Assessment of Heavy Metal Lead (Pb) Contents in Canned Crab Products by Atomic Absorption Spectrophotometry (AAS)

M Agustina¹, Mulyono², and W Tjahjaningsih³*

¹Program Study of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Kampus C Jalan Mulyorejo, Surabaya 60115 East Java, Indonesia.
²Center of Product Quality Testing (BPMHP) Semarang, Central Java, Indonesia
³Department of Marine, Faculty of Fisheries and Marine, Universitas Airlangga, Kampus C Jalan Mulyorejo, Surabaya 60115 Jawa Timur, Indonesia.

*Corresponding Author: wahju_fpk@yahoo.com

Abstract. Crab, a fishery product commodity, is very likely to be contaminated by heavy metals from the aquatic environment due to chemical pollution. The analysis of lead-heavy metal content in canned crabs used a method that referred to SNI 2354.5: 2011 starting from sample preparation to reading the sample using the Atomic Absorption Spectrophotometry (AAS) instrument. The purpose of testing with AAS was to determine the levels of heavy metal Pb in samples of canned crab products carried out at BPMHP Semarang. The analysis results show that the Pb content of the sample of canned crab products is below the threshold of less than 0.5 mg/kg (SNI 6929: 2016) so that the product is safe for consumers. The residual content of heavy metals in a processed fishery product must be minimized to ensure the community's continuity of healthy food.

1. Introduction
Canned crab is a commercial product that can help the community's economy through the crab processing industry. According to Sugeng et al. [1] crabs (Portunus pelagicus) are among the most popular fishery commodities in the international market. The protein contained in the high crab is 16.09%, and the fat content is very low, around 0.84% [2]. However, food safety for processed canned crab products must be considered. Food safety is the main thing that determines the quality of the product, as stated in the Law on Food No.5 of 2012. Food safety assurance is essential for consumers' safety from foodborne diseases [3]. Heavy metals, chemical parasites, fungi, viruses, and bacteria are potential causes of foodborne illness [4]. Several studies have proven the presence of heavy metal contaminants in aquaculture products. Cahyani et al. [5] detected the content of heavy metals (Pb, Hg, Cd, and Cu) in the meat of rejung fish (S. sihama) in the Donan River estuary, Cilacap, Central Java which was obtained from August 2015 to January 2016. Azizah et al. [6] detected Pb's heavy metal content in seaweed Sargassum sp. in Jepara waters ranging from 0.22 to 0.79 mg/kg. According to Okparanta and Daminabo [7], heavy metal Pb detected in shrimp samples was 0.74 ± 0.56 mg/kg. Firdaus et al. [8] proved that the depuration process with activated charcoal could reduce heavy metal Pb in shellfish. Saad et al. [9] also revealed heavy metal Pb in samples of imported canned fish products (canned tuna, sardines, and mackerel) from different supermarkets in Menoufia Governorate.
Lead (Pb) is the most common industrial metal that has spread widely in air, water, soil, and food [10]. The non-biodegradable nature of lead causes its prolonged presence in the environment. Lead contamination in soil is a concern because this metal is very toxic to humans and animals. Pb metal enters human or animal metabolism through the food chain [11].

Lead directly affects the hematopoietic system through a variety of key enzymes involved in the heme synthesis pathway. The lifespan of circulating erythrocytes is reduced as the fragility of the cell membrane increases. Chronic and acute lead poisoning causes heart and blood vessel damage with potentially deadly consequences, including hypertension and cardiovascular disease [12]. Lead is one of the most toxic metals because it affects the brain, cardiovascular system, thyroid gland, blood, kidneys, bones, and the reproductive system [13]. Data from the World Health Organization in 2013 recorded that 143,000 people died from lead poisoning and 600,000 cases of intellectual disability in children each year [14].

Prevention is the best way to manage food toxins. Proper hygienic measures for consuming raw food products and canned food and vegetables should be practiced regularly [4]. One of the canned products, such as canned crab products, should be free from contaminants because it has high commercial value; therefore, it is very important to test whether the heavy metal content in the processed product is still within the permissible level and is safe for consumers. To determine the levels of heavy metals in various materials, the Atomic Absorption Spectrophotometry (AAS) method can be used [15, 16]. The AAS method was used in this study to detect the levels of heavy metal lead in crab product samples at the Fishery Product Quality Testing Center (BPMHP) Semarang, Central Java.

2. Materials and methods

2.1. Materials

Based on SNI 2354.5: 2011, the test material for lead content (Pb) consists of several reagents, including 30% H\textsubscript{2}O\textsubscript{2} reagent, 65% HNO\textsubscript{3}, phosphate modifier, 0.1 M HNO\textsubscript{3}, and Pb standard solution. The use of these reagents has their respective functions and different manufacturing methods. The HNO\textsubscript{3} reagent 65% is a reagent that has a role as a destructive solution, while the 30% H\textsubscript{2}O\textsubscript{2} reagent functions as an oxidizing solution to accelerate the oxidation process [17], so that it can decompose the sample perfectly [18]. HNO\textsubscript{3} 0.1 M reagent functions as a blank solution [19]. The phosphate modifier solution functions as a heavy metal binder, and the standard solution of lead (Pb) serves as a positive control in a testing [20].

2.2. Methods

2.2.1. Wet digestion using a microwave

Before sample analysis, destruction is first carried out to remove/ separate the content of other ions so that errors that occur during study can be reduced [16]. Wet digestion uses acidic reagents to decompose the sample and dry digestion using heating or crushing using very high temperatures [21].

Samples of canned crabs were obtained from the Fishery Product Quality Testing Center (BPMHP) Semarang, Central Java. Sample preparation was started by grinding the canned crab meat sample in a blender. An example of 2 grams of delicate canned crab meat was added with 10 ml of 65% HNO\textsubscript{3} solution and 2 ml of 30% H\textsubscript{2}O\textsubscript{2} solution. According to Rusnawati et al. [21], the addition of HNO\textsubscript{3} as an oxidizing agent and is an excellent metal solvent. Pb is oxidized by HNO\textsubscript{3} to dissolve [21] and is assisted by an H\textsubscript{2}O\textsubscript{2} solution to complete the oxidation reaction [18].

The prepared crab meat samples were put in the microwave for the wet digestion process for 45 minutes at a temperature of 200°C. The sample was allowed to stand for 15 minutes, then transferred from the sample bottle into a 100 ml volume centrifuge tube, and then 0.1 M HNO\textsubscript{3} was added to a volume of 50 ml. In this wet digestion process, they use a closed microwave or the so-called microwave digestion method, which aims to correct the shortcomings of closed digestion using microwaves [22]. The sample to be tested were given a concentrated nitric acid solution to increase the temperature and pressure, which will accelerate the decomposition process. This decomposition process has a function to convert heavy metals in the test sample to dissolve [16].
2.2.2 Sample Reading
Sample reading with AAS at BPMHP Semarang, Central Java, refers to SNI 2354.5: 2011. According to Supriyanto et al. [23], the AAS instrument has high sensitivity, fast and easy at low cost. Also, it can determine the element content with a small concentration [23].

Destruction samples, phosphate modifier solution, standard Pb solution, and HNO3 were put into each vial available in the AAS instrument. Standard Pb solutions were prepared for five concentration points ranging from high concentrations of 50 ppb, 40 ppb, 30 ppb, 20 ppb, and 10 ppb. The sample was put into a vial available in the AAS for testing for the heavy metal lead content. The instrument runs automatically according to predefined settings. The heating process occurs in stages with an initial temperature of 200°C, which can evaporate the reagents in the sample. In contrast, at 400°C, the sample absorption process occurs until the ashes of the sample, and the reagents are easy to ionize. At a temperature of 2000°C, the ionization process occurs correctly to facilitate reading the final result. For Pb, the graphite furnace atomic absorption is at a wavelength of 283.3 nm.

The 283.3 nm wavelength for Pb is the most potent wavelength absorbing the line for the electronic transition from the ground level to the excitation level. Lead has an energy of 7.0134.10^(-8) Joule, where this energy will cause Pb atoms in the ground state (Pb^0) to be excited to a higher energy level (Pb^*). In an excited state, the atom is unstable so that it will return to the basic energy level by releasing a certain amount of energy in the form of light [24].

3. Results and discussion
3.1 Result
The test results showed that the canned crab products containing the highest heavy metal of lead were in the sample code 00509, namely 0.102 mg/kg, while the lowest was in the samples code 00112 and 00216, namely 0.010 mg/kg (Table 1).

| Sample Code | Test Date     | Pb Levels (mg/kg) |
|-------------|---------------|-------------------|
| 00111       | 12/26/2019    | 0.097             |
| 00112       | 12/26/2019    | 0.010             |
| 00113       | 12/26/2019    | 0.096             |
| 00210       | 12/30/2019    | 0.075             |
| 00211       | 12/30/2019    | 0.083             |
| 00212       | 12/30/2019    | 0.061             |
| 00215       | 12/30/2019    | 0.011             |
| 00216       | 12/30/2019    | 0.010             |
| 00508       | 01/06/2020    | 0.072             |
| 00509       | 01/06/2020    | 0.102             |
| 00804       | 01/08/2020    | 0.075             |
| 00805       | 01/08/2020    | 0.085             |
| 00901       | 01/16/2020    | 0.090             |
| 00902       | 01/16/2020    | 0.081             |

3.2 Discussion
Detection of Pb heavy metal in canned crab meat using AAS. According to Helaluddin et al. [25], AAS is a quantitative method that can analyze metals for approximately 70 elements. This method measures the concentration of an element by passing a specific wavelength of light emitted by a particular element's source radiation through the sample's atomic cloud. Atoms will absorb light from an energy source known as a hollow cathode lamp (HCL). A reduction in the amount of light intensity reaching the detector is seen as a measure for the concentration of a particular element in the sample.
The levels of lead detected in samples of canned crabs were still below the predetermined threshold for heavy metal levels of lead. The highest data from the test results for lead content is 0.102 mg/kg contained in code 00509. According to the Indonesian National Standard [26], the threshold is set, namely, SNI 6929: 2016, which regulates pasteurized crab meat (Portunus pelagicus) pasteurized in cans, stated that the maximum heavy metal content of lead is 0.5 mg/kg. The limit of Pb in food allowed, according to FAO [27], is 0.5-0.6 mg kg⁻¹.

The heavy metal Pb detected in canned crab meat was related to the crabs' habitat and biological characteristics. According to Edgar [28], a substrate with textured sand or sandy mud in shallow waters to a depth of 50 m is preferred by adult crabs. Meanwhile, mangrove and muddy areas are preferred by young crabs [29].

Blue crabs are an important organism of the estuary food network. Blue crabs included omnivorously related to sediments so that they have the potential to accumulate significant amounts of metal [30].

According to Mitra et al. [31], aquatic organisms accumulate metals to concentrations several times higher than those in water. The main routes of entry of pollutants into fish and shell-fishes are food or non-food particles, gills, oral water consumption, and skin. Oloolade et al. [32] argued that crustaceans, especially crabs, are good bio-indicators to measure contamination in surface sediments. According to Zainuri et al. [33], heavy metals have properties that easily bind and settle on the bottom of the waters and unite with sediments.

Apart from the aquatic environment, Pb metal contaminants detected in canned crab meat can also be obtained from canned packaging, which is widely used for processed products. According to Dewi [24], lead is used as an alloy in the soldering of canned food caps. Lead in canned food is not harmful to humans in small amounts, but if the quantity exceeds the limit, it will cause acute and chronic poisoning. Fong et al. [34] argued that heavy metal contamination in canned food products could occur during the canning process. The selection and preparation of raw materials for processing are the most important things to get canned food products that meet quality standards [9].

4. Conclusion
The content of heavy metal Pb in canned crab samples was below the threshold of less than 0.5 mg/kg (SNI 6929: 2016) so that it was safe for consumers. The residual content of heavy metals in a processed fishery product must be controlled or minimized for public health.

5. References
[1] Sugeng, Sapto P R, Subiyanto and Hadi P 2003 Budidaya Rajungan (Portunus pelagicus) di Tambak (Jepara (ID): BBPBAP) [in Indonesia].
[2] Jacob A M, Nurjahna and Lenni A B L 2012 JPHPI 15(2), 1-8 [in Indonesia].
[3] Rahmawaty L, Rahayu W P and Kusumaningrum H D 2014 Jurnal Standarisasi 16(2), 95-102 [in Indonesia].
[4] Oni V, Oni A and Esume F 2009 Internet J. Nutr. Wellness 10(1), 1-5.
[5] Cahyanii, N, Batu D T F L and Sulistiono 2016 JPHPI 19(3), 267-276 [in Indonesia].
[6] Azizah R, Malau R, Susanto A B, Santosa G W, Hartati R, Irwani and Suryono 2018 Jurnal Kelautan Tropis 21(2), 155-166 [in Indonesia].
[7] Okparanta S and Daminabo V 2018 Am. J. Environ. Sci. 2(4), 49-55.
[8] Firdaus A R, Mubarak A S and Tjahjaningsih W 2020 IOP Conf. Series: Earth and Environmental Science 441, 1-8.
[9] Saad M S, Hassanian F S and Eldin S S 2014 BMJ 26(2), 119-125.
[10] Raikwar M K, Kumar P, Singh M and Singh A 2008 Vet. World 1(1), 28-30.
[11] Rahman S H, Khanam D, Adyel T M, Islam M S, Ahsan M A and Akbor M A 2012 Appl. Sci. 2(3), 584-601.
[12] Flora G, Gupta D and Tiwari A 2012 Interdiscip. Toxicol. 5(2), 47-58.
[13] Homady M, Hussein H, Jeries A, Mahasneh A, Al-Nasir F and Khleifat K 2002 Environ. Res. 89(1), 43-49.
[14] Rajeswari T R and Sailaja N 2014 JCHPS (Special Issue) 3, 175-181.
[15] Raimon 1993 Lokakarya Nasional Yogyakarta: Jaringan Kerjasama Kimia Analitik Indonesia, pp 5-27. [in Indonesia].
[16] Kristianingrum S 2012 Prosiding Seminar Nasional Penelitian, Pendidikan, dan Penerapan MIPA. Universitas Negeri Yogyakarta, pp 195-201. [in Indonesia].
[17] Ratnawati N A, Prasetya A T and Rahayu E F 2019 Indones. J. Chem. Sci. 8(1), 60-68 [in Indonesia].
[18] Yawar W, Naeem K, Akhter P, Rekana I and Saeed M 2009 J. Saudi Chem. Soc. 1(4), 125-129.
[19] Naschan M, Prasetya A T, and Sumarni W 2017 Indones. J. Chem. Sci. 6(1), 1-8.
[20] Mayaserli D P, Renowati and Biomed M 2017 Sainstek 9(1), 19-25 [in Indonesia].
[21] Rusnawati, Yusuf B and Alimuddin 2018 Prosiding Seminar Nasional Kimia. Universitas Mulawarman, pp 73-76 [in Indonesia].
[22] Taufiq M, Sabarudin A and Mulyasuryani A 2016 Alchemy: Journal of Chemistry 5(2), 31-37 [in Indonesia].
[23] Supriyanto C, Samin and Kamal Z 2007 Seminar Nasional III SDM Teknologi Nuklir Yogyakarta, pp 21-22 [in Indonesia].
[24] Dewi D C 2012 Alchemy: Journal of Chemistry 2(1), 12-25 [in Indonesia].
[25] Helaluddin A B M, Khalid R S, Alaama M and Abbas S A 2016 Trop J Pharm Res. 15(2), 427-434.
[26] Badan Standarisasi Nasional 2016 SNI 6929:2016 tentang Daging Rajungan (Portunus pelagicus) Pasteurisasi dalam Kaleng. (Jakarta: Badan Standarisasi Nasional) pp 1-12 [in Indonesia].
[27] Simanjuntak C P H, Djumanto, Rahardjo M F and Zahid A 2012 Proceedings of the International Seminar (Industrialization of Fisheries and Marine Resources) Universitas Riau, pp 178-196.
[28] Edgar G J 1990 J. Exp. Mar. Bio. Ecol. 139, 23-32.
[29] Smith H 1982 Safic. 6(5), 6-9.
[30] Brouwer M and Lee R F 2007 Responses to toxic chemicals at the molecular, cellular, tissue, and organismal level. In The Blue Crab: Callinectes sapidus. (College Park, MD: Maryland Sea Grant) pp 405-432. gcr.usm.edu/toxicogenomics/docs/BlueCrabChapter11.pdf
[31] Mitra A, Barua P, Zaman S and Banerjee K 2012 Turk J Fish Aquat Sc 12, 53-66.
[32] Ololade I A, Lajide L, Olumekun V O, Ololade O O and Ejelonu B C 2011 J. Environ. Sci. Health Part A 46, 898-908.
[33] Zainuri M, Sudrajat S and Siboro E S 2011 J. Kelaut. 4(2) [in Indonesia].
[34] Fong S S, KanaKaraju D A P and Ling S C 2006 MJChem. 8(1), 10-15.

6. Acknowledgments
The authors would like to thank the Center of Product Quality Testing (BPMHP) Semarang, Central Java for the facility.