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Bioremediation of Lead (Pb) Contaminated Soil Using Metaphire Javanica Earthworms and Eisenia Fetida Earthworms

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A B S T R A C T

Soil contamination by heavy metals, such as lead (Pb), is a serious environmental problem that can reduce soil quality and endanger living organisms. This study aims to analyze the bioaccumulation potential and remediation ability of Eisenia fetida earthworms and Metaphire javanica earthworms in Pb-contaminated soil. This study was conducted on a laboratory scale using four reactors, namely two reactors with earthworm treatment and one reactor with a control without treatment. The study was conducted over 40 days with sampling once every 10 days. The results showed that Eisenia fetida earthworms had the highest Pb reduction effectiveness of 67.19%, followed by Metaphire javanica earthworms at 48.80%. These results indicate that Eisenia fetida earthworms are more effective bioaccumulators than Metaphire javanica and have high potential as bioremediation agents for Pb-contaminated soil

Contribution to Sustainable Development Goals (SDGs):
SDG 15 (Life on Land)
SDG 12 (Responsible Consumption and Production)
SDG 3 (Good Health and Well-Being)

1. INTRODUCTION

1.1. *Research Background*

Heavy metal contamination of soil is currently a significant problem that disrupts ecosystems, human health, and causes damage to the soil. These heavy metals are highly resistant and can persist in the environment for extended periods. Soil contamination is usually caused by human activities, such as industry and agriculture, that misuse fertilisers or pesticides. One of the heavy metals that is particularly dangerous to living organisms is lead (Pb). Lead is a highly toxic heavy metal [1]. Several methods have been developed to reduce the heavy metal content in soil, including soil washing and chemical immobilisation, among others. However, most of these methods are costly and risk damaging the soil structure. A more environmentally friendly alternative is to use bioremediation methods, one of which is using macroorganisms, such as earthworms.

There is still little research conducted to study the relationship between heavy metal levels in contaminated soil and various species of macroorganisms that have different habitats and feeding habits. Earthworms are one type of macroorganism that can improve the physical and chemical properties of soil. Earthworm activity also has a direct effect on the physical properties of soil. Each type of earthworm has a different level of tolerance to its environment [2]. Earthworms are considered important bioindicators for risk assessment and have been utilised in ecotoxicology studies to evaluate their bioaccumulation capacity. Heavy metals are known to accumulate in the body of an organism over a long period of time as accumulated toxins. Earthworms have a high tolerance to heavy metal-contaminated soil and are capable of accumulating heavy metals in their tissues [3]. This occurs because earthworms have a cysteine-rich protein called metallothionein. Metallothionein is a protein that contains a lot of cysteine and functions as a metal binder in earthworms, so that heavy metals do not interfere with cell metabolism. Metallothionein protein is able to absorb heavy metals and store them in certain parts of the body. This study aims to determine the potential of Eisenia fetida and Metaphire javanica earthworms in remediating lead (Pb) and other heavy metals in soil.

2. MATERIALS AND METHODS

2.1. Research Location

Soil samples were taken from the Griyo Mulyo Jabon landfill site in Sidoarjo and analyzed for heavy metal lead (Pb) content prior to soil remediation. Preliminary tests were conducted on heavy metal lead (Pb) parameters.

2.2. Tools And Materials

The tools used were a 5L container measuring 24.5 cm x 16.5 cm x 15 cm, analytical scales, a shovel for soil collection, a soil meter, gloves, and an insect net. The materials used were Eisenia fetida earthworms, Metaphire javanica earthworms, cow manure, and soil contaminated with heavy metal lead (Pb) as a medium whose heavy metals would be reduced by the earthworms.

2.3. Research Design And Variables

This study was conducted using macroorganism variables, namely Metaphire javanica earthworms and Eisenia fetida earthworms. The fixed variables were soil weight: animal weight (4 kg soil: 30 g earthworms), macroorganism nutrient weight of 30 g, with an additional duration of 1x 10 days. The control variable was the environmental conditions, as shown in Table 1, and the research variables are shown in Table 1.

Table 1. Research Control Variables

Types of Earthworms	Environmental Conditions		
	Moisture	Temperature	pH
Cacing Tanah *M. javanica*	60%-72%	$25^0C – 31^0C$	6.8-7.2
Cacing Tanah *E. fetida*	60%-70%	$24^0C – 30^0C$	6.68-7.2

(**Source:** (Martinkosky et al., 2015))

Table 2. Research Variable Details

Reactor Code	Description
CTE	Contaminated soil 4 kg + *M. javanica* 30 g
CTM	Contaminated soil 4 kg + *E. fetida* 30 g
Control	Contaminated soil 4 kg

(**Source:** Analysis Results, 2025)

The remediation process was carried out for 40 days, with soil samples tested every 10 days and heavy metal lead (Pb) testing on macroorganisms conducted after 40 days to determine the bioaccumulation value in each macroorganism. Heavy metal lead (Pb) testing in this study used the AAS (Atomic Absorption Spectrophotometry) method. The research reactor will be stored indoors to block sunlight, and the reactor will be covered with an insect net to prevent disturbance by animals. Temperature, humidity, and pH measurements will be taken daily so that the macroorganisms can live in their natural environment, as described in Table 1. Environmental conditions will be measured using a soil meter. The reactor design used in this study can be seen in Figure 1.

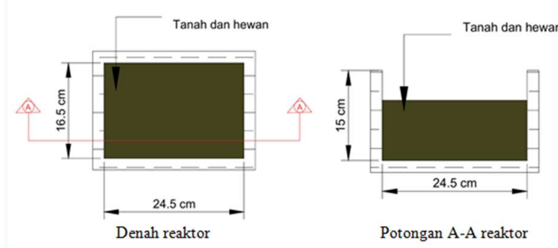

Figure 1. Reactor Design

3. RESULT AND DISCUSSION

3.1. Characteristic of Contaminated Soil

Preliminary testing of lead (Pb) concentration was conducted to determine the concentration of heavy metal lead (Pb) in the soil. Before treatment, a preliminary analysis was conducted to determine the lead concentration in the soil. The results of the preliminary analysis are presented in Table 3. It should be noted that the quality standard for heavy metal lead (Pb) in soil is generally based on international standards and various references, as mentioned by [4] in the book “Heavy Metals in Soils” that the quality standard for heavy metal lead (Pb) is generally 50 mg/kg or 0.05 mg/g.

Table 3. Initial Characteristics of Soil Contaminated Heavy Metal Lead (Pb)

Parameters	Unit	Results
Lead (Pb)	mg/g	0,1082
pH	-	7,1
Temperature	^oC	31
Moisture	%	73

(**Source:** Analysis Results, 2025)

3.2. Characteristic of Contaminated Soil

The acclimatisation process was conducted over a period of 1 week. This process was conducted to adjust the physiological conditions of macroorganisms to the media environment used in the study, thereby minimizing stress and mortality during the study. In this study, acclimatisation was achieved by maintaining Eisenia fetida and Metaphire javanica earthworms in a medium that simulated the experimental conditions to which they would be exposed to heavy metals. Acclimatisation was carried out over 7 days by providing nutrients and maintaining the medium's normal conditions in terms of humidity, pH, and temperature. Metaphire javanica earthworms require a media humidity of between 60-72% [5], and Eisenia fetida earthworms require a media humidity of between 60-70% [6] to maintain their biological activity. During the acclimatisation period, the macroorganisms were observed every 2 days to ensure that there were no significant morphological or behavioural changes.

Acclimatization is considered successful if the macroorganisms do not leave the medium 10]. Similarly, during the acclimatization period, no macroorganisms left the medium for 1 week, indicating that the test macroorganisms successfully adapted to the experimental environment and could be used for research. The acclimatization results are presented in Table 4.

Table 4. Result of Earthworms For 1 Week

Type of Earthworms	Initial Quantity	Final Quantity	Initial Weight (gram)	Final Weight (gram)
M. javanica	50	50	30	30
E. fetida	50	50	30	30

(**Source:** Analysis Results,2025)

3.3. Characteristic of Contaminated Soil

3.3.1. pH

For 40 days, pH measurements were taken using a soil meter, which automatically detects pH, moisture, and temperature values in the soil medium. During the study, pH levels fluctuated within the optimum range, specifically 6.8-7.2 for Metaphire javanica earthworms [7] and 6.68-7.2 for Eisenia fetida earthworms [8]. This occurred because the environmental conditions were still favorable. This pH measurement is a factor in influencing the survival of earthworms. In the treatment of Metaphire javanica and Eisenia fetida earthworms, the soil pH was recorded in the range of 6.6 to 7.3. pH stability can also play a role in reducing Pb levels by increasing interaction with soil organic matter, thereby facilitating the bioremediation of Pb through the use of earthworms. In the control treatment, the pH ranged from 6 to 7.2 and tended to be more stable. This occurred because there was no activity from organisms that could influence pH changes through the decomposition of organic matter. Although stable, the control treatment exhibited a relatively slow decrease in Pb, indicating that the role of earthworms was more significant in accelerating soil remediation.

3.3.2. Temperature

Temperature measurements in this study were similar to pH measurements using a soil meter. These temperature measurements were taken to control and check the temperature during the soil remediation process for each treatment. Over a period of 40 days, the results showed that in the earthworm treatment, the soil temperature during the study ranged from 29°C to 31°C. This condition remains within the optimum tolerance limit for earthworms, which is 24 °C to 30 °C [9]. The soil temperature values obtained indicate values that are in line with the optimum temperature for macroorganisms, which is beneficial for growth and soil remediation. Temperature can also affect the activity and survival of earthworms; if the temperature is too high or too low, it can impact the physiology of these organisms.

3.3.3. Moisture

Moisture measurement is very important for the metabolic activities of macroorganisms. Soil moisture significantly affects the movement of earthworms, as 75-90% of their body weight consists of water. The optimum soil moisture for earthworms is around 60-72% [10]. In this study, soil moisture levels were relatively stable, ranging from 60% to 74%, which is in line with the needs of earthworms, which require a moist environment to maintain oxygen diffusion through their skin. Soil moisture affects the survival of earthworms because if the soil moisture is too high, it will cause the earthworms to turn pale and then die. This can affect the soil remediation process [11].

3.4. Results of Pb Concentration Reduction

This study used three reactors. The first reactor used Eisenia fetida earthworms, the second reactor used Metaphire javanica earthworms, and the third reactor had no treatment. Samples were collected every 10 days for 40 days. During the observation period, manual stirring was also carried out to ensure the oxygen exchange needed by the earthworms. This study found a decrease in the heavy metal lead (Pb) content in the soil. This can be seen in Table 5 and Figure 2

Table 5. Results of The Reduction in Heavy Metal Lead (Pb) Concentration in Soil

Sampling Period (Days)	Pb Concentration (mg/g)		
	CTM	CTE	Control
0	0.1082	0.1082	0.1082
10	0.0869	0.0846	0.1079
20	0.0749	0.0555	0.1075
30	0.0693	0.0490	0.1070
40	0.0554	0.0355	0.1067

(**Source:** Analysis Results, 2025)

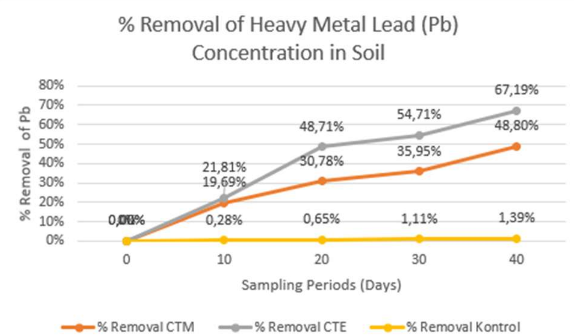

Figure 2. Lead Removal Efficiency Based On Remediation Time For Each Type of Earthworms
(**Source:** Analysis Results, 2025)

Based on the data in Table 5 and Figure 2, it can be seen that the concentration of lead (Pb) in the soil decreased as the contact time increased over 40 days. Treatment using Eisenia fetida earthworms showed good effectiveness because it was able to reduce lead (Pb) concentrations with a removal percentage of 67.19%. Treatment using Metaphire javanica earthworms achieved a removal percentage of 48.80%, while the control treatment (without treatment) only achieved 1.39%.

In the CTM treatment, the percentage of elimination showed a gradual increase, namely 19.69% on day 10, 30.78% on day 20, 35.95% on day 30, and 48.80% on day 40. Meanwhile, the CTE treatment showed a gradual percentage of elimination, namely 21.81% on day 10, 48.71% on day 20, 54.71% on day 30, and 67.19% on day 40. The control treatment showed a percentage of removal on day 10 of 0.28%, on day 20 of 0.65%, on day 30 of 1.11%, and on day 40 of 1.39%. This study shows that the treatment using Metaphire javanica earthworms and Eisenia fetida earthworms has an effect on reducing the level of heavy metal lead (Pb) in the soil. Eisenia fetida earthworms (CTE) were able to reduce heavy metal lead levels more significantly than Metaphire javanica earthworms (CTM). This occurred due to differences in the ecological groups of these two earthworms. Eisenia fetida earthworms belong to the epigeic group, meaning that these earthworms live and feed on the surface layer of soil

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that is rich in organic matter, but are also susceptible to pollutant accumulation. This condition enables earthworms to develop highly efficient mechanisms for metal tolerance and detoxification [12]. In a limited reactor, this can be the primary factor that determines whether Eisenia fetida earthworms remain active in heavy metal-contaminated media and efficiently accumulate heavy metals, such as lead (Pb), in their bodies through the production of metallothionein [13]. Meanwhile, the earthworm Metaphire javanica belongs to the endogeic group. Endogeic earthworms are less adapted to the confined conditions in reactors and heavy metal exposure, resulting in lower removal percentages compared to Eisenia fetida earthworms. These two earthworms also have different ingestion rates, which is the speed at which an organism consumes or takes in material into its body. Epigeic earthworm species such as Eisenia fetida have a higher feeding rate than endogeic earthworm species such as Metaphire javanica, resulting in faster absorption of heavy metal lead (Pb) by Eisenia fetida. However, Metaphire javanica earthworms still have the potential to be used as soil bioremediation agents, but they will require more time compared to using Eisenia fetida earthworms.

The reduction in Pb levels in soil using earthworms is caused by the accumulation of heavy metals (Pb) by earthworms in their bodies. The process of reducing heavy metal (Pb) levels in soil using earthworms is specifically caused by the presence of metallothionein protein in earthworms. The binding carried out by metallothionein protein occurs when soil contaminated with heavy metal Pb enters the earthworm's body. Metallothionein protein, which is rich in cysteine residues with thiol-SH groups, will play a role in binding the heavy metal. These cysteine residues have a high ability to bind heavy metals, forming stable and non-toxic complexes. This Pb-metallothionein complex is then transported to an organ called the chloragogue cell, which functions as a storage and detoxification site for metals. In this organ, heavy metals bound to metallothionein no longer have the potential to cause damage to earthworm cells [14].

In the control treatment, the concentration of lead (Pb) in the soil decreased, but the decrease was relatively small. This could be due to the presence of bacteria in the soil, which can cause a decrease in the concentration of heavy metals such as lead (Pb) in the soil. The decrease in the concentration of heavy metals such as lead (Pb) in the control treatment was due to stirring, which caused an increase in aeration in the soil medium. This is supported by research [15], which shows that soil mixing during the research process can increase aeration, thereby supporting the activity of Pb-degrading bacteria. In the study [16], it was also shown that a decrease in Pb levels also occurred in the control treatment due to the activity of natural soil bacteria and mixing, which increased aeration and microbial activity in the soil.

4. CONCLUSION

Variations in the types of macroorganisms and sampling time affected the reduction in Pb concentration. The macroorganism that was most effective in reducing Pb concentration was the Eisenia fetida earthworm, with a removal rate of 67.19% and a final concentration of 0.0355 mg/g. The earthworm Metaphire javanica is also capable of reducing Pb concentration with moderate effectiveness of 48.80% with a final concentration of 0.0554 mg/g.

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