to the total number of the used organisms within the chosen pH range. The corresponding counting of the immobile *Daphnia similis* is presented in **Table 2**.

It can be seen that chromium (III) at the concentration of 5 mg/l causes total mortality of the organisms. Then, at the concentration of 3.3 mg/l, it decreased to 65% and, finally the level of 2.5 mg/l proved to be non-toxic.

The application of the Trimmed-Spearman-Krabe statistical test [14] allowed establishing that the 48-h CE50 value for this exposition was 3.24 mg/l. For the confidence level $p < 0.05$ it corresponds to the interval

Table 1. Experimental conditions of the tests performed.

Conditions	Preliminary test	Final test
period, h	24	48
minimum volume of test-solution per organism, ml	2	2
number of dilutions	6	7
number of organisms per dilution	10	20
temperature, °C	19.9-20.3	19.9-20.3
pH	6.0-6.5	6.0-6.5
exposition time	24-h	48-h

Table 2. Counting of the immobile *Daphnia similis* in the final test.

Chromium concentration, mg/l	Initial pH	Number of immobile organisms per replicate				Accumulated number of organisms		Immobility %
		1st	2nd	3d	4th	Immobile	Total	
10.0	6.0	5	5	5	5	20	20	100
7.5	6.4	5	5	5	5	20	20	100
5.0	6.4	5	5	5	5	20	20	100
3.3	6.5	3	3	4	3	13	20	65
2.5	6.5	0	0	0	0	0	20	0
control	6.5	0	0	0	0	0	20	0

3.01-3.49 mg/l. The obtained level is superior to the maximum limit of 0.5 mg/l of chromium recommended by the CONAMA 357 Resolution [15].

Thus, the usage of *Daphnia similis* for the evaluation of chromium (III) toxicity has proven to be a simple and accessible test suitable to the conditions of Brazil. Its results are compatible with those obtained by employing a closely related organism, *Daphnia magna*. The acute toxicity obtained in this work was 3.24 mg/l.

4. Conclusions

It was shown that *Daphnia similis* is the most appropriate organism for the evaluation of chromium (III) toxicity. The proposed methodology rendered realistic results and the 48-h CE50 value under the test conditions was 3.24 mg/l. The minimum concentration that did not show any toxicity to *Daphnia similis* was 2.5 mg of chromium (III) per liter. The obtained level is superior to the maximum limit recommended by the Brazilian norms, 0.5 mg/l.

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