Effects of heavy metals on intensity and coefficient of inhibition of firefly bioluminescence

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Abstract. We have analyzed the effects of heavy metals on changes in intensity and the inhibition coefficients of the firefly bioluminescence. The heavy metal compound used is PbNO₃ (Pb); ZnNO₃ (Zn), CuNO₃ (Cu) and FeNO₃ (Fe). The concentration of heavy metals used is 0.5 mg / l, 1 mg / l and 2 mg / l, for each heavy metal. The results found that the coefficient of inhibition and bioluminescence intensity was influenced by the molecular weight of heavy metals. The results explain that the cations participate in the re-distribution of electron density in chemical reactions and in the formation of electron excitation states. This pattern explains that the inhibition of the bioluminescence intensity is observed in the presence of heavy metals with higher molecular weight of heavy metals.

1. Introduction

Industrial developments increase environmental pollution by heavy metals which affect human health. The challenge to detect the presence of heavy metals is very important. Whatever test is done must be fast, easy to interpret and sensitive to various types of heavy metals. Most of the time to detect the presence of heavy metals in environmental samples is to use indicators of organisms such as rats, rabbits and fish. Heavy metal tests using these indicators are usually complex, and require individual expertise to carry them out. For example, additional analysis is needed to determine whether the animal died from toxin or other effects. Then the toxin test in animals takes days to monitor his condition.

The use of other indicators that are faster and more precise that can be used as heavy metal indicators need to be done. Bioluminescent organisms have long been known to be sensitive to heavy metals. The famous bioluminescent organism is fireflies. Rapid response and abundant firefly populations make them a popular choice for the rough detection of the presence of toxic substances.

Bioluminescence requires three main components namely luciferin, an oxygen source (O₂ or hydrogen peroxide H₂O₂) and an enzyme to catalyze a reaction called luciferase. Luciferin is a heat-resistant substrate and produces light, while luciferase is an enzyme that triggers chemical reactions in fireflies and oxygen as fuel [1][2][3]. Luciferin with the help of adenosine triphosphate (ATP) produces luciferyl adenylate. When luciferyl adenylate is reacted with oxygen it will produce oxyluciferin and. is a form of energy produced in the luminance reaction. The stages of bioluminescence reaction in fireflies can be seen in figure 1.
Fireflies produce light as a product of cell respiration. Respiration in these cells is fundamental to cell metabolism and is a living process. A decrease in cell activity (due to toxins) results in a rate of reduction in respiration and is associated with luminescence speed. The presence of a toxic substance can affect enzyme activity. Heavy metals have the ability to bind to enzymes, so the enzyme is easily blocked by its power. Toxic compounds such as heavy metals can cause a decrease in light intensity [4].

The use of bioluminescence organisms has been widely discussed to detect poisons on solid surfaces, such as soil, and in liquids. For example, five bacterial strains have been tested to detect various concentrations and types of heavy metals. The results showed that bioluminescence bacteria were able to detect heavy metals within 60 minutes [5]. Detection of toxins or heavy metals is done by looking at the reduction in light intensity observed from various luminescence strains. However, the detection methods available today have limitations that have not been explained how the type of heavy metal can affect the intensity of bioluminescence. The ability to truly identify heavy metal substances will have a good impact on health and industry. This information is important to know the behavior of heavy metal compounds affects the intensity of bioluminescence. This article discusses the influence of heavy metals on the inhibition coefficient and intensity of firefly bioluminescence.

2. Methods

2.1. Sample Preparation

The main ingredient used in this study is supernatant from fireflies (Pteroptyx tener) originating from west Sumatra and adenosine triphosphate (ATP). Supernatants from fireflies were obtained in a manner that had been explained by previous researchers [3]. The heavy metal compound used is Pb(NO₃)₂ (Pb); Zn(NO₃)₂ (Zn), Cu(NO₃)₂ (Cu) and Fe(NO₃)₃ (Fe) obtained commercially. The concentration of heavy metals used is 0.5 mg / l, 1 mg / l and 2 mg / l, for each heavy metal.

2.2. Characterization

The dependent variable in this study is the relative intensity of light and the inhibition coefficient of fireflies. The intensity of bioluminescence was measured using a UV-Vis Spectrophotometer of the Shimazu type. To see the effect of heavy metal types on decreasing the intensity of light emitting, the maximum intensity value of bioluminescence in the presence of heavy metal compounds with certain molar concentrations (I_max) is compared with the value of the intensity of bioluminescence without heavy metal compounds (I₀,max). Comparison (I₀,max / I_max) is then written as (I/I₀). The effect of the type of heavy metal compounds on the bioluminization system can be analyzed using the maximum ratio of the value of the intensity of bioluminescence with and without the presence of heavy metal compounds. To determine the inhibition coefficient can be calculated using the equation: \[ \ln \frac{I}{I_0} = KC \] [5], where I = maximum intensity value of bioluminescence in the presence of heavy metal compounds; I₀ = maximum intensity value of bioluminescence without heavy metal compounds, K = inhibition coefficient and C = concentration of heavy metal compounds.
3. Results and Discussion

3.1. Effect of heavy metal concentrations on the relative intensity of firefly

Figure 2 is the result of the measurement of the relative intensity of the firefly firefly of fireflies before and after being given various concentrations of heavy metals lead (Pb), copper (Cu), iron (Fe) and zinc (Zn) with a concentration of 0.5 mg/l.

![Figure 2](image)

**Figure 2.** Relative light intensity of fireflies before and after the presence of heavy metals with a concentration of 0.5 mg/l.

Figure 2 shows that the light intensity of fireflies decreases with the presence of heavy metals. The maximum intensity still occurs at a wavelength of 540 nm for each type of heavy metal. This wavelength is classified as visible light in green. From previous studies [6] that fireflies emit various colors of light ranging from green to red (530 nm - 635 nm) the presence of heavy metal lead (Pb) causes the firefly bioluminicity intensity to decrease to 36.92%. Heavy metal copper (Cu) causes the intensity of bioluminization to decrease to 74.25%, heavy metal iron (Fe) causes the intensity of bioluminescence to decrease to 82.63%. Heavy metal zinc (Zn) causes the intensity of bioluminescence to decrease to 68.35%. Of the four types of heavy metals it was found that lead heavy metals (Pb) experienced the greatest decrease in intensity. These results can be explained where the presence of a toxic substance can affect enzyme activity [7]. Heavy metals affect the working system of the luciferase enzyme found in fireflies. The more concentrations of heavy metals accumulated in the firefly's body, the relative intensity of the light will decrease.

Figure 3 is the value of the light intensity of fireflies before and after the presence of heavy metals lead (Pb), zinc (Zn), copper (Cu) and iron (Fe) with variations in concentrations of 0.5 mg/l, 1 mg/l and 2 mg/l, for each type of heavy metal.
Figure 3 describes the effect of being given a heavy metal relative light intensity of fireflies decreases with increasing concentration. The sharpest decrease in intensity occurs in heavy metals lead (Pb). At a concentration of 0.5 mg/l the intensity decreased to 36.92%, the concentration of 1 mg/l to 29.64% and at a concentration of 2 mg/l to 15.62%. Copper (Cu) at a concentration of 0.5 mg/l the intensity decreased to 74.25%, the concentration of 1 mg/l to 66.68% and at a concentration of 2 mg/l to 51.36%. Iron (Fe) at a concentration of 0.5 mg/l the intensity decreased to 82.63, the concentration of 1 mg/l to 71.08% and at a concentration of 2 mg/l to 54.83%. While zinc (Zn) at a concentration of 0.5 mg/l the intensity decreased to 68.35%, the concentration of 1 mg/l to 61.04% and at a concentration of 2 mg/l to 48.65%. The biggest speed of decreasing bioluminensity intensity due to the increasing concentration of heavy metals is Pb metal which is characterized by a slope of a line larger than the others.

3.2. The effect of type and concentration of heavy metals on the relative intensity inhibition coefficient of firefly

Figure 4 describes the effect of heavy metal concentrations on the efficiency of firefly intensity inhibition.

Figure 4. Light inhibition coefficient (M⁻¹) fireflies with heavy metal concentrations.
Figure 4 shows that the light coefficient of inhibition (M$^{-1}$) of fireflies is higher with increasing concentrations of heavy metals. The sharpest increase in inhibition coefficient (M$^{-1}$) occurs in heavy metal lead (Pb). At a concentration of 0.5 mg/l the inhibition coefficient was 1.8620 M$^{-1}$, the concentration of 1 mg/l to 1.1494 M$^{-1}$ and at a concentration of 2 mg/l to 0.8951 M$^{-1}$. Zinc (Zn) at a concentration of 0.5 mg/l inhibition coefficient of 0.6275 M$^{-1}$, concentration of 1 mg/l to 0.4269 M$^{-1}$ and at a concentration of 2 mg/l to 0.3269 M$^{-1}$. Copper (Cu) at concentration 0.5 mg/l inhibition coefficient of 0.4260 M$^{-1}$, concentration of 1 mg/l to 0.3385 M$^{-1}$ and at a concentration of 2 mg/l to 0.2998 M$^{-1}$. While iron (Fe) at a concentration of 0.5 mg/l inhibition coefficient of 0.2481 M$^{-1}$, concentration of 1 mg/l to 0.2747 M$^{-1}$ and at a concentration of 2 mg/l to 0.2671 M$^{-1}$.

3.3. Effect of heavy metal molecules with inhibition coefficients of firefly intensity

The decrease in light intensity of fireflies can also be seen from one of the physical properties possessed by heavy metals, namely molecular weight. Figure 5 describes the relationship of molecular weights of heavy metals lead (Pb), zinc (Zn), copper (Cu) and iron (Fe) with firefly inhibition coefficient of bioluminescence.

Figure 5 (a) can be seen that heavy metal lead nitrate (PbNO$_3$) has the highest molecular weight of 269.20 g/mol, then zinc nitrate (ZnNO$_3$) 127.38 g/mol, copper nitrate (CuNO$_3$) 125.55 g/mol and iron nitrate (FeNO$_3$) 117.86 g/mol. Figure 5 (b) shows that heavy metal lead (Pb) has the greatest inhibition coefficient value, then zinc (Zn), copper (Cu) and iron (Fe), respectively. From Figure 5, the molecular weight relationship with the inhibition coefficient is obtained. The greater the weight of the heavy metal molecule, the firefly inhibition coefficient of bioluminescence will also increase.
The relative light intensity of the firefly bioluminescence reaction decreases if the molecular weight of heavy metals increases. This decrease in light intensity is indicated by the increasing inhibition coefficient \( K (M^{-1}) \). The results obtained are in accordance with what was stated by the previous researchers, even though they use bioluminylated bacteria \([5][10]\), that the light intensity of the bioluminescence reaction decreases if the molecular weight of heavy metals increases. For *Luciola mingrelica* firefly, KCl heavy metal has a small molecular weight compared to KBr and KI so that KCl has a greater intensity value \([8]\). For *Pteroptyx tener* fireflies, heavy metal lead (Pb) has a large molecular weight compared to copper (Cu), iron (Fe) or zinc (Zn). In addition to molecular weight, increasing the concentration of heavy metals also affects the intensity of light produced by fireflies, the higher the concentration of heavy metals, the light intensity produced by fireflies decreases. The difference in intensity values \((I/I0)\) max in species of *Pteroptyx tener* and *Luciola mingrelica* is caused by the enzyme luciferase and pyrophosphate (PPi) which are present in each firefly. Different species of fireflies cause different levels of pyrophosphate (PPi), where (PPi) plays a role in the firefly light emission \([8]\) From these optimum conditions, the *Pteroptyx tener* firefly can be used as an indicator of detecting environmental conditions, especially to detect heavy metal lead (Pb).

4. Summary
The results of the administration of several types of heavy metals in the bioluminescence system of fireflies do not change the wavelength at maximum intensity, which is 540 nm. The type and concentration of heavy metals affect the intensity of bioluminescence from fireflies. The greatest decrease in intensity occurs in the weight of the largest heavy metal molecules. This decrease in light intensity is characterized by the increasing coefficient of inhibition for heavy metals with greater molecular weight. The results explain that the cations participate in the re-distribution of electron density in chemical reactions and in the formation of electron excitation states.

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