PRELIMINARY ASSESSMENT OF COPPER BIOACCUMULATION IN SOIL USING *EUDRILUS EUGENAE*

Julie Anne Pearl Afos[1], Martina Alexandria Balibay[1], Maxine JeloresGeli[1], GaeNamreh Lorenzo[1], John EnrieMaybituin[1], Maria Julia Serino[1], andLouie Ian Mariano[2], RChE, MEN
[1]*Psychology Program, College of Liberal Arts and Sciences*
[2]*Natural Science Area, College of Liberal Arts and Sciences*

ABSTRACT

A wide range of pollutants have been affecting soil for the past years. These pollutants have led to major disturbances in communities and populations of soil organisms, as well as their function in the soil ecosystem. Studies have shown that earthworms are considered to be convenient indicators of land use and soil fertility for they are sensitive to toxic chemicals. According to Weltje (1998 as cited in Dai, 2004), "different factors interact to determine the amounts of pollutants accumulated by earthworms. The affinity of metals for soil constituents is the primary element to take into account. The distribution of metals among the soil phases is important for the bioaccumulation by earthworms as the main pathways for chemical absorption are the skin for soluble elements, gut transit and digestion." This study aims to identify the effectivity of Eudriluseugenae in the bioaccumulation of heavy metals in an artificially contaminated soil and its significant difference in different levels of concentration in each sample. A total mass of 4.3g to 4.5g of 7 starved earthworms exposed for seven days to artificially prepared copper sulfate contaminated soil samples with concentration range of 1000 ppm to 5000 ppm. Soil samples were then tested for copper (Cu^{2+}) ions concentration using Inductively Coupled Plasma (ICP). The results showed that there was a range 92-94 percent removal of copper in the contaminated soil which shows the effectivity of Eudriluseugeniae in bioaccumulating heavy metal. This proves that earthworms are important organisms that may enhance the function of the soil ecosystem. It is also to note that higher concentrations may highly increase the chance of greater consumption of heavy metals.

Keywords: *Eudriluseugenae, Copper, Soil, Contaminants, Bioaccumulation*

INTRODUCTION

Soil pollution has been considered as a serious problem in many countries. Many soil pollutants are so complex that these can extremely give negative effects in our environment. Effects may range from direct toxicity in our soil organisms as well as many indirect effects like affecting solid processes, effect of climate and other predictable major ecosystem distractions. The pollutants directly affect tropic groups of soil organisms, invertebrates, plants and microorganisms (Edwards, 1973 as cited in Edwards, 2002).

In natural environments, there are several stressful factors that are taking place at the same time. The most diffusive chemicals occurring in soil includes heavy metals, pesticides, petroleum, and hydrocarbons. Heavy metals are widely spread in the environment that most of these penetrate the surface of soil layers through pesticides, fertilizers, organic and inorganic amendants, mining, wastes and sludge residues (Capri &Trevisan, 2002 as cited in Lionetto, Calisi & Schettino, 2012.) The effects of soil pollution threaten living organisms; decreases soil fertility, contaminate crops and groundwater, and alter soil structure (Suter, 2006; as cited in Calisi, Lionetto&Schettino, 2011).

In the ecosystem, earthworms play a major part in developing and maintaining soil structure (Lukkari et al, 2003) that has an impact on the soil formation and organic breakdown, and have been considered convenient indicators of land use and soil fertility (Suter, 2006; as cited in Calisi, Lionetto & Schettino, 2011).

Effects of heavy metals on earthworms have been given much focus by many studies (Bundy et al. 2007; Schleifler et al. 2006; Svendsen et al, 2007; Amaral& dos Santos Rodrigues, 2005; Burgos et al. 2005; Reinecke&Reinecke 2004; Lukkari et al., 2004; Sanchez-Hernandez, 2006 as cited in Calisi et al., 2011). Suthar, Singh &Dhawan (2008) asserted that "metal bioaccumulation by soil-dwelling earthworms can be used as an ecological indicator of metal availability in soils."

Given these previous studies, this current paper aims to add to the literature by identifying the effectiveness of *Eudriluseugenae* in the bioaccumulation of heavy metals in an artificially contaminated soil. The paper specifically wants to identify the effect to varying initial concentrations of contaminated soil.

This study only focused on a specific type of red earthwork that is cultivated in the Philippines– the *Eudriluseugenae*, also known as the African Night Crawler. The use of cultivated earthworms may have affected the mortality rate of the study since these earthworms have not been exposed to contaminants prior to the experiment. Results may have differed to actual samples of soil since the sample used in the test was injected with laboratory prepared solution of copper sulfate. The pH and moisture of the soil and the specific age, weight and size of the earthworm were not considered as factors in the study.

MATERIALS AND METHOD

The experiment was conducted to estimate the accumulation ability of the earthworms to the artificial contaminated soil. The contaminated soil used for this study was composed of soil and horse manure. Culture techniques (Lowe & Butt, 2005) and artificial soil test (Journal of European Community, 1988) methods were adapted from previous studies.

Preparation of soil sample

A defined organic soil was used and measured in terms of dry weight. The researchers prepared the soil and the manure by weighing and dividing these into equal samples. After measuring the soil and manure, these were carefully mixed to assure that there was an equal distribution on the container. This was also done to delimit and control the samples. The researchers ensured that each sample was measured at 500g. The sample was then placed in a container with a dimension of 5 inches x 6 inches.

Preparation of Solution

Subsequent to the soil sample preparation, the researchers primed the artificially prepared spike water. There were 5 different concentrations of the solution prepared in the experiment. These were: 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm and 5000 ppm. Using copper sulfate as the base for the artificial spike water, the amount of chemical used was weighed at 1.5g, 3g, 4.5g, 6g and 7.5g, respective of the level of concentration. The solution was dissolved in distilled water. After mixing, the solution was transferred to a glass bottle.

Preparation of Earthworm

The earthworms were starved for one (1) day. They were placed in a sealed container with holes to provide ventilation. The container was kept free from soil or other materials to assure that there was no consumption during the starvation period. Moreover, this process was done to ensure that when the earthworms were placed on the artificially contaminated soil, their stomachs were free from wastes that may have come from their original environment. Hence, allowing greater room for accumulation of contaminants.

Measurement of earthworm mass was made to control accumulation and to avoid variation of results due to the inconsistency in the population of each container. Table 1 shows that a total mass of 4.3g to 4.5g of 7 earthworms were measured regardless of their individual weight and size. After obtaining the desired amount, the earthworms were placed on the prepared contaminated soil.

Table 1: **Mass of earthworm**

Earthworms Group Number	Weight per Group, g
1	4.3273
2	4.4848
3	4.4951
4	4.4575
5	4.3184

Equilibrium analysis

After preparing all the materials for the experiment, the earthworms were exposed to the soil samples for 7 days. Analysis of the heavy metal content was tested on the 7th day. Exposure time was based on the method adopted from the Journal of European Community (1988).

Soil analysis

Before testing the sample and in order to analyze copper content, the targeted analyte was digested through the use of nitric acid. This was accomplished to dissolve the sample. Then, the sample was diluted in distilled water before the test that used the Inductively Coupled Plasma (ICP).

After testing, the percent absorption was computed using the formula:

$$\%_{adsorption} = \frac{C_i - C_{eq}}{C_i} x100$$

where:

C_i = initial concentration

C_{eq} = equilibrium concentration

RESULTS AND DISCUSSION

Results of the study indicate that in terms of the heavy metal content, the experiment upheld the study's assumption that earthworms are effective in the bioaccumulation of heavy metal in contaminated soil. Results further revealed that after the 7-day exposure of the earthworms in the contaminated soil, there was marked difference in the metal content of the tested soil vis-à-vis the exposure days. Results of the soil analysis are shown in Table 2.

Table 2: **Percent Absorption of earthworms at vary initial concentrations**

C_i ppm	C_{eq}ppm	% Absorption
1000	78.36	92.164
2000	115.02	94.249
3000	211.32	92.956
4000	269.30	93.2675
5000	276.13	94.4774

Table 2 shows that concentrations of copper on the soil samples decreased. This implies that earthworms seem to significantly accumulate heavy metal concentrations on contaminated soils. This finding supports the study of Van Gestelet et al. (2009) on the earthworm species *L. rubellus*. The results also showed congruence with the study of Lionetto et. al. in 2012 which stated that high internal metal concentrations were found inside the bodies of these earthworms after being exposed to highly-contaminated soil. However, analysis of internal metal concentrations inside the body of earthworms was not part of this study. It only supported the idea that there is presence of heavy metals inside the body of earthworms which may indicate the increase of its relevance for the evaluation of contaminants' effects on soil organisms (Lionetto et al, 2012).

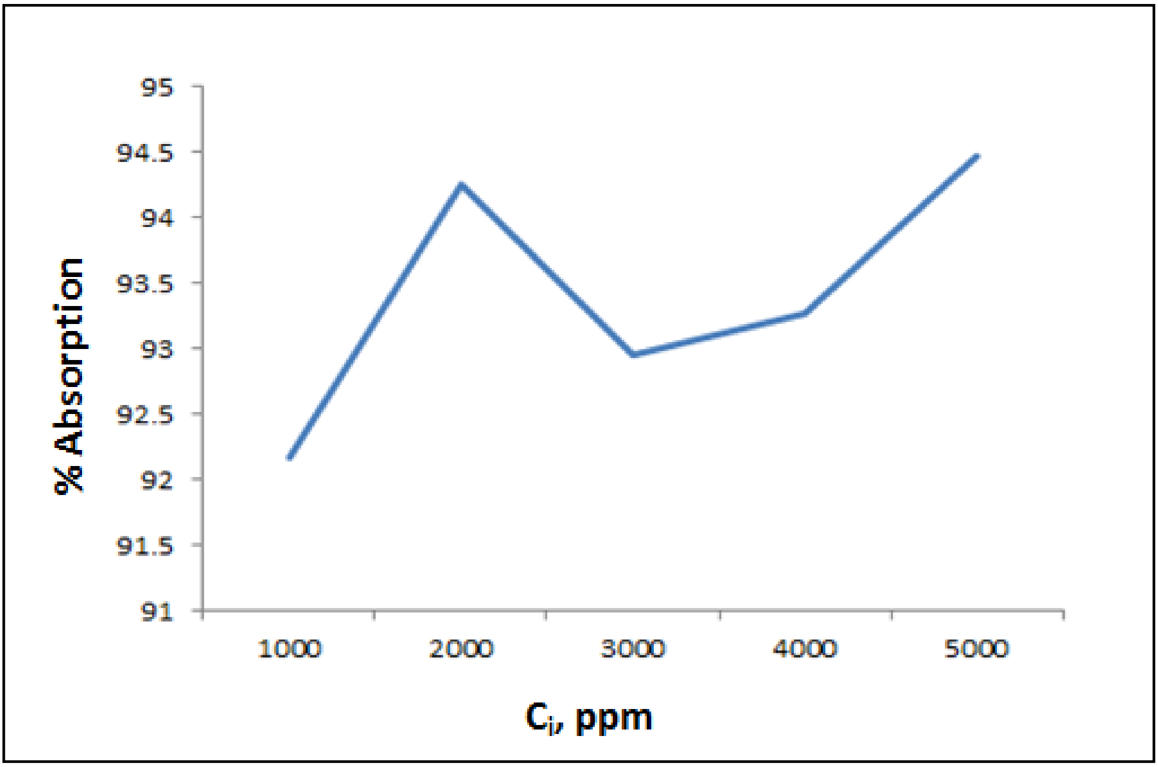

Figure 1: **Percent absorption versus initial concentration**

Figure 1 shows the percent absorption of earthworm versus the initial concentration. In the analysis of heavy metal content, statistics show that there is a large consumption of heavy metal in each sample that vary in initial concentration. It shows that at the maximum concentration, there is 94.5% absorption of copper left in the sample. The study concludes that the higher the concentration, the greater consumption of heavy metal is taken. This is accounted because of the higher concentration for the earthworms to bioaccumulate.

Findings of this research is parallel to the claims of Dendooven&Ramods(2011) that proves adding earthworms to contaminated soil can increase the removal of the contaminant. Moreover, an explanation to this absorption of contaminants is distinguished in Vijver's (2003) study which claims that the uptake of pollutants absorbed to solid particles in the soil plays a role in the ability of earthworms to accumulate contaminants. This is due to the earthworms' behavior to burrow into the soil as well as their capacity to digest organic soil constituents (Edwards & Lofty, 1972 as cited in Vijver et al, 2003).

This phenomenon was further explained by Weltje (1998 as cited in Dai, 2004) who claimed that different factors interact to determine the amounts of pollutants accumulated by earthworms. The affinity of metals for soil constituents is the primary element to take into account. The distribution of metals among the soil phases is important for the bioaccumulation by earthworms as the main pathways for chemical absorption are the skin for soluble elements, gut transit and digestion."

The work of Corter (in Dai 2004) runs parallel with the study especially in determining the relative toxicity of soil based on the bioaccumulation of earthworms.

CONCLUSIONS

Results of the experiment have shown that *Eudriluseugeniae* was likely to contribute to the bioaccumulation of heavy metals in contaminated soils. In the analysis of heavy metal content, especially at the maximum concentration, there was 94.5% absorption found in the analyzed contaminated soil. This has shown the effectiveness of earthworms in absorbing heavy metal content in contaminated soil. The study revealed that higher concentrations of heavy metals in the soil may highly increase the chance of greater consumption by the earthworms.

RECOMMENDATIONS

The researchers recommend that future studies on this topic should also consider using different species of earthworm to further identify, which among them have greater bioaccumulationability. Furthermore, this may also measure the contaminants within material ingested by soil-dwelling earthworms. The study further suggests the use of other types of chemicals aside from copper to test the earthworms' biosorption as this may also yield significant findings. Lastly, the paper wants further study on earthworms as these may add proof to their capacity to enhance their function in the environment.

REFERENCES

Calisi,A.,Lionetto, M. G., &Schettino, T. (2011). Biomarker response in the earthworm Lumbricusterrestris exposed to chemical pollutants. The Science of the total environment, 409 (20): 4456–64.doi:10.1016/j.scitotenv.2011.06.058

Dai, J., Becquer, T., Rouiller, J., Reversat., G., Reversat., F. G., Nahmani, J. & Lavelle, P. (2004). Heavy metal accumulation by two earthworm species and its relationship to total and DTPA-extractable metals in soils.Soil Biology & Biochemistry, 36: 91-98. doi:10.1016/ j.soilbio.2003.09.001

Dendooven, L., Bernal, D. A. & Ramos, S. M. (2011). Earthworms, a means to accelerate removal of hydrocarbons (PAHs) from soil: A mini-review. Pedobiologia, 54S (S187-S192).doi:10.1016/j.pdobi.2011.08.006

Dai, J., Becquer, T., Rouiller, J., Reversat., G., Reversat., F. G., Nahmani, J. & Lavelle, P. (2004). Heavy metal accumulation by two earthworm species and its relationship to total and DTPA-extractable metals in soils.Soil Biology & Biochemistry, 36: 91-98. doi:10.1016/ j.soilbio.2003.09.001

Dendooven, L., Bernal, D. A. & Ramos, S. M. (2011). Earthworms, a means to accelerate removal of hydrocarbons (PAHs) from soil: A mini-review. Pedobiologia, 54S (S187-S192).doi:10.1016/j.pdobi.2011.08.006

Edwards, C. A. (2002). Assessing the effects of environmental pollutants on soil organisms, communities, processes and ecosystems. European Journal of Soil Biology, 38(3-4): 225-231.

Lionetto, M. G., Calisi, A., &Schettino, T. (2012). Earthworm Biomarkers as Tools for Soil Pollution Assessment. Soil Health and Land Use Management. ISBN: 978-953-307-614-0. Retrieved fromhttp://www.intechopen.com/books/soil-health-and-land-usemanagement/earthworm-biomarkers-as-tools-for-soil-pollution-assessment

Lowe, C. N.& Butt, K. R. (2005). Culture techniques for soil dwelling earthworms: A review. Pedobiologia, 49(5): 401–413.doi:10.1016/j.pedobi.2005.04.005

Lukkari, T., Taavitsainen, M., Vaisanen, A. &Haimi, J. (2003).Effects of heavy metals on earthworms along contamination gradients in organic rich soils. Ecotoxicology and Environmental Safety, 59: 340-348. doi:10.1016/j.ecoenv.2003.09.011

Suthar, S., Singh, S. &Dhawan, S. (2008). Earthworms as bioindicators of metals (Zn, Fe, Mn, Cu, Pb and Cd) in soils: Is metal bioaccumulation affected by their ecological category? *Ecological Engineering*, 32: 99-107. doi:10.1016/j.ecoleng.2007.10.003

"Toxicity for earthworms" (1988): Artificial soil test. Journal of European Communities. Retrieved from http://enfo.agt.bme.hu/drupal/sites/default/files/C08web1988.pdf

Van Gestel, C., Koolhaas, J. E., Hamers, T., Hoppe, M., vanRoovert, M., Korsman, C. &Reinecke, S. A. (2009). Environmental pollution, 157: 895-903.Doi: 10.1016/j.envpol.2008.11.002

Vijver, M. G., Vink, J., Miermans, C. &Gestel, C. (2003). Oral sealing using glue: a new method to distinguish between intestinal and dermal uptake of metals in earthworms. Soil Biology & Biochemistry, 35: 125-132. PII: S0038-0717(02)00245-6

Guide to Contributors

Antorcha is a semi-annual scholarly journal of Colegio de San Juan de Letran-Manila published every September and March. As a multidisciplinary research journal, it welcomes researches from the graduate and undergraduate students of the different programs of the Colegio.

The editors recommend that manuscripts conform to the following guidelines:

1. Manuscripts should be endorsed by their respective program chairs together with the consent from the student researchers and research adviser.
2. Authors should submit two versions of the manuscript. One file ("file not for review") should include the names of the authors (adviser and student/s), their contact information (e-mail addresses), and current affiliation (program/area and college). The other file ("file for review") should remove any information that would identify the authors.
3. The paper should include keywords and an abstract of 100 – 200 words.
4. The article should contain approximately 6000 – 7000 words (including abstract, tables/figures, and references) and should be typed in a 12-point font, Times New Roman, double-spaced, with one-inch margin on all sides.
5. Tables/figures and references should follow the APA format style.
6. The editors prefer to have the file in Microsoft Office Word 97-2003 Document (.doc) format and should be sent to antorcha1620@gmail.com.
7. Manuscripts that are already published or in the process of publication in other journals will not be considered in Antorcha.

Review Process

1. The editor screens the submitted manuscripts and selects those deemed suitable for peer reviewing. Selected articles then undergo a rigorous double-blind refereeing.
2. Once accepted, a copyright agreement will have to be sought from the student researchers and research adviser.
3. Attached with the letter of acceptance are the comments and suggestions from the members of the editorial board. Revisions should be in incorporated and returned to the Center within 2 weeks.
4. The language editor ensures that the revisions are properly made. Authors are given another 2 weeks to integrate the comments of the language editor.
5. The editor makes the final decision on the publication of the revised articles.

All communications should be addressed to:

The Editor
Antorcha
Colegio de San Juan de Letran
151 Muralla St., Intramuros,
Manila, Philippines
527-7693 to 97 loc. 122
e-mail: antorcha1620@gmail.com / research@letran.edu.ph