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Phytotoxicity of Natural and Synthetic Coagulants by *Zea mays* Lethality Assays in Treated Waters from the Magdalena River, Colombia

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Abstract : This studyaimed at evaluating lethality effect of natural coagulants (*Moringa oleifera*) and synthetic coagulants (Aluminum Sulfate type B) in treatment of raw waters, through bioassays. A completely randomized experimental design was carried out, based on lethality bioassays of the *Zea mays* species in waters treated with natural and synthetic coagulants. Assays were done during different times using standardized methods. Weather did not affect phytotoxicity of solutions obtained from the flocculation process, both with artificial and naturalcoagulant. Nonetheless, there are significant differences between toxic effects of the artificial and the natural coagulantsfor both seasons. Coagulation residues with *Moringa oleifera* resulted to be significantly less toxic than those obtained with artificial coagulant, except for the natural coagulant supernatant corresponding to the rainy season, which was statistically of similar toxicity as the artificial one. It can be affirmed that the residues obtained from flocculation made with *Moringaoleifera* turn out to have less phytotoxic effects than with Aluminum Sulphate; making it more attractive as a friendly alternative for water treatment.
Key Words : *Phytotoxicity, Magdalena river, Moringaoleifera, Aluminum sulfate, coagulant activity.*

Introduction

Access to clean water is core to health, it is one of the basic human rights and a component of effective health protection policies. Quality of drinking water is a matter of concern in countries all over the world both, developing and developed due to its impact on population health. The World Health Organization emphasizes establishing measures to improve water quality by making the maximum effort to ensure that drinking water safety is as great as possible[1]. This is achieved by adapting a series of traditional technologies to eliminate water turbidity in the domestic environment.

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Currently, use of synthetic coagulants for water purification, such as aluminum sulphate, is questioned due to some unwanted effects of aluminum associated with environmental problems, such as generation of toxic sludge impossible to use in agriculture[2]; due to health problems, since they contribute to the development and progression of Alzheimer's disease[3]; because they are mediators of oxidative stress[4,5],and other reasons. Therefore, development of coagulants less harmful to the environment and harmless to health is necessary.

Natural coagulants, such as *Moringaoleifera* seeds, have shown to be an alternative in turbidity and color removal in raw water in some parts of the world[6]. This natural coagulant is undoubtedly the most studied by the scientific community, since its properties have been widely recognized[7], due to the presence of soluble cationic proteins in the seed. A fraction of protein content in seeds, close to 1%, is made up of active proteins that neutralize and precipitate water colloids, just as industrial coagulants do, but at a lower cost[8].

In Colombia, several studies have been carried out showing that coagulant activity of *Moringaoleifera* seeds is comparable with that obtained by using aluminum sulphate or alum[9-14]. One main advantage of these natural coagulants over synthetic ones is that it does not alter properties of treated water, so its use in rural populations is recommended as an effective substitute, cheap and without risks forhealth[15,16].

There are still no relevant studies on phytotoxicity of coagulants in raw water treated for purification. However, bioassays with plants are increasingly being considered for ecotoxicological diagnosis. They are an excellent tool in environmental risk assessment. This methodology is very convenient in water phytoxic evaluation, becuase it provides information about a substance toxic in the environment, i.e., an agent that can produce an adverse effect on the biological system, damage its structure or function, or cause death. In practice, these methods can not be replaced by chemical analysis[17].

Among standardized methodologies to establish controlled conditions, assays with *Zeamays* (maize) have been recommended by the US Environmental Protection Agency for phytotoxicitytests[18], since it is a very sensitive species. Vascular plants are recommended due to their greater sensitivity, compared to other species[19,20]. The ecotoxicologicalassay with seeds has some advantages over others because it allows quantitative use of root growth.

Radicle germination and elongation test is carried out with soluble constituents of water (surface waters, groundwater, soils, sediments and leachates), taking into account amount of germinated seeds and the average growth of root for the result to calculate the 50% inhibition concentration (IC50) of the parameters analyzed in relation to the blank[21,22].

The purpose of this research study was to verify toxicity of natural and synthetic coagulants in raw water treatment from the Magdalena River, by means of bioassays with Zea mays and to define degree of lethality and phytotoxicity of these.

Materials and Methods

Raw Water Samples

Samples of raw water were taken from the left bank of the Magdalena River, near the catchment of thepotabilization plant from theMaganguéMunicipality, Colombia, see Figure 1. Two simple samplings were carried out, on the region'sdry and rainy season, during August and October of 2017, respectively. Samples were stored in 20 liter plastic containers and taken to the Soils and Water laboratory of the University of Sucre in Sincelejo city, Colombia, where jar tests and measurement of physicochemical parameters were carried out.

Figure 1. Studyarea

Coagulant Extract Preparation

Moringaoleifera seed

Seeds of *M. Oleífera* were obtained in the 2016 region'sharvest. They were dehulled manually and then dried. Dried seeds were passed through a manual mill and sieved in a No. 100 mesh repeatedly until a very fine powder was obtained. Then 10.0 g of the result powder was dissolved in a volumetric flask, up to 1.0 L with 1.0% sodium chloride saline solution (w / v). The solution was mixed with magnetic stirring for 1 hour and filtered under vacuum with cellulose filter paper. Filtrate was labeled as a salt coagulant extract with a 10,000 mg L^{-1}concentration and kept refrigerated at 4 °C until application[23].

Aluminum Sulfate type B.

Aluminum Sulphate type B-Al_2 $(SO_4)_3$.14$H_2$0, was obtained commercially, donated by the aqueduct company of MaganguéMunicipality, Bolívar, and prepared in the same concentrations as the extracts from*Moringaoleifera*.

Jar Test

Extract of *Moringaoleifera* seeds and aluminum sulphatewere applied as coagulantsto each raw water sample, in a 60 mg L^{-1}dose.EyQ F6-300-T jar test equipment with six rotating blades and an equal number of 1,000 mL beakers was used. Rapid mixture was 200 rpm for 1 minute, followed by slow mixture at 40 rpm for 20 minutes and with a of 30 minutessettling time[10,24].

Phytotoxic Evaluation

Selection of Test Organisms

Seeds of *Zea mays*, easily and rapidly germinating, were used as bioindicator species of acute toxicityfor the study, since they can develop in a few days. In addition to being low cost tests, specialized equipments are not required and time of exposure to the toxic is reduced[25]. *Zea mays* seeds were obtained from crops of indigenous communities in the Sucre Department. A 10x3completely randomized experimental design was used, with 10 treatments for the test substances at decreasing concentrations, and the corresponding

untreated control group. Each assay had 3 replicates and 20 seeds were used in each replica (760 seeds in total). The purpose of the assay was to calculate IC50 of the coagulants against *Zea mays*.

Acute Toxicity Test on Zea mays seeds

Acute toxicological bioassays of supernatants and sludge exposure,resulting from water treatment with natural (*M. oleifera)* and synthetic (*Aluminum Sulfate* Type B) coagulant,were carried out in the Natural Products Research Laboratory of the University of Sucre (LIPNUS). The phytotoxicity test is a static test of acute toxicity at 120 hours of exposure, according to the method adapted by[26] from the "Seed Germination/Root Elongation Toxicity Test" Guide from the Environmental Protection Agency[18]. Prior to the test implementation, each batch of seeds was verified to have a germination percentage greater than 90%. Phytotoxic effects of coagulants in the seeds germination process and in the development of seedlings were evaluated during the first five growing days. For the phytotoxic effectsevaluation, inhibition in germination and inhibition or stimulation in elongation of the radicle and hypocotyl were determined.

Preparation of dilutions

Dilutions were prepared for phytotoxicity assays of 10,000; 1,000; 100; 10; 1; 0.1; 0.01; 0.001; 0.0001 g L^{-1}, allowing to establish the convenient concentration interval to obtain effect values between 100% and 0% necessary to calculate IC50. Toxicity evaluation of samples was carried out simultaneously to a control group, using distilled water.

Sowing the seeds

Seed sowing was made in Petri dishes, using Whatman filter paper, 90 mm in diameter, as support and imbibition medium. Sample volume for the test was 5 mL per dish, using distilled water in the negative controls and as a dilution medium. For each Petri dish, and once the filter paper was imbibed, 20 seeds were placed, taking into account to leave enough space between seeds to allow the elongation of roots. Dishes were capped to prevent moisture loss and were immediately covered with light after imbibition started and during the test period to facilitate germination. It was incubated for 120 hours at 22 °C. A replica was made for each dilution tested. Observations and measurements of seedlings were made for statistical analysis.

Inhibition Percentage in Germination

Inhibition percentage of germination was determined according to the methodology proposed by [27], applying this equation:

$$\%Inhibition = \left[\left(1 - \frac{Numberofgerminatedseedsintreatment}{Numberofgerminatedseedsinthecontrol}\right)x100\right] \quad (1)$$

Statistical analysis

All tests were performed in duplicate and using a control. Statistical analysis was done through analysis of variance (ANOVA). Statistical program InfoStat, Version 2017e was used. Adjustments were made to estimate the IC50. A normality test (modified Shapiro-Wilks) and a variance analysis of Kruskal-Wallis were performed at a $p \leq 0.05$significance level.

Results and Discussion

Initial conditions of raw water, taken in the Magdalena River, Maganguéarea, Colombia, are presented in Table 1.

Table 1. Turbidity and pH of raw water samples from the Magdalena River

Parámeters	DrySeason	RainySeason
Turbidity (UNT)	184	513
pH	6.78	6.47

Turbidity of raw water from the Magdalena River, both in the dry season and in rainy season, was high in comparison with other rivers in the Colombian Caribbean region. However, pH did not vary significantly during the sampling period, mainly due to the magnitude of average flow that it handles (7,200 $m^3 s^{-1}$)[9].

Toxicity of supernatants and sludge of water treated with synthetic and natural coagulant at different times were evaluated by the inhibition percentage in the germination process of seedlings and the effect produced by each concentration. A similar effect for theblank and raw water from the Magdalena River on the elongation of *ZeaMays* seedlingswas obtained; 24.13 mm and 28.88 mm, respectively. Thus, there were no phytotoxic effectsin the water from the Magdalena River, usually manifesting itself in the inhibition of elongation of the seedlings.

According to Table 2, there is an inhibition in the elongation of the plant with the highest concentration, and it increases as concentration decreases, similar to the blank. This result was shown in the two monitored seasons (dry and rainy), denoting greater inhibitory effect in the rainy season. Therefore, the higher the concentration of coagulant used for raw water treatment, the greater the growth affectation of the seedling.

Table 2. Phytotoxic effect on the Supernatant (Sup.), on the SyntheticCoagulant Sludge (SC) and on the Natural Coagulant (CN), in the dry and rainy season

Concentration µg/mL	DrySeason				RainySeason			
	Length (mm)				Length (mm)			
	Sup. SC	Sludge SC	Sup. NC	Sludge NC	Sup. SC	Sludge SC	Sup. NC	Sludge NC
0.0001	25.767	24.074	27.173	22.213	23.733	21.023	24.122	22.213
0.001	21.067	21.582	21.645	21.425	19.033	18.531	18.594	21.425
0.01	17.663	20.083	20.708	19.956	15.629	17.032	17.657	19.956
0.1	16.615	18.958	20.086	18.758	14.581	15.907	17.035	18.758
1	15.856	16.361	18.289	17.496	13.822	13.310	15.238	17.496
10	15.074	12.244	17.879	17.378	13.040	9.193	14.828	17.378
100	12.940	11.832	16.734	15.692	10.906	8.781	13.683	15.692
1,000	10.868	10.129	14.794	14.583	8.834	7.078	11.743	14.583
10,000	5.331	5.669	11.579	12.994	3.297	2.618	8.528	12.994

Table 3. Determination of IC50 for the sample in the dry season and rainy season

Treatment	Subtreatment	IC50 (µg mL^{-1})	
		DrySeason	RainySeason
Aluminum Sulfate	Supernatant	3.477	1.281
	Sludges	30.620	2.533
NaturalCoagulant	Supernatant	3,140.896	153.654
	Sludges	$4.29E^{+07}$	$4.64E^{+05}$

Mean inhibitory concentration (IC50) is used to estimate concentration of a toxic substance that can cause an observable adverse effect, by means of a discrete response in a defined percentage of organisms, which is 50% in this case.In this way, it is possible to determine which the optimal dose of coagulant is that can be used safely for the treatment of raw water from the Magdalena River. Average of IC50, for the samples in dry and rainy season, were different in each treatment. Aluminum sulfate showed a slight toxic effect superior to natural coagulant (*Moringaoleifera*), as shown in Table 3.

Results of the Kruskal Wallis test, carried out to determine if there are phytotoxic effects in the IC50 assays, are shown in Table 4.

Table 4. Kruskal Wallis test

Variable	Treatment	Season	N	Means	S.D.	Medians	H	p
IC50	$Al2(SO_4)_3$ -R	Rainy	3	2.53	1.76	1.58	22.01	0.0025
IC50	$Al2(SO_4)_3$-R	Rainy	3	30.62	29.08	14.16		
IC50	$Al2(SO_4)_3$ – D	Dry	3	1.28	1.30	0.63		
IC50	$Al2(SO_4)_3$ -D	Dry	3	3.46	0.91	3.18		
IC50	OM–R	Rainy	3	46,4266.67	460,604.18	462,000		
IC50	OM–R	Rainy	3	42,873,333.33	34,083,957.13	62,1000,000		
IC50	OM–D	Dry	3	153.65	40.81	169.16		
IC50	OM–D	Dry	3	3,140,90	1,0008.74	3,324.32		

No statistically significant differences were found between treatments made to raw water during rainy season and those made during dry season, i.e., the weather did not affect phytotoxicity of solutions obtained from the flocculation process, regardless coagulanttype used. On the contrary, statistically significant differences were observed between toxic effects of artificial and natural coagulant. Organic residues (OM) of coagulation with *Moringaoleifera*, in both sampling seasons, showed to be significantly less toxic than those obtained with the synthetic coagulant, except for the natural coagulant supernatant in the rainy season, which statistically showed, similar toxicity than the synthetic one.

In Table 5, germination percentages of *Zea mays* seeds, subjectto the different treatments, are shown. The germination percentages were carried out to check if used coagulants alter the seeds germination processes.

Table 5.Germination percentage of *Zea mays* seeds in the dry and rainy seasons

InhibitionPercentaje (%)								
Concentration µg/mL	**DrySeason**				**RainySeason**			
	Sup SC	**Sludge SC**	**Sup. NC**	**Sludge NC**	**Sup. SC**	**Sludge SC**	**Sup. NC**	**Sludge NC**
0.0001	5	5	5	5	5	5	5	5
0.001	5.00	6.67	5.00	5.00	5.00	6.67	3.33	5.00
0.01	0.00	1.67	1.67	5.00	0.00	1.67	1.67	5.00
0.1	6.67	0.00	3.33	0.00	6.67	0.00	3.33	0.00
1	0.00	6.67	8.33	10.00	0.00	8.33	6.67	10.00
10	0.00	3.33	5.00	3.33	5.00	8.33	5.00	8.33
100	3.33	8.33	0.00	1.67	8.33	13.33	1.67	5.00
1,000	8.33	6.67	1.67	8.33	13.33	11.67	5.00	11.67
10,000	25.00	6.67	0.00	1.67	30.00	11.67	5.00	6.67

It was observed that for the dry and rainy seasons there was no pattern in the inhibition percentage when concentration of supernatant and coagulant sludge increased, regardless of whichsynthetic or natural coagulant was used. This situation can be explained because sulfates and some metals are essential nutrients for plant normal development [28]. However, when these compounds are found in ecosystems in high concentrations, they can affect viability and cellular functions of plants[29].

On the other hand, results found do not match what it is established by studies on traditional test of seed germination, in which toxic compounds present in low concentration levels are not sufficient to inhibit germination. Nonetheless, they can delay or completely inhibit the elongation processes of the radicle or hypocotyl[26].

Conclusions

Residues obtained from the flocculation made with *Moringaoleifera* seed turned out to have less phytotoxic effects than Aluminum Sulphate in the treatment of medium and high turbidity waters. Water treatment with *Moringaoleifera* was attractive as a more friendly alternative for treatment of raw waters from the Magdalena River.

Although *Zea mays* is not a representative species of aquatic ecosystems, the information generated from this toxicity assay provides data about the possible effect of pollutants in plant communities near the margins of polluted bodies of water. Therefore, this is an interesting species to be considered for phytotoxicity tests due to its agricultural importance.

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