DOI Number: 10.37506/v20/i1/2020/mlu/194651

Modification of Chicken Egg Shell and Lime Extract (*Citrus amblycarpa*) for Minimizing Lead (Pb) Level on Blood Cockles (*Anadara granosa)*

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Abstract

Heavy metal in human body generally is from food consumed by the human either through fish or other aquatic animals in fresh water or seawater which are polluted by heavy metal. The presence of heavy metal in human body in certain level can cause several diseases, such as kidney disease, liver, nerve, and brain disease. Moreover, this research aimed at analyzing the decrease of lead (Pb) level on blood cockles (*A. granosa*) through engineering of stirring time, adsorption temperature, and variation of lime extract (*C. amblycarpa*) concentration by using Stirring Chamber tools through using chicken egg shell adsorbent. The research design used simple experiment in posttest only control group design. Meanwhile, the subject in this research was divided by two groups randomly in which a treatment was given to one group as control group and experiment group. Sample of blood cockles that was used was in 72 samples. Lime extract concentration in 1%, 1.5%, and 2% in stirring time of 5 minutes, 10 minutes, and 15 minutes before and after heating in 35°C utilized stirring speed in 150 rpm. Meanwhile, the chicken egg shell adsorbent was in 50 gram for each treatment. The sampling technique in this research utilized purposive sampling and data was analyzed by utilizing Statistic test of Two-Way Anova. Result showed that Pb level in fresh blood cockles before heating was 0.223 ppm and after heating, it reduced to be 0.062 ppm. Result of hypothesis test was obtained p-value <0.05, which was 0.000, and it indicated that there was a significant enhancement of chicken egg shell adsorption capacity in minimizing Pb level on blood cockles after being conducted the addition of lime extract with temperature variation and stirring time in certain stirring speed through engineering tool of Stirring Chamber. All in all, conclusion of this research was there was a significant enhancement of modified chicken egg shell adsorption capacity and lime extract in minimizing Pb level on blood cockles. Moreover, it was expected that this research could give contribution to food technology in reducing heavy metal of Pb level in pre-treatment of blood cockles (*Anadara granosa*) with modifying lime extract and chicken egg shell through Stirring chamber tool.

Keywords**:** *pH of a solution; adsorption temperature; lime extract*

Introduction

Sea environment in the world has been discovered that it is contaminated by heavy metal. Concentration of heavy metal in environment varies partially or totally as an impact of environmental condition and human activity.[(1)] Condition of aquatic environment that is polluted by heavy metal can impact on biota that lives in it. Pereira, *et al* conducted a research against aquatic biota and it was known that the fish that was tested contained chromium metal, which was 1.5 mg kg−1.[(2)] In the water, through food chain process, first heavy metal is absorbed by phytoplankton, bacteria, fungi and low-level organisms which then, they are eaten by higher-level organisms until they enter into human body if the human consumes them.[(3)] Referring to this statement, it can be meant that one of causes the human can be contaminated by heavy

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metal is from food consumption pattern that is gotten from the waters which are contaminated by heavy metal.

Kenjeran beach Surabaya, East Java Province, Indonesia is one of water areas which has been contaminated by heavy metal. Indication of heavy metal contamination in Kenjeran beach Surabaya has been proven by conducted research by Suryono, *et al* and they stated that the presence of Hg level in 0.582 ppm in blood cockles was taken from Kenjeran beach Surabaya.[(4)] Result of laboratory test that had been conducted by researchers showed that Cd level and Pb level on blood cockles were in the average of 0.93 ppm and 1.92 ppm. Water biota, particularly blood cockles, can be become one indicator of pollution level which is occurred in the water. The blood cockle's body contained heavy metal level which exceeded normal limit that had been determined. Therefore, it could be used as bio indicator of pollution in the environment.[(5)] Conducted research in first stage by Suryono, et al had proven that potency for chicken egg shell waste in reducing Hg metal on blood cockles (*Anadara granosa*) was through stirring chamber tools without controlling stirring speed, adsorption temperature, or pH of a solution. This research was studied further in second research in same year edition in research report that analyzed intervention of temperature and stirring speed of Stirrer chamber in improving potency of egg shell as adsorbent for reducing Cd metal and Pb metal on blood cockles.[(4)]

Result of the research showed that the decrease of Cd metal and Pb metal level was not occurred optimally. Effort in improving chicken egg shell capacity in adsorption process was conducted continuously and one of them was manipulation of pH of a solution. pH in a solution was known that it could influence the presence of metal. Meanwhile, the metal characteristic in low pH generally was as free cation. Whether, on basic pH, metal tended to precipitate as hydroxide, oxide, carbonate or insoluble phosphate.[(6)] This explained that in acidic condition, metal ions could bind easily to the surface of adsorbent. According to conducted research by Abdel-Khalek *et al*, they explained that there was a significant enhancement of capacity of bio-composite chicken egg shell waste in pH 5,23 and contact time in 5 minutes with room temperature. Research result showed that there was maximum adsorption capacity in higher pH 5.23, which was 94,9 mg/g rather than pH 7,09 in which the adsorption capacity was only occurred in 49.5 mg/g.[(7)] Besides, this research explained that pH of a solution which was acidic had potency in influencing the enhancement process of metal adsorption. This research strengthened researcher's argumentation that model in manipulation of solution by adding acid could increase egg shell potency in binding heavy metal. One of substances that could make acidic solution was by using lime extract (*Citrus amblycarpa*).

Lime (*C.amblycarpa*) is one of orange types that can reduce pH value because it contains acid. Acid in orange mostly is citric acid. Kordial in his research evaluated pH value by adding several types of orange extract and one of them was lime extract. Adding lime extract in 1.3% of solution had pH 4.6, meanwhile, concentration of lime extract in 1.5% of solution had pH 3,97. Furthermore, this research aimed at analyzing the decrease of lead (Pb) on blood cockles (*A. granosa*) through manipulation of stirring time, adsorption temperature, and variation of lime extract concentration (*C. amblycarpa*) by using Stirring Chamber tools which used adsorbent of chicken egg shell.

Research Method

This research was simple experiment research by Posttest Only Control Group Design in order to investigate the influence of stirring time, adsorption temperature, and concentration of lime extract against Pb metal level, either in control group or experiment group. In simple experiment, the subject was divided by two groups (or more) randomly. A treatment was given to one (or more) group(s) as treatment group and another group (without any treatment/ giving other treatments) as control group. Furthermore, pattern of this design could be seen as followed:

Pretest	**Treatment**	**Posttest**
Treatment group	X1	O2
Control group		)2

Figure 1. The research design

Findings and Discussion

This research used chicken egg shell as adsorbent that had been through activation process chemically and it used HCL solution. Besides, blood cockles (*A. granosa*) that was become sample in this research was taken from Kenjeran beach Surabaya. Moreover, model development in this research was Stirrer chamber tool which was rearranged its component of stirring speed

settings and adsorption temperature. The setting of stirring speed included: 150 rpm and could be conducted a stirring either in temperature of 35°C or before heating. Besides, it had been conducted laboratory test against Pb level on blood cockles as the sample and the result of the laboratory test was showed in table 1.

Table 1. Recapitulation of Average of Pb level on Blood Cockles (*Anadara granosa*) before Heating based on Lime Extract Concentration in 2019

Sample Code	**Concentration of**	**Stirring Time**	**Average of**
	Lime Extract		**Pb Level (ppm)**
KW1T0	0%	5	0.197
KW2T0		10	0.19
KW3T0		15	0.18
AW1T0	1%	5	0.10
AW2T0		10	0.07
AW3T0		15	0.05
BW1T0	1.5%	5	0.077
BW2T0		10	0.053
BW3T0		15	0.04
CW1T0	2%	5	0.047
CW2T0		10	0.03
CW3T0		15	0.027
Total			1.061
Average			0.088

Table 1 showed that total of Pb level in the average from each treatment before heating was 1.061 ppm with average totally in 0.088 ppm. Data in table above showed that there was a significant enhancement of lime extract concentration and the Pb level on blood cockles reduced significantly. The lowest Pb level was in lime extract concentration of 2% with stirring time in 15 minutes, which was in 0.027 ppm.

Table 2. Recapitulation of Average of Pb Level on Blood Cockles (*Anadara granosa*) based on Lime Extract Concentration after Heating in 2019

Sample Code	Lime Extract Concentration	Stirring Time	Average of Pb Level (ppm)
KW1T1	0%	5	0.17
KW2T1		10	0.16
KW3T1		15	0.14
AW1T1	1%	5	0.07
AW2T1		10	0.03
AW3T1		15	0.02
BW1T1	1.5%	5	0.06
BW2T1		10	0.027
BW3T1		15	0.017
CW1T1	2%	5	0.037
CW2T1		10	0.013
CW3T1		15	0.01
Total			0.754
Average			0.063

Table above showed that Pb level on blood cockles for total average of all treatments was 0.754 ppm with average totally in 0.06 ppm. Besides, based on the table, it could be known that a tendency of Pb level on blood cockles reduced more in 3 variations of stirring time and variation of lime extract concentration after being conducted heating. The highest reduction on the treatment of adding 2% of lime extract concentration in 15 minutes of stirring time was 0.01 ppm.

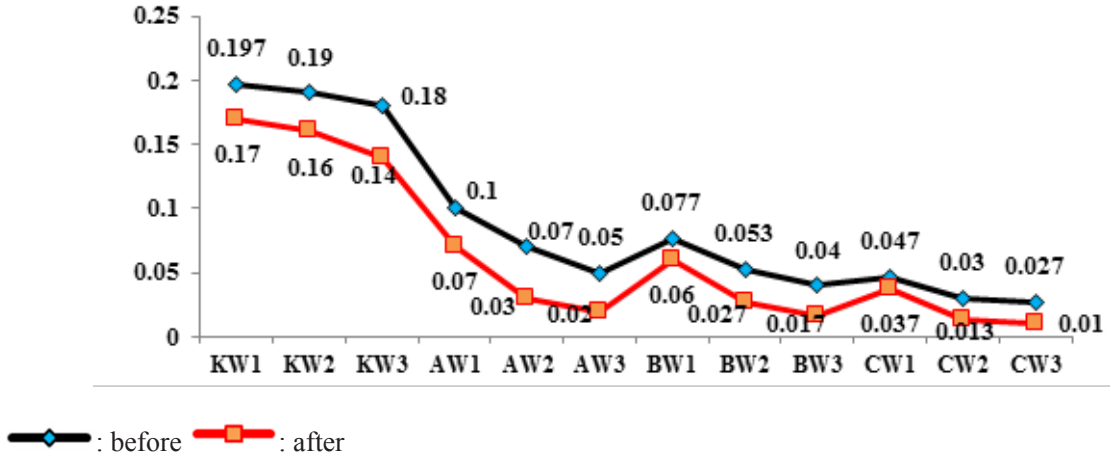

Figure 1. Pb Level (ppm) on Blood Cockles Based on Concentration and Stirring Time Before and After Heating

That graphic showed a tendency of difference of Pb Level on Blood Cockles before and after heating. Pb level after heating was lower than Pb level before heating, which was 0,01 ppm.

According to concentration of adding lime extract before and after heating could be seen the average of difference of Pb level on blood cockles from control group (KW), which was 0.047 ppm, group of AW sample code by giving lime extract in 1% was 0.033 ppm, group of BW sample code by giving lime extract in 1.5% was 0.022 ppm, and group of CW sample code by giving lime extract in 2% had average of difference in 0,017 ppm.

In other words, it could be stated that the higher the lime extract concentration, the bigger the differences of Pb level on Blood Cockles rather than blood cockles that was not added by lime extract anymore.

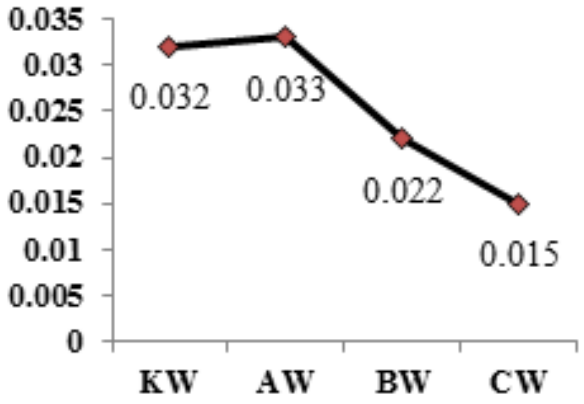

Figure 2. The Difference of Average of Pb Level (ppm) on Blood Cockles Before and After Heating Based on Lime Extract Concentration

That graphic showed that sample which was a control had a tendency of higher Pb level rather than in treatment group which was given lime extract. It was seen that concentration of lime extract in 2% had difference of lower Pb level rather than in sample group of lime extract concentration in 1% and in 1.5%. Hence, it indicated that there was a significant difference of Pb level on blood cockles based on variation of lime extract concentration before and after heating.

Analysis of Difference of Pb Level on Blood Cockles (*A.granosa*) among several treatments

In order to investigate the influence of lime extract and stirring time against Pb level on blood cockles (*A. granosa*) in twice observations, which were before and after heating, the researchers utilized Anova test. Requirements of Two-Way Anova test was conducted if it was completed the homogeneity test. According to the homogeneity test, it showed that all p-value Lavene test >0.05 (p-value before being heated up was 0.690 and after being heated up was 0.348), which meant that the variation of between groups was not significant different either before or after being heated up, thus, data was homogeneous and qualified the homogeneity assumption. Besides homogeneity test, normality test was also conducted in order to fulfill Anova test, which was through Kolmogorv Smirnov normality test with lilliefors correction which showed that anova residuals before and after heating was 0.200 (>0.05) for each, thus, the data contributed normally and it fulfilled the normality assumption.

Moreover, there was a significant influence between lime extract and stirring time against Pb level on twice

observations, which were before and after heating. Besides, it could be showed on table of Anova test and the result showed that there was a significant influence between lime extract and stirring time separately against the average of Pb level before and after heating. This could be proven from p-value of 0.000, and it indicated that there was a significant influence between lime extract and the average of Pb level on blood cockles before and after heating. Besides, there was a significant influence between stirring time and Pb level on blood cockles before and after heating, which was showed by p-value <0.05. In the other hand, there was an addition of lime extract and stirring time together and it was obtained p-value of 0.010. Thus, it indicated that there was a significant influence of lime extract concentration and stirring time together against the average of Pb level on blood cockles.

Furthermore, in order to investigate the difference of average in Pb level between each treatment of lime extract concentration, it could be showed in Post Hoc test by utilizing Tukey test. In Post Hoc test, it showed a significant difference of Pb level average between lime extract concentration that had p value 0,000, which meant that both lime concentrations which were compared with it had significant difference of Pb level value. The difference of the highest Pb level on blood cockles was showed between lime extract concentration of 0% and lime extract concentration of 15%, which was in 0,1461 rather than lime extract concentration of 5% (0.1144) and 10% (0.1278). This indicated that there was a significant influence between lime extract concentration and the decrease of Pb level on blood cockles. Furthermore, data from the table above showed that the higher the lime extract concentration, the bigger the difference of Pb level on blood cockles rather than on blood cockles which was not added by lime extract anymore.

The Post Hoc test (Tukey) in order to assess the difference of Pb level based on stirring time that was obtained data from all variations of stirring time which had significant difference against Pb level on blood cockles. The difference of the highest Pb level on blood cockles was showed on the comparison of stirring time in 5 minutes against stirring time in 15 minutes, which was 0,0346 rather than stirring time in 10 minutes, which was 0,0229. In other words, this data showed that the longer the stirring time in this research, the higher the difference of Pb level.

Conclusion

It could be concluded that manipulation of stirring time, adsorption temperature, and variation of lime extract concentration (*C. amblycarpa*) was through Stirring Chamber tools by utilizing adsorbent of chicken egg shell, which could reduce Pb level on blood cockles.

Source of Funding- Authors

Conflict of Interest- No

Ethical Clearance- Yes

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