Comparative Analysis of Selected Plants as Bioremediant for Lead-Contaminated Water

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Abstract: Lead has been identified by the World Health Organization (WHO) as 1 of 10 chemicals of major public health concern, necessitating action from the Member States for protection of workers, children, and women of reproductive age. It has been known that plants, animals, and sources thereof can be utilized for decontamination of metals (bioremediation). Simulated contaminated water was used for the entire bioremediating analysis. Based on previous studies, the use of peel wastes from *Ananas comosus* (L.) Merr. (Pineapple) and *Citrus aurantium* (L.) (Dalandan), of seeds from *Moringa oleifera* (L.) (Malunggay) and *Lycopersicon esculentum* (Mill.) (Tomato) and of leaves from *Allium sativum* (Garlic) and *Allium cepa* (Onion) as agents in removing lead from water could be cost-effective. This study aimed to compare the capacity of peels, seeds, and leaves as bioremediants in lead-contaminated water. Furthermore, it intended to determine the highest ability (lowest mean concentration value) of each identified plant in removing and chelating lead. The powdered plant samples were added to known amounts of metal in solution with adjusted pH and 10-minute-contact time and were carried out by a batch process. The filtrates were analyzed for residual heavy metal concentration using Flame Atomic Absorption Spectroscopy (FAAS). Optimal results were obtained at pH 5. The amount of lead absorbed increased with pH 5 and 10 % concentration at 10 minute-contact time. Malunggay seeds showed the highest percentage removal of lead and had the lowest mean concentration among the plant samples.

Keywords: Ananas comosus; Citrus aurantium; Moringa oleifera; Lycopersicon esculentum; Allium sativum; Allium cepa; lead

1. INTRODUCTION

1.1 Background

Water, as a basic human need is used everywhere, each day for drinking, cooking, washing clothes, cleaning, gardening, bathing, and the like. Industries have been a major consumer of water as well. It is a natural resource that can be easily be contaminated with bacteria, algae, and heavy metals, of which lead is a common one (Solidum & Solidum, 2012).

Lead is a naturally occurring element in the earth's crust which is about 0.0013%, and it is not considered to be a rare element since it is usually mined and refined (Environmental Protection Agency (EPA, 2015). It occurs as a bluish-gray metal which can be found in air, soil, and water. (Center for Disease Control and Prevention (CDC, 2015). Lead is a soft and malleable material which was used before to make water pipes, as a component of batteries, paints, wire, cable sheaths, ammunition, solders, gasoline, cosmetics and lead which formed alloys with metals like tin, copper, arsenic, antimony, bismuth, cadmium and sodium (Melcakova, I., & H., H., 2010).

Lead contaminates water because of the pipelines which are used in the delivery system. Pipes have high levels of lead, and its corrosion may cause the lead to be transferred into the water (CDC, 2015). Another reason for lead contamination in water is the sewage and manufacturers. It is also known to be toxic heavy metal, attacking the bone marrow, the peripheral and central nervous systems on chronic exposure. It is readily absorbed through inhalation (Solidum, 2012). This toxicity can affect the health of not only people but also animals. Lead has also been identified by the World Health Organization (WHO) as 1 of 10 chemicals of major public health concern, necessitating action from the Member States for protection of workers, children, and women of reproductive age. In 2013, the Institute for Health Metrics and Evaluation (IHME) had estimated around 853 000 deaths due to long-term effects on health due to lead exposure with the highest burden in low and middle-income countries. It also approximated that lead exposure accounted for 9.3% of the global burden of idiopathic intellectual disability, 4% of the global burden of ischemic heart disease and 6.6% of the global burden of stroke (WHO, 2016).

As a potent occupational toxin, lead’s toxicological manifestations are well known (Flora, et al., 2012). The non-biodegradable nature of lead is the prime reason for its prolonged persistence in the environment. The earth is a closed system where only energy can be exchanged with the external environment but not matter (Knebel & Wright 2000). Heavy metals that have been introduced and are still being introduced will stay in the environment for a long time. Lead is popularly known to cause chronic toxicity. Moreover, since cancer is known to be caused by multifactorial factors (heavy metals, included), the clinical detrimental effects of lead cannot be discounted. Complete control and prevention of lead exposure are still far from being achieved, even though lead toxicity is a highly explored and comprehensively published topic. There is no such level of lead that appears to be necessary or beneficial to the body, and no "safe" level of exposure to lead has been found (Flora et al., 2012); Agency for Toxic Substances and Disease Registry (ATSDR, 2017); WHO, 2010). Due to the unwanted side effects and chronic toxicity potentials of lead, the industry nowadays reduced the content of lead usage in some products such as cosmetics and gasoline.

Lead is a heavy metal which is not easily removed even by boiling or heating. Boiling or heating can slightly increase the lead concentration in water (CDC, 2015). Decontamination of water is one possible solution to reduce the toxicity effects. There have been several studies on removing or decontaminating lead in water. One of the methods mentioned is through bioremediation (Archana & Jaitly, 2014).

Bioremediation is a process using plants or microorganisms to decontaminate organic and inorganic xenobiotics. The two types of bioremediation are phytoremediation and micro-remediation. Phytoremediation is performed using plants while micro-remediation uses microbes (Archana & Jaitly, 2014).

There are various researches utilizing the peels or waste materials from plants as bioremediants. This includes peels of *Ananas comosus* (L.) Merr. (Pineapple) and *Citrus aurantium* (L.) (Dalandan), of seeds from *Moringa oleifera* (L.) (Malunggay) and *Lycopersicon esculentum* (Mill.) (Tomato) and leaves from *Allium sativum* (Garlic) and *Allium cepa* (Onion). Peels of *Ananas comosus* (L.) Merr. Have been found to be good biosorbent from lead-contaminated wastewater (Dokprathum, 2010; Solidum, 2013***).*** *Citrus aurantium* (L.) (Dalandan), of seeds and related species, also exhibited bioremediant properties (Habib, Salih, & Muhanad, 2012; Solidum, 2013). The same is true with *Moringa oleifera* (L.) seeds (Araújo, et.al, 2013). A study on phytoremediation potential of Tomato (Lycopersicon Esculentum Mill) in artificially contaminated soils was performed in a local setting and showed a decreasing pattern of the bioconcentration factor (BCF) which implied that the ability of tomato to accumulate heavy metals was reduced as the level of contamination is increased (Andal, F. and Ching, J., 2014). Onion and garlic waste could also be used to purify hazardous heavy metals in contaminated water according to a study conducted by Negi R et al. (2012). Furthermore, maximal extraction was possible for the lead which is one of the most troublesome metallic environmental pollutants (Negi R et al. (2012). As the selected plants have been known to have biosorbent properties, this research aimed to compare the effectiveness of those plant samples as bioremediants for the lead in simulated contaminated water. Specifically, it intended to determine the concentration of the selected plant samples that can reduce the lead content and its optimum pH level. Furthermore, it evaluated which among the selected plant samples have the highest chelating ability in a contaminated water sample, i.e., lowest mean concentration value using Flame Atomic Absorption Spectroscopy (FAAS).

This study proposes to recognize malunggay, garlic, onion, tomato, dalandan and pineapple as chelating/bioremediating agents for the lead in water by providing information to the public on how to decontaminate water by using plant sources which could be cost effective.

Only lead was analyzed in the simulated contaminated water. Determination of the amount of lead was done with the use of instrumental analysis, FAAS.

2. METHODOLOGY

Comparative analysis was used in the study. Plant samples were prepared, acid digested, and analyzed for lead by the used of Flame Atomic Absorption Spectroscopy (FAAS) (Solidum, 2013).

2.1 Sample Collection

Plant samples which are tomato and malunggay seeds were bought in Bureau of Plant and Industry along Quirino Avenue, Manila while the other plant samples, garlic, and onion leaves were bought in Divisoria, Manila. Pineapple peels were collected from the vendors along United Nations Avenue, Manila while the dalandan were bought in Paco, Manila. Plant samples were verified and authenticated by the Botany Department of National Museum, Philippines.

2.2 Period of Collection

Plant samples were collected from February 3 to 26 of 2016.

2.3 Preparation of Samples

The preparation and analysis of the samples were performed mainly based on the study of Solidum (2013).

2.3.1 Moringgaoleifera and Lycopersicon esculentum Powdered Seeds

The seeds of *Moringaoleifera* and *Lycopersicum esculentum* were washed with distilled water to remove any impurities and dried to 65°C for 24 hours. After drying, the shell parts of the seed were removed, and the kernels were grinded into a fine powder using a blender (Devakate, Patil, & Waje, 2009).

2.3.2 Ananas comosus and Citrus aurantium Powdered Peels

The collected peels were washed several times with distilled water to remove extraneous materials. The washed materials were divided into small pieces (10-20mm) then dried in a hot air oven at 60º C until constant weight. After drying, the plant materials were grinded separately using mortar and pestle and screened using a sieve of mesh 80 to an approximate size of 1.5 – 2 mm (Solidum, 2013).

2.3.3 Allium sativum and Allium cepaPowdered Leaves

The leaves were washed with distilled water to remove excess soil and other extraneous matter that might be present in the leaves. The washed materials were divided into small pieces (10-20mm) then dried in a hot air oven at 60º C until constant weight. After drying, the plant materials were grinded separately using mortar and pestle and screened using a sieve of mesh 80 to an approximate size of 1.5 – 2 mm (Solidum, 2013).

2.4 Reagent Preparation

A stock solution of lead (1000mg/L) was prepared by dissolving the desired quantity of lead salt in distilled water (Solidum, 2013).

2.5 Instrumental Analysis

The residual lead concentrations were measured using Flame Atomic Absorption Spectrophotometer (Shimadzu AA-6300) at Department of Chemistry Instrumentation, De La Salle University, Vito Cruz, Manila (Solidum, 2013).

2.6 Quantitative Analysis of Lead

The plant samples were added to 100 mL of the Lead solution at 0.1%, 1% and10% concentrations at pH 3 and five respectively since it has been known that the acidic environments offer the optimum lead chelation. The resulting mixture was agitated for ten minutes continuously in a constant circular motion. After agitation, the samples were filtered. The pH was adjusted using 0.1N nitric acid and 0.1N sodium hydroxide (Solidum, 2013).

2.7 Digestion of Samples

50 mL of the filtrate was collected in a beaker. 2.5 mL of concentrated nitric acid was added to the filtrate, and the resulting solution was immediately covered with a watch glass. Samples were heated in a water bath and evaporated without boiling. After evaporation, the volume was reduced to half, and the sides of the beaker and watch glass were washed with freshly distilled water. Another three mL of concentrated nitric acid was added to each sample and evaporated again to 20 mL. The samples were cooled down and filtered again. The resulting solutions were immediately transferred to 100 mL polyethylene bottles and stand for about 24 hours. They were the subjected to lead content analysis using Flame Atomic Absorption Spectroscopy (FAAS) at De La Salle University-Manila (Solidum, 2013).

2.8 Statistical Analysis

The significant difference of the reduction of lead using selected plant samples of malunggay, garlic, onion, tomato, dalandan, and pineapple was determined using two-way ANOVA. If a significant difference among the groups were established, Tukey's test (pair wise) was used to determine which pair of plant samples is significantly different from each other.

3. RESULTS AND DISCUSSION

The differences between the amounts of the untreated and treated samples were taken as the number of heavy metals removed by the malunggay and tomato seeds, garlic and onion leaves and pineapple and dalandan peels.

Figure 1 showed that tomato and dalandan have lower mean concentration value than the other four plant samples at 10 % in pH 3. It also showed that the trend in 0.1% either in pH 3 and five is increased in mean concentration value. At 10% in pH 5, malunggay had its lowest mean concentration value among other concentrations and pH.

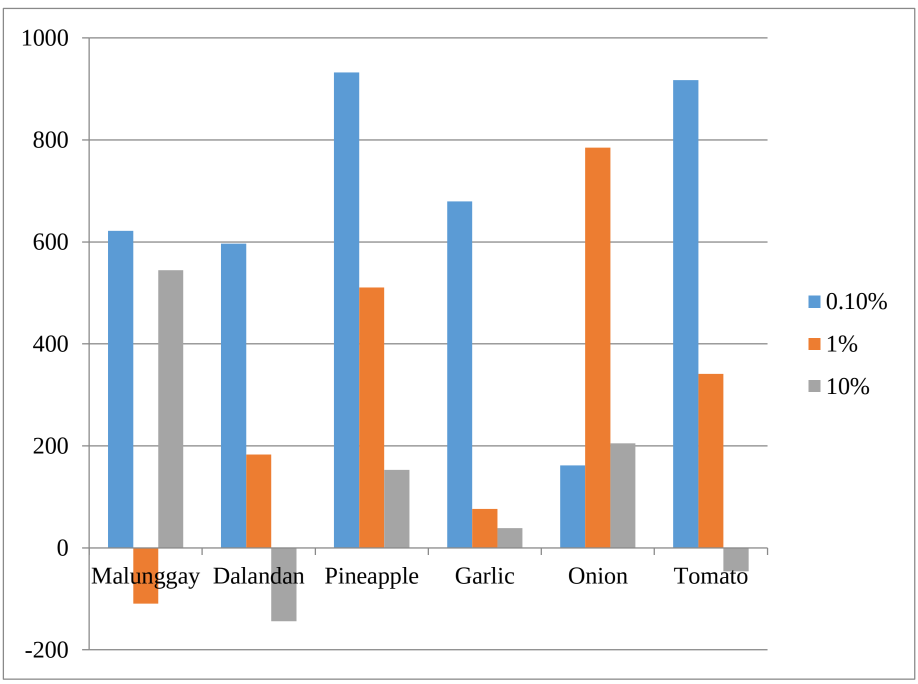

Fig 1. Mean Concentration Values of Each Plant at Sample and Percent Content at pH3

Table 1. Mean (Lowering/Decreased) Concentration Values of Each Plant Samples and % content and Different pH Combinations

	Malunggay	Dalandan	Pineapple	Garlic	Onion	Tomato
0.1%, pH3	621.5617	596.5886	932.5364	679.2377	161.9375	917.0769
1%, pH3	-109.199	182.7484	510.3719	76.3154	785.0761	340.9114
10%, pH3	544.2639	-143.686	153.0185	38.8557	204.7485	-46.1718
0.1%, pH5	650.697	390.2631	325.4519	653.67	725.2199	455.6688
1%, pH5	400.3712	-75.3071	47.7747	68.5856	710.7514	171.451
10%, pH5	-160.335	-129.416	44.8017	117.9372	157.5771	-134.767

Figure 2 presented that the highest ability of malunggay and pineapple to remove lead is at10% at pH five environment. In tomato and dalandan, it showed that the highest ability is 10% at pH three while garlic is 10% at pH 3 and onion is 10% at pH 5. It exhibited that the lowest mean concentration value per plant samples and their respective concentration and pH indicates their highest ability to chelate the lead. Moreover, it displayed that 0.1% concentrations have the highest mean concentration values for malunggay, dalandan and tomato which indicate their lowest ability to chelate the lead. Among all the plants, malunggay had the lowest concentration mean value at 10% in pH 5.

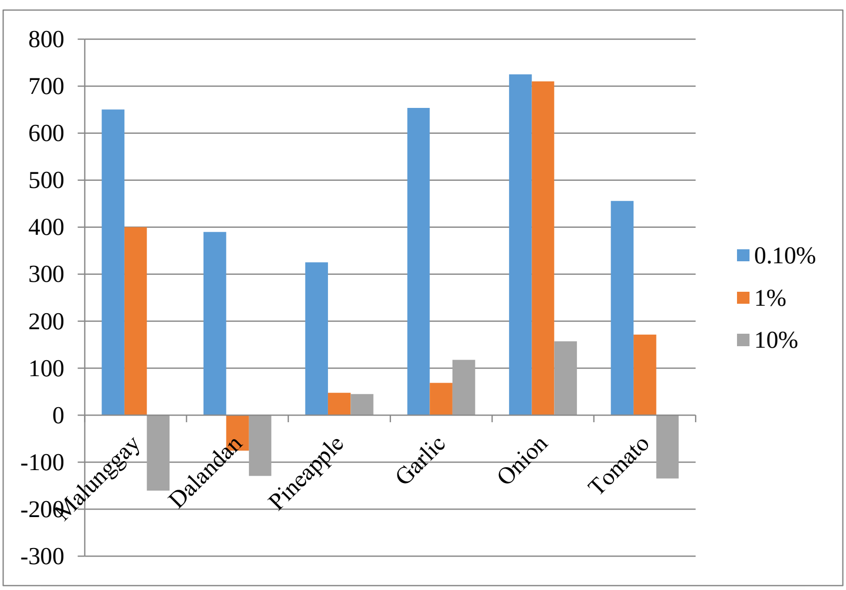

Figure 2. Mean Concentration Values of Each Plant Samples and Percent Content at pH5

Malunggay seeds showed the highest ability to remove lead at 10% and at pH5 which can be seen in Table 4. However, there was no declining trend of lead concentration removal by the malunggay seeds. Tomato seeds show the highest ability to remove lead at 10% and pH five while dalandan peels are at 10% and at pH three which can be seen in Table 10 and five respectively. For onion leaves, it had the highest ability in removing lead at 10% and pH five while garlic leaves are at 1% and at pH five which can be seen in Table 9 and eight respectively. Pineapple peels had the same value of lead removal at 1 and 10% and at pH five which can be seen in Table 6. Among the plant samples, malunggay seeds had the highest ability of chelating lead in water.

Table 2. Mean Concentration Values of each Plant, Regardless of Percent Content at Different pH combinations

Plant	Mean	Standard Error
Malunggay	324.6261	81.18563
Dalandan	136.9313	68.19271
Pineapple	335.6922	75.91476
Garlic	272.4996	67.89722
Onion	457.6508	69.06269
Tomato	284.1934	84.65942

Table 3. Mean Concentration Values of each Percent Content and pH Combination, regardless of Plant Selected

Percent Content and pH combination	Mean	Standard Error
0.1%, pH3	651.5889	62.31392
1%, pH3	297.8691	70.92107
10%, pH3	125.2376	53.5172
0.1%, pH5	533.4951	36.59094
1%, pH5	220.7036	63.91657
10%, pH5	-17.30078	31.28209

Table 4. Percent Removal of Lead by the Selected Plants per pH and Concentration at Ten-minute Contact Time

		0.1 % Sample	**1% Sample**	**10% Sample**
Malunggay Seeds	pH 3	40%	110%	44%
	pH 5	33%	61%	116%
Dalandan Peels	pH 3	35%	80%	114%
	pH 5	61%	108%	113%
Pineapple Peels	pH 3	5%	48%	84%
	pH 5	69%	95%	95%
Garlic Leaves	pH 3	33%	92%	96%
	pH 5	37%	94%	88%
Onion Leaves	pH 3	84%	28%	78%
	pH 5	27%	33%	86%
Tomato Seeds	pH 3	7%	66%	105%
	pH 5	57%	83%	113%

Two-way ANOVA test was employed for statistical analysis. In Table 10, it shows that the mean concentration value is significantly different for at least one pair of plant samples (p-value of 0.0019). The results exhibit the same results that of the mean concentration value for at least one pair of a combination of % content and pH (p-value of 0.0001). Because both null hypotheses are rejected, multiple comparison tests (Tukey's Test) is used to determine which pairs are significantly different from each other. The decision rule of rejecting the null hypothesis (Ho) is if the p-value is less than 0.05.

Table 5. Two-way ANOVA Test for Plants and Combination (% concentration & pH)

Source	**Prob> F**
Plants	0.0019
Combination (% conc. and pH)	0.0001

Table 6 showed that only onion and dalandan have significantly different mean concentration values at a p-value of 0.001. Contrast is the difference between the mean concentration values of two plants. For example, the contrast of onion and dalandan is 320.7195. This means that the difference in mean concentration values of onion and dalandan is 320.7195. Because the value of contrast is positive, this implies that onion has a higher mean concentration value.

Table 6. Tukey's Test for the Plant Samples

Plants	Contrast	Tukey p>t
DalandanvsMalunggay	-187.6948	0.110
Pineapple vsMalunggay	11. 06612	1.000
Garlic vsMalunggay	-52.12643	0.979
Onion vsMalunggay	133. 0248	0.448
Tomato vsMalunggay	-40.43268	0.993
PineappplevsDalandan	198.7609	0.076
Garlic vsDalandan	135.5683	0.426
Onion vsDalandan	320.7195	0.001
Tomato vsDalandan	147.2621	0.332
Garlic vs. Pineapple	-63.19255	0.952
Onion vs. Pineapple	121.9586	0.546
Tomato vs. Pineapple	-51.4988	0.980
Onion vs. Garlic	185.1512	0.119
Tomato vs. Garlic	11.69376	1.000
Tomato vs. Onion	-173.4574	0.169

From the results showed in table 7, the pair combinations that were significantly different from each other has a p-value of <0.05. These are 1% pH 3 and 0.1% pH 3; 10% pH 3 and 0.1% pH 3; 1% pH 5 and 0.1% pH 3; 10% pH 5 and 0.1% pH 3; 10% pH 5 and 1% pH 3; 0.1% pH5 and 10% pH3; 1% pH 5 and 0.1% pH 5; 10% pH 5 and0.1% pH

Table 7. Tukey's Test for Combination (Concentration and pH)

Plants	Contrast	Tukey p>t
1%, pH 3 vs 0.1%,pH 3	-353.7197	0.001
10%,pH 3 vs 0.1%, pH 3	-526.3513	0.001
0.1%, pH 5 vs 0.1%,pH 3	-118.0938	0.581
1%, pH 5 vs 0.1%,pH 3	-430.8853	0.001
10%, pH 5 vs 0.1%,pH 3	-668.8897	0.001
10%, pH 3 vs 1%,pH 3	-172.6316	0.173
0.1%, pH 5 vs 1%,pH 3	235.626	0.019
10%, pH 5 vs 1%,pH 3	-77.16559	0.894
10%, pH 5 vs 1%,pH 3	-315.1699	0.001
0.1%, pH5 vs 10% pH3	408.2576	0.001
1%, pH 5 vs 10%,pH 3	95.466	0.775
10%, pH 5 vs 10%, pH 3	-142.5283	0.369
1%, pH 5 vs 0.1%,pH 5	-312.7916	0.001
10%, pH 5 vs 0.1%,pH 5	-550.7959	0.001
10%, pH 5 vs 1%,pH 5	-238.0043	0.017

The above results substantiate the previous findings that these plants have bioremediant properties. Peels of *Ananascomosus*(L.) Merr. have been found to be good biosorbent from lead-contaminated wastewater (Dokprathum, 2010; Solidum, 2013). *Citrus aurantium* (L.) (Dalandan), of seeds and related species, also exhibited bioremediant properties (Habib, Salih, & Muhanad, 2012; Solidum, 2013). The same is true with *Moringa oleifera* (L.) seeds (Araújo et al., 2013). A study on phytoremediation potential of Tomato (Lycopersicon Esculentum Mill) in artificially contaminated soils was performed in a local setting and showed a decreasing pattern of the bioconcentration factor (BCF) which implied that the ability of tomato to accumulate heavy metals was reduced as the level of contamination is increased (Andal & Ching, 2014). Onion and garlic waste could also be used to purify hazardous heavy metals in contaminated water according to a study conducted by Negi et al. (2012). Furthermore, maximal extraction was possible for the lead which is one of the most troublesome metallic environmental pollutants (Negi et al. (2012).

4. CONCLUSION

The comparison of effectiveness among the six plant samples as bioremediants shows that they all have the ability in the chelating lead in water. Among the selected plants, malunggay has the highest ability in the chelating lead since it has the lowest mean concentration value of -160.335. Also, it also has the highest percentage removal amount of lead which is in 10% concentration at pH 5. The public, industry, health sectors and agencies can then be informed (upon further studies) that malunggay seeds might be used as water-decontaminant.

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