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Application of *Moringa oleifera* coagulant protein as natural coagulant aid with alum for removal of heavy metals from raw water

Mohammad Hadi Mehdinejad[a,*], Bijan Bina[b]

[a]*Environmental Health Research Center, Department of Environmental Health Engineering, Faculty of Health, Golestan University of Medical Sciences, Gorgan, Iran, Tel. +98 1732436107, email: hmnejad@yahoo.com (M.H. Mehdinejad)*
[b]*Department of Environmental Health Engineering, School of Public Health, Isfahan University of Medical Sciences, Isfahan, Iran, Tel. +983137923275, email: bbina123@yahoo.com (B. Bina)*

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ABSTRACT

Presence of aluminum residues in treated water has various health and environmental consequences such as neurotoxicity and possibly Alzheimer's disease. This study investigated the effects of coagulation/flocculation/sedimentation processes using alum as a coagulant in conjunction with *Moringa oleifera* coagulant protein (MOCP) as coagulant aid on alkalinity and hardness as well as removal of metal ions and turbidity from turbid water. In this study, MOCP was synthesized and used as a new method of treating turbid water. A conventional jar test apparatus was utilized for the tests. Optimal dosage for MOCP was found at pH of 7–7.5 for all turbidities. Maximum turbidity removal of 99% was achieved. MOCP significantly reduced the required dosage of alum. The values of TOC in treated water in low, medium and high turbidity were 0.55, 0.5 and 0.65 mg L^{-1}, respectively. The efficiency of metal ion removal in the present study was as follows: $Fe^{2+} > Cu^{2+} > Zn^{2+} > Mn^{2+}$. We demonstrated that this method is an efficient approach for optimization of the coagulation-flocculation process in the treatment of raw water.

Keywords: Coagulant; Metals removal; *Moringa oleifera* coagulant protein (MOCP); Water treatment

1. Introduction

Surface water sources traversing urban areas contain elevated levels of dissolved and suspended materials including organic and inorganic particles and biological organisms from domestic and industrial wastewater and soil erosion. The suspended materials in water are undesirable because they provide attachment surfaces for pathogenic microorganism as well as toxic substances such as heavy metals [1]. Production of potable water from raw water usually requires a coagulation/flocculation process for removal of turbidity in form of suspended and colloidal material [2]. Coagulation is one of the most popular and efficient treatment processes in which coagulants are added to raw water to remove suspended solids and turbidity via several mechanisms such as charge neutralization and sweep coagulation [3]. Charge neutralization is performed since coagulants are usually positively charged, and colloidal particles are normally negatively charged [4]. Use of aluminum salts has raised concerns about the presence of residuals in the treated water and risk of Alzheimer's disease [5]. Although iron salts are cheaper alternatives, the high cost of importing chemicals can cause serious financial problems for developing countries. Another problem is the reaction of alum with natural alkalinity in the water that may reduce pH and efficiency of coagulation in cold waters. Due to disadvantages similar to the case of aluminum salts, other coagulants such as ferric salts and synthetic polymers also limited success [6]. Thus, development of natural coagulants, such as *Moringa oleifera* (MO) and chitosan has received considerable attention in recent years. Utilization of natural coagulants has led to a significant reduction in chemicals and sludge handling costs. MO from the genus *Moringaceae* is a drought-resistant tree native to North

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*Corresponding author.

India. The plant grows in hot semi-arid areas with annual rainfall of 250–1500 mm, and in humid areas with annual rainfall of >3000 mm [7]. Moringa contains several nutrients and essential phytochemicals in its leaves, pods, and seeds [8]. The organic and nutrient release from the seed can be avoided either by purifying the coagulant component of MO or by removing the released substances from the water. The active components of MO seeds are soluble cationic proteins and peptides with a molecular weight ranging from 6–16 kDa and isoelectric pH values around 10 [5]. Its high positive charge (point Isoelectric >10) and small size suggests that adsorption and charge neutralization could be the main destabilization mechanism [9,10]. *Moringa oleifera* cationic protein (MOCP) has a small molecular mass with a high surface charge. The MO seed powder is stable, highly water-soluble, and non-toxic to humans and animals. The protein powder remains active in coagulation even after one year of storage in plastic bottles with no special precaution. They are also efficient in reducing turbidity and microorganisms from raw waters in water softening and sludge conditioning. Thus, this purified protein has far more desirable applications in the water and wastewater treatment industries [6].

Industrial effluent, sewage sludge, and landfill leachate can be considered as secondary sources of materials, especially metals [11–13]. Heavy metals are elements with atomic weights ranging from 63.5 to 200.6 g mole^{-1} and specific gravity higher than 5. Since these non-biodegradable and non-thermodegradable elements are extremely persistent in the environment, they can easily accumulate to toxic levels. Accumulation of heavy metals to toxic levels in soil is due to the long-term application of wastewater and fertilizers [14]. According to the US Environmental Protection Agency (EPA), concentrations of Fe, Cu, Mn and Zn in drinking water should not to exceed 0.3, 1.3, 0.05 and 5 mg L^{-1}, respectively. Even at low concentrations, heavy metals can be toxic to organisms, including humans [15]. Heavy metals are widely present in water and sediments resulting from chemical leaching of bedrocks, water drainage, bank's runoff, and urban industrial and rural agricultural wastewaters [16]. Although many studies have investigated the application of MO coagulant in water treatment, limit information is available on the effects of alum in conjunction with MOCP on removal of turbidity and heavy metals [8,10,17]. Therefore, cost-effective and more environmentally friendly materials for removal of heavy metals from contaminated water and soils are required. The present study aimed to evaluate synthesis of MOCP and its applicability as a natural coagulant in conjugation with alum in the water treatment process.

The main objective of this paper was to investigate the coagulation/flocculation/sedimentation process's performance for removal of turbidity, metal ions and hardness from turbid waters by MOCP in conjunction with alum.

2. Materials and methods

2.1. Preparation of artificial water

First, 10 g of kaolin was suspended in 1 L of distilled water. The suspension was stirred vigorously for 1 h at 200 rpm to disperse kaolin. The suspension was allowed to stand for 24 h for complete hydration of the kaolin. This kaolin suspension was used as the stock solution for preparation of artificial water samples with various turbidities for the coagulation tests (Table 1). Three groups of turbidities were used , including low turbidity (10–20 NTU), medium turbidity (100–120 NTU) and high turbidity (200–220 NTU) [2,18]. A stock solution of alkalinity was prepared using 16.8 g Na_2HCO_3 (Merck) and 1 L distilled water, mixed by a magnetic stirrer, resulting in a concentration of 10000 ppm (alkalinity). One mL of this solution contained 10 mg of alkalinity as calcium carbonate.

2.2. Preparation of alum solution

Alum solution was prepared by dissolving 10 g alum or aluminum sulfate [$Al_2\,(SO_4)_3{\cdot}18H_2O$] in 1 L of distilled water. After increasing the solution volume to 1 L, every 1 mL of the stock solution was equivalent to 10 mg mL^{-1}.

2.3. Synthesis and purification of MOCP

MO pods were collected from Sudan and kept at room temperature. Seeds were shelled just before extraction, and the kernel was powdered using a kitchen blender. Oil was removed by mixing the powder in 95% ethanol (5–10%, w/v) for 30 min [19]. Solids were separated by centrifugation and dried at room temperature. From the dried samples, 5% (w/v) solutions were prepared using distilled water, NaCl solution or ammonium acetate buffer by stirring for 30 min and filtration through Whatman papers No. 3 and 0.45 µm fiberglass. The filtrates are termed as crude extracts [20]. MOCP was purified from the crude extracts by ion-exchange (IEX) column chromatography and bath adsorption. IEX chromatography was carried out in a 1 mL HiTrap CM sepharose fast flow cation exchange column on an akta explorer. The column was equilibrated with ammonium acetate buffer (10 mM, pH 6.7). The optimum ionic strength of NaCl for elution was 0.6 M. Multiple step elusions were carried out to remove the non-coagulant adsorbed protein. All non-coagulant adsorbed proteins were removed using 0.3 M NaCl followed by 0.6 M NaCl, indicating that elution can be performed using 0.6 M NaCl solution [21]. In the ion-exchange column experiment, adsorption equilibrium was achieved after 90–120 min where maximum adsorption of the coagulant protein was observed. Cationic proteins were absorbed and retained in the column, and later eluted from the column using the 0.6 M NaCl solution. The eluted solution was re-dialyzed through the membrane to remove salts. Finally, the solution was lyophilized to obtain a stable white protein powder [2].

Table 1
The composition of the artificial water

Component	Concentration
Alkalinity (mg/L_{CaCO3})	100 ± 20
Magnesium hardness (mg/L_{CaCO3})	50 ± 10
Calcium hardness (mg/L_{CaCO3})	50 ± 10
pH	7 ± 0.5

2.4. Preparation of heavy metals and metal ion concentrations

All reagents and chemicals used in this study were of analytical grade and were obtained from Merck India Ltd. The metal salts including $(NH_4)_2$ $Fe(SO_4)_2{\cdot}6H_2O$, $MnSO_4{\cdot}H_2O$, $ZnSO_4{\cdot}7H_2O$ and $CuSO_4{\cdot}5H_2O$ were used. Double distilled water was used for the preparation of the metal ions solution for the experiments. Later, 1000 mg L^{-1} stock solutions of Fe^{2+}, Mn^{2+}, Cu^{2+} and Zn^{2+} were prepared. Each element was prepared to five different concentrations from 1 to 10 mg L^{-1}.

2.5. Experimental procedures

Jar testing was carried out in a standard jar test apparatus (Phipps and Bird) with six beakers for the coagulation/flocculation process. Synthetic water containing metal ions was prepared by amending the metal ion concentration by addition of the required quantity of the metal salt to fresh water with low, medium, and high turbidity. One liter of the sample was poured into each beaker. The apparatus was working at speed of 100 rpm. The desired doses of alum were added simultaneously, and mixed vigorously for 2 min. The mixing speed was reduced to 40 rpm for 7.5 min and then 20 rpm for 7.5 min. In the next step, different doses of MOCP (mg/L) (as coagulant aid) were added. After settling for 30 min, samples were withdrawn with a pipette from 2 cm depth of the beakers, and residual turbidities were determined. Throughout all experiments, pH was accurately checked by a digital pH meter (EUTECH, 1500) [2]. The calibration of the pH was carried out with standard buffer solutions of pH 7 and pH 10. Coagulated water samples were filtered through a 0.45 mm membrane (Whatman filter paper No.42) to reduce interfering factors such as turbidity [22,23]. The samples were stored in plastic bottles at 4°C until analysis. The samples were analyzed for turbidity, aluminum, iron, manganese, copper, and zinc using the standard methods (APHA, 1998). The turbidity of the samples was measured by a turbidimeter (HACH, 2100P). AAS (Atomic Absorption Spectrometer, Perkin Elmer) was used to determine heavy metals.

2.7. Statistical analyses

All statistical analyses were performed in SPSS (Version 18) at a significance level of 0.05. Independent sample t-test was used to evaluate differences between the means. Correlation between two variables was analyzed using bivariate analysis of variance.

3. Results and discussion

3.1. Determination of optimal pH value

A standard jar-test procedure was used in order to determine the optimal pH value. Experiments were performed as described previously, at pH of 5 to 8 using a coagulant concentration and coagulant aid (Fig. 1). Alum in conjunction with MOCP caused a notable reduction in turbidity only at pH of 7 and 7.5. The final turbidities showed large variations between experiments at pH values close to 5.5–8. The maximum efficiency was observed at pH of 7–7.5. At these pH values, the residual turbidities reached 5 NTU for all turbidities. The white flocs obtained were coarse and settled almost completely in less than 30 min. The lower removal of the studied metal ions below the optimum pH values can be attributed to the effective competition between high concentrations of H^+ and metal ions. In the coagulation process, alum can produce acid in the water that will reduce the pH of solution when there is not enough alkalinity. Therefore, the alkalinity was sufficiently added to synthetic water samples to overcome this issue (Table 1). The zeta potential determines the mechanism of coagulation, which depends on the electrostatic forces between charges carried by the colloidal particles. Many studies have been carried out to determine the zeta potential of kaolin suspensions and MO. Ndabigengesere et al. showed that the zeta potential of the synthetic water was about –46 mV. This means that at a pH of around 7, the kaolin particles were negatively charged [6]. Hence, coagulation of the kaolin suspension using Moringa is caused by the destabilization of negatively charged colloids by cationic polyelectrolytes. Baptista et al. reported that neutralization could be one of the possible mechanisms of coagulation/flocculation. They showed that the zeta potential of MOPC was 6.96 mV at pH of 6.67 [24]. Considering the aforementioned findings, it seems that the primary mechanisms are adsorption and neutralization of charges in the coagulation process by MO [21,24,25].

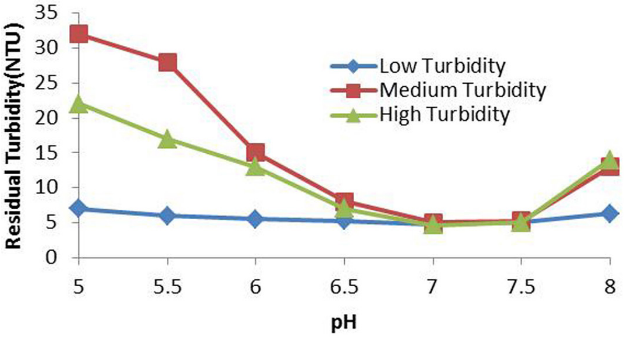

Fig. 1. Optimum pH for alum in conjunction with MOCP (shaking rate = 100 rpm and 40 rpm; rapid mix time= 2 min; slow mix time = 15 min; settling time = 30 min).

3.2. Optimization of MOCP as a coagulant aid in conjunction with alum

The primary goal of the coagulation-flocculation process is turbidity removal. Adding a coagulant aid may be necessary if the efficiency of coagulant alone is not satisfactory. MOCP was used as the coagulant aid to decrease residual Al^{3+} concentrations in treated water, and possible adverse health effects of aluminum in drinking water. Results of optimization of MOCP in conjunction with alum for low, medium, and high turbid water are shown in Fig. 2. The optimum dose of alum in conjunction with MOCP were 15 and 3 mg L^{-1}, 15 and 5 mg L^{-1}, and 17.5 and 1 mg L^{-1} at low, medium and high turbidities, respectively. Residual turbidity was reduced to below 5 NTU without filtration, irrespective of initial turbidity, which is less than the required drinking water standards [26]. The

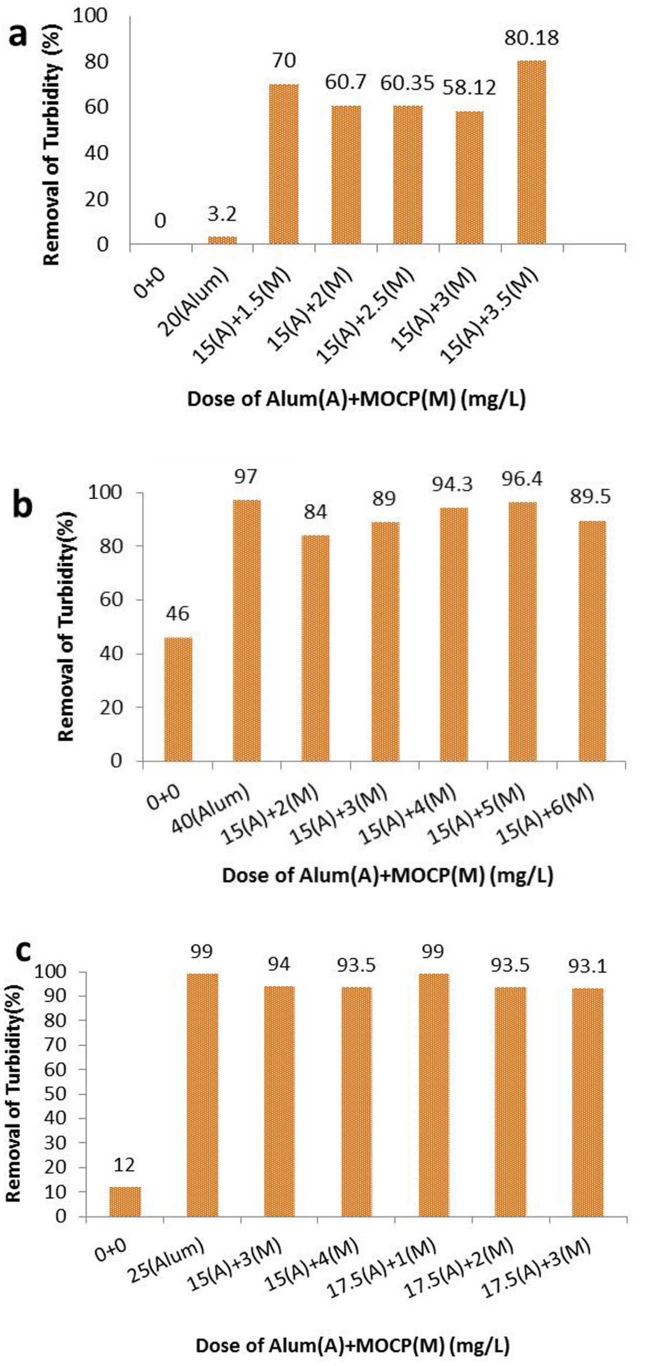

Fig. 2. Optimum dose of alum in conjunction with MOCP: (a) low turbidity = 15 NTU; (b) medium turbidity = 110 NTU; and (c) high turbidity = 200 NTU (pH = 7–7.5; shaking rate = 100 rpm and 40 rpm; rapid mix time = 2 min; slow mix time = 15 min; settling time = 30 min).

flocs formed at low turbidities were significantly less and smaller than the flocs formed at medium and high turbidities. Thus, fewer amount of MOCP were used at low turbidity. It has been suggested to add the coagulation aid one minute after addition of alum. Poor performance was achieved when coagulant aid and alum were added simultaneously.

The obtained results reveal that different doses of MOCP and alum affected the efficiency of coagulation and consequently, the turbidity of settled water. The formed flocs at low turbidities were significantly smaller when compared to those formed at medium and high turbidities. On the other hand, increasing the dose of coagulant and coagulant aid did not improve the efficiency of turbidity reduction. The coagulation/flocculation process is carried out by different mechanisms. The number of particles in medium and high turbidities is more than low turbidity. The electrical double layer compression usually occurs at the high ionic strength, which is named as salting out the colloid. In this condition, electric-double layer and repulsive energy are compressed so long as there is not the energy barrier in the solution. After that, the colloids are affected by Van der Waals forces, resulting in the flocculation or orthokinetic coagulation. Therefore, due to aforementioned facts, the settling of particles was happened without any coagulant (Fig. 2).

Alum is a positively charged inorganic coagulant. When used alone, several mechanisms, such as charge naturalization and sweeping are involved in turbidity removal. The hydrolysis of AS leads to the formation of Al(OH)$_2^+$ and Al(OH)$^{2+}$, which react with colloidal argillaceous particles by neutralization of electrical charges. An exchange occurs between the hydrolyzed species of aluminum and the exchangeable cations of clays (mainly Ca^{2+} for bentonite clays). The colloidal particles with decreased or neutral surface charge can then agglomerate under the effect of stirring. Hydrolysis of aluminum can lead to the formation of an aluminum hydroxide Al(OH)$_3$ precipitate, which has a large surface area. Thus, it entraps colloids in the suspension and facilitates liquid–solid separation during the settling phase. This is the mechanism of flocculation by sweeping [18]. When alum is underdosed, the strongly negatively charged particles repel each other, leading to high residual turbidity. As more coagulants are added, electrostatic forces began to minimize by charge naturalization and adsorption processes, leading to formation of more flocs and a decrease in turbidity. In contrast, when alum is used in conjunction with MOCP, the coagulation mechanism of MOCP seemed to be an sweeping coagulation by the insoluble matters formed by the coagulation active component in MOCP and also intraparticle bridging. This is in line with the findings of Okuda et al. [27]. MOCP has higher capability for agglomeration and flocculation of particles compared with alum. On the other hand, for clays with a low exchange capacity like kaolinites, the flocculation mechanism by sweeping dominates at pH range of 7–8.5 [28]. Our results showed that the values of alum decreased about 87.5% and 96% in medium and high turbidities, respectively. In addition, the turbidity removal efficiency was about 97% and 99% in medium and high turbidities, respectively. Similar results were achieved when using alum alone (Fig. 2b, Fig. 2c). Thus, alum was used in combination with MOCP to reduce residual aluminum to less than 0.2 mg L^{-1}. The removal efficiency of alum combined with MOCP was significantly different in various turbidities ($P < 0.01$). At high turbidity, the removal efficiency of turbidity improved significantly, the supernatant was clear after about 20 min settling, flocs were larger, and settling time was shorter.

The alkalinity and pH of the water did not change during the treatment processes. Alkalinity remained almost constant in the case of MOCP, whereas in the case of alum alkalinity decreased from 100 mg $CaCO_3$ L^{-1} to 50 mg $CaCO_3$ L^{-1}. According to the results, it is not necessary to add any other chemicals when MOCP is used. Therefore, MOCP and alum mixture could be considered as a suitable alternative for partial replacement of alum as a coagulant in the surface water treatment. In addition, MOCP is a natural chemical agent with less or no side effect compared to alum. After jar tests, pH of the treated water was 7.2 ± 0.5 and alkalinity was 100 ± 20 mg $CaCO_3$ L^{-1}. The flocculation and settling time was found to be less than 40 min. Moreover, MOCP significantly reduced the required dosage of alum by 25–62.5% compared to when the coagulant of alum was used alone (Fig. 2). The results showed that the amount of residual Al^{3+} in low, medium and high turbidities were between 0.05 to 0.2 mg L^{-1}. Our results meet the US EPA standards. Thus, use of MOCP can decrease costs of water treatment, and residual aluminum decreases in treated water.

3.3. Determination of total organic carbon (TOC)

The use of natural coagulants in water treatment allows entry of carbon-organic substances into treated waters. The shelled and non-shelled *M. oleifera* seeds tend to increase organic matter considerably in the treated water and after the coagulation process, some of the organic matter remains in the treated water, thus implementation of MOCP as a coagulant aid help reduce the organic matter in treated water. As seen in table 2, after treating turbid water, TOC concentrations in low turbidity, medium turbidity, and high turbidity were 0.55, 0.5 and 0.65 mg L^{-1}, respectively. Increasing the concentration of MOCP as a coagulant aid caused negligible changes in the TOC of treated water. In the other words, components of MOCP have not any organic matter due to the purification of the produced raw extract of Moringa oleifera seed [21].

The results showed that MOCP could be used as natural coagulant aid for drinking water treatment with minimal risk of organic contamination [29]. Furthermore, there was no statistically significant correlation between the initial concentration of MOCP in different turbidities with increase of TOC values ($P < 0.05$).

3.4. Effect of the coagulation process by alum in conjunction with MOCP on hardness removal

As seen in Fig. 3, the rate of hardness reduction was higher at high turbidity and efficiency of hardness removal decreased with increasing the hardness. The maximum rate of hardness removal (99%) was achieved by MOCP at high turbidity. The presence of bivalent cations such as Ca^{2+} and Mg^{2+} in water can significantly increase ion strength of the solution and increase destabilization of colloidal particles. This is due to the existence of the two coagulation mechanisms. With high turbidity and high coagulant doses, coagulation occurs both by adsorption-destabilization and sweep-floc mechanisms [30]. Statistical analysis showed significant differences in hardness removal by MOCP at different turbidities ($P < 0.05$). Inconsistent with the results of the present study, study of Muyibi and Evison showed that MO is capable of softening hard water. However, another study reported that the optimum dosage for turbidity removal may be lower than that for water softening [6].

3.4. Effect of coagulation process by alum in conjunction with MOCP on removal of metals ions

3.4.1. Fe^{2+}

Presence of high concentrations of this ion in groundwater is a common water purification problem worldwide. In this study, removal capability of alum in conjunction

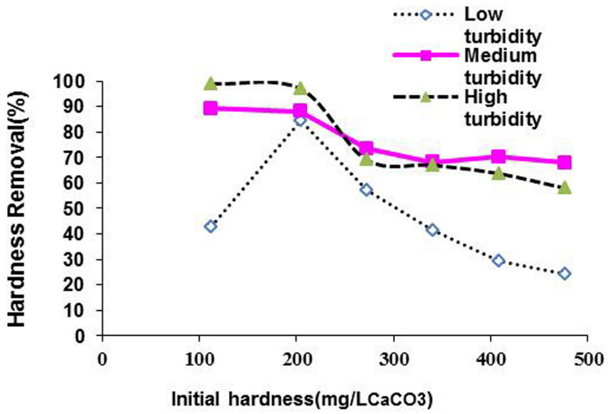

Fig. 3. Effect of alum in conjunction with MOCP on hardness removal.

Table 2
The values of released TOC by using of alum in conjunction with MOCP in treated water after the coagulation, flocculation and sedimentation processes

Types of turbidity (NTU)	Dosage of alum (mg/L) in conjunction with MOCP (mg/L)	TC (mg/L)[1]	IC (mg/L)[2]	TOC (mg/L)[3]
Low turbidity	15 (Alum) + 3 (MOCP)	17.8	17.25	0.55
Medium turbidity	15 (Alum) + 5 (MOCP)	17.3	16.8	0.5
High turbidity	17.5 (Alum) + 1 (MOCP)	19.7	19.05	0.65

1 = Total Carbon ; 2 = Inorganic carbon ; 3 = Total Organic Carbon
(Low turbidity = 15 NTU, Medium turbidity = 110 NTU, High turbidity = 200 NTU; pH = 7–7.5;
Shaking rate = 100 rpm and 40 rpm; rapid mix time = 2 min; slow mix time = 15 min; settling time = 30 min)

with MOCP at initial Fe^{2+} concentrations of 0.3, 0.5, 1, 2 and 5 mg L^{-1} have been investigated, and the results are summarized in Fig. 4a. Metal removal of up to 98% was recorded for 5 mg L^{-1} Fe^{2+} at high turbidity, and 13.3% for 0.3 mg L^{-1} Fe^{2+} at low turbidity. Our results did meet the US EPA drinking water standards since residual Fe^{2+} ions were lower than 0.3 mg L^{-1} at all turbidities and initial concentrations [26]. Final pH value of 7.2 ± 0.5 was obtained for all steps. Interestingly, removal of Fe^{2+} from surface water via the coagulation process has been found to be considerably more efficient than other metal ions. The removal efficiency also increased with increasing the initial concentrations and turbidity. This improved efficiency for Fe^{2+} removal from water can be attributed to coagulation/flocculation/sedimentation mechanisms. Two main mechanisms are generally considered; adsorption and neutralization for low turbidity and sweeping for high turbidity. Okuda et al. reported that the active component from an aqueous salt extraction was not a protein, polysaccharide or lipid, but an organic polyelectrolyte with a molecular weight of about 3.0 kDa [27]. Ndabigengesere et al. purified the coagulant proteins from MO seeds. They observed that under non-reducing conditions, the active agents of coagulation are dimeric cationic proteins with molecular weight of approximately 13 kDa and an isoelectric point between 10 and 11, but under reducing conditions, the molecular weight was found to be 6.5 kDa. They suggested that the active protein is actually composed of two 6.5 kDa subunits, connected with an S-S bond that is cleaved when protein extraction occurs in reducing conditions, and that the mechanism of coagulation with MO consists of adsorption and charge neutralization of the colloidal charges [21]. Thus, it is more likely that adsorption and charge neutralization are the mechanism of interaction between the MO proteins and heavy metals. The MO seed powder has been considered as a potential heavy metal-removing agent because of its oxygen and nitrogen - donating carboxylate and amino groups. MO seed powder extraction with salt increased the removal efficiency. The adsorption of metals using MO is limited to the surface adsorption. This is because MO is a cationic polyelectrolyte with a short chain and low molecular weight. The adsorption of heavy metals is through positive metal ions that form a bridge between the anionic polyelectrolyte and negatively charged protein functional groups on the colloidal particle surface. Structure of polyelectrolyte coagulant aids consists of repeating units of small molecular weight forming molecules of colloidal size that carry electrical charges or ionisable groups, which provide a bonding surface for the flocs [21,31]. In the coagulation process, metal ions react with proteins and destroy them in water. Metal adsorption occurs due to the high protein content of the seeds. The seeds have the capability to adsorb metal cations and attract highly toxic compounds. By increasing positive charges in the solution, sweeping mechanism dominated in the solution and metal ions swept in colloid-polymer flocs and then settled. Moreover, the little solubility and low K_{sp} are affected by the above phenomenon. Chaturvedi et al. showed the favorable potential of coagulants for removal of metal ions from polluted waters [32]. There is currently no information about the removal of heavy metals with MOCP, but limited information is available on MO seeds. Shan et al. reported that heavy metals, such as Fe are fully eliminated from wastewater by applying MO seed [33]. Based on the results of paired t-test, there were statistically significant associations between turbidity removal and Fe^{2+} removal and between initial concentration of Fe^{2+} and Fe^{2+} removal ($p < 0.05$).

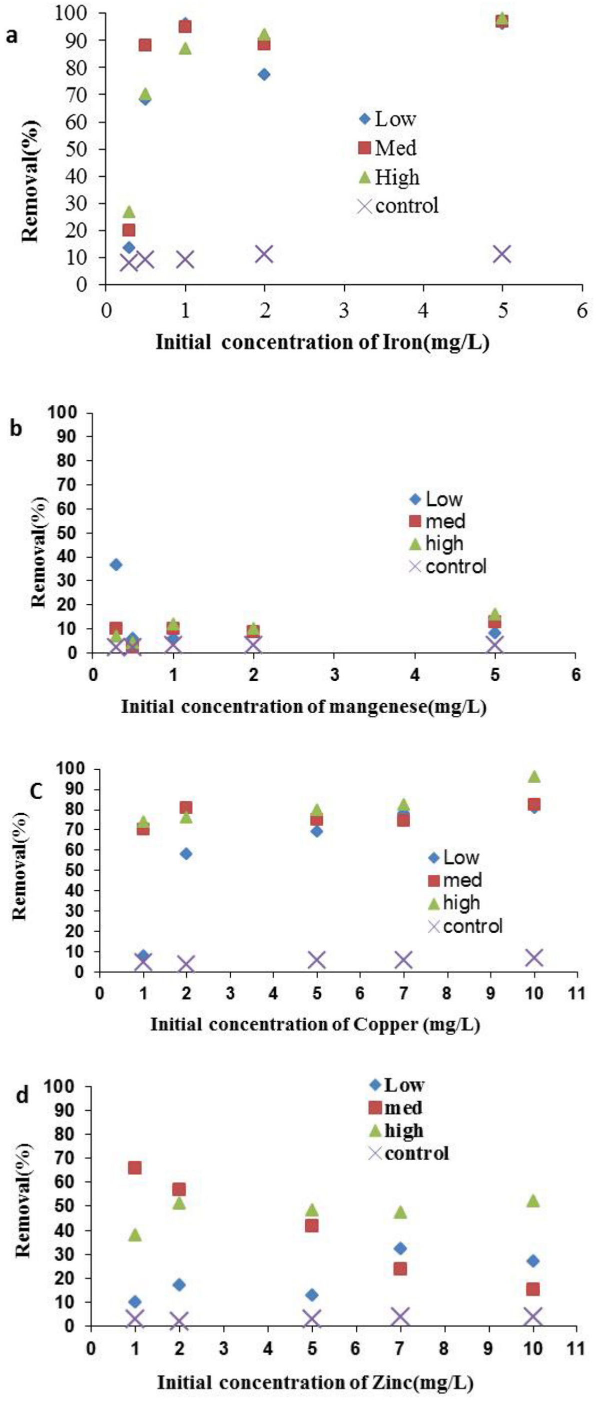

Fig. 4. Effect of coagulation using alum in conjunction with MOCP on metal ions removal; (a) Iron; (b) manganese; (c) copper; and (d) zinc, (Optimum dose of alum in conjunction with MOCP at low turbidity: 15 and 3 mg L^{-1}, medium turbidity: 15 and 5 mg L^{-1} and high turbidity: 17.5 and 1 mg L^{-1}; pH = 7–7.5).

3.4.2. Mn^{2+}

The mean of removal efficiency was very low at about 6.3% for all turbidities. The results showed that concentration of residual Mn^{2+} ion was higher than the US EPA drinking water standard (0.05 mg L^{-1}). Final pH value of 7.2 ± 0.5 was obtained for all steps. This was most probably due to the increase in the total surface area, which provided more sorption sites for the metal ions. Larger and smaller sized metals were the highest and the least removed, respectively [34]. The coagulant in the seed is a protein that acts as a cationic polyelectrolyte. The soluble particles in the water attach to the active agent, binding them together and creating large flocs in the water. This study showed that manganese ions removal was less efficient compared to iron, copper and zinc. It can be concluded that the low manganese removal rate could be attributed to the small size of ions and the high solubility product constant. Overall, the behavior of Mn^{2+} ions in the process was unexpected. The removal rate of Mn^{2+} ions decreased when increasing the initial ion concentration. Based on the results of paired t-test, there was no significant relationship between the initial concentrations of Mn^{2+} and efficiency of removal ($P > 0.05$), but there was a significant correlation between turbidity removal and Mn^{2+} removal ($P < 0.05$).

3.4.3. Cu^{2+}

As shown in Fig. 4c, copper removal efficiency was 86.3% at high turbidity for an initial concentration of 10 mg L^{-1} and 8% at low turbidity for an initial concentration of 1 mg L^{-1}. At high initial concentrations ($Cu^{2+} > 5$ mg L^{-1}), Cu^{2+} residual level was found to be higher than the US EPA drinking water standard limit (1mg L^{-1}). Final pH value of 7.2 ± 0.5 was obtained for all steps. Mean of Cu^{2+} removal was 71.7%, which was directly proportional to the turbidity and initial concentration. The highest removal efficiency was related to Cu^{2+} followed by iron. Efficiency of Cu^{2+} removal by alum and MOCP increased after increasing the concentration of metal ions, which could be due to quick binding of the positively charged Cu^{2+} ions onto the negatively charged functional groups on the coagulant aid surface. The flocs size improved when MOCP was used as the coagulant aid. Thus, it is expected that the dominant mechanism was neutralization followed by adsorption [31]. Among the three copper oxidation states, Cu^{2+} is more common, toxic, carcinogenic and difficult to remove. This toxic metal is commonly found in contaminated water, and exerts adverse effects on the environment and human health. Conventional techniques for removal of such toxic metals from wastewater mainly include ion exchange, membrane filtration, chemical precipitation, adsorption, coagulation/flocculation and reverse osmosis. In study of Nand et al., the Cu^{2+} removal rate by Moringa seeds was 90% [35]. Shan et al. stated that heavy metals, such as Cu can be removed by up to 98% when using *M. oleifera* seeds [33]. We found a statistically significant correlation between Cu^{2+} removal efficiency and the initial concentration ($P < 0.01$).

3.4.4. Zn^{2+}

The effects of initial Zn^{2+} concentration and turbidity are illustrated in Fig. 4d. The average removal efficiency was 36.7%. Increasing the level of initial concentration from 1 to 10 mg L^{-1} enhanced the removal efficiency at low and high turbidities. The maximum removal rate was achieved at medium turbidity (69% for initial concentration of 2 mg L^{-1}). However, our results did not meet the US EPA drinking water standards, and residual Zn^{2+} ions were higher than 5 mg L^{-1} at high initial concentrations. Final pH value of 7.2 ± 0.5 was obtained for all steps. Our study showed that higher ion concentrations could produce more positive charge in water when the adsorption sites are not sufficient. Therefore, the metal ions could not bind to the present composite, resulting in presence of the metal ions as residuals in water. This is in agreement with the study of Bhatti (2007), which showed that the removal efficiency decreases by increasing the initial concentrations of metals. In fact, at low metal ion concentrations, the ratio of sorptive surface area to the total metal ions available is higher, resulting in an increased possibility for metal removal [36]. Although there are other methods such as electrocoagulation, activated carbon, and ion exchange for heavy metal removal from water and wastewater, but use of MOCP seems more applicable. Nand et al. have shown that the rate of zinc removal by Moringa seeds was 50% [35]. We found no significant association between the initial concentration of Zn^{2+} and removal efficiency ($P > 0.05$).

4. Conclusions

The following conclusions have been drawn from the results of the present study:

- Synthesis and purification of MOCP as natural coagulant could be used as a new technique for removal of contaminants from polluted water.
- Alkalinity and pH of the water do not change during the treatment process.
- At high initial turbidity, coagulation performance of alum in conjunction with MOCP is significantly higher for removal of hardness, especially at hardness of 100 to 210 mg $CaCO_3$ L^{-1}.
- The efficiency of metals ions removal in this study is as follows: $Fe^{2+} > Cu^{2+} > Zn^{2+} > Mn^{2+}$.
- Mn^{2+} is the least removed ion in this study, which is related directly to the size and chemical properties of the metals ion.
- Metals ions are removed better at higher turbidities, which could be because of effectiveness of the coagulants and attachment of metal ions to solids in high turbidity.
- MOCP could be used as a natural coagulant aid for drinking water treatment with minimal risk of organic contamination.
- The method used in this study is an efficient approach for optimization of the coagulation-flocculation process for treatment of raw water.

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