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Biaccumulation and tolerance of heavy metals on the tropical earthworm, *Allobophora sp.* after exposed to contaminated soil from oil mine waste

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Abstract. In this study, the impact of contaminated soil from oil mine waste on survival, behavior, tolerance, and bioaccumulation of heavy metals by the tropical earthworm, *Allobophora sp.* has been quantified. Earthworm was isolated from heavy metals-contaminated soil, cultured in laboratory condition, and exposed to contaminated soil from oil mine waste for a couple of months. The behavior and response of earthworms to contaminated soil was monitored for 28 days and evaluated by the response criteria was expressed in scale index (SI) referred to Langdon method. Resistance test of the earthworm (LC_{50}) to heavy metals also conducted with variation soil concentrations of 100%, 50%, 25%, 12.5%, and 6.25%, and 0% (Control). Results showed that contaminated soil extremely affected to the earthworm live, especially length and their body weight. The Lethal Concentration 50% (LC_{50}) of earthworm against contaminated soil was 19.05% (w/w). When exposed to contaminated soil, earthworm accumulated chromium, barium, and manganese at the concentration of 88; 92.2; and 280 mg/kg-DW, respectively. Based on these results, earthworm *Allobophora sp.* has potential to reduce heavy metals from contaminated soil in the field of bioremediation process.

1. Introduction

Excessive anxiety about the presence of heavy metals in environment is caused by very high poisoning levels of heavy metals in all aspects of life [1, 2]. U.S. Environmental Agency (USEPA) has listed 13 heavy metal elements, which are the main element of dangerous pollution, such as Sb, As, Be, Cd, Cr, Cu, Hg, Ni, Pb, Se, Ag, and Zn [2]. These heavy metals in fact harmful to human health and continuity of life in the neighborhood. Although such concentration is low, the effects of heavy metal ions can take effect directly to accumulate in the food chain [3]. Mobilization of heavy metals depend on the characteristics of microorganism and heavy metals in environment [4]. As well as the sources of other environmental pollutants, heavy metals can be transferred within reach very far in the environment, potentially disrupt environmental biota and ultimately affect human health although in a long period of time and far from sources of pollution.

Natural processes such as natural cycles change resulted rocks and volcanoes contribute a very large to environment. Besides, the entering of heavy metals into the environment are derived from other sources which include; mining, oil, gold, coal, hydroelectric, pesticide, ceramics, metal smelting, fertilizer factories, and other industrial activities. In some Asian countries, heavy metal contamination has spread widely as reported by the survey team from National Agriculture and Food Research Organization and Tokyo University of Agriculture and Technology in Japan and University of Madras and Manipal Institute of Technology [5]. Our preliminary research reported that soil contaminated oil as by-product from oil explorations activities in Aceh Province contain a number of heavy metals in high concentrations such as Pb, Cr, Mn, Fe, Zn, and Ba. The problem will occur if soil piled up without being processed or without reducing the heavy metals content. These phenomena will be facing to the world in the future considering activities of oil and natural gas exploration running massively.

In line with these problems, research on the effects of heavy metals on the lives of organisms in environment will continue to be carried out. In the recent years, many researches have focused to study on the acute toxicity effects to earthworms of inorganic and organic pollutants such as the nickel, and so on [6-8]. They are also concerning heavy metals accumulation in earthworms and most are on lead, zinc, and cadmium [9-11]. Earthworms are the representative species of soil animals with the largest terrestrial faunal biomass and constitute a major component in soil ecosystem. They play important roles in element geochemistry cycle since their contribution on organic matter decomposition. In the environment, pollutants in soil such as organic pollutants and metals easily influence earthworms. They are very suitable for monitoring the contaminations effects in field with their limit mobility [12]. Earthworms have a potential as degrading organisms of heavy metals in contaminated soil by bioremediation process. Langdon et al conducted a study of earthworms *L. rubellus* and *L. terrestris* which were exposed on arsenic contaminated soil [13]. Arsenic accumulation by *L. rubellus* in uncontaminated soil smaller compared to the treatment of contaminated soils. Concentrations of arsenic found in the cells of *L. terrestris* and *L. rubellus* was 230 mg-As/kg and 92 mg-As/kg, respectively. Leveque et al reported that metal pollution has a direct impact on earthworm communities (proportion of juveniles, abundance, and diversity) [14]. The response of earthworms, *P. antipodarum* against metal arsenic trivalen (As III) faster compared to arsenic pentavalen (As V), this was caused by metal toxicity levels As III higher compared to As V [15].

Based on the explanation above, few data are available on acute toxicity effects of earthworms to heavy metals from soil contaminated. This paper describes the influence of heavy metals contaminated soil and hydrocarbons on the lives of Earthworm, *Allobophora sp.* isolated from heavy metals contaminated soil from oil mine waste.

2. Materials and Methods

2.1 Contaminated soil

Contaminated soil obtained from oil and gas exploration in East Aceh. The results of preliminary analysis of contaminated soil was used show that soil contains Ba (0.08 mg/g), Cr (0.018 mg/g), Hg (0.65 (g/g), Pb (0.057 mg/g), and Zn (0.314 mg/g), while Co and Cd found a very small concentration in the soil. Meanwhile, hydrocarbon compounds on the contaminated soil found on the average of C between C_{12} to C_{16}. Preliminary analysis results shows that deposits of hydrocarbons in the contaminated soil was hydrocarbons with contains C_{12} (5.28%), C_{13} (6.39%), C_{14} (3.60%), C_{15} (20.77%), and C_{16} (63.96%).

2.2 Growth medium of earthworms

Earthworms require a growing medium containing rich of organic matter for their growth. In this study, growth medium of earthworms was cultivated so that soil conditions can meet the requirements as their natural habitat with the following criteria.

1. Consists of fibrous organic materials that have weathered between 50-60% and has not released the unwanted gas by earthworms;
2. Able to maintain the stability of humidity with a good moisture level (35-50%) for the growth of earthworms. If the water content in the medium is too high, the earthworm body will be pale and become dead;

3. Media is always loose and not easily solid or porous;
4. Media is easy to decompose; and
5. Media temperature is in range 27-30^{o}C with pH about 6.5 – 7.2.

2.3 Isolation of earthworms and resistance test

Preliminary research was conducted to find a species of heavy metal resistant earthworms. Earthworm was collected from contaminated site of Banda Aceh City (such as the area of Darussalam, Kajhu, and Ketapang Dua). Earthworms were kept in soil collected from the same site in brown glass tubes in field and brought to laboratory. Earthworms were picked out with forceps, as soon as possible thoroughly rinsed with distilled water, and put into the brown tubes with one filter paper in, and distilled water were added in few drops to maintain them moist. Earthworms were kept at room temperature ($\pm$ 30^{o}C) for 7 days to reach the adaptation condition and to empty their guts, then weighted, counted and identified, and their body lengths were measured.

Resistance test of the earthworm (LC_{50}) to heavy metals referred to the methods conducted by [13]. Two steps tests were conducted, namely preliminary and actual test. Preliminary test intended to explore and predict the range of concentration test that could lead to the animals' death. While the actual test based on preliminary test results that could be estimated the toxic and nontoxic concentrations, then the actual test of soil concentrations arranged. Concentrations of heavy metals contaminated soil were varied 100%, 50%, 25%, 12.5%, and 6.25%. Control (0%) were also prepared to compare the conditions of earthworm's exposure to the contaminated soil. Six tails of earthworm from stock tubes were separated and transferred into tube of experiment and exposed to contaminated soil already varied over the next 96 hours. The experiment condition was prepared on the same treatments with adaptation phase in tube of shelter. During treatment, clinical symptoms and behavior of earthworm were observed, as well as the number of earthworm live and dead were recorded to earthworms mortality calculation.

Behavior and response of earthworms to contaminated soil was monitored for 28 days and evaluated by response criteria that was expressed in scale index (SI) referred to Langdon, et al [13] as follows: (S=2) good muscle tone, the worm's response to stimulation was very fast, (SI=1) weak muscle tone, normal walking response or good muscle tone, but slow in response, and (SI=0) weak muscle tone, no response to stimulation.

2.4 Heavy metal analysis

Heavy metals concentration in soil and earthworm analysed after destructive based on Toxicity Characteristic Leaching Procedure by using Atomic Absorption Spectroscopy [16]. Heavy metals ion to be specified using gas, air pressure, Hollow Cathode, and specific current lamps.

3. Result and Discussion

3.1 Earthworm characteristics isolated from contaminated soil

Based on the preliminary results, from many places that were taken (Darussalam, Kajhu, and Ketapang Dua), only earthworms from Kajhu area could survive against heavy metal toxid compared to earthworms isolated from other areas. Earthworms isolated from Darussalam area were death after 1 day exposure. The resistance of earthworms against heavy metals toxic are strongly influenced by habitat or earthworms origin place [17,18]. The analysis results showed that earthworms isolated from Kajhu area accumulate manganese 187 mg Mn/kg-WD. This was due to the soil where earthworms live contain low concentration of manganese that not cause toxic to earthworms. The accumulation of manganese in low concentration indirectly influenced to earthworm resist against toxic heavy metals. Physical characteristics of earthworms isolated from contaminated soil have outer and inner segments, hair, having no outer frame; cuticle (outer skin) protects their body, having no motion, and no eyes. To be able to move, earthworms must use the muscles of their long body and thick that circle their body. The presence of mucus produced by epidermal glands of their body can facilitate their movement in dense and rough places. The mucus can also lubricate their body making a hole in soil to easily get in and out of the hole. In addition to these functions, the mucus can be used to defend their self. On their body, there is an organ called seta, which is found on every segment of hair and relatively hard and short-sized. The adhesiveness of these organs is strong so earthworms can cling tightly to surface of the object. Seta can also help earthworms during marriage. Based on these investigation results, the classification of isolated earthworm was Allobophora *sp.*

3.2 Effect of contaminated soil on earthworm behaviour and response

Physical characteristics and behavior of earthworms, *Allobophora sp*. observed were length, weight, color, shape or condition of body, movement, and response to stimuli. The observations conducted before and after earthworms were exposed to contaminated soil. The conditions of earthworms exposed to contaminated soil gradually degenerate and eventually die. Pathologic indications that have been observed were slow responses to stimuli, swelling around the body, red color fades, non-directional movements, and at 100% concentrations most of earthworm conditions were worsening, while earthworm in the control treatment were in good condition.

Furthermore, examination of the effect of heavy metal contaminated soil to earthworms refers to method developed by Langdon et al [13] by setting the Index Score (IS) to the condition of earthworms. Based on the observations, earthworm conditions at various treatments illustrated in Figure 1.

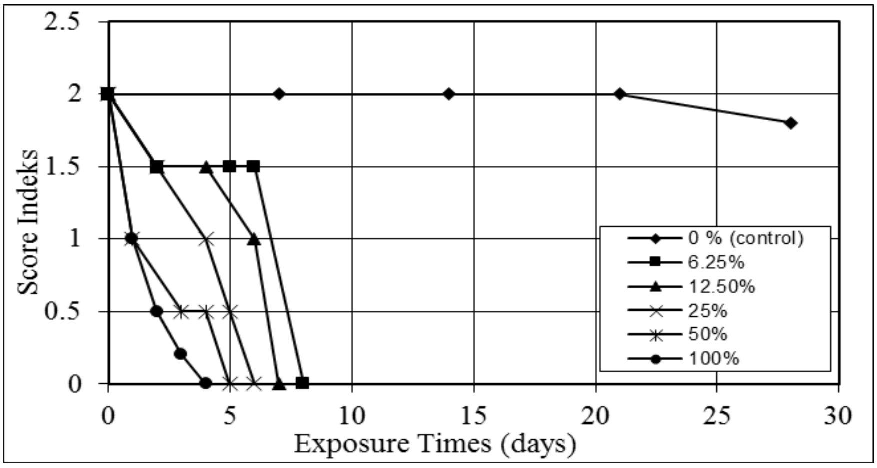

Figure 1. Earthworm conditions at various concentrations of contaminated soil

Figure 1 shows that earthworms exposed to contaminated soil were worsening in line with the increasing of contaminated soil concentration. At the concentrations of 100%, the earthworms only survived in 4 days of exposure because of acute poisoning of heavy metal toxins, whereas at concentrations of 6.25%, earthworms were still able to survive for 8 days of exposure because they have chronic or sub-chronic toxicities. Furthermore, the earthworms on control treatment were still survive in good condition for 28 days. When observed from the influence of exposure time to earthworm conditions, the conditions of these animals appeared weak, slow response, not directed movement since the first day of exposure. This indication was observed at the concentration of 100% (SI=1), whereas at lower concentrations of 6.25% the earthworms can still catch on the quick response, directed movement, although the condition was rather weak (SI=1.5). The second day of exposure for concentration of 100%, two earthworms were found dead and the others were in worsening condition. For concentration of 6.25% on the second day of exposure, no earthworms found died but the condition was weak with slow response and the directional movement (SI=1.5). Further observations conducted on earthworm conditions in line with the length of exposure time indicated that earthworm conditions became worse and eventually die, except for the control treatment was still survive in good condition on 28 days of exposure (SI=2). The results were consistent with the research conducted by Langdon et al [13] that focused on the effect of arsenic contaminated soil against two species of earthworms, *L. rubellus* and *L. terrestris.* They reported that on the first day of exposure, no earthworms died and after 5 days of exposure, an earthworm, *L. terrestris* was died. Furthermore, three earthworms, *L. terrestris* died after 10 days of exposure and six *L. terrestris* and two *L. rubellus* still survived but they were in a weak condition. While earthworms in the control treatment was still survive in good condition on 28 days of exposure (SI=2).

3.3 Toxicity value (LC_{50}) of contaminated soil to earthworms
The methods conducted by Langdon, et al [13] was applied to obtain toxicity value (LC_{50}) of contaminated soil to earthworms. Based on the calculation, LC_{50} was found 19.05%. Its mean that 50% of earthworms died when exposure to contaminated soil with the concentrations of 19.05%.

3.4 Effect of contaminated soils on weight and length of earthworms
To find out Influence of contaminated soils on weight and length of earthworms and to investigate the relationship between concentrations of contaminated soil, the research data were evaluated using statistical methods. Test results show that higher concentrations of contaminated soil cause earthworm weight increases. It was indicated by body weight of earthworms decreased larger at concentration of 100% compared to 50%. Similarly, results occurred to the length of earthworms before and after treatment. Calculation results using statistical methods concluded that contaminated soil greatly affect weight and length of the earthworms, which indicated by $F_0 > F_{Table}$. In the source of variation in length and weight, calculation result for F_0 was 2176.9 while the results of F_{Table} ($F_{0.1}$ and $F_{0.01}$) were 3.94 and 6.92, respectively.

3.5 Accumulation of heavy metals in earthworm after exposed to contaminated soil
Earthworm was exposed to contaminated soil with a concentration of 100; 50; 25; 12.5; 6.26%; and 0% (control). After three days of exposure, earthworm was taken, cleaned, and oven dried (48 h at 60 ^{o}C), ground to homogeneous powder in a quartz bowl, and heavy metal accumulated in cell was analyzed by AAS Shimadzu 630. The results are tabulated in Table 1 and illustrated in Figure 2.

Table 1. Bioccumulation of heavy metals in earthworm after exposed to contaminated soil

Contaminated soil concentration (%)	**Heavy metals accumulated in earthworm (mg/kg-DW)**							
	Cr	**Pb**	**Mn**	**Ba**	**Cd**	**Hg**	**Co**	**Zn**
Control (0)	trace	trace	187	trace	trace	trace	trace	trace
6.25	18	trace	190	18	trace	trace	trace	trace
12.5	88	trace	160	92.2	trace	trace	trace	trace
25	86	trace	280	24	trace	trace	trace	trace
50	27	trace	230	39.6	trace	trace	trace	trace
100	50	trace	220	69.9	trace	trace	trace	trace

Results showed that when earthworms were exposed to contaminated soil, concentration of heavy metals accumulated in earthworm did not correspond to the increasing rate of contaminated soil concentration. Some of accumulated metals were excreted to the soil so the toxic contents of heavy metals present in cell were reduced and toxicant may be in the form of origin, as a metabolite or as a conjugate. Heavy metals that accumulated by the earthworms were Mn, Cr, and Ba with the concentration of 88; 92.2; and 280 mg/kg DW, respectively. Other metals such as Pb, Cd, Hg, Co, and Zn found as trace in earthworm's cell. These results were in line with those reported by Lu [19]. Furthermore, earthworms exposed to control treatment were found accumulate manganese in the concentration of 187 mg-Mn/kg-DW. This was due to soil where the earthworm isolated contains manganese. Due to the presence of manganese in soil from where the earthworm isolated, accumulation of manganese higher compared to other metals. These results support research reported by Langdon et al [13,17]. Accumulation of arsenic on earthworms, *L. rubellus* exposed to the uncontaminated soil was less (<1 mg-As/kg) than that of contaminated soil (230 mg-As/kg).

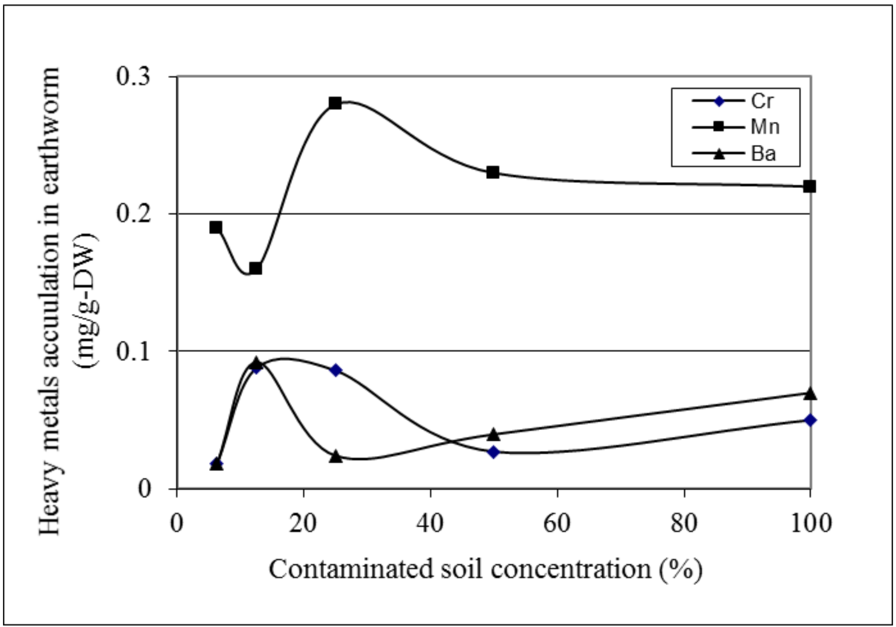

Figure 2. The relationship of heavy metal concentrations accumulated in the earthworm to the concentration of contaminated soil.

4. Conclusions

Based on the results and discussion presented, this research leads some conclusion.

1. The influence of heavy metals and hydrocarbons contaminated soil on the life of earthworms, *Allobophora sp*. increased with the increasing of contaminated soil concentrations.
2. Contaminated soil concentration causing 50% mortality (LC_{50}) on earthworm, *Allobophora sp*. was found 19.05%.
3. Contaminated soils greatly affect to the weight and length of earthworms, Allobophora *sp*. Earthworm, *Allobophora sp*. was able to accumulate heavy metal compounds from contaminated soil such as Cr, Ba, and Mn, with the concentration of 88; 92.2; and 280 mg/kg-DW, respectively.
4. These results reached to conclusion that earthworm, *Allobophora sp*. has a potential for the remediation application and can be effective to remove heavy metals from contaminated soil.

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