Detection of organochlorine pesticide residues in seawater and sediments of Manado Bay

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Detection of organochlorine pesticide residues in seawater and sediments of Manado Bay

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Abstract. The purpose of this study was to determine the type and concentration of organochlorine pesticides contained in sea water and sediments in the waters of Manado Bay. Sampling was carried out at three stations, namely Station 1 - in front of Malalayang Terminal, Station 2 - Megamas, Station 3 - Tumumpa. Samples were analized using the Gas Chromatography Mass Spectrometry method. The results of the analysis found that the type of organochlorine pollutants was the aldrin type in which the highest concentration was found in the substrate at the Megamas location at 1.020 ppm then followed by the substrate at the Tumumpa location at 0.049 ppm and finally the substrate at the Malalayang location at 0.49 ppm. The concentrations in seawater samples were at Tumumpa 0.925 ppm, then followed by 0.899 ppm at the Megamas location and finally at Malalayang location 0.792 ppm. The aldrin concentration for the substrate found at Megamas station and for the water samples found at Tumumpa location had passed the allowable quality limit (0.0624 ppm).
Key Words: organochlorines, pesticides, sediment, sea water, concentration.

Introduction. The waters of Manado Bay in Manado City are one of the coastal areas that are the mainly support of the city. The coastal area of Manado Bay has a length of approximately 18,000 m with a coastal depth between 0.5 and 2000 meters (BAPPEDA 2014). This area is surrounded by 5 rivers namely the Tondano, Bailang, Sario, Bahu, and Malalayang rivers.

Organochlorine pesticides are persistent organic pollutants (POPs) that have slow biodegradation and mobility in both biotic and abiotic environments (Breivik et al 2016; Lui et al 2016). In coastal and marine environments, contamination of pesticide usually comes from rise field activity (Firozjaee et al 2018). In general these pesticides will experience a long journey before arriving in coastal areas (Anasco et al 2010; Gong et al 2020; Hassan et al 2020). Almost all pesticide residues will undergo physicochemical degradation during their passage into the marine environment (Hua et al 2020). Increased pollutant concentrations are generally a consequence of sewage disposal, urban storm water, and agricultural and industrial runoff (Haynes & Johnson 2000).

In North Sulawesi Province, the pollution became the main issue after the floods that carried the surface material into the waters. This has caused side effects for the life of aquatic organisms. The life of fish and other aquatic organisms was badly affected by waters contaminated with pesticides (Junqué et al 2018; Kucuksezgin et al 2016). Pesticide runoff transporting large expanses of water will have deadly consequences for aquatic life and can kill large numbers of fish (Aguayo-Quiroz et al 2020; Trukhin & Boyarova 2020). The use of organochlorines in water can kill the fish when the dead plants rot and the decay takes a lot of oxygen in the water, making it difficult for the fish to breathe (Mahugija et al 2017; Manavi et al 2018). Also polluted water can kill the food of aquatic plants that support fish habitats, which will lead to a reduction in fish populations. Organochlorines in waters can accumulate in the long term and are able to

kill zooplankton, the main food source for small fish (de Vargas et al 2020). Some fish eat insects; the death of fish due pesticides can cause difficulty in obtaining fish as food for human being (Tankiewicz et al 2010; Milun et al 2020). The objective of the research was to determine the organochlorine pollutants in Manado Bay of North Sulawesi Province.

Material and Method

Sampling sites. This research was conducted from June to September 2020 in Manado Bay waters (Figure 1). Samples collected included sea water and sediment. Samples of seawater and sediment were taken at three stations, namely station 1 (Malalayang waters), station 2 (Megamas waters), station 3 (Tumumpa waters).

Water samples were gathered as much as 2 liters using a lamut bottle, then put into a glass bottle and immediately stored in an ice box. Laboratory analysis was carried out at the Pharmacy laboratory of the Faculty of Mathematics and Natural Sciences. Sediment was taken using Ekman grab made of stainless steel. The sediment sample was then put into a clean glass bottle. And the water samples were put into a glass bottle that has been cleaned then closed tightly with aluminium foil coated. The aluminium foil coated glass bottle was then sealed tightly to avoid contamination. Then it was put in a cool box with a temperature below 40°C during transportation to the laboratory (Baird et al 2017).

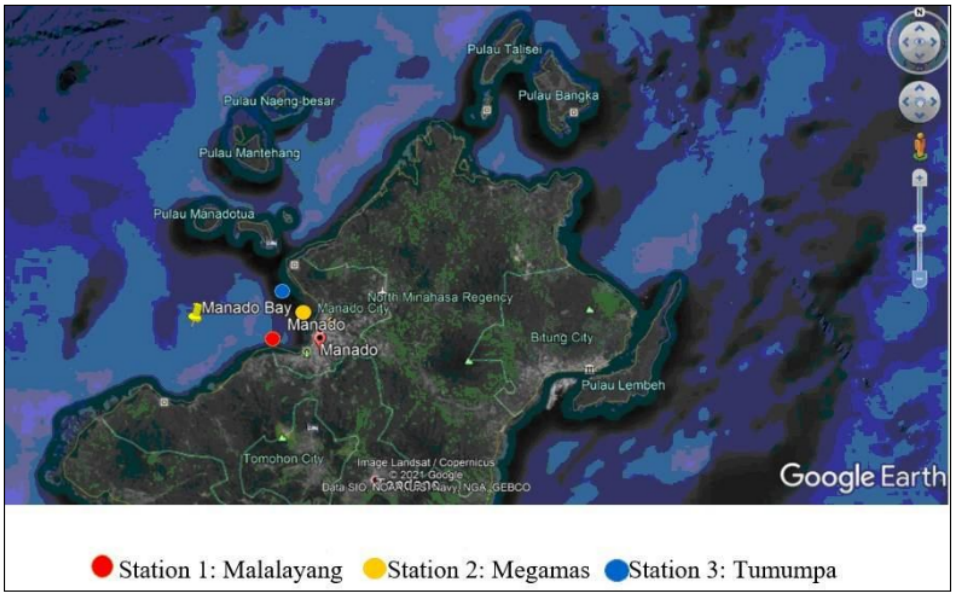

Figure 2. Map of Manado Bay.

Sample analysis. Samples were analysed using Shimadzu UV-Vis Spectrophotometer, glassware, Shimadzu AY 220 analytical balance, 0.45 µm porous Whatman filter paper. The materials used are potassium dihydrogen phosphate (Merck analysis pro), ammonium molybdate (Merck pro analysis), otsuka aquabides, ascorbic acid (Merck technical), bismuth subnitrate (Merck technical), perchloric acid (Merck pro analysis), acetonitrile (pro analysis Merck), hydrochloric acid (Merck technical), nitric acid (Merck technical) (Baird et al 2017).

The sediment and water samples taken were dried by leaving them at room temperature for approximately 48 hours. The dry sample was weighed as much as 10 grams and then added with Chlorophyrifos. Then the sample was dissolved in 50 mL of

acetone and N-hexane as a solvent with a ratio of 1:1. The sample was stirred for about 10 minutes until dissolved. The funnel and glass woll were prepared then anhydrous Na_2SO_4 was added to the glass woll. Then the sample was filtered. The filter results were taken then reconstituted with acetone and N-hexane and filtered again to get optimal results. After being filtered, extraction was carried out using gas nitrogen so that the solvent can be separated until achieve up to 2 mL. The reagent material used was 10,000 ppm ascorbic acid, ammonium molybdate 0.12 M, perchloric acid 5 M, bismuth subnitrate 1460 ppm.

Preparation of KH_2PO_4 standard solution 439.4 ppm. KH_2PO_4 was weighed as much as 0.2197 g then put into a 500 mL volumetric flask and dissolved with aquabidest until a concentration of 439.4 ppm was obtained. From this concentration (439.4 ppm), 0.5; 0.75; 1; 1.25; 1.5 and 1.75 mL were taken using a measuring pipette then put in a 50 mL volumetric flask and diluted with aquabides until concentrations of 5; 7.5; 10; 12.5; 15 and 17.5 ppm were obtained.

Maximum wavelength selection. Determination of the maximum wavelength is done to find out at what wavelength it produces the maximum absorption value in the sample, so that the measurement results are accurate and minimize errors. The UV-Vis spectrophotometer is a tool used to measure the absorption resulting from the chemical interaction between electromagnetic radiation and molecules or atoms of a chemical substance in the UV-Vis region. The tools used in this experiment were the 20D + spectrophotometer and the UV-VIS Shimadzu 1700 PC spectrophotometer. The maximum wavelength was determined with 0.001 M $KMnO_4$ and 0.01 M $K_2Cr_2O_7$ solution in the 400-700 nm wavelength range. The maximum wavelength obtained is used to measure the concentration of the standard solution and the sample. The k values of the measured $KMnO_4$ solution at wavelengths 535 (20D + spektronik) and 525.6 (Uv-Vis Shimadzu 1700 PC) are -424.7 and 586, respectively. The k value of the K2Cr2O7 measurement solution at a wavelength of 430.0 20D +) and 431.0 (Uv-Vis Shimadzu 1700 PC) was 100.3 and 83.86, respectively.

The standard solution with a concentration of 10 ppm was taken as much as 1 mL and added with 2.5 mL perchloric acid, 1 mL ammonium molybdate, 2 mL bismuth subnitrate, 5 mL ascorbic acid using a measuring pipette then put in a 50 mL volumetric flask and diluted with distilled water to the mark, then the absorbance was read at a wavelength of 400-800 nm.

Operating time. The standard solution series with a concentration of 10 ppm was taken as much as 1 mL and added with 2.5 mL perchloric acid, 1 mL ammonium molybdate, 2 mL bismuth subnitrate, 5 mL ascorbic acid using a measuring pipette then put in a 50 mL volumetric flask to be diluted with aquabides up to the mark. The absorbance was read at minute 1, 5, 10, 15, and 20 at the maximum wavelength.

Standard curve creation. About 1 mL from the concentration series of standard solutions (5,10,15,20 and 25 ppm) was taken and added with 2.5 mL perchloric acid, 1 mL ammonium molybdate, 2 mL bismuth subnitrate, 5 mL ascorbic acid using a measuring pipette, put in a 50 mL flask and dilute with aquabides to mark lines. The solution was let stand for operating time then read the absorbance using a UV-Vis spectrophotometer at the maximum wavelength. From the results of absorbent data, then a standard curve is created to achieve an equation of y = a + bx.

Precision. Precision is a measure that indicates the degree of conformity between individual test results, measured through the distribution of individual results from the mean if the procedure is applied repeatedly to samples drawn from a homogeneous mixture.

Precision is measured as standard deviation or relative standard deviation (coefficient of variation). It is expressed as repeatability or reproducibility. About 1 mL of standard solution with a concentration of 12.5 ppm was taken and added with 2.5 mL

perchloric acid, 1 mL ammonium molybdate, 2 mL bismuth subnitrate, 5 mL ascorbic acid using a measuring pipette then put in a 50 mL volumetric flask and diluted with aquabides until mark lines. The solution was let stand for operating time, then the absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength. This procedure was repeated 6 times.

Recovery. Recovery can be established by making a placebo sample (drug excipient, biological fluid) then adding a certain concentration of analyte (usually 80 to 120% of the estimated analyte level), then analyzed by the method to be validated. The sample was powdered and then 10 g of sample was made duplo with the same weight. For the first sample it was not added with standard solution while the second sample was added with 1 mL of KH_2PO_4 standard solution of 12.5 ppm into Erlenmeyer, then the desired substance was taken using a solvent (acetonitrile: aquabides 6.5:3.5) after which it was filtered. Then 100 mL of filtrate was added with 25 mL of HCl. Furthermore, it was decrypted for 2 hours with 5 mL of nitric acid repeatedly until the solution was clear, then filtered. Furthermore, 1 mL of the solution was taken and added with 2.5 mL of perchloric acid, 1 mL of ammonium molybdate, 2 mL of bismuth subnitrate, 5 mL of ascorbic acid using a measuring pipette then put in a 50 mL volumetric flask and diluted with aquabides to the mark. The solution was let stand for operating time, then the absorbance was read using a UV-Vis spectrophotometer at a maximum wavelength for three times. The absorption results were used to calculate the recovery value. The average value of analyte recovery is between 80 and 120% (Gandjar & Rohman 2007).

Organochlorine value setting. The sediment powder was weighed 10 g, then the desired substance was taken using a solvent (acetonitrile: aquabides 6.5:3.5) after which it was filtered. Then 100 mL of filtrate was added with 25 mL of HCl. Furthermore, it was decrypted for 2 hours with 5 mL of nitric acid repeatedly until the solution was clear, then filtered. The sample solution was taken 1 mL and added with 2.5 mL perchloric acid, 1 mL ammonium molybdate, 2 mL bismuth subnitrate, 5 mL ascorbic acid using a measuring pipette then put in a 50 mL volumetric flask and diluted with aquabides to the mark. The solution was let stand for operating time then read the absorbance using a visible spectrophotometer at a maximum wavelength of 722 nm (Gandjar & Rohman 2007; Aamir et al 2017).

Results and Discussion. The results of the analysis using a spectrophotometer found that the type of organochlorine pollutants was the aldrin type. The highest concentration was found in the substrate of Megamas location at 1.020 ppm then followed by the substrate of Tumumpa location at 0.925 ppm and finally the substrate of the Malalayang location at 0.492 ppm. In water the highest concentration of aldrin was detected at Tumumpa, followed by Megamas and then Malalayang (Table 1). The concentrations both in substrate and water had exceeded the threshold of the criteria for assessing the quality of water. According to Baird et al (2017), the acceptable concentration of aldrin in water is 0.004 ppm. Based on LD50, the level of dieldrin toxicity is in the category of "extremely toxic" (LD50: 1 to 50 mg kg^{-1}), while DDT, endosulfan, and lindane are "highly toxic" (LD50: 51 to 500 mg kg^{-1}). The very toxic category is kepone, heptachlor, mirex while in category toxic includes endrin, aldrin, chlordane, and toxaphene. The least toxic includes methoxychlor, perthane, kelthane, chlorobenzilate, and hexachlorobenzene (Unyimadu et al 2018). The potential for acute harm can be sorted (highest to lowest) as follows: endrin, aldrin, dieldrin, chlordane, toxaphene, kepone, heptachlor, DDT and methoxychlor (Pillay 2013; Montory et al 2017; Trukhin & Boyarova 2020). Standard curve on absorbance obtained through UV-Vis spectrophotometer analysis at maximum wavelength was Y = 0.113x + 0.186 (R^2 = 0.988) (Figure 2).

Table 1
Organochlorine concentration of substrate and water samples from Magamas, Malalayang and Tumumpa sites

No.	Samples	Replication			Mean (ppm)
		1	2	3	
1.	Megamas substrate	1.01415	1.03185	1.01415	1.02004
2.	Malalayang subtrate	0.47433	0.49203	0.50973	0.49203
3.	Tumumpa substrate	1.01415	1.03185	0.73097	0.92565
4.	Tumumpa water	1.01415	1.03185	0.73097	0.92565
5.	Malalayang water	0.78407	0.92566	0.66902	0.7929
6.	Megamas water	0.75752	0.92566	1.01415	0.89911

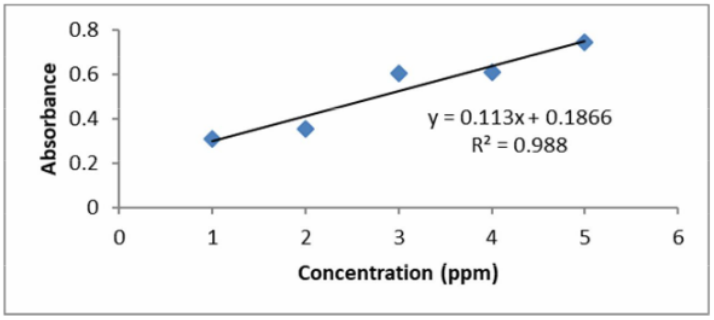

Figure 2. Absorbance value of samples obtained from UV-Vis spectrophotometer analysis at maximum wavelength.

Organochlorine pesticides are persistent organic pollutants (POPs) that have slow biodegradation and mobility in both biotic and abiotic environments (Nemr & El-Sadaawy 2016; Burkow & Kallenborn 2000; Mwevura et al 2020). However, until now there is not known the possibility of pesticides contaminating the coastal and marine environment produced from rice fields. Normally these pesticides will experience a long journey before arriving in coastal areas (Añasco et al 2010; Olisah et al 2019; Unyimadu et al 2018).

Organochlorine insecticides were widely used in the mid-1940s to mid-1960s as insecticides for the control of malaria-carrying mosquitoes and termites. Since organochlorines have been found to persist in the environment and accumulate in a wide variety of organisms, including humans, their use has been dramatically reduced. Many organochlorine compounds have been banned for use in the United States, and the Environmental Protection Agency has restricted the application of other people's applications. One exception is lindane (gamma-hexachlorocyclohexane), which is an insecticide and pharmaceutical preparation used topically as a scabicide and pediculicide (Mason et al 1996; Porter et al 2018). Pesticides from this group are non-biodegradable pollutants, so they tend to stay in the environment for a long time. In addition, the possibility to experience bioaccumulation and biomagnification in the environment is also large (Zhao et al 2009; Wang et al 2015; Aguayo-Quiroz et al 2020).

Conclusions. The organochlorine pollutant found in Manado Bay was aldrin type. The highest concentration in the substrate was detected at Megamas station and the lowest was at Malalayang station. In water sample, the highest concentration was found at Tumumpa station while the lowest was at Malalayang station. Overall, the concentration of aldrin in subtrate and water at Manado Bay had exceeded the threshold concentration.

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Conflict of interests. None reported.

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