Bioaccumulation of heavy metals (Cd, Pb, and Hg) in *wawo* worms (Polychaeta, Annelida) from Ambon Coastal Waters, Indonesia

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Heavy metals contamination in coastal waters is an environmental problem that has recently received serious attention. The accumulation of heavy metals in aquatic organisms can cause cell damage in humans who consume them. One of the organisms that can absorb heavy metals is the *laor* or *wawo* worm (Polychaeta) which is often consumed by the people in Ambon. This research was conducted to analyze heavy metals bioaccumulation in these worms. The heavy metals (Cd, Pb, and Hg) content in the marine waters and *wawo* worms was measured using AAS (Absorption Atomic Spectrophotometer). The results of the present research indicate fluctuation in heavy metals distribution in the water columns and *wawo* worms which were collected from three different locations. There was a significant relationship between heavy metals content in the water columns and the *wawo* worms. The coefficient of determination (0.424) showed that 42.4 % of heavy metals contained in the marine worms were affected by the heavy metals content in the water meanwhile the rest (57.6 %) was regulated by other variables that were not included in the current investigation.

[**Keywords***: Ambon island; Bioaccumulation; Heavy metals; *wawo* worms]

Introduction

Heavy metals contamination in water ecosystems is a serious environmental issue[1]. It can be a result of either natural phenomena or anthropogenic activities. Human activities such as the increased number of industries are the main factor that leads to environmental contamination by heavy metals. According to Perez-Lopez *et al*.[2], industries can produce 100-1000 times higher heavy metals than the layer of earth and when heavy metals are accumulated into the vital organ of the organism, it can damage the organ[3]. Most of the heavy metals will pollute and bring over an impact to aquatic organisms[4-8] and the diversity of marine organisms[1,9]. Heavy metals can be accumulated in all organisms that live in the contaminated environment[6,8,10]. So, contaminated marine organisms can infect humans who consume those[11]. Bazzi[12] showed that there is a correlation between heavy metals content in seawater, sediments, and organisms. Therefore, heavy metals accumulation has become a major concern in environmental research.

Some heavy metals that are found in marine waters ecosystems include cadmium (Cd), lead (Pb), mercury (Hg) and nickel (Ni)[13]. High concentration of heavy metals such as cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn) has been found in fish, squid, and shellfish on the coast of Jeddah Saudi Arabia[14]. In Ambon coastal area, Hg has been accumulated in *Apogon beauforti*[15,16]. Yunus *et al*.[17] explains that heavy metals Pb and Cu originate from anthropogenic activity, in another site these heavy metals were found to be quite high in the mangrove forests of Tanjung Lumpur, Malaysia. Another research by Zagoot *et al*.[18] proved that fish in the coastal areas of Gaza contained zinc (the highest), cobalt and cadmium (the lowest). These heavy metals which are accumulated in marine organisms can be very dangerous for humans once they are consumed.

The heavy metals accumulation process is affected by a number of factors. Metals can be absorbed by a living organism through the skin, or seep into it while it feeds on something or breathes[19]. In fact, the concentration of heavy metals contained in an organism's body highly depends on its habit of looking for food and ways to survive[20,21]. The heavy metals accumulation process in a tissue or an organ is controlled by detoxification and metabolism mechanisms. Therefore, the concentration of the heavy metals varies within organisms. Chiarelli & Roccheri[22] explains that excretion mechanism, accumulation route, and number of heavy metals

found in an environment also affect the bioaccumulation process. Heavy metals exist in waters because a food chain allows interaction between organic and inorganic substances[23]. There is always a chance that these heavy metals can be absorbed by an organism and the amount of the accumulated metals. It can exceed the total number of heavy metals that should be removed from the body through the excretion system. One of the marine organisms that are known to accumulate heavy metals is *wawo* worms (Polychaeta).

Laor or *wawo* worm is an endemic biota to Maluku coast. During the full moon, at the night or the days after in March or April, *wawo* worms usually swarm around the surface of the water for doing *ex-vivo* fertilization. This phenomenon is more popular with the term swarming. People usually make use of this opportunity to catch the worms for high-nutrient diet. Research on *wawo* worms was pioneered by Horst[24] who successfully identified *Lysidice oelo* (Eunicidae) in the coastal area of Ambon. The other related studies have been conducted on common topics such as *wawo* worms' spawning and habitats[25], taxonomy[26], species richness and macro-nutrition content[27], genetic and phylogenetic[28,29], identification and keys to the orders, also families and genera of these worms[30,31]. In contrast, there has not been a lot of research discussing *wawo* worms' ability to accumulate heavy metals. The aim of this study to analyze the bioaccumulation of heavy metals (Cd, Pb, and Hg) in *wawo* worms collected from three different coastal areas on the island of Ambon. This research is important because the *wawo* worms are used by the community in Ambon island as food, so the health hazards caused by consuming *wawo* worms (that have accumulated heavy metals) could be avoided.

Materials and Methods

This research is an exploratory survey that aims to reveal the concentration of heavy metal bioaccumulation in seawater samples and *wawo* worms (Polychaeta) from the coastal waters of Ambon island. The samples were collected from three research sites between 03º-42 S' and 128º-14' E: the village of Alang coastal waters (Station 1), Latuhalat coastal waters (Station 2), and Rutong coastal waters (Station 3) with purposive sampling technique (Fig. 1).
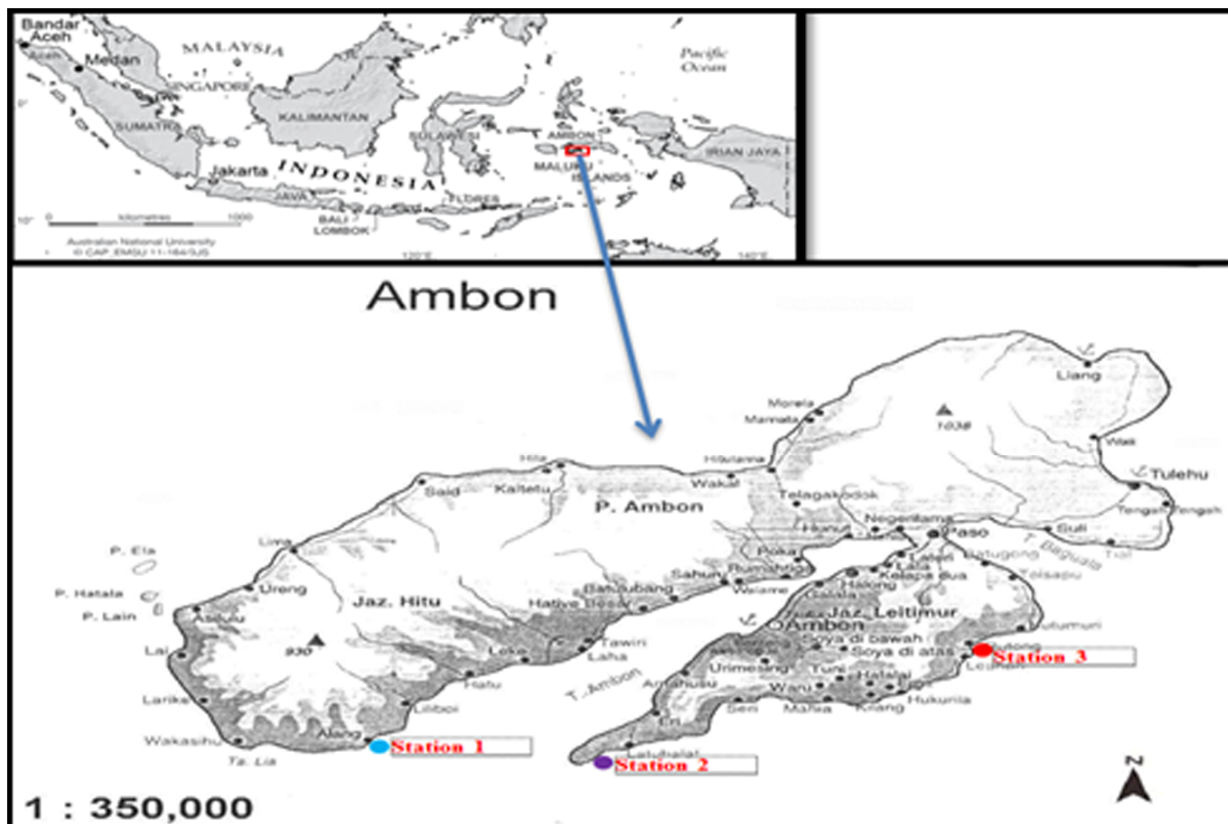

Fig. 1 — Map of research sites (Note: ● station 1, ● station 2, ● station 3)

Samples of seawater and *wawo* worms collected from each location, placed in labeled polyethylene bottle for analysis of metal concentrations of Cd, Pb and Hg[32].

Water samples (2L) were collected from surface water (a depth range of about 0-100 cm) using a Vandorn water sampler, then filtered by using filter paper from Whatman 7184-004 with cellulose membrane. The filtered water sample was stored in a polyethylene bottle and preserved with concentrated HNO_3 to a pH <2. Next, a water sample (300 ml) was fed into the Teflon split funnel, then extracted with APDC/NaDDC/MIBK. After that, it was re-extracted with HNO3. The sample was left for 20 minutes, plus 9.75 ml of distilled water and stirred. The extraction results in the water phase were taken and stored in a polyethylene bottle.

The steps to determine the metal concentration in the *wawo* worm samples are described as follows. The *wawo* worm sample was cleaned with distilled water and then cut into small pieces and dried using a pre-heated oven at 105 °C for 20 hours and followed by 80 °C for 72 hours to equalize sample weight. After that, the sample was destroyed and weighed 500 mg. The sample was digested with HNO_3 solution-$HClO_4$ and heated to a temperature of 100-200 °C until white steam came out, and the volume was measured. Next, concentrations of Cd, Pb and Hg in water samples and *wawo* worms were analyzed using AAS (Atomic Absorption Spectrophotometer)[33] at the chemistry laboratory of Brawijaya University. The research data will be analyzed descriptively to know the concentration of heavy metals in seawater samples and *wawo* worms. In addition, an inferential statistic would be conducted to investigate the correlation between the concentration of heavy metals in a water column and *wawo* worms body.

Results and Discussion
Description of the Heavy Metals Concentration

The average concentration of cadmium (Cd), lead (Pb), and mercury (Hg) in the coastal waters of Ambon Island are presented in Table 1. The results of the analysis on the water column and *wawo* worms collected from three research areas proved that cadmium concentration (Cd) and lead (Pb) is lower than mercury (Hg), however in the coastal waters of Alang village no mercury was found in water samples (0.00 ppm).

The results of heavy metals concentration in Table 1 showed that coastal areas of Alang contained

Table 1 — The average concentration of heavy metals in samples

Sampling Location	Heavy Metal	Water Column (ppm)	*Wawo* worms (ppm)
Alang	Cd	0.08 ± 0.01	1.36 ± 0.03
	Pb	0.03 ± 0.00	1.82 ± 0.06
	Hg	0.00	7.30 ± 1.15
Latuhalat	Cd	0.24 ± 0.01	1.21 ± 0.02
	Pb	0.07 ± 0.01	1.62 ± 0.05
	Hg	5.00 ± 0.28	6.81 ± 4.13
Rutong	Cd	0.09 ± 0.01	2.26 ± 0.01
	Pb	0.09 ± 0.01	1.54 ± 0.11
	Hg	8.80 ± 0.57	6.48 ± 1.53

the highest concentration of cadmium (0.08 ± 0.01). It was followed by lead with an average concentration of 0.03 ± 0.00. There was no sign of mercury in the water column. However, the highest concentration of mercury could be detected in *wawo* worms (7.3 ± 1.15 ppm). The worms also carried out a high concentration of lead (1.82 ± 0.06 ppm) and cadmium (1.36 ± 0.03 ppm). As described by Renieri *et al.*[34] that the increasing concentrations of cadmium (Cd), lead (Pb) and mercury (Hg) could be due to their propensity to accumulate in the fish tissue. Additionally, Ahmed & Bat[35] described that the high concentration of Cd and Pb in the fish body is affected by the continuous exposure of the source of pollution.

In Latulahat area, the highest concentration of mercury was reported in water column (5 ± 0.28 ppm) and *wawo* worms body (6.81 ± 4.13 ppm), respectively. Meanwhile, the concentration of cadmium in the water column (0.24 ± 0.0 ppm) was lower than that in *wawo* worms body (1.21 ± 0.02 ppm). Lead concentration in the water column (0.07 ± 0.01 ppm) was lower than the concentration in *wawo* worms body (1.21 ± 0.02 ppm).

The concentration of heavy metals in Rutong's coastal waters is shown in Table 1. Mercury had the highest concentration among all (8.8 ± 0.57 ppm in water columns and 6.48 ± 1.53 ppm in *wawo* worms body), followed by cadmium (0.09 ± 0.01 ppm in water columns and 2.26 ± 0.01 ppm in *wawo* worms body) and lead (0.09 ± 0.01 ppm in water columns and 1.54 ± 0.11 ppm in *wawo* worms body).

The results of this study showed that the highest concentration of heavy metals was found in the body of the *wawo* worms in Alang and Latuhalat coastal waters as compared to seawater (Table 1 & Fig. 2). The low concentrations of heavy metals in water caused by most heavy metals including Cd, Pb, and
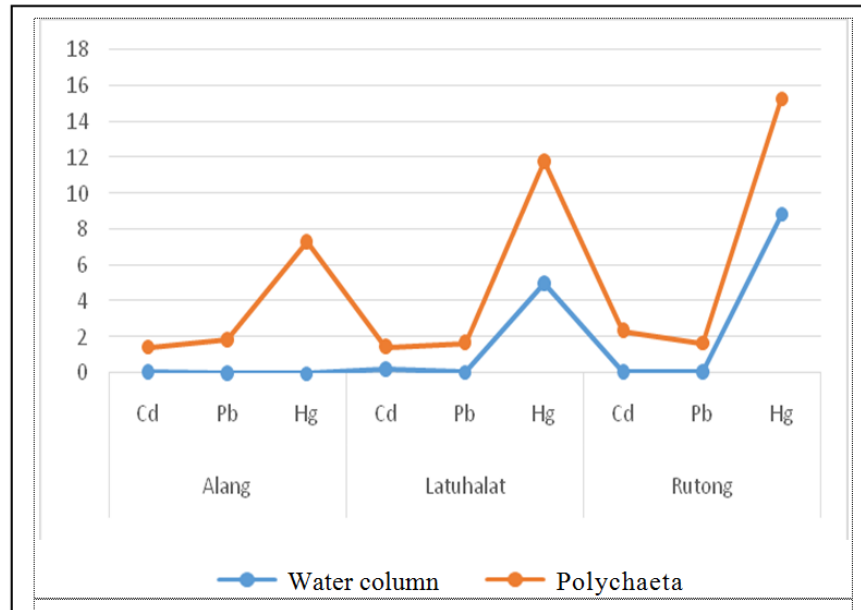
Fig. 2 — Heavy metal concentration in water columns and *wawo* worms body

Hg were derived from the environment. They generally accumulate in the *wawo* worm making it a representative to record the accumulation of heavy metals in marine waters. According to Rumahlatu[36], the presence of heavy metals in the *wawo* worm comes from natural processes such as abrasion of the river and community activities, such as market disposal and household waste, as well as the activity of repairs and painting of vessels in the waters of Ambon Island then carried by the water and accumulated to various aquatic biotas. Furthermore, Nasehi *et al*.[37] explains the high accumulation of heavy metals in fish because of the correlation between the presence of high content of bioavailable metals and concentration in the fish body. This indicates that the accumulation of heavy metal in *wawo* worm can be used as an instrument for monitoring the quality of marine waters.

The results in Figure 2 indicate fluctuation in heavy metals concentration in samples collected from three different locations. This data also shows that the water columns and *wawo* worms body contain relatively high cadmium and mercury. Cadmium is a heavy metal with wide distribution. It can be found in the dust with an average concentration of 0.1mg/kg. The highest concentration of cadmium has been observed in rocky sediments and sea phosphate (15mg/kg). Cadmium has also been used in industries such as ones that produce batteries and dye[38]. Distribution of cadmium in sea waters might come from organic

materials decomposition, deep-sea demineralization, and phytoplankton absorption[39]. Cadmium is known to be dangerous especially for organisms that accumulate it in their body and can harm the tissues by stimulating an oxidative pressure, changing the DNA composition and structure, and also inhibiting cell transport mechanisms. High accumulation of Cd in *Diadema setosum* performs a control mechanism by expressing MTF-1 protein[40]. Cadmium is also able to prevent heme synthesis from occurrence and encourage mitochondrial apoptosis[41]. Shaari *et al*.[42] explained that the various metal content in soft tissue can be caused by the affinities of different metals that bind with proteins metallothioneins.

In another site, mercury is more perilous as it is frequently found in many oxidized forms such as inorganic or organic salt. The instances of the oxidized forms of mercury are Hg^0, mercurous (Hg^{+1}) or mercuric (Hg^{+2}). All these mercury forms are toxic to many organisms. Mercury is usually found in liquid form (pressure = 0.00185mm at 25 °C). This metal is very volatile and harmful[43,44]. The biological, pharmacokinetic, and clinical properties of mercury are different from each other[43]. According to the form of the Hg compound, the toxicity of mercury in humans is categorized into inorganic and organic. An adult who suffers from an inorganic Hg poisoning will experience tremors on face muscles, fingers, and arms. If these symptoms continue in metabolism, the adult will feel his tongue trembling. It will be difficult

for him to speak and walk due to a loss of balance. Organic Hg also can cause deadly toxicity. The methyl mercury on Minamata disease that afflicted both adults and children in Japan is an example of a disaster caused by mercury toxicity. The central nervous system is the target of methyl mercury toxicity. Mercury can also cause paresthesia, decreased sensitivity, stiff fingers and toes, limited sight, decreased hearing power, and pain in the arms and thighs. The patients can also experience motor neurological disorders which result in difficulties to stand up and speak ataxia, tremor, and slow motion. Other related symptoms are mental disorders, terrible headaches, and hypersalivation[45].

The results of the analysis also showed the concentration of lead (Pb) in the water columns and *wawo* worms. Despite its natural formation, lead distribution in sea waters is mostly affected by anthropogenic factors. Various industrial activities such as the use of high temperatures in goods production are the source of lead contamination, not to mention, the use of fossil fuels, radioactive, and gas. Usually, the concentration of lead in the open waters is much lower and less dangerous for humans; however, it still indicates that humans are the culprit[46]. Lead is a poisonous heavy metal. It can damage the humans nervous system, affect the hematopoiesis process in a human's body, and destroy the kidney slowly, corrupt humans reproductive and central nervous system[47,48]. These heavy metals are also known as one of the sources of oxidative pressure which can bring an impact on the human's physiological system.

Based on the results of the analysis, it is also obvious that mercury has contaminated the *wawo* worms body. The concentration of this heavy metal inside the marine animal has surpassed the maximum mercury threshold in foods (1 mg/kg)[49]. Therefore, it can be concluded that *wawo* worms collected from three research sites are not allowed for human's regular consumption. Menon & Mahajan[50] reports that aquatic organisms such as fish that have accumulated Hg, although have low to moderate risks are not feasible for consumption.

The Correlation of Heavy Metals Concentration in a Water Column and *Wawo* Worms Body

The results of an analysis of the correlation of heavy metals concentration in a water column and *wawo* worm's body are summarized in Table 2. The table shows that, with a significance level of $0.005 < \alpha$,

Table 2 — The results of the correlation analysis in brief

R	R Square	Change Statistics				
		R Square Change	F Change	df1	df2	Sig. F Change
.651[a]	.424	.424	5.159	1	7	.005

the correlation between those two variables is positive and significant (0.651). The coefficient of determination (0.424) also indicates that 42.4 % of heavy metals concentration in *wawo* worm's body is determined by the concentration of heavy metals in the water column where the worms dwell. The regression formula which can be generated from the constant (2.505) and the coefficient of water column regression coefficient [(X = 0.651) is Y = 2.505 + 0,651(X)]. If the independent variable remains unchanged, the average heavy metals concentration will equal 2.505. If the concentration of heavy metals in a water column changes by one unit, the concentration of heavy metals in *wawo* worms will increase by 0.651. In other words, the increased concentration of heavy metals in a water column results in inflating the concentration of heavy metals in *wawo* worms body.

Trends in the current research are similar with Mwashote[51] and Tirkey[52] who discovered that the concentration of cadmium (Cd) and lead (Pb) is generally lower in water columns than in sediments or an organism body. Joksimović & Stanković[53] suggested that heavy metals concentration in sea waters has a positive correlation with heavy metals concentration in an organism's body. Therefore, it can be concluded that heavy metals found in the *wawo* worms body could possibly come from the seawater. Due to direct contact made by marine animals with the seawater, bioaccumulation of heavy metals is frequently used as an instrument to monitor the quality of the aquatic environment. Heavy metals found in an organism's body tissue are an indication of a contaminated environment[54]. Heavy metals that exist in nature are mostly particulate or soluble[55]. Unstable heavy metals in particular are the most toxic.

Conclusion

The results of the data analysis have shown that the concentration of heavy metals examined (Cd, Pb, and Hg) in water columns and *wawo* worm bodies fluctuate from one place to another. The results of the correlation analysis also indicate a significant relationship between the two variables (heavy metals concentration in water columns and *wawo* worms

body). The calculated determination coefficient (0.424) even suggests that 42.4 % of heavy metals found in a *wawo* worms originates from the water columns while the rest (57.6 %) is determined by other variables which were not studied in this research. Future researchers, thus, have an opportunity to investigate the concentration of heavy metals in sediments as well as the effects of physical and chemical factors on *wawo* worms' ability in absorbing heavy metals and the rate of the heavy metals absorption. The results of this study indicate that the accumulation of heavy metals in the *wawo* worm can be used as an instrument to monitor seawater quality.

Acknowledgement

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