Farming and food safety analysis of blood cockles (Anadara granosa) from Rokan Hilir, Riau, Indonesia

by Jusup Suprijanto

Farming and food safety analysis of blood cockles (*Anadara granosa*) from Rokan Hilir, Riau, Indonesia

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Abstract. Blood cockles (*Anadara granosa*) have been cultivated and became an export commodity from Panipahan, Rokan Hilir, Riau. The study aimed to determine the condition of the bivalve farming, the water quality of the surrounding area and the food safety aspects of the commodity. Samples were taken by purposive sampling method and selected based on the intertidal zone and on the different human activities around the area. Samplings were carried out 4 times during 5 months of observation in 2 intertidal zones, namely the lower and upper intertidal zones. The farming was started in 2019, using a semi-intensive cultivation system, and in 2018 a production of 6,492.47 tons (49.67% of the total aquaculture production in Rokan Hilir Regency) was recorded. Water quality was classified as good for the cockles' culture and the microbiological analysis of water and the cockles flesh showed that the bacterial densities of *Escherichia coli* and coliforms were still below the thresholds, while the presence of *Salmonella* was negative. Heavy metals were also found in the *A. granosa* flesh: Pb (0.118-0.138 mg L^{-1}), Cd (0.074-0.077 mg L^{-1}) and Hg (0.140-0.163 mg L^{-1}). However, the contamination levels were still far below the thresholds, demonstrating the *A. granosa* suitability for human consumption.

Key Words: bivalve, cockle farming, coliform, heavy metals, mercury.

Introduction. Blood cockles (*Anadara granosa*) are a potential nutritious food with high economic value (Triatmaja et al 2019), containing protein (9-13%), fat (0-2%), glycogen (1-7%), omega-3, vitamin A, vitamin B12 and vitamin C. The bivalve also contains antioxidant minerals such as Zn, Fe, Se and Cu. These invertebrates are intensively captured by fishermen, due to an increasing demand (Dewi et al 2019). *A. granosa* can also be used as bioindicator of sea water pollution because they are non-selective filter feeders and bioaccumulators (Halit et al 2017), storing heavy metals in their bodies, if they live in contaminated waters.

One of the potential areas for the cultivation of *A. granosa* in Riau Province is the Rokan Hilir Regency. Some people have even named this area „the cockles barn of Sumatera". The coastal area of the regency has a coastline length of 263.56 km and has a potential of 6,500 hectares for *A. granosa* cultivation (Research and Development Office of Riau Province 2018). Pasir Limau Kapas District is the largest *A. granosa* producing area in Rokan Hilir Regency, apart from Bangko and Sinaboi Districts (Fisheries Office 2019).

Food safety is the most important aspect in a food industry. The production process and the food products produced must be safe for consumers, workers, and the surrounding community, and also for the environment in which the industry is carried out. Some aspects of food safety have been extensively studied, such as the food hygiene or the microbiological and chemical aspects of food (Norhana et al 2016; Rahayu 2016). WHO and FAO have regulated the food safety aspects, related to the total number of microbial populations in food and to the presence of several species of pathogenic bacteria. From a chemical perspective, food safety is dealing with the presence of several toxic compounds in the food (Velez 2016; Puspitasari 2020). This study aimed to describe the activity of *A. granosa* farming and to analyze the food safety aspects, based on microbiological and heavy metal analysis, in Panipahan waters, Limau Kapas District, Rokan Hilir, Riau, Indonesia.

Material and Method

Research method. This survey research was carried out from August to December 2019 by sampling from Panipahan waters. The samples were collected through purposive sampling techniques and the areas were plotted by intertidal zone and different human activities around the *A. granosa* farming. Samples were taken once a month from the lower, middle and upper intertidal zones, during the 5 months of observation of the population and distribution patterns of the animals. A number of 3 sampling points were selected for every zone. All data were collected and analyzed descriptively.

Blood cockles farming. The used cultivation and the socio-economic aspects of *A. granosa* farming were studied by observing directly the cultivation activities in the field. Among the observed and measured variables, there were: the ponds' length, width and construction, the equipment used and the seedlings. Then the observations were complemented by interviews with employers, workers, government officials, surrounding community leaders and other stakeholders. In addition, the data obtained was combined with secondary data available in the local government offices.

Measurement of water quality. Water quality sampling was carried out in *A. granosa* cultivation areas, in Panipahan waters. Temperature, pH, dissolved oxygen, salinity, alkalinity and turbidity were examined by referring to APHA (2008) and FDA (2020).

Microbiological analysis. Microbiological analysis of sea water and *A. granosa* flesh referred to the Indonesian National Standard Agency methodology, based on APHA (2008), FDA (2020) and Froese (2016). The parameters analyzed were the total number of bacterial cells (TPC; total plate count) at 22^{o}C and 37^{o}C through the total plate count method, the presence of *Escherichia coli*, coliforms and of *Salmonella* bacteria. These analyzes were carried out at the Laboratory of the Fisheries Product Quality Testing Center of the Marine and Fisheries Service of Central Java Province, Semarang, Indonesia.

Heavy metal analysis. The heavy metals determination in the sea water and *A. granosa* flesh referred to the analysis method issued by the Indonesian National Standard Agency, based on APHA (2008) and FDA (2020), and included analysis of levels of lead (Pb), cadmium (Cd) and mercury (Hg).

Results and Discussion

A. granosa are a determinant of the Rokan Hilir Regency people's lifestyle. Apart from being a source of protein for the community's nutrition, the bivalve is a source of livelihood, both as a catch from nature and cultivation production.

A. granosa farming. *A. granosa* farming has a strategic role in the production of aquaculture commodities in Rokan Hilir Regency. In 2018, *A. granosa* represented 6,492.47 tons (49.67%) out of a total of 13,072.21 tons of aquaculture production. The community used to cultivate *A. granosa* since long time ago. However, only in 2010 blood cockles began to be cultivated on a commercial scale. At that time, the community preferred to collect *A. granosa* seeds and then sell them to Malaysia. Some people used to catch *A. granosa* at their consumption sizes and to sell them to meet the needs of the local market and several cities in Riau Province (Yulinda et al 2020).

Catching natural seeds and *A. granosa* for consumption by the community made it difficult for the collectors to obtain consumption-size. Around 2010, the community tried to cultivate *A. granosa* and it turned out that the production was very profitable. Since then, they have been interested in developing *A. granosa* cultivation, considering that *A. granosa* are very suitable for cultivation in waters that have a muddy and sloping substrate base (Chalermwat et al 2003; Saffian et al 2020). Coastal conditions that have such characteristics were found along the coast of Rokan Hilir Regency.

Today, cultivation of *A. granosa* is scattered along the coast of Rokan Hilir Regency, in several districts: Pasir Limau Kapas, Kubu, Kubu Babussalam, Bangko and Sinaboi. The Pasir Limau Kapas District has the largest *A. granosa* farming pond compared to other sub-districts. In 2019, there were 208 *A. granosa* farmers in the Pasir Limau Kapas District, in Panipahan Village (128 farmer), Panipahan Laut Village (33 farmer) and Teluk Pulai Village (47 farmer), implementing a semi-intensive cockle culture (Yulinda et al 2020). In coastal areas that have a muddy and sloping bottom substrate, ponds were made with varying sizes, according to the ability of the pond owner, starting from 3 x 4 m up to 200 x 200 m. To separate between one pond plot and another, a guardrail was made in the form of poles attached with a net surrounding the pond plot. The net height was approximately 0.5 m and the bottom part of the net was buried into the bottom mud of the pond. Besides delimiting the pond ownership, the most important thing was to prevent cultivated *A. granosa* to leave the ponds.

A. granosa seeds with an initial stocking density of about 2,000 individuals m^2 and then spacing up to 200–300 seeds m^2 after 1-2 months period. Harvesting was carried out after 4-5 months of cultivation period and ends around the 9^{th} month. The ideal time to collect the cockles is when the water level is about 0.5 m from the bottom of the pond, so the harvesting process is easier to do by dredging the pond's bottom using a rake. To protect the pond, the owner makes a guard house on top of his pond. A similar technology has also been applied in several countries, for example in Malaysia, Thailand and Vietnam (Saffian et al 2020; Chalermwat et al 2003).

The seeds for *A. granosa* cultivation still depended on seeds from nature. Natural seeds were obtained from the waters along the coast around the *A. granosa* cultivation area. Usually people collect the seeds from February to March. The size of the caught seeds varied from the finer size of sugar to the size of a peanut. *A. granosa* seeds were also purchased from fishermen who collect seeds at various prices. Harvested *A. granosa* were then weighed and sold to collector traders for further sale on the local, regional and export markets. The size varied from 1.0-1.5 cm to >4 cm. Access to the production sites is a serious challenge for the *A. granosa* cultivation in this area. Until now, sea transportation was the only access. This can result in uncompetitive commodity prices, accumulation of production and uncertain price fluctuations, ultimately causing losses for *A. granosa* cultivators (Saffian et al 2020; Taylor et al 2008).

Water quality. The water quality analysis revealed normal conditions for *A. granosa* cultivation, related to the water quality standards in Indonesia: a salinity around 34 ppt, a temperature of 28-29°C, a turbidity of 50-55 cm, a pH of 8, a dissolved oxygen of 4-6 mg L^{-1} and an alkalinity of 120-125 mg L^{-1}. It was noticed that all water quality parameters support the growth and survival of *A. granosa* (Table 1).

Table 1

Average water quality in Panipahan waters

Parameter	*Unit*	*Results*	*Indonesian standard (RMMAF 2016)*
Salinity	ppt	34.0	10-35
Temperature	°C	28.7-29.0	28-30
Turbidity	cm	50.0–55.0	60
pH	-	8.0	7.5-8.5
Dissolved oxygen	mg L^{-1}	4.4-6.1	>3.0
Alkalinity	mg L^{-1}	120-125	80-150

Referring to several existing water quality standards (Pahri et al 2016; Srisunont et al 2020), it can be said that the waters of Panipahan were still suitable for the cultivation of *A. granosa*.

Microbiological analysis. Microbiological analysis aimed to determine the safety aspect of this commodity for human consumption in accordance with the national and international health standards. The Total Plate Count (TPC) test results on the sea water and *A. granosa* flesh showed safe concentrations, ranging from 1,280 to 9,030 cells mL^{-1} and between 2,600 and 4,680 cells g^{-1}, respectively, far under the thresholds (Table 2 and Table 3). TPC can be used as an indicator of the product's hygiene level and environmental conditions. Several bacteria species can be used as an indicator of the hygiene surveillance process and consumption microbiological quality criteria (APHA 2008).

Table 2

Results of microbiological analysis of water samples in the Panipahan waters

Analysis	*Unit*	*Result*	*Quality standards*
Total plate count at 22°C	cell g^{-1}	1,280-5,800	100
Total plate count at 37°C	cell g^{-1}	1,300-9,030	20
Escherichia coli	cell 100 mL^{-1}	0	0
Coliform	cell 100 mL^{-1}	0	0
Salmonella	cell 100 mL^{-1}	Negative	Negative

Table 3

Results of microbiological analysis of *Anadara granosa* flesh in Panipahan waters

Analysis	*Unit*	*Result*	*Quality standards*
Total plate count	cell g^{-1}	2,600-4,680	500,000
Escherichia coli	cell g^{-1}	1.8-3.0	<3
Coliform	cell g^{-1}	0	<3
Salmonella	cell 25 g^{-1}	Negative	Negative

E. coli is a pathogenic bacteria that often causes food poisoning, being used as an indicator of sanitation. *E. coli* infection can cause diseases such as cholera and dysentery in children and adults. Their presence in food may indicate poor environmental sanitation practices. In this study, *E. coli* cells were found only in *A. granosa* flesh, at a density of 1.8-3.0 cells g^{-1}, below the threshold of 3 cells g^{-1}, issued by the Indonesian government regulations. Since bacteria are a part of the microbiome of warm-blooded animals, their presence in a food ingredient is always associated with the animal feces (Zarkasi et al 2018).

Coliform bacteria are defined as rod shaped Gram-negative, non-spore forming and motile or non-motile bacteria, which can ferment lactose with the production of acid and gas, when incubated at 35–37°C. The typical genera are: *Citrobacter*, *Enterobacter*, *Hafnia*, *Klebsiella* and *Escherichia*. Coliforms can be found in the aquatic environment, in soil and on vegetation; they are universally present in large concentrations in the feces of warm-blooded animals. While coliforms themselves are not themselves a cause of serious illness, their presence indicates that other pathogenic organisms of fecal origin may be present.

Salmonella, Vibrio and *Clostridium* are genera of pathogenic bacteria that can contaminate fishery products. These various species of bacteria can break down the nutritional components of fish into foul smelling compounds such as indole acid, H_2S and mercaptans which are harmful to humans if consumed. The presence of coliform bacteria is an indicator that the food is contaminated by organic waste, especially feces (Odonkor & Ampofo 2013). In this study, the results of the analysis of Coliform bacteria in sea water samples and in *A. granosa* flesh were negative. In other words, *A. granosa* from the Panipahan waters were still safe for human consumption.

Salmonella sp. can be found in raw food materials such as fish, shellfish, squid and other fishery commodities. Fish can be contaminated by people, pets, flies or through cross-contamination due to poor handling practices (Desta et al 2014). The presence of *Salmonella* sp. in fish and fishery products are generally in low density, below the diarrhea-causing threshold. However, this small amount of contamination still poses a considerable public health risk. The dangers of *Salmonella* will be higher in foods that are consumed fresh. Symptoms of poisoning in most infected people are diarrhea, stomach cramps, fever, headache, nausea, and vomiting (Černá et al 1991). In this study, the tests conducted for *A. granosa* samples revealed no *Salmonella* contamination.

Analysis of heavy metal. As an export commodity from Rokan Hilir Regency, *A. granosa* must have a heavy metal analysis certificate. Based on the measurement results in this study, heavy metal traces of Pb, Cd and Hg have been found both in water and in *A. granosa* flesh (Table 4 and Table 5).

Table 4

Results of analysis of heavy metal content in Panipahan waters

Heavy metal	*Heavy metal concentration (mg kg^{-1})*
Lead (Pb)	0.065-0.078
Cadmium (Cd)	0.036-0.050
Mercury (Hg)	0.069-0.082

Table 5

Results of analysis of heavy metal content in *Anadara granosa* flesh in Panipahan

Heavy metal	*Heavy metal concentration (mg kg^{-1})*	*Permitted maximum concentration (mg kg^{-1})*
Lead (Pb)	0.118-0.138	1.5
Cadmium (Cd)	0.074-0.077	1.0
Mercury (Hg)	0.140-0.163	1.0

Heavy metal pollution is the introduction of these substances into the environment, which cannot be neutralized by the environment. Most of heavy metal comes from industrial and mining processes, however, heavy metal pollution of natural origin can also occur. For example metals released from volcanic chemical processes, metals transported by fish and from coastal abrasion. The heavy metals include: Al, Hg, Pb, Zn, Cr, Cu, Cd, Co, some of which have a negative impact on the human body, for example the emergence of several dangerous diseases, with an increased dangerosity when found in the water or food (Govind & Madhuri 2014).

In this study, the presence of heavy metals both in the water and in *A. granosa* flesh was not surprising. Several large and small rivers flow into Panipahan waters, located on the East Coast of Sumatra. These waters are relatively open and are part of the Melaka Strait, which is one of the busiest strait in the world. The flow of the Melaka Strait will pass through this place both from the North, West and South of the Melaka Strait. In addition, the east coast of Sumatra Island is a densely populated area. Some cities are located on the lips of the Melaka Strait, either from Indonesia, Malaysia or Singapore. This configuration determines the household waste, mainly from coastal residents, discharge and collection into the strait waters (Mukhtar et al 2020).

Lead (Pb) is widely used in the non-food industry, being extremely poisonous to the biota. Pb is a soft metal of a dark brown color and is easily refined from mining. Pb metal is widely used in the battery, cable, paint (as a coloring agent), gilding, pesticides industry and an antiknock agent in gasoline. Pb is also used as a constituent of solder and as a pipe connector formulation which results in household water having many possible contacts with Pb (Yap et al 2003). As a metal pollutant, the danger posed by the use of Pb is that it often causes poisoning. Pb metal is not required by the human body, so when food is contaminated by this metal, the body will partially excrete it. The rest will accumulate in certain body parts such as the kidneys, liver, nails, fat tissue and hair. Pb metal can enter the body through respiration, food and drink (Puspitasari et al 2020).

In the present study, the concentration of Pb in the sea water and in *A. granosa* was 0.065-0.078 mg kg^{-1} and 0.118-0.138 mg kg^{-1}, respectively. These figures were not very different from those reported in a study carried out by Halit et al (2017) along the estuary of Sungai Tampok and Sungai Sanglang, Malaysia. This amount is still below the thresholds set by the Indonesian Government (1.5 mg kg^{-1}) and also by the Food and Drug Administration of the United States (1.0 mg kg^{-1}), the European Union (1.5 mg kg^{-1}) and Singapore (2 mg kg^{-1}). From these figures, it can be seen that the contaminant levels in the flesh of *A. granosa* were still far below the maximum thresholds.

Cadmium (Cd) is another heavy metal that can pollute seawater and the environment. Cd enters the marine environment as a result of mining processes, industrial waste and metal welding. Consumption of Cd polluted water will have fatal consequences to the body, for example high blood pressure, kidney and testicular tissue damage, and red blood cell damage. A well-known case of Cd poisoning is the emergence of itai-itai disease in Japan which characterized by bone pain and bone loss.

According to the agricultural and livestock WHO and FAO standards (JFAO, WHO-FSP 2011), the maximum Cd level in waters should not exceed 1.0 mg L^{-1}. The maximum level of Cd concentration in water and fishery products, according to the 2020 FDA regulations applicable in the United States, is 3-4 ppm or 0.5-1.0 mg kg^{-1} wet weight, respectively. In this study, the concentration of Cd found in sea water and shellfish was of 0.036-0.050 mg kg^{-1} and 0.074-0.077 mg kg^{-1}, respectively. These figures were not very different from those reported (ESFA 2012) along the estuary of Sungai Tampok and Sungai Sanglang, Malaysia. Similar results were also reported (Yap et al 2003) in a research on heavy metal (Cd, Cu, Pb and Zn) concentrations in the green-lipped mussel *Perna viridis* (Linnaeus) collected from some wild and aquaculture sites in the west coast of Peninsular Malaysia. This figure was still below the thresholds set by the governments of Indonesia (1.0 mg kg^{-1}), Australia, New Zealand, Hong Kong (2.0 mg kg^{-1}), the European Union and Singapore (1.0 mg kg^{-1}).

Mercury (Hg) is the only metal that is liquid at room temperature. Elemental and methyl mercury are toxic to the central and peripheral nervous system. Especially in the short-chain form of alkyl, methyl mercury can cause neuronal degeneration in the brain and result in numbness in the tips of the hands or feet, ataxia (irregular movement), joint pain, deafness and narrowing of visibility. Inhalation of mercury vapors can produce detrimental effects on the nerves, digestive and immune systems, as well as in the lungs and kidneys, which may be fatal. Adverse effects occurring in the kidneys range from increased protein in the urine to kidney failure (Mercola & Klinghardt 2001). Mercury is often absorbed through the digestive tract, lungs and skin. Short-term exposure to high levels of inorganic mercury can cause kidney failure. While long-term exposure at low doses can cause proteinuria, nephrotic syndrome and nephropathy associated with immune system disorders (Krisnayanti et al 2012).

In this study, Hg concentrations found in sea water and shellfish flesh were 0.069-0.082 mg kg^{-1} and 0.140-0.163 mg kg^{-1}, respectively. These figures were not very different from those reported by other researchers (Rahayu et al 2016) in *A. granosa*, in Western Lombok, Indonesia, ranging from 0.020 to 0.070 mg kg^{-1}. This data shows that the metal pollution level in Panipapahn waters was still below the threshold set by the Indonesian government (1.0 mg kg^{-1}), the Food and Drug administration of the United States, Australia, Spain and the Netherlands (1.0 mg kg^{-1}), Italy (0.75 mg kg^{-1}) and Japan (0.4 mg kg^{-1}). In other words, this commodity is still safe for human consumption.

Conclusions. The *A. granosa* farming in Penipahan waters, Limau Kapas District, Rokan Hilir Regency was started in 2010 and was still developing at the time this research was conducted. It was determined that the water quality met the standards for *A. granosa* farming. Microbiologically, the bivalves were still hygienic for human consumption. Heavy metal traces were found in the *A. granosa* flesh, namely Pb (0.118-0.138 mg L^{-1}), Cd (0.074-0.077 mg L^{-1}) and Hg (0.140-0.163 mg L^{-1}). However the levels were still far below the permitted maximum levels set by the government, therefore the current study concluded that the *A. granosa* flesh was still suitable for human consumption.

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Conflict of interest. The authors declare no conflict of interest.

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PRIMARY SOURCES

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2	Ahmad Luqman Halit, Shamila Azman, Mohd Ismid Mohd Said, Noraliani Alias, Nazri Ali. "Cadmium and Chromium Accumulation in Cockles along the Estuary of Sungai Tampok and Sungai Sanglang", Journal of Physics: Conference Series, 2018 Publication	1%
3	Norhan E. Saleh, Fady R. Michael, Mohamed M. Toutou. "Evaluation of garlic and onion powder as phyto-additives in the diet of sea bass (Dicentrarcus labrax)", The Egyptian Journal of Aquatic Research, 2015 Publication	1%

4	Xiangqin Wang, Du Yanhong, Fangbai Li, Liping Fang, Tingting Pang, Weijian Wu, Chuanping Liu, Lei Chen. "Unique Feature of Fe-OM Complexes for Limiting Cd Accumulation in Grains by Target-regulating Gene Expression in Rice Tissues", Journal of Hazardous Materials, 2021 Publication	1 %
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7	"Arsenic in Drinking Water and Food", Springer Science and Business Media LLC, 2020 Publication	1 %
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10	A Wahyudi, W Surono, I Rodliyah, H E Mamby. "Characterization of amalgamation tailings from ASGM in Sekotong area, West Nusa Tenggara, Indonesia, and its potential added value", IOP Conference Series: Earth and Environmental Science, 2021 Publication	<1%
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12	I Effendi, F Feliatra, T Emrinelson, I A Siregar, A Adelina. "Effect of heterotrophic bacteria on the growth of tilapia (Oreochromis niloticus) cultivated in brackish water", IOP Conference Series: Earth and Environmental Science, 2021 Publication	<1%
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