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Acute Toxicity of Natural and Synthetic Coagulants through Bioassays with *Artemia salina* in Treated Waters of Magdalena River in Colombia

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Abstract

Objective: This work aimed at assessing acute toxicity in *Artemia salina* of waters treated with natural coagulants (*Moringa Oleifera*) and with synthetic coagulants (*Aluminum Sulfate* Type B). **Methods/Analysis:** Acute toxicity tests were carried out on *Artemia salina* larvae to solutions with the highest coagulant activity by means of standard eco toxicology techniques and procedures. In addition, adjustments of sigmoid curve models were made to estimate mean lethal concentration (LC50) in this organism. **Findings:** Results obtained showed that the natural coagulant has low toxicity, making it a friendly alternative to replace aluminum sulphate, in water treatment. **Application/Improvements:** *Moringa oleifera* can have advantages over aluminum sulphate due to biodegradability. This is an efficient and non-toxic coagulant that can be used in water treatment for domestic use in urban and rural areas.

Keywords: Acute Toxicity, Coagulant Activity, *Artemia salina*, *Moringa oleifera*, Innocuousness.

1. Introduction

Chemical pollution of water is increasingly worrisome. Large volumes of toxic organic and inorganic substances are released daily directly and indirectly into aquatic systems. Water is essential for life and is the most important non-renewable natural resource worldwide[1]. This vital liquid helps eliminate substances resulting from biochemical processes occurring in living organisms. Nonetheless, water also serves as a means of transporting harmful substances that can hurt people's health[2]. Therefore, potable water treatment systems are necessary to implement, to allow these contaminants' removal and reduce or avoid risks to human health[3].

Coagulants are used in water treatment, however studies conducted by Stauber[4], report that residual aluminum present in water is related to health problems, such as Alzheimer's. This can also generate environmental problems such as production of large volumes of sludge and problems with its management and final disposal. Therefore, it is important to assess coagulants less harmful to the environment and harmless to health, such as the case of *Moringa oleifera* seeds. This natural coagulant has proven to be an efficient alternative in the turbidity and color removal of raw water in some parts of the world[5].

Coagulant toxicity is possible to be determined from bioassays. A bioassay is a test in which power or potency of a substance is measured through the response of living organisms or living systems[6]. They allow a quick response in the direct assessment of toxicity. These methods are fast, inexpensive and sensitive and can be applied in the laboratory or in the field[7]. From this information the mean lethal concentration (LC50) can be determined. According to the Pan American Health Organization (PAHO)[8], LC50 is defined as the concentration derived from a toxin that will cause a lethal effect determined in 50% of a given population of organisms under specific conditions, during a specific exposure period (24, 48 to 96 hours generally)[6,9].

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Artemia salina, one of the common organisms used in aquatic bioassays, is one of the models adapted very well in laboratory tests. Advantages of this model test with this species are: reliability, precision, sensitivity, ease of reproduction, handling, availability in markets and low cost[10]. It has been used for various bioassays of chemical and natural products, determining toxicity of plant extracts. 48 hours larvae are used. At this age, these organisms have sensitivity to various compounds[10]. Similarly, it has been compared with rodents in acute toxicity tests showing that these two models correlate.

Artemia salina is an alternative model to replace rodents. In an independent study, acute toxicity of 20 plant extracts was evaluated, where the extracts turned out to be toxic for both rodents and *Artemia salina*[11]. Therefore, animal rights advocates have not refuted use of these invertebrates for experimental work[12]. Nonetheless, there is no completely harmless substance, no matter how natural it might be[13-15].

The objective of this research was to verify acute toxicity of the use of natural and synthetic coagulants in the purification process of raw waters of the Magdalena River through bioassays.

2. Materials and Methods

2.1 Sampling Site Location and Preparation of Coagulants

Samples of raw water used in the assays were taken from the Magdalena River, near Magangué Municipality, Colombia, at coordinates 9° 14´ 28″ N and 74° 44´ 30″ W out of the Greenwich meridian. A simple sampling was carried out in August of 2017, corresponding to the region's dry season. Samples were collected, stored, and transported following standard protocols to the campus of University of Sucre, Colombia, where the respective treatability and toxicity tests were carried out; following the parameters established by ASTM D2035-08[16] and the measurement protocols established in the standardized methods for the analysis of drinking and residual water according to the American Public Health Association[17], respectively.

Coagulants of *Moringa oleifera* and Sulfate of Aluminum Type B ($Al_2(SO_4)_3.14H_20$), were prepared in the same concentrations, following the methodology described for[18] to obtain coagulant extracts.

2.2 Toxicological Assessment

2.2.1 Selection of Test Organisms

For this study, *Artemia salina* larvae, which have served worldwide for more than 40 years in toxicological and ecotoxicological studies, were used as bioindicator species of acute toxicity[12,19]. This species has been widely studied and due to its multiple potential uses, the technique of using it as a toxicity bioindicator is considered as a practical and economical method for bioactivity determination of synthetic compounds and natural products[20,21].

Artemia salina is a crustacean species which belongs to the Phylum Arthropoda family, class Crustaceae, subclass Branchiopoda. It is transparent to light and is distributed in saline waters rich in chlorides, sulfates and carbonates; it feeds on algae, organic particles and bacteria[12]. It can live in extreme conditions[10]. Females produce eggs that, in favorable external conditions, hatch producing larvae of 1 mm approximate size. Eggs can also form cysts and remain like this for a year or more. *Artemia* become adults after 6 to 8 weeks, reaching a 7mm average size and can grow at temperatures between 6 and 35 ^{o}C[12].

2.2.2 Acute toxicity test in Artemia salina

Acute toxicity was determined by inhibiting the mobility of the crustacean *Artemia salina*, after 24 and 48 hours of exposure, according to the procedure established by the OECD (Organization for Economic Cooperation and Development).

A completely randomized experimental design was used. Five experimental groups were formed for each coagulant, 4 groups with treatments of the test substance at decreasing concentrations, and the corresponding untreated control group. 30 larvae were randomly assigned to each group, distributed in 3 replicates of 10 individuals each. Mortality data allowed to construct the concentration-effect graph, to determine the Average Lethal Concentration (LC50) of coagulants against *Artemia salina*.

2.2.3 Preparation of dilutions

Dilutions of 1, 3, 5 and 10 mg L^{-1}; and 10, 100, 500 and 1000 mg L^{-1} for supernatants and sludge, were prepared respectively. The purpose of his experimental arrangement, was to establish the convenient concentration range to obtain effect values between 100% and 0% necessary to calculate LC50.

2.2.4 Test protocol

Artificial seawater (ASW) was prepared with pH = 9. 23 g sodium chloride, 11g Magnesium Chloride hexahydrate, 4g sodium sulfate, 1.3g calcium chloride dihydrate, 0.7g potassium chloride were used in 1 liter of distilled water. A 25 ± 2 °C constant temperature was maintained. Hatching of *Artemia salina* was started by adding 45 mg of cysts in 450 mL of artificial seawater -ASW.

Aliquots of 100µL containing at least 10 larvae were added to each prepared test sample. Larvae incubation was carried out for 24 hours and then mortality was evaluated 24 hours later.

2.2.5 Lethality Percentage

Larvae were observed with magnifying glass 24 hours after incubation, and dead larvae were recorded. Individuals unable to move for 10 seconds were considered dead. The response variable measured was mortality, and a dose-response curve was projected. The lethality percentage for each group was calculated with the following equation:

$$\textbf{\% Lethality} = \textbf{(Total Dead / Total)} \times \textbf{100.} \qquad (1)$$

Where response between 0-10% of lethality is considered as non-toxic, 11-50% moderately toxic, 51-90% highly toxic and 100% extremely toxic.

Toxicity degree was defined according to the range in which LC50 values were found according to the following categories[22]: extremely toxic (LC50 <10 µg mL^{-1}); very toxic (10 <LC50 <100), Moderately toxic (100 <LC50 <1000) and non-toxic (LC50> 1000 µg mL^{-1}).

2.6 Statistical Analysis

For determining the LC50, the EPA probit analysis program, version 1.5, was used with the statistical software InfoStat, Version 2017e, at a 0.05 significance level.

3. Results and Discussion

Raw water of the Magdalena River in dry season, presented a 184 UNT turbidity and a pH of 6.78, due to the sedimentological load of the Magdalena River, because of deforestation occurring along the basin; to the excessive mining exploitation; and to the wastewater and industrial waste discharges of the riverside towns[23].

Tables 1-2 show results of lethality grade of supernatants and sludges of raw water of the Magdalena River treated with synthetic and natural coagulant, using *Artemia salina*.

No effect vs concentration was observed from the 60.0 mg L^{-1} optimal dose of coagulants, as shown in Tables 1 and 2. With the concentrations assessed it was not possible to determine toxicity, thus demonstrating innocuosness, and therefore difficult affectation to that aquatic organism.

Table 3 shows promising results of the lethality test in *Artemia salina*, applied to the stock solution (10,000 mg L^{-1}) of *Moringa* seeds, about the preliminary biological activity.

LC50 values of 375.26; 370.95 and 377.56 ug L^{-1} were found for 24 hours of exposure, which classify *Moringa oleifera* seed extract as moderately toxic[22] (100 <LC50 <1 000) ug L^{-1}.

These results are like those found in other studies, where complex components of *Moringa oleifera* have also raised concerns about their potential toxicity[24,25]. The toxicity of aqueous extraction of *Moringa oleifera* was estimated since the 1990s[14,26] and both acute and chronic impacts were reported.

Due to the limited dissolution of the hydrophobic components, other researchers[27] showed that the water

Table 1. Acute Toxicity with *Artemia salina* of the supernatant Natural Coagulant (NC) and Synthetic Coagulant (SC)

Concentration µg mL^{-1}	Number of Dead Individuals						
	R1 SC	R2 SC	R3 SC	R1 NC	R2 NC	R3 NC	Exposed/ replicate
Control sample	0	0	0	0	0	0	10
1	1	1	2	0	0	0	10
3	3	2	3	0	1	1	10
5	1	3	1	3	3	1	10
10	1	2	2	2	0	3	10

There is no evidence of an effect vs. concentration trend

Table 2. Acute Toxicity with *Artemia salina* from Natural Coagulant Sludge (NC) and Synthetic Coagulant (SC)

Concentration µg mL^{-1}	Number of Dead Individuals						
	R1 SC	R2 SC	R3 SC	R1 NC	R2 NC	R3 NC	Exposed/ replicate
Control sample	0	0	0	0	0	0	10
10	0	0	0	0	0	2	10
100	0	0	0	1	2	0	10
500	0	0	0	2	2	1	10
1000	0	0	0	2	2	3	10

There is no evidence of an effect vs. concentration trend

Table 3. Toxicity of Stock Solution (10,000 mg L^{-1}) of Moringa oleifera seeds

Concentration ug L^{-1}	R1	R2	R3
Control sample	1	2	1
10	0	0	0
100	2	3	3
500	6	5	6
1000	8	9	8
LC50 (ug L^{-1})	375.26	370.95	377.56

extraction toxicity and the soluble proportion can be presented mainly as genotoxicity, but of slight cytoxicity. Similarly, results suggest that the water-soluble fraction of *Moringa oleifera* has low toxicity and the dominant toxicity comes from hydrophobic components[27,28].

On the other hand, some studies have confirmed that the root, bark and seed contain substances such as moringin, moringinin, spirochin and benzyl isothiocyanate that are potentially toxic for the organism[29].

4. Conclusions

Mean lethal concentration (LC50) of stock solution of *Moringa oleifera* seeds presents a moderately toxic effect to *Artemia salina*. Nonetheless, no effect vs concentration was observed from the optimum dose of coagulants tested in the water treatment of the Magdalena river. With the tested concentrations, toxicity was not possible to be determined, thus demonstrating that both coagulants are harmless, and therefore, difficult to affect human health due to biotoxicity.

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