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Assessing the impacts of catchment land-uses and coastal urban developments on heavy metals and microplastics in commercial mangrove crabs as measures for food safety

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1. Introduction
1.1. Background

Land-use changes are inevitable result of human nature. The great changes in land use had occurred in early agricultural era (Ruddiman, 2013). Rapid increase in food production in the farming age resulted in human population growth to 250 million from 6 million between 10,000 years BP and 1,000 BP (Stipp, 2011). This made a new land demand for agriculture and settlements, causing further forest conversion. Mechanisation in farming practices in the industrial era continued cutting forest for farming (Ruddiman, 2013). Moreover, this era enhanced the forest cover changes into various land uses or covers, including farms, plantations, pastorals, dams, open mining pits, mining tiling ponds, rural and urban settlements, road networks, and urbanized area.

Land use changes have transformed the earth system, mostly terrestrial and this change then affected on coastal and marine system. Large deforestation for agriculture when human left hunting and gathering era has been hypothesised to increase the greenhouse gases (GHGs)--CO_2 and CH_4 (Ruddiman, 2013). Along with fossil fuel burning, forest clearing for farming and urban expansion in the industrial age is clearly documented causing GHGs increases, as mentioned in numerous scientific papers and popular articles. The GHGs is very well known as a cause of climate change. Land-use changes have reduced soil quality and health; increased pesticide, veterinary medicine, and antibiotic residue; caused soil acidification, salinisation and degradation; and caused carbon loss and water scarcity (Smith et al., 2016). Land-use changes and its human activities have also impacted terrestrial water; through surface and ground water movement all wastes and pollutants enters water bodies. It includes fertilizers, pesticides, agrochemicals, domestic and municipal wastes, sewage sludges, oils, organic compounds, heavy metals, trace elements, and plastics (Islam and Tanaka, 2004)

Once wastes and pollutants enter the water system, they will end up in coastal and ocean. The pollutants have contaminated sediments and waters including biota and it affected the structure and function of marine communities (Islam and Tanaka 2004). Since related to public health, the food safety of fisheries products is of the major concerns on the coastal and marine pollutions, which heavy metals and pesticides residues are among notified hazard category (Piglowski, 2017). Also, microplastic contamination in seafood is another emerging issue in food safety (Barboza et al. 2018).

Being situated between land and ocean, mangrove ecosystems also experience pollution exposure, being a possible hotspot for the accumulation of pollutants and microplastics. Mangrove ecosystems with the complex structure of its roots perfectly trap marine plastic debris; and promote sedimentation and heavy metals depositions. Microplastic deposits in mangrove sediments have been reported (Li et al., 2018; Garces-ordonez et al. 2019; Naji et al., 2019), including in Indonesia (Cordova et al., 2021), where the mangrove forests are located close in proximity to the urbanized areas. There is also a strong and positive correlation between heavy metal concentration in mangrove sediments with the size of the surrounding cities (Branoff, 2017).

Heavy metal and microplastic depositions in mangrove ecosystems could contaminate mangrove- based fisheries products through direct exposures or bioaccumulation and bio-magnificent. Collected from mangrove areas, *Scylla* spp. could be a good example to demonstrate food safety risks, because the crabs are an important export commodity, heavily relying on the wild catches (Hungria et al. 2017). Yogeshwaran et al. (2020) reported that *Scylla serrata* crabs from polluted mangrove areas have high concentration in heavy metals. A further 41 peer reviewed papers, indexed by Scopus, published since 1975 indicated heavy metal accumulations in *Scylla* spp. Microplastic particles have also been recorded found in the digestive system of *Scylla* spp. (Adidharma, 2019). This is strong indication that *Scylla* spp. are possible to accumulate microplastics from its preys, for example mangrove snails (Fitri and Patria, 2019; Li et al., 2020).

Being the second largest exporter of crabs, including *Scylla* spp., Indonesia should consider early on the food safety of this product. This is important to be anticipated if the importers may change their regulations on the quality of crabs, otherwise we would lose the market. Therefore, we proposed our research agenda regarding food safety on fisheries products, land use analysis and environmental management. This may contribute to provide basis data for food safety decision making and land use management to control coastal and marine pollution and plastic debris. In this study, part of our agenda will be proposed in this research project.

1.2. Objectives

This study will investigate heavy metals and microplastic content in *Scylla* spp. The samples will be collected from all around Indonesia at least 15 locations. Then, spatial analysis of land-uses where *Scylla* spp. are collected will be explored to examine its effect on the heavy metal and microplastic accumulation. Together with this research, we will establish a monitoring site in one of the locations and this will be part of monitoring networks.

1.3. Expected Output

1. Publication-1: “urbanized coastal areas surrounding mangrove forests increase food safety of *Scylla* spp.”, will be submitted in *Marine Pollution Bulletin*
2. Publication-2: “Risk assessment of heavy metal accumulation in *Scylla* spp. will be submitted in *Chemistry.*
3. Long term monitoring sites.

2. State of the art of the research

To my knowledge, there is limited studies on heavy metal concentrations in *Scylla* spp. and only one has been reported in a Ph.D thesis regarding microplastic content in *Scylla* spp. (Adidharma, 2019). By using keywords (“Scylla” AND “heavy metal”), Scopus database displays less than 50 peer-reviewed papers. These papers represent the current state of the investigations of the topic in the world. Further search of literatures published in Indonesian is conducted through Google Scholar using keywords (“Scylla” AND “logam berat”). The results showed less than 30 papers.

Regarding to the topic of heavy metal, most papers in the literature investigated the concentration of heavy metal species. A few papers addressed the toxicity of heavy metals to *Scylla* spp. (Zhu et al., 2018; Vasanthi et al. 2014; Zhang et al., 2010; Chen et al., 2000; Balaji et al., 1989). There is an old paper published in 1978 but showing invaluable information about metal binding protein (Olafson et al., 1978). *Scylla serrata* were commonly investigated to study heavy metal accumulation in the crabs of *Scylla* spp. but *Scylla olivaceae and Scylla* *paramamosain* are the other two species *Scylla* sp. regarding heavy metal investigations (Jiang et al., 2014; Zhu et al., 2018; Saher and Kanwal, 2019; Ateshan et al., 2018; Yusni and Melati, 2020). The heavy metal and trace element concentrations assessed in *Scylla* spp. meats include Cu, Zn, Pb, Cd, Cr, As, Hg, Mn, Fe, Ni, Sn, Co, and Se. The concentration of these metals varies greatly from paper to paper. For example, Harris et al. (2018) and Ateshan et al. (2018) reported that Cd was under the limit detection, but Borrel et al. (2016) and Jiang et al. (2014) found high accumulation of Cd in *Scylla* spp. tissues. Pb is another heavy metal that reported higher than the permissible concentrations based on the available standard (Batvari et al., 2013; Phuong and Khoa, 2013; and Purnamasari et al., 2014).

One of the key papers in this research project is that has been written by Gilby et al. (2020). This paper concluded that high coastal urbanisation did not always contaminate the fisheries products with harmful levels of heavy metals. They explained that better waste and water sewage management in the study locations may reduce coastal water pollutions. Nevertheless, this paper gives an idea for investigation the same issue in Indonesia, where there are lack water treatments and municipal and industrial sewage. The results of the investigation would be different. Moreover, very different stage of development among cities in Indonesia could give an opportunity to test the effect of the catchment land uses on the heavy metal accumulation in *Scylla* spp. The same story could be found in microplastic accumulation which has been detected in mangrove sediments in Muara Angke, Jakarta. This is the place where waste and pollutants from Jakarta and the hinterland cities accumulate in the estuarine, becoming a top benchmark for the most polluted mangrove area in Indonesia (Cordova et al., 2020).

3. Methods

This research was undertaken into six steps, including: 1) establishment of monitoring site, 2) sample collections, 3) heavy metal measurements, 4) microplastic determination, 5) spatial analyses of land-uses, and 6) statistical analysis and paper writing.

3.1. Monitoring site establishment

Monitoring site was established in the Banten Province and Segara Anakan-Central Java, where *Scylla* spp. are collected (Figure 1). In this site, we set up permanent plots in the middle of mangrove forests. In the plots, dendrometer bands were installed permanently on the tree stems and its stem diameter was measured regularly, including heavy metals and microplastics.

3.2. Sample collections

The crab samples were collected from 10-15 local markets around Indonesia (Figure 1.). We have the contact number of local people who can assist to purchase the crabs from the local markets. We purchased 4-5 crabs from each market regardless the species, but it must be *Scylla* spp. Standardisation of species and size is the best choice, but it may not be practical. Therefore, we just requested to buy 4 kg or around 4-5 crabs. The life crabs were sent to Jakarta with special handling by courier services. Once it arrives at Jakarta, all the samples were labelled and then stored in a freezer until further analysis. The label contains the following information: arrival date, location, sample number, species name, weight, and carapace width and length.

Figure 1. Locations for sample collections and monitoring site.

3.3. Heavy metal measurements

Heavy metal measurements were undertaken using ICP-MS 7400 Thermo. This instrument could detect various heavy metal species in single run. The instrument requires analytical-reagents grade and Double Distilled Deionized Water (DDDW) for the preparation of reagent solutions. The reagents include Nitric acid (65% HNO3, Merck), HCl 37% (Merck), multi element standard solution 100 mg/L (As, Be, Cd, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Mo, Ni, Se, Sr, Sb, Tl, Ti, V, Zn from Merck), and Single element standard solution 1.000 mg/L (Na and P, Merck).

We use a modified method from USEPA 3051a for total metals analysis, using a mixture of concentrated HNO_3 and HCl and a microwave oven. Firstly, acid mixture (9 mL HNO_3 and 3 mL HCl) was drop-added into 0.2 g of samples. The mixture was heated using microwave oven CEM MARS 5 Express at 185 °C for 15 min and hold for 30 min. Then, the solution was filtered using Whatman filter paper No. 41, and the filtrate was diluted into 25 mL using DDDW. Two replicates of dried samples were also applied for measurements. The samples are ready to be measured using ICP-MS 7400 Thermo. This measurement will be conducted in Food Safety Laboratory, Research Division for Natural Product Technology, Indonesian Institute of Sciences.

3.4. Microplastic determinations

Microplastic determinations on crabs were carried out following a method documented in Dehaut et al. (2016). First, sample was rinsed with Milli-Q water several times and covered to prevent any airborne contamination. The gastrointestinal tracts and gills of crabs were extracted from the biota. The microplastic extraction procedure was conducted based on digestion with 10 % KOH followed by density separation with a NaCl/ZnCl hypersaline solution and filtration. A series of procedural blank without samples was performed simultaneously during extraction and inspection to analyze the airborne plastic particle contamination. The microplastic was observed under a stereo-zoom light microscope. The abundance, shapes (e.g., fiber, fragment, film, pellet) and color of collected MPs were recorded. Microplastic particles were further identified by using Fourier Transform Infrared (FTIR) spectroscopy.

3.5. Spatial analysis

Spatial analysis of land-uses/covers was undertaken using QGIS software. Shapefile data of land-uses/covers in Indonesia has been available, provided by Direktorat Jenderal Planologi dan Tata Lingkungan, Ministry of Environment and Forestry. The analysis includes catchment delineations and data extractions.

3.6. Data analysis and paper writing

Once data has been collected, statistical analysis will be conducted using R and R Studio. It includes data exploration, correlation analysis, and statistical testing with linear and non-linear models, depending on the nature of the data. Yaya Ihya Ulumuddin (Research Center for Oceanography, Indonesian Institute of Sciences) and Suyadi (Research Center for Biology, Indonesian Institute of Sciences) undertake this component of projects. All the team members contribute proportionally in the paper writing.

4. Results and Discussion

4.1. Sampling Location and Monitoring Site Establishment

The initial plan for collecting crab samples was from 10-15 locations as shown in Figure 1. However, there were several locations that were not selected as sample sources. This is because there are several obstacles, such as: no local contact, delivery problems, or the planned visit was immediately canceled.

However, the number of locations that became the sample source in this study exceeded the initial target, which was 19 locations (See Figure 2). And it's still possible to add a few more locations. Samples from 13 sites (Figure 2) were worked out for analysis of heavy metals and microplastics. The total samples that have been dissected are 105 crabs Scylla spp. Meanwhile, there are still a few of samples from 6 locations that are stored at -5ºC. This sample was obtained within the last month.

Figure 2. Sampling location and monitoring sites (Banten and Segara Anakan)

4.2. Spatial Analysis

The results of the spatial data analysis of land cover showed that from 13 locations of crab sample sources, watersheds with very different width variations were found. The smallest watershed area is Semarang (6824 Ha), while the largest is Bintuni (1,691,702 Ha). The dominance of land cover also varies, for example in contrast, the Berau watershed is dominated by dry land forest (Figure 3), while the Citanduy watershed is dominated by agriculture (dry land and rice fields) (Figure 4). This variation is expected to provide contrasting information, so that it can see the influence of development and other human activities in the upstream part of the river on the intensity of pollution downstream or in the mangroves. This will be indicated by the accumulation of pollutants, either in the form of heavy metals, microplastics, or perhaps other pollutants.

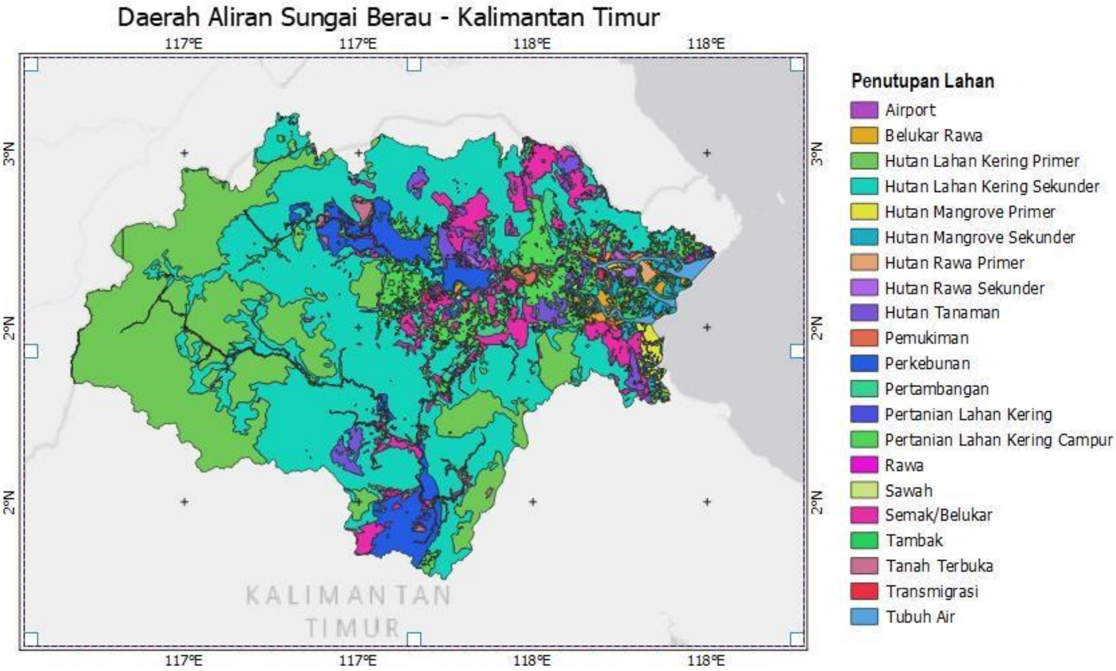


Figure 3. Land uses in Berau Catchment, East Kalimantan

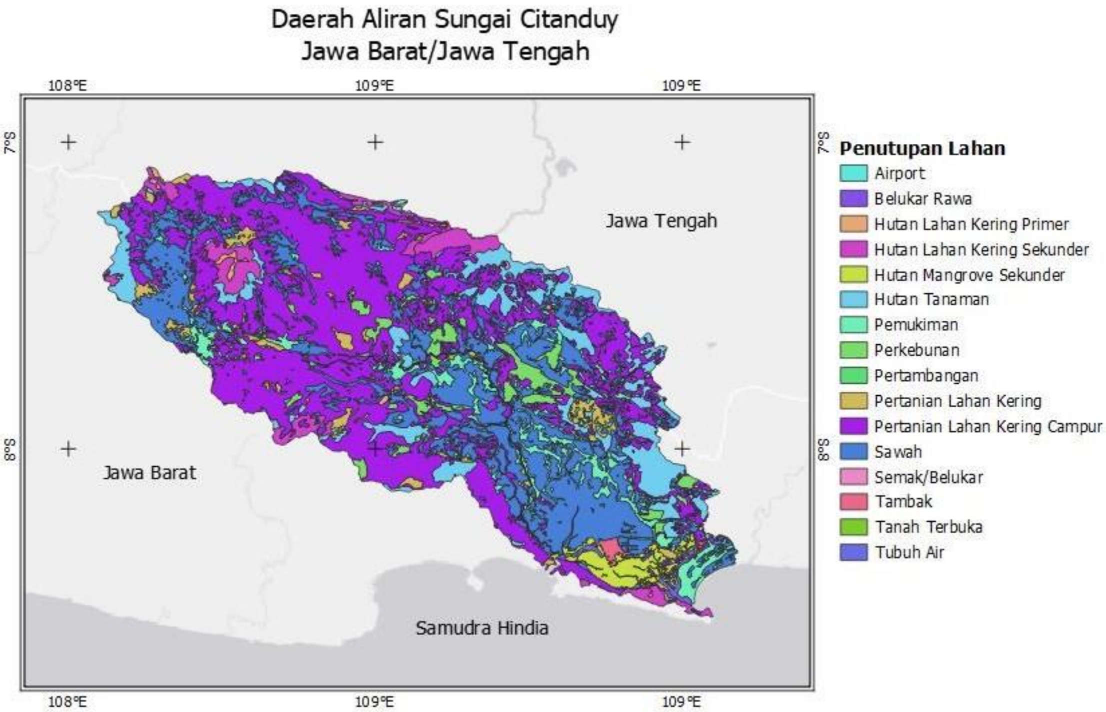


Figure 4. Land Uses in Citanduy Catchment, West and Central Java

From 13 locations, there are four groups distinguished based on the dominant land use classes. Bintuni-West Papua and Konawe Utara-Southeast Sulawesi represent relatively pristine mangroves, dominated by Primary Mangrove Forest and Primary Swamp Forest. Berau-East Kalimantan is a home for mining industry and plantation, although secondary dryland forest remains. Indramayu-West Java and Segara Anakan-East Java are typical of rural landscape in Java Island, which dominated by ricefield, dryland agriculture and settlement. The last group is typical of urban landscape, which Semarang, Banten, and Kendari are obvious representing cities in Java and Sulawesi, while Mentawai, Biak, and Buton Utara are port cities.

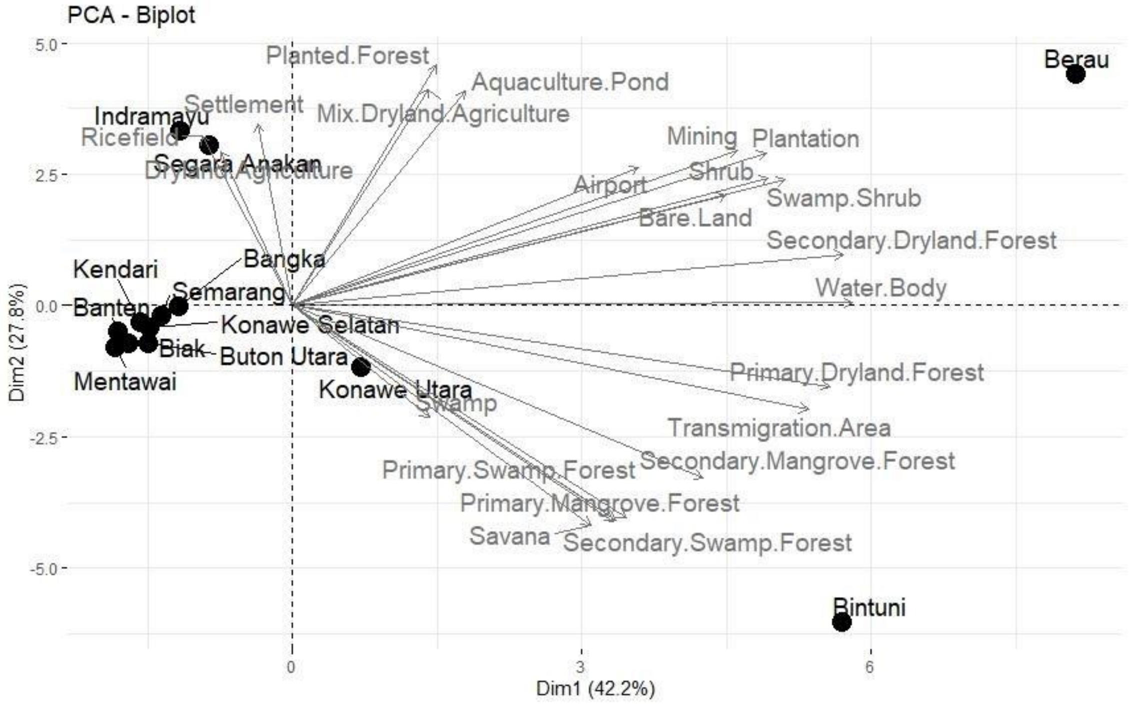


Figure 5. Characterization of catchments based on the dominant class of land uses

4.3. Sampel preparation for measurement of trace metals and microplastic

For the measurement of heavy metals and microplastics, crab samples must be dissected. Prior to that, the morphometry of each sample was measured (weight, carapace length and carapace width). The following is the size distribution of the crabs that were sampled in this study (Figure 6). Scylla serrata and S. paramamosain were the crab species with the most samples (84 individuals), the rest were S. tranquebarica and S. olivacea. The samples collected are those that are ready for sale (>200 g), but there are also some samples that are too small

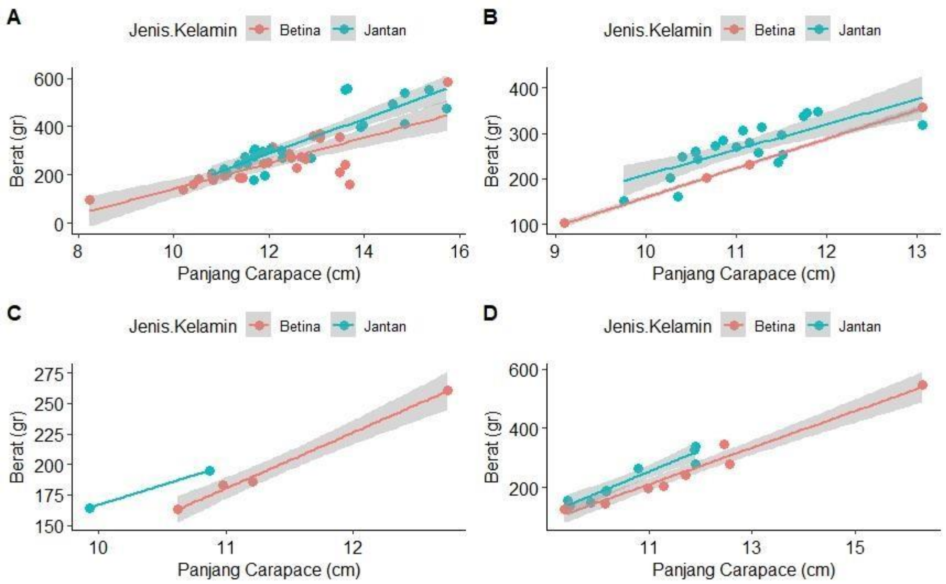


Figure 6. Mud crab morphology. (A) *Scylla serrata*, (B) *Scylla paramamosain*, (C) *Scylla tranquebarica*, and (D) *Scylla olivacea*

The crab samples that had been measured morphometrically were then dissected. The flesh is removed from the carapace (Figure 7a). The digestive tract and gills of crabs were removed under conditions that minimized microplastic contamination of air and surgical equipment (Fig. 7b). Each sample was then stored in a petri dish for further analysis.

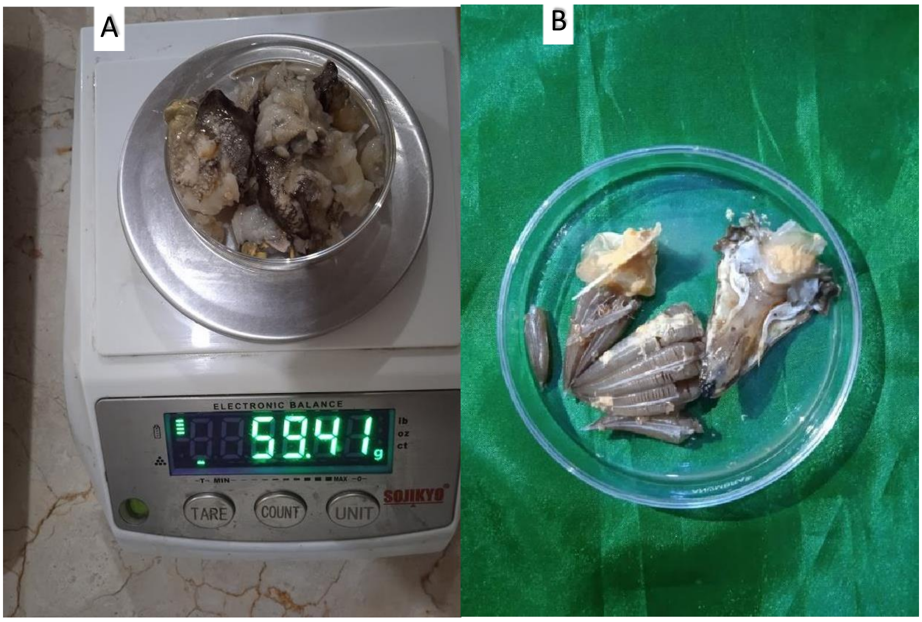


Figure 7. Samples ready for analysis. (A) muscles; (B) digestive systems and gills

4.4. Trace Metal Measurement

The measurement of heavy metals was slightly hampered due to several obstacles, namely the delay in procuring metal standards and oxygen gas for HPLC-HRMS. This is because the Covid-19 condition makes it difficult to procure these materials. In addition, the solution to using other laboratories is also constrained by Covid19. The mobility of researchers is also constrained by restrictions on community activities. So far, only total mercury has been measured (Figure 8).

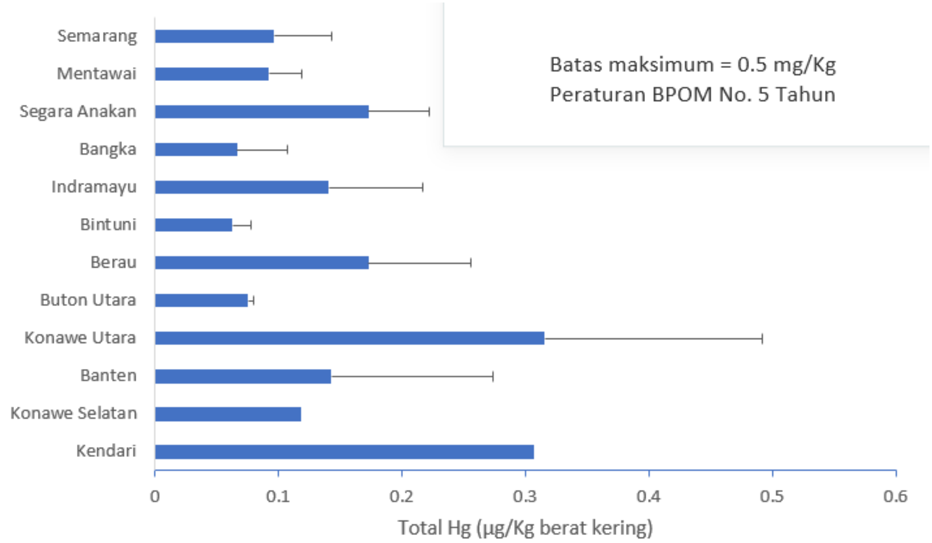


Figure 8. Mercury total

Mercury measured from the sample is less than 0.5 g/Kg dry weight. This means that the mercury content in the crab samples is very small. Much smaller than the threshold set by BPOM in 2017 (0.5 mg/Kg). Among the crab source locations, Kendari and North Konawe showed crab samples containing more mercury than other locations (See Figure 8).

Currently, we have undertaken trace metal analysis. This study may be the first attempt in screening 18 trace metal elements from mud crabs (*Scylla* spp.) collected from multilocation around Indonesian Archipelago. To my knowledge, previous studies reported lesser than 10 elements and only one study location (Zonggonao, 2005; Ardianto et al. 2019; Aris et al., 2018; Meirikayanti et al., 2018; Hasi et al., 2020). The concentration of the toxic trace elements in this study are highly variable among individuals, species, or locations. The range can be from zero to as high as four orders of magnitudes (Table 1). Pb (0.001-0.59 µg/mg) and Cd (0-0.2 µg/mg) are lower than the maximum allowed level, according to Australia and New Zealand standard, ANZFC (2012), 2 µg/mg for either Pb or Cd., but Pb is a bit higher based on Indonesia standard, BPOM (2018), i.e., 0.1 µg/mg. Those ranges are similar to those observed in the previous studies (Table 1). Total arsenic is high (1.71-18.53 µg/mg) and even higher than the maximum allowed level, 0.25 µg/mg BPOM (2018) or 1 µg/mg ANZFC (2012). However, this is not surprising, because marine organisms are generally high in total arsenic contents (Kato et al., 2020), as high as 5580 ± 14 µg/mg in mollusc *Alviniconcha hessleri* (Price et al., 2016). It can be toxic to human body if in inorganic forms, but 100 times less toxic if it is organic arsenic compounds. Here, distinguishing arsenic into inorganic and organic forms is not the main concern of this study, rather, the objective is to detect the correlation between pollution content (trace elements and microplastic) in *Scylla* spp. and land use in the catchment and coastal area.

Table 1. Trace metal content in the muscles of mud crabs (*Scylla* spp.)

No.	Location	Cu (µg/g)	Fe (µg/g)	Mn (µg/g)	Zn (µg/g)	Ni (µg/g)	As (µg/g)	Cd (µg/g)	Pb (µg/g)	Method	References	Notes
1	Indonesia	13.61-128.19 (62.10)	4.11-70.56 (27.64)	1.52-56.9 (12.12)	85.5-241.98 (163.25)	0.002-1.31 (0.13)	1.71-18.53 (6.40)	0-0.2 (0.036)	0.001-0.59 (0.17)	ICPMS	This study	dry weight
2	Terengganu, Malaysia	89			137			0.25	0.22	ICPMS	Chuan et al. 2017	
3	North Coast of Java, Indonesia	22.48 ± 3.76	60.26 ± 36.05					0.3 ± 0.04		AAS	Zonggonao, 2005	
4	Malaysia	21.54±7.14			376.62±21.91			0.42±0.05	6.27±0.75	ICPMS	Kamaruzzaman et al. 2012	
5	Borneo, Malaysia	32.85		2.55	379.6	2.3		0.25	2.25	AAS	Anandkumar et al. 2019	male
		98		1.8	384.2	2.45		0.25	2.15			female
6	Maluan Bay, China	2.68-4.91			14.58-21.39			0.21-0.25	0.03-0.07	ICP-OES	Wang et al. 2010	
7	Chennai, India	0.026	0.542		0.262			0.136	2.316		Batvari et al. 2012	
8	Terengganu, Malaysia	89			137			0.25	0.22	ICPMS	Chuan et al. 2017	
9	Tanzania		12-43.2	1-15.9	16.9-27.6	0.01-0.14	1.0-6.0	0.001-0.13	0.01-0.07	ICPMS	Rumisha et al. 2017	wet weight samples
10	Surabaya, Indonesia							0.032±0.008		AAS	Ardianto et al. 2019	
11	Southeast Sulawesi, Indonesia									AAS	Aris et al., 2018	
12	Surabaya, Indonesia	0.02-0.08								AAS	Meirikayanti et al., 2018	
13	Southeast Sulawesi, Indonesia									AAS	Hasi et al., 2020	
14	China	56.7-69			72.5-81.3		0.475-0.83	0.009-0.180	0.089-0.4	AAS	Jiang et al. 2013	
15	Punnakayal, India	0.41±0.05			39.92±1.2			0.13±0.001	0.09±0.01	AAS	Yogeshwaran et al. 2020	
16	Queensland, Australia	16.4-637	7.5-286	6.7-48.9	86-249	2.4-15.7	4.4-46.9	0-16.8	0-1.7	ICPMS	Mortimer, 2000	

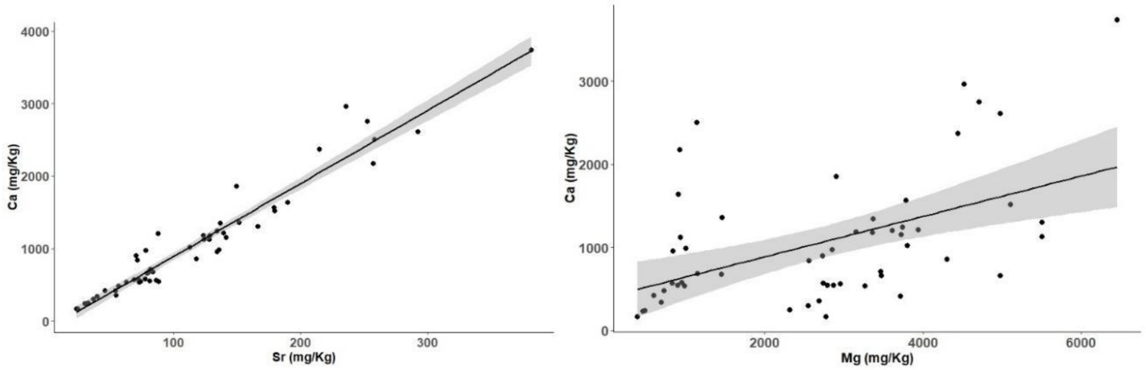
Ten other trace elements presented in Table 1, separately from Table 2 because of lacking studies for comparison. Among the toxic trace elements, Sb and Ti in this study are in the same range as the reference (Mortimer, 2000), but there is no data in literature regarding Li content in *Scylla* muscle. Li content ranges between 0.05-1.04 µg and this should be an alarm that lithium has been detected in *Scylla* spp., which also found in a stone crab, *Menippe merceneria*, collected from South Carolina coastal waters (USA). The content of essential trace elements in Table 1 and 2 has a wide range of values, but they are within the same ranges as those found in the literature.

Table 2. Trace metals in mud crabs *Scylla* spp.

No.	Heavy Metals	This study (µg/g dry weight)	Queensland, Australia (µg/g dry weight)
1	Co	0.01 - 0.30 (0.09)	0-3.69*
2	Se	0.43 - 7.86 (1.76	0.5-11.4*
3	V	0.02 - 0.26 (0.08)	0-32.5*
4	Mo	0.02 - 0.51 (0.10)	0-1.6*
5	Sb	0 - 0.03 (0.005)	0-0.0455*
6	Li	0.05 - 1.04 (0.36)	0.05-0.1+
7	Ti	3.49 - 73.7 (19.74)	0.4-54.8*
8	Mg	395.25 - 6457.31 (2716.29)	
9	Ca	164.03 - 3738.06 (1058.37)	
10	Sr	23.35 - 381.44 (116.32)	

* Mortimer (2000)
\+ Reed et al. (2010)

Mg, Ca, and Sr are macro-elements, which are important for carapace development in brachyuran species, e.g., shrimps and crabs (Gibbs and Bryan, 1972), including *Scylla* sp. (Triajie et al. 2021). A relationship between Ca and Sr content in *Scylla* muscles gives a good fit (R^2 = 0.93, n=55), while Mg and Sr have a weak relationship (R^2 = 0.24, n=55). This finding complements with those reported by Gibbs and Bryan (1972). They found that if unmoulted, the Sr/Ca ratio in the carapace remains constant because reabsorption does not occur. Ca moves to the muscle crabs when they are moulted. From this explanation, it makes sense if Sr and Cr have a strong linear relationship that means Sr/Ca ratio in the muscles is the same for all individual crabs. The crabs are unmoulted having Sr/Ca ratio not only in the carapaces but also in the muscles. However, Mg distribution in relation to Ca and Sr cannot be predicted, as Gibbs and Bryan (1972) reported as well.



Gambar 9. Hubungan linier Sr dan Ca; dan Mg dan Ca

4.5. Microplastic measurement

Exposure to microplastics in the body of biota can occur through the process of taking food in their habitat. Plastic can be mistaken for food or prey. In addition, plastic can also enter the body of biota because it preys on organisms that have been exposed to plastic (Derraik et al. 2016). From several research results it has been reported that the entry of plastic into the body of biota can affect the eating behavior, growth, physiological function of the body and the immune system of the biota (Cole et al., 2013). Microplastics can also act as agents for the entry of toxic materials into the body of biota (Rochman et al., 2013). The level of plastic exposure in the body of marine biota is influenced by feeding behavior and conditions of plastic pollution in the habitat of biota (Setala et al. 2016). Based on the habitat and eating behavior of crabs, mud crabs are very susceptible to contamination by plastic, both from their habitat and from their food. Mangrove crab Scylla sp. live in mangroves and estuaries and prefer muddy bottoms which have been reported to be under high anthropogenic pressure, including plastic waste (Khoironi et al. 2020; Suyadi & Manullang et al. 2020). This mangrove crab is also a type of omnivorous biota that eats a mixture of animals and plants as well as detritus (Paul et al. 2018). Mangrove crabs also prey on shellfish, which are filter feeders (Paul et al. 2018).

In this study, 68 species were examined from 15 sites. From the first visual search of plastic during the destruction process, it was found that 5 out of 68 samples were confirmed to contain plastic waste with a size of more than 5 mm. The type of plastic found was fiber with black color (Figure 10).

Figure 10. Microplastic particle >5mm in *Scylla* spp.

Meanwhile, microplastics were found in all locations, where 55 out of 68 samples were contaminated with microplastics. The average concentration of microplastic per individual (n/ind) from 15 locations is shown in Figure 11. The highest concentration of microplastic was found in Semarang (13.25 n/ind), while the lowest concentration of microplastic was found in Bintuni (1 n/ind). Previous research conducted in Semarang confirmed that the concentration of microplastics found in mangrove areas in Semarang reached 444 000 n/kg, where this concentration was the highest concentration of microplastics in Indonesia (Khoironi et al., 2020).

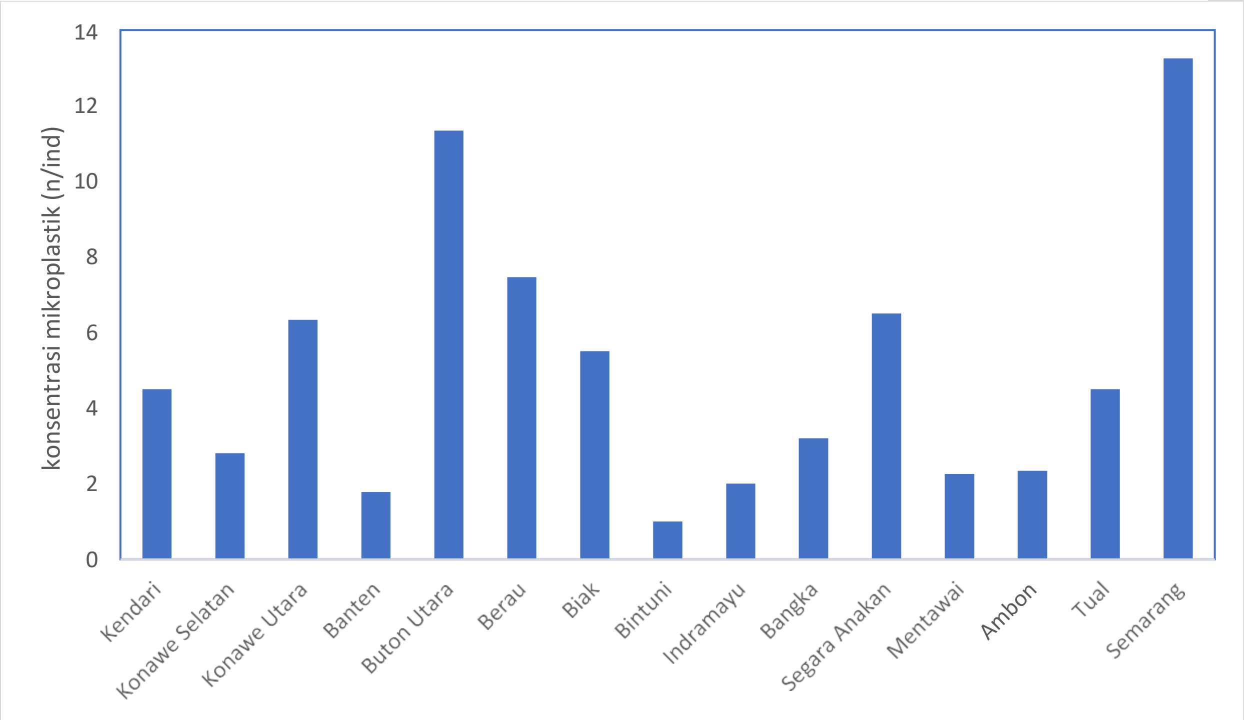


Figure 11. Microplastic content in the digestive systems of mud crabs, *Scylla* spp. collected from 15 location in Indonesia

The most common type of microplastic found was fiber (76%) (Figure 12). This type is a type of microplastic that is commonly found in Indonesia. Meanwhile, very few pellet types were found in this study (8%).

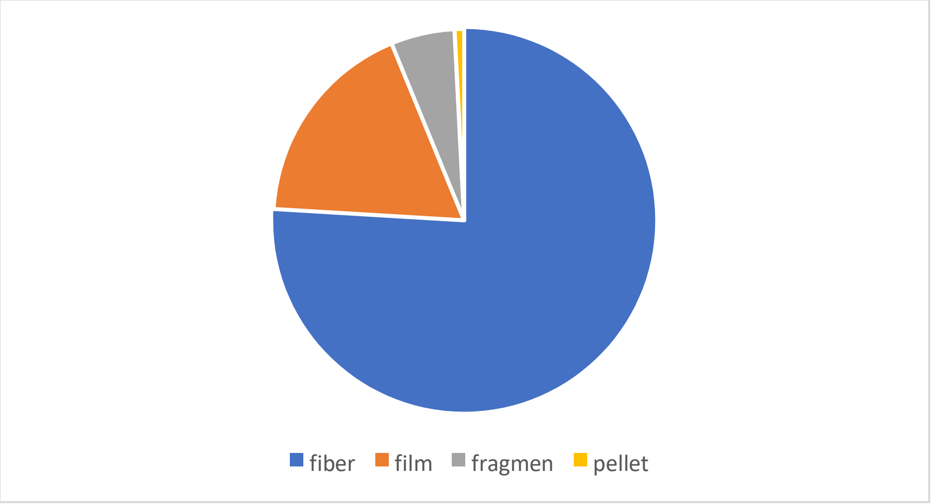


Figure 12. Proportion of microplastic types found in the digestive systems of mud crabs *Scylla* spp. collected from 15 location in Indonesia

In this study, it was confirmed that the smaller the size of the microplastic, the higher the amount of plastic found (Figure 13). The most amount of plastic found was in size: 100-200 m (74%), while microplastic size >1000 m was found the least (1%).

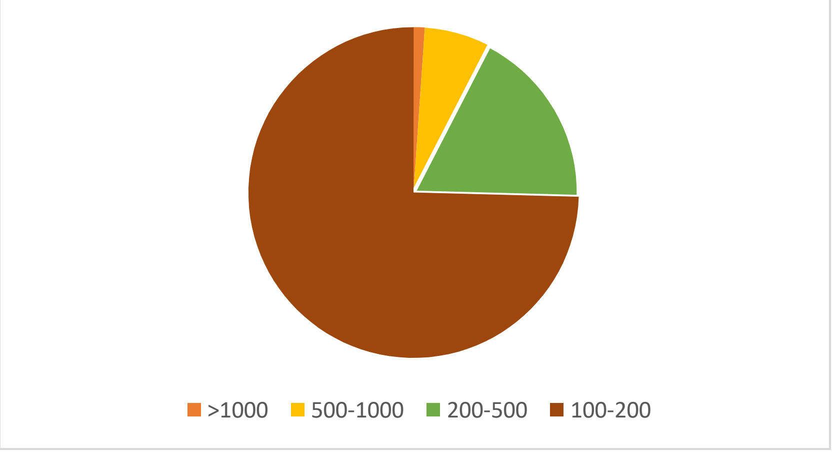


Figure 13. Proportion of size of microplastic found in the digestive systems of mud crabs *Scylla* spp. collected from 15 location in Indonesia

This study also observed the average concentration of microplastics based on the mangrove crab species Scylla sp. from 15 locations in Indonesia. Of the 4 species of mangrove crab Scylla sp. observed, it is known that the species S. serrata has a higher concentration

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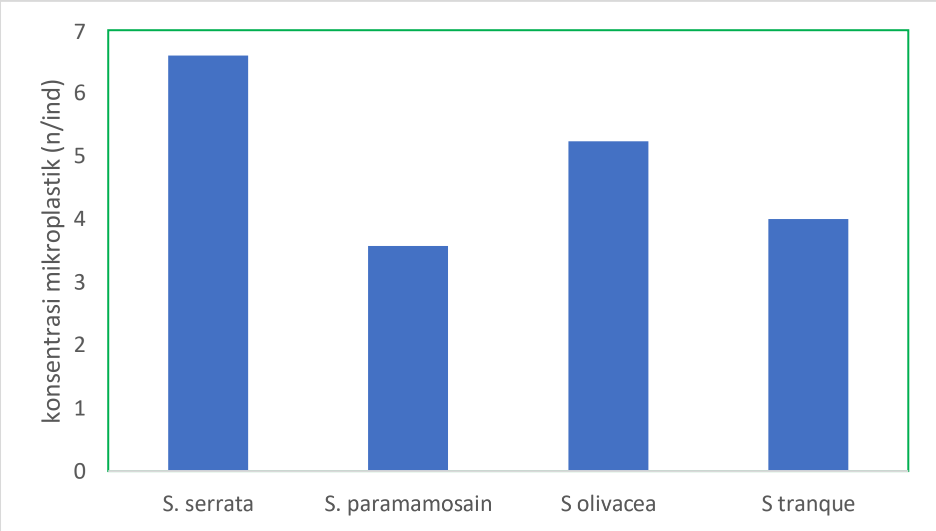

Figure 14. Average of microplastic content among species of mud crabs *Scylla* spp. collected from 15 location in Indonesia

4.6. Land uses indicate the accumulation of trace metal elemenets and microplastic

Although scatterplot results of PCA do not exactly follow the grouping in the locations based on land use of the catchment area, there is a strong indication of relationship between land use and pollution (trace metal elements and microplastic). For example, mud crabs collected from Banten, Semarang, and Segara Anakan contain various trace elements (Cu, Mo, Fe, V, Cd, Co, Pb, and Mn), while Bintuni and Mentawai are in the opposing state (Figure 15). Bangka and Konawe Utara have a similar character of trace metal pollutions, dominated by Ti, Ca, Sr, Mg and Ni, although they have different landscape character. Bangka has not a dominant land use class, while Konawe Utara is considered as pristine mangrove area. This study, to some extent, confirms the hypothesis proposed by Gilby et al. (2021), that land use practices may lead to the accumulation of various pollutants in seafood, but in terms of magnitude, mud crabs in our samples remains lower than the thresholds (ANZFC, 2012 and BPOM, 2018) or within the same range of available data (see Table 1 and 2).

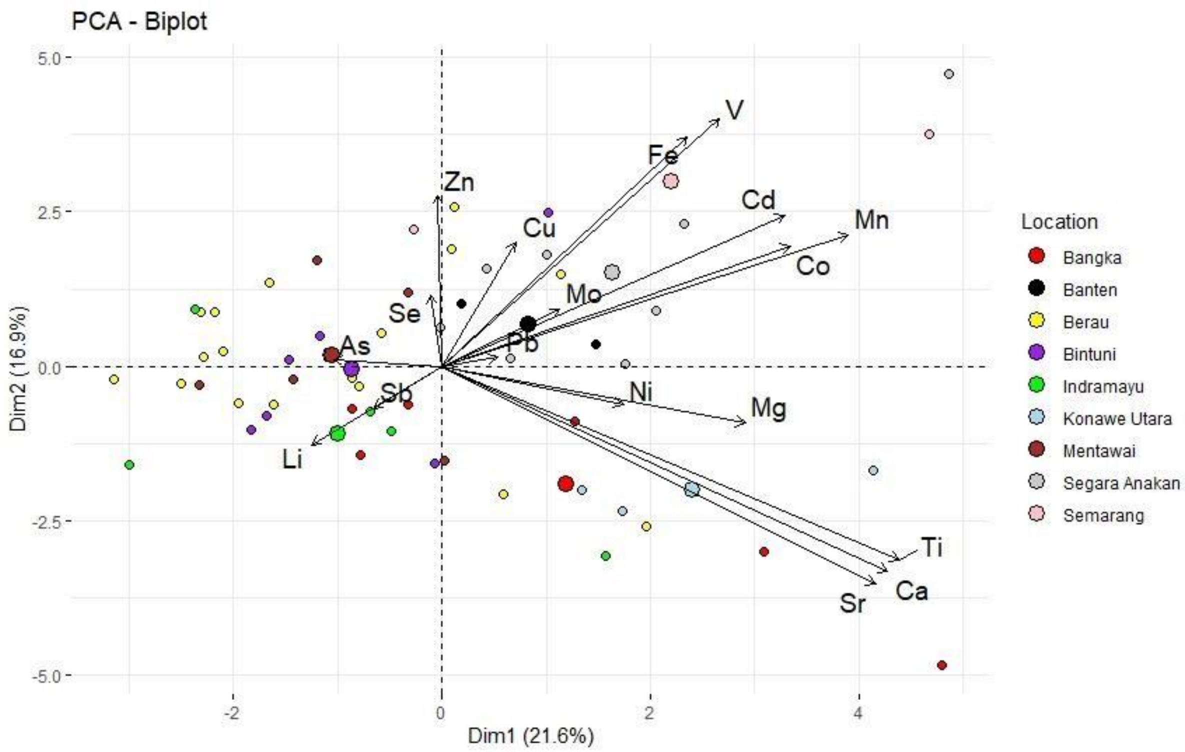

Figure 15. Multivariate analysis for trace metal elements

In contrast, microplastic content in mud crab samples does show a clear pattern. Although not all our samples contain microplastic, this contaminant presence at least at one sample of each location. Some locations are obvious having great number of microplastic, i.e., Semarang and Segara Anakan (Figure 16). The samples collected from Semarang contain microplastic with the dominant size between 100 and 200 µm, while the dominant size of microplastic in Segara Anakan's samples range from 200 to 1000 µm. These are the sampling locations considered to have diverse pollutants. Other locations having relatively high in microplastic content are Buton Utara-Southeast Sulawesi, Berau-East Kalimantan, and Biak-West Papua. These locations have different characteristics of land use, but the land uses are potential to derive anthropogenic activities that produce plastic waste to the environment, e.g., settlements, mining, and plantation. In addition, small islands, like Buton and Biak, may receive plastic waste from the surrounding cities through ocean currents, trapped by mangrove forests (Martin et al., 2019).

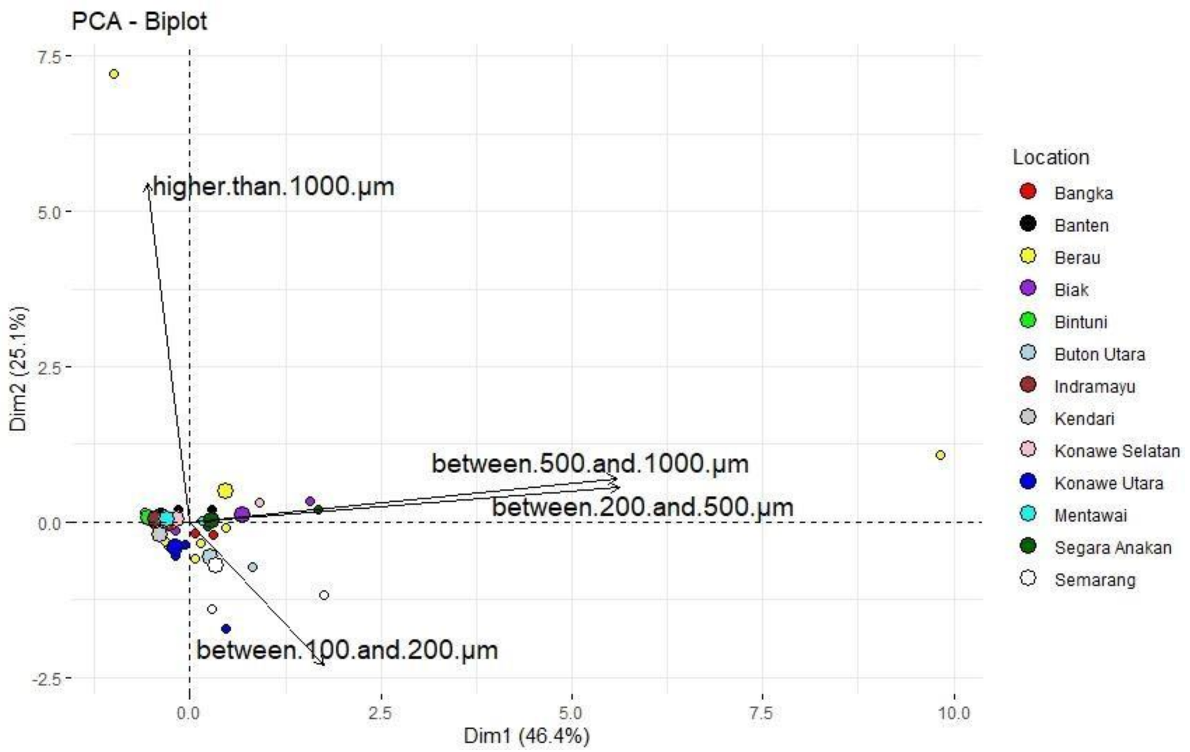

Figure 16. Multivariate analysis for microplastic content

The data of land uses and pollutants (trace elements and microplastic) in mud crab's muscles explained before shows different ways of causal relationships. Indeed, there is a link between modified land uses and diverse human activities (Bosch et al., 2016; Landos, 2013; Tchounwou et al., 2012, that can lead to additional of substances to the environment from those activities, e.g., fuel burning (Pb), paints (Pb and Ti), glass (Pb), manufacturing (trace elements), and wastewater (all trace elements Bosch et al., 2016; Cempel and Nikel, 2006; Tchounwou et al.,2012), Once these pollutants enter aquatic environment, reaching estuaries, and end up in mangrove forests or sea floor (Branoff, 2017). Plastic wastes may have a wider distribution when entering the ocean, but mangrove is a good trap for solid wastes (Martin et al., 2019) and microplastic in mangrove sediment has been extensively reported (Li et al., 2018; Garces-ordonez et al. 2019; Naji et al., 2019, Cordova et al., 2021).

Microplastic might have a high risk for human health in the future through mud crab consumption, if there is no proper plastic waste management. But trace elements might have different stories, because mud crab (*Scylla* spp.) have mechanisms to release the trace elements while moulting (Mohapatra et al., 2009 and Triajie et al., 2021). Nevertheless, either microplastic or trace elements may contaminate our seafoods through bioaccumulation and biomagnification in various marine organisms with different trophic levels (Rumisha et al., 2017; Nelms et al., 2019; Harris et al., 2018; Sun et al., 2020).

5. Conclusion

Modified land uses are snapshots to record the diversity of human activities and it can be a general indicator for environment problem of pollution and safety seafood. This can be true if there is no waste management before it enters to the aquatic environment. While a previous study found that poor indicator of land uses for pollutant detection in seafood, our data shows the link between land uses and pollutant accumulation in mud crabs. In addition, the distance to urbanized area should also be considered as land use indicators due to the spreading of marine pollutants is not only through watershed but also ocean currents.

6. Personal Investigator and other researchers

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		3. Suratno, M.Sc.
		4. Corry Yanti Manullang, M.Si.
		5. Suyadi, Ph.D

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Annex

Table 1. Morphometric data of mud crab samples, *Scylla* spp. and mercury content

No Sample	Location	Species	Sex	Weight (gr)	Carapace Width (cm)	Carapace length (cm)	THg (µg/kg dw)
1	Kendari	S. serrata	Jantan	491.36	10.46	14.60	0.307
2	Kendari	S. serrata	Jantan	398.76	9.36	13.91	
3	Konawe Selatan	S. serrata	Betina	212.54	9.17	13.47	
4	Konawe Selatan	S. serrata	Jantan	304.88	8.06	11.71	
5	Konawe Selatan	S. serrata	Betina	288.50	8.70	12.41	
6	Konawe Selatan	S. serrata	Jantan	268.58	8.60	12.27	
7	Konawe Selatan	S. serrata	Jantan	178.65	7.92	11.68	0.118
8	Konawe Selatan	S. serrata	Jantan	271.94	8.52	12.87	
9	Banten	S. paramamosain	Jantan	256.96	8.00	11.25	
10	Banten	S. serrata	Betina	96.35	5.97	8.25	0.103
11	Banten	S. paramamosain	Jantan	318.74	8.32	13.07	
12	Banten	S. paramamosain	Betina	356.95	8.67	13.07	
13	Banten	S. paramamosain	Jantan	253.97	7.90	11.52	0.371
14	Banten	S. paramamosain	Jantan	202.65	7.07	10.27	0.087
15	Banten	S. paramamosain	Jantan	161.87	7.05	10.35	0.040
16	Banten	S. paramamosain	Jantan	150.68	6.65	9.75	0.112
17	Banten	S. paramamosain	Betina	102.71	6.26	9.10	
18	Konawe Utara	S. olivacea	Jantan	263.45	7.20	10.80	
19	Konawe Utara	S. serrata	Betina	355.20	6.67	13.07	
20	Konawe Utara	S. olivacea	Jantan	279.77	8.17	11.90	
21	Konawe Utara	S. serrata	Betina	229.79	8.20	12.57	
22	Konawe Utara	S. olivacea	Jantan	339.56	7.76	11.91	
23	Konawe Utara	S. serrata	Jantan	196.52	7.92	11.91	0.191

24	Konawe Utara	S. tranquebarica	Betina	260.76	8.21	12.75	0.440
25	Konawe Utara	S. serrata	Betina	160.04	8.65	13.70	
26	Konawe Utara	S. serrata	Betina	242.49	8.52	13.60	
27	Buton Utara	S. serrata	Jantan	538.58	9.92	14.85	0.072
28	Buton Utara	S. serrata	Jantan	557.87	8.91	13.65	
29	Buton Utara	S. serrata	Jantan	411.96	10.14	14.85	0.079
30	Buton Utara	S. serrata	Jantan	551.81	9.62	13.60	
31	Berau	S. serrata	Betina	188.75	7.15	11.37	
32	Berau	S. tranquebarica	Betina	185.98	7.41	11.21	
33	Berau	S. serrata	Betina	158.64	6.91	10.40	
34	Berau	S. serrata	Betina	351.57	8.52	13.07	
35	Berau	S. serrata	Betina	180.61	7.15	10.82	
36	Berau	S. serrata	Jantan	207.79	7.45	11.12	
37	Berau	S. serrata	Betina	356.70	9.15	13.49	
38	Berau	S. serrata	Betina	184.58	7.07	10.52	
39	Berau	S. serrata	Jantan	274.48	7.77	11.50	
40	Berau	S. serrata	Jantan	291.48	8.21	11.85	
41	Berau	S. serrata	Betina	316.98	8.27	12.07	
42	Berau	S. serrata	Jantan	256.34	8.05	11.52	
43	Berau	S. serrata	Jantan	207.21	7.21	10.81	
44	Berau	S. olivacea	Jantan	146.92	6.50	9.87	
45	Berau	S. serrata	Betina	187.73	7.12	10.85	
46	Berau	S. olivacea	Betina	344.31	6.35	12.46	
47	Berau	S. serrata	Betina	267.62	8.20	12.47	
48	Berau	S. serrata	Betina	186.87	7.57	11.45	
49	Berau	S. serrata	Betina	263.37	8.19	12.77	
50	Berau	S. serrata	Jantan	244.02	7.87	11.35	
51	Berau	S. serrata	Betina	177.60	7.27	10.82	
52	Berau	S. serrata	Betina	194.72	7.15	11.05	
53	Berau	S. serrata	Betina	198.23	7.15	10.81	
54	Berau	S. serrata	Betina	194.55	7.12	11.07	
55	Berau	S. olivacea	Jantan	157.17	6.32	9.43	
56	Berau	S. serrata	Betina	360.84	8.65	12.92	0.102
57	Berau	S. serrata	Jantan	245.23	7.65	11.55	

58	Berau	S. serrata	Betina	246.62	8.19	11.88	0.103
59	Berau	S. serrata	Jantan	281.63	7.22	11.71	0.220
60	Berau	S. serrata	Betina	274.99	8.10	12.68	0.266
61	Biak	S. serrata	Jantan	551.16	10.35	15.36	
62	Biak	S. serrata	Betina	583.81	10.63	15.75	
63	Bintuni	S. serrata	Jantan	224.87	7.32	11.05	0.055
64	Bintuni	S. serrata	Jantan	299.80	8.22	12.25	
65	Bintuni	S. serrata	Jantan	295.31	7.52	11.87	0.055
66	Bintuni	S. serrata	Jantan	296.85	7.89	11.68	0.078
67	Bintuni	S. serrata	Jantan	273.13	7.70	11.69	0.046
68	Bintuni	S. serrata	Jantan	307.32	8.02	12.02	0.080
69	Indramayu	S. paramamosain	Betina	230.60	7.65	11.15	0.196
70	Indramayu	S. paramamosain	Jantan	247.71	6.82	10.40	0.309
71	Indramayu	S. paramamosain	Jantan	236.33	7.45	11.47	0.120
72	Indramayu	S. paramamosain	Jantan	242.44	7.07	10.57	0.098
73	Indramayu	S. paramamosain	Jantan	270.19	7.40	11.00	0.113
74	Indramayu	S. paramamosain	Jantan	271.02	7.37	10.77	0.073
75	Indramayu	S. paramamosain	Jantan	285.44	7.42	10.85	0.148
76	Indramayu	S. paramamosain	Jantan	257.77	7.37	10.55	0.070
77	Indramayu	S. paramamosain	Betina	201.40	7.27	10.67	0.092
78	Bangka	S. tranquebarica	Jantan	164.39	6.57	9.93	0.045
79	Bangka	S. tranquebarica	Betina	182.82	7.27	10.98	0.092
80	Bangka	S. tranquebarica	Betina	162.71	6.78	10.62	0.050
81	Bangka	S. tranquebarica	Jantan	194.79	7.45	10.87	0.080
82	Bangka	S. olivacea	Jantan	183.92	6.75	10.17	0.064
83	Bangka	S. olivacea	Jantan	189.83	7.00	10.18	0.157
84	Segara Anakan	S. olivacea	Betina	202.74	7.25	11.28	0.219
85	Segara Anakan	S. olivacea	Betina	278.02	8.25	12.57	0.176
86	Segara Anakan	S. olivacea	Betina	198.64	7.18	10.98	0.186
87	Segara Anakan	S. olivacea	Betina	136.60	6.25	9.47	0.182
88	Segara Anakan	S. olivacea	Betina	243.18	7.86	11.72	0.250
89	Segara Anakan	S. olivacea	Betina	124.83	6.17	9.37	0.122
90	Segara Anakan	S. serrata	Betina	138.30	6.55	10.20	0.150
91	Segara Anakan	S. olivacea	Betina	146.40	6.77	10.15	0.098

92	Mentawai	S. olivacea	Betina	546.81	11.21	16.32	0.073
93	Mentawai	S. serrata	Jantan	408.91	9.60	13.97	0.073
94	Mentawai	S. serrata	Betina	369.12	9.15	13.07	0.075
95	Mentawai	S. serrata	Betina	251.43	7.87	11.97	0.130
96	Mentawai	S. serrata	Jantan	474.08	10.75	15.71	0.111
97	Semarang	S. paramamosain	Jantan	344.57	7.87	11.78	0.137
98	Semarang	S. paramamosain	Jantan	312.33	7.55	11.28	0.066
99	Semarang	S. paramamosain	Jantan	279.25	7.21	11.15	0.039
100	Semarang	S. paramamosain	Jantan	338.78	7.75	11.75	0.121
101	Semarang	S. paramamosain	Jantan	259.62	7.24	10.55	0.109
102	Semarang	S. olivacea	Jantan	327.39	7.87	11.88	0.171
103	Semarang	S. paramamosain	Jantan	347.27	7.75	11.90	0.089
104	Semarang	S. paramamosain	Jantan	296.22	7.55	11.50	0.027
105	Semarang	S. paramamosain	Jantan	306.80	7.72	11.07	0.108