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Effects of Zinc Accumulation on Earthworm *Aporrectodea caliginosa* (Haplotaxida: Lumbricidae)

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ABSTRACT

Earthworms are key to the earth's ecosystem, which helps the soil increase its fertility and repair its existing elements, as well as remove contaminants. This study investigated the accumulation of Zn in the earthworm *Aporrectodea caliginosa* after 15 days of exposure. The worms were grown in media with different concentrations of Zn between 750 and 1500 ppm. Each treatment consisted of three replicates, containing 30 worms. A control group without Zn was also used. Data were analyzed by using Duncan Multi–Range. The results revealed that *A. caliginosa* had a strong ability to accumulate Zn in its tissue compared to the control group. It was noticed that the increase of the heavy metal in the worm´s tissue is associated with the elevation of the metal in its media. The results show a significant loss of weight in the worm´s body and loss in the growth rate; they also shown a decrease in specific growth rate. Moreover, there was a significant decrease in the worm length, showing a high effect, especially after 15 days of breeding with all concentrations used in this study. The researchers recommend using earthworms to purify the soil from contaminants because earthworm has a great ability to get rid of all pollutants, whether metals or pesticides or parasites, especially in industrialized countries and agricultural lands.

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INTRODUCTION

Earthworms are key to the earth's ecosystem, which helps the soil increase its fertility and repair its existing elements, as well as remove contaminants. The zinc element is the second important element of the body in addition to it is considered a toxic element if used with certain concentrations and time periods. One of the most peculiar and special behavior of earthworms is to accumulate heavy metals in their tissues and is quite sensitive to contaminants, which make them good bio-indicators (Nahmani & Lavelle, 2002). Soil is a complex system functioning as habitat group for microorganisms, plants, animals, and humans. Contaminated soils have become a nowadays problem since they lead to groundwater contamination and use excessive in agriculture of biochemical compounds in the food and industry (Loureiro et al., 2005).

Bioremediation represents an interesting alternative of decontamination processes of the environmental factors, which increasingly gain attention in the last decades. Sources of Heavy metals (Cd, Pb, Zn, Cu) from agricultural and mining industry. In terms of the sources in the agricultural sector, these can be categorized into, pesticides, manure of animal, and Sewage (Li et al., 2014). Use of polluted wastewater for irrigation purposes has led to increase soil heavy metal concentration effect non-target objects distribution and physiology, especially used Zn because Zn t enters the metabolism process for living organisms (Latha & Mahaboob-Basha, 2016). Heavy metals are known as dangerous pollutants because they are toxic and non-degradable, thus accumulation of heavy metals in soil causes many risks to the ecosystem and human life (Odoh et al., 2011).

Utilizing living organisms in decontamination and restoring environmental factors can be efficient and feasible compared with other physical and chemical processes (Iordache & Borza, 2012). Among these organisms are earthworms which have the ability to store heavy metals in their bodies, including zinc and cadmium, for example, and this is done by building a group of proteins known as metallothiones, which are characterized by their low molecular weights and being rich in cysteine, as well as building proteins associated with minerals that are formed as a result of urging certain sites that build new proteins. It is believed that one of the functions of these proteins is to reduce the toxic effect of metals through their association with these proteins and the decrease in the concentrations of minerals present in a free state within the bodies of these worms (Hilmy et al., 1988).

The implication associated with heavy metal contamination is of great source, specifically in agricultural production system (Uzoma et al., 2013). The capacity of earthworms to assimilate metals into their bodies has led to use for cleaning soil from metal (Iordache & Borza, 2012). The aim of the current study was to assess the efficiency of *Aporrectodea caliginosa* in accumulation Zn in the body tissue from polluted soil. Compared to other species of earthworms, it was noted in previous research that they have the ability to accumulate heavy metals (Latha & Mahaboob-Basha 2016) .The study also aims at showing the effect of the key biological processes on the weight and growth rate These findings weight and growth rate also point to a possibility to restore their quality by maintaining in appropriate levels the earthworm populations in the agricultural lands, as well as the specific growth rate of the earthworms, which were estimated to rid the soil of contaminants and increase soil fertility.

MATERIALS AND METHODS

Experimental Organism

Earthworms (*Aporrectodea caliginosa*) were used as test material in the current study. To determine the effect of zinc and its accumulation in worms' tissue and its comparison with soil, in addition to its impact on the weight of worms and their relative growth and specific growth rates and lengths.

Methods of Collection

Earthworm was collected from the upper layer of the earth about 30-50 cm in depth from the gardens of Mosul University. The appropriate time for the sampling in spring (April) is early morning. The collected live worms were stored in plastic bags filled with a suitable amount of wet soil.

Preparation of Tested Compounds

The heavy metal Zn used in the study was $ZnSO_4.7H_2O$ with three different concentrations containing 750, 1000, and 1500 ppm of Zn. Each treatment consisted of three replicates, containing 30 worms. A control group without Zn was also used.

Determination of Growth Performance

In this study, after taking the weights of the worms treated with zinc concentrations, the mean weight and relative growth were calculated according to the time period using Sogbesan & Ugwumba (2006) method.

Calculation of the Number of Heavy Metals Accumulated in Worms and Soil

In order to determine the number of heavy metals accumulated in experimental worm bodies (worm tissues) for all the concentrations used in the current study as well as the control group, as well as the soil and their concentrations, these worms were digested by (Dai et al., 2004) and (Al-Safwii 2006). The earthworms were dehydrated at 40°C. after that, a sample of earthworms was taken after grinding, 100 mg were weighed and mixed with 4 ml of concentrated nitric acid and 2 ml of hydrochloric acid, which were left for 12 hours in covered glass bottles and then heated by placing it on a hot plate at high temperature for two hours, the samples were filtered after cooling and then completed to 25 ml with distilled water and then measured by an Atomic Absorption Photometer (APHA, 1985).

The soil samples were digested. Then, 2 g of soil were added to the soil: A mixture of sulfuric acid: nitric: perchlorate 1: 1: 3 and 10 ml of this mixture then heated on a hotplate for two hours. The samples were covered with an hour bottle after the second day, the color became milky then 5 ml of the previous mixture was added back to the samples without heating and left for 30 minutes, the samples were then nominated and sized to 50 ml with distilled water and the samples were ready for measurement with the Atomic Absorption Photometer.

Data Analysis

All data recorded were computed and analyzed for difference using Duncan Multi–Range. The error rate was less than < 0.05 (Al-Zubaidy & Al-Falahy 2016).

RESULTS AND DISCUSSION

The accumulation of Zn in earthworm tissues

This study found that there was significant effect of the heavy metal (Zn) on earthworm tissues. Results shown in (Table.1) and (Figure1) showed the effect of three concentrations (750, 1000, and 1500 ppm) of zinc on earthworm tissues. There is a significant effect between the different treatments and the control group after 15 days of breeding. The highest accumulation of Zn was 1086.602 ppm at concentration of 1500 ppm in the earthworm tissues, and it was significantly different from the lower amount that was noticed in the control 256.801 ppm. The study also showed that the highest accumulation of Zn was 642.645 ppm in worm tissue, while it was 498.693 ppm in breeding soil. The results showed the highest accumulation of Zn was 1564.505 ppm in worm tissue at a concentration of 1500 ppm while it was less in the soil at the same concentration (608.7 ppm), the reason due to special mechanism in worm body that could accumulate the heavy metals in the tissues of epidermis and digestive tract. This study showed positive accumulated metal increased in earthworm tissues with increasing concentration.

The results of this study confirmed the results of Tischer (2009) and Akber et al. (2011), in which the highest metal amounts of Zn and Cd were accumulated in the body tissue of earthworm and this accumulation increased with increasing concentration in the chloragogen tissue. In a similar study conducted by Iordache & Borza (2012), they found in their study the effect of soil polluted by heavy metals (Zn, Cu, Mn, Pb) which showed Zn accumulation in three species of earthworms (*Allolophora rosea*, *Lumbricus castaneus* and *Lumbricus rubellus)* that collected 123.22, 89.58, and 104.52 ppm of Zn in earthworm tissue. Similar observation was recorded by Usmani and Kumar (2015) their studies refer to the important role various species of earthworms can tolerate and bio-accumulate high concentrations of heavy metals like Cd and Zinc in their tissues which range from 20.4 to 1420 for lead, it is accumulated in nerve cord muscles, cerebral ganglion, seminal vesicles and chloragogen tissue.

Table1. The accumulation of different concentration of Zn in soil and earthworm tissues *Aporrectodea caliginosa*

Place	The mean of accumulated Zn		Mean of the concentration
Concentration	Tissue	Soil	effect
750	228.4 e	489.934 c	359.167 c
1000	608.677 b	551.535 b-c	580.106 b
1500	1564.5 a	608.7 b	1086.602 a
Control	169 e	344.603 d	256.801 d
Mean place	642.645 a	498.693 b	

*Duncan test, significance level < 0.05

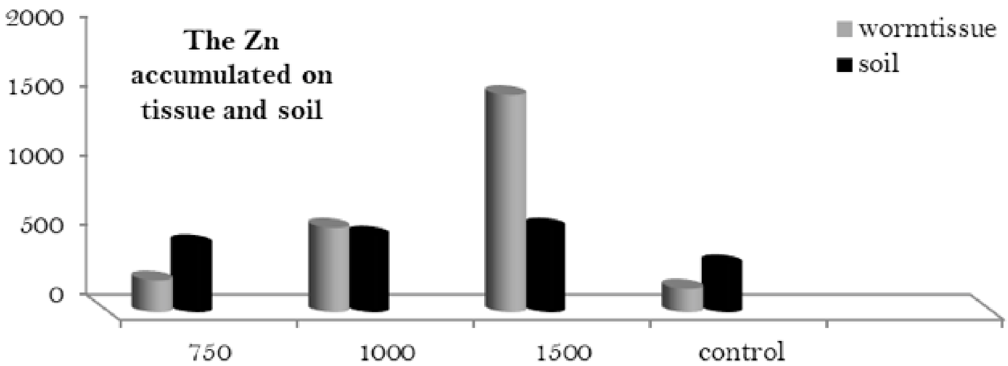

Figure 1. The accumulation of different concentration of Zn in soil and earthworm tissues

The effect of Different Concentrations of Zinc with Three Exposure Period on the Weight of A. caliginosa Earthworm

Results in (Table 2) and (Figure 2) show the effect of the concentrations (750, 1000, and 1500 ppm) of Zn for three period of exposure (5, 10, 15 days). It was found that there was a significant difference between the treatments and the control group. The earthworm's weight decreases with the increase of Zinc concentration. The lowest weight was 0.171, 0.208 g in the concentration 1500, 750 ppm, respectively. The research also indicates the effect of the exposure period on the earthworm's weight. It was 0.259, 0.231 g in the second and the theird period of exposure (10, 15 days, respectively). The interaction between the Zinc concentration and period of the breeding (exposure) has a significant effect as compared with the control treatment during the three periods of exposure. The result shows a decrease in earthworm weight during the third period (15 days of breeding), and weight was 0.134, 0.126 g at the concentrations of 750 and 1500mg/ kg respectively.

The conclusion of these results is that the earthworm's weight decreases with the increasing exposure. Song et al. (2005) found that there was a reduction in earthworm weights when applied to soil contaminated with industrial and domestic waste in western Shenyang, China. Moreover, Akber et al. (2011) also made a similar observation in their study, finding that the increasing amount of heavy metal in the soil caused a positive correlation, which determined between increased metal and increased loss in earthworms' weight. Iordache and Borza (2012) found soil polluted with heavy metals (Zn, Cu, Mn, and Pb) that had an effect on the accumulated Zn in three different species of earthworms: *Allolophora rosea*, *Lumbricus castaneus*, and *Lumbricus rubellus*, which affect the earthworms' weight.

This is attributed to the ability of earthworms to store heavy metals in their bodies, including zinc and cadmium, for example, and this is done by building a group of proteins known as metallothiones, which are characterized by their low molecular weights and rich in cysteine, as well as building proteins associated with minerals that are formed as a result of urging certain sites that build new proteins, It is believed that one of the functions of these proteins is to reduce the toxic effect of metals through their association with these proteins and the decrease in the concentrations of minerals present in a free state within the bodies of these worms (Hilmy et al.,1988).

Table 2. Effect of different concentrations of Zn associated with three intervals of breeding on earthworm weight

Time / Concentration	Weight/g First time 5 days	Weight/g Second time 10 days	Weight/g Third time 15 days	Mean of the concentration effect
750	0.256 c-e	0.234 e	0.134 e	0.208b
1000	0.538 a	0.277 c-e	0.218 c-e	0.344a
1500	0.221 e	0.164 d-e	0.126 e	0.171 b
control	0.292 c	0.362 b	0.448 ab	0.367 a
Mean of time effect	0.326 a	0.259 b	0.231 b	

*Duncan test, significance level < 0.05

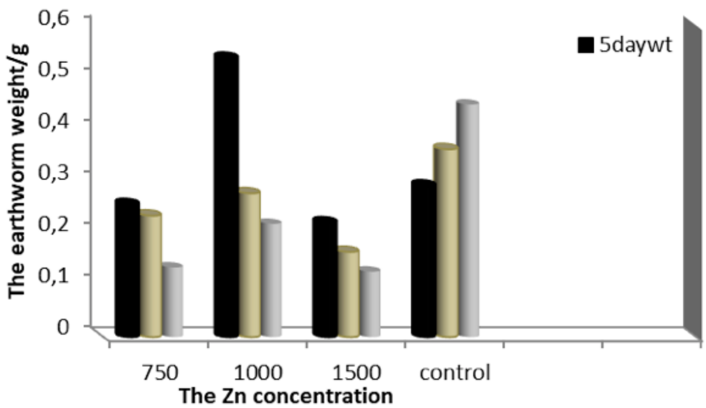

Figure 2. Effect of different concentrations of Zn associated with three intervals of breeding on earthworm weight

The effect of Zinc on the relative of A. caliginosa Earthworm Growth Rate

Results on (Table 3) and (Figure 3) show the effect of three concentrations (750, 1000, and 1500 ppm) of Zinc during three periods of breeding or exposure (5, 10,15 days) on the mean of A. *caliginosa* earthworm relative growth rate. The results show that all concentrations used in the experiment decreased the earthworm's relative growth rate, so the lowest effect on relative growth was 61.376 % at the concentration of 1000 ppm. The effect of exposure period did not appear in the periods of 5 and 10 days, but the effect was 79.88% in the 15-day period. Also, the results on the above Table and Figure 3 showed the effect of the interaction between the Zinc concentration and period of breeding, as well as the interaction between the periods of 10 and 15 day with all concentrations gave a significant effect, so the relative growth rate decreased in comparison with the control treatment, and it was 42.8%, and 1000 ppm and 15 days of exposure in comparison with the control treatment was 169.5%.

The results show that the relative growth rate decreases with increasing exposure periods. A study by Morgan and Morgan (1993) also found a significant decrease in body mass when they bred the earthworm *A. calginosa* in soil contaminated with Zn. This is in agreement with results of other researchers Homa et al. (2003) show reduction in body mass when breeding earthworms in soil polluted with Zn and another heavy metal. A study by Morgan and Morgan (1993) also found a significant decrease in body mass when they bred the earthworm *A. calginosa* in soil contaminated with Zn. Study Zaltauskaite and Sodione (2010) show effect of Cd and Pb on *Eisenia fetida* earthworm growth, which reduced growth of the worm.

Results in (Table 4) and (Figure 4) indicate the effect of the concentrations of 750, 1000, and 1500 mg/kg of Zinc for three intervals of breeding (5, 10, and 15 days) on the specific growth rate of earthworms was 2.533%. Results also indicate that the period of growth has significant effects on the specific growth rate, which was 9.675, 4.487, and 2.6907% in the three periods (5, 10, and 15 days) respectively. The interaction between concentrations and period of breeding also shows a significant effect, the concentration of 1000 mg/kg gives significant decrease of 4.7, 1.92 and 0.98% during the intervals (5, 10, and 15 days) respectively. Similar observation was made by Liesch et al. (2010) on the use of biochar containing potentially toxic micronutrients, including As, Zn, Cu, Fe, and on *Eisenia fetida* applied to an artificial soil, which caused loss of weight reaching 100% and the growth rate of earthworm loss reaching 100%. Other results confirmed, the results of Belmeskine et al. (2012) study, which used the *Eisenia andrei* earthworm in their research. They found toxicity of PCDD/Fs mixtures to earthworms during 28-day exposure, there was less effect when earthworms were exposed to C1 (0.1 ng 2, 3, 7, 8-TCDD/g soil), while the worms exposed to C2 (1 ng 2, 3, 7, 8-TCDD/g soil) presented pronounced effects and were significantly different from the control. In fact, a reduction in growth rate was obtained at 7, 14 and 21 days and was stable at 28 days. A Similar observation was made by Latha and Mahaboob-Basha (2016), who found in their study the effect of Zn on many species of earthworms, which changed in body mass and had a steep decrease in body weight.

Table 3. Effect of Zn metal on the relative of *A. caliginosa* earthworm growth rate

Stage / Concentration	Mean growth rate: First time 5 days	Second time 10 days	Third time 15 days	Mean Concentration effect
Control	110.7 b-c	136.7 b	169.5 a	138.966 a
750	93.13 c-d	85.16 c-e	53.16 e-f	77.15 b
1000	24.33 c-d	54 e-f	42.8 f	61.376 b
1500	91.93 c-d	69.73 d-f	54.04 e-f	71.906 b
Time effect	97.522 a	84.647 a-b	79.88 b	

*Duncan test, significance level < 0.05

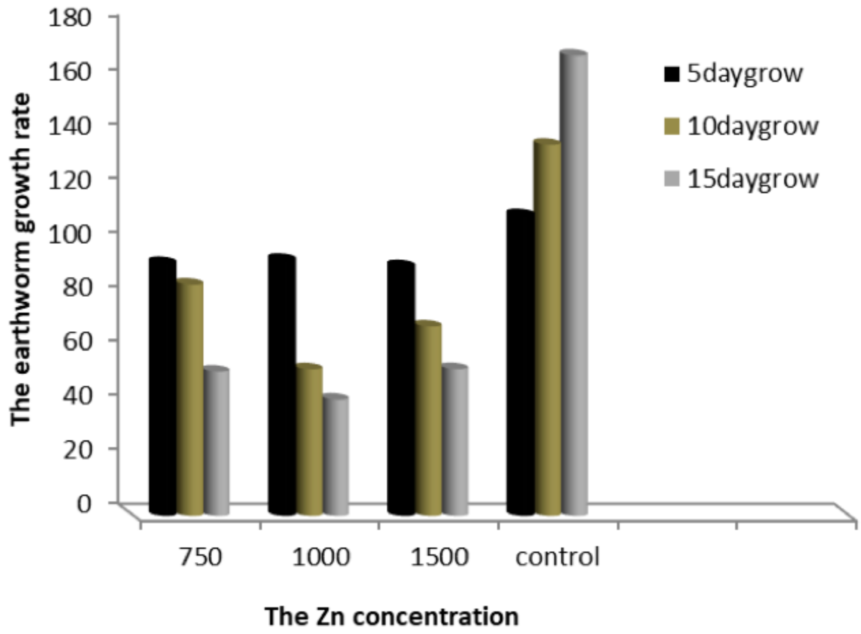

Table 4. Effect of Zn metal on specific of *A. caliginosa* earthworm growth rate

Time / Concentration	The mean of specific growth rate: First time 5day	Second time 10day	Third time 15 days	Mean of the concentration effect
Control	11.026 a	5.336 b	3.5 b	6.620 a
750	10.636 a	4.986 b	2.87 c	6.164 a
1000	4.7 b	1.92 c	0.98 c	2.533 b
1500	12.34 a	5.706 b	3.413 c	7.153 a
Time effect	9.675 a	4.487 b	2.6907 c	

*Duncan test, significance level < 0.05

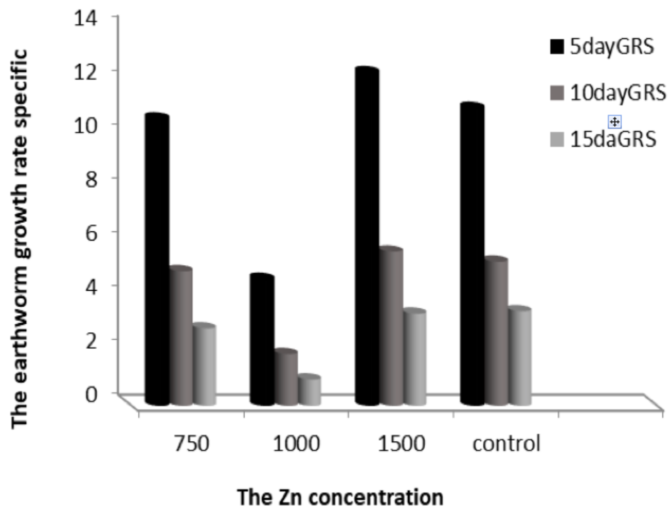

Figure 4. Effect of Zn on growth rate specific of earthworm

The Effect of Zinc on Length of A. caliginosa Earthworm

Results on (Table 5) and (Figure 5) indicate the effect of different concentrations (750, 1000, and1500 ppm) of Zinc for the period of breeding (5, 10, and 15 days) on the worm length. Our results showed that there were significant effects of Zinc concentrations on earthworm length, but the breeding periods have no effect. It is also shown on Table 5 and Figure 5. The interaction between the Zinc concentration and period of breeding decreased the length significantly, and it was reached at -1.16, - 1.31 and – 1.05 cm under the concentrations of 750, 1000, and 1500 ppm and the times of breeding of 5 , 10 and 15 days respectively . Similar observations were made by Hasan (2011), who used type of nutrition, which caused reduction in the length of earthworm *Lumbricus terrestris*. The results in this study are in accordance with Ogbonna and Berebon (2013), which compared the length of worms collected in different areas, such as trees, grass cover, scanty grass cover, and bare grass.

Table 5. Effect Zn metal on length of *A. caliginosa* earthworm

Time / Concentration	Difference in lengths of worms / cm			Mean of the concentration effect
	First time 5 days	Second time 10 days	Third time 15 days	
control	0.42 a-b	0.22 a-c	0.663 a	0.434 a
750	-1.026 b-d	-1.683 d	-1.16 d	-1.289 b
1000	-0.736 b-d	-1.48 d	-1.31 d	-1.1755 b
1500	-0.52 a-d	-1.05 c-d	-1.05 c-d	-0.873 b
Time effect	-0.465 a	-0.998 a	-0.714 a	

*Duncan test, significance level < 0.05

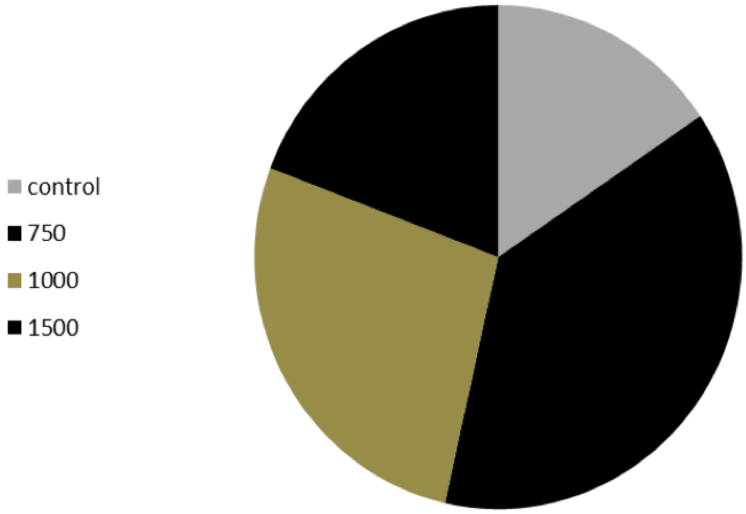

Figure 5. Effect of Zn on earthworm length

CONCLUSION

The results of this study revealed that worms were sensitive to the detection and assessment of soil contamination. Therefore, earthworms can be used as bioindicators to measure soil contamination. According to this study, the addition of Zinc metal reduces weight, both relative and specific growth rates which increased with increasing concentration and period. The earthworm reduces the concentration of metal on the soil. Therefore, the results of this study sustain the bioremediation potential of earthworms for soils polluted with heavy metals as a possibility to restore their quality by maintaining adequate levels of earthworm populations in agricultural lands.

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REFERENCES

Al-Rawi, K.M & Khalafullah, A. (1980). Design and analysis of agricultural experiments. Mosul: Dar Al Kutb.

Al-Safawi, A.Y.T. (2006). Environmental pollution of Mosul city and treatment methods. PhD dissertation, University of Mosul. Mosul, Iraq.

Akber, M. M., AL-Saad, H. T., & Ghadban, A.M. (2011). Bioaccumulation and effect of some heavy metals on worm *Namalycastis indica* (Annelida: Polychaeta). *Journal of Research Basra (Science)*, 37 (15B), 46-51.

A.P.H.A. 1985. Standard methods of the examination of waste and wastewater. 16thedu. American Public Health Association. Washington, D. C.

Belmeskine, H., Haddad, S., Vandelac, L., Sauve, S. & Fournier, M. 2012. Toxic effects of PCDD/Fs mixtures on Eisenia andrei earthworms. *Ecotoxicology and Environmental Safety*, 80, 54–59. https://doi.org/10.1016/j.ecoenv.2012.02.008

Dai, J., Becquer, T., Rouiller, J.H., Reversat, G., Bernhard-Reversat, F., 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. https://doi.org/10.1016/j.soilbio.2003.09.001.

Hasan, M.S. (2011). Effect study on nutrition media and different temperature on earthworm growth *Lumbricus terrestris.* (Oligochaeta: Annelida). *Journal of Babylon University/Pure and Applied Sciences*, 19 (2), 617- 622.

Hilmy, A.M., Abd El–Hamid, N.F. & Ghazaly, K.S. (1988). Biochemical and physiological changes in the tissues and serum of both sexes in *Portunus pelagicus* (L.) following acute exposures to zinc and copper. *Folia Morph*, 1, 79-94. PMID: 3391461.

Homa, J., Niklinska, M. & Plytyez, B. (2003). Effect of heavy metals on coelomocytes of the earthworm *Allobophpora chlorotica.* Pedobiologia, 47(5-6): 640-645. https://doi.org/10.1078/0031-4056-00239.

Iordache, M. & Borza, I. (2012). The bioremediation potential of earthworms (Oligochaeta: Lumbicidae) in a soil polluted with heavy metals. *Journal of food Agriculture & Environment*, 10(2),1183-1186.

Latha, V. & Mahaboob-Basha, P. (2016). Extent of heavy metal accumulation in sewage irrigated soils and their impact on distribution of earthworm communities: linking Chromium and Zinc toxicity on growth and reproduction in selected earthworm species. *Current World Environment* ,11(1), 279-29. http://dx.doi.org/10.12944/CWE.11.1.34.

Liesch, A.M., Weyers, S.L., Gaskin, J.W., and Das, K.C. (2010). Impact of two different biochars on earthworm growth and survival. *Annals of Environmental Science*, 1 4: 1-9. https://openjournals.neu.edu/aes/journal/article/view/v4art1.

Li Z., Ma Z., van der Kuijp T.J., Yuan Z. & Huang L. (2014). A review of soil heavy metal pollution from mines in China: Pollution and health risk assessment. *Science Total Environment*, 468–469, 843–853. https://doi.org/10.1016/j.scitotenv.2013.08.090.

Loureiro, S., Soares, A.M.V.M., & Nogueira, A.J.A. (2005). Terrestrial avoidance behavior tests as screening tool to assess soil contamination. *Environ Pollution*, 138, 121-131. https://doi.org/10.1016/j.envpol.2005.02.013.

Morgan, J.E., & Morgan, A.J. (1993). Seasonal changes in the tissue-metal (Cd, Zn, and Pb) concentration in two eco physiologically dissimilar earthworm species: Pollution- monitoring implications. *Environ Pollution*, 82 (1), 1-7. https://doi.org/10.1016/j.scitotenv.2013.08.090.

Nahmani, J. & Lavelle, P. (2002). Effects of heavy metal pollution on soil macrofauna in a grassland of northern France. *Euro Journal Soil Biology*, 38,297-300. https://doi.org/10.1016/S1164-5563(02)01169-X.

Odoh, R., Agbaji, E.B., Kagbu, J.A., & Thomas, S.A. (2011). Heavy metal speciation in agricultural farmland in some selected local government areas of Benue state, Nigeria. *Archives. Appl. Sci. Research*, 3, 560-573.

Ogbonna, C.B., & Berebon, D.P. (2013). Observations on the ecology of earthworms and effect on the population dynamics of cellulolytic bacteria in soils across selected habitats in imo state University Main Campus Owerri, Nigeria. *Journal of Environmental Science, Toxicology & Food Technology*, 5(4), 17-22.

Sogbesan, A.O., & Ugwumba, A.A.A. (2006). Effect of different substrates on growth and productivity of Nigeria semiaridzone earthworm (*Hyperiodrilus euryaulos* Clausen1842) (Oligochaeta: Eudrinae). *World Journal of Zoology*,1(2),103-112.

Song, Y., Zhou, Q., Gong, P., & Sun, T. (2005). Eco toxicity of soil contaminated with industrial and domestic wastewater in western Shenyang, China. *Sci. Chem. Sci. C. Life. Sciences*, 48(1), 48-56. https://doi.org/10.1007/BF02889801.

Tischer, S. (2009). Earthworms (Lumbricidae) as biondicators: The relationship between in soil and in tissue heavy metal content. *Journal Polish of Ecology*, 57(3), 513-523.

Usmani, Z., & Kumar, V. (2015). Review paper open access role of earthworms against metal contamination: a review. *Journal of Biodiversity and Environmental Sciences*, 6(1), 414-427. https://innspub.net/role-of-earthworms-against-metal-contamination-a-review/.

Uzoma, K.O., Iroha, A.E., Chinyere, C.G., Sunday, E.A., Kelechukwu, D.M., & Ahuwaraeze, N.L. (2013). Effects of mining effluent contaminated soil treated with fertilizers on growth parameters, chlorophyll and proximate composition of *Cucurbita pepo* vegetable. *J. Biodiver. Environ. Sci*, 3,1-8.

Zaltauskaite, J., & Sodiene, I. (2010). Effects of total cadmium and lead centrations in soil on the growth, reproductionand survival of earthworm *Eisenia fetida. Ekologija.* 56(1-2): 10-16.