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Avoidance Behavior of *Eisenia fetida* to Contaminated Soil by Pesticides and Heavy Metals around Benghazi City, Libya

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***Abstract* – Avoidance tests has been developed and validated for some years. Earthworm *Eisenia fetida* able to avoid contaminated soils. It has a great potential as early screening tools. This rapid response enhances the utility of the test to evaluate contaminated sites. It has been indicated that earthworm avoidance behavior is an ecologically relevant parameter for assessing harmfulness of field contaminated soils. The objective of this study to evaluate soil contamination around Benghazi city by using avoidance test as rapid, inexpensive, and easy tools. Series percentage (100, 75, 50, 25%) of soil which collected from locations around Benghazi city (Bouatni, Hawari, Lowifia, and Jarotha) used in avoidance test under control conditions for 24 hours. The locations soil was mixed with artificial soil to get the desired percentages. Earthworm completely avoid all soil100% from all locations as well as Bouatni soil 75%. However, Hawari, Lowifia, and Jarotha soil 75% were avoided by means 6±1.2, 6±3.6, 9±1.2 respectively. At 50% soil in all locations were no avoid behavior accept that in Bouatni soil 50% were avoided by 6±3.6. Earthworm expose to concentrations below these mentioned above showed no avoid behavior. Our result show that Bouatni area have the highest heavy metal concentrations. The outcomes from avoidance behavior tests might bring rapid information for future decisions on the evaluation procedure of contaminant sites, terrestrial risk assessment and soil quality.**

***Keywords* – Earthworm, Avoidance Behavior, Soil, Heavy Metals, Benghazi, Libya.**

I. INTRODUCTION

Earthworms perform many essential and beneficial functions in soil ecosystems, including decomposition, nutrient mineralization, and soil structure improvement [5] and their ability to perform these functions can be inhibited upon exposure to harmful substances [4]. Many study have addressed that earthworm can be very useful tools to assess chemicals pollution in soil [16]. Earth worm has been used as bio indicator for a long time, because many aspects of their life can response to the change in the surrounding environmental and could be measured easily such as avoidance behavior, growth rate, enzyme activity level, mortality, and reproduction patterns [14].

This 14-day LC_{50} test using the earthworm *Eisenia fetida* has been important for risk assessment and regulation of new and existing chemicals [2]. The end point of the 'earthworm acute toxicity test' is mortality. However, mortality is unlikely to be either the most sensitive or ecologically relevant parameter for predicting effects on field populations. The acute earthworm avoidance test was first developed in 1996 [14]. The International Standards Organization (ISO) has established earthworm avoidance test guidelines for rapid screening and evaluation of soil function and influence of contaminants and chemicals on earthworm behavior [8].

Eisenia fetida is an ultra epigeic species (living almost entirely in organic matter) currently used as the standard earthworm in terrestrial ecotoxicology tests in the European Union ([15; 7]. The current Organization of Economic and Cooperative Development acute earthworm toxicity test [10] also uses the earthworms' *E. fetida* as biological monitors for testing effects of contaminants on soil biota. This species are readily available through suppliers and are easy to culture in laboratories. Avoidance tests have higher sensitivity to contaminants and require less experimental time than other earthworm toxicity tests [12; 14], making it ideal for a rapid screen of emerging substances or materials that may be deliberately or incidentally applied to soils. Benghazi (32_10¢N, 20_06¢E), the second largest city in Libya, it is colonized by many soil invertebrates such as earthworms, Isopoda, etc. Pollution of terrestrial ecosystem is a serious environmental problem worldwide. Soil pollutions have enormously increased during the last decades due to the intensive use of pesticides and fertilizers in agriculture. The increase in soil pollution levels due to pesticides as well as heavy metals has endangered both the environment and human life [3].

The objective of this study to evaluate soil contamination around Benghazi city by using avoidance test as rapid, inexpensive, and easy tools. As well as screen test for heavy metals in the same areas. The outcomes from avoidance behavior tests might bring rapid information for future decisions on the evaluation procedure of contaminant sites, terrestrial risk assessment and soil quality criteria studies.

II. Material and Methods

Soil collected from locations around Benghazi city (Bouatni, Hawari, Lowifia, and Jarotha), used in avoidance test under control conditions for 24 hours. The locations soil was mixed with artificial soil to get the desired percentages.

Earthworms were maintained and reared for three months under laboratory conditions in plastic bedding (41cm long, 35cm wide, 20cm high) containing commercial soil. Then moved to the plastic containers (22cm L*18cm W*9cm H) with two divider for avoidance experiment. This two chamber test unit is a version of the two chamber test unit [12]. The earthworms were carefully brought to the laboratory with moist soil and cultured at temperature 18± 2C^o and humidity 75±5 on the same moist commercial soil were used in breeding culture. Dechlorinated tap water was given to reach 60% of maximum water holding capacity (WHC), at light cycle of 12h\12h and fed on powder of barley grains.

Four stations located within the municipality of Benghazi were selected for the study (Fig. 1). These stations were (1) Bouatni, (2) Hawari, (3) El-Guarsha, and (4) Jarotha Figure (1). The soil, were mixed with artificial soil to get the desire concentrations which were 25, 50, 75 and 100%.

Each treated soil was mixed thoroughly with one batch of soil in stainless steel pan before being introduced into the test chamber while the separator is still in place. The same volume of plain distilled water was added to control treatment soil. Then the chambers were filled with treated soil up to a height of about 4-5cm (125g soil for each part of two-chamber) the two-divider was removed and 10 worms of *E. fetida* were placed on the center of the hole, and all the chambers were closed with performed plastic film to allow air circulation and maintained at approximately 20 c^o under 12h\12h of light.

Each container in this case contained one replicate of each treated soil and control for the four concentrations tested.

After 24h test period, the two-divider was reintroduced in the marked position and the individuals were counted by hand -sorting in each compartment of chamber containing the control and treated soil. Animals that were cut by divider were considered as being in the soil to which the animal's head was directed. The avoidance to the different soil treatments was calculated by counting the mean number of earthworms in treated with the mean number of worms in the untreated control soil. The numbers of earthworms was converted to percentage of individuals of total number of worms in each compartment of chamber.

P (%) = [I\N]*100, where P= individuals percentage; I=number of worms in each compartment; N= total number of worms.

Selected heavy metals were determined in biota samples (microwave digestion with nitric acid and hydrogen peroxide). However, heavy metals in soils for "pseudototal" content with aqua-regia extraction (ISO 11466) and bioavailable fraction with 1M ammonium nitrate extraction (ISO 19730) followed by ICP-MS analysis. Total content of Hg in soils and biota was determined by thermo-oxidation solid sampling AAS method.

III. Result and Discussion

Although the avoidance test may not be considered as an alternative to acute test yet, where LC_{50} or LD_{50} is the endpoints of certain toxicants. However, the avoidance test still considered an important and valid test for measuring the direct effects of such toxicant in the form of behavioral response of the animals.

Series percentage (100, 75, 50, and 25%) of soil which collected from locations around Benghazi city (Bouatni, Hawari, Lowifia, and Jarotha). These soil were used in avoidance test under control conditions for 24 hours. The locations soil was mixed with artificial soil to get the desired percentages. Earthworm completely avoid all soil 100% from all locations. Earth worm did not avoid Bouatni soil 25% as well as earthworm did not completely avoid the 50%. However, no earth worm were found in the 75,100% Figure (1). In Hawari soil, Earth worm did not avoid the soil 25% as well as 50% nor 75%. However, no earth worm were found in the 100% Figure (2). In Lowifia soil, Earth worm did not avoid the soil 25% as well as 50% nor 75%. However, no earth worm were found in the 100% (3). In Jarotha soil, Earth worm did not avoid the soil 25% as well as 50% nor 75%. However, no earth worm were found in the 100% Figure (4).

Earthworm completely avoid all soil 100% from all locations as well as Bouatni soil 75%. However, Hawari, Lowifia, and Jarotha soil 75% were avoided by means 6±1.2, 6±3.6, 9±1.2 respectively. At 50% soil in all locations were no avoid behavior accept that in Bouatni soil 50% were avoided by 6±3.6. Earthworm expose to percentages below these mentioned above showed no avoid behavior. Bouatni area is more commonly used in agriculture, which makes it more expose to pesticides and heavy metals.

The pseudototal concentration in soil of all heavy metals were higher in Buatany area than other areas, Zn concentration was 321.8 mg/kg which was the highest concentration followed by Cu with concentration 44.8 mg/kg. Meanwhile, Hg was the lowest concentration 0.239 mg/kg followed by Cd with concentration 0.43 mg/kg. The

bioavailable fraction concentration of all heavy metals were higher in Buatany area than other area, Cu was 1.0mg/kg which was the highest concentration followed by Zn with concentrations 0.8. Meanwhile, Cd was the lowest concentration 0.003 mg/kg table (1).

The concentration of heavy metals in earthworm was almost higher in Buatany earthworm than other earthworm collected from another areas. Zn was the highest concentration 576 mg/kg which was even higher than the pseudototal concentration of Zn mentioned above. The second highest concentrations were Cu with concentrations 9.04 mg/kg.

The behavioral changes in earthworms and other macroinvertebrates due to the presence of contaminants in soils can be detected by the avoidance test. The main advantages of using the avoidance behavior to evaluate the ecological risks are the short duration of the test 24h and its easiness of set up [1]. The results observed here came in support of avoidance behavior of the earthworm *Eisenia andrei* from soils treated with the insecticide Cypermethrin reported by [13]. This test also support the finding of [6] who found that the avoidance of the earthworm *E. fetida* was higher toward the insecticide Lambda-cyhalothrin and Carbendazin compared to the fungicide Benomyl. Similar results by [9] confirmed the value of avoidance response as a measure of soil pollutants. In their study they report that avoidance response of *E. Andrei* was at 320mg copper per kg of soil.

It has been concluded from the avoidance test that earthworms, *E. fetida* can be dependent on as bioindicators for pesticides and heavy metals contamination in soil. The worms expressed high degree of avoidance response to insecticide, fungicide and herbicide present. However, despite of these reasons avoidance response behavior can still be considered as a good indicator for pesticide pollution for its short duration test and easiness of perform. The outcomes from avoidance behavior tests might bring rapid information for future decisions on the evaluation procedure of contaminant sites, terrestrial risk assessment and soil quality criteria studies.

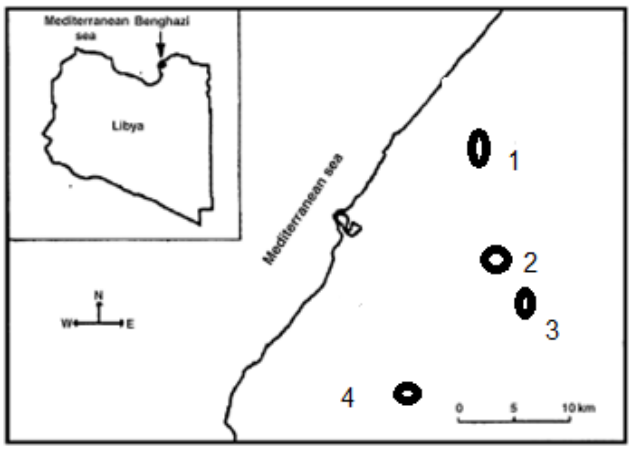

Fig.1. Location of study stations in Benghazi (up to down) (1) Bouatni, (2) Hawari (3) El-Guarsha, and (4) Jarotha,

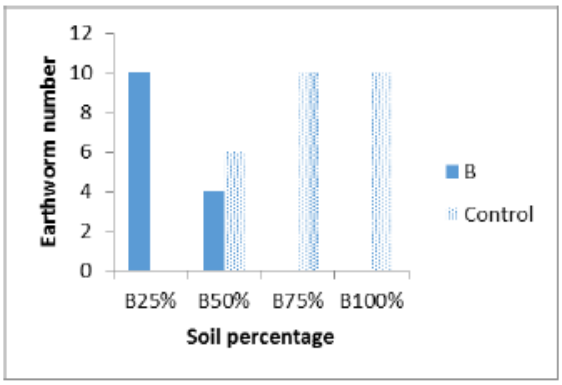

Fig.2. Effect of mixture Bouatni (B) soil (25, 50, 75 and 100%) with control soil on earthworm avoidance behavior.

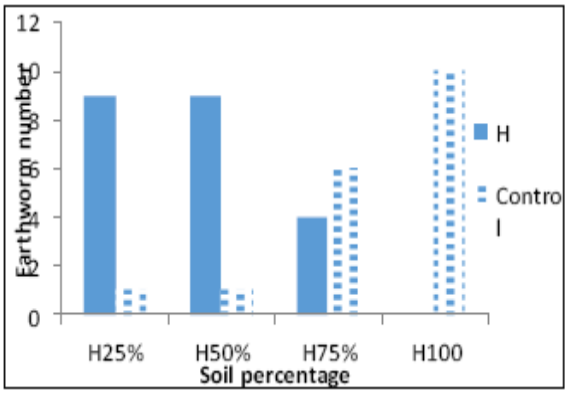

Fig.3. Effect of mixture Hawari (H) soil (25, 50, 75 and 100%) with control soil on earthworm avoidance behavior.

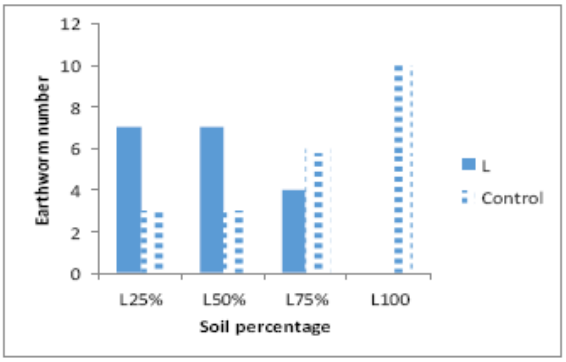

Fig.4. Effect of mixture Lowifia (L) soil (25, 50, 75 and 100%) with control soil on earthworm avoidance behavior

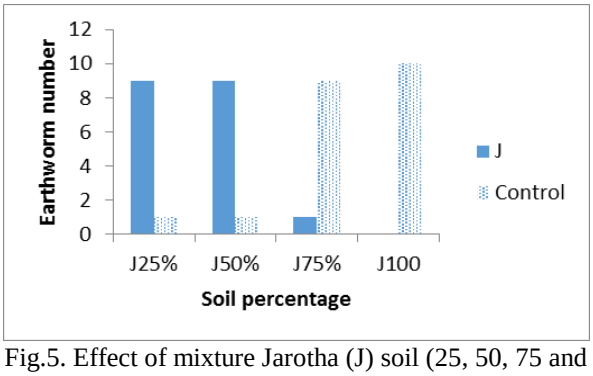

Fig.5. Effect of mixture Jarotha (J) soil (25, 50, 75 and 100%) with control soil on earthworm avoidance behavior.

Table 1: Shows means of Heavy metals concentrations (mg/kg) in different compartments (Soil and Biota)

	SOIL SAMPLES - aqua regia leachate - mg/kg						
Stations	Pb	Cd	As	Zn	Cu	Ni	Hg
BUATANY	33.1	0.43	6.73	321.8	44.8	24.6	0.239
Hawari	19.6	0.26	4.54	52.3	12	16.9	0.01
El-Guarsha	17.6	0.2	5.1	87.7	16.1	21.5	0.012
Jarotha	13.5	0.2	5	115	15.3	18.6	0.224
	SOIL SAMPLES - 1M Ammonium nitrate leachate						
BUATANY	0.008	0.003	0.08	0.8	1	0.2	-
Hawari	< 0,002	0.00065	0.018	< 0,04	0.12	0.03	-
El-Guarsha	< 0,002	0.001	0.012	0.1	0.15	0.03	-
Jarotha	< 0,002	0.001	0.03	0.1	0.24	0.07	-
BUATANY	4.0	6.9	5.4	576.5	14.9	4.96	0.197
Hawari	-	-	-	-	-	-	-
El-Guarsh	4.13	3.25	6.59	367	9.42	3.07	0.07
Jarotha	2.78	1.63	9.94	446	9.06	2.64	0.118

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AUTHOR PROFILE

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I have experience in toxicity testing, working on *Daphnia magna*, Frog Embryo Teratogenesis Assay Xenopus (FETAX), and *Lemna minor* as aquatic toxicology models and I have received good training using aquatic insects to monitor freshwater at Highlands Biological Station, Highlands, NC. Also, I have good experience with toxicity testing using soil organisms such as earthworms and Isopoda. I have good training on earthworm during my stay at the Research Centre for Toxic Compounds in the Environment (RECETOX), Masaryk University, Brno, Czech Republic, and I have started cultures of earthworms and Isopoda in the Zoology Department at Benghazi University, which is going very well. Also, I have got good teaching experience: I have taught toxicology, general zoology and invertebrate zoology courses.