

# Proteins from Natural Coagulant for Potential Application of Turbidity Removal in Water

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**Abstract:** Coagulation-flocculation followed by sedimentation and filtration is the most commonly used water treatment process, in which turbidity or particles removal is strongly dependent on proper coagulant dosage, effect of pH, effect of time, jar test and settling column tests were performed. In the method the seeds were treated with different solvents of NaCl and NaOH to extract the active coagulant agent has been investigated. In this study to evaluate the turbidity removal of the synthetic and real turbidity water has been investigated. The jar test was conducted on kaolin as a model wastewater. 0.5M Sodium chloride extract was found to provide a high turbidity removal of > 99% compared to NaOH and distilled water extract. The optimum turbidity removal at different values of initial synthetic wastewater turbidity from 100 to 500 NTU were investigated. The performance of natural coagulant after oil extraction was also investigated.

**Key words:** Coagulation, Oil extraction, Synthetic wastewater, Turbidity Removal.

## I. INTRODUCTION

The application of a coagulation/flocculation process is applied in water and wastewater treatment to remove turbidity color and natural organic matter. Chemical treatment techniques are effective for color removal but use more energy and costly than biological processes [1]. They also concentrate the pollution into solid or liquid it requires additional treatment of disposal [2][3]. Turbidity is the principle parameter, which is caused by the suspended matters or impurities, interfering with the clarity of the water. Positive correlation between turbidity and pathogens has been reported in the previous studies, and high residual turbidity in the treated water may promote the regrowth of pathogens in the distribution system, leading to the water borne disease outbreaks. Aluminum and iron salts are the chemicals most commonly used together with synthetic organic polymer. Moreover, some studies have reported that residual aluminum sulphate (alum) and polyaluminium chloride may induce Alzheimer's disease [4] [5]. Whereas the synthetic organic polymers, such as acrylamide have neurotoxic and carcinogenic effects.

A major problem in membrane filtration processes in water treatment plants is membrane fouling, which causes deterioration of both the quantity and quality of the treated water, reduces the membrane life and consequently results in higher treatment cost. A part of possible solution of these problems might be development of new coagulants, preferably natural and renewable sources which having safe for human health as well as

biodegradable. In recent years in the development of new coagulants which are preferably extracted from natural and renewable sources [6] [7], such as microorganisms, animals and plants. The numerous studies on a variety of plant materials which can be used as source of natural coagulants have been reported. For (e.g.) natural coagulants from *Moringa oleifera* (Drumstick) [8], Common bean (*Phaseolus vulgaris*) and Nirmali seed (*Strychnos potatorum*) have been investigated. The material which has received the greatest degree of attention is the seed of *Moringa oleifera*. *Moringa oleifera* [MO] seeds contain between 30-35% (w/w) of vegetable oil known as Behen (or) Ben oil. *Moringa oleifera* (Drumstick) treatment, due to the presence of water soluble cationic coagulant protein able to reduce the turbidity of the water treated [9]-[13]. The seed extracts of *strychnos potatorum* (Nirmali) contains the anionic polyelectrolyte, which contains carboxylic (COO<sup>-</sup>) and hydroxyl (OH<sup>-</sup>) as main active groups. The presence of divalent cations in can greatly increase the anionic polyelectrolyte to aggregate negative colloids. The crude extracts of common bean (*Phaseolus vulgaris*) seed showed to ability to act as a natural coagulant. The common bean seed has a food grade nature and contains no oil. The three agro based seeds *Moringa oleifera* (Drumstick)[14] [15], *Strychnos potatorum* (Nirmali), *Phaseolus vulgaris* (common bean) [16][17] are non-toxic and effective coagulant aids useful for removing turbidity and bacteria from water.

The mechanism of coagulation with MO that extracted with distilled water appears to consist of adsorption and charge neutralization of the colloidal charges. The active agents of the MO seeds have been determined to be cationic peptides that has molecular weight between 6-16 k-Da[18] Previous study reported that MO seed in dry form contained a significant amount of the active agent for coagulation. The *strychnos potatorum* seed (Nirmali) contains Anionic polyelectrolyte it is to remove the turbidity from drinking water. The common bean (*phaseolus vulgaris*) is food grade nature. It contains no oil. The three agro based seeds such as *Moringa oleifera* (Drumstick), *Strychnos potatorum* (Nirmali), *Phaseolus vulgaris* (Common bean) are used to remove the turbidity from drinking water [19]. The present investigation studied the ability of the different concentration of coagulant in jar test method, the effect of settling column test, effect of time, effect of pH, the effect of defatted crude oil extract, comparison of coagulant in alum and natural coagulant, the surface morphology structure of dried seed

powder and combined coagulant in seed powder and wastewater were studied and reported here.

## II. EXPERIMENTAL

### A. Materials

Sulphuric acid (98%), Glycerol (95%), Hydrochloric acid (35.4%), Ethyl alcohol (95%), Silver nitrate (99.5%) were obtained from Merck (India). Seeds were purchased from local markets.

### B. Instrumentation

Turbidity was measured using a turbidity meter (ELICO CL 52D NEPHELOMETER) and it was expressed in nephelometric turbidity units (NTU). pH was measured using a pH meter (ELICO LI 120 pH meter). Analytical instrument (ELICO PE 135 DO Analyser) was used to determine the Dissolved oxygen. Conductivity meter (ELICO CM 180) was to measure the water conductivity, Chloride and Sulphate concentration was determined according to standard titrimetric methods (APHA-2012), using (ELICO SL 159) UV-VIS Spectrophotometer. Morphologies of the air-dried flocks were examined and measured spectroscopically using a SEM (S-3500N, Hitach) under a 20kV voltage.

### C. Preparation of Synthetic Turbid water

In this study, synthetic turbid water was prepared by adding kaolin, in distilled water for all coagulation experiments. The kaolin suspension was prepared by dissolving 10g of kaolin powder in 1L of distilled water. The suspension was stirred slowly at 20rpm for 1h to achieve uniform dispersion of the kaolin particles. The suspension was then permitted to stand for 24 h to allow for complete hydration of the kaolin. This suspension was used as a stock solution for the preparation of water samples of varying turbidity for the coagulations tests. The initial pH was adjusted with 0.1M NaOH (or) 0.1M HCl to obtain desired values of turbidity and pH of the synthetic turbid water.

### D. Preparation of natural coagulants seed powder

Sample 1: Moringa oleifera  
Sample 2: Strychnos potatorum  
Sample 3: Phaseolus vulgaris

These husks of the three seeds were removed manually. Good quality seeds were selected and the kernels were ground to a fine powder (63-500 $\mu$ m) using an ordinary food processor. The seed powder was then used in each experiment.

### E. Extraction of active component from seed

Natural coagulants were obtained from three types of seed

Sample 1: Moringa oleifera  
Sample 2: Strychnos potatorum  
Sample 3: Phaseolus vulgaris

Seeds were ground and sieved through the sieve of pore size 0.4mm. 250mg of seed powder was blended with 1000ml of solvent using an ordinary food processor (Model BL333, Khind) for 2 min to extract the active

coagulating agent from the seeds. The solvents used were distilled water (DW), Sodium chloride (NaCl) and Sodium hydroxide (NaOH) solution. The solvent concentration selection was based on preliminary laboratory results. The suspension was filtered through a filter paper and the filtrate solution was used in a subsequent jar flock test. To prevent any ageing effects, such as pH and coagulation activity due to microbial decomposition of organic compounds during storage, fresh coagulants agent was prepared and used immediately for each sequence of experiments.

### F. Coagulation experiments

The jar test was performed to evaluate the performance of the coagulants agent extracted from the various processes as described above based on standard methods. Six 500ml beakers were filled with 200ml of turbid water and placed in the slots of a jar tester which was equipped with an illuminator. Varies dosages of seed extracts were added to each beaker and agitated for 4min at 100rpm for rapid mixing. The mixing speed was reduced to 40rpm for another 25 min. All the suspensions were then left for sedimentation, the clarified samples were collected from the top of the beaker and filtered using filter paper to remove any remaining sediment. The turbidity of each clarified sample was then measured using turbidity meter. The initial turbidity was studied from 100 to 500NTU and three types of solvent namely NaOH, NaCl and distilled water were employed. All the experiments were repeated at least twice for consistency and results averaged.

### G. Settling column test

Settling column tests were carried out using the optimum dosage of coagulants to see the turbidity removal efficiency, at different settling times. The effect of different types of coagulants used for the effective sedimentation, settlement characteristics of the turbid water of different type of initial turbidity samples; diameters of settling columns are also studied. The settling column test was carried out for no coagulant and for different types of coagulants such as Moringa oleifera [drumstick], Phaseolus vulgaris [Common bean] and Strychnos potatorum [Nirmali]. The optimum dosage found in the jar test was used in this settling columns test, depend upon the settling columns. Dose calculation required for that particular volume of a settling column was carried out. The optimum dosages are added in the settling column and at a constant time interval the samples are drawn from the sampling ports of settling column and their turbidity was measured. The measured turbidities of each sample from each sampling port at constant time are tabulated.

### H. Defatted crude oil extract

Defatted crude seed extract was prepared by mixing crude seed extract with 70% ethanol. A mass/volume ratio of 1:4 was used, where two grams of powder was mixed with 8mL of 70% ethanol for 20 minutes with a magnetic stirrer. The solution was left to stand still for approximately 1 hour where by the ethanol /oil phase

had separated from the coagulant phase and could be removed with a syringe. The defatted crude seed extract was spread on a plastic plate and left to dry for at least one night, until completely dry. Dried crude seed was there after ground with mortar and pestle to create a fine powder.

### III. RESULT AND DISCUSSION

#### A. Effects of using different solvents as the extracting agent of the coagulant from natural seeds

In order to study the improvement of the extraction process and hence the amount of coagulant agent extracted from the seeds, different molar concentration of NaCl and NaOH were used. The effect of using different NaCl concentrations on turbidity percentage removal of synthetic wastewater was shown in Fig.1. The investigation was conducted at pH 3 and at dosage of 120mg/l as determined in previous work. Turbidity removal was found to increase as the salt concentration increased until up to 0.5M. Similar trends were also observed for all other initial turbidity values of the synthetic wastewater studied. Since the coagulant agent was a protein, when the salt concentration increased, the solubility of the coagulation efficiency can occur, thus leading to a higher percentage of turbidity. Above 0.5M, the percentage of turbidity removal started to decrease as the NaCl concentration increased. This attributed to the salting out effect where by the solubility of the proteins decreases with salt concentration. The percentage removal increased as the initial turbidity increased M.O -0.5M NaCl showed a greater percentage reduction in turbidity removal using different concentrations of NaOH for extracting the coagulant agent from seeds at different coagulant agent of synthetic wastewater. Here the range of NaOH increased the coagulation efficiency of wastewater also increased until it reached an optimum level at 0.05M was shown in Fig. 2. After that, the percentage removal started to decrease. The decreasing in coagulation activity upon reaching the optimum level suggested that some protein may be denatured at NaOH concentration higher than 0.1M and hence this reduced the protein solubility in the crude extract solution. These results were similar to those obtained when NaCl as the extracting agent. In the determination of best solvent to be used for the extraction of the active coagulant agent from M.O seeds, the effects of M.O 0.5 NaCl and 0.5M NaOH were compared with M.O distilled water. Fig. 3, shows the turbidity removal of synthetic waste water using these three types of solvents. At 500 NTU M.O 0.5M NaCl could effectively coagulate more than 99% of the initial turbidity, while the M.O 0.5M NaOH provided 95% turbidity removal. Seeds extracted using NaCl demonstrated a greater performance compare to the other solvents. Fig. 4, shows that Phaseolus vulgaris seed shows the turbidity removal of synthetic wastewater using three types of solvent. At 500 NTU (Phaseolus vulgaris) P.V-DW and P.V 0.5M NaCl

could effectively coagulate more than 85% of the initial turbidity, while the P.V 0.5M NaOH provided 85% of turbidity removal. Seeds extracted using NaCl demonstrated a greater performance compare to the other solvents.

In Fig. 5 Strychnos Potatorum (S.P) seed shows the turbidity removal of synthetic waste water using three types of solvents. At 500 NTU S.P-DW and S.P 0.5M NaCl could effectively coagulate more than 95% of the initial turbidity, while the S.P 0.5M NaOH achieved 93% of turbidity removal. Seeds extracted using NaCl demonstrated a greater performance compared to the other solvents.

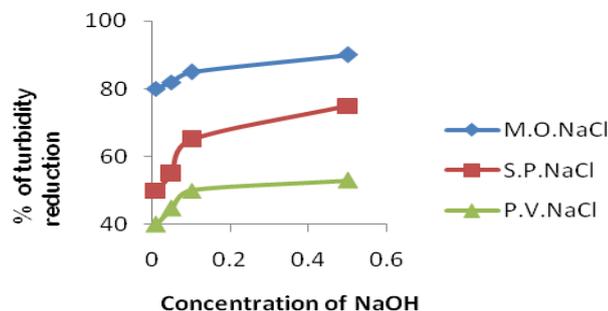
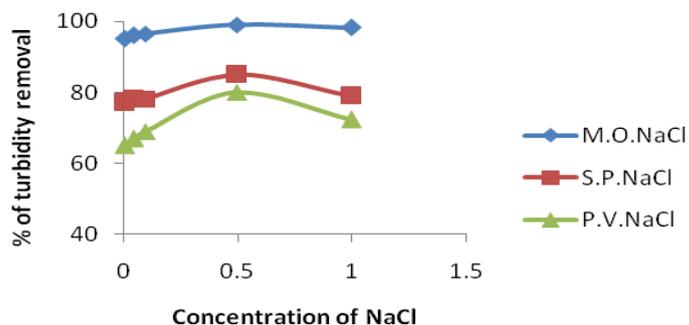


Fig. 1 & Fig.2: Effect of NaCl and NaOH concentration. (Experimental condition Different concentrations of NaCl and NaOH solution, pH=7.4)

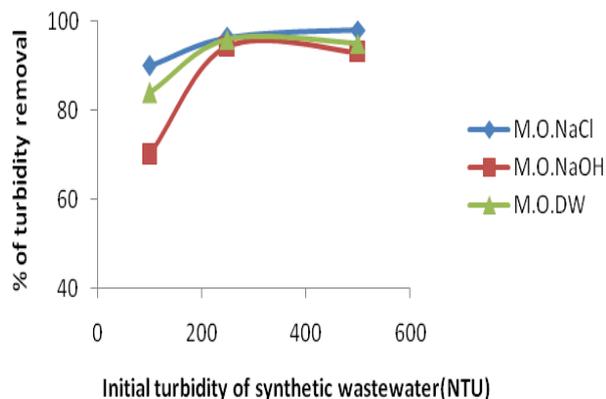


Fig. 3: Effect of Moringa oleifera seed coagulant (Experimental condition: NaCl concentration=0.5M, NaOH concentration=0.5M and distilled water, pH=7.4)

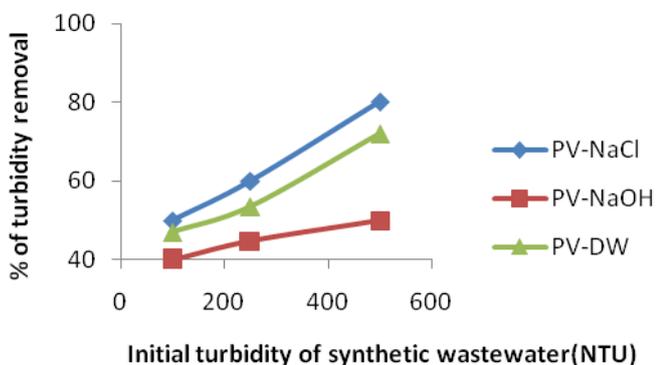
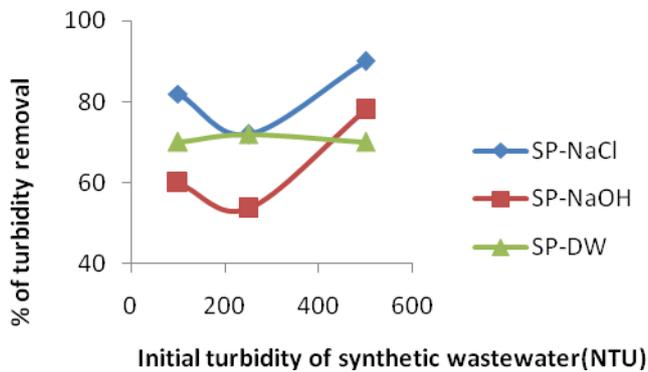


Fig .4, Fig .5: Effect of Phaseolus vulgaris seed coagulant and strychnos potatorum seed coagulant. (Experimental condition: NaCl concentration=0.5M, NaOH concentration=0.5M and distilled water, pH=7.4)

**B. Settling Column Test**

Settling column analysis was carried out using optimum dosage of coagulants. A Comparative study of turbidity reduction efficiency of different coagulants in different turbidity ranges are shown in Table 1. The settling column test was carried out for without coagulant and for different types of coagulants like Moringa Oleifera, Phaseolus vulgaris and Strychnos potatorum using the optimum dosage. The maximum turbidity removal efficiency obtained for no coagulant at 12 hrs retention time in the settling column for 100 NTU, 250 NTU and 500 NTU initial turbidity removal efficiency are as 71.44%, 75.10% and 78.06% respectively. The maximum turbidity removal efficiency obtained for Moringa oleifera at 12hrs retention time in the settling column, for 100 NTU, 250 NTU, 500 NTU initial turbidity removal efficiency are as 95.93%, 95.10% and 99% respectively. The maximum turbidity removal efficiency obtained for Phaseolus vulgaris at 12hrs retention time in the settling column for 100 NTU, 250 NTU and 500 NTU initial turbidity removal efficiency are as 77%, 85% and 87% respectively. The maximum turbidity removal efficiency obtained for Strychnos potatorum at 12 hrs retention time in the settling column

for 100 NTU ,250 NTU and 500 NTU initial turbidity removal efficiency are as 80%, 89% and 96% respectively. From all these values observed that maximum turbidity removal efficiency was obtained by Moringa oleifera. Then the overall removal efficiency decreases in the order of Moringa oleifera and Strychnos potatorum and then Phaseolus vulgaris and finally it is least for no coagulant.

**Table I: A Comparative study of turbidity reduction efficiency of different coagulants**

Coagulants	Dose used mg/l	% of turbidity reduction 100 NTU	% of turbidity reduction 250 NTU	% of turbidity reduction 500 NTU
Moringa oleifera	250	82.5	91.2	96.7
	500	80.7	88.4	94.2
	750	76.3	92.1	95.2
	1000	80.1	93.2	96.3
Phaseolus vulgaris	250	70.7	72.8	80.5
	500	66.5	80.9	85.8
	750	75.5	82.1	86.5
	1000	76.1	84.3	87.3
Strychnos potatorum	250	80.2	90.3	86.5
	500	85.2	86.8	84.4
	750	82.3	88.9	88.6
	1000	84.2	86.7	90.6

**C. Effect of equilibrium time**

The residual turbidity observed as a function of contact time for the range of natural coagulants used in this experiment. At a coagulant dose of 500mg/l the residual turbidity was observed to decrease as the contact time increased. The highest residual turbidity 500NTU for the 500mg coagulant dose occurred at a contact time of 12hrs. Fig.6 , shows that the percentage of turbidity removal in Moringa oleifera seeds were 98.2% Phaseolus vulgaris 96% and Strychnos potatorum was 86% of turbidity reduction takes place. Moringa oleifera coagulant to remove the turbidity of synthetic wastewater at short time compared to the other coagulants.

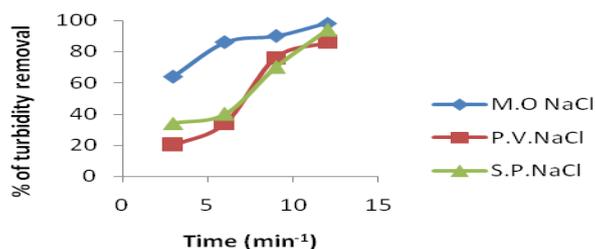
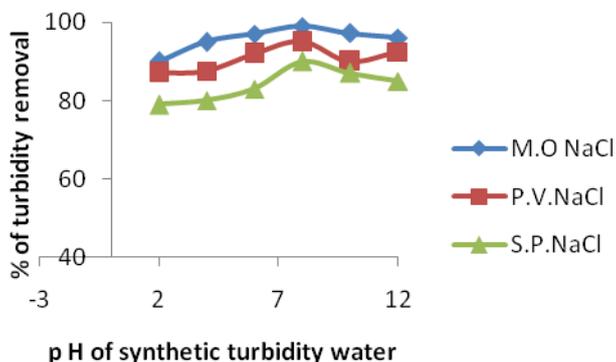


Fig . 6:Effect of time (Experimental condition: NaCl concentration=0.5M, Time =12 hrs, pH=7.4)

**D. Effect of pH and coagulant dosage on M.O-0.5M NaCl, P.V-0.5M NaCl, and S.P-0.5 M NaCl**

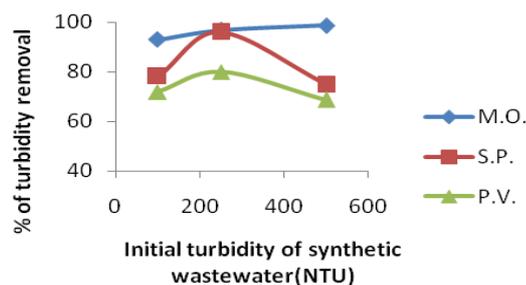
Fig.7, shows that the effect of pH on turbidity removal using M.O-0.5M NaCl, P.V-0.5M NaCl and S.P-0.5 M NaCl. As the pH increased the percentage of turbidity removal decreased. The highest turbidity removal using M.O-0.5M NaCl, P.V-0.5M NaCl, S.P-0.5M NaCl was observed to occur at pH 7 with a percentage of turbidity removal 99%, 95% and 90% respectively, while it is clear that pH changes do alter the final turbidity in terms of the overall removal, the changes are not appreciable and suggest that there is no reason to consider using Phaseolus vulgaris and strychnos potatorum at a pH 5 other than neutral. In comparison, M.O-0.5M NaCl exhibits better turbidity removal under alkaline conditions for which the percentage removal was on average greater than 95%.



**Fig.7: Effect of Ph (Experimental condition NaCl concentration=0.5M, Time =12hrs, various pH)**

**E. Defatted crude seed extracts**

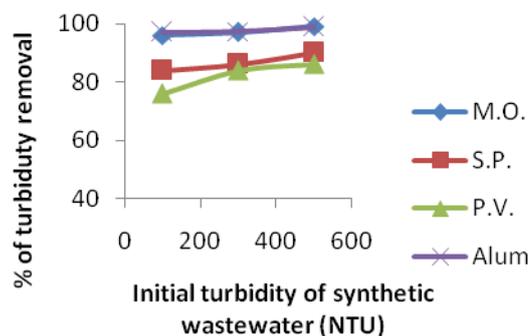
Coagulant solution of defatted Moringa oleifera, Strychnos potatorum and Phaseolus vulgaris was filtered once before a coagulation, flocculation test, with the consequence that the residual turbidity 100NTU, 250NTU, 500NTU were tested. Fig.8, shows that Defatted Moringa oleifera in distilled water seed extract to remove the turbidity of wastewater was greater than 96%.The strychnos potatorum seed shows that the turbidity reduction greater than 87%.The phaseolus vulgaris seed shows that the reduction of turbidity greater than 75%.



**Fig. 8:Effect of Defatted crude oil extract (Experimental condition: seed powder distilled water extract, pH=7.4)**

**F. A Comparison of the performances of aluminum sulphate and natural coagulants**

Fig. 9, which shows a typical set of data for aluminum sulphate and vegetable coagulant, such as Moringa oleifera, Strychnos potatorum and Phaseolus vulgaris the final plateau turbidity values were very similar although the dose of aluminum sulphate which was required to achieve this was greater than that of the Moringa oleifera and other coagulant. This was comparable with previous work which found that the natural coagulant gave a better performance than alum (20).



**Fig. 9: Effect of comparison of alum and natural coagulant (Experimental condition: seed powder weight=0.5g, pH=7.4)**

**IV. CONCLUSION**

It can be concluded from this study that natural coagulants technique can be a useful method for the treatment of turbidity water. This method is very simple, low equipments and investment. The influence of variables such as different solvent as extracting agent, settling column test, effect of equilibrium time, effect of pH and coagulant dosage, defatted crude seed extract, comparison of aluminum sulphate and natural coagulants, scanning of electron microscopy has been determined. Under these optimal conditions, The maximum turbidity removal efficiency obtained for Moringa oleifera at 12hrs retention time in the settling column, for 100 NTU, 250 NTU, 500 NTU initial turbidity removal efficiency are as 95.93%, 95.10% and 99% respectively. Turbidity values were very similar although the dose of aluminum sulphate which was required to achieve this was greater than that of the Moringa oleifera and other coagulant. The preliminary results made in this paper will be used as the basis for further investigations into the pilot and full scale treatment processes of this type of source water.

**REFERENCES**

[1] Y.Gao, Q.Y.Yue, B.J.Wang, Y.B.Chu, "Poly-aluminum – silicate-chloride (PASIC) – a new type of composite inorganic polymer coagulant," Colloids and Surfaces A: Physicochemical. Eng.Aspects pp. 121-127, July 2003.

[2] Wen Po Cheng , Fung Hwa Chi ,Chun Chang Li , Ruey Fang Yu, " A Study on the removal of organic substances from low-alkalinity water with metal-polysilicate

- coagulants,” Colloids and Surfaces A:Physicochem.Eng.Aspects pp.238-244 July 2007.
- [3] Wei –Lung Chou , Chin-Ta Wang , Shin-Yu Chang , “Study of COD and turbidity removal from real oxide-CMP wastewater by iron electro coagulation and the evaluation of specific energy consumption,” Journal of Hazardous Materials, Vol. 168, pp.1200-1207 , Sep 2009.
- [4] Gurusamy Annadurai, S.S.Sung, Duu-Jong Lee, “Simultaneous removal of turbidity and humic acid from high turbidity storm water,” Advances in Environmental Research , Vol.8, pp.713-725, Mar 2004.
- [5] Hassabia Zemmouri , Madani Drouiche , Amna Sayeh , Hakim Lounicia, Nabil Mameria, “ Coagulation Flocculation Test of Keddara’s Water Dam Using Chitosan and Sulphate Aluminum,” Proscenia Engineering , Vol 33 , pp.254-260, Mar. 2012.
- [6] P.Canizaries, C.Jimenez, F.Martinez, M.A.Rodrigo, and C.Saez ,The pH as a key parameter in the choice between coagulation and electro coagulation for the treatment of wastewaters,” J . Hazard.Mater, Vol 163, pp.158-164, Feb 2009.
- [7] A.Olesin, “Low technology water purification by bentonite clay and Moringa Oleifera seed flocculation as performed in Sudance villages: effects on Schist soma Mansoni Cerceriae. In: Water research, Vol.21, pp.517-522, May 1978.
- [8] M.R.Giddle, A.R.Bhalerao, Mr. C.P.Pise, “Turbidity removal by blended coagulant alum and Moringa oleifera” pp.18-20, Dec. 2009.
- [9] A.Ndbigengesere and K.S.Narasiah, “Quality of water treated by coagulation using Moringa oleifera seeds,” Water Res .Vol 32, pp.781-791, Mar.1998.
- [10] Krishnan, “Column Settling test for flocculation suspension,” Journal of Environmental Engg. Div., EEI,pp.227-229, Feb 1976.
- [11] J.Thomous, Overcamp, “Type II settling data analysis” Journal of Environmental Engg., ASCE, Vol.132, pp.137-139, Jan, 2006.
- [12] Zuria Z.Abidin, Nur S. Mohd Shamsudin, Norhafizah Madehi, Shafreeza Sobri, “Optimization of a method to extract the active coagulant agent from Jatropha curcus seeds for use in turbidity removal,” Vol.41, pp.319-323, May 2012.
- [13] Sonal Choubey, S.K.Rajput, K.N.Bapat, “Comparison of efficiency of some Natural Coagulants – Bioremediation,” Int.J. Of Emerging Tech.Adv. Engg. Vol 2, pp.2250-2259, Oct 2012.
- [14] Zuria Z.Abidin, N.Ismail, R.Yanus, I.S.Ahamad and A.Idris, “A preliminary study on Moringa oleifera as coagulant in wastewater treatment,” Environ. Tech. Vol.32, pp.971-977, July 2011.
- [15] J.Sanchen-Martain, K.Ghebremichel, J.Beltrain-Heredia, “Comparison of single step and two-step purified coagulants from Moringa oleifera seed for turbidity and DOC removal,” Bioresource Tech. Vol .101, pp.6259-6261, March 2010.
- [16] Mirjana G.Antov, Marina B.Sciban, Nada J.Petrovic, “Proteins from common bean (Phaseolus vulgaris) seed as a coagulant for potential application in water turbidity removal,” Bioresearch Tech. Vol .101, pp.2167-2172, Nov.2009.
- [17] A.Tepic, B.Vujicic, M.Vasic, and A.Lucic: “Amino acid and phytic acid in some Serbian varieties of Dry Beans (Phaseolus vulgaris); 2<sup>nd</sup> International congress on Food and Nutrition, Istanbul,” pp.24-26, Oct 2007.
- [18] M. Sciban, M.Klasnja, M.Antov, B.Skrbic, “ Removal of water turbidity by natural coagulants obtained from chestnut and acorn,” Bioresource . Technology, Vol .100, pp.6639-6643, July 2009.
- [19] T. Nkurunziza, J.B.Nduwayezu, E.N.Banadda, I.Nhapi, “ The effect of turbidity levels and Moringaoleifera concentration on the effectiveness of coagulation in water treatment,” Water Sci Technol . ,Vol 59, pp.1551-1558.
- [20] S.A.Muyibi and C.A.Okufu, “Coagulation of low turbidity surface water with Moringa oleifera seeds,” Int.J.Envirion.stud, Vol .48, pp.263-273, Feb 2007.