Application of Corncob Powder Adsorbent for Removal of Heavy Metal Ions

Su Su Aung[1] , Khin Ei Ei Thein[2]

Abstract

The present research work is the preparation of corncob powder to be used as the effective sorbents for the removal of heavy metal lead and cadmium. The corncobs were collected from the Shwe Kyi Narr village, Banmaw Township in Kachin State. Thermal activation of corncob powder (80-mesh size) was prepared by heating process of 100˚C at 2 hr. The elemental content of corncob powder was determined by EDXRF analysis. The removal of heavy metal lead and cadmium from aqueous solution on thermal activated corncob powder were carried out by titrimetric method. The effects of sorption parameters (concentration of metal ions solution, contact time and sorbent dose) were investigated. For concentration effect, 100, 200, 300, 400, 500, and 600 mg L^{-1} of each metal ions were used on sorbent dosage 0.50 g at 60 min contact time for shaking at 150 rpm . For contact time method, the initial concentration 300 mg L^{-1} of each metal ions solution and sorbent dosage 0.50 g were used with various content time 15, 30, 45, 60, 75, 90 , 105, 120, and 135 min respectively. For dosage method, the amount of sorbents 0.25 g, 0.50 g, 0.75 g, 1.00 g , 1.25 g, and 1.50 g and initial concentration 300 mg L^{-1} were used at 60 min contact time for shaking at 150 rpm Sorption isotherm studies corresponded to Langmuir and Freundlich isotherms were used. And then, significant sorption parameters of these isotherms were also evaluated. Based on R^2 values the Freundlich isotherm is more favorable and multilayer adsorption is possible. Based on the percent removal results, the sorption capacities of sorbent for the removal of lead are greater than that of cadmium. The kinetics of adsorption could be described using pseudo-first order and pseudo-second order kinetic model. In all cases, the kinetics follows the pseudo-second order rate equation.

Keywords : corncob powder, adsorption , lead, cadmium, isotherm ,kinetics

Introduction

Heavy metal pollution is one of the most significant environment problems since they are non-degradable and can accumulate in the food chain, and hence in the human body (Zhu *et al.*, 2012). Industrial and mining wastewaters are important sources of heavy metal pollution (Quek *et al.*, 1998). As a result of rapid industrialization, toxic metals and metalloid such as cadmium, lead, chromium, mercury, arsenic and copper are released into the environment resulting in damage in ecosystems and human health. Unlike organic pollutants, heavy metals are non-biodegradable in the environment and can accumulate in living tissues particularly in human bodies causing significant physiological disorders such as damage of central nervous system and blood composition, production of energy and irreversible damage of vital organs of body. Hence, the presence of heavy metals in natural or industrial wastewaters is a subject of great interest

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in environmental science which is one of the most serious worldwide environmental problems (Axtell *et al*., 2003; Amarasinghe *et al*., 2007).

Toxicological effects of acute cadmium poisoning are manifested in a variety of symptoms including high blood pressure, kidney damage, and destruction of red blood cells. Cadmium is used in a wide variety of industries such as the electroplating industry, nickel-cadmium batteries, pigments, plastics, pesticides, dyes and textile operation. Lead is known to damage the kidney, liver and reproductive system, basic cellular processes and brain functions. The toxic symptoms are anemia, insomnia, headache, dizziness, irritability, weakness of muscles, hallucination and renal damage (Naseem and Tahir, 2001). The main sources of human exposure to lead include the uses of leaded gasoline, industrial sources such as lead mining, smelting and coal combustion, the use of lead-based paint and lead containing pipes in water supply systems. Therefore, a systematic study of the removal of cadmium and lead from wastewater is of considerable significance from an environmental point of view. Many treatment processes such as oxidation, reduction, precipitation, membrane filtration, ion exchange and adsorption have been used to remove metal ions from aqueous solutions (Gupta *et al.,* 2001). Among the above methods, adsorption is simple and cost-effective and it has been widely used (Boparai *et al*., 2011). Corncob is one of the low-cost adsorbents and it is used as a type of biowaste such as firestarter for cooking, as a biofuel, charcoal production. Chemical composition of corncob ash could be suitably used in blended cement production. (Kaliyan and Vance, 2008)

Materials and Methods

General

The corncobs were collected from the area of Shwe Kyi Narr village, Banmaw Township at Kachin State. The collected corncobs were dried in air and ground with mechanical blender. The finely powdered corncob samples were sieved with 80-mesh size. Then, the corncob samples were taken to carry out the experiment. The chemicals used in this experimental work were from the British Drug House Chemical Ltd., Kanto Chemical Co. Ltd. All chemicals are of reagent grade and were used as received. In all investigations, the methods and techniques involve both conventional and modern methods. This research work , the absorption property of prepared corncob samples were investigated due to the effect of initial metal ion concentration of metal ions , effect of adsorbent dose and effect of contact time .The phenomena of adsorption is generally applied for the removal of heavy metal lead and cadmium from solution. The sorption studies of the corncob powder can be carried out by the sorbent-sorbate interaction with the lead nitrate solution and cadmium sulphate solution. In the sorption studies initial concentration effect, dosage method and contact time method were used.

Preparation of Sample

The corncob sample was cut into small pieces and dried in air, finely powdered and sieved with 80-mesh size. An accurately weighed sample (30 g) was put into a pre-dried and cooled dish with a cover. The uncovered dish was placed in an electric oven and heated for 2 hr at 100°C. After heating, the resultant sample was cooled at room temperature and stored in a sealed bottle.

ED XRF Analysis

The elements in the corncob powder sample were determined by EDXRF 8000 spectrometer, Shimadzu, Japan at Department of Chemistry, West Yangon University. The finely powdered and dried sample was placed in the EDXRF 8000 spectrometer and measured according to instruction manual. The EDXRF spectrometer was recorded by spectrum and table. The EDXRF spectrum is shown in Figure 1.

Adsorption Properties of Lead and Cadmium from Aqueous Solution by Corncob Powder

Effect of Initial Concentration

Each 0.50 g corncob powder was added to 100 mgL^{-1}, 200 mgL^{-1}, 300 mgL^{-1}, 400 mgL^{-1}, 500 mgL^{-1} and 600 mgL^{-1} of $Pb (NO_3)_2$ solution. Each sample solution was shaken with a shaker at 150 rpm for one hour at room temperature. Then, the mixture was filtered through filter paper and Pb^{2+} ion in the filtrate was determined by EDTA titrimetric method.5 mL of filtrate was placed in a conical flask. 1 drop of xylenol orange was added and the color become to red. Then, 1 drop of 1M HNO_3 was added and the red color changed to yellow color. And then 3 drops of 5% hexamine powder solution was added and the solution was changed to red colour. Finally, the mixture was titrated with 0.001208 M EDTA. The end point colour was yellow. The same procedure was carried out for removal of cadmium but 1M sulphuric acid was substituted for 1M nitric acid and the concentration of 0.002415M EDTA was used for this titration.

Effect of Dosage

Each 25 mL of 300 mgL^{-1} $Pb (NO_3)_2$ solution was added to 0.25g, 0.50g, 0.75g , 1.00g, 1.25g, and 1.50g corncob powder. This sample solution was shaken with a shaker at 150 rpm for one hour at room temperature. Then, the mixture was filtered through filter paper and Pb^{2+} ion in the filtrate was determined by EDTA titrimetric method.

5 mL of filtrate was placed in a conical flask. 1 drop of xylenol orange was added and the color become to red . Then 1 drop of 1M HNO_3 was added and the red color was changed to yellow color. And 3 drops of 5% hexamine powder solution was added and the solution was become to red. Finally , the mixture was titrated with 0.001208 M EDTA. The end point colour was yellow. The same procedure was carried out for removal of cadmium but 1M sulphuric acid was substituted for 1M nitric acid and the concentration of 0.002415M EDTA was used for this titration.

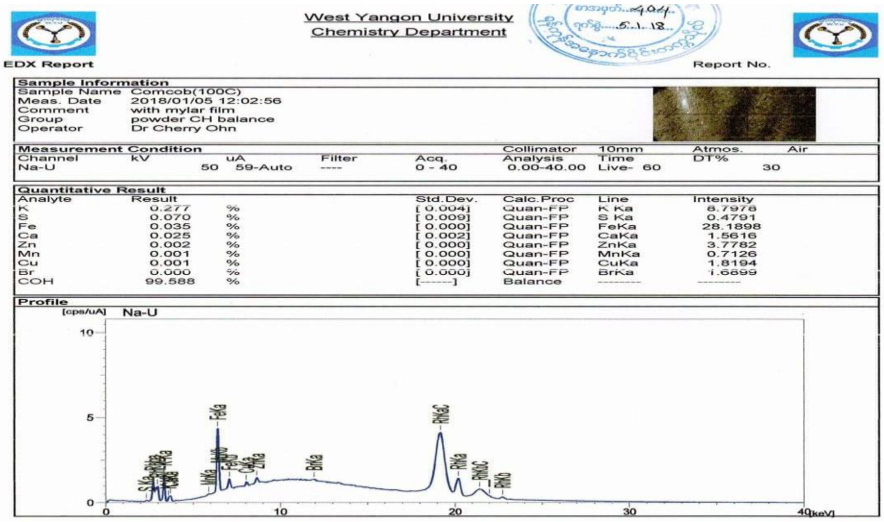
Effect of Contact Time

25 mL of 300 mgL^{-1} $Pb (NO_3)_2$ solution was added to 0.50g corncob powder. This sample solution was shaken with a shaker at 150 rpm for 15 min at room temperature. Then, the mixture was filtered through filter paper and Pb^{2+} ion in the filtrate was determined by EDTA titrimetric method. 5 mL of filtrate was placed in a conical flask. 1 drop of xylenol orange was added and the color was changed to red color. Then 1 drop of 1M HNO_3 was added and the red color was changed to yellow color. And then 3 drops of 5% hexamine powder solution was added and the solution was changed to red colour. Finally, the mixture was titrated with 0.001208 M EDTA. The end point colour was yellow. Similarly, the shaken time periods was changed 30 min, 45 min, 60 min, 75 min, 90 min, 105 min, and 120 min respectively. The same procedure was carried out for

removal of cadmium but 1M sulphuric acid was substituted for 1M nitric acid, and the concentration of 0.002415M EDTA was used for this titration.

Results and Discussion

The elemental contents of the corncob powder was determined by energy disperses X-ray fluorescence (EDXRF) method. The EDXRF spectrum was represented in Figure 1. From the experimental data, organic compounds (99.588%) were observed. Then, K as the major constituent was observed. And then, S, Fe, and Ca were observed as the minor constituents. EDXRF spectrum quantitatively shows the presence of the trace amounts of elements such as Mn, Zn and Cu in the corncob sample.



West Yangon University
Chemistry Department

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EDX Report

Report No.

Sample Information

Sample Name Comcob(100C)
Meas. Date 2018/01/05 12:02:56
Comment with mylar film
Group powder CH balance
Operator Dr Cherry Ohn

Measurement Condition

Channel	kV	uA	Filter	Acq.	Collimator Analysis	10mm Time	Atmos. DT%	Air
Na-U	50	59-Auto	----	0 - 40	0.00-40.00	Live- 60	30	

Quantitative Result

Analyte	Result		Std.Dev.	Calc.Proc	Line	Intensity
K	0.277	%	[0.004]	Quan-FP	K Ka	8.7978
S	0.070	%	[0.009]	Quan-FP	S Ka	0.4791
Fe	0.035	%	[0.000]	Quan-FP	FeKa	28.1898
Ca	0.025	%	[0.002]	Quan-FP	CaKa	1.5616
Zn	0.002	%	[0.000]	Quan-FP	ZnKa	3.7782
Mn	0.001	%	[0.000]	Quan-FP	MnKa	0.7126
Cu	0.001	%	[0.000]	Quan-FP	CuKa	1.8194
Br	0.000	%	[0.000]	Quan-FP	BrKa	1.6899
COH	99.588	%	[------]	Balance	--------	--------

Profile

Figure 1 EDXRF spectrum of corncob powder

Adsorption Study

The phenomena of adsorption is generally applied for the removal of heavy metal lead and cadmium from solution. The sorption studies of the corncob powder can be carried out by the sorbent-sorbate interaction with the lead nitrate solution and cadmium sulphate solution. In the sorption studies initial concentration effect, dosage method and contact time method were used.

Effect of Initial Metal Ion Concentration

Table 1 shows the percent of removal of lead and cadmium by a constant amount of sorbents sample with initial concentration in the range from 100 mgL^{-1} to 600 mgL^{-1} of lead nitrate solution and cadmium sulphate solution. Figure 2 shows the corresponding data in terms of the percent of removal with respect to initial concentration. It can be seen that as the percent of removal of lead and cadmium decreases with increases in initial concentration. Therefore it was evident from the results that lead and cadmium adsorption were dependent on the initial metal concentration. In addition, it was observed that the percent of removal lead was more than that of cadmium on the corncob powder sample.

Table 1 **Effect of Initial Concentration of Lead and Cadmium on Corncob Powder**

No	Dose (g/25 mL)	C_0 (mgL^{-1})	Lead		Cadmium	
			C_e (mgL^{-1})	Removal (%)	C_e (mgL^{-1})	Removal(%)
1	0.50	100	20.02	79.78	32.58	67.42
2	0.50	200	65.08	67.46	70.58	64.71
3	0.50	300	110.13	63.29	135.80	54.73
4	0.50	4000	195.23	51.19	184.60	28.85
5	0.50	500	265.32	46.94	423.49	15.30
6	0.50	600	335.40	44.10	553.80	7.70

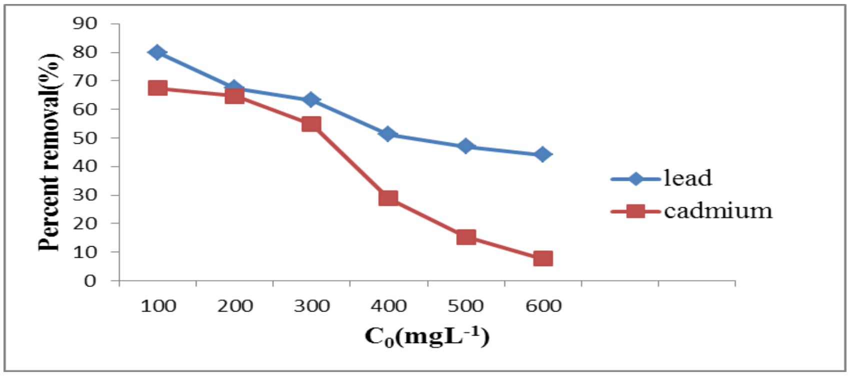


Figure 2 **Effect of initial concentration of lead and cadmium on corncob powder**

Effect of Adsorbent Dose

Adsorption efficiency of lead and cadmium were studied by varying the amount of adsorbent from 0.25 g to 1.50 g with constant other parameters (initial concentration and contact time). The results were shown in Table (2) Figure (3) shows that the removal efficiency of metal ion usually progressed on increasing adsorbent doses. This may occur due to the fact that the higher dose of adsorbent in the solution provides the greater availability of exchangeable sites for the ions. The maximum percent of the removal of lead was about 93.33% and cadmium was 85.52% at the dosage of 1.50 g.

Table 2 **Effect of Dosage on the Removal of Lead and Cadmium by Corncob Powder**

No	Dose (g/25mL)	C_0 (mgL^{-1})	Lead		Cadmium	
			C_e(mgL^{-1})	Removal(%)	C_e(mgL^{-1})	Removal (%)
1	0.25	300	175.21	41.60	190.04	36.65
2	0.50	300	110.13	63.29	135.80	54.73
3	0.75	300	65.08	78.31	97.74	67.42
4	1.00	300	45.03	84.99	76.02	74.66
5	1.25	300	30.04	89.99	54.30	81.90
6	1.50	300	20.02	93.33	43.44	85.52

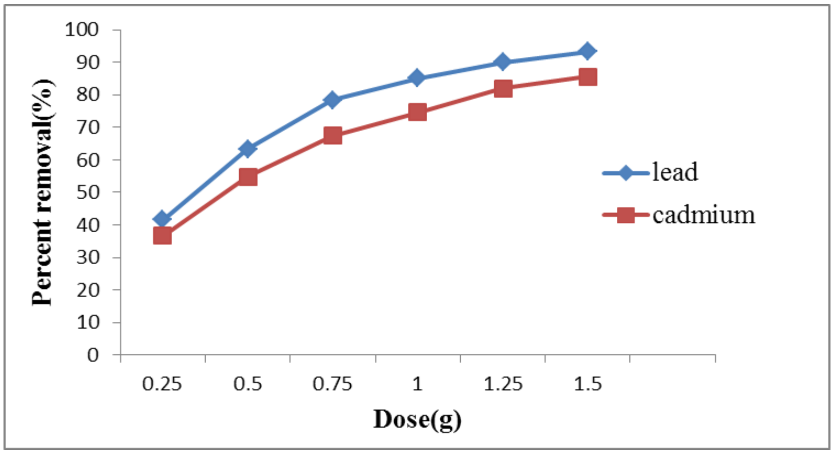


Figure 3 **The effect of dosage on the removal of lead and cadmium by corncob powder**

Effect of Contact Time

Figure (4) indicated that metal ions removal was increased with increase in contact time before equilibrium was reached. Dose of adsorbent and concentration of metal ions solution were kept constant. The results showed that lead ion removal was increased from 31.59% to 84.98% with the contact time variation from 15 to 120 min.

The percent of the removal of lead was constant at 120 and 135 min, which showed that equilibrium was reached at 120 min itself. For cadmium ion, the removal percent was changed from 34.85% to 81.90% with the contact time interval from 15 to 135 min. The maximum percent of the removal was observed at 135 and 150 min, which showed that equilibrium was reached at 135 min itself.

Table 3 Effect of Contact Time on the Sorption of Lead and Cadmium by Corncob Powder

No	Contact Time (min)	C_0 (mgL^{-1})	Lead		Cadmium	
			C_e(mgL^{-1})	Removal(%)	C_e (mgL^{-1})	Removal (%)
1	15	300	205.24	31.59	195.46	34.85
2	30	300	185.22	38.26	176.50	41.17
3	45	300	150.18	49.94	152.02	49.33
4	60	300	110.13	63.29	135.80	54.73
5	75	300	90.11	69.96	124.88	58.37
6	90	300	75.09	74.97	97.74	67.42
7	105	300	55.07	81.64	65.16	78.28
8	120	300	45.05	84.98	59.72	80.09
9	135	300	45.05	84.98	54.30	81.90
10	150	300	-	-	54.30	81.90

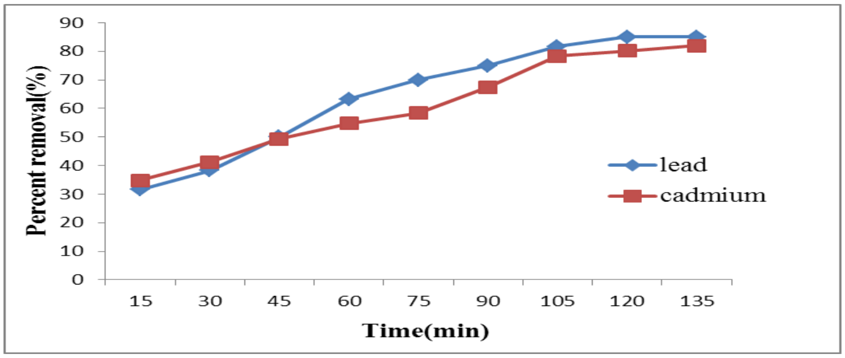


Figure 4 The effect of contact time on the removal of lead and cadmium by corncob powder

Adsorption Isotherm Studies

The equilibrium adsorption isotherms are one of the promising data to understand the mechanism of the adsorption. Various isotherm equation are well known and two different isotherms are selected in this study, which are the Langmuir and Freundlich isotherms. The Langmuir adsorption isotherm assumes that adsorption takes place at specific homogeneous sites within the adsorbent and has found successful applications in many adsorption processes of monolayer adsorption. The Langmuir isotherm is more meaningful and a potential plot. It is based on theoretical concepts. The parameter constants reflect the significance of sorption phenomena.

Tables (4 and 5) represent the data and Figures (5 and 6) showed the Langmuir and Freundlich isotherms for two metal ions solution on corncob powder. The Langmuir sorption equation was used to fit the experimental sorption data.

$$q_e = \frac{X_m\, b\, C_e}{1 + b\, C_e}$$

Where, q_e = the amount of sorbate adsorbed per unit mass of sorbent

C_e = equilibrium concentration of the sorbate in solution

X_m= the maximum monolayer amount of sorbate per unit mass of sorbent

b = Langmuir constant related to the affinity between the sorbent and sorbate

The linear equation is

$$\frac{C_e}{q_e} = \frac{1}{X_m b} + \frac{C_e}{X_m}$$

Therefore, the plot of $\frac{C_e}{q_e}$ versus C_e gives a straight line of slope $1/X_m$ and intercept $1/X_m$b.Regarding the above equation, Figures gave the linear plot. The value of the Langmuir constants X_m and b with the correlation coefficient are listed in Table 3.10.

The Freundlich isotherm is an empirical equation employed to describe heterogeneous systems. The linearized form of the Freundlich isotherm equation is:

$$q_e = KC_e^{1/n}$$

Where, q_e is the sorption capacity and C is the concentration of substance remaining unadsorbed at equilibrium. The two constant K and 1/n are particular to the system, i.e., to both sorbent used and nature of the sorbent. The equation in logarithmic form is:

$$\text{Log } q_e = \frac{1}{n} \log C_e + \log K$$

The calculated results of the Langmuir and Freundlich isotherms parameters are presented in Table 3.8. According to the correlation coefficient (R^2) of the two isotherms, the adsorption of lead and cadmium on corncobpowder can be modeled well.The essential feature of Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor or equilibrium parameter, R_L which is used to predict if an adsorption system favorable or unfavorable .

The separation factor, R_L is defined by:

$$R_L = \frac{1}{1 + b\, C_o}$$

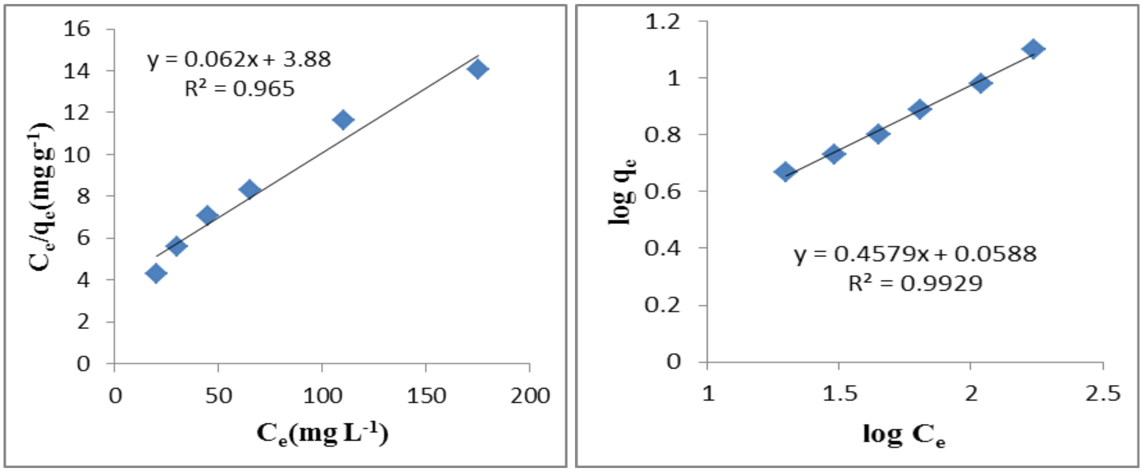
Where, C_o is the initial concentration and b is the Langmuir adsorption equilibrium constant. Based on the effect of separation factor on isotherm shape, the R_L values are in the range of $0 < R_L < 1$, which indicates that the adsorption of lead and cadmium on corncob powder were favorable.

Table 4 **Data Treatment of Langmuir and Freundlich Isotherms for Sorption of Lead on Corncob Powder (Dosage Method)**

Initial Concentration = 300mgL^{-1}

Contact time = 60 min

Dose (g/25mL)	C_e (mgL^{-1})	q_e (mg g^{-1})	Ce/qe (gL^{-1})	log C_e	log q_e
0.25	175.21	12.48	14.04	2.24	1.10
0.50	110.13	9.45	11.65	2.04	0.98
0.75	65.08	7.83	8.31	1.81	0.89
1.00	45.03	6.37	7.07	1.65	0.80
1.25	30.04	5.40	5.56	1.48	0.73
1.50	20.02	4.67	4.29	1.30	0.67



(a) (b)

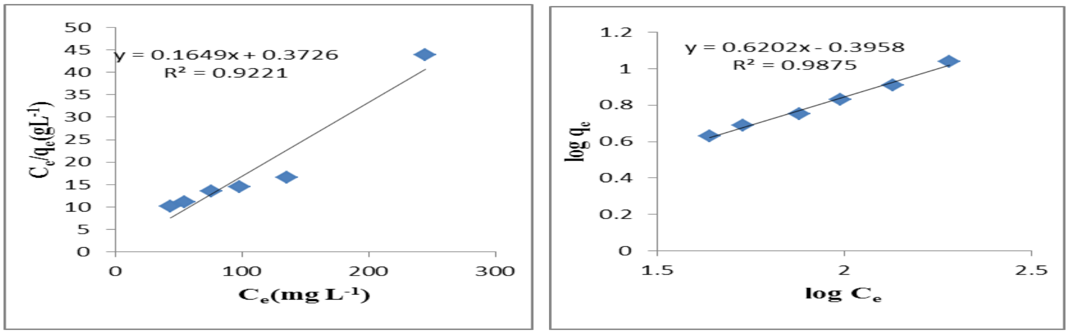
Figure 5 **(a) Langmuir isotherm and (b) Freundlich isotherm on the removal of lead by corncob powder**

Table 5 Data Treatment of Langmuir and Freundlich Isotherms for Sorption of Cadmium on Corncob Powder (Dosage Method)

Initial Concentration = 300mgL^{-1}

Contact time = 60 min

Dose (g/25mL)	C_e (mgL^{-1})	q_e (mg g^{-1})	Ce/qe (gL^{-1})	log C_e	log q_e
0.25	190.04	10.96	17.34	2.28	1.04
0.50	135.80	8.21	16.54	2.13	0.91
0.75	97.74	6.74	14.50	1.99	0.83
1.00	76.02	5.60	13.58	1.88	0.75
1.25	54.30	4.91	11.06	1.73	0.69
1.50	43.44	4.28	10.15	1.64	0.63



(a) **(b)**

Figure 6 (a) Langmuir isotherm and (b) Freundlich isotherm on the removal of cadmium by corncob powder Adsorptions Kinetics

The kinetic study of adsorption plays an important role because it affords important insight into the reaction pathways and into the mechanism of the reaction. Kinetic models have been proposed to explain the mechanism of a solute sorption from aqueous solution onto an adsorbent:

- Pseudo first order kinetic model.
- Pseudo second order kinetic model.

The pseudo first-order kinetic model has been widely used to predict the metal adsorption kinetics. The metal adsorption kinetics following the pseudo first-order model is given by (Ho and Mckay, 1999): $\frac{dq}{dt} = k_1 (q_e - q_t)$

Where k_1 (min−1) is the rate constant of the pseudo-first-order adsorption, q_t (mg/g) denotes the amount of adsorption at time t (min) and q_e (mg/g) is the amount of adsorption at equilibrium. After definite integration by application of the conditions $q_t = 0$ at $t = 0$ and $q_t = q_t$ at $t = t$, pseudo first-order equation becomes.

$$\log(q_e - q_t) = \log q_e - \left(\frac{k_1}{2.303}\right) t$$

By plotting $\log(q_e - q_t)$ versus t, the adsorption rate can be calculated.

The adsorption kinetic data can be further analyzed using Ho's pseudo second-order kinetics (Ho and Mckay, 1999). This is represented by: $\frac{dq}{dt} = k_2(q_e - q_t)^2$

Integration of the above equation and application of the conditions $q_t = 0$ at $t = 0$ and $q_t = q_t$ at $t = t$, gives

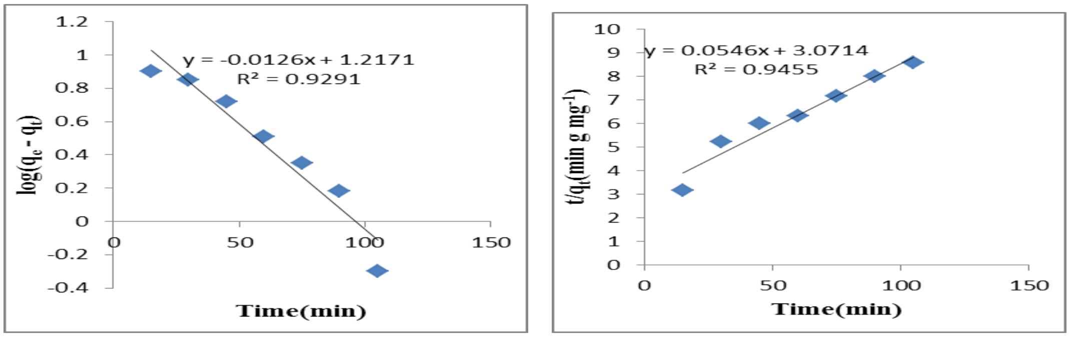
Where k_2 (g/(mg min)) is the rate constant, k_2 and q_e can be obtained from the intercept and slope.

Table 6 Kinetic Study for Sorption of Lead on Corncob Powder

Initial Concentration = 300mgL^{-1}

Dose = 0.5g/25mL

No	Time (min)	C_e (mgL^{-1})	q_t (mg g^{-1})	$q_e - q_t$ (mg g^{-1})	t/q_t (min g mg^{-1})	$\log(q_e - q_t)$
1	15	205.24	4.74	8.01	3.16	0.90
2	30	185.22	5.74	7.01	5.22	0.85
3	45	150.18	7.49	5.26	6.01	0.72
4	60	110.13	9.49	3.26	6.32	0.51
5	75	90.11	10.49	2.26	7.15	0.35
6	90	75.09	11.25	1.50	8.00	0.18
7	105	55.07	12.25	0.50	8.57	-0.30
8	120	45.05	12.75	0.50	8.57	-0.30
9	135	45.05	12.75	0.50	8.57	-0.30



(a) (b)

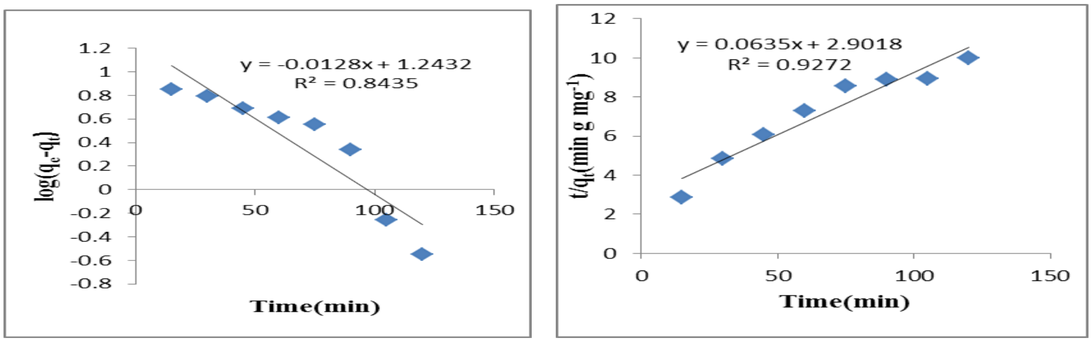
Figure 7 (a) Pseudo-first order and (b) Pseudo-second order kinetics plot for lead adsorption

Table 7 **Kinetic Study for Sorption of Cadmium on Corncob Powder**

Initial Concentration = 300mgL^{-1}

Dose = 0.5g/25mL

No	Time (min)	C_e (mgL^{-1})	q_t (mg g^{-1})	q_e - q_t (mg g^{-1})	t/ q_t (min g mg^{-1})	log(q_e - q_t)
1	15	195.46	5.23	7.06	2.87	0.85
2	30	176.50	6.18	6.11	4.85	0.79
3	45	152.02	7.40	4.89	6.08	0.69
4	60	135.80	8.21	4.08	7.31	0.61
5	75	124.88	8.76	3.53	8.56	0.55
6	90	97.74	10.11	2.18	8.90	0.34
7	105	65.16	11.74	0.55	8.94	-0.26
8	120	59.72	12.01	0.28	9.99	-0.55
9	135	54.30	12.29	0.28	9.99	-0.55
10	150	54.30	12.29	0.28	9.99	-0.55



(a) (b)

Figure 8 **(a) Pseudo-first order and (b) Pseudo-second order kinetics plot for cadmium adsorption**

Table 8 Langmuir and Freundlich Parameters for the Adsorption of Lead and Cadmium

Sorbent	**Metal Ions**	**Langmuir model**				**Freundlich model**		
		X_m (mg/g)	**b (L/mg)**	**R^2**	**R_L**	**K (mg/g)**	**n (L/mg)**	**R^2**
Corncob powder	**Lead**	16.130	0.016	0.965	0.170	1.140	2.190	0.992
	Cadmium	6.10	0.440	0.922	0.0075	0.400	1.610	0.987

Table 9 Adsorption parameters of kinetic for the adsorption of Lead and Cadmium on Corncob Powder

Metal Ions	**$q_{e,exp}$ (mg g^{-1})**	**Pseudo-first order**			**Pseudo-second order**		
		q_e (mg g^{-1})	**k_1 (min)**	**R^2**	**q_e (mg g^{-1})**	**k_2 (g mg min^{-1})**	**R^2**
Lead	12.75	16.48	0.028	0.929	18.52	9.49×10^{-4}	0.945
Cadmium	12.29	17.50	0.028	0.843	15.87	1.37×10^{-3}	0.927

Conclusion

In this research, the corncobs were collected from the Shwe Kyi Narr village, Banmaw Township at Kachin State. Thermal activation of corncob powder was prepared by heating process of 100°C at 2 hr. The elemental content of corncob powder was determined by ED XRF analysis. Potassium was found to be the highest quantity in this sample. In addition sulphur, iron, calcium, manganese, zinc and copper were observed in the elemental analysis.The effects of sorption parameters (concentration of metal ions solution, contact time and sorbent dose) of each sorbent upon the removal of specified metal ions were investigated. For concentration parameter, the decreased in removal percent for lead and cadmium on corncob powder were observed with increased in concentration. It was found that the removal percent of lead and cadmium on corncob powder were increased with the increased in adsorbent dosage. For contact time method, the removal percent for lead and cadmium on corncob powder were steadily increased with increasing contact time. In addition the amount of removal percent of lead was greater than that of cadmium for sorption parameters (concentration of metal ions solution, contact time and sorbent dose). It can be seen that, the sorption ability of the metals were in the following order; $Pb^{2+} > Cd^{2+}$. Sorption isotherm studies using relevant isotherm equation corresponded Langmuir as well as Freundlich were applied and significant sorption parameters were evaluated. Based on R^2 values the order of isotherm in both the surfaces are Freundlich greater than Langmuir. Therefore, multilayer adsorption is possible. For kinetic study the correlation factor, R^2, showed that the pseudo-second order model, fits better the experimental data (R^2 = 0.945 for lead and 0.927 for cadmium) than the pseudo-first order model (R^2 = 0.929 for lead and 0.843 for cadmium). According to these results, the corncob powder could be applied for removal of heavy metal in waste water treatment.

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The Use of *Moringa oleifera* Lamk. (Dant-Da-Lun) Seed Powder as Natural Coagulant for Water Treatment

Kyi Kyi San*

Abstract

Myanmar has been facing considerable challenges in the management of the environment due to increasing domestic and foreign investments in the industrial and development sectors. Water pollution and management is one of the main concerns for the development and also environmental management. The present study was carried out to confirm the effectiveness of seed powder extracted from mature-dried *Moringa oleifera* seeds which are commonly available in most rural communities. The main objective of this work is to evaluate the efficiency of a natural coagulant from *Moringa oleifera* seeds in treating river water. During this study, surface water samples were collected for treatment by watery extract of air dried powdered *Moringa oleifera* seeds, resulting in an effective natural coagulant agent for highly turbid and untreated pathogenic water. Various doses of *Moringa oleifera* seed powdered extract viz. 60, 80 and 100 mg/L were taken and checked for the efficiency dose on raw water. After treatment of seed powder with water samples were analyzed for some physico-chemical parameters like pH, temperature, TDS, DO, hardness and conductivity. All parameters were reduced with increasing dose of 60, 80 and 100 mg/L seed powdered extract. Application of this low cost *Moringa oleifera* seeds is recommended for eco-friendly, nontoxic, simplified water treatment where rural and peri-urban people living in extreme poverty.

***Key words*:** *Moringa oleifera* seeds, river water treatment, watery extract, natural coagulant, physico-chemical parameters

Introduction

Water is an important resource for life. Water is used by human for various purposes, therefore the cleanliness of water consumed is very important since water is known to affect the health. Today, the quality of water becomes a major problem that needs serious attention. Good quality water has become an expensive item because many water sources have been polluted by waste coming from the various human activities (Hendrawati, 2016).

Access to safe and clean drinking water free of contaminants is one of the main problems facing rural people in developing countries. Water treatment before use is another challenge, as the use of chemical coagulants requires exorbitant amounts of money, in addition to the health side effects associated with the use of these chemical materials. The use of organic materials such as *Moringa oleifera* and other materials locally available in many developing countries are becoming preferred alternative solution for the treatment of drinking water (Awad, 2013).

Various methods are used to make water safe and attractive to the consumer. Among all the plant materials that have been tested over the years, powder processed from the seeds from *Moringa oleifera* has been shown to be one of the most effective as a

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primary coagulant for water treatment and can be compared to that of Alum (conventional chemical coagulant) (Mangale, *et*., 2012).

Moringa oleifera is among organic materials that are being used for water urification by several communities in many developing countries. *Moringaoleifera* has been proven as an efficient natural coagulant for watertreatment physically, chemically and biologically and it could be available alternative to the chemical coagulants. In this research, experiments have been carried out to confirm these limitations to have a better understanding regarding *Moringa oleifera* use in water purification (Awad, 2013).

Coagulation

Coagulation is one of the most common ways to reduce the pollutant contents in the water body that are presented as turbidity, color and organic matters. Coagulation is also used to reduce the metal ion content in water. Separation of these colloids can be done by the addition of synthetic coagulant or biocoagulant followed by slow agitation (flocculation) that causes coagulation of colloidal particles so they can be separated by sedimentation (Hendrawati, 2016).

Chemical coagulants like Aluminium sulphate (alum) and $FeCl_2$are used in municipal drinking water treatment plant for purification process. This excess use of amount of chemical coagulants can affect human health. In rural and undeveloped countries, people living in extreme poverty are presently drinking highly turbid and microbiologically contaminated water as they lack of knowledge of proper drinking water treatment and also not afford to use highly cost of chemical coagulants .

Naturally occurring coagulants are usually presumed safe for human health. The use of *Moringa* has an added advantage over the chemical treatment of water because it is biological and has been reported as edible.

Moringa oleifera seeds act as a natural absorbent and antimicrobial agent as their seeds contain 1% active polyelectrolyte's that neutralize the negatively charged colloid in the dirty water. The protein powder is stable and totally soluble in water (Mangale, *et al*., 2012).

Treatment of river water

River water is mostly the exclusive drinking water source for many tropical developing countries and treatment processes heavily depend on the use of chemical water treatment agents, which are mostly imported and are usually very expensive. Several chemical coagulants have been used in conventional water treatment processes for potable water production that includes inorganic, synthetic, organic polymer and naturally occurring coagulants. Many water treatment plants resort to under dosing of chemicals so as to meet the increasing water demand of a fast growing population, which resulted to supply of low quality water especially during the rainy season when rivers carry highly turbid water (Yusuf, 2015).

Moringa oleifera Lamk. (Dant-Da-Lun)

Moringa oleifera Lamk.(Dant-Da-Lun) whole plant, flowers and fruits, the pods and seeds are as shown in Figures 1, 2 and 3, respectively.

Figure 1 The whole plant of *Moringa oleifera* Lamk.

Figure 2 The flowers and fruits of *Moringa oleifera* Lamk.

Figure 3 The pods andseeds of *Moringa oleifera* Lamk.

Some Physiochemical Parameters of Water

pH

Conductivity : µS/cm

Total Hardness : mg/L $CaCO_3$

Dissolved oxygen : mg/L O_2

Total dissolved solids : mg/L solids (Dried at stated temperature)

Aim and Objectives

Aim: To study water pollution control and management using watery extract of *Moringa oleifera* Lamk. seeds as natural coagulant

Objectives:

☑ To collect water samples from three different sites, near Zabwet Chaung Bridge at Minbu Township, Ayeyarwady River water near Municipal Water Supply at MinBu & Magway, Magway Region

☑ To prepare watery extract of air dried powdered *Moringa oleifera* Lamk.seeds

☑ To analyze some physicochemical properties of water samples before treatment

☑ To analyze some physicochemical properties of water samples after treatment

Materials and Methods

Sample Collection

The seeds of *Moringa oleifera* Lamk. (Dant-Da-Lun) were collected from Kan Thar Quarter, Magway Township, Magway Region in the month of July, 2019 as the active constituents were found to be more during these months. The seeds without coating of *Moringa oleifera* Lamk. (Dant-Da-Lun) are shown in Figure 4.

Figure 4 The seeds without coating of *Moringa oleifera* Lamk.

Botanical Identification of Collected Plant

The collected plant was identified at Department of Botany, University of Magway.

Sample Preparation

Moringa oleifera (Dant-Da-Lun) seeds were cleaned and shade-dried at room temperature. The seeds were washed with distilled water. The coated layers of cleaned seeds were removed. The seeds powder was obtained by motor and pestle and then 20 g seeds powdered were weighed by analytical balance.Treatment to water was given by directly using seed powder extract (20%). The water quality parameters were checked before and after treatment. Doses of seed powder extract i.e., 60, 80 and 100 mg/L were selected for treatment by low cost water treatment technologies.

Chemicals and Reagents

All chemical reagents are not used in the investigation and used without chemical coagulant for water purification. But, ethylene diamine tetra acetic acid (EDTA) and

Eriochrome Black T (EBT) were used for hardness determination of water samples. EBT is used as an organic indicator and purchased from Yangon Chemical Store. Distilled water is used throughout the experiments.

Apparatus and Equipment

Glass wares (Pyrex) were mainly used for these experiments. Some were locally fabricated. Other equipments used included:

1. Electronic Balance
2. Magnetic Stirrer
3. pH Meter(MP-551)
4. Orbital Shaker
5. Motor and Pestle
6. Conductivity meter
7. Dissolve Oxygen meter
8. TDS meter (MP-551)

Coagulant Preparation

Three different clean beakers labeled A to C were placed on a working desk, a dose of 60, 80 and 100 mL of the stock solution were measured and transferred into the flasks respectively. Each beaker was mixed with 100 mL water samples. And then, the mixture was done for 15 minute at a speed of 150 rpm, followed by stored mixing for one week. The seeds powder extract of *Moringa oleifera* (Dant-Da-Lun) is shown in Figure 5.

Water Sample Collection

Water samples were collected from three different sites at Ayeyarwady river water from Magway and Minbu cities for the study purpose. Sample (I) was collected from near Zabwet Chaung Bridge at Minbu Township. Sample (II) was collected from Ayeyarwady River near Municipal Water Supply in MinBu, Magway Region. Sample (III) was collected from Ayeyarwady River near Municipal Water Supply at Magway, Magway Region. The collected water sample (I) from Zabwet Chaung Bridge in Minbu is as shown in Figure 6. The collected water sample (II) from Ayeyarwady River near Municipal Water Supply in Minbu, Magway Region is as shown in Figure 7. The collected water sample (III) from Ayeyarwady River near Municipal Water Supply in Magway, Magway Region is as shown in Figure 8. The collected water samples of three different sites are as shown in Figure 9. After treatment of river water by natural coagulant using *Moringa oleifera* seeds extract (Dant-Da-Lun) is as shown in Figure 10.

Figure 5 **The seeds powder extract of *Moringa oleifera***

Determination of Some Physiochemical Parameters

Temperature

This test was determined using the mercury-in-glass thermometer. The temperature of water samples (I),(II) and (III) are measured by using mercury-in-glass thermometer (Before treatment).

pH measurement

The pH of water samples (I), (II) and (III) were measured by using a pH meter (Electrode meter). The pH measurements of water samples by pH meter before treatment and after treatment are as shown in Figures 10 and 16, respectively.

Conductivity

This test was determined using conductivity meter model (MP-551) for water samples. The conductivity measurement of water samples before treatment is as shown in Figure 11.

Total dissolved solids (TDS)

This test was determined using conductivity meter model (MP-551) for water samples. The conductivity measurement of water samples before treatment is as shown in Figure 12.

Figure 6 Water sample of Zabwet Chaung Bridge at Minbu

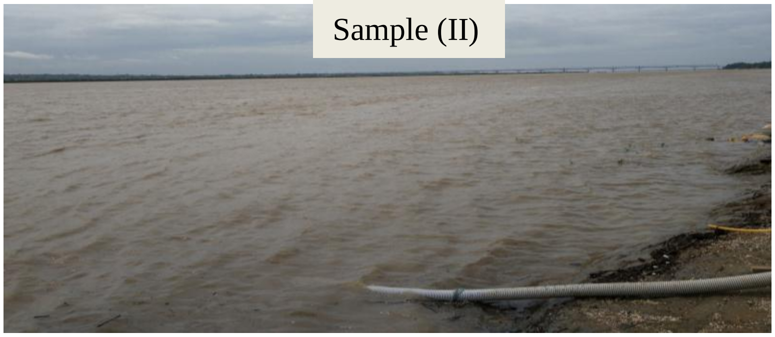

Figure 7 Ayeyarwady river water sample of Municipal Water Supply at Min Bu

Figure 8 **Ayeyarwady river water sample of Municipal Water Supply at Magway**

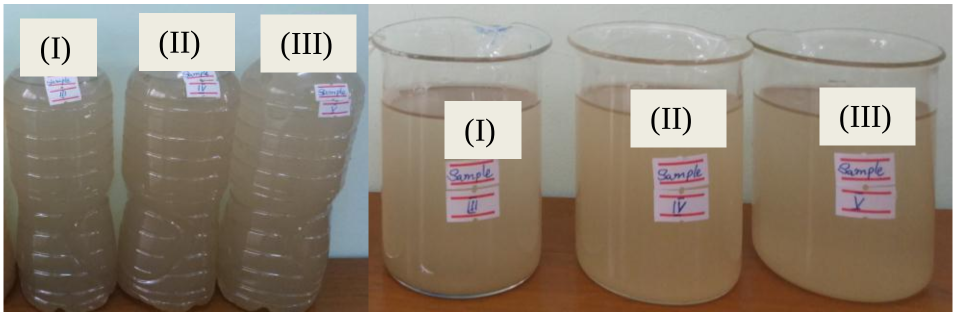

Figure 9 **Water samples of three different sites**

Dissolve oxygen (DO)

The test was determined using Dissolve Oxygen (DO) meter model. DO measurements of water samples by DO meter before treatment and after treatment are as shown in Figures 13 and 17, respectively.

Hardness

50 ml of water sample was measured and transferred into a beaker, two drops of Eriochrome Black T (EBT) indicator was added and the colour changed to wine red. A standardized Ethylene Diamine Tetra Acetic acid (2 M EDTA) was run into the sample until the sample changed to blue which indicated the endpoint. Total hardness $CaCO_3$ in mg/L was calculated from the hardness obtained from the relation. Determination of hardness of water sample (I) by EDTA titration method (Before treatment) is as shown in Figure 14. After treatment of river water by natural coagulant using *Moringa oleifera* seeds extract is as shown in Figure 15.

Turbidity

This test was not determined by turbidity meter, before and after treatment of the water samples. But, the turbidity of water samples was also visually examined.

Results and Discussion

Some Physiochemical Parameters

Colour

The initial straw colour of the river water sample was completely removed after the treatment of *Moringa oleifera* seed powder. This suggests that the *Moringa oleifera* seeds show absorbent properties.

Turbidity

It was observed that the initial turbidity was not cleared by visually examination. In the present study, it was observed that the use of *Moringa oleifera* seed powder showed decreased turbidity with increased dose from 60, 80 and 100 mg/L respectively.

Temperature

From the temperature measurements of water samples (I), (II) and (III) were found to be 29.5 ^{o}C for sample (I), 28.0 ^{o}C for sample (II) and 28.5 ^{o}C for sample (III), respectively. These results are presented in Table 1.

pH

During the present study, treatment of *Moringa oleifera* seed powder was given to river water in different doses. During the analysis, it was observed that after treatment with *Moringa oleifera* seed powder extract, pH was decreased at 60, 80 and 100 mg/L dose. After treatment the range of pH was 6.62 – 7.66 and it is within the limit. The recommended acceptable range of pH for drinking water specified by WHO is between 6.0 and 8.0. The treatments gave a pH range of 6.62 – 7.66 which falls within the reducing trends as the concentrations of the dosing solutions were increased. These pH results for water samples before treatment and after treatment are presented in Tables 1 and 2, respectively.

Dissolve oxygen

From the DO measurements of water samples (I), (II) and (III), it was found to be decreased for all water samples. These DO results for water samples before treatment and after treatment are presented in Tables 1 and 2, respectively.

Total dissolved solids

In the present study, it was observed that, the initial TDS were in the range of 0.9-1.1 mg/L for the river water sample. After the treatment of *M. oleifera* seed powder extract, the total dissolved solids were reduced from the river water. *Moringa oleifera* is known to be a natural cationic polyelectrolyte and flocculent with a chemical composition of basic polypeptides with molecular weights ranging from 6000 to 16,000 daltons, containing up to six amino acids of mainly glutamic acid, methionine and arginine. These total dissolved solids result for water samples before treatment are presented in Table 1.

Hardness

Hardness ranges from 140-250 mg/L after treatment which is within the limits of WHO standards. As a polyelectrolyte it may therefore be postulated that *Moringa oleifera* removes hardness in water through adsorption and inter-particle bridging. This implies that as the number of hardness decreases, the required dosage of *Moringa oleifera* seed powder *increases*. These hardness results for water samples before treatment are presented in Table1.

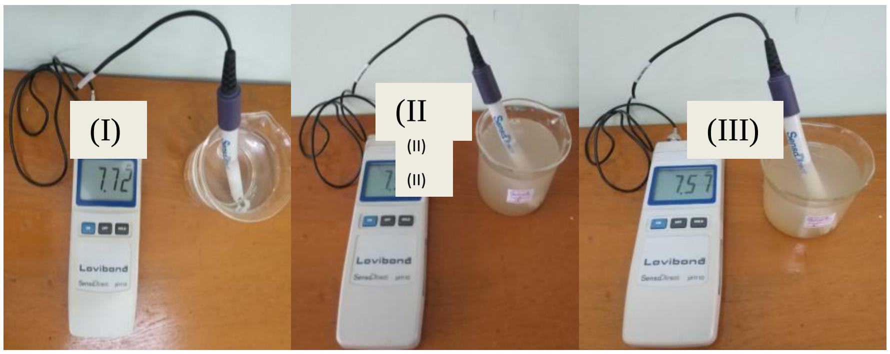

Figure 10 **pH measurement of water samples by pH meter (Before treatment)**

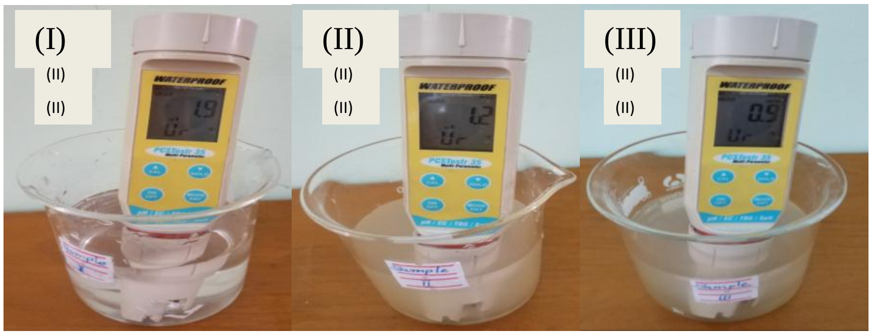

Figure 11 **Conductivity measurement of water samples by Conductivity meter (Before treatment)**

Figure 12 **Total dissolved solids measurement of water samples by TDS meter (Before treatment)**

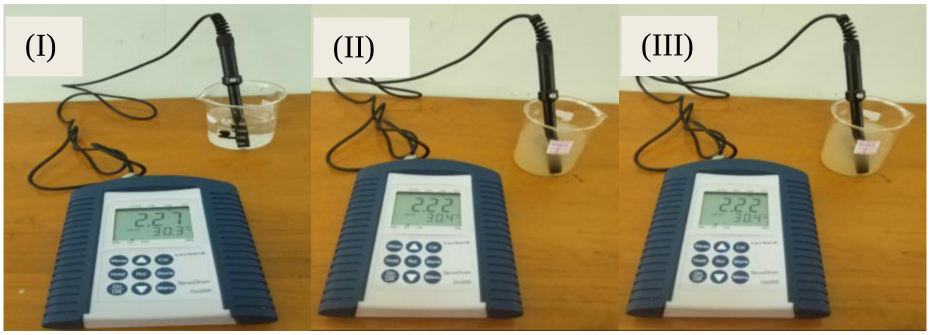

Figure 13 DO measurement of water samples by DO meter (Before treatment)

Figure 14 Determination of hardness of water samples by EDTA titration method (Before treatment)

Figure 15 Hardness measurement of after treatment of river water by natural coagulant

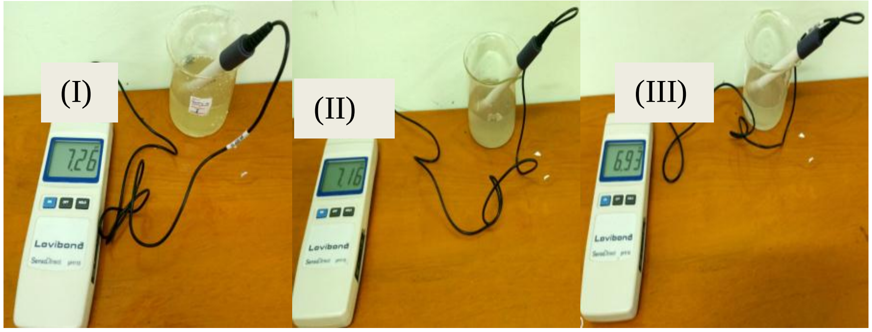

Figure 16 **pH measurement of water samples by pH meter (After treatment)**

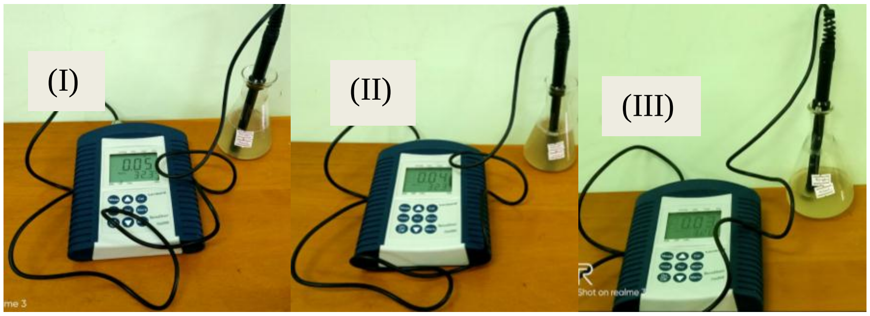

Figure 17 **DO measurement of water samples by DO meter (After treatment)**

Table 1 **Physicochemical Properties of River Water before Treatment of *Moringa oleifera* Lamk. Seeds Powder Extract**

Parameters	Untreated			
	Sample (I)	Sample (II)	Sample (III)	WHO Standard
Temperature (^{o}C)	29.5	28.0	28.5	
pH	7.72	7.56	7.57	6.0- 8.0
DO (ppm)	2.27	2.22	2.22	> 5
TDS (ppm)	1.1	1.0	0.9	4.7-5.8
Conductivity(Sm^{-1})	1.9	1.2	0.9	0.05 - 200
Hardness(ppm)	248	148	140	500

Table 2 Physicochemical Properties of River Water afterTreatment with Various Doses of *Moringa oleifera* Lamk. Seeds Seeds Powder Extract

Parameters	Treated								
	Sample (I)			Sample (II)			Sample (III)		
	60 mL	80 mL	100 mL	60 mL	80 mL	100 mL	60 mL	80 mL	100 mL
pH	7.66	7.26	7.59	7.31	7.16	7.21	7.59	6.93	6.62
DO (ppm)	0.06	0.05	0.04	0.05	0.04	0.03	0.04	0.03	0.03
TDS (ppm)	0.34	0.23	0.19	0.38	0.29	0.21	0.41	0.22	0.14
Conductivity (Sm^{-1})	1.8	1.7	1.3	0.6	0.5	0.5	0.8	0.8	0.7
Hardness(ppm)	121	98	98	133	94	83	116	88	76

Conclusion

This research investigates the efficiency of *moringa oleifera* seed as a natural coagulant for domestic water purification. Some physiochemical parameters (temperature, pH, conductivity, dissolve oxygen, total dissolved solids, hardness,) were also determined.

From these measurements, a dose of 80 mg/L of *Moringa oleifera* seeds extract were found to be the best result for the river water samples. The treated water showed the lowest value of DO, closest pH value to 7, and the lowest value of conductivity. *Moringa oleifera* seed was used acts as a natural coagulant, flocculent, absorbent for river water treatment. It reduces the pH values, turbidity, DO and conductivity values after the treatment.

From the results of water treatment processes, all of DO values were found to be lowest for all water samples. After the treatment of *Moringa oleifera* Lamk. seeds powder extract, the electrical conductivity was reduced for river water samples. The amount of conductivity was found to be the lowest value (0.7 Sm^{-1}) for mixture of 100 mL *Moringa oleifera* Lamk. seeds extract with water sample (III). *Moringa oleifera* seed is not giving any toxic effect. It is eco-friendly and cheaper method of purification of water and therefore can be used in the rural areas where no facilities are available for the treatment of river water.

The quality and accessibility of drinking water are of paramount importance of human health. Therefore, more research work needs to be done on the optimum dosage determination, elemental determination and some physicochemical parameters that give water portability for drinking.

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I would like to express my gratitude to Dr Aung Kyaw Thin, Rector, Banmaw University and Dr Aye Aye Han, Pro-rector, Banmaw University for their interest and encouragement on my research work. We also wish to express my thanks to Dr Myint Myint Sein , Professor and Head Department of Chemistry , Banmaw University and Dr Thidar Aung , Professor and Head Department of Chemistry, Magway University for their kind help and invaluable guidance for this research work and providing with necessary facilities.

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Determination of Total Phenolic Content, Total Flavonoid Content and Antioxidant Activity of Lemon Plant

Nu Nu Nge[*]

Abstract

The three different parts of Lemon extracts (leaf, bark and root) were analyzed for total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity. TPC and TFC of Lemon extracts were determined as mg of gallic acid equivalents per gram (mg GAE/g) and mg of catechol equivalents per gram (mg CAE/g) by using their calibration curves. The antioxidant activity was determined by the reducing power assay. This assay based on the electron transfers to oxidative ions or free radicals. Ascorbic acid was used as a standard for the comparison of IC_{50} values in the reducing power assay.

Key words: Lemon, TPC, TFC, mg CAE/g, mg GAE/g, antioxidant activity, reducing power assay, IC_{50}

Introduction

Antioxidant is also defined as a substance which is capable of inhibiting a specific oxidizing enzyme of a substance that reacts with oxidizing agents before causing damage to other molecules or a substance that sequesters metal ions or even a substance capable of repeating system such as iron transporting protein. As such, production of free radicals and other reactive oxygen species (ROS) in the human body by numerous physiological and biochemical processes is reported (MALATHI *et al*., 2018).

Plants constitute a rich source of a wide variety of therapeutic molecules and therefore hold a great promise for new medicines. Natural antioxidants are required to prevent and /or cure the disorders caused by free radicals. The free radicals are highly reactive chemical species produced in the body and have the potential to damage cells, organelles, DNA, and other biomolecules, resulting in diseases such as cancer, and cardiovascular and neurodegenerative ailments. The treatment of such diseases has serious efficacy and safety issues. In addition, it is often highly expensive and many people cannot afford it. This necessitates efforts to discover safe and effective remedies, readily available to common people. Treatment of infectious diseases is also becoming a challenge due to the problem of multi-drug resistance. As pathogens soon develop resistance to existing antibiotics, new alternatives are inevitable to treat infectious diseases (Ahmed *et al*., 2015).

Some antioxidants have been shown to possess additional pro-oxidant effects. Diverse methodologies exist for studying redox properties of synthetic and natural chemicals. Plants synthesize flavonoids, phenolic compounds recognized as counter-agents to coronary heart disease. Their antioxidant activities are affected by their hydroxylation patterns (Chobot *et al*., 2013).

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In this study, Lemon plant extracts choose in attempts to explore their redox properties by chemical methods in contrasting hydrogen peroxide environments.

Botanical Description of Lemon

Family name - Rutaceae

Botanical name - *Citrus limon* (L.) Osbeck

English name - Lemon

Myanmar name - Shauk

Part used - Leaf, Bark and Root

Medicinal uses - Treatment of indigestion, constipation, dental problems, Throat infections, fever, internal bleeding, rheumatism, burns, obesity, respiratory disorders, cholea, and high blood pressure

Figure (1) Plant and three parts of Lemon (*Citrus limon* (L.) Osbeck)

Material and Methods

Sample Collection

The three different parts of Lemon were collected from Namphar Quarter, Banmaw Township, Kachin State. The samples were washed with distilled water, cut, air dried and grind into powders at room temperature. The sample powders were stored in air tight bottle. And then, samples were refluxed with water for six hours to obtain aqueous extracts.

Determination of Maximum Wavelength for the Determination of TPC

The ethanol 0.5 mL, 10 % FCR 2.5 mL and 75mg/mL of Na_2CO_3 2 mL were mixed and it was heated at 50°C for 10 minutes. And then, hot solution was cooled in the dark room for 30 minutes. It was determined to obtain the maximum wavelength between 670 nm and 850 nm ranges.

Determination of Total Phenolic Content (TPC)

The total phenolic content of Lemon plant extracts were measured spectrophotometrically according to the Folin-Ciocalteu's method, as described by Siddiqui *et al*., 2016. Dry extracts were solubilized with ethanol to a final concentration of 200µg/mL. Aliquots of these samples (0.5 mL) were mixed with 2.5 mL of the Folin-Ciocalteu reagent (diluted 10 times with distilled water) and 2 mL of an aqueous sodium carbonate solution (75 mg/ mL). The final mixture was heated at 50°C for 10 min, after which time the absorbance was read at 760 nm against a blank (solution with no extract added). Gallic acid was used to prepare a calibration curve and results were expressed in gallic acid equivalents (mg GAE/ g dry extract) (Kasangana *et al*., 2015). TPC values was calculated from the following gallic acid calibration equation: $y = 0.0046x + 0.0197$ and $R^2 = 0.9979$, where y is absorbance at 760 nm and x is the amount of gallic acid equivalent (µg/mL).Formula of TPC is **$C = C_1V/m$**, where C is total phenolic content (mg/ g) in GAE (gallic acid equivalent), C_1 is the concentration of gallic acid established from the calibration curve in mg/ mL, V is volume of extract in mL, and m is the weight of the plant extract in g (Siddiqui *et al*., 2016).

Determination of Maximum Wavelength for the Determination of TFC

A mixture of 0.5 mL ethanol and 0.3 mL 5% $NaNO_2$ was allowed to stand for 5 minutes. 10 % $AlCl_3$ 0.3 mL was added to it and stood for 6 minutes again. And, 2 mL of 1M NaOH was added and adjusted to final volume 5 mL with ethanol. Finally, it was determined to get the maximum wavelength between 465 nm and 560 nm ranges.

Determination of Total Flavonoid Content (TFC)

Total flavonoid content was determined by aluminium chloride method described by Siddiqui *et al*., 2016, with minor modification. 0.5 mL of the sample was mixed with 0.3 mL of 5 % sodium nitrite. After 5 min 0.3 mL of 10 % aluminium chloride was added. After 6 min, 2.0 mL of 1 M sodium hydroxide was added and the total volume was made up to 5.0 mL with distilled water. The absorbance of the mixture was measured at 510 nm against a reagent blank. Catechol was used as standard. The flavonoid content was expressed as milligram of catechol equivalence (CAE) per gram of extract (Sasikumar and Kalaisezhiyen, 2014). TFC values calculated from the following catechol calibration equation:$y = 0.001x + 0.0393$ and $R^2 = 0.9971$, where y is absorbance at 510 nm and x is the amount of catechol equivalent (µg/mL).Formula of TFC is **$C = XV/m$**, where C is total flavonoid content (mg/ g) in CAE (catechol equivalent), X is the concentration of catechol established from the calibration curve in mg/ mL, V is volume of extract in mL, and m is the weight of the plant extract in g (Siddiqui *et al*., 2016).

Determination of Maximum Wavelength for the Determination of Reducing Power Assay

The 2.5 mL of 0.2 M phosphate buffer and 2.5 mL 1 % $K_3Fe(CN)_6$ were mixed to incubate at 50℃ for 20 minutes. 2.5 mL of 10 % TCA was added to it and centrifuged 3000 rpm for 10 minutes to get clear solution. The supernatant solution 2.5 mL, distilled water 2.5 mL and 0.1 % $FeCl_3$ 0.5 mL were mixed. And then, it was determined to obtain the maximum wavelength between 630 nm and 770 nm ranges.

Determination of Reducing Power for Lemon Plant extracts

Substances which have reduction potential, react with potassium ferricyanide (Fe^{3+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

$$\text{Potassium ferricyanide} + \text{Ferric Chloride} \longrightarrow \text{Potassium ferrocyanide} + \text{Ferrous chloride}$$

The different concentrations of sample extracts (2.5 mL) were added to 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] solution. The reaction mixture was vortexed well and then incubated at 50℃ for

20 min using vortex shaker. After incubation, 2.5 mL of 10% TCA was added to the mixture and centrifuge at 3000 rpm for 10 min. The supernatant solution 2.5 mL was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride. The colored solution was read at 700 nm against the blank with reference to standard using UV-Spectrophotometer. Ascorbic acid was used as a standard (Vijayalakshmi and Kuckmani *et al*., 2016). A blank was prepared without adding standard or test compound. Increased absorbance of the reaction mixture indicates increase in reducing power (Khanam *et al*., 2004).

The percent increase in reducing power was calculated using the following equation

$$\text{Increase in reducing power (\%)} = \frac{\text{A test} - \text{A blank}}{\text{A blank}} \times 100$$

Where “A test” is absorbance of solution, “A blank” is absorbance of blank.

Results and Discussion

Determination of Maximum Wavelength for Total Phenolic Content (TPC)

A maximum wavelength (760 nm) was measured using the (10 %) Folin-Ciocalteau reagent and (75mg/mL) sodium carbonate solution. The result datas are the following table (1) and figure (2).

Table (1) Maximum Wavelength for TPC

No.	Wavelength (nm)	Absorbance	No.	Wavelength (nm)	Absorbance
1	670	0.014	11	770	0.152
2	680	0.027	12	780	0.142
3	690	0.034	13	790	0.097
4	700	0.041	14	800	0.089
5	710	0.057	15	810	0.078
6	720	0.071	16	820	0.065
7	730	0.081	17	830	0.051
8	740	0.090	18	840	0.039
9	750	0.111	19	850	0.032
10	**760**	**0.154**			

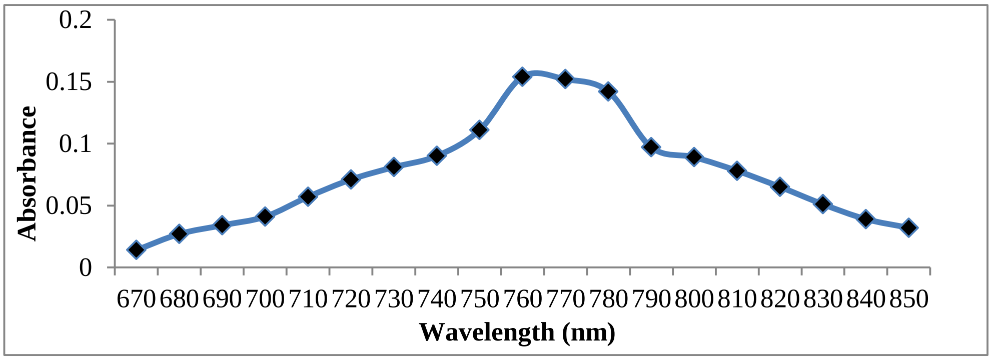


Figure (2) A plot of absorbance and wavelength (nm) for TPC

A maximum wavelength of TPC was found at 760 nm where the absorbance is 0.154. Therefore, TPC values were continuously measured at this wavelength.

A Standard Calibration Curve of TPC

The total phenolic content of a standard gallic acid was determined to calculate TPC values and it was expressed as milligram of gallic acid equivalent (mgGAE/ g). TPC value of three samples extracts was calculated as mg GAE/g, using the following equation based on the calibration curve: $Y = 0.0046x + 0.0197$ $R^2 = 0.9979$. The absorbance values of gallic acid in the various concentrations were shown below in table (2) and figure (3).

Table (2) Concentration and Absorbance of Gallic Acid

Concentration (µg/mL)	Absorbance (mean ± STDEV)
200	0.913±0.009
180	0.840±0.007
160	0.758±0.038
140	0.677±0.049
120	0.573±0.025
100	0.471±0.051
80	0.382±0.044
60	0.283±0.038
40	0.186±0.032
20	0.125±0.028

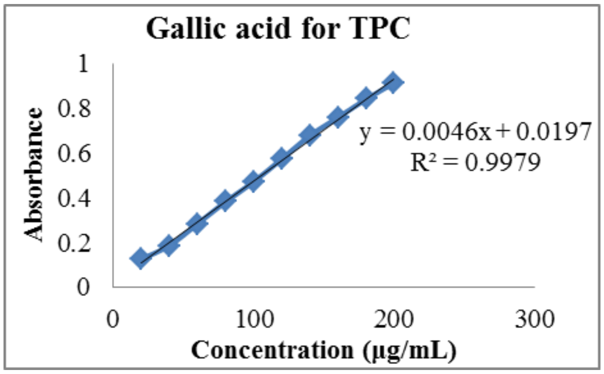


Figure (3) Standard calibration curve (mgGAE/g) of gallic acid

In the 200 µg/mL, the TPC value of standard gallic acid contains 970.98 mg GAE/g.

Table (3) Absorbance and TPC Values of Lemon Plant Extracts at 200µg/mL

Samples	Absorbance (mean ± STDEV)	TPC (mgGAE/g)
Lemon leaf (LL)	0.416±0.029	430.8
Lemon bark (LB)	0.476±0.337	**496.0**
Lemon root (LR)	0.348±0.232	356.9

According to table (3), the highest TPC order of Lemon plant was found to be LB (TPC=496.0 mg GAE/g) > LL (TPC= 430.8 mg GAE/g) > LR (TPC= 356.9 mg GAE/g), respectively.

Determination of Maximum Wavelength for Total Flavonoid Content (TFC)

The maximum wavelength of TFC was found to be 510 nm and the absorbance value is 0.301. That value was determined between 465 nm and 560 nm ranges. The results are shown in table (4) below.

Table (4) Maximum Wavelength for TFC

No.	Wavelength (nm)	Absorbance	No.	Wavelength (nm)	Absorbance
1	465	0.072	11	515	0.292
2	470	0.099	12	520	0.280
3	475	0.111	13	525	0.265
4	480	0.130	14	530	0.251
5	485	0.150	15	535	0.238
6	490	0.171	16	540	0.208
7	495	0.196	17	545	0.197
8	500	0.216	18	550	0.164
9	505	0.171	19	555	0.141
10	**510**	**0.301**	20	560	0.130

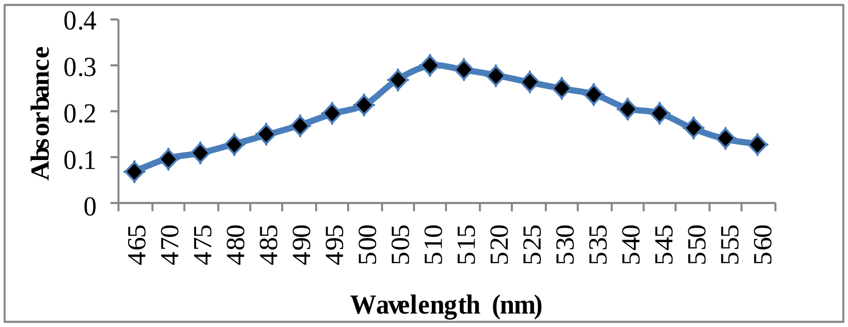


Figure (4) A plot of absorbance and wavelength (nm) for TFC

A Standard Calibration Curve of TFC

The total phenolic content of a standard catechol was determined by linear regressive equation and it was expressed as milligram of catechol equivalent (mg CAE/ g). TFC values of three samples were calculated as mg CAE/g, using the following equation based on the calibration curve: $Y = 0.001\ x + 0.0393 \quad R^2 = 0.9971$. The absorbance values of catechol in the various concentrations were shown in table (5) and figure (5) below.

Table (5) Concentration and Absorbance of Catechol

Concentration (µg/mL)	Absorbance (mean ± STDEV)
200	0.251±0.025
180	0.228±0.043
160	0.206±0.039
140	0.181±0.030
120	0.160±0.021
100	0.142±0.019
80	0.124±0.020
60	0.102±0.020
40	0.077±0.010
20	0.066±0.008

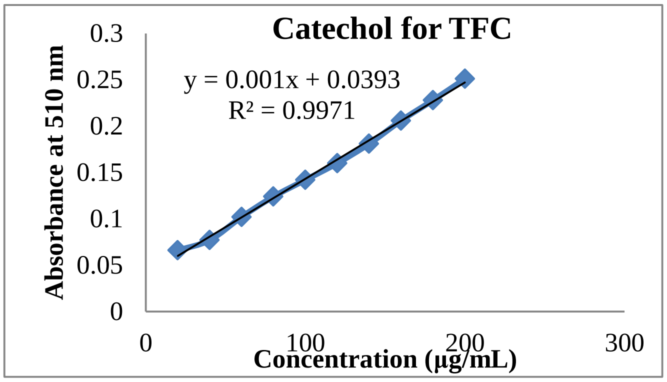


Figure (5) Standard calibration curve (mg CAE/g) of catechol

In the 200 µg/mL concentration, TFC value of standard catechol was found to be 1058.5 mg CAE/g).

Table (6) Absorbance and TFC Values of Lemon Plant Extracts at 200µg/mL

Samples	Absorbance (mean ± STDEV)	TFC (mg CAE/g)
Lemon leaf (LL)	0.081±0.346	208.5
Lemon bark (LB)	0.085±0.010	**228.5**
Lemon root (LR)	0.076±0.008	183.5

In the determination of TFC in Lemon plant, TFC value of LB (228.5 mg CAE/g) was found to be the highest contents among the three samples.

Table (7) TPC and TFC Values of Lemon Plant Extracts

Samples	TPC values (mg GAE/g)	TFC values (mg CAE/g)
Lemon leaf (LL)	430.8	208.5
Lemon bark (LB)	**496.0**	**228.5**
Lemon root (LR)	356.9	183.5

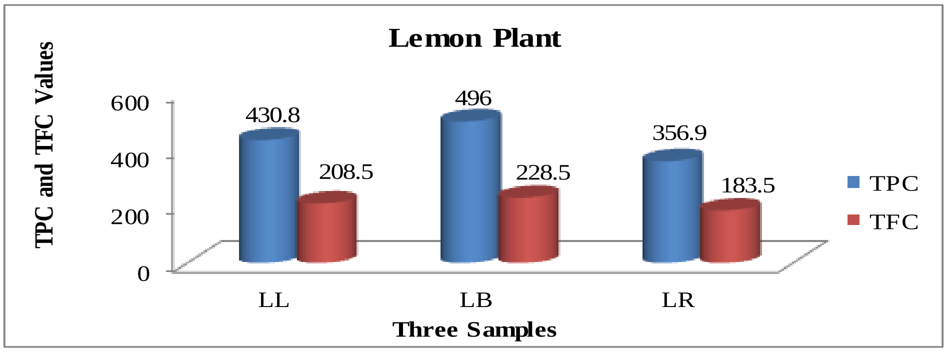


Figure (6) TPC and TFC values of Lemon plant extracts

According to these comparative results, TPC and TFC values of three samples are related to each other.

Determination of Maximum Wavelength for Reducing Power Assay in Lemon Plant Extracts

The maximum wavelength of reducing power assay was observed at 700 nm and the absorbance value was 0.250. The nanometer ranges and absorbance values were shown in table (8) below.

Table (8) Maximum Wavelength for Reducing Power Assay

No.	Wavelength (nm)	Absorbance	No.	Wavelength (nm)	Absorbance
1	630	0.181	9	710	0.248
2	640	0.195	10	720	0.240
3	650	0.206	11	730	0.232
4	660	0.218	12	740	0.228
5	670	0.230	13	750	0.225
6	680	0.236	14	760	0.218
7	690	0.242	15	770	0.210
8	**700**	**0.250**			

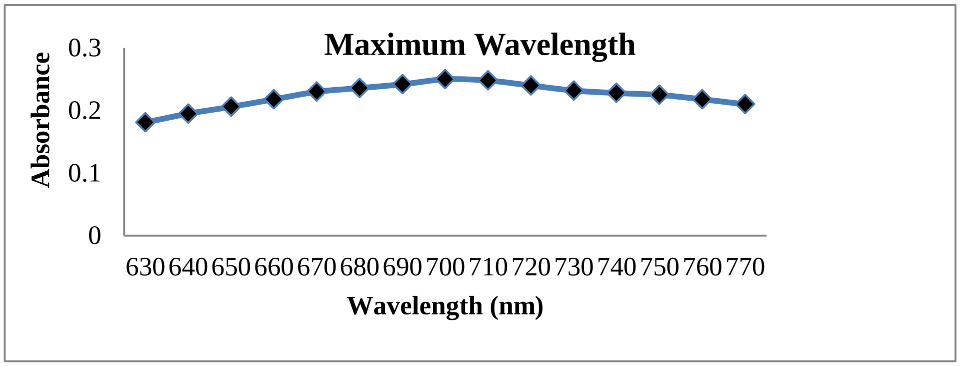


Figure (7) A plot of absorbance and wavelength (nm) for control solution reducing power assay

Determination of Reducing Power Assay for Standard Ascorbic Acid at 700 nm

The reducing power of standard ascorbic acid was determined at 700 nm. The highest percent inhibition of standard ascorbic acid was shown in IC_{50} values. The results were described in table (9) and figure (8).

Table (9) Percent Inhibition of Standard Ascorbic Acid for Reducing Power Assay

Concentration (µg/mL)	**Absorbance (mean ± STDEV)**	**% inhibition**
100	0.493±0.095	97.2
80	0.465±0.011	86.0
60	0.432±0.008	72.8
40	0.383±0.017	53.2
20	0.352±0.010	40.8

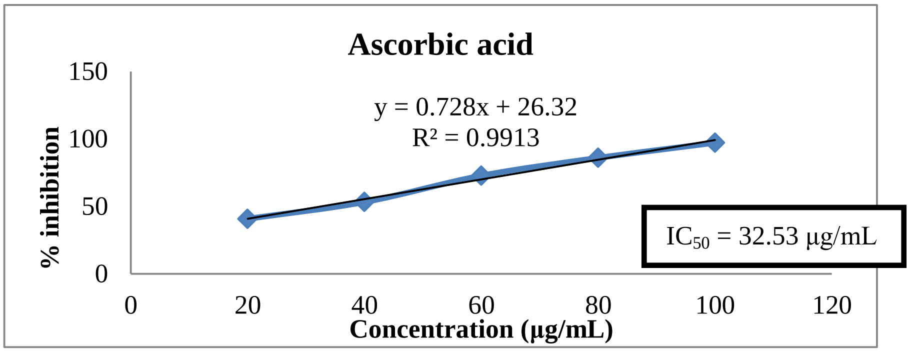


Figure (8) Percent inhibition of standard ascorbic acid for reducing power assay

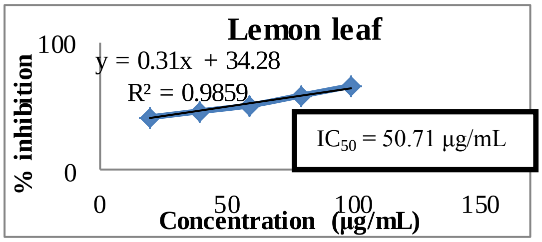
According to the results, the higher percent inhibition of standard ascorbic acid was found to be the higher concentration. The IC_{50} value of ascorbic acid was 32.53µg/mL.

Determination of Reducing Power Assay for Three Extracts of Lemon (LL, LB and LR) at 700 nm

In this assay, the ability of sample extracts to reduce Fe^{3+} to Fe^{2+} was determined. The antioxidants cause the reduction of the ferric cyanide complex (Fe^{3+}) to the ferrous cyanide form (Fe^{2+}), thereby changing the solution color from green to blue depending on the reducing power of the sample extracts. It was observed that the higher the absorbance of the reaction mixture become the higher the reducing power will be. The aqueous extracts of Lemon samples showed some degree of electron donation. The reducing power of Lemon extracts was found to be in order of LB (IC_{50} = 34.33 µg/mL) > LL (IC_{50} = 50.71 µg/mL) > LR (IC_{50} = 68.48 µg/mL). The reducing power of standard ascorbic acid (IC_{50} = 32.53µg/mL) was found to be higher than all the tested extracts. These extracts may act as electron donors or hydrogen donors and could react with oxidative ions or free radicals to convert them into more stable compounds, and then terminate the free radical chain reactions. The results were described in table (10) and figure (9), table (11) and figure (10), and table (12) and figure (11), respectively.

Table (10) Percent Inhibition of Lemon Leaf for Reducing Power Assay

Concentration (µg/mL)	Absorbance (mean± STDEV)	% inhibition
100	0.416±0.009	66.4
80	0.397±0.014	58.8
60	0.378±0.018	51.2
40	0.366±0.013	46.4
20	0.354±0.011	41.6



Figure(9) Percent inhibition of Lemon leaf for reducing power

Table (11) Percent Inhibition of Lemon Bark for Reducing Power Assay

Concentration (µg/mL)	Absorbance (mean± STDEV)	% inhibition
100	0.437±0.014	74.8
80	0.420±0.012	68.0
60	0.413±0.013	65.2
40	0.375±0.014	50.0
20	0.359±0.017	43.6

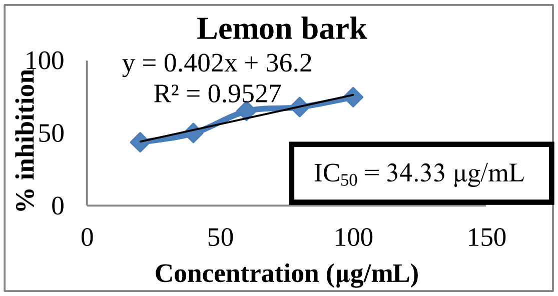


Figure (10) Percent inhibition of Lemon bark for reducing power

Table (12) Percent Inhibition of Lemon Root for Reducing Power Assay

Concentration (µg/mL)	Absorbance (mean± STDEV)	% inhibition
100	0.392±0.013	56.8
80	0.388±0.016	55.2
60	0.383±0.017	53.2
40	0.373±0.013	37.2
20	0.335±0.011	34.2

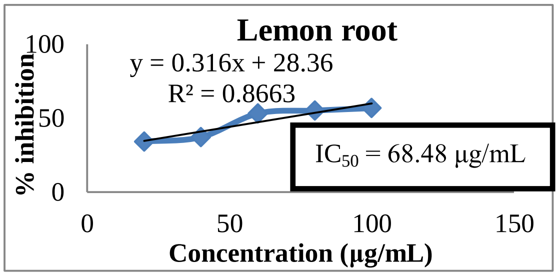


Figure (11) Percent inhibition of Lemon root for reducing power

The IC_{50} values of LL, LB, LR and ascorbic acid were 50.71, 34.33, 68.48 and 32.53µg/mL, respectively. These comparison values were shown in the following figure (12) and (13).

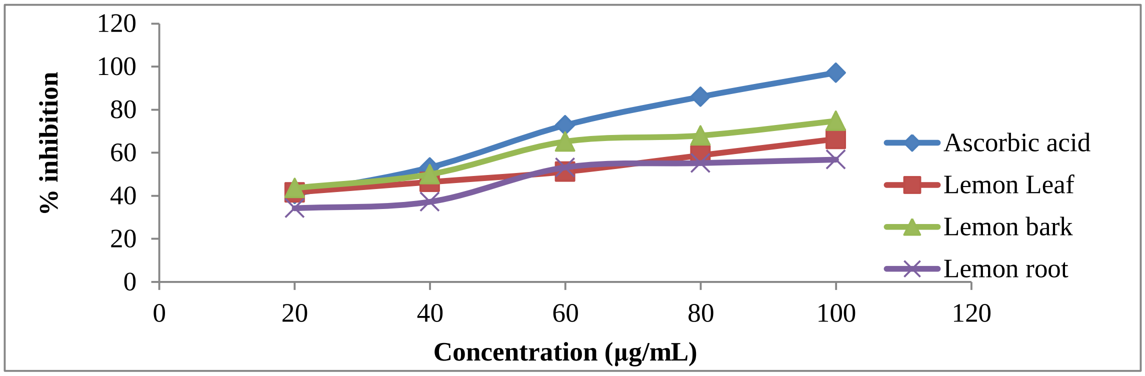


Figure (12) Reducing power of three Lemon extracts and standard ascorbic acid

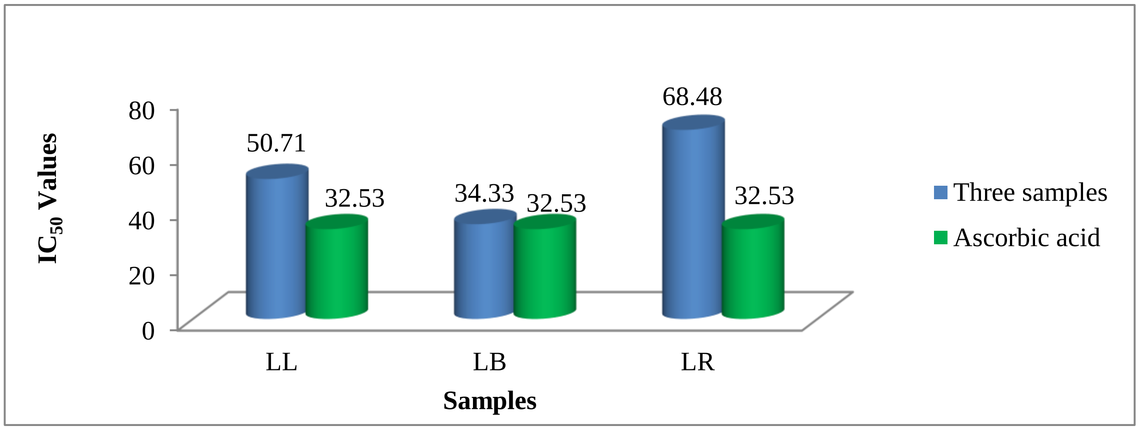


Figure (13) IC_{50} values of three Lemon extracts and standard ascorbic acid for reducing power assay

Conclusion

In this research work, total phenolic content (TPC) and total flavonoid content (TFC) of Lemon plant extracts were determined. TPC values of Lemon plant extracts were expressed as gallic acid equivalent (mg GAE/g) and TFC values were also showed as catechol equivalent (mg CAE/g), respectively. From gallic acid standard curve, Lemon extracts of the highest TPC order were found to be LB (495.9 mg GAE/g) > LL (430.8 mg GAE/g) > LR (356.85 mg GAE/g). In the catechol standard curve, the order of TFC values for Lemon plant extracts was LB (228.5 mg CAE/g) > LL (208.5 mg CAE/g) > LR (183.5 mg CAE/g). The reducing power assay of Lemon plant extracts showed that the highest antioxidant power (lowest IC_{50} values) with the highest percent inhibition results. The most active results were found to be LB (IC_{50} = 34.33 µg/mL) in the Lemon plant extracts. The extracts of LB displayed the presence of high amount of TPC and TFC values. Hence, Lemon bark extract can reduce a radical or a metal compound.

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