A Novel, Eco-Friendly Bioremediation Of Lead-Contaminated Water

ABSTRACT

A Novel, Eco-friendly Bioremediation of Lead-Contaminated Water

Lead exposure is a global problem, especially in developing countries where lead poisoning accounts for 853,000 annual deaths. The objective of this experiment was to design an eco-friendly method for the bio-remediation of lead-contaminated water which would alleviate this problem. The Moringa Oleifera (MO) seeds were the main component of the bio-remediation system because they are locally available and have adsorbing properties that could be used in purifying heavy metal-contaminated water. It was hypothesized that with this method it would be possible to significantly reduce lead in effluent water. The procedure included designing and constructing a filter, de-oiling Moringa seeds, preparing heavy metal-contaminated water, experimenting with Moringa treatment and sand filtration, and testing samples with an AA-Spectrometer. The variables tested were dosage of Moringa, pH, treatment time, and effects of initial concentration and 2-Stage Filtration. The hypothesis was supported by results. The pre-treatment with Moringa and filtration through the sand filter was most efficient - 100% removal of lead after one treatment and filtration. The treatment with only Moringa seeds was also very effective – over 96% of lead removal. The optimal conditions for lead removal are 3g/100mL of Moringa at pH=8 and 20min pre-treatment time. Moreover, this pre-treatment/filtration method for lead removal is simple, extremely cost-effective, and environmentally-friendly. It has great potential to be the ultimate bio-remediation method for lead removal in developing countries.

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1. **INTRODUCTION**

1.1 The Need for Low Cost, Eco-Friendly Bioremediation of Lead-Contaminated Water

240 million people are still exposed to Pb, with lead exposures accounting for 853,000 annual. Developing countries are the most affected by lead poisoning, where 15 to 18 million children suffer permanent brain damage because of Pb poisoning. The typical water treatment system requires expensive chemicals; therefore, so many people in the world cannot afford purified water.

1.2 Research

Lead in Drinking Water

The presence of heavy metals in drinking water is a result of mining, agriculture, and other industrial activities. Furthermore, when the concentration of heavy metals in drinking water is elevated, it can cause serious health problems. One of the most common heavy metals is lead, which is very toxic even at trace levels. When lead concentration is above the safe limit, it is toxic to everyone, but it is most hazardous for pregnant women and children under 6 years old. It can cause pre-mature birth, kidney damage, seizures, hearing loss, behavioral problems, brain damage, learning disabilities, and lower IQ.

The major sources of lead in developing countries are from mining and smelting of Pb, battery recycling, and leaded gasoline and paint. On the other hand, in the United States lead can be found in leaded paint in old houses and corroded Pb water pipes. Lead contamination remains a main concern in developing countries because there are many opportunities for exposure, not many regulations on lead industries, and there are no health screening programs present.

The Moringa Oleifera plant

The Moringa Oleifera plant, which is widely available in developing countries, is considered "Nature's Gift." In addition to many benefits such as animal food, production of oil, and use in medicine, it has also been found to have coagulating, antimicrobial, and adsorbing properties, which could be used for water purification. There are many reasons why Moringa Oleifera should be considered for water purification - it is inexpensive, sustainable, a natural coagulant, and as effective as chemical coagulants. The Moringa seed is also non-toxic, as opposed to aluminum salts, which are linked to possible health concerns, such as Alzheimer's.

Figure 1
Moringa Oleifera Tree, Pods and Seeds
"Moringa Tree", www.learnaboutnature.com

The mechanism for heavy metal removal with Moringa Oleifera seeds is based on ion exchange and Van der Waals forces on the surface of the biomass. The group responsible for adsorption of heavy metals is the Carboxyl group (Cleide S. Araujo et al).

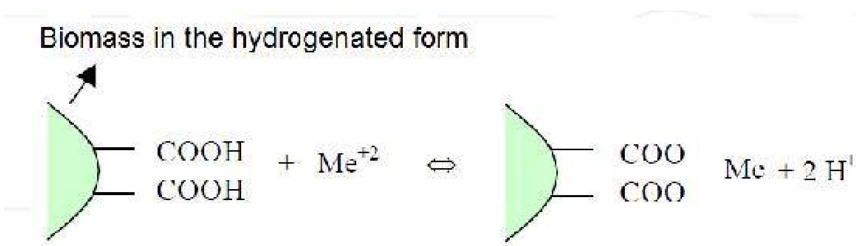


Figure 3 Schematic Diagram of an ion exchange mechanism (Cleide S. Araujo et al.)

Moringa Oleifera is a biomass with amphoteric character. Depending on the pH of the solution, its surface can become either positive or negative. Moringa Oleifera has a Zero Point Charge at a pH between 6 and 7. At pH < PZC the surface charge becomes positive, at pH=PZC – neutral, and at pH > PZC - negative, favoring adsorption of cationic species.

1.3 Hypothesis

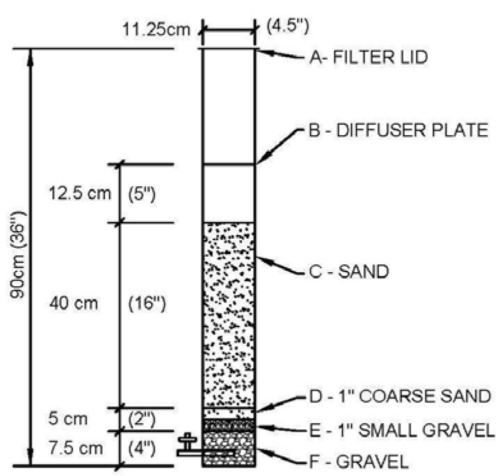
The objective of my experiment was to design a method of eco-friendly remediation of lead-contaminated water. It was hypothesized that by using Moringa Oleifera seeds and a sand filter, it will be possible to significantly reduce the concentration of lead in contaminated water.

2. MATERIALS AND METHODS

2.1 Filter Design

The total height of the sand column and total filter media was designed based on Darcy's Law for water flow of 8oz/min: Q = KA * (depth of filter media + 8" depth of excess water)/depth of sand
Q= flow rate (8oz/min), K= hydraulic conductivity (0.5"/min), A=filter cross sectional area. The total depth of filter media was designed at 22" (4" gravel, 1" small gravel, 1" coarse sand and 16" sand).

Figure 4 - FILTER DESIGN (Competition Entrant)



A - PREVENTS CONTAMINANT ENTERING FILTER
B - PROTECTS SAND SURFACE FROM DAMAGE
C - SAND ABSORBS SUSPENDED PARTICLES & HEAVY METALS
D - PREVENTS SAND FROM ENTERING SMALL GRAVEL
E - PREVENTS SAND FROM ENTERING GRAVEL
F - PREVENTS OUTLET PIPE FROM CLOGGING

2.2 Procedure *(Competition Entrant)*:

The procedure included the following steps:

1. Assembling the filter (acrylic tube, end plate, lid, water valve).
2. Building a filter based on design to include gravel, small gravel, coarse sand, regular sand, and dispersion plate
3. De-oiling of Moringa with Soxhlet apparatus after seeds were shelled, grounded, and dried (160 F).
4. Treating the lead-contaminated water with Moringa to establish optimum conditions:
   - Dosage of Moringa (0.1, 0.2, 1, 3 & 4g/100mL), pH 7, 350rpm, 20min, 5ppm of Pb
   - pH (5, 6, 7, and 8), 3g/100mL of Moringa crushed seeds, 350rpm, 20min, 5ppm of Pb
   - Time of treatment (10, 15, 20, and 25min), 3g/100mL MO at 350rpm, 5ppm of Pb
5. Testing the effects of initial Pb concentration on the efficiency of lead removal with Moringa seeds
   - Concentrations of 0.03, 0.3, 3, and 5ppm of Pb were tested with 3g/100mL MO at 350rpm and pH 7 for 20 min.
6. 2-Stage Filtration: 8L of HMW were pre-treated (first time) at pH=8 with 240g (30g/L) of de-oiled crushed Moringa seeds for 20min, then run through the filter. First 3.5L were discarded, additional 4L were treated it with MO (120g) second time, then run through the filter again, using the same procedure as for the first pre-treatment/filtration. Samples were obtained after each step of pre-treatment and filtration.
7. All samples were tested with the Flame Atomic Absorption Spectrometer- Varian AA240FS. (Spectrometric Analysis). Five standards were prepared for Pb with concentrations of 0.05, 0.1, 1.0, 3.0, and 6.0 ppm. Based on the known standards concentration, the absorbances were calculated and assigned to each standard, and the Peak Absorbance Calibration Curves were created according to Beer's Law: $A = e \times L \times c$ (A – Absorbance of light energy from light source by free atoms, e - molar absorptivity constant, L – path length, c – concentration). Using the Peak Absorbance Calibration Curves, the concentrations were calculated for all tested samples.

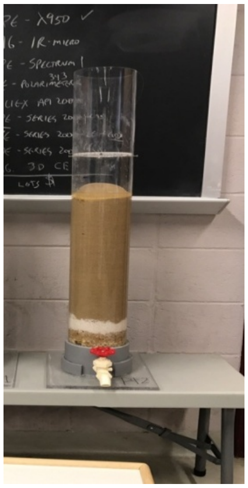


Fig. 7 - Filter

Fig. 8 - De-Oiling of MO seeds

Fig. 9 - Preparation of Pb water

Fig. 10 - Adjusting pH

Fig. 11 - Pre-Treatment with MO

Fig. 12 - Pre-Treatment with Moringa (2-Stage Filtration)

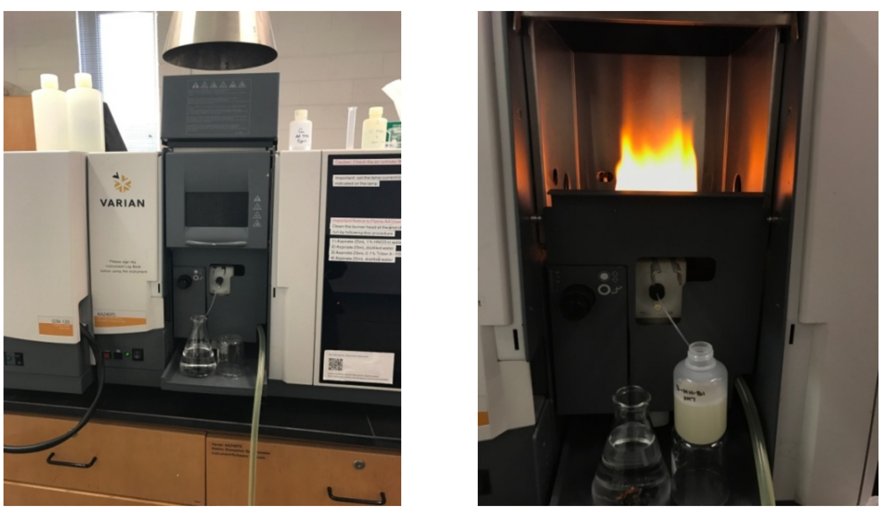


Fig. 13 - Testing with Atomic Absorption Spectrometer

3. RESULTS:

Legend (Samples tested):

C – Control Sample (before treatment with Moringa), 5 ppm of Pb

D1, D2, D3, D4, D5 – Moringa dosage - 0.1, 0.2, 1, 3, and 4/100mL respectively at pH=7, 20min

pH5, pH6, pH7, pH8 – pH of the treated water - 5, 6, 7 and 8 respectively with 3g/100mL, 20min

T10, T15, T20, T25 – Treatment time – 10, 15, 20 and 25min respectively with 3g/100mL, at pH=7

IC1, IC2, IC3, IC4 – Initial Concentration of Pb – 0.03, 0.3, 3 and 5 respectively with 3g/100mL, 20min

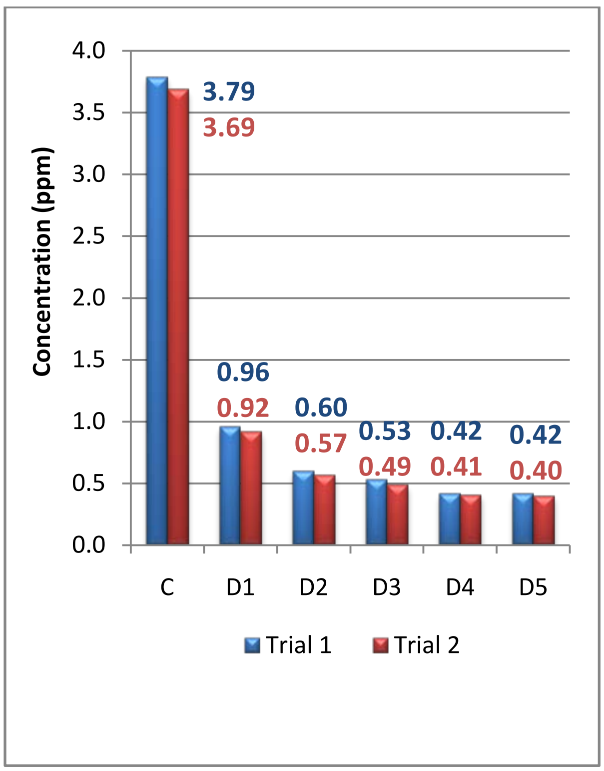
P1 - First Pre-treatment with MO (30g/L); pH=8

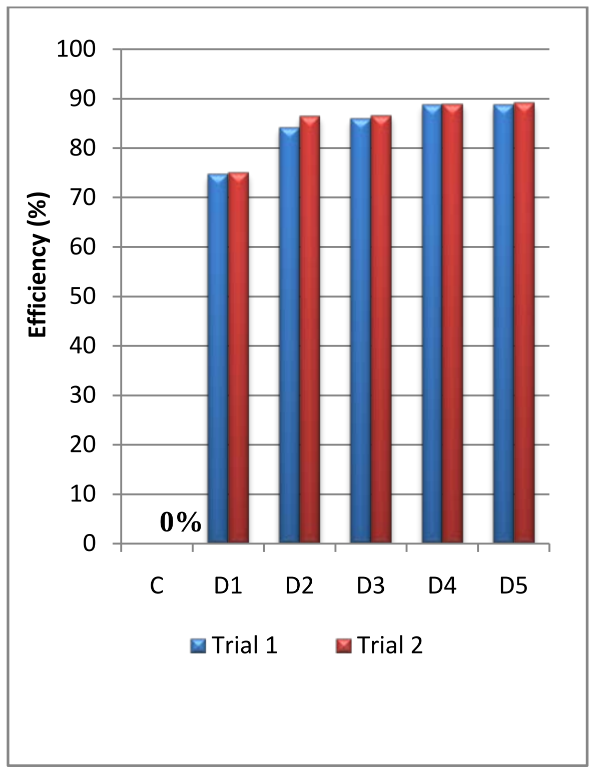
F1 – First filtration

P2 - Second Pre-treatment with MO (30g/L); pH=8

F2 – Second filtration

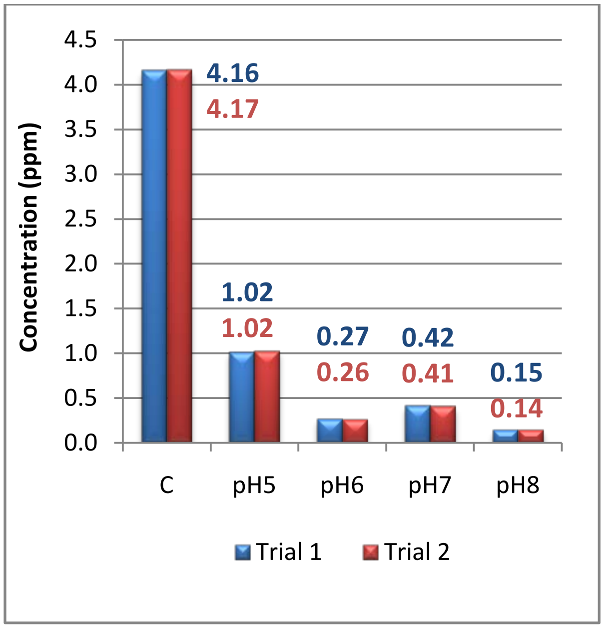
Figure 14 - Effect of Moringa Dosage on Pb Removal

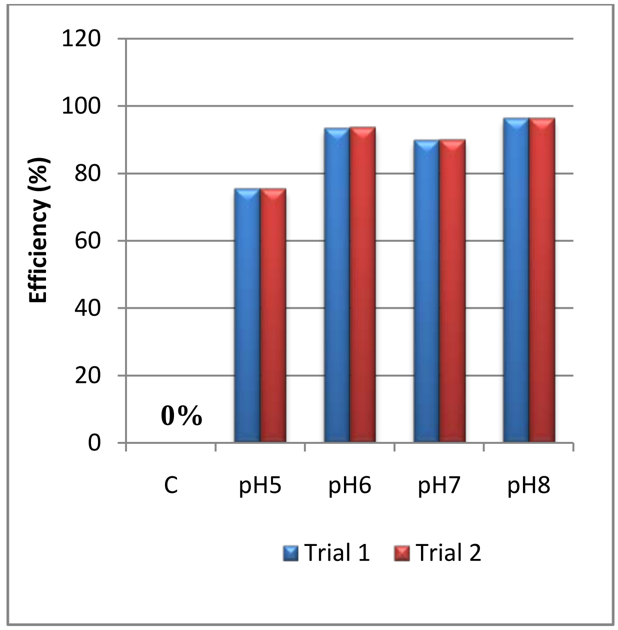




Efficiency of Pb removal increased with dosage up to 3g/100mL, and after that, it remained constant.

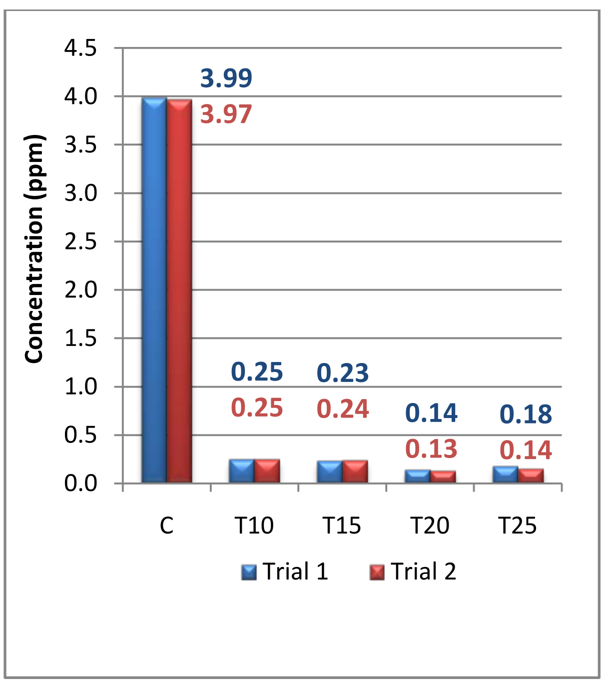
Figure 15 - Effect of pH on Pb Removal

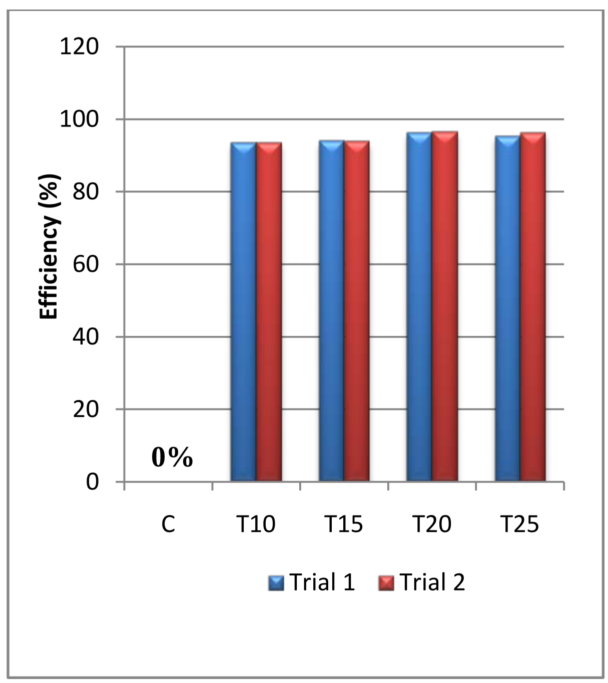




Efficiency of Pb removal was affected by pH of the treated water with maximum removal obtained at pH=8 with 96.4% of Pb removal.

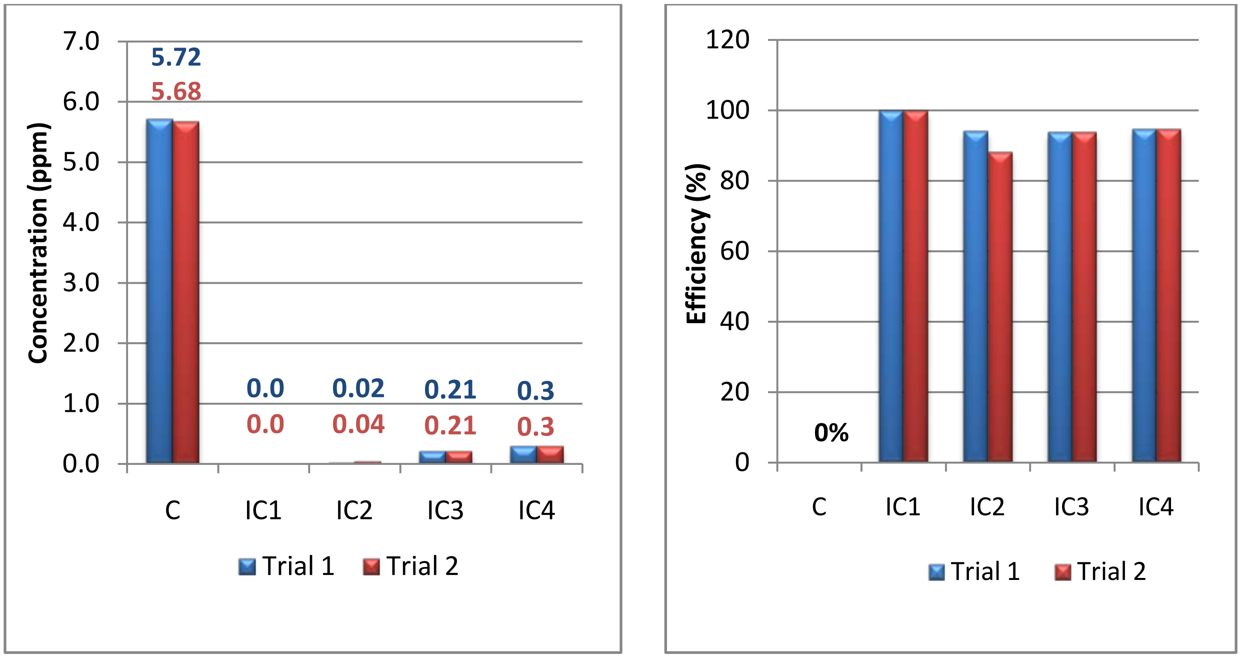
Figure 16 - Effect of Treatment Time on Pb Removal





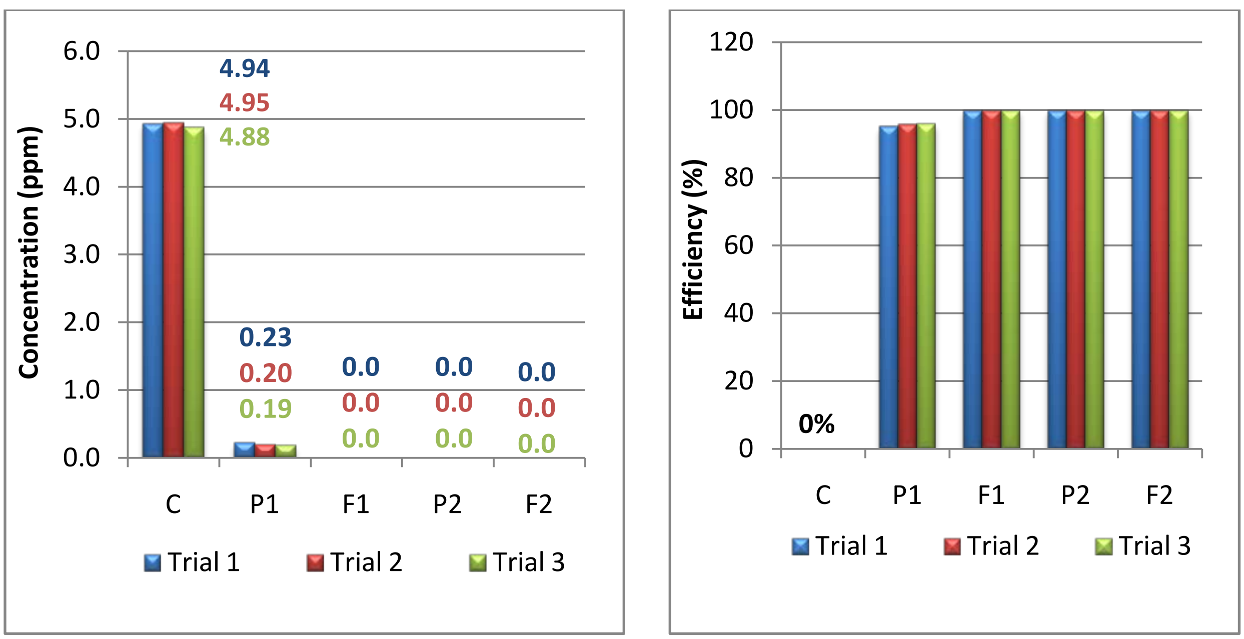
Efficiency of Pb removal increased with time of treatment up to 20min, and after that, there was no significant change in Pb removal.

Figure 17 - Effect of Initial Concentration of Pb on Efficiency of Lead Removal with Moringa



Initial concentration of Pb was not significantly affected by the efficiency of treatment with Moringa. The efficiency of removal was approx. 94%, regardless of initial concentration with exception to the very small concentration of 0.03ppm, when there was a complete removal of lead.

Figure 18 - Results of Pb Removal in 2-Stage Filtration



The 2-Stage filtration method was very effective with 96% of lead removal after just one treatment with Moringa and 100% removal after first filtration.

Figure 19
Peak Absorbance Calibration Curve
Samples tested after Treatment with Moringa

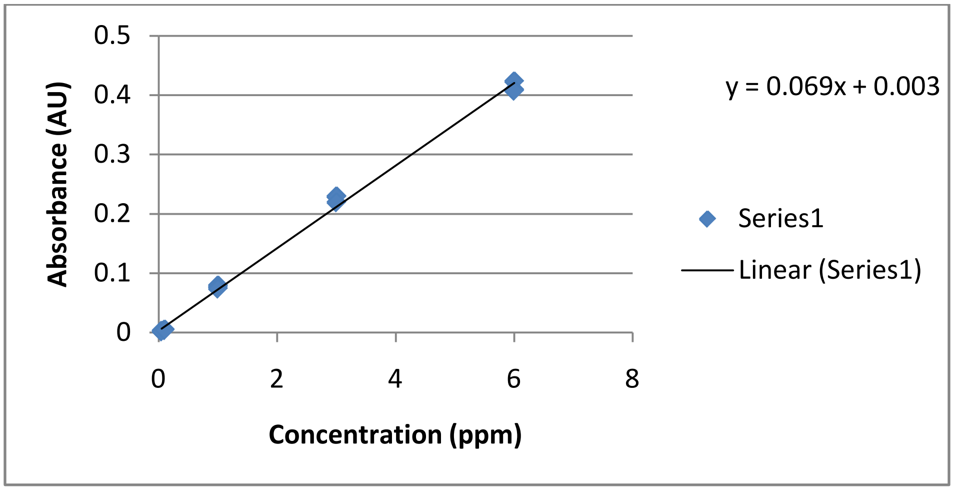
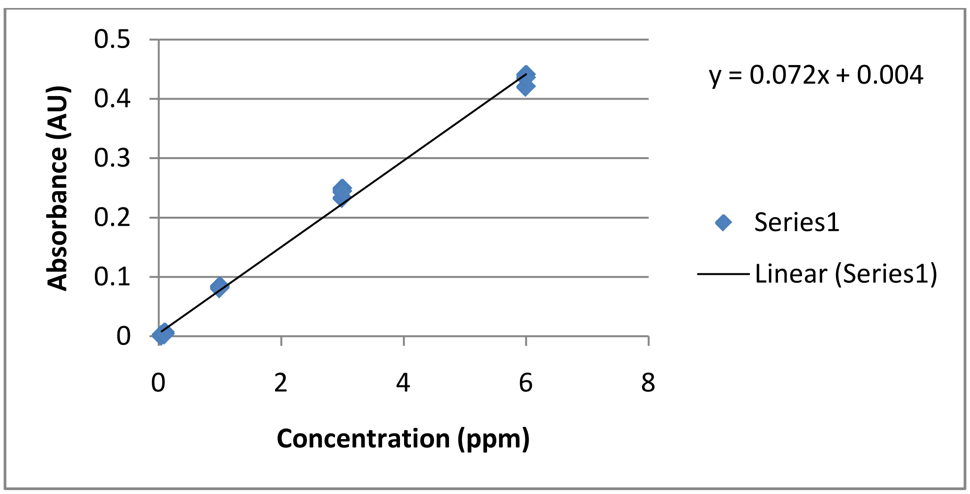


Figure 19
Peak Absorbance Calibration Curve
Samples tested after 2-Stage Filtration



5 standards were prepared for Pb with concentrations 0.05, 0.1, 1, 3, and 6ppm. Based on the known standards concentration, absorbance was calculated and assigned to each standard, and the Peak Absorbance Calibration Curves were created. Using the Peak Absorbance Calibration Curves, the concentrations were calculated for all tested samples.

4. DISCUSSION

Since this method of lead-contaminated water purification is one-of-a-kind, there is not much data available to compare with other similar bioremediation systems. The 2-Stage filtration produced great results – 100% of lead removal and clean water to drink. The treatment with only Moringa seeds resulted in an efficient removal of 96% for Pb, while in another study with Moringa Oleifera for the removal of lead, (Alfonso et al. 2013) the results were much lower - 69.2% removal of Pb. Unexpected results were obtained after experimenting with the sand filter. It appears that sand also has adsorbing properties that could aid in the proposed bioremediation of lead-contaminated water. A possible explanation could be due to the binding of lead cations with anionic silicon dioxide (sand).This theory was confirmed in another study conducted by M. Ali Awan et al. (2003). Although a different procedure was used (adjusted to pH=6 and the heavy metal contaminated water with the sand was shaken), according to that research, silica has the ability to adsorb metal cations. It has properties that support hydrolysis, such as high charge, small radius, and polarizability. Based on these properties, Pb should have an affinity for the adsorption to sand. There is not much research available that addresses the possibility to use sand filters for heavy metal removal, and therefore, there is no data to compare.

Based on other studies, there were conflicting results as far as pH and treatment time. One study indicated pH=5, 20 min (Ongulu et al. 2015) and another indicated pH=7, 60 min (Cleide et al. 2015). A set of experiments was done to establish the optimum dosage, pH, and treatment time for this experiment. Based on the results, the optimum pH for lead removal was 8, with a dosage of 3g/100 mL of Moringa and a 20 min treatment time. The results related to pre-treatment time agreed with research done by Ongulu et al.(2015), where they concluded that the adsorption rapidly increased up to 20 min, but then there was only insignificant difference in the pre-treatment with Moringa. Although these optimum conditions produced the best results, the treatment was also very effective at pH=6, pH=7, 0.2g/mL of Moringa and only 10 min treatment time.

A set of experiments was performed to establish the effect of initial concentration of Pb on lead removal with Moringa. The initial concentration of Pb did not significantly affect the efficiency of treatment with Moringa. The efficiency of removal was approx. 94%, regardless of initial concentration, with the exception to a very small concentration of 0.03ppm where there was complete removal of lead.

Although the pre-treatment with Moringa crushed seeds was very effective (96% of lead removal), the water after the treatment was turbid and not clear enough to drink. It was decided to adjust the procedure to include pre-treatment of lead-contaminated water with Moringa crushed seeds first, and then filter it through the sand filter, which was very effective in the removal of suspended particles in the water.

The effectiveness of heavy metal removal with Moringa crushed seeds depends on the bio-sorbent dosage, which initially increases with the added amount of bio-sorbent, but then decreases (Hossein et al.). The main idea behind the proposed 2-Stage filtration was to pre-treat the contaminated water with Moringa, filter it to remove the bio-sorbent material from the water, treat this cleared water again with Moringa to remove more heavy metals, and then filter it once more through the filter, so the effluent water would be clear enough for drinking. This 2-Stage filtration produced great results, made the water clear enough to drink, and had an efficient removal of **100% for Pb removal after just one pre-treatment and one filtration.**

5. CONCLUSION

5.1 Findings

The hypothesis was supported by the results – I was able to obtain significant to complete removal of lead in contaminated water.

- Highest Pb removal was obtained with 3g/100mL MO at pH=8 and 20 min treatment time
- Efficiency of lead removal with Moringa pre-treatment was not affected by the initial concentration
- Treatment with only Moringa seeds was very effective – over 96% of lead removal
- 2-Stage filtration was most effective -**100% removal** of lead after just one treatment and one filtration and clear water to drink

Pre-treatment/filtration method for lead removal is simple, extremely cost-effective, environmentally-friendly, and has great potential for the removal of lead in developing countries. This method of pre-treatment/filtration can also be used in the US in areas with elevated concentrations of lead in drinking water.

5.2 Future Research

The results obtained with this novel, bioremediation method of lead-contaminated water are very promising, but more research needs to be done to address the following:

- Effectiveness of treatment with Moringa and filtration through sand filter on removal of other heavy metals
- Saturation time before the filter needs to be renewed
- Recovery of heavy metals from biomass
- Effect of sand alone on removal of heavy metals

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5. APPENDIX A: SUPPLEMENTARY DATA TABLES

TABLE A1 EFFECT OF MORINGA DOSAGE ON Pb REMOVAL						
SAMPLES	ABSORBANCE		CONCENTRATION (ppm)*		EFFICIENCY (%)**	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
MP1-CN	0.2669	0.2599	3.79	3.69	0.0	0.0
MP1-A	0.07	0.0677	0.96	0.92	74.4	75.1
MP1-B	0.0452	0.0429	0.60	0.57	84.2	86.5
MP1-C	0.0399	0.0375	0.53	0.49	86.0	86.7
MP1-D	0.0324	0.0311	0.42	0.40	88.9	89.2
MP2-C	0.0324	0.0321	0.42	0.41	90.0	90.1

* Concentration calculated from Absorbance Calibration Curve (y=0.069x+0.003)
y=Absorbance; x=Concentration; x=(y-0.003)/0.069

** Efficiency =100% - (A/C)x100
A= Concentration after treatment with Moringa
C= Concentration in control sample “C”

C (MP1-CN) – Control sample – pH 7, 5 ppm of Pb

D1 (MP1-A) – 0.1g/100mL at 350 rpm, 20min, pH 7, 5ppm of Pb

D2 (MP1-B) – 0.2g/100mL at 350 rpm, 20min, pH 7, 5ppm of Pb

D3 (MP1-C) – 1g/100mL at 350 rpm, 20min, pH 7, 5ppm of Pb

D4 (MP2-C)– 3g/100mL at 350 rpm, 20min, pH 7, 5ppm of Pb

D5 (MP1-D) - 4g/100mL at 350 rpm, 20min, pH 7, 5ppm of Pb

TABLE A2 EFFECT OF pH ON Pb REMOVAL						
SAMPLES	**ABSORBANCE**		**CONCENTRATION (ppm)***		**EFFICIENCY (%)****	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
MP2-CN	0.2929	0.2934	4.16	4.17	0.0	0.0
MP2-A	0.0744	0.0741	1.02	1.02	75.5	75.5
MP2-B	0.0219	0.0217	0.27	0.26	93.5	93.8
MP2-C	0.0324	0.0321	0.42	0.41	90.0	90.1
MP2-D	0.014	0.0135	0.15	0.15	96.4	96.4

* Concentration calculated from Absorbance Calibration Curve (y=0.069x+0.003)
y=Absorbance; x=Concentration; x=(y-0.003)/0.069

** Efficiency =100% - (A/C)x100
A= Concentration after treatment with Moringa
C= Concentration in control sample "C"

C (MP2-CN) – Control sample – pH 7, 5 ppm of Pb

pH5 (MP2-A) – sample treated at pH 5, 3g/100mL, 350 rpm, 20min, 5ppm of Pb

pH6 (MP2-B) – sample treated at pH 6, 3g/100mL, 350 rpm, 20min, 5ppm of Pb

pH7 (MP2-C) – sample treated at pH 7, 3g/100mL, 350 rpm, 20min, 5ppm of Pb

pH8 (MP2-D) – sample treated at pH 8, 3g/100mL, 350 rpm, 20min, 5ppm of Pb

TABLE A3 EFFECT OF TREATMENT TIME ON Pb REMOVAL						
SAMPLES	**ABSORBANCE**		**CONCENTRATION (ppm)***		**EFFICIENCY (%)****	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
MP3-CN	0.2811	0.2792	3.99	3.97	0.0	0.0
MP3-A	0.0208	0.0209	0.25	0.25	93.7	93.7
MP3-B	0.0194	0.0198	0.23	0.24	94.2	94.0
MP3-C	0.013	0.0125	0.14	0.13	96.5	96.7
MP3-D	0.0159	0.0129	0.18	0.14	95.5	96.5

*Concentration calculated from Absorbance Calibration Curve (y=0.069x+0.003)
y=Absorbance; x=Concentration; x=(y-0.003)/0.069

** Efficiency =100% - (A/C)x100
A= Concentration after treatment with Moringa
C= Concentration in control sample "C"

C (MP3-CN) – Control sample – pH 7, 5 ppm of Pb

T10 (MP3-A) – sample treated at pH 7, 3g/100mL, 10 min, 350 rpm, 5ppm of Pb

T15 (MP3-B) – sample treated at pH 7, 3g/100mL, 15 min, 350 rpm, 5ppm of Pb

T20 (MP3-C) – sample treated at pH 7, 3g/100mL, 20 min, 350 rpm, 5ppm of Pb

T25 (MP3-D) – sample treated at pH 7, 3g/100mL, 25 min, 350 rpm, 5ppm of Pb

TABLE A4 EFFECT OF INITIAL CONCENTRATION OF Pb ON LEAD REMOVAL WITH MORINGA						
SAMPLES	**ABSORBANCE**		**CONCENTRATION (ppm)***		**EFFICIENCY (%)****	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
MP4-CN	0.4013	0.3983	5.72	5.68	0.0	0.0
MP4-CN-A MP4-A	0.0031	0.0037	0.0343 0.00	0.034 0.00	0.0 100.0	0.0 100.0
MP4-CN-B MP4-B	0.0048	0.0059	0.3432 0.02	0.34 0.04	0.0 94.2	0.0 88.2
MP4-CN-C MP4-C	0.0178	0.0182	3.432 0.21	3.4 0.21	0.0 93.9	0.0 93.8
MP4-CN-D MP4-D	0.4013 0.0241	0.3983 0.0241	5.72 0.30	5.68 0.30	0.0 94.8	0.0 94.7

* Concentration calculated from Absorbance Calibration Curve (y=0.069x+0.003)
y=Absorbance; x=Concentration; x=(y-0.003)/0.069

** Efficiency =100%-(A/C)x100;
A= Concentration after treatment with Moringa
C= Concentration in control sample "C"

C (MP4-CN) – Control sample – pH 7, 5 ppm of Pb

IC1 (MP4-A) – 0.03 ppm Pb sample treated at pH 7, 3g/100mL, 350 rpm

IC2 (MP4-B) – 0.3 ppm Pb sample treated at pH 7, 3g/100mL, 350 rpm

IC3 (MP4-C) – 3 ppm Pb sample treated at pH 7, 3g/100mL, 350 rpm

IC4 (MP4-D) – 5 ppm Pb sample treated at pH 7, 3g/100mL, 350 rpm

TABLE A5 2-STAGE FILTRATION (LEAD-CONTAMINATED WATER)									
SAMPLES	**ABSORBANCE**			**CONCENTRATION (ppm)***			**EFFICIENCY (%)****		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
1P-C-A	0.371	0.372	0.367	4.94	4.95	4.88	0.0	0.0	0.0
1P-M-A	0.021	0.019	0.018	0.27	0.25	0.25	94.5	95.0	94.9
1P-F-A	0.001	0.002	0.002	0.0	0.0	0.0	100.0	100.0	100.0
2P-M-A	0.001	0.001	0.001	0.0	0.0	0.0	100.0	100.0	100.0
2P-F-A	0.002	0.002	0.002	0.0	0.0	0.0	100.0	100.0	100.0

* Concentration calculated from Absorbance Calibration Curve (y=0.072x+0.004)
y=Absorbance; x=Concentration; x=(y-0.004)/0.072

** Efficiency =100% - (A/C)x100
A= Concentration after treatment with Moringa
C= Concentration in control sample "C"

C (1P-C-A) – Control sample (before pre-treatment and filtration)

P1 (1P-M-A) - First Pre-treatment of contaminated water with MO (30g/L); pH 8

F1 (1P-F-A) – First Filtration

P2 (2P-M-A) - Second Pre-treatment of contaminated water with MO (30g/L); pH 8

F2 (2P-F-A) – Second Filtration