

Spatial and Seasonal Distribution of Cadmium and Lead in Sediment, Water and Its Response of Metal Transcription Factor-1 in Cardinal Fish *Apogon beauforti*

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Abstract

Cadmium (Cd) and lead (Pb) are known to have high toxic effects and have been found polluting marine environment. Regular monitor the concentration of heavy metals in marine waters is necessary by combining chemical and biological analysis. This research was conducted to analyze the levels of heavy metals in sediment, water column, cardinal fish *Apogon beauforti*, and to measure the concentration of MTF-1 protein in *A. beauforti*. This research was conducted at four sampling stations at Ambon bay for 2 years. The levels of Cd and Pb metals were analyzed using AAS instruments (Atomic Absorption Spectrophotometer), while the concentration of MTF-1 protein in the body of *A. beauforti* was analyzed using indirect ELISA. The results showed that the seasonal and spatial distribution of heavy metals Cd and Pb at Ambon bay did not show any variation. The results of the ANOVA proved that the concentrations of heavy metals Cd and Pb in the four research stations were not significantly different in the year 2013 and in the year 2014 in the samples of sediment, the water column, and the body of *A. beauforti*. In addition, it was revealed that the concentration of MTF-1 protein of *A. beauforti* in 2014 was higher than that in 2013, with an average of the concentration of MTF-1 protein of *A. beauforti* was higher at station 2, which was at Ferry Galala Harbor.

Keywords: distribution, spatial, seasonal, cadmium, lead, MTF-1, *Apogon beauforti*

Introduction

The contamination of heavy metal in aquatic environments is a global concern today. Various human activities, such as industrial, urban and agricultural activities, which continue to increase along with the development of population, have resulted in high levels of heavy metals in the aquatic environment. Various types of heavy metals which get into the water system will settle into sediment, and capable of contaminating aquatic organisms. The amount of heavy metals absorbed by aquatic organisms will magnify and cause various diseases in humans (Yasran and Yipel, 2013).

Heavy metals of concern today are cadmium (Cd) and lead (Pb). Cadmium is a type of heavy metals that is widely used for the industrial activities of battery, dyes and plastics. Cadmium can be found in soils, sediments, air and water, and widely distributed in nature as a result of the erosion of rocks, soil erosion, and volcanic eruptions. The

average concentration of cadmium in the earth layer ranges from 0.1 to 0.5 ppm and mostly accumulated in sedimentary rocks. Waisberg *et al.* (2003) explained that although cadmium is basically found in nature, the contamination originating from human activity is much higher, 2-10 times, than that in nature. This means that most of the cadmium contained in the aquatic environment is from human activities. Cadmium is known to have high toxicity even in low doses, and known to have a long half-life 15-20 years in the human body (Benton *et al.*, 2011). Cadmium is known to disrupt a variety of physiological functions such as the immune system, and reproductive and developmental processes. Moreover, cadmium can cause genetic changes very quickly, so it is classified as carcinogenic agents in a number of tissues (Waalkes *et al.*, 2000).

Lead (Pb) is a type of heavy metal in small amounts in nature (Cheng and Hu, 2010). The presence of lead in nature mostly comes from the

activity of burning using fossil fuels, mining, and manufacturing industries. The use of lead in industrial activity, agricultural and household activities is a major source of contamination in aquatic ecosystems of the sea (Tchounwou *et al.*, 2012). Although the use of lead in industrial activity has been reduced substantially over the past few years, Jacobs *et al.* (2002) reported that approximately 25% of homes in the United States were contaminated with lead from the use of paint, dust and soil. Lead is known to cause a variety of negative effects on the nervous system, kidneys, immune system, reproductive and developmental system, cardiovascular system, and can cause abnormal behavior, deterioration of learning ability and intelligence (IQ) (Gillis *et al.*, 2012). Thus, efforts to address and monitor the presence of heavy metals such as cadmium and lead in the environment are required, particularly in the marine environment. It is because both heavy metals can be absorbed by marine organisms which are a source of food for humans.

The problem of heavy metal contamination on aquatic organisms and the bio-concentration has been the focus of the study of various disciplines. Alam *et al.* (2012) explained that the analysis of the biological response of aquatic organisms will be able to explain the water quality better than chemical analysis of the sediment and the water column. The measurement of heavy metals in the tissues of marine organisms is an indirect measurement on the content and the presence of heavy metals in the marine environment (Kucuksegin *et al.*, 2006). However, the selection of the organisms used as a monitoring instrument also needs attention. One of the organisms commonly used in monitoring heavy metal contamination is fish. Elevated levels of Cd and Cr in fish are significantly influenced by increasing abundance of parasites in the stomach cavities of fish samples (Oyoo-Okoth *et al.*, 2010). The concentrations of heavy metals in the gills of *Anodonta cygnea* and sediments show a decreasing sequence, ie Pb, Cu > Cd for gills, and Cu > Pb > Cd for sediments (Pourang *et al.*, 2010).

Research on Apogon species in the last decade found the fish spreads in various waters, namely in the waters of the island of Rhodes (Foka *et al.*, 2004), in Lake Sentani, Northern New Guinea (Umar and Makmur, 2006; Allen and Boeseman, 1982), in Indo-Pacific (Goren *et al.*, 2009). One type of fish that widely spreads in almost every area at Ambon bay is *A. beauforti* included in the family of Apogonidae, Actinopterygii class, phylum Chordata (Fraser and Lachner, 1985).

The fish can accumulate toxic materials, such as heavy metals, directly from other fish as well as the residues of contaminants with hundreds, even thousands of times of concentration in the water, sediment, and food (Labonne *et al.*, 2001; Goodwin *et al.*, 2003; Osman *et al.*, 2007). According to Weber *et al.* (2013), monitoring of heavy metal contamination in aquatic environments using the fish tissue may help explain the ecosystem quality. Heavy metals can get into fish tissue via four main routes, namely food, gills, water and skin. Adeyeye *et al.* (1996) explain that the concentration of heavy metals in various types of fish differs. This means that each species has the ability to accumulate heavy metals in different ways. A number of researches confirm that the ability of fish to accumulate heavy metals from the environment is affected by the concentration and duration of exposure, salinity, temperature and metabolism (Cusimano *et al.*, 1986; Karthikeyan *et al.*, 2007). Therefore, the fish is the most common organisms used in monitoring the presence of heavy metal contamination in sediment or to explain the problem of water quality (Barak and Mason, 1990; Mansour and Sidky, 2002).

The fish is able to accumulate high levels of heavy metals because the reaction is supported by physiological system which is able to detoxify heavy metals. It is known that various types of proteins are involved in the physiological reactions of heavy metal adaptation. One of the molecules that play an important role in the adaptation of heavy metal is metal-responsive transcription factor 1 (MTF-1). Protein MTF-1 is also known to have an important role in the process of embryonic liver development. Structurally, the MTF-1 protein has 6 elements of type C₂H₂ which is able to bind zinc. The C-terminal domain of the zinc finger is 3 different activation domains, which are, acidic, proline rich and serine/threonine rich domains. According to Daniels *et al.* (2002), all the three domains are very conservative and play a role in the regulation of heavy metals as well as the attachment of DNA (DNA-binding) in response to zinc. MTF-1 protein is usually found free in the cytoplasm and will be translocated into the nucleus when it is under pressure of heavy metals. The study is thus a contribution to the potential use of Metal Transcription Factor-1 in Cardinal Fish *A. beauforti* for evaluating the level of cadmium and lead pollution in marine environment.

Materials and Methods

This research is an exploratory survey to investigate the levels of Cd and Pb in water, sediment, and *A. beauforti* in the waters of Ambon

bay. This research was conducted in two different seasons namely in east season of 2013 and in the west season of 2014. The samples were taken by using purposive sampling, simultaneously to the sample of sediment, seawater and *A. beauforti*. The samples in 2013 were taken during the east season, which was in June and July. The samples in 2014 were taken during the west season in August and September. The samples were collected in four stations, namely Stasion 1 in Yos Sudarso harbor, Station 2 in Ferry Galala harbor, Station 3 in coastal waters of Passo village, and Station 4 in the coastal water of Waiyame village (Figure 1).

The data of the levels of heavy metals Cd and Pb in water, sediment, and *A. beauforti* at the four stations of data collection in Ambon bay was collected by picking out a small portion. The sample of sea water at the sea floor was collected by using a bottle with the volume of 3 liters. The samples of *A. beauforti* were collected by using nets (mesh 1 × 1 cm). 50 individuals of *A. beauforti* were collected. 50g of sediment was collected by using Ekman dredge. Each activity of picking out the samples was repeated three times in the east season and three times in the west season. The collected samples were then burnt into ash, and then the levels of metal of Cd and Pb were analyzed by using Absorbion Atomic Spectrophotometer (AAS) instrument (Bielymyer *et al.*, 2005) at the chemical laboratory of Brawijaya University.

The measurement of the concentration of MTF-1 protein by using the method of indirect ELISA (Enzyme Linked Imunoassay) was conducted at the Physiology Laboratory of Medicine Faculty of Brawijaya University following Lequin (2005). The

samples were prepared by smoothing the body of *A. beauforti* with thawing. After that, the testing process with ELISA reader was done, by making the ELISA plate layout and coating buffer based on the sample code and location of the sample. After that, Coating Antigen was performed with the levels 1:40 diluted with buffer coating and incubated at a temperature of 4°C overnight. The next day the plate was washed with a solution of 0.2% PBS Tween as much as 100 µl and repeated for 6 times. After that, 100µl of primary antibody anti MTF-1 (1:400) was added into the assay buffer. After that, the ELISA plate was incubated at room temperature for 2 hours while being shaken with ELISA plate shaker. In the next stage, the washing was done with a solution of PBS Tween 0.2% as much as 200 µl for 6 times, and then 100 µl anti-rabbit Biotin IGG secondary antibody (1:800) was added in assay buffer and incubated at room temperature for 1 hour while being shaken. After that, the plate was washed again with PBS Tween 0.2% for 6 times. After that, 100µl of SAHRP solution (1:800) was added into the assay buffer and incubated at room temperature for 1 hour while being shaken. After that, the solution was washed with PBS Tween 0.2% as much as 200µl for 6 times. And then, 100µl was added for each well substrate sure blue TMB microwell, incubated for 20-30 minutes in a dark room. At this stage, if there was a reaction between the antigen and the antibody, the solution would turn blue. Next, 100µl of HCl 1N was added as the stopping reaction. At this stage, the solution which was formerly blue turned yellow. After that, the sample was read by using an ELISA reader at a wavelength of 450 nm. The results of absorbance were then converted to a standard curve and so that the levels of MTF-1 of each sample can be found out.

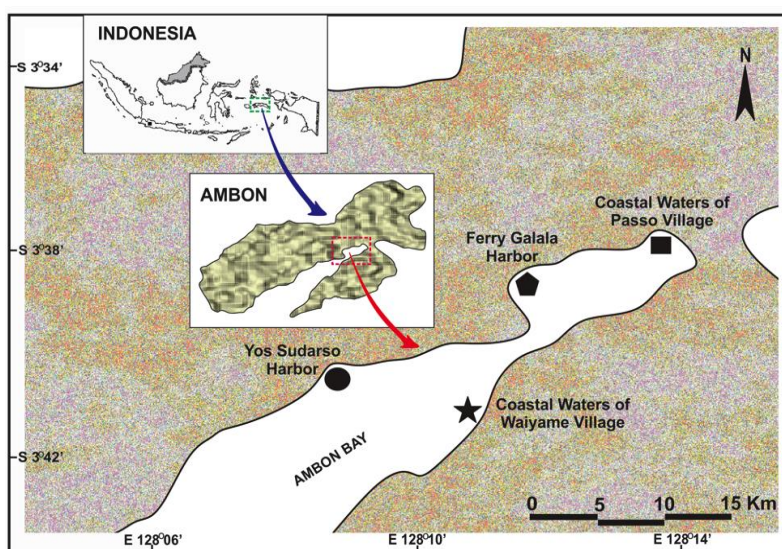


Figure 1. Research location with four stations, 1) Yos Sudarso harbor, 2) Ferry Galala harbor, 3) coastal waters of Passo village, and 4) coastal water of Waiyame village

Results and Discussion

Spatial and seasonal distribution of cadmium

The results showed that the average concentration of heavy metals cadmium was high in the sediment, body of *A. beauforti* and sea water (Table 1). This pattern was seen in all sampling stations, namely station 1 (Yos Sudarso harbor), station 2 (Ferry Galala harbor), station 3 (Coastal water of Passo Village), and station 4 (Coastal water of Waiyame Village). The concentration of cadmium in July was higher than in June. In 2013, the average concentration of heavy metals cadmium appeared to be higher in the sediment at station 1 with an average of 0.59 ± 0.02 (ppm) in June and increased to 1.39 ± 0.02 (ppm) in July. The concentration of cadmium was also high in the body of *A. beauforti* during the sampling in June. A high concentration was also observed at station 2 with an average of 0.56 ± 0.02 (ppm) and was still higher than sediments 0.39 ± 0.01 (ppm). Furthermore, in July, the concentration of heavy metals cadmium seemed to be even in the sediment in the four stations. However, the highest concentration average in station 3 was 1.42 ± 0.01 (ppm) and station 1 was 1.39 ± 0.02 (ppm). The results of the analysis also showed that the average concentration of heavy metals in the body of *A. beauforti* was higher than that in the water column, which was relatively low. This means that the heavy metal cadmium accumulates in the sediment and the body of *A. beauforti*.

In August 2014, the concentration of cadmium in the body of *A. beauforti* collected at station 3 with an average of 1.83 ± 0.09 (ppm) was higher than another three stations with an average of 2.39 ± 0.12 (ppm) at station 4, 1.83 ± 0.09 (ppm) at station 2 and 1.83 ± 0.09 (ppm) at station 1. This pattern changed in September 2014. The highest concentration average of cadmium was found in the sediment at station 4 with an average of 1.82 ± 0.09 (ppm). It is followed by station 1 with an average of 1.81 ± 0.02 (ppm). The concentration of cadmium at stations 2 and 3 showed a different pattern. The concentration of cadmium more accumulated in the body of *A. beauforti* from station 3 and 2 were 1.65 ± 0.09 and 1.61 ± 0.09 (ppm), respectively.

On the other hand, there was a significant difference in the cadmium levels of sediments, water column, and body of *A. beauforti* ($P < 0.05$). The results of LSD also showed that the average of the concentration of cadmium found in sediments was higher and significantly different from that found in the water column. Similarly, the concentration of cadmium in the body of *A. beauforti* was significantly different from that found in the water column. The average of the concentration of cadmium in sediments was not significantly different from that in the body of *A. beauforti*. The variable of sampling station difference did not have any effect on the difference of cadmium concentration. There was no significantly different ($P = 0.971$) which means that the levels of heavy metals in all sampling stations were the same.

Table 1. Concentration of Cadmium (Cd) in sediment, water and cardinal fish *Apogon beauforti* in 2013 and 2014 (ppm)

Month	Stations	Sediment (ppm)	Water column (ppm)	<i>Apogon beauforti</i> (ppm)
June (2013)	St. 1	0.59 ± 0.02	0.19 ± 0.01	0.23 ± 0.02
	St. 2	0.39 ± 0.01	0.26 ± 0.01	0.56 ± 0.02
	St. 3	0.16 ± 0.01	0.25 ± 0.01	0.28 ± 0.01
	St. 4	0.30 ± 0.01	0.27 ± 0.01	0.10 ± 0.02
July (2013)	St. 1	1.39 ± 0.02	0.05 ± 0.00	0.87 ± 0.01
	St. 2	1.31 ± 0.01	0.07 ± 0.00	0.81 ± 0.02
	St. 3	1.42 ± 0.01	0.07 ± 0.00	1.18 ± 0.01
	St. 4	1.26 ± 0.02	0.08 ± 0.00	1.29 ± 0.03
August (2014)	St. 1	1.29 ± 0.06	0.16 ± 0.01	1.46 ± 0.06
	St. 2	0.35 ± 0.02	0.10 ± 0.00	1.83 ± 0.09
	St. 3	0.40 ± 0.04	0.05 ± 0.00	2.54 ± 0.04
	St. 4	1.07 ± 0.02	0.01 ± 0.00	2.39 ± 0.12
September (2014)	St. 1	1.81 ± 0.02	0.30 ± 0.00	1.39 ± 0.09
	St. 2	1.30 ± 0.06	0.31 ± 0.00	1.61 ± 0.09
	St. 3	1.15 ± 0.06	0.35 ± 0.00	1.65 ± 0.09
	St. 4	1.82 ± 0.09	0.29 ± 0.00	1.34 ± 0.03

Note: St. 1 = Yos Sudarso Harbor, St. 2 = Ferry Galala Harbor, St. 3 = coastal water of Passo Village, St. 4 = coastal water of Waiyame Village

These results also showed that the distribution of cadmium does not always follow the sources of contamination. Station 1 and 2 were the harbor area with a high activity, making it one of the sources of heavy metal contamination. The oil and gas coming out from the marine transportation are a major source of cadmium in station 1 and station 2. Lu *et al.* (2007); Storelli and Marcotriggiano (2001) explains that the oil and gas from transportation activities are a source of heavy metal contamination in the water. Although the activity of sea transportation was high at station 1 and station 2, cadmium also could spread to other areas away from the main source of contamination. It was proven by the high concentration of cadmium at Station 3 and Station 4. Cadmium is a type of heavy metal that easily spreads in the aquatic environment. Mwashote (2003) explains that cadmium has a high mobility nature compared with the other types of heavy metals, such as lead. The heavy metal cadmium has a tendency to separate from the complex dissolved inorganic and organic materials, which are relatively stable at a neutral pH. The spread of cadmium is also influenced by climate and subsequently attached to the mineral deposits containing iron hydroxide and organic substance (Lai *et al.*, 2002; Namieśnik and Rabajczyk, 2010).

The results of the analysis also showed that cadmium more accumulated in sediment and the body of *A. beauforti* than water column. According to Chen *et al.* (2012), cadmium has a low solubility in aqueous solution, so that it is more easily suspended in particle form. Therefore, cadmium accumulated more, so that the concentration in the sediment reflected the level of water pollution. It is also in line with the explanation by Muohi *et al.* (2003) stating that the sediment acted as a container for pollutants contaminating the water. As a result, the sediments containing pollutant contaminants may affect the concentrations of heavy metals in the water column and marine biota. Trujillo-Cárdenas *et al.* (2010) explains that the migratory behavior and bio-concentration of heavy metals is determined by the way in which the metal ions are distributed in a particulate or liquid form. Thus, not only is the sediment contamination transport media, but also it is a secondary source of contamination in the water system. The changes in environmental conditions such as pH, redox potential and the concentration of metal in solution can cause heavy metals to experience remobilization of sediment and pose a risk to the environment. In addition, factors such as tides, storms and human activities such as dredging and fishery can also cause remobilization of sediment containing heavy metal contaminants (Eggleton and Thomas, 2004; Nascimento *et al.*, 2006).

Spatial and seasonal distribution of lead

The results showed that the lead more accumulated in sediment and in the body of *A. beauforti* than in the water column (Table 2). In contrast to cadmium, lead was higher in station 1 (Yos Sudarso Harbor) followed by station 2 (Ferry Galala Harbor), station 4 (Coastal area of Waiyame Village) and station 3 (coastal area of Passo village) respectively. The concentration of lead in 2013 was generally higher than in 2014. In June 2013, the highest concentration of lead was seen in the sediment at Station 1 with an average of 67.60 ± 0.06 (ppm). Similarly, in July 2013, the highest concentration of lead in sediments was found in station 1 with an average of 6.62 ± 0.04 (ppm) and in station 2 with an average of 6.37 ± 0.02 (ppm). The concentration of lead was higher in the body of *A. beauforti* than in the water column. The high concentration of lead was found in the body of *A. beauforti* which was collected from station 1 and station 2. The higher concentration occurred in July 2013, where the amount that accumulated in the body of *A. beauforti* was collected in station 2 as much as 1.15 ± 0.04 (ppm) and stations 1 as much as 1.08 ± 0.20 (ppm).

The same pattern was also observed during the sampling in 2014. The concentration average of lead was seen to be higher in sediment at station 1 and station 2 than station 3 and station 4. A higher concentrations was seen in August, where the average of lead in the sediment at Station 1 was at 15.23 ± 0.12 (ppm), followed by station 4 with an average of 1.63 ± 0.09 (ppm). In September, a higher concentration was also observed in the sediment at Station 1 with an average of 3.89 ± 0.09 (ppm), followed by station 2 with an average of 3.54 ± 0.08 (ppm). The concentration of lead was also still higher accumulated in the body of *A. beauforti* than in the water column. In August, a higher concentration was observed in *A. beauforti* which was collected at station 1 with an average of 1.75 ± 0.18 (ppm) followed by station 4 with an average of 1.21 ± 0.07 (ppm). In September, the concentration of heavy metal lead was observed higher in the body of *A. beauforti* which was collected at station 3 with an average of 1.74 ± 0.07 (ppm) followed by station 2 with an average of 1.36 ± 0.06 (ppm). On the other hand, the results of ANOVA showed that there was not any significant difference in the levels of lead in all four sampling stations ($P = 0.450$). Similarly, the variable of the source of accumulation where F_{count} of X_2 variable was 1.743 with a significance level of 0.253. Thus, it can be concluded that there were not any variations in the spatial and seasonal distribution of heavy metal lead. Although it did not show any statistically significant differences, visualization of lead

distribution showed that the level of concentration was very high in station 1 (Yos Sudarso Harbor) on the sampling in June 2013 and in August 2014 (Figure 3).

The results of this research show that the spatial distribution of lead is relatively narrow. Lead is more deposited in the surrounding sediments of the sources of contamination such as station 1 and station 2. Both sampling stations were an area of harbor with high transportation activities. The contamination of heavy metal may come from combustion and paint of ships in both observation

stations (Tchounwou *et al.*, 2012). Mwashote (2003) explains that the lead is a type of heavy metal that has low mobility compared to cadmium. According to Ward *et al.* (1986), the spatial distribution pattern of heavy metals can be explained in the context of the distance from the main source of pollutants. This means that the further the source of pollutants the smaller the concentration of lead. Moreover, the spatial distribution is also determined by the nature of the heavy metal. Muzuka (2007) and Maggi *et al.* (2006) also found that heavy metals lead accumulated more in the harbor area with high transportation activities.

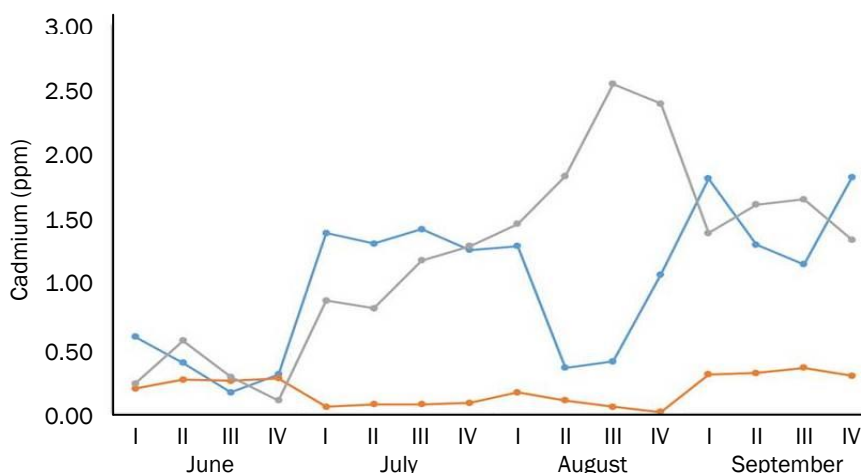


Figure 2. Spatial and seasonal distribution of Cadmium in sediment, water and *Apogon beauforti* at Ambon bay
 Note : ● = sediments; ● = water column; ● = *A. beauforti*; I = the first week; II = the second week; III = the third week; IV = the fourth week,

Table 2. Concentration of Lead (Pb) in sediment, water and body of *Apogon beauforti* at Ambon bay in 2013 and 2014 (ppm)

Months	Stations	Sediment (ppm)	Water column (ppm)	<i>Apogon beauforti</i> (ppm)
June (2013)	St. 1	67.60 ± 0.06	0.18 ± 0.01	1.04 ± 0.06
	St. 2	3.48 ± 0.06	0.12 ± 0.00	1.77 ± 0.11
	St. 3	0.19 ± 0.01	1.51 ± 0.68	0.68 ± 0.24
	St. 4	1.06 ± 0.05	0.30 ± 0.01	1.21 ± 0.07
July (2013)	St. 1	6.62 ± 0.04	0.05 ± 0.01	1.08 ± 0.20
	St. 2	6.37 ± 0.02	0.10 ± 0.01	1.15 ± 0.04
	St. 3	3.25 ± 0.79	0.13 ± 0.01	0.52 ± 0.15
	St. 4	2.99 ± 0.05	0.14 ± 0.00	0.56 ± 0.06
August (2014)	St. 1	15.23 ± 0.12	0.08 ± 0.01	1.75 ± 0.18
	St. 2	1.05 ± 0.08	0.01 ± 0.07	0.63 ± 0.00
	St. 3	1.08 ± 0.14	0.07 ± 0.01	0.65 ± 0.15
	St. 4	1.63 ± 0.09	0.04 ± 0.01	1.27 ± 0.16
September (2014)	St. 1	3.89 ± 0.09	0.08 ± 0.00	0.92 ± 0.05
	St. 2	3.54 ± 0.08	0.01 ± 0.00	1.36 ± 0.06
	St. 3	1.27 ± 0.16	0.08 ± 0.00	1.74 ± 0.07
	St. 4	2.34 ± 0.07	0.04 ± 0.00	1.01 ± 0.03

Note: St. 1 = Yos Sudarso Harbor, St. 2 = Ferry Galala Harbor, St. 3 = coastal water of Passo Village; St. 4 = coastal water of Waiyame Village

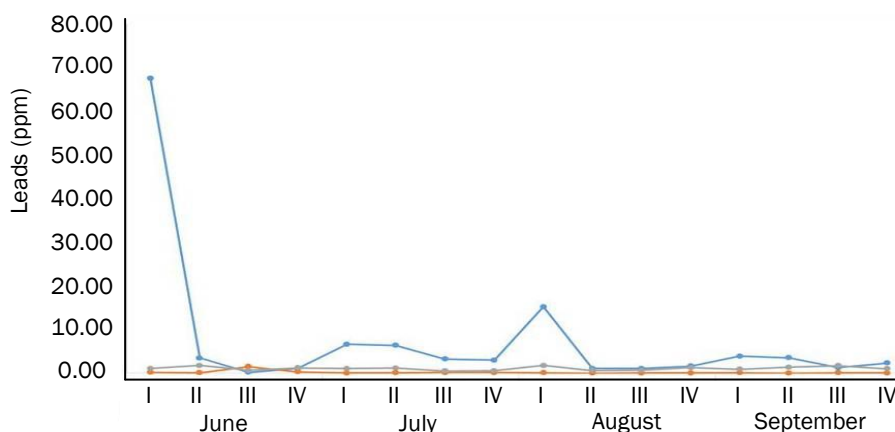


Figure 3. Spatial and seasonal distribution of Lead in sediment, water and *Apogon beauforti* at Ambon bay
 Note : ● = sediments; ● = water column; ● = *A. beauforti*; I = the first week; II = the second week; III = the third week; IV = the fourth week,

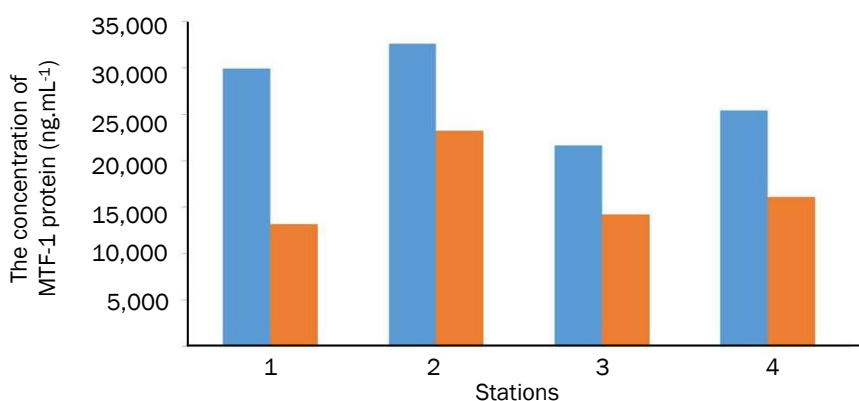


Figure 4. The concentration of MTF-1 protein of cardinal fish *Apogon beauforti*
 Note. ■ = 2013; ■ = 2014

The expression of MTF-1 protein

The analysis results of the concentration of MTF-1 protein showed a pattern similar to the distribution of heavy metals (Figure 4). The highest expression of MTF-1 protein was expressed by *A. beauforti*, which was collected from station 2 with an average of 32,600 ng.mL⁻¹. Although it seemed to decrease on sampling in 2014, the concentration of MTF-1 protein in *A. beauforti* which was collected from station 2 was still higher (23,210 ng.mL⁻¹) than in the other observation stations. The concentration of MTF-1 protein was also high at station 1 with an average of 29,940 ng.mL⁻¹. The concentration of MTF-1 protein of *A. beauforti* which was collected at station 1 seemed to decline in 2014 with an average of 13,160 ng.mL⁻¹.

On the other hand, the results of ANOVA showed that there was a significant difference in the concentration of MTF-1 protein in 2013 and 2014, where F_{count} of season variable (year) was 29 266

with a significance level of 0.000. The concentration average of MTF-1 protein in 2013 was higher than that in 2014. Similar results were also seen at the observation station variable. F_{count} of station variable was 4.507 with a significance level of 0.007. It shows that there was a significant difference in the

This results of this research show that the concentration of MTF-1 protein was higher in the areas having high levels of heavy metals, such as at station 1 and station 2. Both of the sampling locations were the areas of harbor. Having high activities of transportations, these two research stations were also known to contain high levels of heavy metals cadmium and lead in the sediment. The high concentration of MTF-1 protein is a physiological response of *A. beauforti* against the pressure of heavy metals from the environment. Günther *et al.* (2012) explains that the MTF-1 acts as an intracellular metal sensor, so that an organism can adapt to the pressure of heavy metals or other various environmental stresses,

such as hypoxia or oxidative stress. The higher expression of MTF-1 in the area of pollutant sources (station 1 and station 2) means that this protein has the potential to be used as a biomarker in monitoring the heavy metals in aquatic ecosystems. According to Blackmore and Wang (2004), the use of biomarkers, such as biochemical or physiological component, is indispensable as an *early warning system* to monitor the destructive effects that may be caused by some pollutants such as heavy metals.

Conclusion

There is no significant variations in the seasonal distribution of the heavy metals cadmium and lead in Ambon bay. The average concentration of cadmium and lead was not significantly different between 2013 and 2014. There is no significant variation in the spatial distribution of heavy metals cadmium and lead in Ambon bay. The concentration average of heavy metals of the four stations, Yos Sudarso Harbor (Station 1), Ferry Galala Harbor (Station 2), coastal area of Passo Village (Station 3) and coastal area of Waiyame Village (Station 4) was not significantly different. There is a significant difference in the concentration of heavy metal cadmium in sediment, water column and the body of cardinal fish *Apogon beauforti*. The concentration of heavy metal cadmium was highest in sediment and followed by *A. beauforti*, water column. There is a significant difference in the concentration of MTF-1 protein in 2013 and 2014. The concentration of MTF-1 protein was higher in 2014 than 2013. There was also a significant difference in the concentration of MTF-1 protein at the four sampling stations. The higher concentration average of MTF-1 protein was Ferry Galala harbor.

Acknowledgment

The researcher was very grateful to the Head of the Chemistry Laboratory and the Head of the Physiology and Histology Laboratory Faculty of Medicine, University of Brawijaya that has provided facilities for the author to conduct this research until the final stages of writing this article, and to DP2M Higher Education Ministry of Education and Culture who has Provided the funding for the Researchers to conduct this research using a grant with the contract number: 02/UN13.2/SP-HB/VII /2013.

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