

Moringa Oleifera Seed Extract in the Clarification of Surface Waters

J.R. Rodríguez-Núñez¹, D.I. Sánchez-Machado², J. López-Cervantes^{*3}, J.A. Núñez-Gastélum⁴, R.G. Sánchez-Duarte⁵, M.A. Correa-Murrieta⁶

Departamento de Biotecnología y Ciencias Alimentarias, Instituto Tecnológico de Sonora, P.O. Box 541, Ciudad Obregón, Sonora, México

¹jesus.rodriguez@itson.edu.mx; ²dalia.sanchez@itson.edu.mx; ^{*3}jaime.lopez@itson.edu.mx; ⁴jose.nunez@itson.edu.mx; ⁵reyna.sanchez@itson.edu.mx; ⁶maria.correa@itson.edu.mx

Abstract- *Moringa oleifera* is a plant whose seeds have protein or polypeptide with coagulant properties. The present study evaluated the effect of *Moringa oleifera* seed (MOS) extract as primary coagulant and its mixture with aluminum sulfate in the treating of superficial waters with turbidities between 72 and 118 NTU obtained from Náinari lagoon located in Northwestern México. An average of 88.9% turbidity removal was achieved when MOS extract was used as primary coagulant with a dosage of 250 mg/l. In combination with aluminum sulfate reached 96.5% of clarification, and moreover is obtained saving 66% of chemical coagulant. The MOS extract is a viable alternative to diminish the use of chemical coagulants in the clarification of surface waters.

Keywords- *Coagulants; Wastewater; Polypeptide; Water Treatment; Turbidity*

I. INTRODUCTION

The access to safe drinking water is fundamental to ensure the well-being of humanity. However, the water resources of the world are being polluted through various anthropogenic activities ^[1]. The use of fertilizers, manure, cattle slurry and fungicides used in agriculture are the main pollutants in surface waters ^[2]. Wastewater treatment is a priority at the global level, because it is important to have enough water of good quality, which will allow an improvement of environmental, health and life quality ^[3, 4]. According to [5], water borne diseases are one of the main problems in developed countries; about of 1.6 million people are compelled to use contaminated water. Moreover, in less developed countries, increasing population, urban migration and environment strain have increased the necessity for drinking water treatment ^[6].

Mexico has a great problem with the treatment of wastewater. According to the Mexico's National Water Commission ^[7] in 2008 only the 40% was treated and by 2010 it aims to treat 60% of the total flow of sewage systems. In this context, the shortage of water has promoted the development of new technologies for treatment and reuse of water in Mexico ^[8].

Among principal operational costs in the functioning of wastewater treatment, plants are the coagulant agents as aluminum sulfate and ferric sulfate ^[9, 10]. These products are a limitation for the countries in process of development due to their high cost, and the management of sewage sludge is complicated by the high content of aluminum sulfate.

Similarly, the formations of aluminum hydrides affect the turbidity, due to the precipitation. Furthermore the coagulation with aluminum salts change the natural alkalinity of the water, diminishing pH and the coagulant effectiveness, also, and its efficiency is reduced on cold water ^[11]. According to [12] the use of metals such a copper in agriculture can reach waterbodies and the metals are considered as major pollutants causing cytotoxic, mutagenic and carcinogenic effects in animals. Recent studies had shown that Al^{+3} and Fe^{+3} salts accumulated in the sludge are stretchy related to diseases like Alzheimer and pre-senile dementia ^[13]. This has promoted the development of the new clean, simple and cheap technologies that emerging countries need ^[14, 15].

A viable alternative to minimize environmental impact and reduce operational costs of wastewater treatment is the application of vegetal products (cellulose, polysaccharide gum, starch) which work as helpers on the coagulation-flocculation processes ^[16, 17].

Naturally occurring coagulants are usually presumed safe for human health. *Moringa oleifera* is a tropical multipurpose tree known as the miracle tree, originally grows at the north of India and today abounds throughout the Tropic ^[18]. This tree is well known due to its high nutritional content (proteins, peptides, linolenic acid, oleic acid, linoleic acid, calcium, iron, vitamin C, and tocopherol) and applications in medicine ^[19]. In the same way, previous investigations reported the efficiency of MOS extract on wastewater treatment as a coagulant agent, softening agent and bactericidal agent ^[20, 21]. This property is caused by a polypeptide that acts as a cationic polymer with a molecular weight of about 13 kDa and an isoelectric pH point of 10 ^[22].

Several studies have reported that the MOS polypeptide can be a viable alternative to partially or totally replace of chemical products used on water treatment as aluminum sulfate and iron chloride ^[23, 24]. Moreover, this extract reduces operating costs in the water treatment and decreases the concentration of aluminum in the sludge sedimented ^[20]. The aim of this investigation was the characterization of *Moringa oleifera* seed and testing the seed extract as a primary coagulant and combined with aluminum sulfate by the clarification of surface waters.

II. MATERIALS AND METHODS

A. *Moringa Oleifera* Seed in Powder

Moringa oleifera seeds were collected from a field of about 40 ha located in the State of Sonora in Northwest Mexico. The healthy Seeds were collected and transported in plastic bags at room temperature. The seeds were dried, peeled by hand and grounded in a mortar until get a fine powder. Finally, the resulting powder was sieved (212, 106 and 75 μm) and stored in plastic bags at room temperature.

B. Extraction of *Moringa Oleifera* Seed Oil

Reference [25] mentioned the extraction of the oil. Specifically, in a 500 ml flask where added 70 g of MOS in powder and 200 ml of hexane, this was mixed for 20 min and recovered the solvent in a rotary evaporator (BUCHI, Flawil, Switzerland). Finally, the residue was dried at 60°C in the oven (Felisa, model FE-291D, México D.F, México) until get an amber-colored powder, without oil.

C. Proximate Composition and *Moringa Oleifera* Seed Extract

Reference [26] pointed the analysis of moisture, ashes, protein (Kjeldahl) and lipids (Soxhlet). The active compound was obtained by mechanical extraction, mixing 5 g of MOS in 200 ml of distilled water and stirred for 1 min. The suspension was filtered with Whatman paper No. 42 and the filtrate was diluted to 1 l with tri-distilled water to obtain a stock solution of 5000 mg/l [19].

D. Coagulation-Flocculation Studies

The water samples were collected at Náinari lagoon, located in Ciudad Obregón, Sonora, México, in July 2008. The sampling was made in plastic tanks of 20 l and stored at room temperature until use.

The analyses of coagulation-flocculation were evaluated through jars tests at room temperature (25°C) [6, 21]. The extracts of MOS in solution (50, 100, 150, 150, 200, 250, 300, 350 and 400 mg/l) and aluminum sulphate (10, 30, 50, 70, 90, 110, 130 and 150 mg/l) were evaluated as a primary coagulant at different concentrations. Besides, the extract of MOS was tested in combination with aluminum sulfate, as shown in the Table 1.

TABLE I CONJUNCTIVE USE TEST: ALUMINUM SULFATE AND *MORINGA OLEIFERA* SEED EXTRACT DOSE MATRIX

Aluminum Sulfate	+	<i>Moringa Oleifera</i> Seed Extract (mg/l)
30 + 50		40 + 50
30 + 100		40 + 100
30 + 150		40 + 150
30 + 200		40 + 200
30 + 250		40 + 250

Specifically, 500 ml of sample water was poured into beakers of 1 l and placed on jars equipment. The unity was started and adjusted to quick mix (120 rpm during 4 min). The dosages of the coagulant agents were added;

subsequently the revolutions were diminished to 20 rpm by 20 min, and let it rest over 10 min. Finally, the residual turbidity was registered with a turbidimeter (VWR Scientific, USA).

E. Statistical Analysis

Batch experimental were conducted in triplicate (N = 3) and data represent the mean value. The statistical significance of differences between means ($p < 0.05$) was estimated by ANOVA using Statgraphic plus 4.0 (StatPoint Technologies, Inc, Warrenton, VA, USA).

III. RESULTS AND DISCUSSION

A. Proximate Composition of *Moringa Oleifera* Seed

The lipid content in MOS powder without defatted was $35.96 \pm 0.186\%$ while defatted MOS powder presented $1.04 \pm 0.20\%$, these results indicated a removal efficiency of 97.1% compared with the MOS powder without defatted. The values of the lipids content in MOS samples are similar to those reported by [27, 28], who pointed a percentage in the range of 40.39 to 41.58.

According to [29] the variation in the lipids content can be attributed to the region from planted and the growing conditions of the plant, and may diverge in a range from 30 to 42% of lipids. Also, the extraction efficiency can differ from the reported values as in [11] who reported a lipid remotion percentage of 83 and 65, respectively. Table 2 gives the proximate composition of MOS defatted in powder.

TABLE II PROXIMATE COMPOSITION OF *MORINGA OLEIFERA* SEED DEFATTED

Analysis	Results (%)
Moisture	7.63 ± 0.06
Ashes	6.05 ± 0.03
Lipids	1.04 ± 0.20
Protein	59.58 ± 0.20

Means values of n = 3, triplicate determination \pm standard deviations

The percentage of protein obtained in the present work is similar to reported values by [30] who mentioned a 60% of protein in MOS defatted. Furthermore, [27] pointed that MOS defatted in the area of Nigeria presents 69% of protein, while [28] reported 49.25% of protein in the region of Pakistan. This shows that the content of protein of our investigation is within the range and the variations are related to the geographic region.

B. *Moringa Oleifera* Seeds Extract and Aluminum Sulfate as Primary Coagulants

The MOS extract used as a primary coagulant reached a clarification efficiency of 88.9% on water samples with an initial turbidity of 118 NTU, using a dosage of 250 mg/l and

sedimentation time of 10 min (Figure 1). These results are similar than those reported by [31], who pointed that MOS extract applied at dosages of 250 mg/l to water with initial turbidity of 270 to 380 NTU gave a residual turbidity below to 4 NTU. Likewise, [24] found that a dosage of 250 mg/l of MOS extract was effective to reduce 98.2% surface water samples with initial turbidity of 109 to 190 NTU.

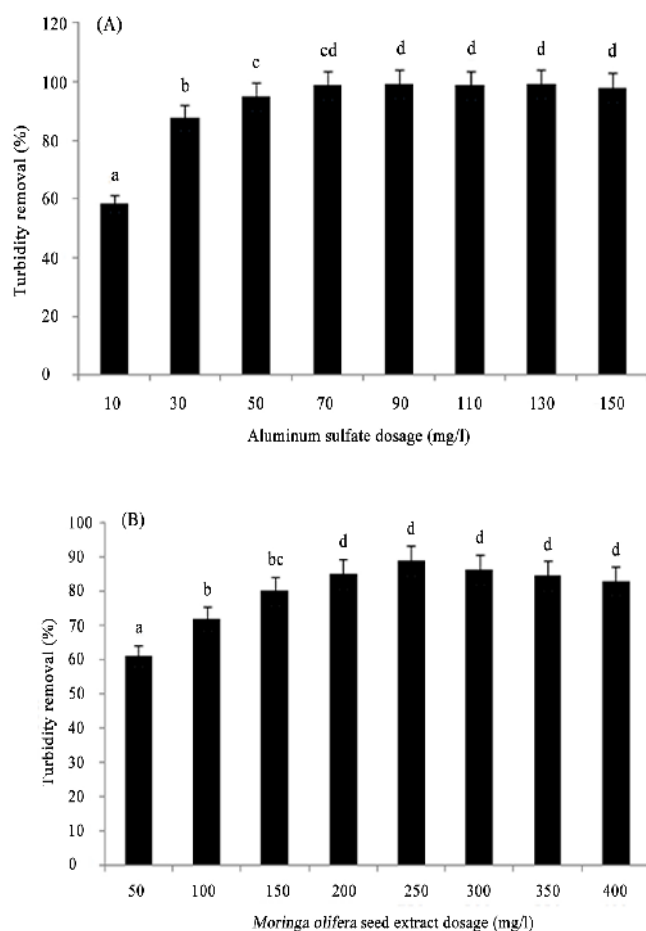


Fig. 1 Comparative effect of turbidity removal between MOS extract (a) and aluminum sulfate (b) in surface water. Bars with the same letter are not significantly different according to the Tukey's-b test ($p < 0.05$).

Reference [20] recorded that defatted MOS achieves a removal efficiency of 87% on surface water samples with an initial turbidity of 56 NTU with a dosage of 250 mg/l, also mentioned that MOS without defatted reached only 81% of clarification with the same dosages. This variation can be attributed to the content of lipids on the MOS, because these modify the solubility, affecting the coagulation-flocculation ability [22].

Otherwise, the aluminum sulfate reached a removal efficiency of 99.2% with a dosage of 90 mg/l in samples from Nánari lagoon, with an initial turbidity of 130 NTU. These values are similar than those reported by [32], who mentioned that a combination of aluminum sulfate, ferric chloride and polychlorinated aluminum, manages to remove a 98 and 91% of turbidity and color, respectively. In similar studies [33] indicated a turbidity removal efficiency of

88.4% with a dosage of 9.5 g/l of aluminum sulfate in lixiviated samples.

These results show that aluminum sulfate is more efficient as a primary coagulant than the MOS extract, possibly related to the electrostatic mechanism and the high density charge. Furthermore it was observed that the flocs formed with MOS extract were small, fragile and the settling process was slower compared with aluminum sulfate. According to [20] the MOS extract has a short-chain cationic polypeptide; therefore the turbidity removal would be mainly through electrostatic patch mechanism, which is a surface phenomenon.

The electrostatic mechanism depends mostly on surface contact of charges between the cationic polypeptide of the MOS and turbidity particles. Therefore, this is affected by the oil residues in the powder forming an emulsion or coating that avoid the contact with the surface from reaction reducing flocs formation. Instead, aluminum sulfate is very efficient because it has a high valence (Al^{+3}) providing it a superior capacity of charge exchange, generating a swapping while settles [34].

C. Conjunctive Use of Moringa Oleifera Seed Extract with Aluminum Sulfate

A dosage of 250 mg/l of MOS extract combined with 30 mg/l of aluminum sulfate reached a clarify efficiency of 96.5% on water samples with an initial turbidity of 72 NTU, these results are similar to those achieved with aluminum sulfate as a primary coagulant (99.2%) and reduced its use in a 66%, showing that MOS extract is effective when is applied in conjunction with aluminum sulfate (Figure 2).

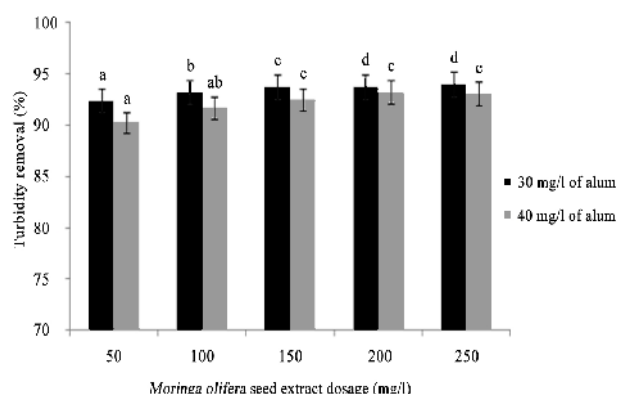


Fig. 2 MOS extract in conjunctive use with aluminum sulfate in surface water

Bars with the same letter are not significantly different according to the Tukey's-b test ($p > 0.05$).

The results are consistent to those reported by [8] who mentioned that it can be possible to make savings up to 80% of aluminum sulfate when mixed with MOS extract. Similarly, [24] pointed that a dosage of 30 mg/l of aluminum sulfate and 40 mg/l of MOS extract achieved the maximum turbidity removal capacity, saving 40% of the use of aluminum sulfate in the waters treatment.

Furthermore it was observed that the increasing of aluminum sulfate concentration to 40 mg/ml with the same dosage of MOS extract reduced the clarification efficiency, achieving only a 92.35% of clarification. Previous studies have mentioned this behavior, in which it was observed that low concentration of the coagulants resulted in incomplete flocculation, while an excess caused stabilization of the suspend particles and some of which may become over saturated with the coagulant agent, causing ineffective flocculation^[9, 24].

IV. CONCLUSIONS

The present study demonstrated the effectiveness of the application of MOS extract as primary coagulant and conjunctive use with aluminum sulfate in the turbidity removal of surface waters. In conjunctive form, this extract saves more than half of coagulant chemical. Research should therefore continue in this area in order to develop new technologies for the treatment industrial or natural waters in developing countries.

ACNOWLEDGMENT

This research was financed under Project no. 95935 from FONCICYT C002-2008-1/ALA – 127 249, and by AGRINOS AS Bio Tech Company. The first author is grateful to CONACYT (213712). Michael A. Bryan, MBA and Native English Professor offered suggestions and improvements in the wording of this manuscript.

REFERENCES

- [1] P. Kumari, P. Sharma, S. Srivastava, and M. Srivastava, "Arsenic removal from the aqueous system using plant biomass: a bioremedial approach," *Journal of Industrial Microbiology and Biotechnology*, vol. 32, pp. 521-526, Oct. 2005.
- [2] A. Bermúdez-Couso, M. Arias-Estévez, J.C. Nóvoa-Muñoz, E. López-Periago, B. Soto-González, J. Simal-Gándara. "Seasonal distribution of fungicides in soils and sediments of small rivers basin partially devoted to vineyards," *Water Research*, vol. 41, pp. 4515-4525, Jun. 2007.
- [3] M. Romero-Aguilar, A. Colín-Cruz, E. Sánchez-Salinas, and M.A. Ortiz-Hernández, "Tratamiento de aguas residuales por un sistema piloto de humedales artificiales: Evaluación de la remoción de la carga orgánica," *Revista Internacional de Contaminación Ambiental*, vol. 25, pp. 157-167, Jan. 2009.
- [4] M. Arias-Estévez, E. López-Periago, E. Martínez-Ceballos, J. Simal-Gándara, J.C. Mejuto, L.García-Rios. "The mobility and degradation of pesticides in soils and the pollution of groundwater resources," *Agriculture, Ecosystem and Environment*, vol. 91, pp. 232-242, Jan. 2008.
- [5] F.K. Amagloh and A. Benang, "Effectiveness of Moringa oleifera seed as coagulant for water purification," *African Journal of Agricultural Research*, vol. 41, pp. 119-123, Feb. 2009.
- [6] A.G. Liew, M.J. Noor, S.A. Muyibi, A.M.S. Fugara, T.A. Muhammed, and S.E. Iyuke, "Surface water clarification using M.oleifera seeds," *International Journal of Environmental Studies*, vol 63, no. 2, pp. 211-219, Apr. 2006.
- [7] (2010) CONAGUA. México licitará la mayor planta de tratamiento de aguas del mundo en 2009. Available at: http://www.agua.org.mx/index2.php?option=com_content&d_o_pdf=1&id=6778.
- [8] P. Gevara-Ruiz, and M. Ortiz-Pérez, "Adaptación a microescala del método potenciométrico con electrodo ión selectivo para la cuantificación de fluoruro," *Revista Internacional de contaminación ambiental*, vol. 25, no. 2, pp. 87-94, Dec. 2009.
- [9] S.A. Muyibi, and C.A. Okuofu, "Coagulation of low turbidity surface waters with Moringa oleifera seed," *International Journal of Environment Studies*, vol. 48, pp. 263-273, Nov. 1995.
- [10] B. Shi, G. Li, D. Wang, C. Feng, and H. Tang, "Removal of direct dyes by coagulation: The performance of preformed polymeric aluminum species," *Journal of Hazardous Materials*, vol. 146, pp. Sept. 567-574, 2007.
- [11] S.A. Muyibi, and A.M.S. Alufugara, "Treatment of surface water with Moringa oleifera seed extract and alum a comparative study using a pilot scale water treatment," *International Journal of Environment Studies*, vol. 60, pp. 617-626, Dec. 2003.
- [12] D. Fernández-Calviño, J.A. Rodríguez-Suárez, E. López-Periago, M. Arias-Estévez, J. Simal-Gándara, "Copper content of soils and river sediments in a winegrowing area, and its distribution among soil or sediment components," *Geoderma*, vol. 145, pp. 91-97, Apr. 2008.
- [13] Z. Yang, B. Gao, and Q. Yue, "Coagulation performance and residual aluminum speciation of Al₂(SO₄)₃ and polyaluminum chloride (PAC) in yellow river water treatment," *Chemical Engineering Journal*, vol. 165, pp. 122-132, Aug. 2010.
- [14] Y.W. Chiang, K. Ghyselbretcht, R.M. Santos, J.A. Martens, R. Swennen, V. Cappuyns, and B. Meesschaert, "Adsorption of multi-heavy metals onto water treatment residuals: Sorption capacities and applications," *Chemical Engineering Journal*, vol. 200-2002, pp. 405-415, Jun. 2012.
- [15] G. Crini, "Non-conventional low-cost adsorbents for dye removal: A review," *Bioresourse Technology*, vol. 97, pp. 1061-1085, June. 2006.
- [16] M.R. Pérez-Gregorio, M.S. Gacía-Falcón, E. Martínez-Carballo, J. Simal-Gándara, "Removal of polycyclic aromatic hydrocarbons from organic solvents by ashes wastes," *Journal of Hazardous Materials*, vol. 178, pp. 273-281, Jan. 2010.
- [17] N.W.S. Wan, and M.A.K.M. Hanafiah, "Removal of heavy metal ions from wastewater by chemically modified plant wastes as adsorbents: A review," *Bioresourse Technology*, vol. 99, pp. 3935-3948, July. 2008.
- [18] J. Sánchez-Martín, K. Ghebremichael, and Beltrán-Heredia, "Comparison of single-step and two-step purified coagulants from Moringa oleifera seed for turbidity and DOC removal," *Bioresourse Technology*, vol. 101, pp. 6259-6261, Mar. 2010.
- [19] S. Katayon, M.J. Noor, M. Asma, L.A. Abdul Ghani, A.M. Thamer Azni, J. Ahmad, B.C. Khor, and A.M. Suleyman, "Effect of storage condition of Moringa oleifera seeds on its performance in coagulation," *Bioresourse Technology*, vol. 97, pp. 1455-1460, Oct. 2006.
- [20] S.A. Muyibi, M.J. Noor, T.K. Leong, and L.H. Loon, "Effect of oil extraction from Moringa oleifera seed on coagulation of turbid water," *International Journal of Environment Studies*, vol. 59, no. 2, pp. 243-254, Mar. 2002.
- [21] M. Pritchard, T. Craven, T. Mkandawire, A.S. Edmonson, and J.G O'Neill, "A comparison between Moringa oleifera and chemical coagulants in the purification of drink water-An alternative sustainable solution for developing countries," *Physics and Chemistry of Earth*, vol. 35, pp. 789-805, Jul. 2010.

- [22] S.A. Muyibi, M.J. Noor, F.R. Ahmadun, and E.S.M. Ameen, "Bench Scale Studies for pretreatment of sanitary landfill leachate with *Moringa oleifera* seed extract," *International Journal of Environment Studies*, vol. 59, no. 5, pp. 513-535, Aug. 2002.
- [23] S.A. Shehata, S.A. Badr, and S.Z. Wahba, "Drinking water treatment option for eliminating freshwater algae," *International Journal of Environment Studies*, vol. 59, pp. 679-688, Nov. 2002.
- [24] S.A. Muyibi, and L.M. Evison, "Coagulation of turbid water and softening of hardwater with *Moringa oleifera* seed," *International Journal of Environment Studies*, vol. 49, pp. 247-259, Apr. 1996.
- [25] R. Maikokera, and H.M. Kwaambwa, "Interfacial properties and fluorescence of a coagulating protein extracted from *Moringa oleifera* seeds and its interaction with sodium dodecyl sulphate," *Colloids and Surfaces B: Biointerface*, vol. 55, pp. 173-178, Dec. 2007.
- [26] AOAC. Official Methods of analysis of the Association of Official Analytical Chemistry. Edited by Sidney Williams. Fourteenth edition. USA, 1984.
- [27] B.A. Anhwange, V.O. Ajiobala and S.J. Oniye, "Chemical studies of seed of *Moringa oleifera* (Lam) and deuterium microcarpum (Guill and Sperr)," *Journal of Biological Sciences*, vol. 4, pp. 711-715, 2004.
- [28] F. Anwar, and M.I. Bhanger, "Analytical characterization of *Moringa oleifera* seed oil grown in temperate regions of Pakistan," *Journals of Agricultural and Food Chemistry*, vol. 51, pp. 6558-6563, Apr. 2003.
- [29] T. Okuda, A.U. Baes, W. Nishijima, and M. Okada, "Improvement of extraction method of coagulation active components from *Moringa oleifera* seed," *Water Research*, vol. 33, pp. 3373-3378, Jan. 1999.
- [30] H. Ben Salem, and H.P.S. Makkar, "Defatted *Moringa oleifera* seed meal as a feed additive for sheep," *Animal Feed Science and Technology*, vol. 150, pp. 27-33, July. 2009.
- [31] J.P. Sutherland, G.K. Folkard, M.A. Mtawali, and W.D. Grant, "*Moringa oleifera* as a natural coagulant," in 20th WEDC Conference. Affordable Water Supply And Sanitation., Colombo, Sri Lanka, 1994, pp. 297-299.
- [32] E. Marañón, L. Castrillón, Y. Fernández-Nava, A. Fernández-Méndez, and A. Fernández-Sánchez, "Coagulation-flocculation as a pretreatment process at a landfill leachate nitrification-denitrification plant," *Journal of Hazardous Materials*, vol. 156, pp. 538-544, Jan. 2008.
- [33] S. Ghafari, H.A. Aziz, M.H. Isa, and A.A. Zinatizadeh, "Application of response surface methodology (RSM) to optimize coagulation-flocculation treatment of leachate using poly-aluminum chloride (PAC) and alum," *Journal of Hazardous Materials*, vol. 163, p. 650-656, Apr. 2009.
- [34] M. Rodríguez, U. Lugo, C. Rojas, and C. Malaver, "Evaluación del proceso de la coagulación para el diseño de una planta potabilizadora," *Umbral científico*, vol. 1, pp. 1692-3375, Nov. 2007.