Effectiveness of Liquid Smoke as a Source of Acetic Acid in Lowering Heavy Metals Levels in Blood Cockle (*Anadara granosa*)

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Abstract. Blood cockle (*Anadara granosa*) can accumulate heavy metals in the waters. The purpose of this study was to analyze the heavy metal content of lead, cadmium, and arsenic in blood cockle in Jepara waters through fish landing area Kedung and to determine the effectiveness of liquid smoke as a source of acetic acid in reducing heavy metal in blood cockle. The addition of liquid smoke concentrations of 0%, 4%, 5%, and 6% in distilled water, then the blood cockle soaked for 60 minutes and repeated 3 times. Control and blood cockle soaked in liquid smoke were tested for heavy metals (ICP-MS), protein, water, pH, and organoleptic levels. Heavy metal levels of lead, cadmium and arsenic in blood cockle in Jepara were 0.41±0.08 (mg/kg), 1.25±0.07 (mg/kg), and 1.28±0.17 (mg/kg). The results showed a significant difference *P*<5% in the reduction of heavy metals with liquid smoke. The lowest decreasing value at concentration of 4% of 25 until 27%, while the highest decrease was at a concentration of 6% 57 until 78%. Soaking blood cockle with liquid smoke as source of acetic acid effectively reduces heavy metal levels and maintains the quality of blood cockle.

1. Introduction

Blood cockle (*Anadara granosa*) are organisms that live on the bottom of the water or are attached to the substrate that is widely found in Indonesian waters. Blood cockle are in great demand by the Indonesian people because they have a savory taste and low prices. Indonesian people consume shellfish by boiling or processing them into dishes. The value of shellfish production in Indonesia in 2017 was 48,141.51 kg, in 2018 it was 94,247.27 kg[1]. This shows that the production of shellfish in Indonesia has increased significantly from year to year. In addition to increasing the high production value, shellfish also have high nutritional value for humans. However, cockle have filter feeder properties that can accumulate contaminants in the waters. One of the contaminants in the waters is heavy metals.

Mercury (Hg), lead (Pb), cadmium (Cd), Tin (Sn), and Arsenic (As) are heavy metals found in the substrate or mud at the bottom of the water. Heavy metal pollution in waters is caused by organic and inorganic waste, causing environmental pollution. Heavy metals can enter the waters either directly or as a by-product of industry. Waste disposed of in waters containing heavy metals can bind organic matter and will settle in the bottom sediments of the waters so that it accumulates by shellfish living on the bottom of the waters. Increased levels of heavy metals can cause adverse effects on humans [2].

Heavy metals have a maximum level by accordance with the Regulation of the Food and Drug Supervisory Agency No. 13 of 2014 the limit of cadmium contamination is 0.3 ppm in herbal medicines. Efforts to reduce levels of heavy metal cadmium can use acidic materials. Liquid smoke is the result of vapor condensation from the combustion of materials containing lignin, cellulose, and hemicellulose. Liquid smoke contains phenol, acid, and carbonyl [3]. The benefits of liquid smoke, among others, as chelating agents are food additives that can bind metal ions contained in shellfish.
Liquid smoke contains acetic acid and other carboxylic acids which cause acidic properties in liquid smoke. Acetic acid comes from the pyrolysis of cellulose and hemicellulose found in plants [4].

This study aims to determine the effectiveness of different concentrations of immersion in liquid smoke to reduce levels of lead, cadmium, mercury, tin, arsenic, and to determine their effect on water content, protein content, pH, and organoleptic values.

2. Methods
The material used in this study was blood cockle (Anadara granosa) from Kedung Jepara waters. The tools used in this research are ICP-MS (Inductive Coupled Plasma – Mass Spectrometry), oven, analytical balance, Kjeldigester, desiccator and pH meter. The method used in this study is experimental laboratories with a completely randomized design model with three repetitions. The concentrations used were 0%, 4%, 5% and 6%.

2.1. Boiling Process
The process of boiling cockle, first clean scallops are boiled in boiling water with a ratio of 200 grams of cockle and 400 ml of distilled water, then the cockle are drained after being washed with distilled water 3 times. The boiling process aims to make it easier to take cockle meat.

2.2. Making Liquid Smoke Solution
The process of making liquid smoke solution, calculating the need for liquid smoke calculated from the volume of 100 ml against 4%, 5% and 6% liquid smoke with the formula, then measuring 4 ml, 5 ml and 6 ml of liquid smoke. ml and then dissolved in 100 ml of distilled water then the solution was homogenized. The soaking process is done by soaking the cockle meat in a liquid smoke solution for 60 minutes. Each treatment was repeated 3 times.

2.3. Heavy Metal Concentration Test ICP-MS Method
The test for heavy metal cadmium in blood cockle by the ICP-MS method using [5] procedure are, the sample is mashed with a blender until it becomes smooth then placed in a clean and closed container. Wet sample is weighed 1.5 g in the vessel and the weight is recorded. The sample in the vessel is added with 10 mL of concentrated HNO₃ and allowed to stand for 15 minutes. Sample put into a microwave digester to be destroyed using a multilevel temperature of up to 150°C for 10 minutes and followed by destruction at 150°C (hold) for 15 minutes. The solution of the digested sample is cooled and put into a 50 mL volumetric flask. Vessel was rinsed with distilled water and combined the rinse results with the destruction results in a 50 mL volumetric flask. Sample solution in 50 mL volumetric flask plus 0.4 mL of internal standard mixture of Ge for analytes As, for analytes Cd and Sn, and Bi for analytes Hg and Pb 10 mg/L. Sample solution in a 50 mL volumetric flask is diluted with distilled water to the mark and then homogenized. The sample solution was filtered using a 0.20 μm filter RC/GHP filter. The intensity of the sample solution is measured in the ICP MS system. As analytes use the internal standard of Ge, analytes of Pb and Hg use the internal standard of Bi, and the analytes of Cd and Sn use the internal standard of In.

2.4. Protein Level Test
The steps carried out in the protein test based on [6] consist of three stages, namely destruction, distillation and titration. Measurement of protein content was carried out by the Kjeldahl method. The sample is weighed 0.5 g, then put into a 100 ml Kjeldahl flask, then 0.5 g selenium and 3 ml concentrated H₂SO₄ are added. Samples were crushed at 410°C for approximately 1 hour until the solution was clear and then cooled. After cooling, 50 ml of distilled water and 20 ml of 40% NaOH
were added to the Kjeldahl flask, then the distillation process was carried out with a distillation temperature of 100°C. The results of the distillation are accommodated in a 125 ml Erlenmeyer flask containing a mixture of 10 ml of 2% boric acid (H$_3$BO$_3$) and 2 drops of pink methyl red indicator. After the distillate volume reaches 40 ml and is bluish-green in color, the distillation process is stopped, then distillate with 0.1 N HCL until a pink color change occurs. The titrant volume is read and recorded.

2.5. Moisture Test
The procedure for testing water content based on [6]. The porcelain cup is dried in an oven at a temperature of 100°C for 15 minutes and then cooled in a desiccator and then the weight of the cup is weighed. Sample of 2 g is put into a cup, weighed and dried in an oven for 16 hours at a temperature of 100°C. The sample is removed from the oven and cooled in a desiccator. After cooling, it is weighed to a constant weight.

2.6. pH Level Test
Measurement of pH was carried out with a digital pH meter, using the method of [6] Samples that have been chopped into small pieces are weighed 20 g and put into a blender and added 40 ml of distilled water, then mashed for 1 minute. The sample is then poured into a 100 ml beaker whose pH is measured with a pH meter. Before using the pH meter, the sensitivity of the pH meter needle was calibrated with pH 4 buffer, then with pH 7 buffer. The magnitude of the pH value is the reading of the pH needle after 1 minute.

2.7. Organoleptic Test
According to [7] to calculate the average quality value interval of each panelist, the following formula is used:

$$\left( \bar{x} - \left( 1.96 \frac{s}{\sqrt{n}} \right) \right) \leq \mu \geq \left( \bar{x} + \left( 1.96 \frac{s}{\sqrt{n}} \right) \right) \cong 95\%$$

3. Results and discussion
3.1. Level of Pb
The results of testing the levels of Pb of blood cockle with liquid smoke immersion treatment at concentrations of 4%, 5% and 6%.

| No. | Treatment | Levels of Pb (ppm) | Decrease (%) |
|-----|-----------|--------------------|--------------|
| 1.  | 0%        | 0.41±0.08$^a$      |              |
| 2.  | 4%        | 0.29±0.05$^{ab}$   | 27.64        |
| 3.  | 5%        | 0.19±0.03$^{bc}$   | 52.03        |
| 4.  | 6%        | 0.09±0.02$^c$      | 78.04        |

Information:
- The data in the table followed by different superscript letters showed a significant difference (P<0.05)
- Data is the average of three replications with different concentrations of liquid smoke immersion ± Standard deviation (SD)

Based on the test results, the reduction of Pb using liquid smoke is very effective. According to [8], the decrease in heavy metal Pb by liquid smoke is due to the large number of hydroxyl, carboxyl and carbonyl groups in liquid smoke. This is because the functional groups in acid, phenol and carbonyl compounds will form bonds that can bind to metals. Phenol compounds and hydroxyl groups will form metal cation bonds.
The maximum limit for Pb compounds in blood cockle according to the [8] is 1.5 mg/kg. According to [9], the maximum level of Pb in shellfish is 1.5 mg/kg so that Pb levels in blood cockle can be consumed but are close to the maximum threshold.

The concentration of 6% liquid smoke has the lowest Pb levels. Difference in Pb levels in the control and 6% liquid smoke immersion decreased by 78.04%. In another study by [10], decreased heavy metal Pb by 96.5% by UV depuration after 48 hours. According to [11], under acidic conditions Pb metal can be released. The chelating content in the acid will cause the confinement of the heavy metal cation content in the material until an equilibrium occurs. Therefore chelating efficiency is very important in reducing metal content. The mechanism of Pb metal transfer is by binding Pb metal ions with deprotonated acid groups so that metal ions are released from shells and bind to acid groups. Metal ions Pb will undergo a transition and form bonds with acidic compounds due to the presence of free electrons in the citric acid functional group in the form of deprotonated -COOH [12]. The acid group is deprotonated due to the presence of hydroxide ions (OH) so that the carboxylic group turns into a negatively charged (COO-) which has been deprotonated and will bind to Pb metal.

3.2. Level of Cd

Data on the results of testing Cd levels in blood cockle with liquid smoke immersion in concentrations of 4%, 5% and 6%.

| No. | Treatment | Levels of Cd (ppm) | Decrease (%) |
|-----|-----------|--------------------|--------------|
| 1.  | 0%        | 1.28±0.17<sup>a</sup> |              |
| 2.  | 4%        | 0.95±0.07<sup>ab</sup> | 25.52        |
| 3.  | 5%        | 0.79±0.06<sup>ac</sup> | 38.28        |
| 4.  | 6%        | 0.46±0.16<sup>c</sup>  | 63.54        |

Information:
- The data in the table followed by different superscript letters showed a significant difference (P<0.05)
- Data is the average of three replications with different concentrations of liquid smoke immersion ± Standard deviation (SD)

The maximum limit of Cd heavy metal content in shellfish according to the [8] is 1 mg/kg. Based on [9] the maximum limit for heavy metal Cd is 1 mg/kg. The concentration of heavy metal Cd of blood cockle in the control treatment exceeded the determination of BSN and EC, which was 1.14 ppm, while after immersion using liquid smoke the levels of heavy metals decreased below SNI and EC.

Based on the test results of heavy metal levels of Cd in blood cockle showed that the control treatment was significantly different from the liquid smoke immersion treatment. Immersion of liquid smoke with a concentration of 4%, 5% and 6% reduced levels of heavy metal Cd in blood cockle. The decrease in Cd heavy metal levels in blood cockle was due to the content of carboxyl groups and acids contained in liquid smoke. According to [13], acid content in liquid smoke is acetic acid obtained from the cellulose content found in wood.

The lowest level of cadmium in blood cockle with immersion in liquid smoke with a concentration of 6% was 0.46± 0.16 reduction in cadmium levels of 63.54%. Immersion of liquid smoke is more effective than research by [14], the reduction of heavy metal levels of Cd by soaking green cockle with 25% of aceric acid above 37.88%. According to [15], ions in the acid will bind to the metal so that it can remove the metal ions that accumulate in the shells. The higher the concentration of acid in a solution, the faster the solution will react with metal ions. Likewise with the immersion time. The longer a substance interacts with other compounds, the faster the reaction between the acid and the metal.
3.3. Level of As
The data on the test results for heavy metal levels of As Blood Cockle with liquid smoke immersion at concentrations of 4%, 6% and 7%.

| No. | Treatment | Levels of As (ppm) | Decrease (%) |
|-----|-----------|--------------------|--------------|
| 1.  | 0%        | 1.25±0.07          |              |
| 2.  | 4%        | 0.93±0.04          | 25.06        |
| 3.  | 5%        | 0.82±0.05          | 34.13        |
| 4.  | 6%        | 0.53±0.12          | 57.33        |

Information:
- The data in the table followed by different superscript letters showed a significant difference (P<0.05)
- Data is the average of three replications with different concentrations of liquid smoke immersion ± Standard deviation (SD)

The maximum limit of arsenic heavy metal content according to the [8], is 1 mg/kg. According to [16], the maximum limit for heavy metal As contamination in shellfish is 1 mg/kg. The concentration of heavy metal As in the control blood cockle (without immersion in liquid smoke) was 1.25 ppm, thus exceeding the maximum limit of heavy metal As according to BSN or SFA. The immersion treatments of 4%, 5% and 6% resulted in heavy metal concentrations of As 0.93 ppm, 0.82 ppm and 0.53 ppm meeting the maximum recommended limits according to BSN and SFA.

Based on the results of the As content test, it shows that with the increase in the concentration of liquid smoke, the As content value will decrease. The highest concentration of heavy metal As was found in the control treatment where without immersion using liquid smoke. This is because the acetic acid content in liquid smoke is able to reduce the levels of heavy metals As in blood cockle. According to [17], to reduce the levels of heavy metals is done by increasing the number of carboxyl groups by using acid. Acidic compounds show excellent adsorption for some heavy metal ions due to the large amount of carboxyl content that can adsorb heavy metal arsenic. This is confirmed by [18], the decrease in heavy metals is due to the release of protein metal complex bonds so that the metal ions come out. Metal ions found in the body of organisms are almost all bound to proteins.

3.4. Level of Protein
The value of the results of testing protein levels in blood cockle (Anadara granosa) with liquid smoke immersion concentrations of 4%, 5% and 6%.

| No. | Treatment | Protein Content (%) |
|-----|-----------|---------------------|
| 1.  | 0%        | 15.39±0.49          |
| 2.  | 4%        | 14.39±0.44          |
| 3.  | 5%        | 13.95±0.59          |
| 4.  | 6%        | 13.68±0.44          |

Information:
- The data in the table followed by different superscript letters showed a significant difference (P<0.05)
- Data is the average of three replications with different concentrations of liquid smoke immersion ± Standard deviation (SD)

Based on the results of the protein content test of blood cockle, it showed that the control treatment was significantly different from the liquid smoke immersion treatment. Liquid smoke immersion 4%, 5% and 6% can reduce the protein value of blood cockle. Decreased protein levels in blood cockle due to the acid content in liquid smoke can damage protein.[19], proteins bind to metal ions, but the
equilibrium can be reduced in an acidic environment. Heavy metals have the property of binding to proteins, carbohydrates and fats so that heavy metals can settle in tissues.

Protein is a network that binds to metal ions. The bond formed between protein and metal is metalloprotein, this bond is an unstable bond. According to [20], metalloprotein bonds are metallic bonds that are labile so that it is easy to exchange between ions and the environment.

3.5. Water Content
The results of testing the water content of blood cockle (Anadara granosa) with liquid smoke immersion in concentrations of 4%, 5% and 6%.

Table 5. Blood Cockle Water Content

| No. | Treatment | Water Content (%) |
|-----|-----------|-------------------|
| 1.  | 0%        | 77.31±0.4a        |
| 2.  | 4%        | 76.53±0.57a       |
| 3.  | 5%        | 76.13±0.56a       |
| 4.  | 6%        | 74.82±0.38b       |

Information:
- The data in the table followed by different superscript letters showed a significant difference (P<0.05)
- Data is the average of three replications with different concentrations of liquid smoke immersion ± Standard deviation (SD)

The high water content is caused by the process of boiling and soaking the blood cockle. This is due to the boiling process that uses plain water and the immersion of liquid smoke in the form of this liquid which can cause an increase in the water content of blood cockle and can help reduce heavy metal levels in blood cockle. The boiling process with distilled water can also reduce heavy metal levels, although not significantly. Boiling process of cockle can cause protein to be degraded, one of the broken bonds is metallothionine which can bind heavy metals. The use of distilled water can damage the heavy metal complex bonds but is not effective. This is confirmed by [21], the decrease in heavy metal levels due to boiling is due to the disposal of heavy metals as free salts into the cooking water which is closely related to dissolved amino acids and uncoagulated proteins.

3.6. pH level
The value of the results of testing pH levels on blood cockle (Anadara granosa) with liquid smoke immersion in concentrations of 4%, 5% and 6%.

Table 6. Blood Cockle pH Level

| No. | Treatment | pH levels         |
|-----|-----------|-------------------|
| 1.  | 0%        | 7.09±0.37a        |
| 2.  | 4%        | 6.35±0.5ab        |
| 3.  | 5%        | 6.36±0.42ab       |
| 4.  | 6%        | 5.72±0.45b        |

Information:
- The data in the table followed by different superscript letters showed a significant difference (P<0.05)
- Data is the average of three replications with different concentrations of liquid smoke immersion ± Standard deviation (SD)

Based on the results of the pH test, it shows that the higher the concentration of liquid smoke given, the lower the pH level of the blood cockle. The average obtained is 6.38. The highest pH was obtained from blood cockle without immersion in liquid smoke of 7.09 while the lowest pH was obtained from immersion in blood cockle with liquid smoke with a concentration of 6% of 5.72. The addition of liquid smoke has a significant effect on the pH level of blood cockle. This is caused by the
content of organic acids in the liquid smoke so that the pH of the blood cockle meat decreases. According to [22], increasing the concentration of citric, oxalic or malic acid can increase Cd adsorption at low pH. The amount of heavy metal adsorption decreases significantly with increasing pH value.

The low pH content is related to the decreasing value of heavy metal content. According to [23] at pH 4 the carboxylate begins to form a complex in the presence of metal ions. When the pH increases, the decarboxylation of the carboxylate groups increases so that more ionized carboxylate groups can bind to heavy metals.

3.7. Organoleptic
The results obtained from organoleptic tests on blood cockle (Anadara granosa) with liquid smoke immersion in concentrations of 4%, 5% and 6%.

Table 7. Blood Cockle Organoleptic Test Results

| Parameter | Treatment | A         | B         | C         | D         |
|-----------|-----------|-----------|-----------|-----------|-----------|
| Appearance|           | 8.73±0.69 | 8.26±0.98 | 7.93±0.98 | 7.6±1.4   |
| Smell     |           | 8.53±0.86 | 7.6±0.93  | 7.06±0.36 | 7±0.9     |
| Taste     |           | 8±1.01    | 7.73±0.98 | 7.06±0.82 | 7±1.17    |
| Textur    |           | 8.46±0.89 | 7.8±0.99  | 7.6±1.3   | 7.3±1.39  |
| Overall   |           | 8.43±0.55 | 7.85±0.58 | 7.41±0.56 | 7.23±0.65 |

Convidence Interval: 8.23<µ<8.62 7.64< µ<8.05 7.2< µ<7.61 7< µ<7.45

Information:
- The data in the table followed by different superscript letters showed a significant difference (P<0.05)
- Data is the average of three replications with different concentrations of liquid smoke immersion ± Standard deviation (SD)

3.7.1. Appearance
The organoleptic test on the appearance of blood cockle gave an average value ranging from 8.13. The highest value was obtained from treatment A (control) which was 8.73 and the lowest was from treatment D (6% liquid smoke immersion) which was 7.6. Based on this value, it is known that the blood cockle from all treatments were favored by consumers. According to [24], appearance is one of the organoleptic parameters that consumers value through the sense of sight. Consumers generally like food that has an attractive appearance.

3.7.2. Smell
The data from the organoleptic test on the aroma of blood cockle showed an average value of around 7.54. Treatment A had the highest value of 8.53 while treatment D had the lowest value of 7. The higher the concentration of liquid smoke, the lower the aroma value. This is because liquid smoke has a pungent aroma. According to [25], liquid smoke generally has a strong aroma or sharp, sour, charred smell. This is due to the volatile compounds contained in liquid smoke.

3.7.3. Taste
The organoleptic test on the taste of blood cockle gave an average value of around 7.4. The highest value was obtained from treatment A (control) and the lowest was from treatment D (6% liquid smoke immersion). Based on this value, it is known that the taste of blood cockle from all treatments is favored by consumers. The higher the concentration of liquid smoke, the lower the organoleptic value. This is because the taste of the product becomes more sour and smoke-specific. According to [26], the
first thermal degradation of cellulose during the manufacture of liquid smoke will form glucose, acetic acid, water and phenolic compounds that affect the taste of the product.

3.7.4. Texture
The data from the organoleptic test on the texture of the blood cockle showed an average value of around 7.73. Treatment A has the highest value of 8.46 while treatment D has the lowest value of 7.33. The higher the concentration of liquid smoke, the lower the texture value. This is because there is a boiling and soaking process that causes the texture of the blood cockle to become softer. According to [27], the boiling and soaking process causes the water content of the product to increase so that the texture of the food becomes mushy. The texture of food ingredients is closely related to the water content of food ingredients.

4. Conclusion
In this study, the heavy metal content of lead in the waters of Jepara has levels below the SNI while the levels of heavy metals cadmium and arsenic are above the SNI and the heavy metal content of Pb in the waters of Jepara has levels below the SNI while the levels of heavy metals Cd and As are above the SNI.

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