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Concentration of Heavy Metal Hg, Au, and Fe in Sediments, Water, and Tissue Damage of Golden Sea Cucumber *Stichopus herrmanni* (Semper, 1868) (Holothuroidea; Stichopodidae) in Kayeli Bay, Indonesia

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Research Article

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Abstract

Heavy metals concentrated in waters can also accumulate in sediments and various biota. This research was conducted to examine the concentration of heavy metals in seawater, sediment, intestine and meat of golden sea cucumber *Stichopus herrmanni* in Kayeli bay, Buru Island, Indonesia. The concentrations of heavy metals Hg, Au, and Fe were analyzed using the AAS, and the tissue damage of *S. herrmanni* used the HE staining method. Correlation analysis and multiple linear regression were used to examine the correlation and the effect of the independent variables (heavy metals Hg, Au, and Fe) partially on the dependent variable (damage on intestinal villi of *S. herrmanni*) with a statistically significant at α= 0.05. The results showed that the highest to lowest heavy metal concentrations in water was Hg>Fe>Au, while the concentration of heavy metals in water, intestine and meat was of sea cucumber was Fe>Hg>Au. There was a correlation between heavy metals in sediments, intestines, and meat which caused damage to intestinal villi, while the multiple linear regression tests showed a probability of 0.012<0.05 for Fe, a probability of 0.000< for Hg, while probability >0.05 for Au on the damage of intestinal villi of *S. herrmanni*. This shows that Fe has a significant potential to cause damage to the intestinal villi of *S. herrmanni*. Fe is highly concentrated in sediments, intestines and meat showed a positive correlation to the damage of intestinal villi of *S. herrmanni*.

Keywords: Heavy metals, *Stichopus herrmanni*, tissue damage, Kayeli Bay

Endonezya Kayalı Körfezi'nde Bulunan *Stichopus herrmanni*'nin (Semper, 1868) (Holothuroidea; Stichopodidae) Dokuları İle Su Ve Zeminde Hg, Au, Fe Ağır Metalleri Birikimi

Özet

Bu araştırma, Endonezya'nın Buru Adası, Kayeli koyunda deniz suyu, sediment ile Stichopus herrmanni (deniz salatalığı) bağırsak ve kaslarında ağır metallerin konsantrasyonunu incelemek için yapıldı. Hg, Au ve Fe konsantrasyonları AAS kullanılarak analiz edildi ve *S. herrmanni*'nin doku hasarı için HE boyama yöntemi kullandı. Korelasyon analizi ve çoklu lineer regresyon, bağımsız değişkenler ile (ağır metaller Hg, Au ve Fe) kısmen bağımlı değişken (*S. herrmanni*'nin bağırsak villusundaki hasar) üzerindeki korelasyonunu ve etkisini α = 0.05. istatistiksel önem seviyesinde incelemek için kullanıldı. Sonuçlar, sudaki en yüksek ve en düşük ağır metal konsantrasyonlarının Hg> Fe> Au olduğunu, su, bağırsak ve etteki ağır metal konsantrasyonunun ise deniz salatalıklarında Fe> Hg> Au olduğunu göstermiştir. Sediman, bağırsak ve etlerdeki ağır metaller arasında bağırsak villerine zarar veren bir korelasyon vardı, ancak çoklu lineer regresyon testleri, Fe için 0.012 <0.05, Hg için 0.000 < olasılık, *S. herrmanni*'nin bağırsak villusunun hasarına dair Au için> 0.05 olasılığı göstermiştir.

Bu Fe'nin S. herrmanni'nin bağırsak villerine zarar verme potansiyeli olduğunu göstermektedir. Fe, tortular, bağırsaklar ve ette yüksek oranda konsantre edilir ve *S. herrmanni*'nin bağırsak villusunun hasarı ile pozitif bir korelasyon göstermiştir.

Anahtar Kelimeler: Ağır metaller, *Stichopus herrmanni*, doku zararı, Kayeli Körfezi.

INTRODUCTION

Heavy metals are one type of pollutant that pollutes the environment and causes degradation in the aquatic environment and endangers the lives of various aquatic biota (Kumara et al., 2013; Lorenzon et al., 2001). The presence of heavy metals in an organism indicates human activity (Mohiuddin et al., 2011). Heavy metals that pollute the aquatic environment due to human activities originate from household waste, industrial, agricultural activities, fuel combustion, and mining activities (Al-Naggar et al., 2018; Jakimska et al., 2011).

Heavy metals including copper (Cu), cobalt (Co), molybdenum (Mo), zinc (Zn), iron (Fe) and manganese (Mn) at low concentrations are nutrients for organism, while cadmium (Cd), Arsenic (As), lead (Pb) and mercury (Hg) are toxic elements, although they are found at very low levels (Khaled, 2004; Jakimska et al., 2011; Liu et al., 2015). Hg is one of the metals that pollute the environment due to agricultural and industrial activities (Al-Nagar et al., 2018). Whereas Nindyapuspa and Ni'am (2018) argue that heavy metal Fe is a natural pollutant in marine ecosystems. Aquatic ecosystems consist of water, sediments, and various biota. Heavy metal pollution in biota tissue is strongly influenced by the level of bioavailability of heavy metals, both external bioavailability; which depends on the ability of heavy metals to dissolve and be released in certain media and internal bioavailability; which is the ability of heavy metals to be absorbed and at the same time toxic for the tissue of organisms (Caussy et al., 2003). Therefore, heavy metals concentrated in waters can also accumulate in sediments and various biota (Ziyaadini et al., 2017). According to Jinadasa et al. (2014), marine biota can accumulate heavy metals such as Hg, Zn, Cu, Cd, and Pb. In addition, Chen et al. (2000) explain that sediments are rich in dissolved organic material, especially the protein content that is able to bind metals. It is confirmed by (Gbrauko and Friday, 2007) that the amount of heavy metals accumulated in the body of aquatic biota depends on the chemical effects of the heavy metals and tends to bind to proteins and lipids in the biota tissues. Metal-binding proteins are metallothionein (Rumahlatu and Leiwakabessy, 2016) and metal transcription factors (Rumahlatu, 2017).

Research on the use of aquatic biota as a pollution indicator species to monitor heavy metals in the waters has become the current research trend. Flammang et al. (1997) used *Diadema setosum* to monitor heavy metals Zn, Pb, Cd, Fe, Cr, Cu, and Ti. Rumahlatu and Huliselan (2016) used *Apogon Beauforti* as a bioindicator species of Hg pollution in the waters of Ambon Island. Khalaf et al. (2012) used three species of fish *Decapterus macrellus*, *Decapterus macrosoms* and *Decapterus russelli* to monitor heavy metals Cu, Ni, Mg, Pb, Zn, Cd, and Fe in Aqaba Bay, Red Sea, Jordan. This means that aquatic biota can be used as an indicator of heavy metal pollution, one of which is a sea cucumber. Currently, sea cucumbers receive attention as the subject of heavy metal pollution research and have been proposed as bioindicators to monitor heavy metal pollution in marine ecosystems (Ahmed et al., 2018). The sea cucumbers of *Actinophyga bannwarth* and *Holothuria impatiens* are used as pollution indicator species of heavy metals Cd, Cu, Ni, Mn, Zn, Fe and Pb in the Red Sea, Gulf of Aqaba (Al-Najjar et al., 2018). Golden sea cucumber (*Stichopus herrmanni*) can be used as an indicator of pollution in marine waters, because of his life habits that immerse themselves in sediment, as well as eating habits that decompose organic matter. Purcell et al. (2016) added the way of its eating is deposit-feeding, and it eats diatoms mixed with sediments. Its habitat is in reef areas and lagoons, in seaweed ecosystems, and muddy sand (Conand, 2006). In addition, *S. herrmanni* is an animal that easily absorbs heavy metals through its cell membrane during the bioabsorption process. This characteristic allows *S. herrmanni* to be contaminated with heavy metals (Ismail et al., 2004). On the other hand, *S. herrmanni* is a species of sea cucumber that is used as a medicine, and *S. herrmanni* is rich in protein and low in lipids and contains a large number of sulfated glycosaminoglycans (Pangestuti and Arifin, 2017).

Kayeli Bay waters are one of the habitats of *S. herrmanni*. This is influenced by the physical properties of the sediments in the Kayeli Bay waters which are of muddy sand. Kayeli Bay is located in the North-East of the Buru sub-district close to the gold mining area on Mount Botak. The gold mining on Mount Botak is geographically located between $127^0$03'28" east longitude and $3^0$24'33" south latitude. According to Male et al. (2014) that mining activities in Mount Botak are a trigger for heavy metal pollution on Buru Island.

The mining activities carried out at Mount Botak use simple mining methods with simple tools. Salatutin et al. (2015) explained that the processing of gold required large amounts of water so that the gold processing unit placed on the riverbank resulted in mercury waste being absorbed in the sediments of the Wamsai river and Kayeli Bay. The research conducted by Male et al. (2014) on mercury levels in sweet shells in Kayeli Bay showed that high levels of mercury were caused by mercury waste which was disposed into the river bodies due to "drum" activities along the slopes of Mount Botak. This caused the Kayeli Bay as the estuary of several rivers on the island of Buru to be affected by mercury heavy metal pollution. The research conducted by Naickera et al. (2003) shows that the groundwater in the mining district is highly contaminated and acidified due to the oxidation of pyrite (FeS_2) contained in mining wastes, and it increases the concentration of heavy metals. Meanwhile, the deposition of heavy metals Fe in water flow is a consequence of the oxidation of the pyrites. Based on these facts, the traditional gold mining process which occurs in Mount Botak can accumulate heavy metals Hg, Fe, and the rest of Au. According to Cobbina et al. (2013), the waste from gold mining is mercury which is discharged into the environment, so that it can contaminate water bodies through the flow of surface water. The research conducted by Nurcholis et al. (2017) reported that gold mining in the Gunung Mas, Boto village, and Wonogiri area had resulted in the exposure of heavy metals to the environment, and the soil around the mining area had been contaminated by heavy metals Mn, Fe, Pb, Hg and As.

The sea cucumbers have a distinctive morphology with a soft, cylindrical body, extending from the mouth to the anus, and lying on the seafloor (Conand, 2006). Ahmed et al. (2018) report that heavy metal Hg dissolves in fat so that it easily accumulates in the body of the biota and can even accumulate in the nerves and brain. Based on this opinion, the pollution of heavy metals due to traditional mining activities in Mount Botak can accumulate in the meat and intestinal tissues of *S. herrmanni*. Therefore, in this research, we examined the effect of concentrations of heavy metals Hg, Au, and Fe on intestinal villi damage, and examined the histopathology of intestinal villi of *S. herrmanni* which were exposed to heavy metals.

MATERIALS and METHODS

Study Area

This research was carried out in the waters of Kayeli Bay, Buru Island, Indonesia (Figure 1). The selected location was the coast in the Kayeli Bay with coordinates Southern Latitude 3^{0}16'32"-3^{0}20'10" and Eastern Longitude 127^{0}7'18"-127^{0}9'42", which were Kodim Asrama Beach (station 1), Kota Namlea Beach (Station 2), Sailong Beach (Station 3), Siahoni Beach (Station 4), Sanleko Beach (Station 5), Muara Waeapo (Station 6), Anahoni Estuary (Station 7), Kayeli Beach (Station 8), Masarete Beach (station 9) and Waelapia Beach (station 10).

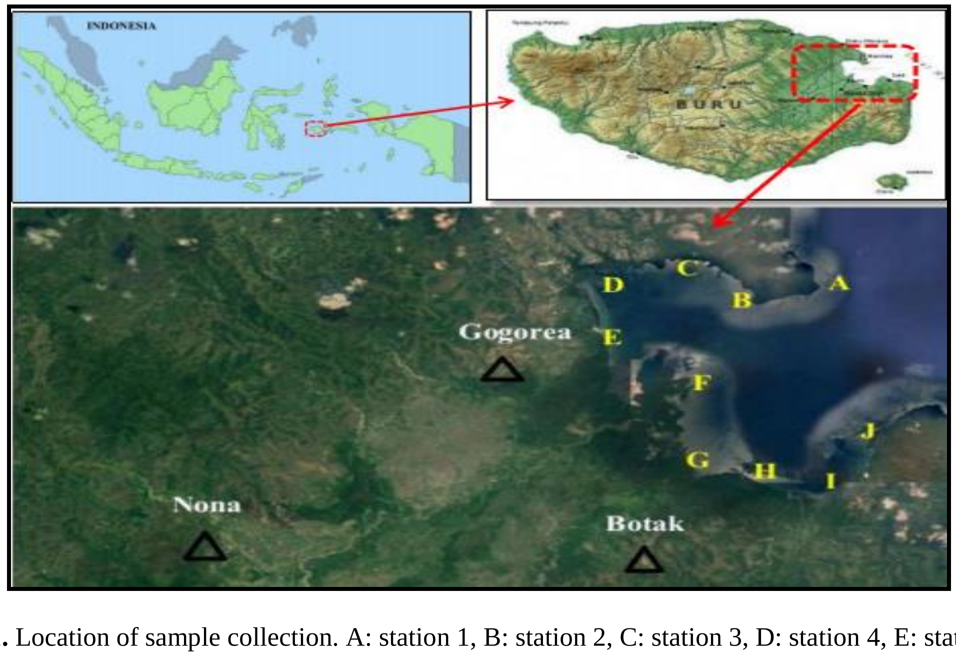

Figure 1. Location of sample collection. A: station 1, B: station 2, C: station 3, D: station 4, E: station 5, F: station 6, G: station 7, H: station 8, I: station 9, J: station 10.

Sample Collection

The samples of water, sediment, and specimens of *S. herrmanni* were taken from each station. The samples of *S. herrmanni* sampled randomly at each station as many as 3 individuals (Figure 1). After that, the samples were put into ethylene plastic and labeled. After that, the sample *S. herrmanni* was put into the icebox. And then, all samples were taken to the laboratory.

Sample Preparation and Analysis of Heavy Metals Hg, Au and Fe

The testing of heavy metals Hg, Au, and Fe for all samples were carried out at the Bandung Geological Survey Central Laboratory, Indonesia. The samples were prepared, reconstructed, and calibration curves were made and then analyzed using the Atomic Absorption Spectrophotometer (AAS) (Warnau et al., 2006). The heavy metal content in the sample (wet weight) was calculated by the equation:

$$ppm = \frac{a}{b}$$

a = the amount of metal μg from the measurement results with AAS

b = sample weight (5.0 g).

Histopathology of Intestine Tissue of *Stichopus herrmanni*

The histological preparations followed Ken (1985), with the following steps. (1) The sample was washed, then incised into the abdomen, and then the intestine was taken, (2) The intestine was put into 4% formalin and fixed for 24 hours, (3) After fixation, the tissue was dehydrated into alcohol at 70%, 80 %, 90%, absolute, xylol 1 and xylol 2 for 1 hour each, (4) Intestinal tissue was inserted into a vacuum to remove air from the tissue, (5) The tissue was printed with paraffin blocks, then thinly incised using a microtome , (6) The results of the incision were placed above the waterbath then lifted using a glass object and placed on a hot plate, (7) Then the tools and materials for coloring were prepared, including xylol 1, xylol 2, alcohol absolute, multilevel alcohol, Hematoxylin solution, eosin solution, (8) The results of the incision were arranged in a shelf, then put into xylol 1, xylol 2, absolute alcohol, 90% alcohol, 80% alcohol, 70% alcohol, hematoxylin solution, rinsed with flowing water for 1 week, eosin solution, rinsed with with flowing water for 1 week, 80% alcohol, 90% alcohol, absolute alcohol, xylol 1, xylol 2 each solution was put in for 3 minutes, (9) an adhesive solution was dropped into a glass object and then covered with a glass cover and observed under a microscope. Specifically for intestinal histopathological observations, researchers used one individual *S. herrmanni* to represent each station, so the total number of individuals used for histopathological observation was 9 individuals. Determination of the intestinal tissue damage of *S. herrmanni* used the Hematoxylin Eosin (HE) staining method carried out at the Zoology Laboratory, Faculty of Mathematics and Natural Sciences, Pattimura University.

Data Analysis

The obtained data were descriptively analyzed to describe the conditions of the water, sediment and the histology of the tissue of the body of *S. herrmanni* which had been exposed to heavy metals. Furthermore, to analyze the effect of concentration of heavy metals on the damage of the villi of *S. herrmanni* in Kayeli bay, the research data were analyzed using correlation analysis and multiple linear regression to determine the correlation and the effect of the independent variables (heavy metals) partially on the dependent variable (villous damage) with a significant level (α= 0.05). The type of correlation used is the Pearson correlation to analyze the relationship between heavy metal concentrations in water, sediment, intestines, and meat with villous damage. Damage to the intestinal villi of *S. herrmanni* was determined based on observations of the histological intestine that experienced necrosis on the surface of the villi. Intestinal necrosis is determined by the characteristics of unclear cell boundaries, and the loss of some or all of the tissue in the intestinal villi. Villi damage was focused on one villi cell in epithelial tissue, then observed 5 times of view and calculated damage in the form of necrosis of *S. herrmanni* is intestinal villi. Regression analysis was used to predict the concentration of heavy metal Hg against villous damage, while three-predictor regression analysis was used to predict the concentration of heavy metals Au and Fe in sediment, water, intestines, and meat against villi damage.

RESULTS and DISCUSSION

The Concentration of Heavy Metal in Kayeli Bay

The average measurements of heavy metals Hg, Au, and Fe on the sampled sediment, water, intestine and meat of *S. herrmanni* from Kayeli bay are presented in Table 1.

Table 1. The average concentration of heavy metals in the Kayeli bay

Station	Concentration of Heavy Metals (ppm)											
	Sediment			Water			Intestine			Meat		
	Hg	Au	Fe	Hg	Au	Fe	Hg	Au	Fe	Hg	Au	Fe
1	0.112	0.004	0.080	0.00008	0.0005	0.05	0.148	0.001	46	0.607	0.0005	11
2	0.154	0.006	1.690	0.00023	0.0001	0.06	0.156	0.003	111	0.812	0.0039	14
3	0.185	0.004	0.730	0.00008	0.0005	0.13	0.178	0.002	139	0.923	0.0012	17
4	0.148	0.003	0.520	0.00009	0.0003	0.03	0.155	0.001	121	0.615	0.0005	12
5	0.260	0.004	0.970	0.00008	0.0006	2.57	7.305	0.004	1974	2.467	0.0008	16
6	6.855	0.092	0.251	0.00880	0.0007	0.05	0	0	0	0	0	0
7	5.361	0.106	0.255	0.00023	0.0030	0.34	7.163	0.010	6510	14.48	0.0045	27
8	6.595	0.006	0.204	0.00008	0.0002	0.36	2.800	0.009	3603	10.922	0.0041	23
9	0.921	0.008	2.110	0.00008	0.0002	0.58	1.028	0.002	2474	6.310	0.002	22
10	0.133	0.003	1.360	0.00008	0.0020	0.10	0.043	0.001	51	0.404	0.0007	4
Average	2.0724	0.0236	0.817	0.000983	0.00081	0.427	1.8976	0.0033	1502.9	3.754	0.00182	14.6

The concentration of heavy metals in the sediment sample was Hg>Fe>Au, while in the water sample was found to be Fe>Hg>Au (Table 1). The concentration of Hg and Fe was the highest in the sediment sample. The concentration of Au in sediment and water was very low. The results of this study are also supported by the results of research by Asha et al. (2010) that the concentration of heavy metal Fe was found to be higher in sediments when compared to seawater in the Tuticorin coastal area. Abdel-Baki et al. (2011) reported that the concentration of heavy metal Hg was found to be higher in sediments by 14.7 ppb, whereas in seawater it was 0.26 ppb.

Praveena et al. (2008) state that heavy metals dissolved in water bodies or bound together with sediment layers are influenced by the nature of heavy metals, and physicochemical factors such as pH and salinity. High heavy metal concentrations in sediments are caused by sediments that are sedentary at the base as well as being a place to store concentrations of various xenobiotics including heavy metals (Chakraborty and Owens, 2014).

The concentration of heavy metals in intestinal and meat tissues varies. The concentrations of heavy metals in the intestinal and meat samples are Fe>Hg>Au (Table 1). The concentration of Fe found in the intestine of *S. herrmanni* was very high compared to the concentration of Fe in the meat of *S. herrmanni*. According to Warnau et al. (2006), the concentration of heavy metal Fe was found the highest in the intestine of Holothuria tubulosa. Meanwhile, the concentration of heavy metal Au in the intestines and meat of *S. herrmanni* was very low. The concentration of heavy metal in the tissue of *S. herrmanni* was influenced by trophic level processes in the food pyramid (Wang, 2002). Nott and Nicolaidou (1989) showed that the highest concentration of heavy metals was found in the digestive gland due to the presence of granular $Mg_3\ (PO_4)_2$ compounds that function as ligands to bind metal ions. Meanwhile, the concentration of heavy metals Cd, Cu, Pb, and Fe was in intestinal tissue very high when compared to that in other tissues (Al-Najjar et al., 2018). Several types of research also show that muscle is a tissue that accumulates lower concentrations of heavy metals when compared to other tissues such as the liver, gills, and intestines (Zhang et al., 2007; El-Moselhy et al., 2014; Annabi et al., 2017).

Tissue Damage of *Stichopus herrmanni* Due to the Concentration of Heavy Metals

Based on the accumulation of heavy metals in the intestine of *Stichopus herrmanni*, it was followed by observation of intestinal histology to find villous damage as a physiological response to the exposure of these heavy metals. Villi damage was observed in the form of necrosis (Figure 2)

The tissue damage is characterized by lysis of the intestinal villi of *S. herrmanni* (Figure 2). The lysis of the intestinal villi at each station was different in appearance. At station 1, it can be seen that the appearance of villi was still normal with epithelial cells lining the outside of the mucosa. Meanwhile, at stations 2, 3, and 4, villous damage was seen. At station 5, 7, 8 and 9, high damage to villi was observed, lysis which was characterized by the loss of some epithelial cells in the intestinal mucus layer. At station 10, the villi reappeared clearer than before, but it still had damage caused by lysis. This means that the concentration of heavy metals tends to increase damage marked by lysis in villi. The results of this research showed that concentrations of heavy metals in intestinal tissue were higher, which was influenced by the eating behavior and the food eaten by *S. herrmanni*. The type of food eaten by *S. herrmanni* is organic material that accumulates in sediments (Navarro et al., 2013). Moreover, the tentacles of *S. herrmanni* in the form of a shield (peltate) make it easier for sea cucumbers to eat sediments (Tehranifard et al., 2011). Jakimska et al. (2011) added that the highest metabolic activity occurs in duodenal organs and allows the biota to absorb the largest amount of metal with the assistance of binding proteins such as metallothionein.

The lysis of villi was calculated from the intestinal preparations of *S. herrmanni* taken from each sampling station (except station 6) (Table 2, Figure 3). The number of damaged intestinal villi of *S. herrmanni* where lysis occurred on each station in Kayeli Bay ranged from 5-15. The most damaged villi were found at station 7, and the least damaged villi were found at station 1. No species of *S. herrmanni* was found at station 6 so that no observation of damage villi was carried out at this station (Table 2, Figure 3).

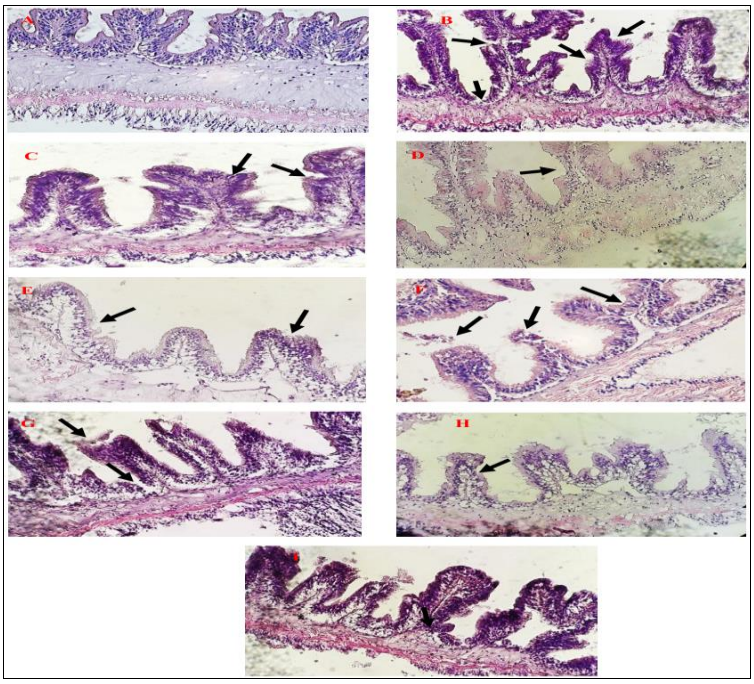

Figure 2. Results of the staining of the intestinal tissue *of S. herrmanni* by using Hematoxylin Eosin (HE) staining. Observations using an olympus microscope for shooting slides with 1000x magnification. Picture with notation: A) station 1; B) station 2; C) station 3; D) station 4; E) station 5; F) station 7; G) Station 8; H) station 9; and I) station 10. Note: black arrows indicate lysis of villi.

Table 2. The number of damaged villi of *S. herrmanni* at each station in Kayeli Bay

Station	The number of damaged villi					Total
	I	II	III	IV	V	
1	0	1	2	1	1	5
2	1	2	1	1	2	7
3	2	0	2	2	1	7
4	1	1	1	1	2	6
5	1	1	3	2	2	9
6			n.f			
7	3	2	3	4	3	15
8	2	3	2	2	3	12
9	3	1	2	3	2	11
10	1	2	0	2	1	6

Note: n.f: not found *S. Herrmanni*

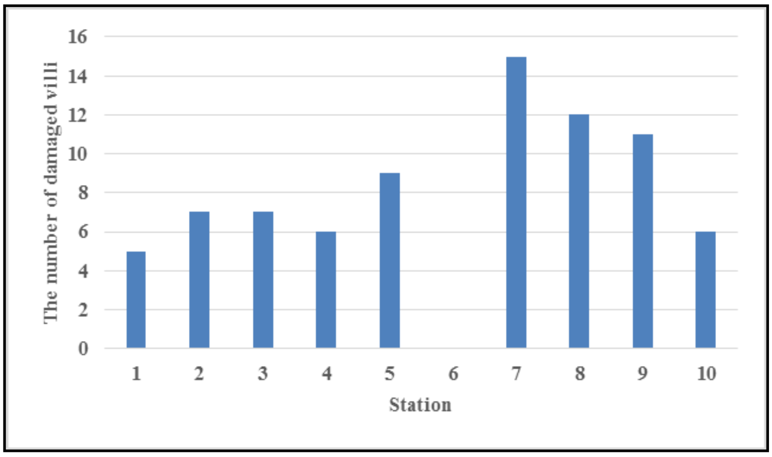

Figure 3. The number of intestinal villi of *S. herrmanni* which experienced cell damage at each station in Kayeli Bay.

At station 1, there were 5 damaged intestinal villi of *S. herrmanni*, while at station 2 and 3 there were 7 damaged villi. Station 4 had 6 damaged villi, and at station 5 there were 9 villi that experienced lysis (Figure 2, Table 2). Furthermore, station 7 had the highest lysis rate in villi reaching 15, followed by station 8 with 12 villi. The number of damaged villi decreased again at station 9 with 11 villi and continued to decline at station 10 with 6 villi experiencing lysis (Figure 2, Table 2). The results of this research showed that the intestinal cells of *S. herrmanni* were very sensitive to heavy metals concentrated in the waters of Kayeli bay. This caused lysis (necrosis) in the intestinal tissue. Sultana et al. (2016) explained that the stages of necrosis in intestinal cells began with an erosion of the intestinal mucosal layer, collection of cells that had undergone inflammation, swelling of the intestinal mucus layer, followed by bleeding, and the peak was cell degeneration and necrosis.

The Effects of the Concentration of Hg, Au, and Fe on the Intestinal Villi Damage of *S. herrmanni*

The results of the partial correlation analysis of the concentration of heavy metals in sediments, water, intestines and meat toward the damage of intestinal villi of *S. herrmanni* showed that several variables had a significant correlation with a heavy metal concentration in the intestine of *S. herrmanni*. Of the four variables tested, only the water factor did not have a significant correlation with the damage of the intestinal villi of *S. herrmanni* (Table 3).

Table 3. The results of the correlation analysis between the concentration of heavy metals in water, sediments, intestines, and meat with the damage to villi.

Variant sources		Pearson Correlation				Damage to Villi	
		Sediment	Water	Intestine	Meat	P. correlation	Sig.
Hg	Sediment	1				0.76	0.017
	Water	0.641	1			0.383	0.31
	Intestine	0.802	0.365	1		0.964	0.000
	Meat	0.959	0.537	0.935	1	0.896	0.001
Au	Sediment	1				0.862	0.003
	Water	0.628	1			0.425	0.254
	Intestine	0.932	0.419	1		0.882	0.002
	Meat	0.767	0.226	0.821	1	0.745	0.021
Fe	Sediment	1				0.852	0.004
	Water	0.004	1			0.217	0.575
	Intestine	0.769	0.207	1		0.973	0.000
	Meat	0.665	0.159	0.847	1	0.899	0.001

Heavy metals Hg, Au, and Fe found in sediments, intestines and meat had a positive correlation because of the sig. value $<\alpha= 0.05$, while heavy metals Hg, Au, and Fe found in seawater had a negative correlation because of the sig. value $>\alpha= 0.05$ (Table 3). If it is associated with how strong a correlation is, then the heavy metals in sediments, intestines, and meat partially have a strong correlation because the value of the correlation coefficient (P-correlation) is almost 1 (Table 3). According to Ahmed et al. (2018), heavy metals Hg that enter the marine ecosystems accumulate in sediments so that they can pose risks to benthic biota and further risks to consumers. This means that a high concentration of heavy metals in the sediment is correlated with the concentration of heavy metals in the intestine and meat. Madkour et al. (2011) reported that *Galatea paradoxa* is a species whose habitat is on the sedimentary water surface, thus heavy metal contamination in sediments can also contaminate tissue significantly.

On the other hand, partially sediment, intestine and meat factors have a strong correlation with the damage of intestinal villi of *S. herrmanni*, while the water factor is not correlated with the damage of intestinal villi of *S. herrmanni*. Thus, it can be concluded that the high concentration of heavy metals (Hg, Au, and Fe) in sediments has a correlation with the high concentration of heavy metals (Hg, Au, and Fe) in meat and intestines; and the high concentration of heavy metals (Hg, Au, and Fe) in the intestine has a correlation with the high concentration of heavy metals (Hg, Au, and Fe) on the intestinal villi damage. This research is supported by the research conducted by Asha et al. (2010) that the concentration of heavy metal Fe in sediments is found to be higher than the other heavy metals in sediment. The concentration in this sediment has a correlation with the concentration in bivalve animals. Another research conducted by Lin et al. (2018) on the sea cucumber *Acaudina leucoprocta* in the East China sea showed that the part that has very high concentrations of heavy metal Pb is the intestine and body compartment. This is because sea cucumbers eat by swallowing seawater and mud which has been contaminated by heavy metal Pb, besides the body wall of the sea cucumbers secretes sticky mucus which allows it to absorb heavy metals in the mud. This means that there is a correlation between the concentration of heavy metals in sediments and the concentration of heavy metals in the intestine of *S. herrmanni.*

Table 4. The results of the regression analysis on the effect of heavy metal concentration in water, sediment, intestine, and meat on the damage of villi of *S. herrmanni*

Variant sources		**Model Summary**			**Sig.**	**Equation**
		R	**R_{square}**	**$Sig.F_{change}$**	**Regretion**	
Hg	Sediment	0.965	0.935	0.012	0.689	Y= 6.100+0.001 (X3)
	Water				0.63	
	Intestine				0.56	
	Meat				0.741	
Au	Sediment	0.89	0.792	0.112	0.726	Y=8.610+0.008 (X1)+0.002 (X3)+0.016(X4)
	Water				0.948	
	Intestine				0.549	
	Meat				0.905	
Fe	Sediment	0.998	0.995	0.000	0.008	Y=8.610+0.008 (X1)+0.002 (X3)+0.016(X4)
	Water				0.146	
	Intestine				0.002	
	Meat				0.016	

The significance test of the multiple correlations from Table 4 obtained the value of R_{square}= 0.935 with a probability value (Sig. F_{Change}) of 0.012<α = 0.05. This shows that there is a significant effect on the damage of Villi. In addition, the regression equation obtained was (Y)= 6.100 + 0.001 (X3), this indicates that the damage of Villi was affected by the third factor, the heavy metal Hg factor in the intestine.

Metal Au shows that the simultaneous effect of sediment, water, intestine and meat factors on the damage of Villi has a correlation coefficient of 0. 890, R_{square}= 0.792 with probability value (Sig. F_{Change}) is 0.112>α = 0.05. This indicates that overall the metal Au does not have an effect on the damage of villi even though it has a big R-value.

The significance test of the effect of the concentration of metal Fe on the damage of intestinal villi of *S. herrmanni* (Table 4) shows that the effect of sediment, water, intestine and meat factors on the damage of cell has a correlation coefficient of 0.998, R_{square} value= 0.995 and probability value (Sig. F_{Change}) 0.000<α = 0.05. This indicates that there is a significant effect on the damage of intestinal villi. The regression equation obtained is (Y= 8.610 + 0.008 (X1) + 0.002 (X3) + 016 (X4). This indicates that the damage of villi is simultaneously affected by heavy metals concentrated in sediments, intestines, and meat. This test proves that the damage of the intestinal villi is caused by high concentrations of heavy metal Fe in the intestine and meat. Accumulation of heavy metals in the intestine is influenced by food sources. Jinadasa et al. (2014) explained that sea cucumbers could accumulate heavy metals through food consumption. Meanwhile, Kumar et al. (2012) added that Fe metal is one type of essential metal that functions as an enzyme cofactor in cell metabolism so that its concentration is found to be higher in organisms. The heavy metals belonging to non-essential elements have relatively lower concentrations, for example, Hg. This indicates that the heavy metal Fe is an essential element in the tissue of living organisms because it is a material for hemoglobin synthesis so that it increases higher in the tissue of *S. herrmanni* compared to heavy metals Hg and Au.

CONCLUSION

This research was conducted to provide information that heavy metals Hg, Fe, and Au on 10 stations in Kayeli Bay, Buru Island were concentrated on water, sediment, intestines, and meat of *Stichopus herrmanni*. The results showed that the highest Hg concentration in water was 0,000983 ppm, while the highest Fe metal concentration in sediment, intestine, and meat were 0.817 ppm, 0.427 ppm, and 1502.9 ppm, respectively. The accumulation of heavy metals in the intestine *S. herrmanni* caused damage to villi in the form of necrosis, the most severe villi damage was in *S. herrmanni* at station 7, and the least damage was at station 1. Correlation analysis showed that there was a positive relationship between heavy metals Hg, Au, and Fe in sediment, intestine, and meat, while heavy metals Hg, Au, and Fe in seawater have a negative relationship. Meanwhile, one predictor regression analysis showed that the factor that caused damage to villi was the concentration of heavy metal Hg in the intestine. Three-predictor regression analysis showed that the factors that caused damage to villi were the simultaneous effects of heavy metal concentrations of Fe on sediment, intestine, and meat. While the analysis of three predictors for heavy metal Au did not show the effect of damage to the villi, due to the low concentration of heavy metal Au. Heavy metal accumulations are toxic, accumulating in seawater, sediments, intestines, and meat from *S. herrmanni*.

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