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Application of symbion spons bacteria as a bioindicator of heavy metal contamination in the waters of manado bay

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Abstract
A study was conducted on the use of symbiont sponge bacteria as bioindicators of heavy metal contamination in the waters of Manado Bay. The objective of this research was to determine the accumulation levels of heavy metal pollution in Manado Bay waters by utilizing symbiont sponge bacteria as bioindicators. The research method employed was descriptive, two laboratories were involved: the biological laboratory for isolating symbiont sponge bacteria and the chemistry laboratory for harvesting, destruction and heavy metal analysis. Sponge samples were collected from three sites. Symbiont bacteria were taken from the surface and inner parts (flesh) of the sponge and cultured on nutrient agar for 2 x 24 hours. Heavy metal analysis was performed using Atomic Absorption Spectrophotometry (AAS) with standards for Pb (SNI 06-6989.8-2004), Zn (SNI 6989.7:2009) and Hg (SNI 6989.78:2011). The results showed that Pb content at the three sampling points was very high, both on the sponge surface ranging from 52.125 µg/g to 86.959 µg/g and inside the sponge ranging from 63.316 µg/g to 87.622 µg/g. Zn levels were also high at all sampling points with surface concentrations ranging from 8.053 µg/g to 8.464 µg/g and inner sponge concentrations ranging from 9.703 µg/g to 9.589 µg/g. In contrast, Hg levels were comparatively lower than Pb and Zn with surface values ranging from 0.042 µg/g to 0.254 µg/g and inner sponge concentrations between 0.036 µg/g and 0.050 µg/g. Based on these data, it can be concluded that symbiont sponge bacteria can be used as effective bioindicators of heavy metal content in the waters of Manado Bay.

Keywords: Sponges, simbion bacteria, bioindicators, heavy metals

Introduction
Sponges (Porifera) are simple multicellular invertebrates that inhabit depths of up to 50 meters below the sea surface and attach to solid substrates [1]. Sponges are known to produce a variety of secondary metabolites with cytotoxic, antibiotic, anti-inflammatory and antiviral properties [2, 3]. As filter feeders, sponges can filter water and surrounding materials through pores (ostia) on their body surface [4]. This filter-feeding characteristic also allows sponges to host various symbiotic bacteria [3]. Symbiont bacteria reside on the sponge surface (outer layer) as well as intracellularly (within host cells or nuclei). These bacteria use the sponge body as a habitat and a place of protection [5]. Manado Bay serves as the estuary for several rivers, including the Bailang, Maasing and Tondano Rivers. River water entering Manado Bay carries various wastes from household sources, such as laundry and latrine effluents, waste disposal sites, transportation facilities and industrial activities [6]. These conditions have led to contamination of Manado Bay waters by pollutants, including heavy metals [6, 7]. This study aims to analyze the concentrations of heavy metals Pb, Zn and Hg in symbiotic microorganisms within sponges to provide information on heavy metal pollution in the waters of Manado Bay.

Research Methodology
Sampel Preparation in Nutrient Broth
Sponge samples weighing 15 g were rinsed with 30 ml of physiological NaCl solution, 20 ml of the rinse water were then transferred into 20 ml of sterilized Nutrient Broth. The mixture was shaken using a shaker at room temperature for 24 hours. The rinsed sponges were ground using a mortar and pestle until finely homogenized. Physiological NaCl solution was added and the mixture was placed into 20 ml of Nutrient Broth. It was shaken using a shaker at room temperature for 24 hours.

Bacterial Cultivation on Nutrient Agar
Isolates from the inner and outer parts of the sponge were separately inoculated on Petri dishes containing Nutrient Agar and incubated at room temperature. Bacterial growth was observed for 2 x 24 hours.

Determination of Heavy Metal Content in Simbiont Bacteria
The incubated bacteria were harvested by drying the mixture of Nutrient Agar and bacteria in Petri dishes at 80°C. The harvested bacterial mass was weighed to 0.5 g and then digested on a hotplate using a mixture of HNO_3 and H_2SO_4 (1:1). After cooling, the sample was transferred into roll film bottles. The heavy metal content was analyzed using Atomic Absorption Spectrophotometry (AAS) with an AA 900 F Perkin Elmer flame spectrometer located in the chemistry laboratory at UNIMA.

Data Analysis
Standard solution variations were prepared using the dilution formula:

$$V_1 \times M_1 = V_2 \times M_2$$

Where:
- V_1 = volume of standard solution before dilution (ml)
- M_1= concentration of standard solution before dilution (mg/L)
- V_2= volume of standard solution after dilution (ml)
- M_2 = concentration of standard solution after dilution (mg/L)

Samples from the digestion process were transferred into a 25 ml volumetric flask and diluted up to the mark. The concentration was then measured using Atomic Absorption Spectrophotometry (AAS). The total heavy metal content was calculated using the formula:

$$\text{Concentration} = \frac{D \times W}{V}$$

where:

- D = concentration obtained from AAS reading,
- V = final volume of the solution (ml),
- W = weight of the sample (g).

Results and Discussion

Sample Preparation

Symbiont bacteria in sponges inhabit both the surface and the inner parts (sponge flesh) [3,5]. Based on this, symbiont bacteria from the inner and outer parts of the three sponge samples collected from three sampling sites were cultured to observe the accumulation of metals in the bacterial samples and their use as bioindicators of heavy metals. The surface and inner bacteria were extracted and then rejuvenated in Nutrient Broth. According to [8] Nutrient Broth (NB) is used for bacterial rejuvenation and revitalization, aiming to enhance bacterial growth and facilitate bacterial propagation on Nutrient Agar media. Bacterial growth was indicated by a color change in the broth after homogenization.

Bacterial Cultivation on NA Media

After rejuvenation, bacteria were inoculated on Petri dishes containing Nutrient Agar (NA). The inoculation was done using the zigzag method and incubated at room temperature for 2 x 24 hours [9]. The incubation results showed bacterial growth during the incubation process, except for the inner part of the sponge sample from Manado Port, where no bacterial growth was observed.

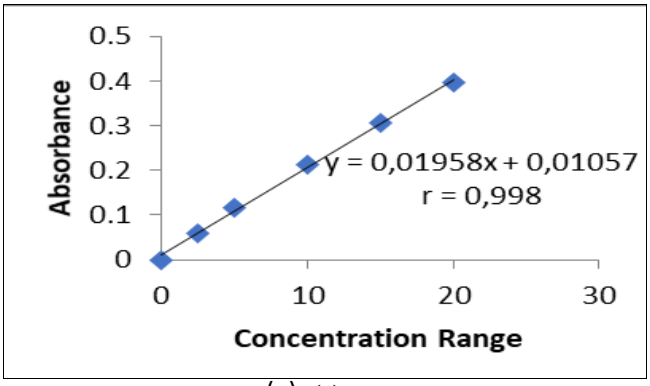
Harvesting and Digestion Process

The incubated bacteria were harvested by drying the mixture of NA and bacteria on Petri dishes at 80°C. The harvested bacteria, weighing approximately 0.5 g, were digested on a hotplate using a mixture of HNO_3 and H_2SO_4 (1:1) until the sample turned clear yellow. The solution was then removed and cooled [9], transferred into roll film bottles to await completion of the digestion process for all samples. The digestion method used was open acid digestion. The purpose of digestion is to obtain a homogenous solution of the analyte, complete decomposition of solids, and to prevent loss or contamination of the analyte [10].

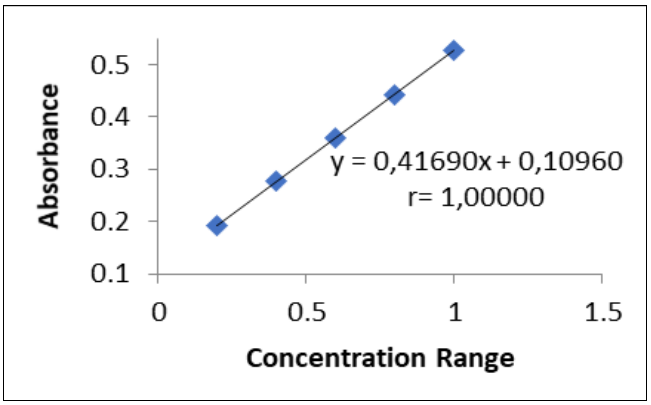
Metal Analysis in Samples

Calibration Curve Preparation

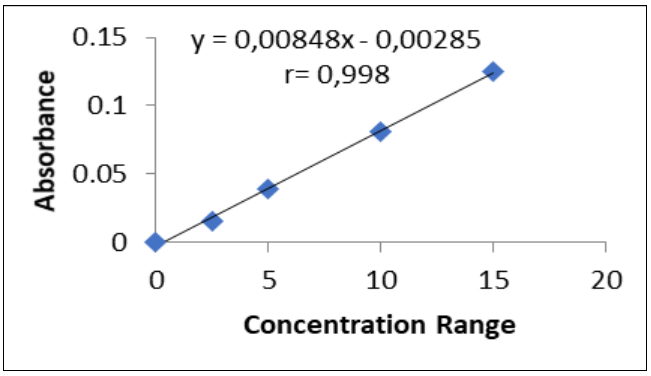
This study analyzed three metals: Pb, Zn and Hg. Standard solutions with varying concentrations were prepared as follows: Pb (2.5 ppm, 5 ppm, 10 ppm, 15 ppm, 20 ppm), Zn (0.2 ppm, 0.4 ppm, 0.6 ppm, 0.8 ppm, 1 ppm) and Hg (2.5 ppb, 5 ppb, 10 ppb, 15 ppb). Prior to analyzing the metal content in the samples, calibration curves were established using these standard concentrations. The calibration curves yielded linear equations with regression values used as references for measuring metal concentrations in the samples.



(a) (a)



(b)



(c)

Fig 1: Calibration Curve (a). Pb (b). Zn (c). Hg

From the calibration curve data above, linear equations were obtained as shown in the figure with correlation coefficients (r) approaching 1. Therefore, these calibration curves can be used as reliable references for measuring the metal concentrations in the samples.

Determination of Pb, Zn and Hg Metal Concentrations

Heavy metals by their nature are pollutants. Heavy metal pollution affects the growth, morphology and metabolism of microorganisms by disrupting cellular functions, altering proteins or damaging cell membranes [9], since microorganisms are living organisms that are highly sensitive to heavy metal exposure [11].

The concentrations of Pb, Zn and Hg metals were measured by their absorbance at wavelengths of 217.00 nm, 213.86 nm and 253.65 nm, respectively. Absorbance measurements were used to calculate the heavy metal concentrations in the samples, expressed in µg/g (ppm). The results are presented in the following figure.

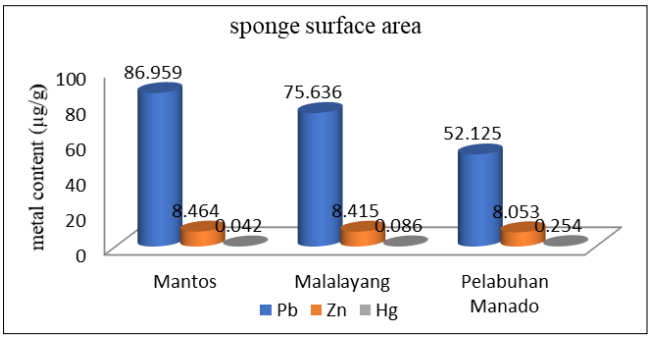


Fig 2. (a) Comparison diagram of Pb, Zn and Hg concentrations on the sponge surface (outer part) and inner part (flesh) at three sampling sites.

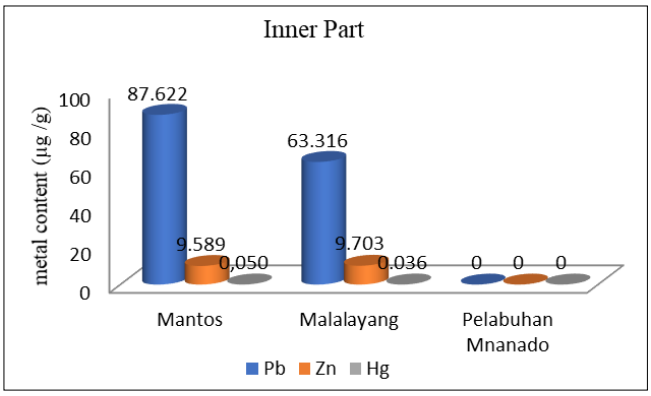


Fig. 2 (b) Comparison of Pb, Zn and Hg Metal Concentrations in Symbiont Bacteria from the Inner Part (Flesh) of Sponges

Figures 4.2 (a) and (b) illustrate the comparison of three metals at three sampling sites. Based on the sampling locations, the accumulation of heavy metals lead (Pb), zinc (Zn) and mercury (Hg) in symbiont bacteria of sponges in Manado Bay waters varies.

The accumulation of Pb in symbiont bacteria indicates that the environmental condition of the waters at the three study locations shows a high level of Pb contamination. The high Pb levels are attributed to sampling sites being near the densely populated Manado city center and the trading activities along Manado's coastal area. The sources of Pb accumulated in sponges (Porifera) and their symbiont bacteria may also originate from motorized boats used as transportation that utilize motor fuels containing additives such as tetraethyl lead ($(C_2H_5)_4Pb$) as an anti-knock agent. Additionally, gasoline often contains ethylene dibromide ($C_2H_4Br_2$) and ethylene dichloride ($C_2H_4Cl_2$), which during combustion produce Pb byproducts that enter the aquatic environment [12].

The accumulation of Zn in symbiont bacteria also suggests that Zn levels in the waters of the three sampling sites are high. The contamination of Zn in symbiont bacteria in Manado Bay is primarily due to human activities around the water area. Zn sources may come from household or industrial wastes containing metals, batteries, plastic paints and rubber products [12].

Mercury (Hg) is the last heavy metal analyzed in this study. Based on mercury content analysis around Manado Bay at the three sampling points, the accumulation of Hg in symbiont bacteria on the sponge surface and inner parts is shown in Figure 4.2. Hg accumulation in samples is lower compared to Pb and Zn. The accumulation of mercury in Malalayang is associated with the coastal area being a livelihood source for some residents of Manado, who have established residential and commercial areas such as shops, restaurants, boat repair facilities and others. Hg accumulation in Manado Port has been reported. According to [13] suspected to originate from fishermen frequently performing boat maintenance activities using materials containing heavy metals.

Conclusion

Based on the results of this study, the content of heavy metal Pb at the three sampling points was found to be very high, both on the sponge surface, ranging from 52.125 µg/g to 86.959 µg/g, and in the inner part of the sponge, ranging from 63.316 µg/g to 87.622 µg/g. The heavy metal Zn concentrations at the three sampling points were also high with surface levels ranging from 8.053 µg/g to 8.464 µg/g and inner sponge levels ranging from 9.703 µg/g to 9.589 µg/g. In contrast, the Hg levels at the three sampling points were lower compared to Pb and Zn with surface concentrations ranging from 0.042 µg/g to 0.254 µg/g and inner sponge concentrations between 0.036 µg/g and 0.050 µg/g. From these data, it can be concluded that symbiont sponge bacteria can be used as bioindicators of heavy metal content in the waters of Manado Bay.

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