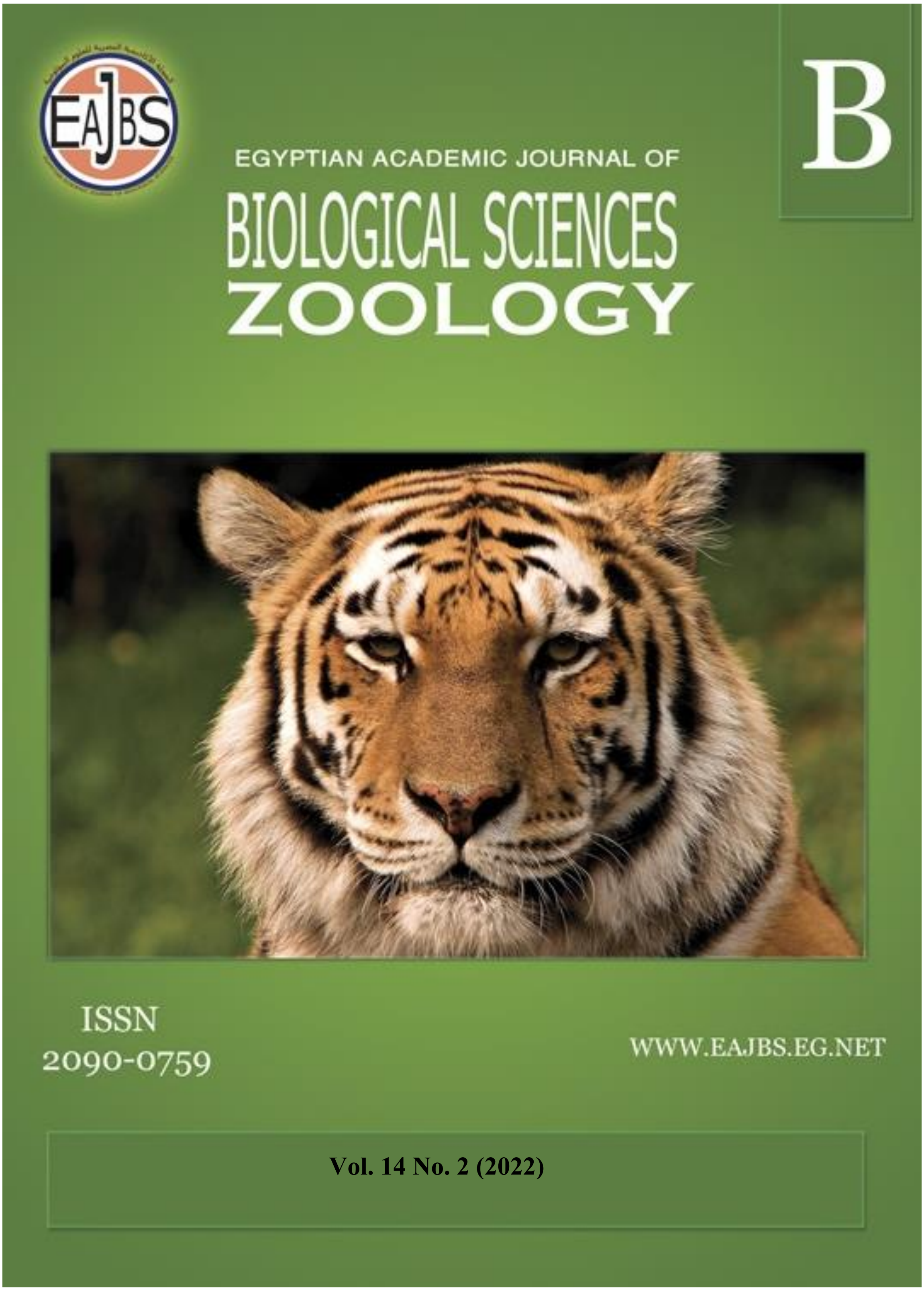


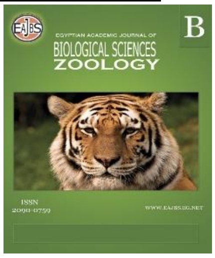
.

www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci. (B. Zoology) Vol. 14(2) pp: 173-181(2022)*
DOI: 10.21608/EAJBSZ.2022.259407

Egypt. Acad. J. Biolog. Sci., 14(2):173-181 (2022)

Egyptian Academic Journal of Biological Sciences
B. Zoology
ISSN: 2090 – 0759
http://eajbsz.journals.ekb.eg/



The Toxic Influence of The Fungicide Remilitine and Chromium Ion on Reproduction of Earthworm, *Eisnia feditia*

Ali A. Ali[1], and Abeer A. Farag [2]

1- Department of Zoology, Faculty of Science, University of Sirte, Libya

2- Department of biology, Faculty of Science, University of Ghemines, Libya.

E-mail*: Alibinomar_1991@yahoo.co.id

REVIEW INFO

Review History
Received:21/6/2022
Accepted:29/8/2022
Available:2/9/2022

Keywords:
Eisenia fetida, artificial soil lead, Remiltine, growth, Annelida.

ABSTRACT

In The present study, The *E.fetida* has maintained in a glass aquaria on a culture media as described by Organization for Economic Cooperation and Development (OECD, 2004), at room temperature of 20±2 ºC. The food consisted of artificial soil mixed with barley grains powder as a food supplement every week throughout the test period. The moisture conditions of the rearing soil were started at approximately 60% water holding capacity. The moisture, thereafter, was maintained by regularly sprinkling water on the soil. Fungal growth was removed when observed on the soil surface. Rearing soil was changed every eight weeks until the worms required for the experiment were with an average weight of 7 to 9 grams. In this experiment, the adult worms were exposed to the artificial soil (Van EGmond, Postbus 24, 2230AA Rijnsbury) contaminated with one concentration of 50 mg/L chromium + Remilitine 500mg/L., respectively. The artificial soil used (OECD, 2004) consisted of 70% quartz sand, 20% kaolin clay, 10% sphagnum peat and calcium carbonate to adjust the pH to 5-6.5. A weight of 250 grams of soil weighted by (Electronic scale soil) was transferred into glass containers (12 cm width, 15cm length, 20 cm high) to which 100 ml of chromium + Remilitine were added and mixed thoroughly. Each treatment was replicated three times and a control site with three replicates were set using plain water. the number of cocoon production by control worms were compared with the cocoon numbers produced by the chrome-Remiltine treated worm using t-test, the results clearly showed a significant difference (F=8.47 P<0.04) after 28-day period, the mean ± S.D of cocoon produced by control worms were 46.67±2.082 compared to 37.00 ± 9.29 of chrome-Remiltine at the 28-day post treat.

INTRODUCTION

Earthworms, the "earth annelids", having super-streamlined and stripped-down bodies are fairly highly evolved critters. Charles Darwin accentuated the role of earthworms in the history of the world and also referred to earthworms as "nature ploughs" because of the mixing of soil and organic matter. Earthworms (phylum Annelida, class Oligochaeta) are also called megadriles (or big worms) as opposed to the microdriles (or small worms) in the families Tubificidae, Lumbriculidae, Enchytraeidae and others. The importance of earthworms has been highlighted by several workers in the fields of waste management, environmental conservation, organic farming and sustainable agriculture (Talashikar and Powar, 1998; Senapati, 1992). Earthworms are burrowing in nature and forming tunnels by literally eating their way through the soil. Their distribution in soil depends on factors like soil moisture, *p*H, and availability of organic matter. They prefer to live in dark and moist places. Cattle dung, humus, kitchen waste and other organic materials are highly attractive sites for some species. Earthworms are very sensitive to touch, light and dryness. Worms can tolerate a temperature range between 5 and 29ºC (Sinha *et al*., 2008; Edwards and Bohlen 1996).

Heavy metals are the main pollutants in the environment and are a big problem due to their toxicity and accumulation in the environment. Soils contaminated with heavy metals are one of the environmental issues considered to be a serious threat to human health and other organisms (Chen *et al.*, 2005; Blaylock *et al.*,1997). Chromium and cadmium are two dangerous heavy metals. For example, exposure to chromium causes lung and digestive organ cancer, severe diarrhea and nausea (Cefalu and Hu, 2004).

Earthworms' uptake metals from contaminated soil, fly ash, and slag through gut uptake. Earthworms accumulate heavy metals and other cations. They are known to be potential bio accumulators and therefore they have been successfully demonstrated in mitigating the toxicity of industrial and municipal waste by vermicomposting technology. A number of mechanisms are followed by the earthworms for uptake, immobilization and excretion of other metals (Sinha *et al.*, 2008).

Earthworms are numerous large-bodied individuals, resistant enough and sensitive enough to contaminants; which make them good bioindicators. They are important micro-organisms in terms of soil functionality (Brown *et al.*, 2000) and consequently play a key role in terrestrial ecotoxicological risk assessment (Weeks *et al.*, 2004; Sheppard *et al.*, 1997). Tiunov and Scheu, (2000) mineralize the organic matter therein and release nutrients that contribute to plant nutrition. Various species of earthworms can tolerate and bio-accumulate high concentrations of heavy metals like cadmium (Cd), mercury (Hg), lead (Pb), copper (Cu), manganese (Mn), calcium (Ca), iron (Fe) and zinc (Zn) in their tissues without affecting their physiology and this particularly occurs when the metals are mostly non-bioavailable.

Since the agricultural products are directly linked to the soil and because of the widespread human use of these products and the high potential of these soils for contamination with heavy metals, especially in industrial areas, the need to purify contaminated soils will be important. In the decontamination of soils contaminated with heavy metals, stability and high resistance to degradation of the metals are considered to be one of the most challenging issues. Traditional methods of refining for the recovery of contaminated sites, mainly due to the high cost and lack of compatibility with ecosystems, are not very efficient (Blaylock *et al.*, 1997).

Hence the need for new methods with high efficiency and low cost for refining contaminated soils is high. The use of earthworms for soil bioremediation is a biological method so that the pollutant concentrations in the soil are reduced through bioaccumulation mechanisms in the body of the earthworms (Matscheko *et al.*, 2002, Slizovskiy and Kelsey, 2010).

These organisms can accumulate high concentrations of heavy metals in their body (Li *et al.*, 2010). Because they are the main components of biomass, earthworms are the most important food source for other organisms higher in the food pyramid (Nahmani *et al*., 2007).

The aim of the study: is a continuous assessment of soil pollutants in the soil animals represented by *E. fetida* (Fig.1) which aims to assess the impact of soil contamination at sub-lethal concentrations at different endpoints including:

Fig. 1: European *Eisenia fetida* (Savigny, 1826).

Earthworms (Annelida, Oligochaeta) are relatively large detritivores (Sims and Gerard, 1985) as well as soft-bodied, cylindrical, long, narrow, segmented and symmetrical organisms. Their body is dark brown, glistening, and covered with soft cuticles. They generally range in weight from 1400-1500 mg after 8-10 weeks. The lifespan of earthworms varies from 3-7 years depending upon the type of species and the ecological conditions prevailing there. The earthworm body contains 65% protein (70-80% high quality 'lysine-rich protein' on a dry weight basis), 14% fats and 14% carbohydrates. They grow throughout their life and the number of segments continuously proliferates from a growing zone just in front of the anus (Sinha *et al*., 2008) .

MATERIALS AND METHODS

Equipment:

Thermometer, pH meter, suitable equipment for humidity control, incubator or small room with air conditioner, water bath, drying cabinet Tongs, hooks and loops, electronic glass containers, soil meter, power source.

Test Organism:

A sample of *Eisenia fetida* was brought from the Czech Republic as a research sample that was tested for this study because it has a greater reproductive potential compared to the local species. Worms breeding: || *Eisenia fetida* was maintained in a glass tank on a culture medium at room temperature. The food was from industrial soil mixed with barley grain powder as a nutritional supplement every week throughout the testing period. Moisture conditions in the soil. Breeding with a water absorption capacity of 60%. After that, the humidity was maintained by spraying water regularly on the soil.

Worm Rearing:

The *E.fetida* has maintained in a glass aquaria on a culture media as described by Organization for Economic Cooperation and Development (OECD, 2004), at room temperature of 20±2 ºC. The food consisted of artificial soil mixed with barley grains powder as a food supplement (Fig. 2) every week throughout the test period. The moisture conditions of the rearing soil were started at approximately 60% water holding capacity. The moisture, thereafter, was maintained by regularly sprinkling water on the soil. Fungal growth was removed when observed on the soil surface. Rearing soil was changed every eight weeks until the worms required for the experiment were with an average weight of 7 to 9 grams.

Fig. 2: Glass container contine artificial soil.

Soil Test:

In this experiment, the adult worms were exposed to the artificial soil (Van EGmond, Postbus 24, 2230AA Rijnsbury) contaminated with one concentration of 50 mg/L chromium + Remilitine 500mg/L., respectively. The artificial soil used (OECD, 2004) consisted of 70% quartz sand, 20% kaolin clay, 10% sphagnum peat and calcium carbonate to adjust the pH to 5-6.5. A weight of 250 grams of soil weighted by (Electronic scale soil) was transferred into glass containers (12 cm width, 15cm length, 20 cm high) to which 100 ml of chromium + Remilitine were added and mixed thoroughly (**Fig.3**). Each treatment was replicated three times and a control site with three replicates was set using plain water. Five grams of barely grain powder were spread on top of each test container as food, supplement and soil moisture content was checked once a week and five ml of water was added when needed.

Fig. 3: Glass containers for a soil test.

Study of Growth and Reproductive Parameters:

Adult forms of *Eisnia fetida* were obtained from the stock culture maintained at the laboratory. The selected worms (10 in number), were washed and weighed by Balance worm (KERN and Sohn GmH). These worms were placed into each of three replicate trays and after 3 hours, they were checked to ensure that all the worms had burrowed into the soil. This was designated as day one of the experiment. Glass containers were covered with a muslin cloth to avoid moisture loss. Optimum growth conditions of temperature, pH and moisture were maintained and worms were hand sorted from all the soil treatments and control was monitored weight change present on days 28, 49 and 70 of the experiment, cocoons were collected and counted from each container by hand sorting at 28 and 70 days. The number of juveniles was also recorded in all treatments at 70 days.

Statistical Analysis:

The data were statistically analyzed by analysis of variance (ANOVA) was used to find the significant difference and t. test for the mean differences using computer software Spss.

RESULTS

Cocoon Production:

The effect chrome-Remilitne on the worm cocoon production and the number of cocoon production by control worms were compared with the cocoon numbers produced by the chrome-Remiltine treated worm using a t-test, and the results clearly showed a significant difference ($F=8.47$ $P<0.04$) after 28-day period, the mean ± S.D of cocoon produced by control worms were 46.67±2.082 compared to 37.00 ± 9.29 of chrome-Remiltine at the 28-day post-treatment.

At the end of the exposure of 70 days, no significant difference was reported ($F=4.43$ $P>0.10$) the mean ± S.D of cocoon was 123.67±7.095 for control compared to 121.33 ± 6.60 for chrome Remiltine treated (Fig. 4).

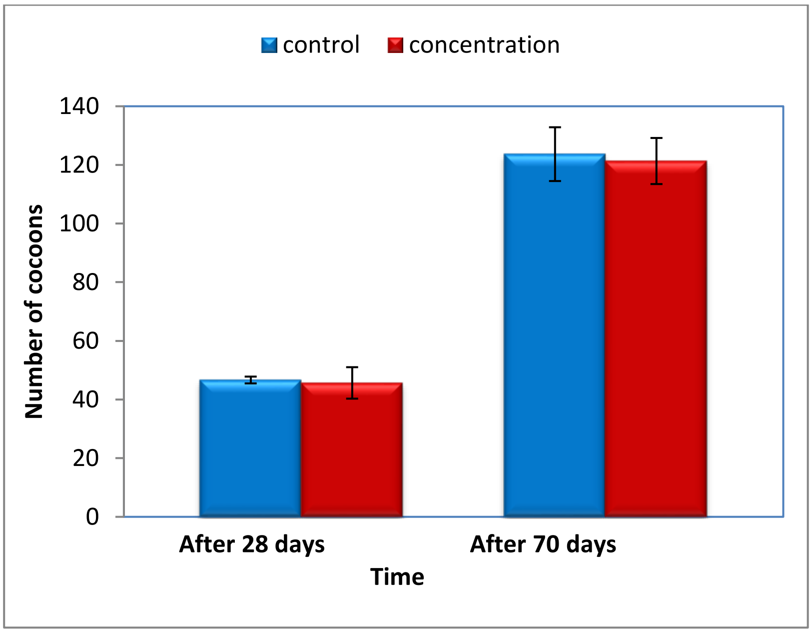


Fig. 4: The mean ± S.D of the number of cocoons per worm in control and chromium-Remiltine treated soils, after 28- and 70-days post-treatment. *Significantly different from controls at $p<0.05$, ** $p<0.01$.

Juvenile number:

The juvenile number produced by cocoons of the control worms and chrome-Remilitine treated worms was further counted.

The t-test revealed a significant difference in juvenile numbers between treatments (F=10.93 P<0.03). The t-test revealed that cocoons produced by control worms were significantly greater than those produced by chrome-Remilitine worms. The mean ± S.D of these mean ± S.D were 94.00 ± 35.53 for control juvenile and 57.67 ± 10.69 for chrome-Remilitine at 70 days post treated (Fig. 5).

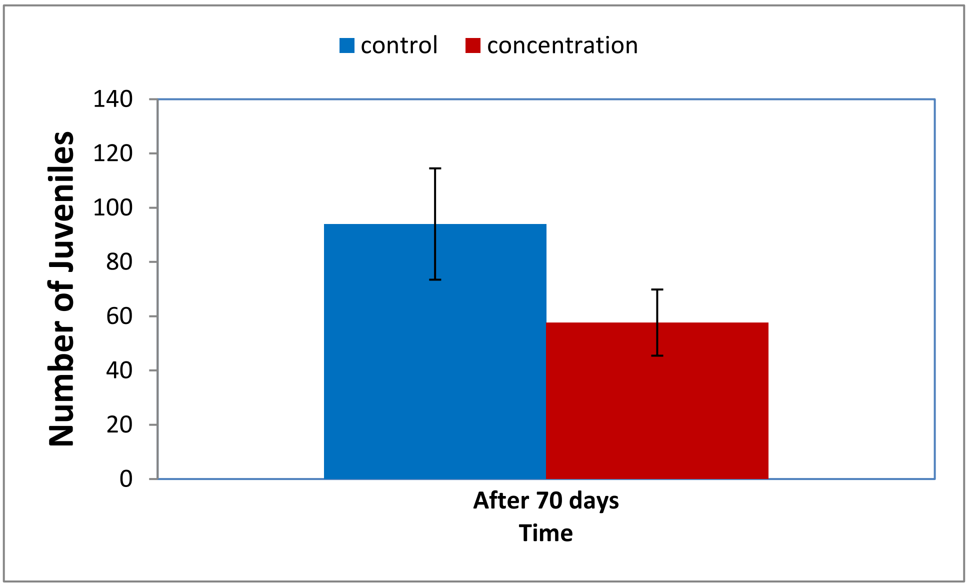


Fig.5 : The mean ± S.D of the number of juveniles per worm in control and chromium-Remiltine treated soils. *Significantly different from controls at p < 0.05, ** p<0.01.

DISCUSSION

Earthworms are regularly used as a fragment of sequences of indicator species to test the effects of pollutants on ecosystems. A wide array of substrates (including artificial substrates like OECD soils), test designs, and endpoints are exploited and guidelines have been considered to regulate the assessment of adverse effects on earthworms. Apart from laboratory testing, terrestrial model ecosystems (TMEs) (Koolhaas *et al* ., 2004) The species most frequently tested in laboratory settings are the compost worms Eisenia andrei and E. fetida as more field-appropriate species unlike L. rubellus and A. caliginosa that are difficult to rear.

Chromium is a dangerous heavy metal, where it causes respiratory and digestive cancers, severe diarrhea and nausea. Moreover, the toxicity of chromium in soil organisms is not studied enough (Cefalu and Hu, 2004). Morgan *et al*., (1990) found discrete alterations in the distribution of various metals throughout the earthworms' body, whereby the sequestration of chlorogocytes played a dominant role, causing changing patterns of tissue accumulation, on the other hand, different tolerances to metals were reported by (Morgan and Morgan, 1998). Metals such as Cu and Cd are mainly bound to metal-binding proteins (St¨urzenbaum *et al*., 2001) and with these proteins, the metal travels through the body to the organs and tissues in which it is deposited inorganic forms. Cd was retrieved in great amounts from the nephridia and to a lesser extent from the body wall of earthworms (Prinsloo *et al*., 1990), and Pb is located in waste nodules found in the coelomic fluid (Andersen and Laursen, 1982).

Other studies on the collaboration between heavy metals have been carried out, where Pan and Yu (2011) found that the combination of Cd and Pb had synergistic toxicological effects on enzyme activities in soil. Aebeed *et al*. (2019) established significant effects on body weight and cocoon production, while the high significant juvenile number was described in Eisenia fetida worms. The combined toxicity tests on earthworms in this research displayed that the interaction of Remilitine fungicide and Chromium was antagonistic.

The proposed objectives of this study are in agreement with that of (Aebeed and Amer 2018) and (Aebeed *et al*., 2019) who stated that mortality is in general accepted to be a somewhat insensitive parameter, whereas sub change in body weight and reproduction were more vital to assess. However, *E. fetida* body weight decrease due to heavy metals was detected in some studies, (Berthelot *et al*., 2008). Whereas, no impact on body weight was detected in other studies (Van Gestal *et al*., 1993). The relationship between metals and body weight has been clarified by (Spurgeo and Hopkin, 1996), who specified that the worms living in metal-contaminated soil reach a lower weight or require more time to reach the maximum weight than in non-polluted sites.

Based on the statistical analysis performed in this study, it was found that, due to the high toxicity of Remilitine fungicide for the body weight, the toxicity of Chromium was less on the body weight. In addition, this study showed that the toxicity of the Remilitine - Chromium mixture during the time is moderate body weight which is greeted by other studies (Aebeed and Mohamed,2018), (Aebeed et al., 2019) and (Dou and Hu, 2014).

CONCLUSION

In the present study, the toxicity of Remilitine and Chromium is more severe in juvenile numbers than in cocoon production. The mechanisms of effects combined between Remilitine and Chromium are complex, which may have been influenced by the competitive adsorption of Chromium and Remilitine in soil and biomembrane and their bioavailability.

REFERENCES

Aebeed S., Mohamed. I and Amer. A. H, (2019). The combined effect of Remilitine and copper on the growth and production of the earthworm, *Eisenia fetida*,. *Libyan Journal of Basic Sciences,* Vol: 9, No: 1, pp 1–12.

Aebeed, S and Mohamed, I (2018). Effect of some heavy metals on the growth, cocoon production and juvenile number of the earthworm Eisenia fetida. *Libyan Journal of Science & Technology,* 7;2 p 91 -95.

Aebeed, S. and Amer, A. H, (2018). Combined toxicity of cypermethrin, a pyerthroid insecticide and metal to earthworms *Eisenia fetida*. *Medical Toxicology Current Research,* 1, 4 .

Andersen C, Laursen J., (1982) Distribution of heavy metals in Lumbricus terrestris, Aporrectodea longa and A. rosea measured by atomic absorption and X-ray fluorescence spectrometry. *Pedobiologia*, 24: 347–356.

Berthelot, Y., Valton, E., Auroy, A., Trottier, B. and Robi-doux,. Y. (2008). PIntegration of toxicological and chemical tools to assess the bioavailability of metals and energetic compoundsin contaminated soils. *Chemosphere,* 74: 66–177.

Blaylock, M. J., Salt, D. E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., Ensley, B. D., Raskin, I., (1997). Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environmental Science & Technology,* v. 31, p. 860865

Brown GG, Barois I, Lavelle P. 2000. Regulation of soil organic matter dynamics and microbial activity in the drilosphere and role of interactions with other edaphic functional domains. *Eurasian Journal of Soil Biology,* 36(3-4), 177-198.

Cefalu, W. T., Hu, F. B., (2004). Role of chromium in human health and in diabetes. *Diabetes Care,* v. 27, p. 2741-2751

Cefalu, W. T., Hu, F. B., (2004). Role of chromium in human health and in diabetes. *Diabetes Care,* v. 27, p. 2741-2751.

Chen, T.-B., Zheng, Y.-M., Lei, M., Huang, Z.-C., Wu, H.-T., Chen, H., Fan, K.-K., Yu, K., Wu, X., Tian, Q.-Z., (2005). Assessment of heavy metal pollution in surface soils of urban parks in Beijing, China. *Chemosphere,* v. 60, p. 542-551

Chen, T.-B., Zheng, Y.-M., Lei, M., Huang, Z.-C., Wu, H.-T., Chen, H., Fan, K.-K., Yu, K., Wu, X., Tian, Q.-Z., (2005). Assessment of heavy metal pollution in surface soils of urban parks in Beijing, China. *Chemosphere,* v. 60, p. 542-551

Dou. J. and Hu. S, (2014). Individual and combined toxicity of chromium and cadmium on the earthworm *Eisenia fetida, BTAIJ,* 10(17) P : [9737-9742]

Edwards CA, Bohlen PJ. 1996. Biology and Ecology of Earthworms. Springer science and business media, Vol. III. Chapman and Hall, London, 426.

Koolhaas JE, Van Gestel CAM, R¨ombke J, Soares AMVM, Jones SE (2004). Ring-testing and field-validation of a terrestrial model ecosystem (TME) – An instrument for testing potentially harmful substances: Effects of carbendazim on soil micro arthropod communities. *Ecotoxicology,*13: 75–88.

Li, L., Xu, Z., Wu, J., Tian, G., (2010). Bioaccumulation of heavy metals in the earthworm Eisenia fetida in relation to bioavailable metal concentrations in pig manure. *Bioresource Technology,* v. 101, p. 3430-3436

Morgan JE, Morgan AJ (1990) The distribution of cadmium, copper, lead, zinc and calcium in the tissues of the earthworm Lumbricus rubellus sampled from one uncontaminated and four polluted soils. *Oecologia,* 84: 559–566.

Morgan JE, Morgan AJ., (1998). The distribution and intracellular compartmentation of metals in the endogeic earthworm Aporrectodea caliginosa sampled from an unpolluted and a metal contaminated site. *Environmental Pollution,* 99: 167–175.

Nahmani, J., Hodson, M. E., Black, S., (2007). A review of studies performed to assess metal uptake by earthworms. *Environmental Pollution,* v. 145, p. 402424

OECD 2004. Guideline for testing of chemical n 222. Earthworm reproduction test (Eisenia fetida/Eisenia andrei), acute toxicity tests, adopted 13 April 2004

Pan. J and Yu. L, (2011). Effects of Cd or/and Pb on soil enzyme activities and microbial community structure. *Ecology of England,* 37, 1889–1894.

Prinsloo MW, Reinecke SA, Przybylowicz WJ, Mesjasz-Przybylowicz J, Reinecke AJ., (1990). Micro-PIXE studies of Cd distribution in the nephridia of the earthworm Eisenia fetida (Oligochaeta). *Nuclear Instruments and Methods Physics Research B,* 158: 317–322.

Senapati BK. 1992. Biotic interactions between soil nematodes and earthworms. *Soil Biology and Biochemistry, 2*4(12), 1441-1444.

Sims RW, Gerard BM. 1985. Earthworms: In Synopses of the British Fauna (New Series) 31 Kermac DM, Barnes RSK, eds. The Linnean Society of London, 171.

Sinha RK, Bharambe G, Chaudhari U. 2008. Sewage treatment by vermifiltration with synchronous treatment of sludge by earthworms: a low-cost sustainable technology over conventional systems with potential for decentralization. *Environmentalist,* 28(4), 409-428.

Slizovskiy, I. B., Kelsey, J. W., (2010). Soil sterilization affects aging-related sequestration and bioavailability of p, p′-DDE and anthracene to earthworms.

Environmental Pollution, v. 158, p. 32853289

Spurgeon, D. J. and Hopkin S. P. (1996). Effects of metal-contaminated soil on the growth, sexual development and early cocoon production of the earthworm Eisenia fetida, with particular reference to zinc. *Ecotoxicology and Environmental Safety,* Vol. 35. P. 86 – 95.

St¨urzenbaum SR, Winters C, Galay M, Morgan AJ, Kille P., (2001). Metal ion trafficking in earthworms – identification of a cadmium specific metallothionein. *Journal Biological Chemistry,* 276: 34013–34018.

Talashikar SC, Powar AG. 1998. Vermibiotechnology for eco-friendly disposal of wastes. Ecotechnology for pollution control and environment management. *Indian Journal of Environmental Ecoplanning,* 7, 535-538.

Tiunov AV, Scheu S. 2000. Microbial biomass, biovolume and respiration in Lumbricus terrestris L. cast material of different age. *Soil Biology and Biochemistry,* 32(2), 265-275.

Van Gestel, C. A. M., Dirven – Van Breemen E. M., Baerselman R. (1993). Accumulation and elimination of cadmium, Chromium and Zinc and effects on growth and reproduction in Eisenia Andrei (Oligochaeta, Annelida). *The Science of the Total Environment,* P. 585 – 597.

Weeks JM, Spurgeon DJ, Svendsen C, Hankard PK, Kammenga JK, Dallinger R, Kohler H-R, Simonsen V, Scott-Fordsmand N. 2004. Critical analysis of soil invertebrate biomarkers: a field case study in Avonmouth, UK. *Ecotoxicology,* 13(8), 819-824.