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ANALYSIS OF HEAVY METAL CONTENT IN *Anandara granosa* (Linnaeus, 1758): A CASE STUDY OF 10 MARKETS IN SEMARANG, CENTRAL JAVA, INDONESIA

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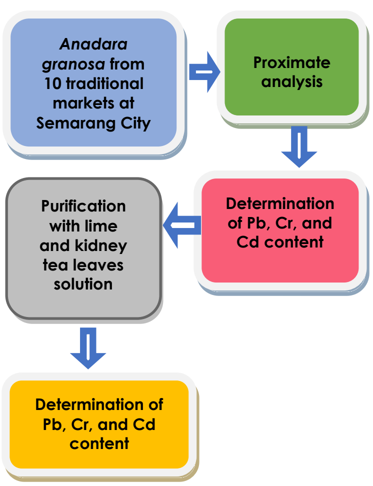
Graphical abstract



Abstract

The purpose of this study was to evaluate Pb, Cr, and Cd levels inside the *Anadara granosa* and to investigate the effect of submersion using *Citrus aurantifolia* (lime) and *Orthosiphon aristatus* (kidney tea leaves) solution on Pb, Cr, and Cd levels contained in the blood cockles taken from 10 markets in the city of Semarang, Central Java, Indonesia (Johar, Genuk, Gayamsari, Jati, Peterongan, Karangayu, Mangkang, Pedurungan, Boom Lama, and Ngaliyan). The concentration of heavy metals of *Anadara granosa* was analyzed using Atomic Absorption Spectrometer (AAS). Analysis of variance (ANOVA) showed that heavy metals (Pb, Cd, and Cr) of blood cockle taken from 10 markets provided no significant difference ($P < 0.05$) on the content of Pb, Cd and Cr. Purification was carried out using *Citrus aurantifolia* (lime) and *Orthosiphon aristatus* (kidney tea leaves) solution. The lowest Pb (0.00 mg kg^{-1}) was obtained from Boom Lama and Peterongan market with purification using kidney tea leaves solution, while the lowest Cr [(0.30 ± 0.00) mg kg^{-1}]) was obtained from Genuk market with lime solution. In addition, the lowest Cd (0.00 mg kg^{-1}) was obtained from Boom Lama market with purification using kidney tea leaves solution. Accumulation of heavy metal contamination in blood cockle could affect the micronutrient status and consumer health.

Keywords: *Anadara granosa*, heavy metals, micronutrients inhibitor, traditional

1.0 INTRODUCTION

Heavy metals such as lead (Pb), chromium (Cr), and cadmium (Cd) are toxic contaminants that can cause biochemical changes and bioaccumulation in aquatic and its organisms. Not only adversely affect aquatic organisms, but also impact on humans who consume metal toxic of fish products. For example, heavy metals Pb are simply accumulated in the body of children [1]. The recent study pointed out that the accumulation of Pb is not only in the organs or in the blood, but also in the adipose fat tissue [2]. Pb and Cd poisoning in children can cause severe malnutrition due to micronutrients deficiency and impaired brain development [3].

Anadara granosa (Linnaeus,1758) is a fishery product which is able to accumulate heavy metals. *A. granosa* is one type of blood cockle that has the potential and economic value to be developed as a source of protein and essential minerals to meet the nutrient adequacy in public health situation. *A. granosa* lives in a way to immerse in the mud, beneath the surface in shallow water [4]. The accumulation of Pb, Cr, and Cd in *A. granosa* through human consumption can be detrimental to health such as decreased renal function, memory loss, respiratory tract disorders, impaired liver function, and cancer [5–10].

The accumulation of Pb, Cr, and Cd inside *A. granosa* is caused by the nature, the location and the way to obtain the food as it is categorized as filter feeders that can accumulate metals from environment. In addition, due to differences in substrate where life and the life of older blood cockles also allow metals accumulate more than younger blood cockles [11]. A study in rats showed that *Citrus aurantifolia* (lime) reduced blood lead levels [12]. *Citrus aurantifolia* (lime) contains antioxidants and some studies have confirmed such antioxidants could prevent metal toxicities [13, 14].

The purpose of this study was to evaluate Pb, Cr, and Cd levels inside the *Anadara granosa* and to investigate the effect of submersion using *Citrus aurantifolia* (lime) and *Orthosiphon aristatus* (kidney tea leaves) solution on Pb, Cr, and Cd levels of *A. granosa*.

2.0 EXPERIMENT

2.1 Materials

Materials used in this study were blood cockles of *Anadara granosa* taken from 10 traditional markets mentioned above. Blood cockles were collected for 1 kg each from every market with the size from 3 cm to 5 cm and weight of 10 g. Samples were taken using a plastic bag, a camera, a label, and a sterofoam box.

2.2 Research Methods

This study was conducted in two stages. The first stage was determined to asses the level of proximate, Pb, Cd, dan Cr in *A. granosa*. The second stage was to investigate the effect of *Citrus aurantifolia* (lime) and *Orthosiphon aristatus* (kidney tea leaves) solution on Pb, Cr, and Cd levels of *A. granosa*.

2.2.1 Sampling of the Blood Cockles

About 500 g sample was taken directly from the traditional markets located in Semarang. The sample was then washed to remove dirt and then boiled to separate the blood cockles and blood cockle meat.

2.2.2 Sample Preparations

About 50 g sample was deluged inside 20 % solution of *Citrus aurantifolia* (lime) and *Orthosiphon aristatus* (kidney tea leaves) solution for 60 min. Concentration and time of submersion were determined using previous study with concentration of 10 %, 15 %, 20 %, 25 % and time of submersion of 30 min, 60 min and 90 min.

2.2.3 Testing of Heavy Metals by Atomic Absorption Spectrometer [15]

About 50 g sample was taken and then dried at a temperature of approximately 100 ˚C, then mashed into powder. The sample was dissolved into the vessel of 500 mg and added with nitric acid and perchloric acid in 1 mL and 2.5 mL of distilled water. Then it was put in a microwave digestion. Subsequently, the sample was analyzed using Atomic Absorption Spectroscopy (AAS). The working of this method was conducted by comparing the absorbance of the sample solution with the standard solution to obtain the concentration of the sample. Scale absorbance of AAS was calibrated with a standard series of known concentration. The result of the analysis of AAS was shown in a calibration curve. After measuring the absorbance the solution, the calibration curve was used to determine the concentration of sample solution. Factors that may affect the calibration process were AAS standard solution and AAS instrument.

2.2.4 Data Analysis

Heavy metal test results of *A. granosa* from 10 traditional markets in the city of Semarang, then, were analyzed by using analysis of variance or one–way ANOVA with IBM SPSS 22. This analysis was used to determine differences in the concentration of heavy metals such as Pb, Cd, and Cr in *A. granosa* taken from the 10 traditional markets in the city of Semarang.

3.0 RESULTS AND DISCUSSION

3.1 Proximate

Result of proximate analysis of *Anadara granosa* taken from 10 markets in Semarang city is shown in Table 1. It can be seen that the content of moisture ranged from (66.61 ± 0.11) % to (72.25 ± 0.15) %, while protein was from (21.91 ± 0.08) % to (25.52 ± 0.33) %. In addition, the content of lipid ranged from (2.27 ± 0.22) % to (3.91 ± 0.07) %, while ash from (1.22 ± 0.13) % to (2.93 ± 0.07) %.

Table 1 Analysis of *Anadara granosa*'s proximate from 10 traditional markets in Semarang city

No	Samples code	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
1	Ps. Pedurungan	71.72 ± 0.05	21.91 ± 0.08	2.67 ± 0.08	2.32 ± 0.04
2	Ps. Genuk	71.28 ± 0.11	22.52 ± 0.11	3.12 ± 0.07	1.85 ± 0.11
3	Ps. Gayamsari	69.64 ± 0.19	23.40 ± 0.38	2.57 ± 0.05	2.86 ± 0.10
4	Ps. Johar	69.89 ± 0.02	22.85 ± 0.08	2.94 ± 0.02	2.82 ± 0.04
5	Ps. Jati	70.24 ± 0.02	22.62 ± 0.09	2.71 ± 0.13	2.89 ± 0.06
6	Ps. Peterongan	69.92 ± 0.05	22.95 ± 0.01	2.92 ± 0.10	2.91 ± 0.04
7	Ps. Boom Lama	72.25 ± 0.15	22.56 ± 0.47	2.27 ± 0.22	1.22 ± 0.13
8	Ps. Karangayu	68.82 ± 0.05	22.92 ± 0.10	3.91 ± 0.07	2.93 ± 0.07
9	Ps. Mangkang	70.83 ± 0.06	22.60 ± 0.05	2.94 ± 0.03	2.77 ± 0.26
10	Ps. Ngaliyan	66.61 ± 0.11	25.52 ± 0.33	3.50 ± 0.09	2.86 ± 0.07

Note: Average value of duplicate measurement ± standard deviaton.

A study of blood cockle taken from Teluk Tomini, Gorontalo, Indonesia obtained 65.69 % moisture, 23.23 % protein, 7.01 % lipid and 2.57 % ash [32]. This might be caused by the differences in enviromental condition, gender, age and fishing season.

3.2 Lead (Pb)

Lead (Pb) or black tin is a natural substance found in the earth's crust and often used in chemically manufactured industries (e.g. batteries industry, stationery industries, electrical wiring and coloring paint). Waste of lead (Pb) can often be found in the form of sediment, which can contaminate the waters and organisms such as blood cockle (*A. granosa*). Table 2 presents the results of lead (Pb) analysis of *A. granosa* at 10 traditional markets in Semarang city.

Table 2 illustrates lead (Pb) contained in *A. granosa* taken from the traditional markets of Pedurungan, Genuk, and Gayamsari were the highest levels among the other traditional markets. Lead is a substance that is highly toxic or poisonous if absorbed into the body. Lead poisoning can be experienced by people of various ages especially in high–risk groups e.g. pregnant women. Exposing lead (Pb) during pregnancy could be a threat as it could flow through the placenta. For the fetus to be at risk of micronutrient deficiencies, malnutrition, to the chronically impaired brain development [3]. Lead poisoning is able to influence brain development by reducing the IQ, hyperactivity, hearing damage and impaired the child growth [16]. However, the content of lead (Pb) in the consumption of fishery products at generally northern coastal communities should remain a concern because of the nature of accumulation.

Table 2 Results of the analysis of *Anadara granosa*'s lead (Pb) from 10 traditional markets in Semarang city

No.	Samples code	Lead (mg kg^{-1})				
		I	II	III	Mean	SD
1	Ps. Pedurungan	0.24	0.23	0.23	0.23	0.01
2	Ps. Genuk	0.22	0.23	0.22	0.22	0.00
3	Ps. Gayamsari	0.20	0.20	0.20	0.20	0.00
4	Ps. Johar	0.18	0.18	0.18	0.18	0.00
5	Ps. Jati	0.17	0.18	0.17	0.17	0.00
6	Ps. Peterongan	0.14	0.15	0.15	0.15	0.00
7	Ps. Boom Lama	0.11	0.13	0.13	0.12	0.01
8	Ps. Karang ayu	0.12	0.12	0.11	0.12	0.00
9	Ps.Mangkang	0.10	0.10	0.10	0.10	0.00
10	Ps. Ngaliyan	0.09	0.09	0.09	0.09	0.00

Note: SD= Standard Deviation

3.3 Chromium (Cr)

Chromium (Cr) is a metal that has been used extensively in human life such as industry, textiles, tanning, and explosives [17]. The chromium waste of industrial products generally is often discharged into waters which contaminate water and organisms such as blood cockle. Table 3 illustrates result analysis of chromium of *A. granosa* taken from 10 traditional markets in Semarang city.

Table 3 Results of *Anadara granosa's* chromium (Cr) analysis from 10 traditional markets in Semarang city

No.	Samples code	Chromium (mg kg^{-1})				
		I	II	III	Mean	SD
1	Ps. Pedurungan	0.07	0.07	0.07	0.07	0.00
2	Ps. Genuk	0.08	0.08	0.08	0.08	0.00
3	Ps.Gayamsari	0.09	0.09	0.09	0.09	0.00
4	Ps. Johar	0.06	0.06	0.06	0.06	0.00
5	Ps. Jati	0.05	0.05	0.05	0.05	0.00
6	Ps. Peterongan	0.05	0.05	0.05	0.05	0.00
7	Ps. Boom Lama	0.04	0.04	0.04	0.04	0.00
8	Ps. Karangayu	0.06	0.06	0.06	0.06	0.00
9	Ps. Mangkang	0.06	0.06	0.06	0.06	0.00
10	Ps.Ngaliyan	0.04	0.04	0.04	0.04	0.00

Note: SD= Standard Deviation

Chromium (Cr) is an element that plays an important role in everyday life. At low concentrations in the form of Cr^{3+} (trivalent), chromium is an essential micronutrient for humans [18]. However, at high concentrations in the form of Cr^{6+} (hexavalent), it has been known to be carcinogenic [19]. Chromium in foods is mostly found in coral and sea water [18]. The sufficient quantity of chromium in the form of Cr^{3+} has been known to be able to improve the ability of insulin in the metabolism of glucose [20]. In addition, in the treatment of chromium parenteral nutrition, it has become one of the essential nutrients [18]. Nevertheless, the accumulation of the chromium in the tissue should not form Cr^{6+} as it is toxic and could be carcinogenic in the long–term exposure.

3.4 Cadmium (Cd)

Cadmium (Cd) is a toxic heavy metal elements often found in sewage pollution of waters and blood cockle other than lead (Pb) [21]. Result of analysis of cadmium (Cd) of *A. granosa* taken from 10 markets in Semarang city is shown in Table 4.

A. granosa, obtained from Genuk traditional market, contained the highest cadmium [(0.16 ± 0.00) mg kg^{-1}], while the blood cockles from Ngaliyan market contained the lowest amount [(0.06 ± 0.00) mg kg^{-1})]. Cadmium will be transported by an enzyme transporter, which has trivalent in valency number and has a potential reduction into 2^{+}. It may then be accumulated in the kidneys and liver. If the concentration reaches 200 µg g^{-1} or more, it will cause kidney damage. Cadmium metal source can be derived from the metals that may be plated with cadmium. Based on the WHO, cadmium consumption threshold is between 57 mg d^{-1} and 71 mg d^{-1}[22].

Table 4 Analysis of *Anadara granosa*'s cadmium (Cd) from 10 traditional markets in Semarang city

No	Samples code	Cadmium (mg kg^{-1})				
		I	II	III	Mean	SD
1	Ps. Pedurungan	0.09	0.09	0.09	0.09	0.00
2	Ps. Genuk	0.16	0.16	0.16	0.16	0.00
3	Ps. Gayamsari	0.14	0.14	0.14	0.14	0.00
4	Ps. Johar	0.13	0.13	0.13	0.13	0.00
5	Ps. Jati	0.13	0.13	0.13	0.13	0.00
6	Ps. Peterongan	0.10	0.10	0.10	0.10	0.00
7	Ps. Boom Lama	0.09	0.09	0.09	0.09	0.00
8	Ps. Karangayu	0.08	0.08	0.08	0.08	0.00
9	Ps. Mangkang	0.09	0.09	0.09	0.09	0.00
10	Ps. Ngaliyan	0.06	0.06	0.06	0.06	0.00

Note: SD= Standard Deviation

In the biochemical process of human body, there are three main mechanisms of how the heavy metal interact and cause a variety of biochemical disturbances [23, 24]. The first mechanism is by entering the human body through food intake, through the digestive process, and is absorbed through the intestinal villi to the blood circulation. The second mechanism is after they enter the circulation, there is a trivalent metal receptor, which is unrecognized whether it is a metal that is essential or not essential to human body. The effect is there will be a complex competition between essential trace elements against non essential metal [25]. The third mechanism is in the long term effect, since imbalanced competition between non essential metal and essential trace elements, may cause essential micronutrients deficiency and functional disorders e.g. child growth and impaired brain development [26].

3.5 Lead, Cadmium, and Chromium Levels after Purification using *Citrus aurantifolia* (lime) and *Orthosiphon aristatus* (kidney tea leaves) Solution

3.5.1 Lead (Pb)

Figure 1 and Figure 2 depict the decrease of lead in *A. granosa* after purification with *Citrus aurantifolia* (lime) and *Orthosiphon aristatus* (kidney tea leaves) solution. Submersion using *Orthosiphon aristatus* showed significant reduction of lead concentration compared to *Citrus aurantifolia* solution.

After purification of *Anadara granosa* with *Orthosiphon aristatus* solution, the highest amount of lead [(0.57 ± 0.08) mg kg^{-1})] was obtained by those from Jati market, while the blood cockles obtained from Boom Lama and Peterongan market contained the lowest amount of lead (0.00 mg kg^{-1}). Purification of *A. granosa* from Gayamsari traditional market using *Citrus aurantifolia* solution resulted in the highest content of lead [(1.82 ± 0.00) mg kg^{-1})], whereas the blood cockles obtained from Mangkang traditional market contained the lowest lead [(1.57 ± 0.00] mg kg^{-1})]. In addition, *A. granosa* taken from the market of Muara Angke provided lead that exceeded the threshold (3.5 ppm to 6.21 ppm) [29] (ppm = part per million = µg g^{-1}). The permissible levels of Pb set by the Commission Regulation of EU (2006) for human consumption was 1.00 µg g^{-1} or 1.00 ppm [28].

A study about *Avicennia marina* showed that 25 % *Citrus aurantifolia* solution reduced the level of lead from 1.78 ppm to 0.71 ppm [27]. In addition, the other study of boiled beef liver using 10 % kidney tea leaves solution resulted in the reduction of lead from 0.069 ppm to 0.017 ppm [33]. The decrease in the content of lead might be caused by the denaturation of protein of blood cockles with regard to acid treatment. This could cause the release of metal complex bonds of the meat along with body fluids [30].

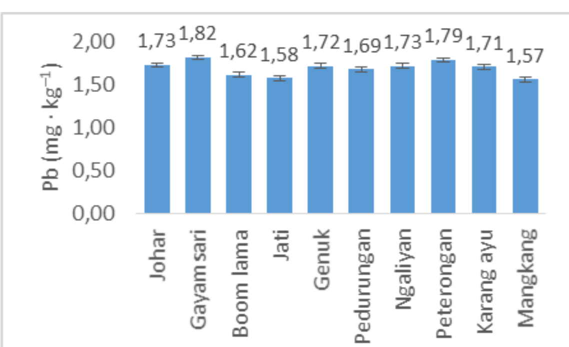


Figure 1 *Anadara granosa's* lead (Pb) from 10 traditional markets in Semarang City after purification using *Citrus aurantifolia*

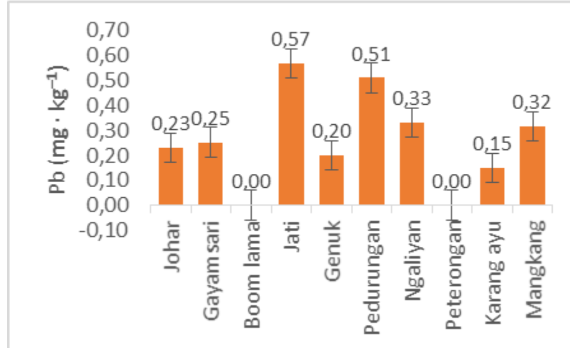


Figure 2 *Anadara granosa's* lead (Pb) from 10 traditional markets in Semarang City after purification using *Orthosiphon aristatus*

3.5.2 Chromium (Cr)

The submersion of *A. granosa* using *Citrus aurantifolia* (Figure 3) significantly decreased the chromium level compared to that using *Orthosiphon aristatus* (Figure 4). The purification of *A. granosa* obtained from Jati traditional market using *Orthosiphon aristatus* solution resulted in the highest level of chromium [(6.26 ± 0.03) mg kg^{-1})], while those obtained from Peterongan traditional market provided the lowest chromium [(3.87 ± 0.03) mg kg^{-1})].

In addition, the purification of *A. granosa* obtained from Mangkang traditional market using *Citrus aurantifolia* solution presented the highest chromium [(0.54 ± 0.00) mg kg–1)], whereas the blood cockles obtained from Genuk traditional market showed the lowest level of chromium [(0.3 ± 0.00) mg kg–1)]. Meanwhile, *A. granosa* taken from Muara Sayung river was found to contain chromium between 0.13 ppm to 0.16 ppm [31]. A study about boiled beef liver revealed that 10 % kidney tea leaves solution could reduce chromium from 0.732 ppm to undetected value [33].

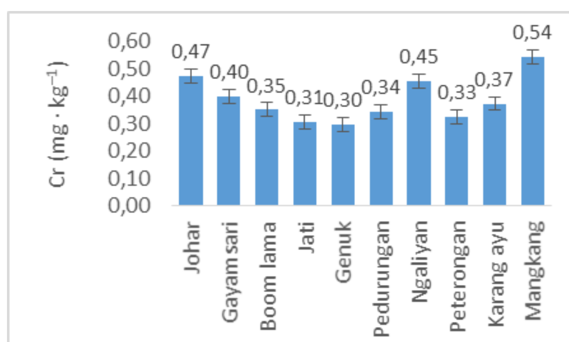


Figure 3 *Anadara granosa's* chromium (Cr) from 10 traditional markets in Semarang city after purification using *Citrus aurantifolia*

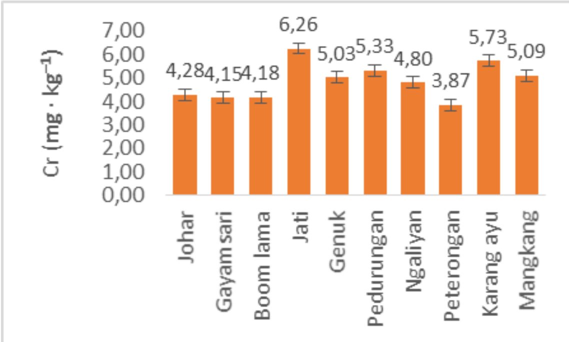


Figure 4 *Anadara granosa's* chromium (Cr) from 10 traditional markets in Semarang City after purification using *Orthosiphon aristatus*

3.5.3 Cadmium (Cd)

Figure 5 and Figure 6 show that *Orthosiphon aristatus* solution significantly reduced cadmium (Cd) levels of *A. granosa* compared to *Citrus aurantifolia* solution. After purification of *A. granosa* from Jati traditional market with *Orthosiphon aristatus* solution, the highest level of cadmium [(0.47 ± 0.02) mg kg^{-1})] was obtained, while the blood cockles from Boom Lama traditional market showed the lowest cadmium level (0.00 mg kg^{-1}).

In addition, the purification of A. granosa obtained from Ngaliyan traditional market with Citrus aurantifolia solution resulted in the highest cadmium level [(0.81 ± 0.00) mg • kg–1)], while the blood cockles obtained from Genuk traditional market provided the lowest level [(0.6 ± 0.00) mg • kg–1)]. These results met the maximum standards of cadmium content in bivalvia regulated by Badan Standarisasi Nasional (2009), which is 1.00 mg • kg–1 [34]. A. granosa taken from the market of Muara Angke was found to contain cadmium between 0.25 ppm and 0.83 ppm [29]. According to a study about boiled beef liver [33], the use of 10 % kidney tea leaves solution reduced cadmium level from 1.283 ppm to undetected level.

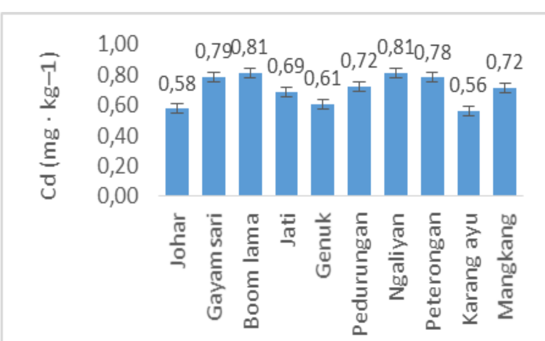


Figure 5 *Anadara granosa's* cadmium (Cd) from 10 traditional markets in Semarang city after purification using *Citrus aurantifolia*

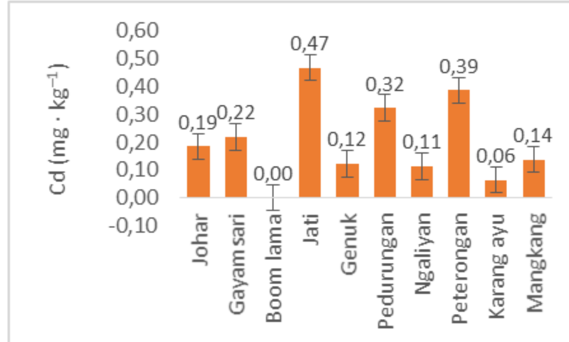


Figure 6 *Anadara granosa's* Cadmium (Cd) from 10 traditional markets in Semarang city after purification using *Orthosiphon aristatus*

4.0 CONCLUSION

High level of lead (Pb), chromium (Cr), and cadmium (Cd) were found in *A. granosa* taken from 10 traditional markets in the city of Semarang. Based on the criteria of food quality and considering of the characteristics of heavy metals that can accumulate in the human body, it could be emphasized that *A. granosa* is unfavorable for consumption in the long–term. This study recommends dissolution using *Citrus aurantifolia* (lime) and *Orthosiphon aristatus* (kidney tea leaves) solution that could be applied on food processing for reducing the level of Pb, Cd, and Cr of *A. granosa*.

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