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Metal ions removal from wastewaters using *Moringa oleifera* seeds

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Abstract. Heavy metal contamination and its consequences for human health and the environment have gained interest in developing economical, easy-to-use and environmentally friendly methods in wastewater treatment. One such aspect is the utilization of natural materials collected from plants in coagulation-flocculation process. This study outlines the potentialities of metal ions removal from wastewater through the use of *Moringa oleifera* seeds. Water samples were taken from the entrance and exit of a sewage treatment plant and the quality of water was examined before and after the treatment using aqueous extracts from powdered seeds with different dosages between 1 and 6 g/l. The determination of Chloride and Fluoride was performed by an automatic Mettler Toledo titrator with an electrode 141 SC and Fluoride selective electrode, ICP-OES determined the concentration of 12 heavy metals, all calibrated before use. The removal efficiency of the extracted bio flocculants was influenced by the type and initial concentration of water pollutants as well the dosage used. The highest percentage of removal was 98% for Fe, 95% for Cu and 72% for Zn, whereas this effect was less notable for residual Cl ions even at high dosage and have no influence of fluoride concentration.

1 Introduction

Water is used for numerous activities and purposes in all industries. The uncontrolled discharge of industrial wastewater which often consists of various types of waste generated (dissolved and fine suspended solids, organic and inorganic particles, chemical additives, dyes, grease, oil, and other impurities) into the environment could increase the turbidity, pH, alkalinity, COD, and BOD of water. These may lead to both surface and groundwater contamination which may initiate several other hazards that are detrimental to human health [1].

Among potential contaminants, metal ions are of particular concern due to their non-biodegradable property; hence making them accumulate to living organisms' tissues [2]. Some metals, including copper, zinc, and nickel, are essential for cellular metabolism but can be hazardous at excessive doses. Other metals, such as lead and cadmium, are harmful even at trace quantities. Fluorine is also a highly toxic pale-yellow gas with a pungent suffocating odor. Specific for it is extremely high chemical activity. It is due to its very high electronic affinity and the weak uniform chemical bond in its molecules. After the isolation of fluorine and the possibility of easy synthesis of its compounds, fluorine is considered one of the most dangerous pollutants in the environment. In human body, fluoride is important for integrity of teeth and bones. In suitable concentrations (approximately 0.5 mg/l), it increases bone density, promotes the re-mineralization of enamel and prevents dental caries. Excessive concentration above 1.5 mg/l can cause fluorosis, insulin resistance, impair brain function, decreasing of fertility, increase risk of chronic kidney disease and so on [3].

Water treatment or purification can be done in many different physical, chemical and biological ways, depending on the severity of the contamination and intended use, and could be classified into adsorption-, membrane-, chemical-, electric-, and photocatalytic-based treatments [4].

Coagulation-flocculation is commonly included either as a pretreatment phase or as a post-treatment step, depending on the nature of the sample being treated, e.g., various types of water or wastewater [1, 5, 6]. Its application includes removal of particulate and dissolved organic and inorganic contaminants from water via addition of conventional chemical-based coagulants/ flocculants, with Al, Fe salts and organic polymers being the most prominent. While the effectiveness of these chemicals is well-recognized, their usage is associated with several problems such as reaction of alum with natural alkalinity present in the water leading to a reduction of pH and a low efficiency in coagulation of cold water; generation of excessive chemical sludge in addition to the suspended solids to be removed; thus, the handling of sludge becomes another issue to resolve. In addition, they can have some long-term harmful impact on the environment and health. For example, it has been reported that Al concentration of more than 50 μg/l becomes potentially toxic for aquatic life and above 200

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μg/l in the treated water can cause Alzheimer's or other neuro-degenerative diseases [3].

Current research is oriented toward the application of natural coagulants/flocculants of vegetable and mineral origin aiming to counteract or minimize the aforementioned drawbacks The seeds of *Moringa oleifera* and their water-soluble *Moringa* seed proteins are among the most studied plant-based coagulants that possess coagulating properties similar to those of al and synthetic cationic polymers [6, 7, 8].

Although a number of papers are found on the water clarification properties of *Moringa oleifera* seeds, only a few were devoted to simultaneous removal of multiple heavy metal ions; some examples are reported in Table 1. Wastewater composition can vary widely depending on the chemicals used in the upstream processes and the nature of treatment it has undergone. Thus, coagulant dose applied could be very different and several operating factors such as extraction method, stirring speed and time, settling time, and temperature should be studied to optimize the treatment performance via coagulation-flocculation for a given case.

Table 1. Application of Moringa oleifera seeds for removing heavy metals from different wastewater types

Metals/ Initial concentration	Coagulant dosage	Ref.
Cu (3.97-5.80 mg/l) Co (1.90- 2.85 mg/l) Fe (1.10 1.49 mg/l) Pb (1.40- 1.90 mg/l) Mn (1.99-3.00 mg/l) Zn (9.93- 11.20 mg/l)	125 - 750 mg/l	[5]
Mn (8.216 mg/l) Fe (63.14 mg/l) Cu (0.823 mg/l) Pb (0.026 mg/l) Cd (0.007 mg/l) Zn (3.554 mg/l)	2000 mg/l	[7]
Cu (1.401 mg/l) Cd (1.887 mg/l) Fe (1.414 mg/l) Pb (1.854 mg/l	10 000 - 20000 mg/l	[8]
Fe (1.361 mg/l) Zn (0.251 mg/l) Cu (0.086 mg/l) Cd (0.013 mg/l) Pb (0.285 mg/l) Mn (0.309 mg/l) Mg (12.867 mg/l)	5000 mg/l	[9]

The purpose of this work is to show whether bio flocculants are suitable for wastewater treatment and to what extent they are suitable in a real setting of a treatment plant.

2 Materials and methods

2.1 Preparation of *Moringa oleifera* seed extract

Seeds from *Moringa oleifera* were bought from commercial network. They were cleaned from the outer shell. The isolated core was weighed to two grams. This amount was grinded in domestic grinder for 30 s until a homogeneous fine powder was produced. Thus, obtained powder was mixed with 200 ml distilled water and stirred for 2 min in high speed of 100 rpm and 3 min in slow mixing of 30 rpm on magnetic stirrer and allowed to settle for 1 min to prepare stoke solution of 1% w/v. The resulting suspension was filtered through filter paper. A certain amount was taken from the filtrate obtained and added to the sample, mixed and precipitated up to 120 min. The samples, to be tested for the change of metal ions, were taken a few ml from the surface of the suspension and filtrated; the samples, tested for removal of chloride ions were not filtrated.

2.2 Samples from water treatment plant

Treatment plant used a mix of purification methods. First the water entered in the treatment plant passed through a gravity gravel trap, that captures the incoming pebbles, gravel and other heavy particles. After that, a self-cleaning metal grid with 50 mm permeability of the holes, was situated. Filtrated water enters in pump tank at inlet pump station, consisted of four submersible pumps. Three of them were used all the time and one was used for reserve. The next process was fine filtration and final, the water enters in pools, named "active sludge tank I" and "active sludge tank II" where water if purified by sedimentation.

To determine the ability of bio flocculants, isolated from *Moringa oleifera* seeds, to remove the concentration of metal ions, samples were taken from the entrance, after the first rough filtration and in the exit, where wastewater flows into a local river. Taken sample from the entrance of treatment plant was with high turbidity, so the sample was homogenized before experiment.

2.3 Calibration standards and used apparatus

The determination of Chlorides was performed using an automatic Mettler Toledo titrator with an electrode DMI 141-SC – combined silver ring electrode, Fluoride Ion Selective Electrode to determine the fluoride in samples and ICP-OES - Thermo Fisher Scientific ICAP 6300, to determine concentration of metal ions, all of them, calibrated or checked immediately before use.

Quality checks and calibration standards were prepared from a different mother with concentration 1000 mg/l, respectively quality checks were with a concentration near to received results. Used formula was:

$$C_1.V_1 = C_2.V_2 \quad (1)$$

Where&
C_1 is the concentration of standard, we are taking form,
V_1 is its volume,
C_2 is the wanted concentration and
V_2 is its volume.

For Chlorides quality check was 20 mg/l, for metal ions, calibration standards were with concentrations 0.01 ppm, 0.03 ppm, 0.1 ppm, 1 ppm and 5 ppm, quality checks were with concentrations 0.01 ppm, 0.03 ppm and 2 ppm and for Fluorides, calibration standards were 0.1 ppm and 10 ppm and quality checks were 0.1 ppm and 10 ppm.

Millivolts of DMI-141 SC were checked by using $AgNO_3$ and an internal standard with a concentration of 20 mg/l was tested immediately afterwards. The result was 20.6 mg/l (only 2.92 % deviation), calculated by the formula:

$$D\ (\%) = (C_{std}-C_{exp})/C_{exp} \times 100 \qquad (2)$$

where C_{std} and C_{exp} are the initial and final values of target parameter for measurement, and D is the removal efficiency after the coagulation-flocculation-decantation experiments in percentage.

Quality checks used to test the calibration of Thermo Fisher Scientific ICAP 6300 was in range of 0.008 - 0.012 ppm, 0.025 - 0.035 ppm, 1.95 - 2.05 ppm. Results were different for different elements.

Quality checks for fluoride electrode were 0.09 ppm and for high standard from 9.94 ppm.

To determine the change in the values of BOD parameter, Thermostat cabinet for BOD Direct Plus HACH LZQ080 was used. One of the requirements for this method was to choose correct volume of the sample, as it can vary from 21.7 ml to 428 ml according to the expected BOD concentration in mg/l – from 40 mg/l to 4000 mg/l.

The possible volumes that can be used in BOD methodology were: 428 ml if expected BOD is in the range of 0 - 40 mg/l, 360 ml – 0 - 80 mg/l, 244 ml – 0 - 200 mg/l, 157 ml – 0 - 400 mg/l, 94 ml – 0 - 800 mg/l, 56 ml – 0 - 2000 mg/l and 21.7 ml – 0 - 4000 mg/l BOD. All of volumes were tested and the most of possible options, after the last day of measurement, showed the message “UFL-The values are below the permitted measurement”.

The most suitable amount of sample, after examining all possible options, turned out to be 360 ml. For this volume, methodology recommend usage of 10 drops ATH Nitrification inhibitor, put in the samples and 3 - 4 drops of KOH solution put in the dry seal cup before the installation of automatic detector. pH of samples should be in range 6.5 - 7.5. pH of samples was measured by electrode DGI 111-SC, calibrated immediately before usage, the value was 7.344 pH.

For the experiment, about 2500 ml of the influent water was irradiated for 5 min with UV light, in every minute, except the initial one, which was not irradiated, an individual sample was loaded for BOD measurement. Results were reported after the end of the program – 5 d.

3 Results

3.1 Removal of metal ions

In order to determine the influence of bio flocculants extracted from *Moringa oleifera* seeds on the concentration of metal ions, two types of experiments were set up and performed. First was variable precipitation time and constant amount of extract and the second one was increasing amount of extract with constant time for precipitation.

For the first experiment initial sample was, taken from the entrance of the treatment plant, placed in 7 different Beaker cups. To all of them, except the first one, was added 2 ml of extract, suspension was mixed for 5 min in low speed of magnet stirrer (30 rpm) and leave to precipitate. In every 5 min, sample was taken of the upper site of the solution, filtrated and explored on ICP-OES. Results are shown in Table 2.

Table 2. Concentration of metal ions in sample (in ppm), treated with constant amount of bio flocculants and different time of precipitation

t, min	**As**	**Fe**	**Cu**	**Zn p**	**Cd**	**Pb**
0	0.021	3.182	1.707	0.098	nd*	0.028
5	*0.002*	0.042	0.077	0.027	nd	*0.002*
10	*0.005*	0.482	0.831	0.036	nd	*0.004*
15	*0.007*	0.27	0.807	0.028	nd	*0.002*
20	0.013	0.048	0.113	0.026	nd	*0.002*
25	*0.003*	0.088	0.089	0.031	nd	*0.005*
30	0.01	0.256	0.166	0.048	nd	*0.009*
t, min	**Se**	**Ni**	**Mn**	**Co**	**Bi**	**Sb**
0	*0.007*	*0.006*	0.054	*0.001*	nd	*0.003*
5	*0.004*	0.078	0.041	nd	nd	*0.001*
10	*0.005*	0.059	0.043	nd	nd	*0.002*
15	*0.004*	0.177	0.041	nd	nd	*0.001*
20	*0.004*	0.12	0.038	nd	nd	*0.002*
25	*0.006*	0.143	0.041	*0.001*	nd	*0.003*
30	*0.008*	0.334	0.037	*0.001*	nd	*0.004*

*nd - not detected
**data in italic are under first calibration standard

Most visible reduction of concentration of metal ions is in Fe, Cu and Zn ions, shown in Fig. 1.

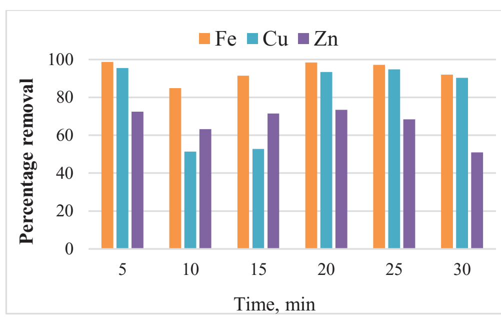

Fig.1. Percentage removal of Fe, Cu and Zn in sample, treated with constant amount of bio flocculants and different time of precipitation (for entrance in treatment plant)

The percentage of removal for Fe ions in first 5 min of precipitation, according to eq. (2) is 98.68%, for Cu - 95.49%, Zn - 72.45%, Mn -24.78%. With the extension of precipitation time the removal efficiency decreases, for Fe – 84.85%, Cu – 51.32%, Zn – 63.27, Mn – 20.37% before become relatively stable after 20 min.

For the second experiment, samples of entrance and exit of treatment plant were taken. To 40 ml of samples, situated in different Beaker cups, were added different amount of *Moringa*’s extract between 4 ml and 24 ml, corresponding to 1 and 6 g seed/l waste water.

The mixture was homogenized for 5 min in fast speed (100 rpm) and 25 min in slow speed (30 rpm) of magnetic stirrer. After 120 min of precipitation, 10 ml of sample was analyzed in titrator for chlorides, 10 ml in ICP-OES and 1 ml in Fluoride selective electrode. Results can be seen in Table 3 and 4.

Table 3. Concentration (in ppm) of metal ions in initial sample of entrance of treatment plant, treated with different amount of bio flocculants and 120 min of precipitation

C, ml	As	Fe	Cu	Zn	Cd	Pb
0	*0.004*	0.165	0.142	0.047	nd	*0.006*
4	0.01	0.046	0.152	0.038	nd	*0.003*
8	*0.005*	0.055	0.153	0.041	nd	*0.003*
12	*0.006*	0.063	0.158	0.043	nd	*0.002*
16	0.016	0.064	0.182	0.046	nd	*0.002*
20	*0.009*	0.267	0.750	0.062	nd	*0.002*
24	*0.009*	0.161	0.210	0.064	nd	*0.003*
C, ml	**Se**	**Ni**	**Mn**	**Co**	**Bi**	**Sb**
0	*0.008*	0.004	0.05	nd	nd	*0.001*
4	*0.007*	0.120	0.043	nd	nd	*0.003*
8	*0.004*	0.05	0.037	nd	nd	*0.002*
12	*0.004*	0.039	0.033	nd	nd	*0.004*
16	*0.005*	0.208	0.032	*0.001*	nd	0.011
20	*0.006*	0.04	0.033	nd	nd	*0.003*
24	*0.004*	0.077	0.032	nd	nd	*0.002*

Results in Table 3 and 4 show that higher dosages did not further reduce metallic ions concentration.

Table 4. Concentration of metal ions (in ppm) in initial sample of exit of treatment plant, threatened with different amount of bio flocculants and 120 min of precipitation

C, ml	As	Fe	Cu	Zn	Cd	Pb
0	*0.003*	0.084	0.024	0.143	nd	*0.004*
4	*0.006*	0.115	0.075	0.712	nd	*0.002*
8	*0.005*	0.106	0.028	0.929	nd	*0.001*
12	*0.003*	0.093	0.026	1.068	nd	*0.001*
16	*0.004*	0.155	0.089	0.754	nd	*0.007*
20	*0.004*	0.136	0.055	1.107	nd	*0.004*
24	*0.006*	0.087	0.054	1.093	nd	*0.002*
C, ml	**Se**	**Ni**	**Mn**	**Co**	**Bi**	**Sb**
0	*0.008*	0.002	0.027	nd	nd	*0.001*
4	*0.004*	0.063	0.026	nd	nd	*0.001*
8	*0.005*	0.035	0.023	nd	nd	*0.001*
12	*0.005*	0.039	0.026	nd	nd	*0.001*
16	*0.005*	0.033	0.026	nd	nd	nd
20	*0.004*	0.077	0.026	nd	nd	*0.001*
24	*0.005*	0.387	0.024	nd	nd	*0.003*

Possible cause is the very low metal contents presented by the effluents collected. The release/return of the metal ions back into the suspension during the process of coagulation can be attributed to the destabilization of the flocks due to excess positive ions in the *Moringa oleifera* seed extract or non-proper selection of mixing speed and time. Possible explanation, for the recorded increase in Ni concentration (Tables 2, 3 and 4) is the "Drifting effect" in Thermostat cabinet happening when the glass of the device is not sufficiently ventilated.

So, we can conclude, that bio flocculants are effective in high heavy metals concentrations.

3.2 Removal of chlorides

Sample of entrance of treatment plant was with concentration of 31.8 mg/l Chlorides and the outlet sample was with concentration 27.1 mg/l Chlorides. Four ml, 8 ml, 12 ml, 16 ml, 20 ml and 24 ml of extract was added to 40 ml of initial samples (taken from entrance and exit). This amount of samples and extract was homogenized for 5 min in fast speed (100 rpm) and 25 min in slow speed (30 rpm) of magnetic stirrer. After 120 min of precipitation, 10 ml of all samples was taken and analyzed in titrator Mettler Toledo. As titrant for DMI141-SC electrode was used 0.01 N $AgNO_3$. Titration curve is made of potentials on X, consumption of $AgNO_3$ on Y. Chlorides in the sample made a complex with Ag and electrode detects change in mV. Results can be seen in Fig. 2.

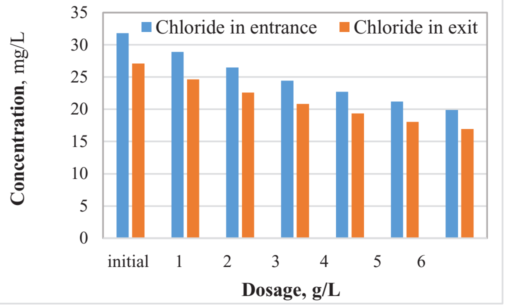

Fig. 2. Concentration of chlorides in entrance and exit of treatment plant at different bio flocculants dosages

In this case within the domain of studied flocculants range, the removal efficiency for was not as high as was the case with the metallic ions treatment. Indeed, natural flocculants extracted could remove only 9% using dosage of 1 g/l and the obtained maximum removal efficiencies did not exceed 30-38% at a dosage of 6 g/l, even the residual concentration was decreasing with every time we increased the dosage.

3.3 Decreasing of BOD

After the check of the pH value, and irradiation, 6 samples were situated in BOD Direct Plus HACH LZQ080. Nutrition inhibitor suppresses the activity of the bacteria by enzymatic inhibition, so that only the breakdown of organic substances in the sample will be measured as the BOD value. By adding a clean magnetic stirring rod to each sample bottle, we guarantee the homogenization of the sample and 3 - 4 drops of 45% potassium hydroxide solution to the seal gasket (this will absorb the carbon dioxide). Then insert the seal gasket in the neck of the bottle. After 5 d, results were reported. Initial sample was with concentration 30 mg/l, after one-minute irritation, this concentration drops to 19 mg/l and after 3 min of irritation, BOD was only 1 mg/l. Results are shown in Fig. 3.

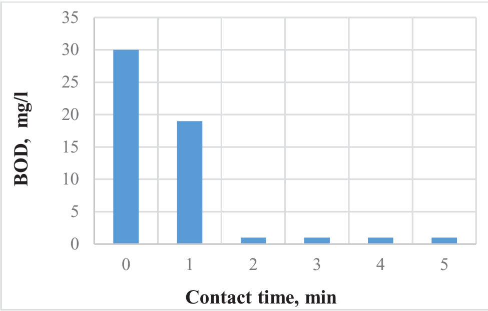

Fig. 3. BOD reduction using UV radiation

Taken into account that water with BOD values of 3 - 5 mg/l is considered fairly clean, while concentration about 10 mg/l indicate water pollution, the treatment is very effective in reducing BOD values.

Treatment plant is constructed of two big activated sludge tanks. The outside one is with height 6 m, diameter 25 m, inside one is with diameter 15 m. The flow is different for 24 h, according to the hour of a day, but average for 10 d is 230 m^3/h. According to the flow, volume of inside and outside activated sludge tanks, the water stands around 8 h in treatment plant, so UV light is also appropriate for decrease of BOD concentration.

3.4. Fluorides

Only the sample, taken from the entrance of treatment plant was tested for the presence of fluorides. The initial sample was with concentration 0.095 mg/l, extract from *Moringa oleifera* was with concentration 0.026 mg/l and mixed sample (bio flocculants and sample from the entrance) was with concentration 0.096 mg/l. There is no reduction in concentration of fluorides in analyzed samples.

Few findings comparable to the results presented in this paper could be found in the literature [9, 10, 11]. Studies have so far shown that the seeds of this tropical multipurpose tree contain high amount of positively-charged proteins (up to 30 - 40%) with molecular weights ranging from 6000 to 16000 Da which provides effective coagulation/ flocculation properties similar to those of alum and synthetic cationic polymers in some case by using less dosages [8]. Several explanations for both the coagulation and the flocculation activity have been offered, including electrostatic attraction (reduction of the repulsive potential of electrical double layers of colloids), sorption (related to protonated amine groups), bridging, related to polymer high molecular weight), or a combination of distinct mechanisms depending on the chemical nature of the pollutant involved [12, 13].

Due to the complexity of wastewater composition and the numerous factors that can influence coagulation-flocculation activity, including the variability of the extracted compounds from natural sources, it is necessary to determine the treatment conditions for achieving satisfying results for each specific waste water treatment process.

4 Conclusion

Considering the results, the following can be concluded:

Moringa oleifera's seeds are rich in bio flocculants and the extraction step is easy to apply due to the hydrophilic properties of water-soluble proteins;

The crude extracts have the capacity to treat the wastewater with high-level metal concentrations and are selective for some metal ions, as Fe, Mn and Zn;

UV light offers the opportunity to decrease of BOD in real wastewater treatment plant.

Organic matter might consume additional chlorine in the treatment plant during the disinfection with chlorine. For these reasons, it is suggested that *Moringa oleifera* seeds could be used more advantageously after a suitable purification or modification of the cationic active proteins.

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