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Superoxide dismutase (SOD) and metallothionein (MT) *Tubifex tubifex* at the acute mercury exposure

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Superoxide dismutase (SOD) and metallothionein (MT) *Tubifex tubifex* at the acute mercury exposure

I M Widiastuti¹, A M S Hertika², M Musa³ and D Arfiati³

¹Doctoral Program Faculty of Fisheries and Marine Science Universitas Brawijaya Malang, Indonesia

²Aquaculture Study Program Faculty of Animal Husbandry and Fisheries Tadulako University Palu, Indonesia

³Faculty of Fisheries and Marine Science Brawijaya University Malang, Indonesia

*Corresponding author: irakarno88@gmail.com

Abstract. Oxidative stress is a condition of over production of free radicals that can cause cell damage, pathological conditions and cell death. This study aims was to determined the activity of superoxide dismutase (SOD) and metallothionein (MT) levels in *Tubifex tubifex* worms exposed to HgCl₂ in various concentration levels. The concentration of HgCl₂ used was based on LC50-48 h values in acute toxicity test (15,85 µg / L; 25,12 µg / L; 38,81 µg / L; 63,09 µg / L; 99,98 µg / L). The method used was experiment. Determination of SOD activity done using spectrophotometry and MT measurement using ELISA method. The results was showed that HgCl₂ exposure to *Tubifex tubifex* caused increased levels of superoxide dismutase enzyme and metallothionein levels. SOD and MT levels were directly proportional to the concentration of HgCl₂, the higher the concentration, SOD and MT levels will increase accordingly.

1. Introduction

Mercury includes heavy metals that are highly toxic. When entering into the body of the organism will cause metabolic disorders, tissue damage and growth disorders. Mercury is a heavy metal that causes pollution of aquatic ecosystems. The presence of mercury in aquatic environments is largely human activity [1].

Invertebrates (gastropods, annelids, shells) are organisms that live in aquatic ecosystems. Water invertebrates are metal accumulators. The absorbed metal will accumulate in the tissues of the body [2]. *Tubifex tubifex* is one of the Annelids classes used as organisms in laboratory toxicity testing [3].

Oxidative stress is a state of imbalance between free radicals and antioxidants, where the amount of free radicals is more than the amount of antioxidants [4]. Oxidative stress is a condition of imbalance in the production of Reactive Oxygen Species (ROS) and endogenous antioxidants [5]. Oxidative stress arises from metabolic reactions that use oxygen and leads to a disruption of the balance between oxidants and cell antioxidants. If free radical production exceeds the ability of intracellular antioxidants then cause cell damage [4]. Increased oxidative stress causes damage to membrane lipids (malonaldehyde formed), damage to proteins, carbohydrates and DNA [6]. Oxidative damage caused by free radicals caused damage, tissues and organs such as liver, kidneys, heart in both humans and animals. The damage that happens can cause death [6] [7].

Organisms have a body's defense system to balance the formation of free radicals with intracellular antioxidants or endogenous antioxidants. Superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) include intracellular antioxidants. Superoxide dismutase (SOD) to catalyze the



reaction of superoxide anion free radical dismutation into hydrogen peroxide and hydrogen molecules [4].

Metallothionein (MT) is formed in the body of an organism in response to the entry of metal into its body. Metallothionein (MT) is a non-enzyme protein containing both essential and non-essential metals [8] [9]. Metallothionein (MT) is formed by induction of UV exposure [8] [10].

Metallothionein serves to reduce free radicals formed in the body and to detoxify metals to achieve homeostatic conditions [9]. Metallothionein also has a role in the process of adaptation to metallic exposure [11]. This study aims was to determined the levels of superoxide dismutase (SOD) and metallothionein (MT) in *Tubifex tubifex* worms exposed to HgCl_2 in various concentration levels.

2. Material and methods

The experimental method was experimental with six (6) HgCl_2 concentration treatments and each replicated three times. The test animal is *Tubifex tubifex* worm that comes from cultivation. Analysis of SOD activity using Nebot method [12][13] with the following procedure 150 μl of worm supernatant in 400 ml of chloroform / ethanol (37.5: 62,5 v / v) centrifuged 4,000 rpm for 10 min. Supernatant was taken for analysis of SOD content. A 50 μl sample solution was added 2.9 ml of xanthine and cytochrome C (ratio 1:10) and vortex was then added 50 μl xanthine oxidase and divortex. Furthermore, the absorbance value is read by using a spectrophotometer at a wavelength of 550 nm.

Determination of Metallothionein (MT) using Enzyme-linked Immunosorbent Assay (ELISA) method. ELISA methods include immunoassay type (immune test) which is a rapid test in detecting or measuring the amount of antibody or antigen against viruses, bacteria or other substances. The ELISA method consists of qualitative and quantitative techniques. Qualitative techniques are based on antibodies determined by absorbance values. Metallothionein (MT) levels were determined based on the ELISA method using the MT-2 Fish ELISA Kit.

3. Result and discussion

3.1. SOD level

Table 1 shows that at concentrations of HgCl_2 99.89 $\mu\text{g/L}$ yielded the highest SOD content (15.9 ± 0.46 U/ mL) compared with other concentrations. Exposure to concentrations of 63.09 $\mu\text{g/L}$ resulted in the same SOD concentration of 99.89 $\mu\text{g/L}$ (12.78 ± 0.11 U / mL). However, other HgCl_2 concentrations show low SOD levels and similar values. *Tubifex Tubifex* which was not exposed to HgCl_2 contains SOD 1.43 ± 0.15 U / mL.

Table 1. SOD levels of *Tubifex tubifex* worms exposed to HgCl_2 .

HgCl_2 concentration ($\mu\text{g/L}$)	SOD concentration (U/mL)
99.89	15.9 ± 0.46
63.09	12.78 ± 0.11
38.81	1.85 ± 0.53
25.12	1.79 ± 0.06
15.85	1.68 ± 0.26
0.00	1.43 ± 0.15

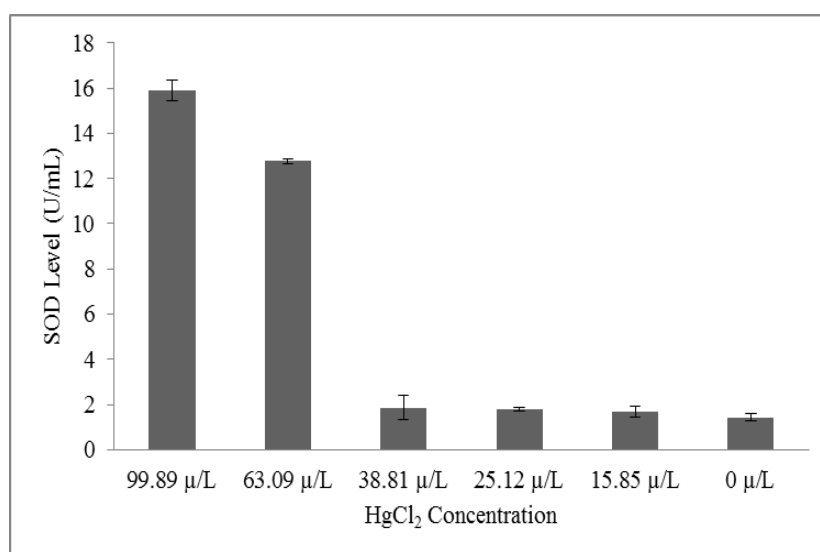


Figure 1. SOD levels of *Tubifex tubifex* exposed to HgCl₂.

Table 1 show the SOD level increases with the increase of HgCl₂ concentration. *Tubifex Tubifex* worms produce high SOD on high HgCl₂ exposure. This suggests that exposure to HgCl₂ with higher concentrations causes the SOD produced *Tubifex tubifex* to be higher as well. SOD produced *Tubifex tubifex* worm is a response from the body to neutralize the formation of oxidative stress against the metal.

Tubifex Tubifex worm that was not exposed HgCl₂ produces the lowest SOD. It was associated with oxidative stress. Sources oxidative stress, in addition to metallic exposure, also occurs by unfavorable environmental conditions for the organism. Environmental factors (eg UV radiation, ionizing radiation, xenobiotic, tobacco smoke) and cell receptor activation may lead to increased ROS production in the organism [14][15]. ROS is an oxygen species that is reactive due to the reduced number of electrons, one of which is superoxide which is the primary ROS. Superoxide is formed by the reduction of one electron from the oxygen molecule [16].

Superoxide Dismutase (SOD) is a superoxide scavenger or oxygen scavenger (O₂⁻), which catalyzes superoxide radicals (•O₂⁻) into hydrogen peroxide (H₂O₂). Superoxide dismutase (SOD) plays a role in protecting cells against oxidative stress disorders to cope with stress that can lead to several diseases and degeneration processes [17]. As ROS increases, the body responds by producing CAT, GPx and SOD enzymes [18].

Superoxide dismutase, glutathione peroxidase and catalase enzymes include intracellular antioxidant enzymes produced in the body that are essential for the body to absorb free radicals that prevent cell damage. The superoxide dismutase enzyme as one of the intracellular antioxidant enzymes works by clearing free radicals or reactive oxygen species (ROS) with enzymatic reactions and converting them into more stable products. SOD catalyzes the reaction of superoxide anion free radical (O₂⁻) into oxygen and hydrogen peroxide molecules so they are harmless to cells [4].

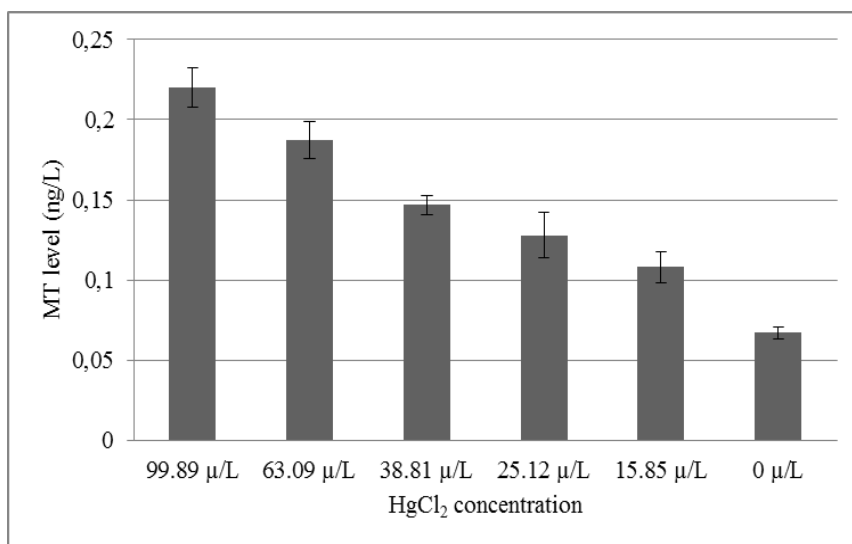
3.2. Metallothionein (MT) levels of *Tubifex tubifex* exposed to HgCl₂

Metallothionein (MT) levels of *Tubifex tubifex* exposed to HgCl₂ with varying concentrations showed that HgCl₂ concentrations of 99.98 µg / L resulted in the highest MT levels compared with other concentration treatments. In *Tubifex tubifex* worms that were not exposed to HgCl₂, the MT level was 0.067 ± 0.004 ng / L, the lowest level in *Tubifex* (Table 2).

Table 2. MT level of *Tubifex tubifex* worms.

HgCl ₂ Concentration (μg/L)	MT Concentration (ng/L)
99.98	0.22±0.01
63.09	0.18±0.01
38.81	0.14±0.01
25.12	0.12±0.01
15.85	0.11±0.01
0	0.06±0.00

Figure 2 shows that the MT produced by *Tubifex tubifex* after HgCl₂ exposure for 48 hours was directly proportional to the increase in HgCl₂ concentration. The higher the concentration of HgCl₂ the more MT produced by *Tubifex tubifex*.

**Figure 2.** MT levels of *Tubifex tubifex* exposed to HgCl₂

The results of the determination of MT levels show that *Tubifex tubifex* worms were exposed to HgCl₂, resulting in increased MT levels in accordance with the increase in HgCl₂ concentration. This shows that the higher the concentration of HgCl₂, the higher the MT level produced by *Tubifex tubifex* worm.

Heavy metals entering the waters will be absorbed by *Tubifex* into the body so that more heavy metal content entering the waters will increase the metal content in the body of *Tubifex* worms. The MT concentration in organisms is influenced by the concentration of metal contaminants [11][19][20]. That heavy metals in the body of an organism may exceed the threshold of the adaptive capability of the organism. Heavy metals can enter the body of the organism through water or through the digestive tract [21].

Metallothionein has a major role in the intracellular regulation of various metals such as copper, zinc, and cadmium. Improved MT synthesis is associated with increased capacity to bind metals and to protect the binding of metallic toxicity [23]. Increased levels of MT in certain organisms will reduce the susceptibility of the organism to heavy metals or become less sensitive and become resistant [11]. Metallothionein is the only biological compound that interacts with metals in the organism's body [22]. The interaction between proteins with metal ions will form thiolate-platelet groups. MT may increase with increasing cadmium exposure (Cd) [23]. Directly, metallothionein plays an important role in the mechanism of detoxification, facilitating the distribution and excretion of various metal compounds [24][25].

Metallothioneins (MTs) are considered proteins involved in the detoxification of unimportant and excessive essential metals. The hydroxyl radical reaction with MT is approximately 340 times higher than in GSH [26]. MTs are expressed in a special way on the tissues, especially in parts that accumulate metals. Copper binding to MTs causes an accumulated copper luminescence in a midgut copper cell. Likewise other heavy metals will be bonded in such a way that, in a short time, they can trigger the activation of MT genes, resulting in negative feedback on MT gene expression [27].

4. Conclusion

The results showed that HgCl₂ exposure to *Tubifex tubifex* caused increased levels of superoxide dismutase (SOD) enzyme and metallothionein (MT) levels. SOD and MT levels were directly proportional to HgCl₂ concentration, the higher the mercury concentration, the higher the SOD and MT levels.

5. References

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