ENTRY INTO THE STOCKHOLM JUNIOR WATER PRIZE 2020

Using naturally derived poly glutamic acid in association with *Moringa oleifera* seeds as a coagulant for treating water

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Abstract

The end goal of the project was to find the flocculation properties when seeds from Moringa were mixed with naturally produced Poly-glutamic acid (y-PGA). Poly-glutamic acid, typically produced industrially are rather expensive. To carry out flocculation with y-PGA in the current system, some clarifying agents like ferric chloride is required since it follows an ionexchange charge neutralization flocculation (anion). Previously, the flocculation characteristics of Moringa seed has been observed which also work with the same ion-exchange flocculation (cation). The goal was to see the outcome when y-PGA produced from soybean, which were fermented by *Bacillus Subtilis* existing in rice straw, were mixed with moringa seeds. The naturally produced y-PGA which was verified with melting point and UV-vis spectroscopy. At the end, a successful flocculation with y-PGA and crushed Moringa seeds was observed. Turbidity reduced by 91.6% and a maximum 86.03% reduction in Color of the water samples.

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Acknowledgements

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We would also like to thank Aninda Kumar Chowdhury for helping us get lab support in BUET.

Biography

Adittya Kumar Chowdhury is a first year college student at Mastermind English Medium School. He is currently studying Mathematics, Chemistry, Physics. Amongst his rather vast interests, graphics designing, travelling and learning new things and applying them in various competitions and real life situations intrigues him the most. His basic moto comes from a quote that says "if you want to change the word, start with yourself" by Mahatma Gandhi. And so this is his first step in his journey.

Iftekhar Khaled is a first year college student currently studying in Mastermind English Medium School. Being an avid traveller and a lover of the STEM field, he explored almost every field and loves to challenge himself with problems in the Status quo and coming up with cheap and efficient methods and correction, he also participated in Google Science Fair 2018, where he became one of the few qualifiers from the country. Currently he is studying on Physics, Chemistry, Mathematics and Economics and aspires to stay in the STEM field.

Introduction

Bangladesh has been currently undergoing some massive economic changes and the river bodies are being affected through pollution. Unethical practices are typically not taken into account and the rise in economic growth translates to more industries, which is good for the economy but bad for the water bodies.

According to World Health Organization (WHO), of the 97% of the population having access to water, about 60% of the population is yet to have access to safe drinking water[1]. Furthermore, a World Bank report suggests the fact that about 41% of all improved water sources are actually contaminated with various bacteria[2]. Moreover, another study claims that in the average turbidity in river bodies around Bangladesh are 8018 mg/L. However, the maximum allowed turbidity for drinking water is roughly 10 NTU.

In the current system, in order to tackle such issues, people resort to many type of water purification measures. With about 63.37% of total population living in rural areas[17] for people living in rural areas and at a poverty rate of 24.3%[18], expensive purification means like reverse osmosis is not an option for them. So the most common alternative they tend to use is alum or potassium aluminium sulphate $KAl(SO4)_2.12H2O$ [34] However, using aluminium sulphate comes with its health concerns. The primary concern with alum is that the long-term exposure to low levels of the chemical can cause degeneration of nervous system tissue. Alum has to be manufactured which is complex whereas our method uses household items to purify water.

Therefore, the best solution would be using a flocculant which can be made naturally and can perform successful flocculation by precipitation of all suspended particles.

Our proposed solution to the problem would be the implementation of using naturally produced poly glutamic acid in combination with moringa oleifera seeds in order to perform flocculation and the reasons why they complement each other are further described in the Mechanism section. The added benefit with using the seeds is that they have shown anti-bacterial properties[14]. Poly glutamic acid can be produced by fermenting soybean[7] with bacteria called *bacillus subtilis* which is available in rice straws[8]. This gives us an upper hand since moringa oleifera is abundant in the tropical region[9]. Soybeans are usually used to make oil and thereby after removing fatty acids from soy bean oil, the soy beans are disposed of. Being an agricultural rice producing country also allows us access to great amounts of rice straw since it is a waste product and using two waste products is surely economical. More importantly both the soybean and moringa oleifera seed are classified as agricultural waste products thereby, are typically discarded. Our solution is unique. This is because our solution is cheap and attained from agricultural bi-products. The solution can be mass produced and it is sustainable for the foreseeable future.

Methodology:

2.1) <u>Sample Collection</u>: 500mL water samples were collected from Dhanmondi lake in Dhaka, Bangladesh (W-1). In addition to these samples 50g generic mud was added to 500ml of water (W-2) in order to suspend mud particles and the flocculation properties on these particles were observed.

W-1

W-2

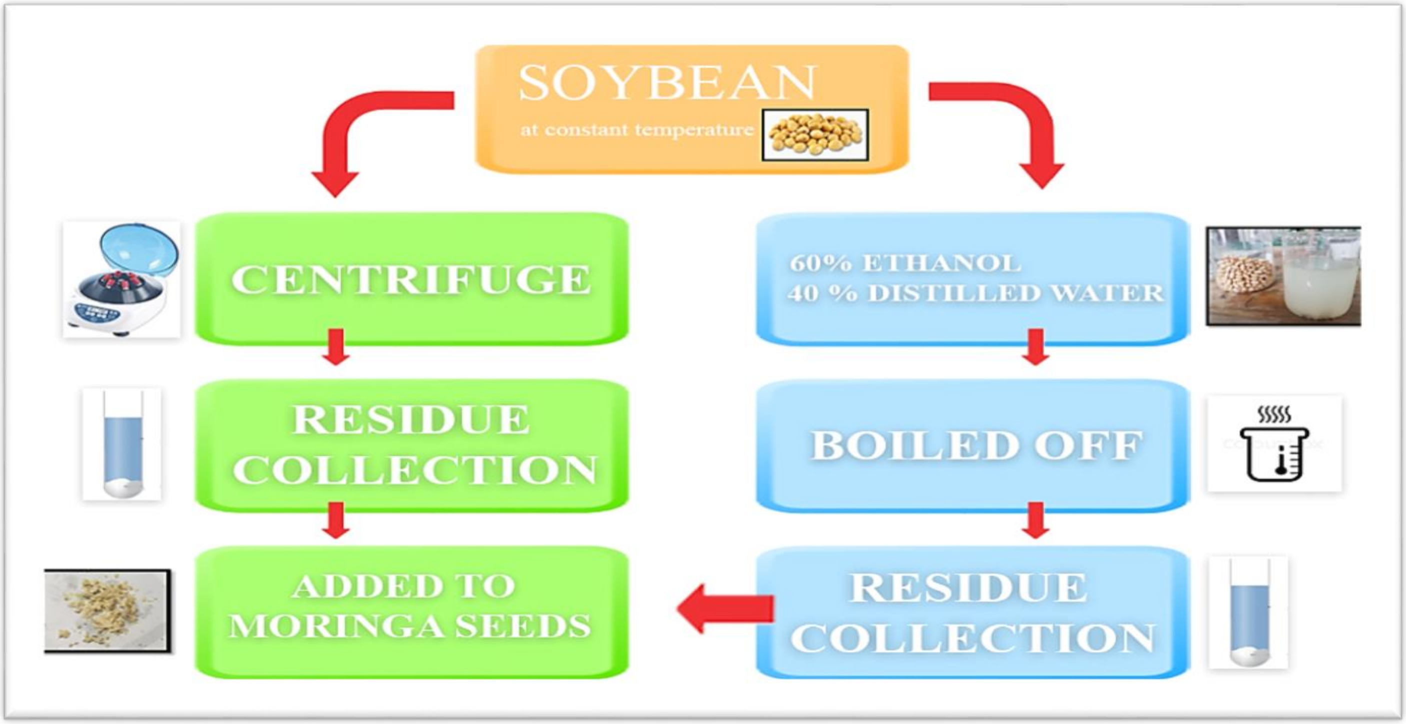
2.2) <u>Producing Polyglutamic acid</u>:

Around 74.99g (fig 2b) of fresh soy bean were added and covered with rice straw (fig 2a and fig 2c) and kept at 34°C for 2 days at a constant temperature since it was the best temperature for the bacteria and the best fermentation would be obtained at this temperature. 3 batches of such were made.

Fig.2a

Fig.2b

Fig.2c



<u>Production of polyglutamic acid</u>

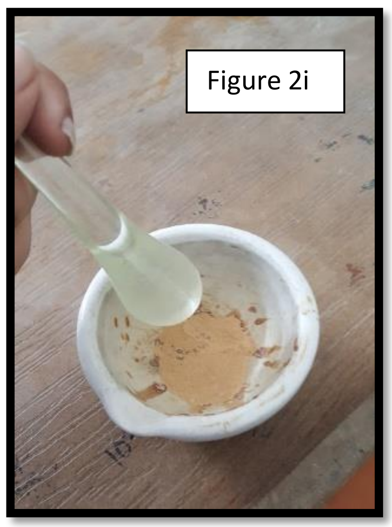
2.3) Extraction of polyglutamic acid: Method 1

Fig 2d :fermented Soy beans

(fig 2e) ethanol dissolved polyglutamic acid

(Fig 2f) solution at 78 degrees celcius

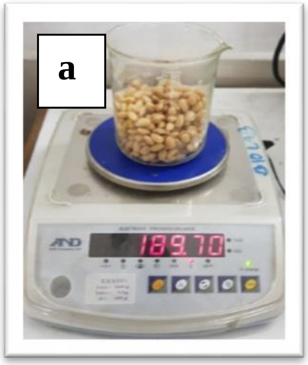
(fig 2g) polyglutamic acid and ethanol solution.



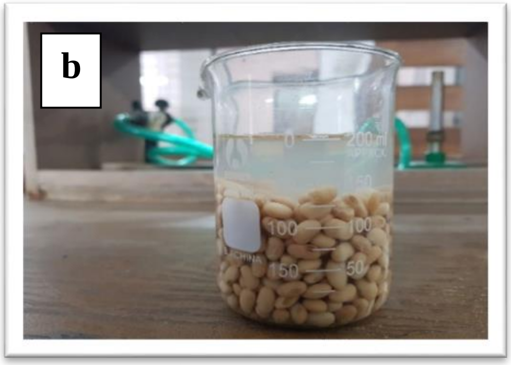
After the fermentation was over 75.38g of soy bean (fig 2d) were taken for poly glutamic acid to be extracted. At first it was dissolved in ethanol and diluted with distilled water in a 60% ethanol ratio. A 212.5mL of the solution was obtained which comprised of 127.5ml of ethanol and 85ml of water. The solution was added to the fermented soybean. Resulting in a ratio mass of fermented soybeans to solution millilitres ratio of 1:2:8. This immediately dissolved the poly glutamic acid produced and after stirring for 5 minutes, the solution was decanted to remove any suspended particle (figure 2e). The solution was later heated with Bunsen burner until a constant temperature of 78°C (figure 2f) was observed and boiling started to occur. This allowed all the ethanol to evaporate off leaving only the sticky poly glutamic acid behind. After all the ethanol was boiled off, precipitate remained which was heated in an oven at 100°C so that a powder was produced which was ground by a mortar and pestle (figure 2h)

At the end, around 8g of poly glutamic acid were made from 75.38g of soybean. In order to ensure that the product was the one intended the boiling point was checked by putting some powder inside capillary tube and heating to melting point of 206°C which is the melting point of polyglutamic acid (figure 2 j,k,l).

Extraction of polyglutamic acid: Method 2



a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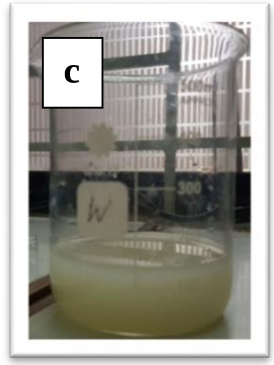

c

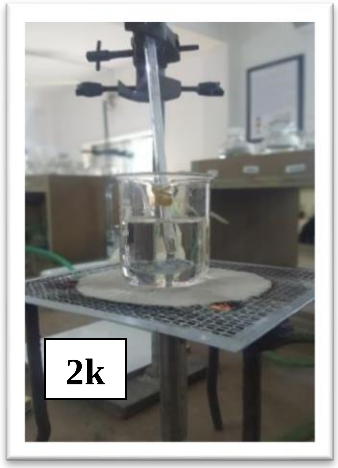
1. 189.70 g of fermented soybean was taken in a beaker. (figure a)
2. Water was filled to 200 ml mark while the beaker contained the beans. It was then stirred very slowly for 5 minutes. This transferred most of the acid from the soy surface to the water. (figure b)
3. The acid was decanted and about 130 ml of the acid and water was obtained. (figure c)
4. This water was divided into equal samples and put in a centrifuge for 5 minutes.
5. The acid sediment at the bottom of the tubes and the water was decanted.
6. All the acid was poured in a test tube and 0.49 g of acid was obtained. (figure d.i, d.ii and d.iii)

d.i

d.ii


d.iii

The melting point of the sample obtained using method 1 was found to be 195 degrees Celsius. The melting point of pure polyglutamic acid is 206 degrees Celsius. The discrepancy in melting point may have emerged from the slight amount of fructone present. But this is not a problem since fructone is not harmful for health.



UV Spectroscopy:

1g of poly-glutamic acid was also taken and mixed with deionized water and UV-vis spectroscopy was carried out on the sample from the range of 190nm to 220nm wavelength. The maximum absorption given was roughly at 0.3 absorbance mark. This spectroscopy matched the one obtained from pure polyglutamic acid.

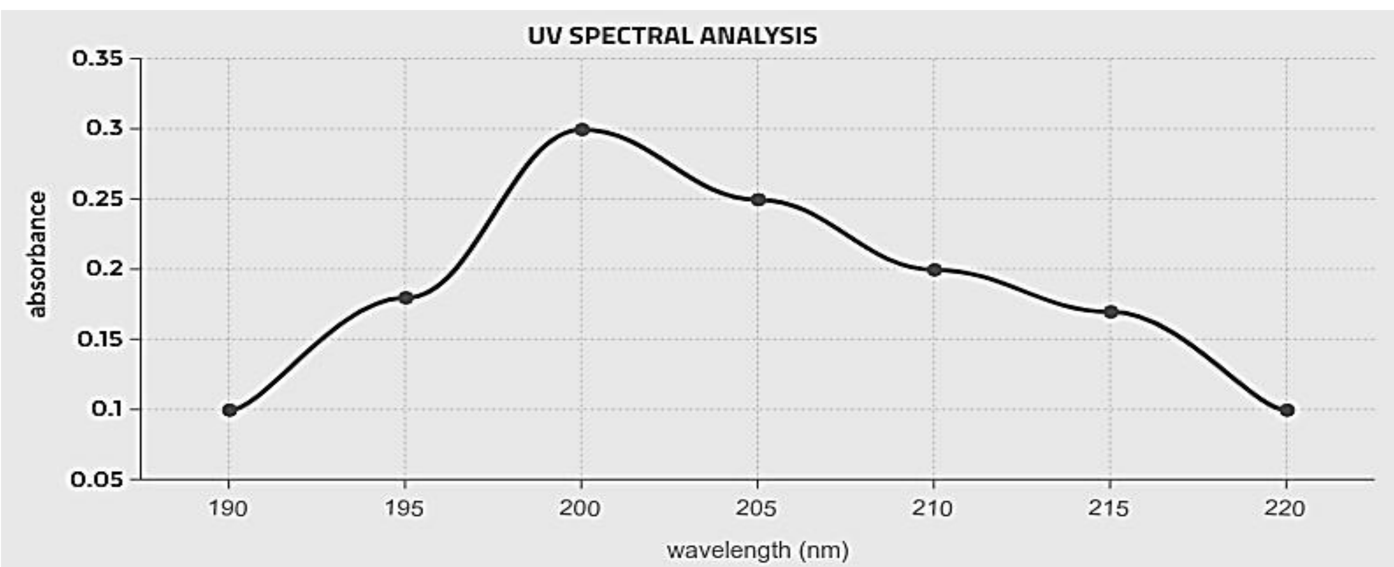


Figure 2P

In order for maximum flocculation to occur 6 moringa seeds were taken and crushed and then dissolved in 50ml of distilled water

Fig 2m (1st photo) **Fig 2n (2nd photo)** **Fig 2o (3rd photo)**

2.4) <u>Purification of water:</u>

2g of poly glutamic acid was taken and mixed with 50 ml sample of moringa-distilled water solution and was added to around 200 ml of all types of water samples. The containers were then vigorously shaken in order to get the flocculation process to get started.

After that the reagents were added to W-1 and the container was vigorously shaken to get the flocculation process started. Alongside the same water sample was kept without any reagents to examine the difference at the end of the experiment. 2 hours after the addition of the reagents, some particles were seen to precipitate down and after 3.5 hours of the experiment dust particles completely precipitated at the bottom of the beaker. This point marked the end of the flocculation process as no further visible change was noticed after this point. Figure 3a shows the original sample on the left and the flocculated water sample on the right.

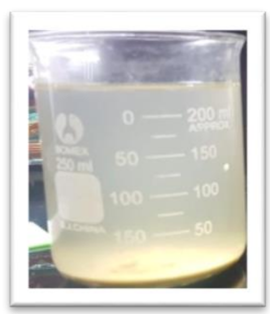
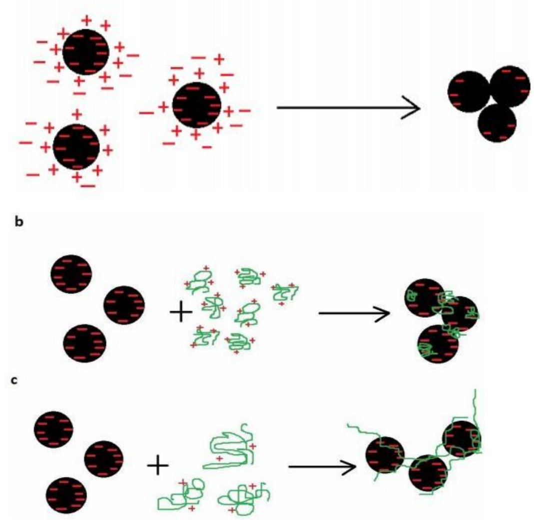


Fig 3a Original water (left) flocculated water (right) **Fig: 3b: condition after 2 hours** **Fig 3c condition after 3.5 hours**

2.5) **Mechanism**:

The main mechanism through which particles sediment to the bottom is by the process of flocculation. Both poly glutamic acid and moringa oleifera can perform successful flocculation but they have different roles in the process. Flocculation works by flocculants aggregating colloidal particles via charge neutralization, patching, and bridging mechanisms as shown in Figure 4. In the charge neutralization mechanism, the charges of colloidal particles are neutralized by adsorption of flocculants, and thus repulsion force existing between adjacent particles is reduced, which causes these particles to agglomerate via developing Van der Waals interaction, as in Figure 4a. In the patching mechanism, flocculants adsorb on a colloidal particle due to their opposite charges and they neutralize a part of the particle's surface. Due to their opposite charges, the adsorbed flocculants will act as anchoring points for another adjacent particle for aggregation (Figure 4b). In the bridging mechanism, high molecular weight flocculants adsorb on particles. Due to their large sizes, the flocculants bridge particles and hence generate large flocs (Figure 4c)[12].

Fig 4(a,b, c)



Moringa oleifera contain significant quantities of low molecular-weight, water-soluble proteins which carry a positive charge. When the crushed seeds are added to raw water, the proteins produce positive charges acting like magnets and attracting the predominantly negatively charged impurities (such as clay, silk, bacteria, and other toxic particles in water)[13]. Alongside flocculation, moringa oleifera seeds have also shown anti-microbial properties which adds up here and can reduce bacteria infestation in water[14]. Poly glutamic acid on the other hand contains in an anionic polymer and contains carboxyl and amino groups. The presence of carboxyl group allows them to become deprotonated ($–COO^-$), whilst amino groups remain fully protonated as (–NH3+). After the surface charges of particles are neutralized and micro-flocs are formed, flocculants are often added to bring the micro-floc particles together[15]. In addition to making the flocculation process faster the presence of carboxylic and amine groups also allows poly glutamic acid to act like a bio absorbent[16] as it has been successfully proven that through the adsorption properties and the fact that it can bind to heavy metals like Cr3+, Ni2+, Cu2+, Mn2+, Al^{3+}[19]. The visual representation of adsorption is shown below (fig 4d).

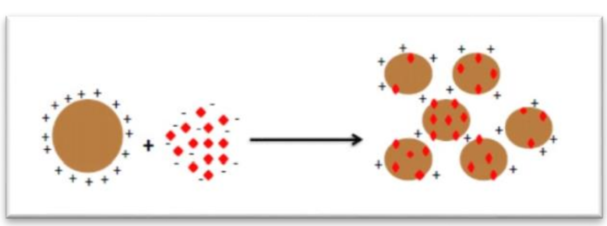


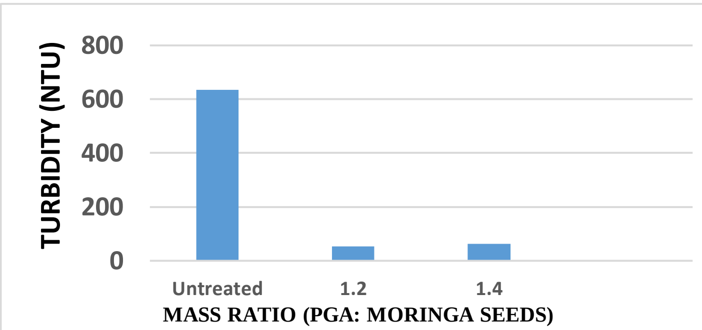
Fig 4d

Results:

3.1) TURBIDITY TEST

First stage testing, water samples were collected, one sample was untreated and two samples were taken in a 1:2 (4 seeds) and 1:4 (6 seeds) mass of polyglutamic acid: mass of moringa seeds ratio. Then they were allowed to rest for 1 hour 30 minutes and then decanted into sterilized bottles and finally sent for turbidity testing. These two ratios reduced turbidity by 91.57% and 89.92% respectively.

Results (Turbidity test): After the report came in. it was seen that the untreated water sample had a turbidity of (635) NTU and the one with 1:2 had a 53.5 NTU and the 1:4 had a 63 NTU.



Turbidity for 2 different ratios of PGA: Moringa seeds graphically

Water appearance for different 2 different ratios of PGA: Moringa seeds. Starting from left Untreated, 1:2, 1:4

Sample	Parameters	Concentration (NTU)	Percentage Reduction
1: Untreated water	**Turbidity**	**635**	**N/A**
2: 4 Seeds	**Turbidity**	**53.5**	**91.57%**
3: 6 Seeds	**Turbidity**	**63**	**89.92%**

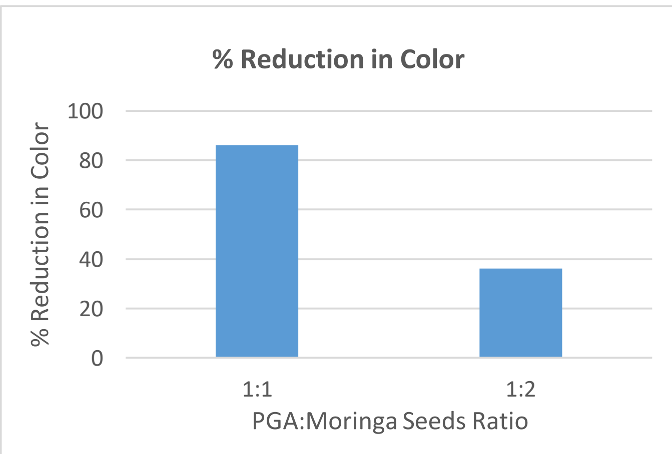
Turbidity for different 2 different ratios of PGA: Moringa seeds in tabular form.

3.2) WATER QUALITY TESTS (second stage)**:**

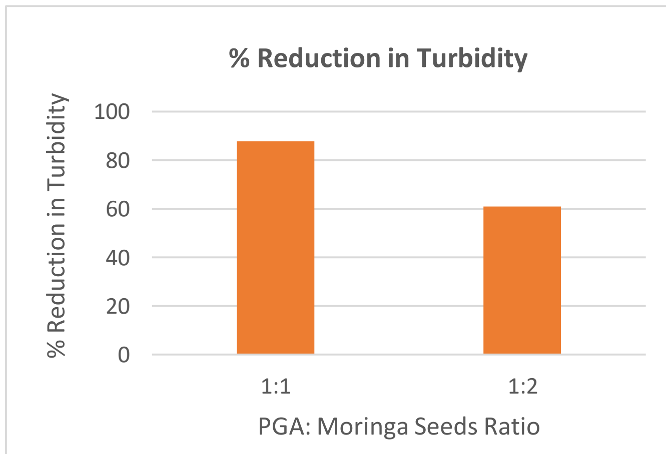
Water samples were collected later again and more tests were carried out. The bar graph below shows the remaining turbidity and colour of the solution after the flocculation process was carried out. Upon getting the first set of results it was evident that a higher amount of moringa seeds would not benefit the reagent. Thereby another batch of samples were tested in a 1:1 and in a 1:2 ratios with more data points and the result is shown below in the table. They are summarised as follows:

Sample Ratio	TSS (mg/l)	Color (Pt/Co) scale (PCU)	pH	Turbidity (NTU)	Percentage Reduction in Color	Percentage Reduction in Turbidity	Mass of Poly-glutamic acid (grams)	Mass of Moringa seeds (grams)
Untreated	420	3020	8.08	933.98	0	0	0	0
1:1	54	422	7.54	115.04	86.03	87.68	2	2
1:2	168	1930	7.22	364.19	36.09	61.01	2	4

Water quality parameters for different ratios of PGA: Moringa seeds.



% Reduction in Color for 2 different ratios of PGA: Moringa seeds graphically



% Turbidity reduction for 2 different ratios of PGA: Moringa seeds graphically

Discussion:

There are some limitations of our experiment. We met our goal in proving that polyglutamic acid works with in complement of moringa to do successful flocculation, we did not reach WHO and or Bangladesh government drinking water standards. However, it is to be noted that our solution used in carrying out turbidity test was unfiltered thereby filtering it with filter papers would reduce the turbidity further, we also achieved the added benefit of a reduction of Color in the water and even after treating the water with polyglutamic acid the pH of the water remained within drinking water standards. A journal from University of Extremadura does reveal that a reduction in microorganism colonies (up to 96% and 94% in the case of total and fecal coliforms and almost 100% in the case of fecal streptococcus) were found using moringa seeds. The next step would be to carry out a professional identification of bacteria present and the ones that has been reduced in population and carrying out Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) test to quantify the reduction of organic content in water.

Moringa seeds and polyglutamic acid are more effective than other two ways of purification which are the common in the following ways:

1. **Turbidity**: Alum reduces turbidity by 86.3% in 500 NTU water in optimum conditions, ferric chloride reduces turbidity by 93.5% in 500 NTU water in optimum conditions and our method of using polyglutamic acid and moringa reduces it by 91.6% in 635 NTU water.[26]
2. **Cost**: About 100ml water is treated by 22g of alum. 100g of alum which costs about 422.35 taka[22]
   -Ferric chloride is very corrosive and hazardous to humans but 100g of it costs about 342.76 taka.[23]
   -1kg of moringa seed pots costs about 60 Taka so each seed pod costing about 2.5 taka that contains at least 10 seeds or more averagely and 2.5 kg of soybean cost about 80 taka. About 1 liter of Extreme turbidity NTU >250 water can be treated by 2 seeds.[29]
3. **Availability**: Both alum and ferric chloride are rarely found as free compound in nature. For example, alum is normally found mixed with other elements and forms: kalinite, alunite, and leucite, which can be treated with sulfuric acid and ammonia to obtain crystals of the alum. Ferric chloride can be obtained by doing direct chlorination by reacting iron and chlorine in their elemental configuration but iron is not obtained in elemental form. On the other hand, moringa trees grow within 8 months of planting that can tolerate a wide range of soil conditions for growing and soybeans grow within 45 days to 65 days of planting and they also grow in a broad range of well-drained soil types.
4. **Nature**: Alum when present in high concentrations, has for long been recognized as a toxic agent to aquatic freshwater organisms, i.e. downstream industrial point sources of Al-rich process water. Today the environmental effects of alum are mainly a result of acidic precipitation; acidification of catchments leads to increased Al- concentrations in soil solution and freshwaters. Large parts of both the aquatic and terrestrial ecosystems are affected. In the aquatic environment, alum acts as a toxic agent on gill-breathing animals such as fish and invertebrates, by causing loss of plasma- and haemolymph ions leading to osmoregulatory failure.

   -The ferric chloride sludge also exhibited high heavy metal concentrations revealing a further potential for pollution and harmful chronic effects on the aquatic biota when the sludge is disposed of without previous treatment.

Our produced polyglutamic acid by reaction by dissolving in ethanol was not 100% pure and contained fructone, whilst the one which was centrifuged did give a pure sample of poly glutamic acid. But it did not affect the flocculation process whatsoever. On the other hand, the sample with W2 had less microbes and impurities since it contained clay and mud particles and had comprised of tap water only. In order to reduce the experimental errors, we could use method 2, that is, the fermented beans are dissolved in water and then the decanted water is used in centrifuged to have PGA. This is more effective than method 1 and produces purer acid.

Possible implementation:

Since from the test results it is evident that the reagent with 1:1 was the best performer, we made it into a capsule form as shown in the image below, with the intention that a capsule is easier to distribute and use than actually mixing the individual reagents. Thus the resulting capsule was comprised of 4g of polyglutamic acid and 4g of moringa seeds and they were stored inside a capsule which can be opened and the purifier be released into the water.

Making capsules

Capsules

Raising awareness: We had talked to eighteen people from various districts of Bangladesh, including rickshaw pullers, fruit and vegetable vendors and our respective building guards. According to them, the current sources of water are unprotected ponds, tanks, wells, streams and rivers, which are often quite far from their home. And the problem that stops them from getting clean water is the cost of the pumps and the poor management of services and lack of infrastructure. When we told them about our method they graciously acknowledged that such an initiative would make their daily lives a lot easier.

Interviewing one of the rickshaw pullers

Review of literature: Islamic Azad University in Iran researched the effects of moringa and alum on wastewater treatment in the Bandar Abbas oil refinery. They had reduced turbidity by 86.14%, COD by 50.41% and TSS by 81.52%. The paper was published in 2016[32]

The faculty of Science and Engineering in the University of Wolverhampton in the U.K. researched into polyglutamic acid production, properties and its application. Field of wastewater treatment mentioned were biopolymer flocculent, heavy metal removal and dye removal. The paper was published in 2015[33]

Challenges:

Fermented soybeans cannot last more than 2 weeks before decomposing.

Conclusions:

While the carrying out the experiment, the lab conditions were ensured and the measurements were taken accurately, judging by the experimental outcome as seen from the photographs from figure 3a to 3c, it can be concluded that there was a visual reduction in turbidity upon introduction of our reagents. There was about a maximum 91.6% drop in turbidity of the water sample in the first stage and a 87.68% in the second stage and with our version of the first stage of the experiment, it was also noted that the 1:1 ratio outperformed the 1:2 ratio reagent which was discovered in the second stage of the experiment. This directly shows the fact that adding excess of moringa seed might not actually be beneficial to the water bodies. Moreover, the result of the second stage pointed out another important fact that there was a maximum 86.03% reduction in Color of the water samples.

This proves our point and concludes the fact that our theory of using both moringa and polyglutamic acid to carry out flocculation and the fact that they can work in complement to each other was true which was not documented before.

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