UNIVERSITY OF BENGHAZI

FACULTY OF SCIENCE

DEPARTMENT OF ZOOLOGY

The combined effect of the Remiltine the Cyperkill and Cadmium on the adult, cocoon and juvenile of the earthworm *Eisenia fetida*.

A thesis submitted in partial fulfillment for the requirements of the Degree of Master of Science.

Presented By

Abid S. Abid

Supervisor

Prof. Dr. Abdalla I. Mohamed

August, 2013

UNIVERSITY OF BENGHAZI

FACULTY OF SCIENCE

DEPARTMENT OF ZOOLOGY

The combined effect of the Remiltine, the Cyperkill and Cadmium on the adult, cocoon and juvenile of the earthworm *Eisenia fetida*.

BY

ABID . S . ABID

Approved by the examination committee on the **29**th of July, **2013**:

Advisor:

Prof. Abdalla Ibrahim Mohamed ….....

External examiner:

Dr. Ahmed Farag Mhgoub Ahmed ……....

Internal examiner:

Dr. Maher Hamed Haiba …….

Countersigned by:

Dr. Jalal Ahmed Bojwari	Dr. Ahmed Mammi
(Head, Zoology,Department)	(Deen, Faculty of science)

الإهداء

إلى من زرعت في قلوبنا الحب والخير

والاحترام

أمي الحبيبة

إلى من شجعني حتى وصلت إلى هذه المرحلة

أبى الغالي

إلى من وقفوا معي وشدو بأزري

أخوتي و أخواتي

ACKNOWLEDGEMENT

In the beginning, my great appreciation to AIIah for his blessing and his help to complete this study.

I offer my gratitude and thanks to **Prof.** Abdalla**. I. Mohamed** for his advice and guidance.

Thanks are extended to Dr. Ahmed Farag Mhgoub Ahmed, the External examiner and **Dr. Maher Haiba** the Internal examiner for reviewing the thesis and for their suggestions.

Thanks are also due to **Dr. Gebriel M Shamia** for his help in the statistical analysis.

I am also thankful to all staff members of the Department of Zoology for their co-operation and encouragement, special thanks to **Mr walid A. Awgi** for his advice.

All my regards, thanks, and appreciation for **my family** specially my brother **Dr. Abdelhamid.**

Further, I am thankful to all **my friends** who always appeared helpful to me, whenever, I needed them.

Abid S. Abid

CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

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 (Beeby, 2001). Consequently attempts have been made to reduce and / or prevent such pollutants from the environment, where many biological methods can be used to achieve this objective (Jain and Singh, 2004).

Heavy metals can enter the soil from different sources such as pesticides, fertilizers, organic and inorganic materials, which mainly come from mining, wastes and sludge residue (Capri and Trevisan, 2002). On the other hand, pesticides have been used to control agriculture pests (insects, weeds and fungi) are normally directed to plants. However, the major portion is deposited on the surface of the soil (Cremlyn, 1978).

The increased use of various types of pesticides in the modern world has led to much greater emphasis on the possibility of serious environmental contaminations arising from their use in soil. These uses of such chemicals on soils causes decrease in soil fertility, alteration of soil structures, disturbance of the balance between flora and fauna residing in the soil, leading to a threat for living organisms.

There are billions of organisms that make up the soil food web. These include bacteria, fungi, protozoa, nematode, and invertebrates. Each type of organism plays an important role in keeping the soil healthy. However, a great proportion of biomass of terrestrial invertebrates is represented by earthworms which play an important role in structuring and increasing the

nutrient content of the soil. Therefore, they can be suitable bioindicators of chemical contamination.

Earthworms are excellent biological indicators of soil pollution, as they can ingest large amounts of soil or specific fractions of soil, in addition they have shown that their skin is significant route of contaminate uptake as well (Sanchez-Hernandez, 2006). Although investigation of earthworms can be helpful to provides information on conditions of the environment by its presence or absence as well as by their behaviour (Georgesca *et al*., 2002). Earthworm may represents good sensitive organisms of soil chemicals pollution because they are in direct contact with soil in contrast to many vertebrates that are indirectly exposed through the food chain (Kammenga *et al*., 2000).

Among the earthworms, two species are well known as soil animals for testing soil pollutants including pesticides and heavy metals, these are the European Species *Eisenia fetida* and the *Aporrectodea caliginosa* which is widely found in Libyan habitat. Although, several researches were conducted on this later species in the zoology lab and have been published in several journals.

However, in the present study *Eisenia fetida* savigny, 1826 was selected because it is a standard test organism used in terrestrial ecotoxicology and because it can be easily bred on a variety of organic wastes with short generation time, therefore, different endpoints can be observed in short time such as mortality, change in body weight, cocoon numbers as well as fecundity. Furthermore, *E.fetida* was chosen for this study because its growth and reproduction are well documented (Venter and Reinceke, 1988;

Presleg *et al*., 1996) and it is considerer suitable model species (spurgeon and hopkin, 1996).

The objective of this study is a continuation assessment of soil pollutants to the soil animals represented by *E.fetida* which aims to assess the impact of soil contaminates at sub lethal concentrations at different endpoints including:

1. The effects of pesticides and metals on cocoons production and hatchability.
2. The effects of pesticides and metals on worm growth, and reproduction.
3. Evaluating toxicity mixtures of pesticides- cadmium combinations.

REVIEW OF LITERATURE

Soil contaminations become a serious problem worldwide. An effort has been done to evaluate, and reduce pollutants from the environment by chemicals analyses, which are expensive and do not give good pictures about the toxicity of pollutants. Recently, many scientists encourages using biota for assessing chemicals availability in soil. Main kinds of the contaminants can be pesticides and heavy metals especially in agricultures, which occur as single chemicals as well as mixtures.

There are billions of organisms that make up the soil food web. Earthworms which belonging to the phylum annelida class oligocheata take a special place in this respect as not only they eat almost every other particle in the soil, but also when they eat they leave behind "casting" which are high in organic matter and plant nutrients' and are a valuable fertilizer (Fragoso *et al.*, 2004), as well as other terrestrial vertebrates which prey upon earthworm (Dell'Omo, 1999). Soils get in a better condition and their fertility is further improved by earthworm presence (Fragoso *et al*., 2004).

Earthworms move through the soil creating tunnels, thus areas that can be filled by air and water. Field that is "tilled" by earthworm tunneling can absorb water at the rate of 4-10 times that of fields without earthworm, nutrients enter the sub soil at faster rate and opens up pathways for roots to grow into soil. Furthermore, earthworms are the major catalyst for decomposition in a healthy vermicomposting system (Edwards, 2004).

A greater proportion of biomass of terrestrial invertebrates is represented by earthworms which play an important role in structuring and increasing the nutrient content of the soil. These make earthworm suitable bioindicators of chemicals contamination of the soil (Culy and Berry, 1995).

Darwin considered earthworms as one of our plant's most important caretakers (Darwin,1809-1882) and was the first to describe how earthworms tilled the soil, swallowing and ejecting soil as castings, or worm manure (Lee, 1985). He estimated that an acre of garden soil could contain over 50,000 earthworms and yield 18 tons of organic castings per year (Lee, 1959). However, scientists later figure estimated that worms can number over million per acre. Therefore, earthworm helps to keep the soil healthy by moving organic matter from the surface into the soil.

More than 16000 publications have been written on different subject of the earthworms. Righi published about 100 papers on earthworms taxonomy, ecology, physiology and biogeography (Fragoso *et al.*, 2004). However, more than 400 papers have been published about using earthworm as indicator of soil contaminations. Meanwhile, very few articles have been found about using earthworm as bioindicator in North Africa especially in Libya. Earthworm species are often used as test organisms to determine the effect and accumulation of chemicals from soil (Lokk *et al.*, 1998 and Lanno *et al*., 1997). Due to their behavior and morphology, earthworms are in close contact with the aqueous and solid phases of the soil as well.

The increase in soil pollution levels particularly by heavy metals and pesticides has endangered human life. Therefore, attempts have been made to monitor, reduce and eliminate pollutants from soil. To achieve this objective,

physical, chemicals and biological methods have been used. (Jain and Singh, 2004). Experimental studies have shown that earthworm could be indicator for both inorganic (Vijver *et al*., 2003) and organic (Jager *et al.*, 2003). Earthworms are frequently used as part of batteries of indicator species to test the effects of pollutants on ecosystems (Boyle and Fariched, 1997).

Some metals are essential for life and others have no known biologic function but both can lead to serious toxic hazard, still other metals have the potential to produce disease (Schroeder *et al*., 1970b). The availability of some metals must be determined because they are beneficial at certain concentrations, (Jain and Singh, 2004). However, this metals availability affect by vermicompost, by soil characteristics such as pH and salinity, as well as earthworm is known to be able to accumulate heavy metals in their body (Jain and Singh, 2004).

Cadmium ranks close to lead as a metal of current toxicologic concern it, having identified as a distinct element only in 1817. It occurs in nature in association with zinc and lead. The usual sources of Cadmium for the general population are mainly food and inhaled tobacco smoke (Friberg, 1948). The major toxic phenomena in man are respiratory and renal toxicity, Seen principally in industrial workers, the itai- itia disease complex reported from Japan, (CEC, 1978).

Cadmium is toxic to the tests of rats and mice probably as a result of toxicity to the vasculature. Cadmium also causes hyperglycemia and glucose intolerance in animals, possibly as a result of decreased secretary activity of pancreatic beta cells, (Perry and Erlanger, 1947, Nechay *et al.*, 1978). Numerous other effects of cadmium may be cited , as examples of the former

cadmium causes anosmia and yellow staining of the teeth in heavy industrial exposure and Cadmium causes cerebral and cerebellar damage to newborn animals , whereas, adult are resistant to these effects .

Cyperkill (Cypermethrin), is a pyrethroid insecticide. It was first synthesized in 1974 (WHO, 1989) (Appendix 1.1). Cyperkill is a synthetic chemical similar to the pyrethrins in pyrethrum extract (which comes from the chrysanthemum plant). Pyrethroids, including Cyperkill were designed to be effective longer than pyrethrins (WHO, 1989). Some products that contain Cyperkill include (termiticide, household insecticides, outdoor insecticides, Ammon^{tm}, cybush^{R}, cynoff^{Tm}, Cyperkill, Demon^{R}).

The typical half – life of Cyperkill in the soil is 30 days, although it can range from two to eight weeks (USEPA, 1989 and Knisel, 1993). Soil microbes rapidly break down Cyperkill. Cyperkill is stable in sunlight (USEPA, 1989). The average half – life of Cyperkill on foliage is 5 days (Knisel, 1993).

The effect of Cyperkill on wildlife is very evident as it is highly toxic to fish. Cyperkill is highly toxic to bees, very highly toxic to water insects and very low in toxicity to birds. Some products for agricultural and commercial outdoor applications are limited to use by certified Applicators (USEPA, 1989).

Toxicity of Cyperkill to cockroach brain cells exposed to very small doses up to 0.02 micrograms per gram of brain weight. Cyperkill exhibited a nervous system response, which in cockroaches would result in restlessness, incoordination, prostration, and paralysis in the laboratory testing (Gammon, 1981). Mice exposed to small doses (0.3 to 4.3 µg/g) of Cyperkill displayed

symptoms including writhing, convulsions, and salivation (Lawrence and Casida, 1982). Rats exposed to Cyperkill exhibited similar symptoms including termors, seizures, writhing, and salivation as well as burrowing behavioer (Klaassen, 1996) Cyperkill may be a week skin sensitizer in guinea pigs (Tomlin, 1994. USEPA, 1989). Newborn rats were more sensitive to Cyperkill than adult rats; the liver enzymes that break down Cyperkill in the body are not completely developed in the newborn rats (Cantalamessa, 1993). People handling or working with pyrethrins and pyrethroids (including Cyperkill) sometimes developed tingling, burning, dizzinss and itching (WHO,1989 and Klaassen, 1996). Mice fed high doses (up to 1600 mg /g) over a lifetime did not develop cancer (Maliganant tumors) (WHO, 1989).

The fungicide Remiltine, a formulation of 8% Cymoxanil and 64% mancozeb Constituents of Remiltine is currently being used as a section 18 emergency exemption material to control late blight fungi on potatoes (US EPA, 1997 and Reviewed by DuPont Agricutural Products). Cymoxanil was first introduced in 1977 (Appendix1.2), it is an acetimide compound used as both acurative and preventative foliar fungicide (Thomson, 1997. Farm chemicals Handbook, 1997).

Technical Cymoxanil has low acute toxicity; the acute oral LD_{50} is 960 mg/kg in rats. The acute dermal LD_{50} is > 2,000 mg/kg in rabbits. The 4-hour rat inhalation LC_{50} is > 5.06 mg/L. Minimal transient irritation of the skin and eyes was observed in rabbits. Cymoxanil did not cause skin sensitization in guinea pigs. Cymoxanil should be classified as Toxicity Category III for oral and dermal toxicity and Toxicity Category IV for inhalation toxicity and skin and eye irritation potential (USEPA, 1997). 12-month chronic feeding study was conducted in male dogs at dietary levels of 0, 50, 100 and 200ppm and in

female dogs at 0, 25, 50 and 100ppm. The no- observable-effect-levels for chronic toxicity were 100ppm in male dogs (3.0 mg/kg/day) and 50ppm in female dogs (1.6 mg/kg/day), based on body weight and food consumption effects in both sexes and decreased red cell parameters in males. No gross or histopathological effects were observed (USEPA, 1997).

Mancozeb is a fungicide with protective action can control many fungal diseases in a wide range of field crops, fruit, nuts, vegetables and etc (Appendix 1.3). Mancozeb is one commonly used fungicide in vineyards. (Edwards and Bohlen, 1992; Hogger and Ammon, 1994).

However, if a population of earthworm is exposed to an additional stress through elevated metal concentrations such as copper based fungicides, that will increase copper concentrations in soils leading to reductions in abundance, diversity and biomass of earthworms (Morgan and Bowden, 1993). The increasing presence of heavy metals, which may accumulate in earthworms (Fleckenstein and Graff, 1983), could pose a problem if the production and earthworm biomass is consider. Fungicides are used extensively by deciduous fruit farmers in the North Africa; very little information is available about their effects on beneficial non target organisms such as earthworms.

Cluzeau *et al*., (1992) reported detrimental effects of mancozeb on *Lumbricus terrestris* while (Roark and Dale, 1979) concluded that the compound had no negative effect on *E .fetida*.

Mohamed *et al*., (1995) Published their findings on the impacts of chemical pesticides including fungicides on the survival and body mass of *Aporrectodea Caliginosa* trapezoids, where various effect were defected.

Potter *et al*., (1990) reported the toxicity of Pesticides to earthworms and their degradation functions in bluegrass turf. Haimi, in the same year, published his findings on the growth and reproduction of the compost –living earthworms *Eisenia Andrei* and *Eisenia fetida* .

Reineke and vilijoen, (1990). Published their results on the influence of worm density and cocoon production of *Eisenia fetida.* (Callahan *et al.,* 1994) and (Potter *et al.,* 1994). Reported the comparative toxicity of chemical pesticides to different species of earthworms.

The effect of a toxic compound is assumed to depend on its concentration within the organism more than on the exposure concentration (Moriarty, 1983). Metal concentrations found in the body tissues reflect the bioavailability of these metals at the specific sampling area (Depledge *et al*., 1994).

Earthworm populations can be modified by arrange of factors ,these include soil conditions (Lee, 1985). food availability (Fraser *et al.*, 1994) altered predator abundance (Churchfied *et al.,* 1991), and climate (Lofs, 1992). All biological activity of earthworms is influenced by temperature, not only by mean values but also by extremes and fluctuations (Lofs-Holmin, 1985).

Earthworm growth and fecundity is temperature dependant and both can be affected by the increasing temperatures above the threshold value. (Butt,

1997). *A. caliginosa* is a highly plastic species with many morphological variants (Sims & Gerared, 1999).

Laboratory –based experiment have shown that earthworm growth, adult mass and fecundity are significantly influenced by earthworm biomass and density in culture e.g. for *A. chlorotica*. (Butt, 1997)

Widely respected ecologists like Darwin and Righi were among the first scientists to recognize the importance of species in general and earthworms in particular. (Bouche, 1977).

Studies of accumulation kinetics and the factors that influence uptake and loss have a key role in ecotoxicology as they can be used to predict the physiological fate of a pollutant (Walker *et al.*, 1996).

Loh *et al*., (2004) reported that biomass gain and cocoon production by *Eisenia Foetida* was more in cattle waste than goat waste. The growth, fecundity and mortility of the same species were arranged in the different wastes cattle manure solids, pig manure solids and super market waste for more than one year (Gunadi and Edwards, 2003).

Singh *et al.*, (2004) studies the optimum moisture requirement during vermicomposting, where they found that the moisture content of 80% was optimum for stabilization of waste in minimum processing time.

MATERALS AND METHODS

The test organism:

The two model earthworms available in the zoology (Toxicology) Lab are the local *Aporrrctodea caliginosa* collected from several locations in Benghazi and reared for several years now, and the European strain *Eisenia fetia* brought from the Czech Republic as a research sample and reared in the lab for more than two years (Appendix, 2.1). *E.fetida* was chosen for this study was as it has a more reproductive potential as well as more literature were found concerning them.

Worm rearing:

The worm *E. fetida* was maintained in a glass aquaria on a culture media as described by OECD (2004), at room temperature of 20 ± 2C° the food consisted of an artificial soil mixed with barley grains powder as a food supplement every week throughout the test period (Appendix,2.2).

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 were required for experiment. Adult worms with an average weight of 7 to 9 grams were required for the experiment. Cocoon of close ages were required for cocoon hatchability test (Appendix 2.3).

Scientific classification(savigny)

Kingdom : Animalia

Phylum : Annelida

Class : Oligochaeta

Order : Haplotaxida

Suborder : lumbricina

Superfamily : lumbricoidea

Family : lumbricidae

Species : *Eisenia*

Subspecies : *fetida* (misspelled foetida)

The Chemicals:

Pesticides in general are widely used in Benghazi farming system with very usual misuse in dose and interval applications. The two compounds selected in this study were:

1. The pyrethriod insecticides Cyperkill, these groups of pesticides are used for both Agriculture and public health (Appendix, 3.1).
2. The fungicide Remiltine a compound consisting of two active compounds (Mancozeb and Cymoxanil). The Compound is known to be very effective for soil fungi in both open and closed (greenhouses) farming system (Appendix, 3.1).

Both pesticides can pose negative hazardous to the soil organisms and soil micro flora if misused in terms of quantity and timing.

The heavy metal:

Cadmium, the third compound selected for the study is cadmium chloride as a source of the heavy metal cadmium. Cadmium is a nonessential element for any organisms which mean it can be toxic even in small quantity. The source of cadmium to soil is thought to be with the organic manure (Appendix, 3.2).

The selected dosages of pesticides used in this study were based on their recommended filed use as well as on a trail experiment.

The experiments:

Two experiments were undertaken in this study they were:

1. Adult *E.fetida* growth and reproduction under three concentrations of the pesticides and cadmium as well as pesticides-metal mixtures.

2. Cocoon hatchability-under five concentrations of the pesticides and Cadmium as well as their pesticides-metal mixtures.

Experiment- 1:

Growth and reproduction test:

In this experiment, young adult worms were exposed to the artificial soil contaminated with three concentrations of Cyperkill, Remiltine and Cadmium each as well as the combinations of the paired compound at one concentration each

Table (1): The pesticides Cyperkill, Remiltine and cadmium concentrations as well as their paired combinations in adult *E. fetida* growth and reproduction test.

The Chemicals	**Concentrations**
Cyperkill	
Concentration-1	50ppm
Concentration-2	25ppm
Concentration-3	12.5ppm
Remiltine	
Concentration-1	1000ppm
Concentration-2	500ppm
Concentration-3	250ppm
Cadmium	
Concentration-1	50ppm
Concentration-2	25ppm
Concentration-3	12.5ppm
Mixture treatments	
Cadmium + Cyperkill	25+25ppm
Cadmium + Remiltine	25+500ppm
Cyprerkill + Remiltine	25+500ppm

The artificial soil used OECD (1984) 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 was transferred into glass containers (12cm W, 15cm L, 20cm H) to which 100 ml of each of pesticide, cadmium or their mixtures were added and mixed thoroughly (Appendix 4).

Each treatment (concentration) was replicated three times and control treatment with three replicates were set using plain water. Ten adult *E. fetida* were then transferred into each test container, after their initial body weight as a whole replicate were taken by Balance for worms, KERN and Sohn GmbH. The further worms body weight were again taken after 28, 49 and 70 days post treatment. Five grams of barely grain powder were spread on top of each test container as food, and soil moisture content was checked once a week as regularly and five ml water was added when needed.

The parameters measured in this experiment were:

A. Worm growth body weight change.
B. Cocoon production by worms after 28 and 70 days post treatment.
C. Number of juveniles hatched from the cocoon after 70 days post treatment.

Experiment -2:

Cocoon hatchability:

This test intended to check the effects of the pesticides, cadmium and their combination on the cocoon of *E.fetida*.

Adult *E.fetida* were isolated from the rearing stock and transferred into plastic pans containing 2.5kg artificial soil moistened with 1600 ml tap water and spread on top with 50 grams barley grain powder. After one week the cocoons were isolated and transferred into Petery dishes containing wet filter paper at the rate of 5 cocoons per each dish and were assigned as age one week cocoon.

Each five cocoons were then transferred into each of five concentrations of Cyperkill, Remiltine and cadmium treated 40 grams, 60% water holding capacity soil, each treatment was replicated three times. Control treatment was treated with plain water.

The parameters measured were:

A. Cocoon hatching after 28 days of cocoon treatment.
B. Juvenile numbers after 70 days of cocoon treatment.
C. Young worms body weight after 70 days of cocoon treatment.

Table (2): The pesticides Cyperkill, Remiltine and cadmium their paired concentrations used in cocoon hatchability test.

Concentrations	Cadmium	Cyperkill	Remiltine
1	100ppm	100ppm	2000ppm
2	40ppm	50ppm	1000ppm
3	20ppm	25ppm	500ppm
4	10ppm	12.5ppm	250ppm
5	5ppm	6.25ppm	125ppm

Statistics:

All Data were subjected to spss where ANOVA were used to find the signinficant difference, t. test for mean difference.

THE RESULTS

The effects of pesticides, Cyperkill, (pyrethroid insecticide), Remiltine (fungicide) and the heavy metal cadmium as well as their combinations were tested at three concentrations each on the adult European earthworm *Esinia fetida* for 70 days. The measured parameters were the worms body weight at different time intervals, the cocoon production of the treated worms and the juvenile count from these cocoons.

Further study included the effect of the same compounds at five different concentration of each on the cocoon hatchability, number of juveniles from the treated cocoons and the total juvenile weight after 70 days of cocoon treatment.

Body weight of treated adult worms:-

The insecticide Cyperkill treated worms:

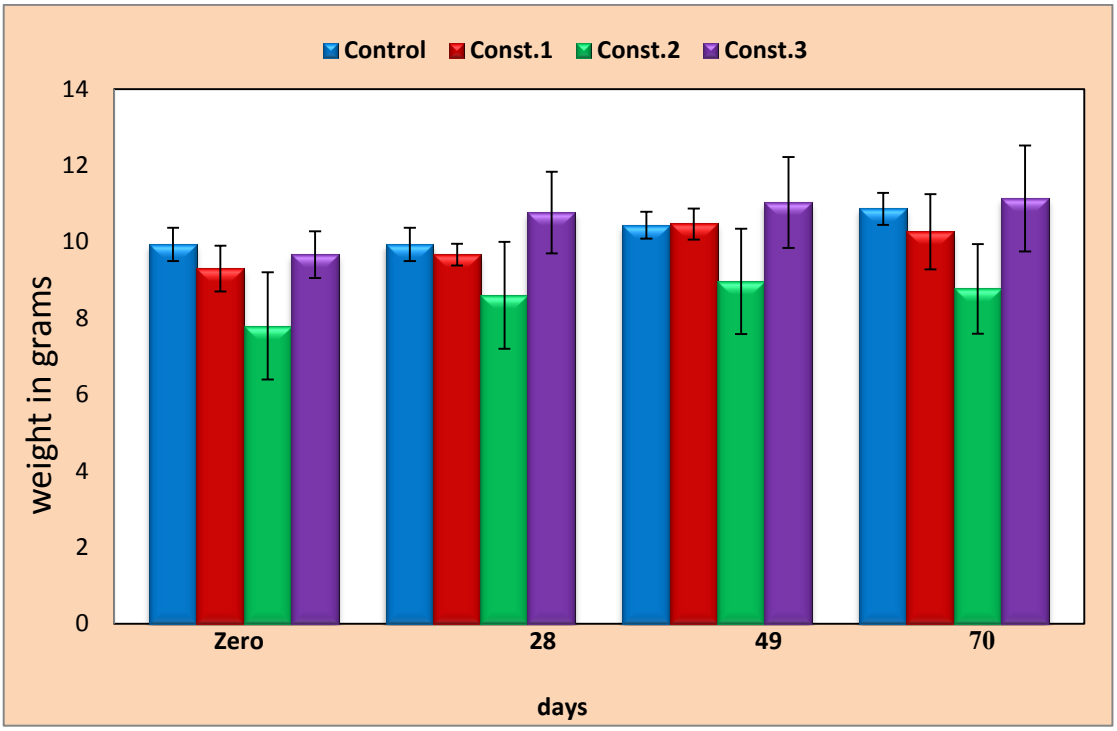
Worms exposed to the three concentrations of Cyperkill reveled insignificant change in body weight compared to control along the time interval up to 70 days ($F=1.15$, $P > 0.05$). However, there were significant differences ($F=3.83$, $P < 0.05$), when Cyperkill concentrations were compared to the control worm body weight Table (3), Fig (1) after 70 days post treatment.

The mean ± SD of the worm body weight in grams were 10.87 ± 0.72 for control, 10.267 ± 1.703 for Cyperkill 50ppm, 8.76 ± 2.03 for 25ppm and 11.13 ± 2.40 for 12.5ppm.

The results clearly shown that the worms body weight in the lowest Cyperkill concentration 12.5ppm was almost comparable to that of control, whereas, the 25ppm and 50ppm reported lower worm body weight compared to that of control and the 12.5ppm Cyperkill.

Table (3): The mean ± S.D of *E .fetida* body weight in grams of control and Cyperkill treated soils.

Time	Control	50ppm	25ppm	12.5ppm
Zero	9.933±0.750	9.300±1.039	7.800±2.426	9.667±1.059
After 28	9.933±0.750	9.667±0.493	8.600±2.426	10.767±1.850
After 49	10.433±0.611	10.467±0.702	8.967±2.386	11.033±2.064
After 70	10.867±0.723	10.267±1.703	8.767±2.0306	11.133±2.402



Control=0, Const.1=50ppm, Const.2=25ppm, Const.3=12.5ppm, (Appendix, 4)

Figure (1): The mean ± SD of worms body weight in grams in control and three concentrations of the Cyperkill treated soil.

The fungicide Remiltine treated worms:

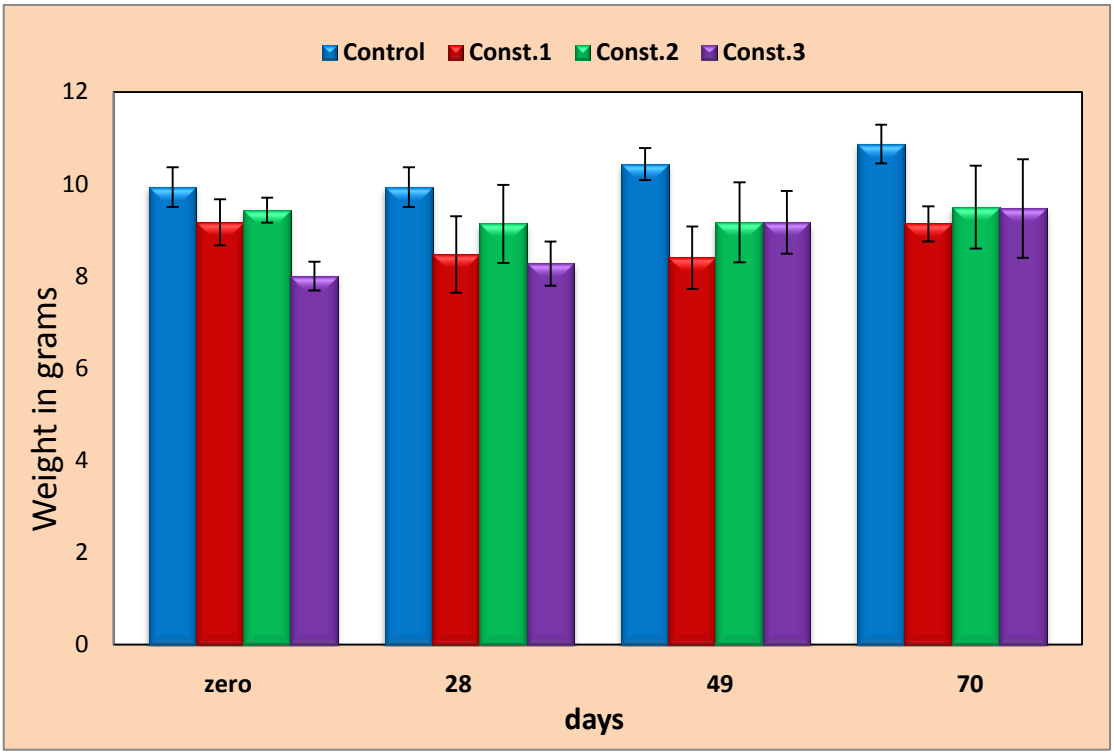
The fungicide Remiltine showed relatively different effect on the treated worms body weight compared to that of control Table (4), Fig (2). The worms of control treatment reported significantly greater body weight ($F=5.4$, $P < 0.05$), than that of Remiltine concentrations treated worms. However, the change in worms body weight over time was almost comparable in control to that of the different concentrations treated worms.

The mean ± SD of the worms body weight of control and Remiltine different concentrations treated worms after 70 days post treated in grams were 10.87±0.73 for control, 9.13±0.66 for 1000ppm, 9.5±1.56 for 500pp

and 9.46±1.85 for 250ppm. The results clearly shown that control worms gained greater weight after 70 days compared to Remiltine treated worms.

Table (4): The mean ±S.D of *E. fetida* body weight in grams in control and Remiltine treated soils.

Time	control	1000ppm	500ppm	250ppm
zero	9.933±0.750	9.167±0.862	9.433±0.472	8.00±0.529
After 28	9.933±0.750	8.467±1.436	9.133±1.464	8.270±0.832
After 49	10.433±0.611	8.400±1.179	9.167±1.504	9.167±1.171
After 70	10.867±0.723	9.133±0.665	9.500±1.562	9.467±1.858



Control=0, Const.1=1000ppm, Const.2=500ppm, Const.3=250ppm

Figure (2): The mean ± SD of worms body weight in grams in control and three concentrations of the Remiltine treated soil.

The heavy metal cadmium:

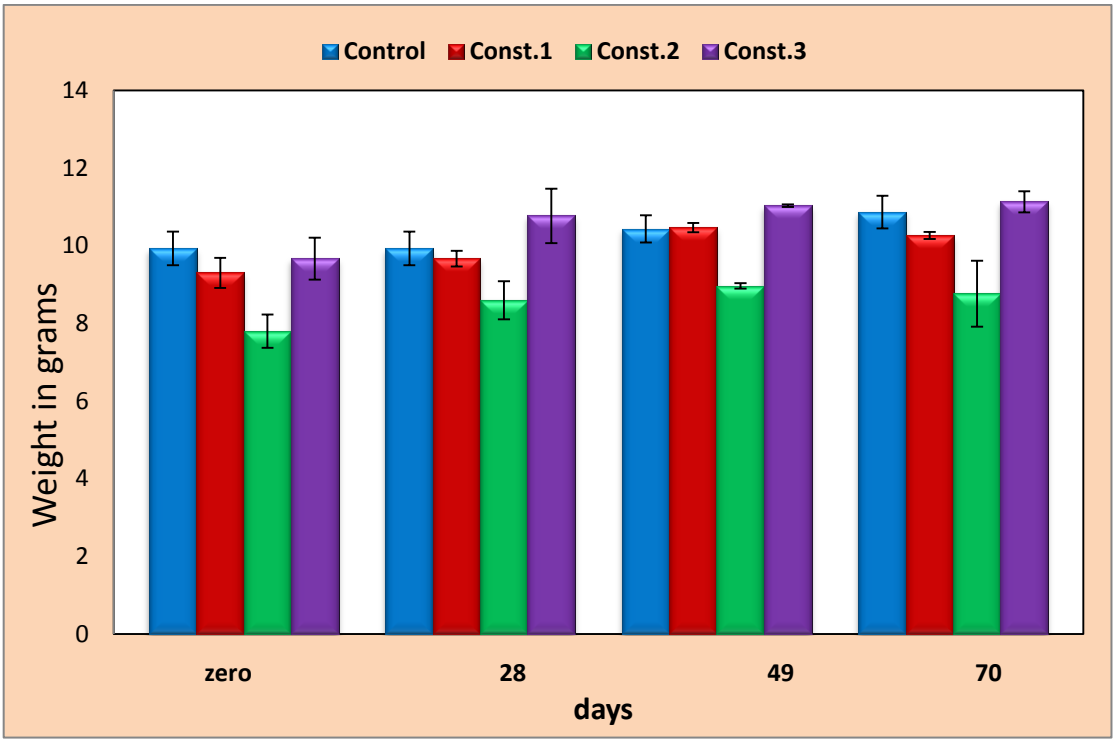
The nonessential cadmium metal has been known to be toxic to many organisms including soil animals such as earthworms, Table (5), Fig (3) revealed insignificant differences in worms body weight between the control worms and the different cadmium concentration treated worms at the different time intervals.

However, the results clearly shown that at 70 days post treatment all control and treated worms gained body weight increase, where the mean ± SD of body weight in grams were 10.87± 0.72 for control, 11.67± 0.12 for

50ppm, 10.80 ± 1.48 for 25ppm and 9.93 ± 0.46 for 12.5ppm. The results indicated that cadmium 50ppm and 25ppm came comparable to control, whereas cadmium 12.5ppm reported lower worms body weight gain at 70 days

Table (5): The mean ± S.D of *E. fetida* body weight in grams in control and Cadmium treated soils.

Time	control	50ppm	25ppm	12.5ppm
zero	9.933±0.750	9.867±0.680	8.967±0.750	9.533±0.929
After 28 days	9.933±0.750	10.867±0.351	10.133±0.850	10.133±1.205
After 49 days	10.433±0.611	11.400±0.200	10.167±0.115	10.133±0.577
After 70 days	10.867±0.723	11.667±0.152	10.800±1.479	9.933±0.461



Control=0, Const.1=50ppm, Const.2=25ppm, Const.3=12.5ppm

Figure (3): The mean ± SD of worms body weight in grams in control and three concentrations of the Cadmium treated soil.

Pesticides- Cadmium combination:-

Cadmium -Cyperkill:

Worms exposed to the mixture of Cyperkill and Cadmium mixture at (25 + 25ppm) each for 70 days, revealed no significant different in their body weight compared to that of control Table (6), Fig (4). The mean ± SD of worms body weight in grams have shown very slight change over time intervals. However, reduced body weight was observed by the end of the experiment.

The mean ± SD values of body weight were 9.4± 0.56 at 28 days, 8.90±1.13 at 49 days and 8.10±1.8 at 70 days post treatment. The treated

worms body weight reported a reduced body weight over all time intervals compared to control.

Cadmium -Remiltine:

Worms exposed to Remleltin -Cadmium mixture at (500 + 25ppm) did not shown significant differences compared to that of control through the test intervals up to 70 days post treatment. ($F=2.30$, $P < 0.05$). The mean ± SD of worms body weight in grams were 9.23 ± 0.42 after 28 days, 9.5 ± 0.52 after 49 days and 8.43 ± 0.42 after 70 days of exposure.

The results showed that there were slight increase from the initial worm weight (day 0), along the time. However, by day 70 a clear reduction of the worms body weight was evident Table (6), Fig (5).

Cyperkill-Remiltine:

Worms exposed to the combination of Cyperkill- Remiltine mixture at 25+500ppm respectively revealed no significant different ($F=2.99$, $P > 0.05$) in body weight along the time intervals Table (6), Fig (6).

The mean ± SD of the control and that of treated worms in grams during the test durations were 8.03 ± 0.20 after 28 days, 8.36 ± 0.55 after 49 days and 7.69 ± 0.17 at the final weight. The results indicated an evident body weight reduction after 70 days post treatment

Table (6): The mean ±S.D of *E. fetida* body weight in grams in control, Cyperkill + Remiltine, Cadmium+ Cyperkill and Cadmium + Remiltine treated soils.

Treatment	Time			
	Day0	**Day28**	**Day49**	**Day70**
control	9.933±0.750	9.933±0.750	10.438±0.611	10.867±0.73
Cadmium+ Cyperkill (25 + 25ppm)	8.767±0.493	9.400±0.556	8.900±1.135	8.100±1.852
Cadmium+ Remiltine (25 + 500ppm)	8.067±0.862	9.233±0.416	9.500±0.519	8.433±0.416
Cyperkill+ Remiltine (25 + 500ppm)	6.967±0.404	8.033±0.208	8.367±0.550	7.692±0.173

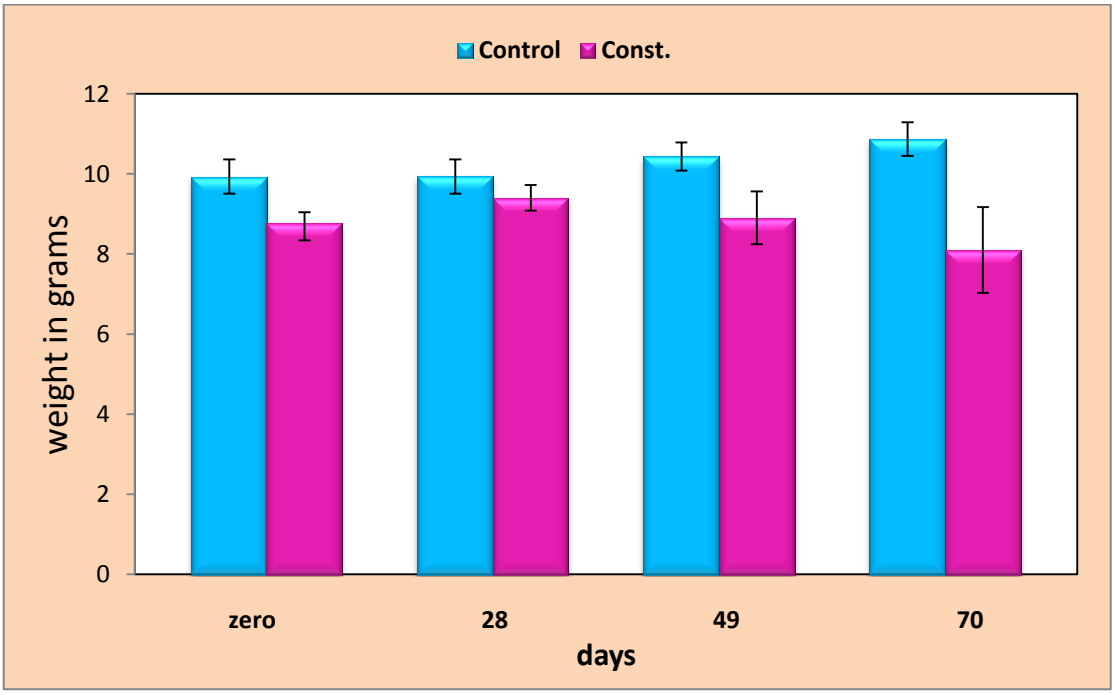


Figure (4): The mean ± SD of body weight in grams in control and three concentrations of the Cadmium+ Cyperkill treated soil.

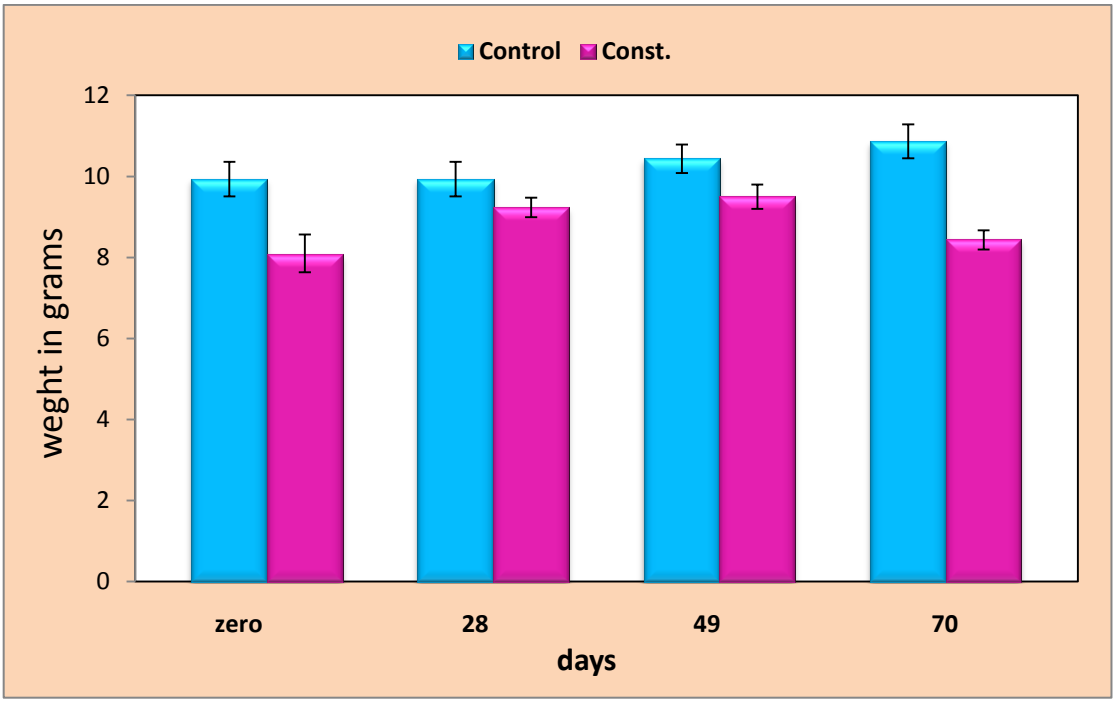


Figure (5): The mean ± SD of body weight in grams in control and three concentrations of the Cadmium+ Remiltine treated soil.

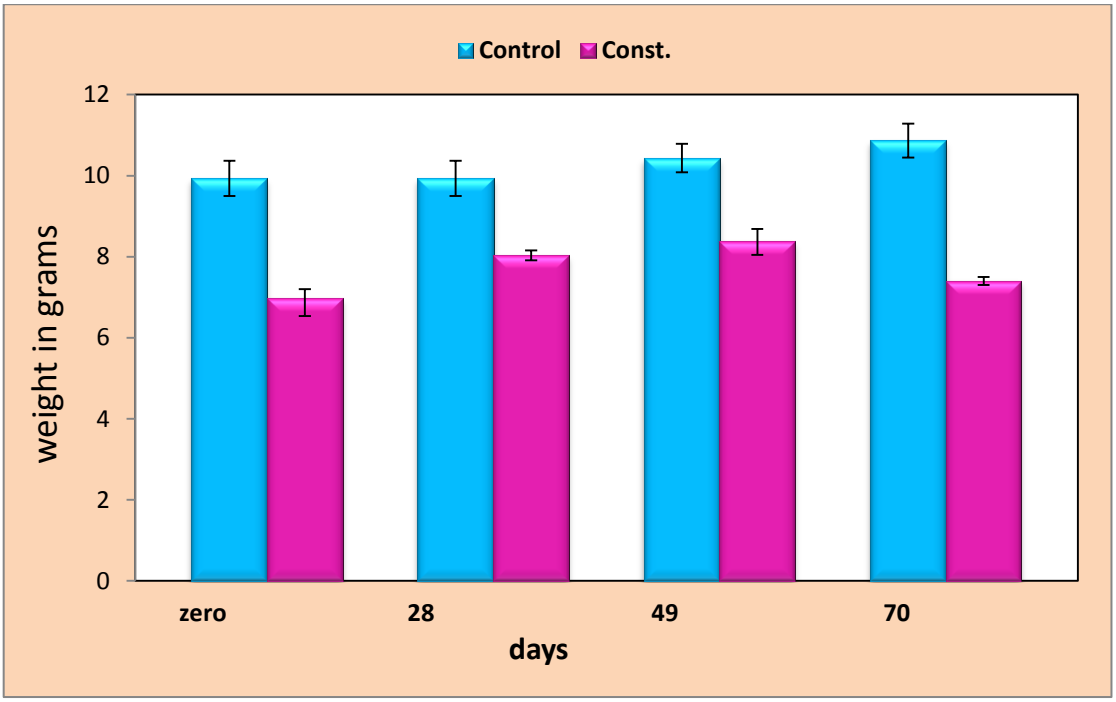


Figure (6): The mean ±SD of worms body weight in grams in control and three concentrations of the Cyperkill + Remiltine treated soil.

Treated worms Cocoon production:-

Cyperkill:

The effect of the insecticide Cyperkill on the worm cocoon production was evaluated at 28 days and 70 days post treatment Table (7), Fig (7). When the number of cocoons produced by control worms were compared with the cocoon number produced by the three Cyperkill concentrations 50, 25 and 12.5ppm respectively, the results clearly showed a significant difference ($F=4.64$, $P < 0.05$) at 28 day period, but such difference were not detected at the 70 days, post treatment .

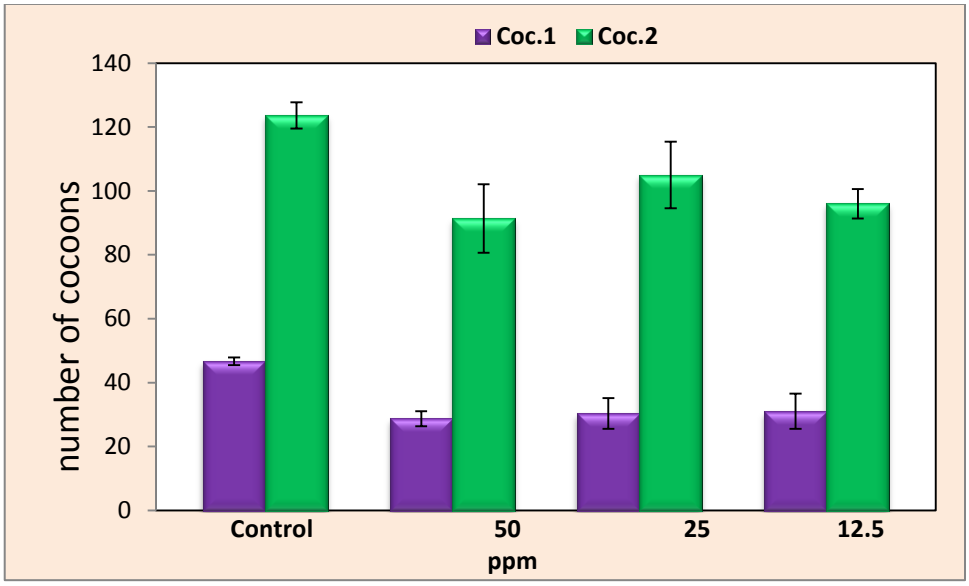
The means ± SD of cocoons from control worms were 46.67±2.08 compared to only 28.67±4.04 30.33±8.36 and 31.00± 9.53 for 50, 25and 12.5ppm Cyperkill treated worms at 28 days post treatment.

At the end of the 70 days, there were clear increase in cocoon number in all treatments, however, no significant difference reported ($F= 3.12$, $P > 0.05$). The mean ± SD of cocoon were 123.67±7.09 for control worms compared to 91.33±18.58, 105±18.58 and 96±7.93 for Cyperkill treated worms at 50, 25 and 12.5ppm respectively Fig (7).

It is generally seems that the insecticide Cyperkill has mild negative effect on worms and that was reflected in the number of cocoon produced by the treated worms compared to control worms.

Table (7): The mean ± SD of number of cocoon per worms in control and three concentration of the Cyperkill treated soils, after 28 and 70 days post treatment.

Time	Concentration			
	control	**50ppm**	**25ppm**	**12.5ppm**
After 28 days	46.67±2.082	28.67±4.041	30.33±8.386	31.00±9.539
After 70 days	123.67±7.095	91.33±18.583	105.00±18.583	96.00±7.939



Coc.1=Days 28, Coc.2=Days 70 (Appendix, 4).

Figure (7): The mean ± S.D of number of cocoon per worms in control and three concentration of the Cyperkill treated soils, after 28 and 70 days post treatment.

Remiltine:

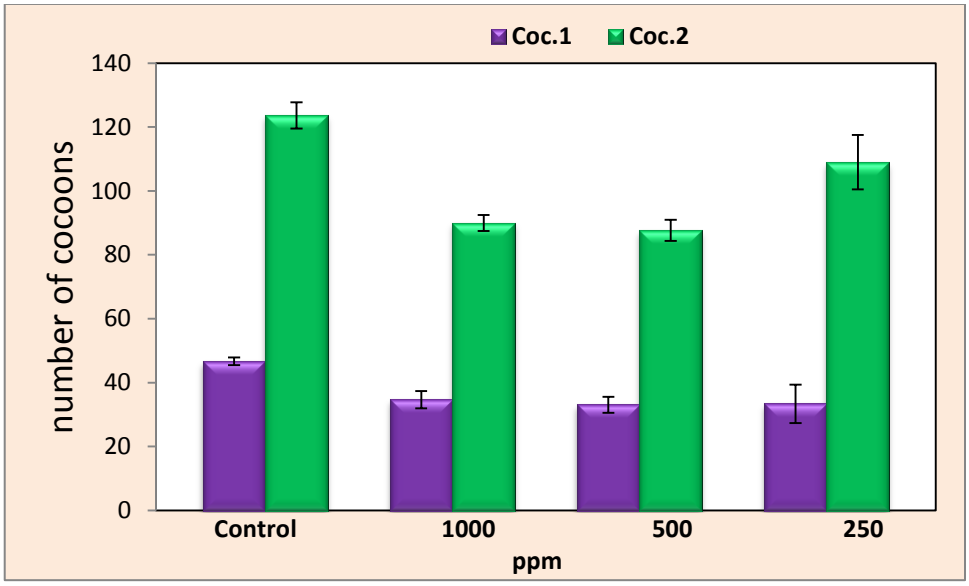
The effect of Remiltine different concentrations 1000, 500 and 250ppm on *E.fetida* were further evaluated through the number of cocoons produced compared to that of control table (8).

The analysis of variance for cocoons produced revealed significant difference ($F=3.33$, $P > 0.05$), in cocoon numbers between control and that of Remiltine treated worms. Control worms delivered significantly greater cocoon number compared to Remiltine treated worms.

The mean ± SD of cocoon number 28 days Post treatment were 46.67 ± 2.08 for control compared to 34.67±4.72, 33.0±4.35 and 33.33±10.40 for Remiltine concentration respectively. However, more cocoons were delivered by the control as well as the treated worms after 70 days ($F=10.84$, $p < 0.05$). The mean ± SD of these cocoon were 123.67±7.0 for control compared to 90±4.35, 87.67±5.77 and 109±14.73 for 1000, 500 and 250ppm respectively it was very evident that the lowest Remiltine concentration 250ppm treated worms produced comparable cocoon number to that of control, but the two higher concentrations 1000 and 500ppm treated worms produced significantly lower cocoon number than that of control worms Fig, (8).

Table (8): The mean ± S.D of number of cocoon per worms in control and three concentration of the Remiltine treated soils, after 28 and 70 days post treatment.

Time	Concentration			
	control	**1000ppm**	**500ppm**	**250ppm**
After 28 days	46.67±2.082	34.67±4.726	33.00±4.359	33.33±10.408
After 70 days	123.67±7.095	90.00±4.359	87.67±5.774	109.00±14.731



Coc.1=Days 28, Coc.2=Days 70.

Figure(8): The mean ± SD of number of cocoon per worms in control and three concentration of the Remiltine treated soils, after 28 and 70 days post treatment.

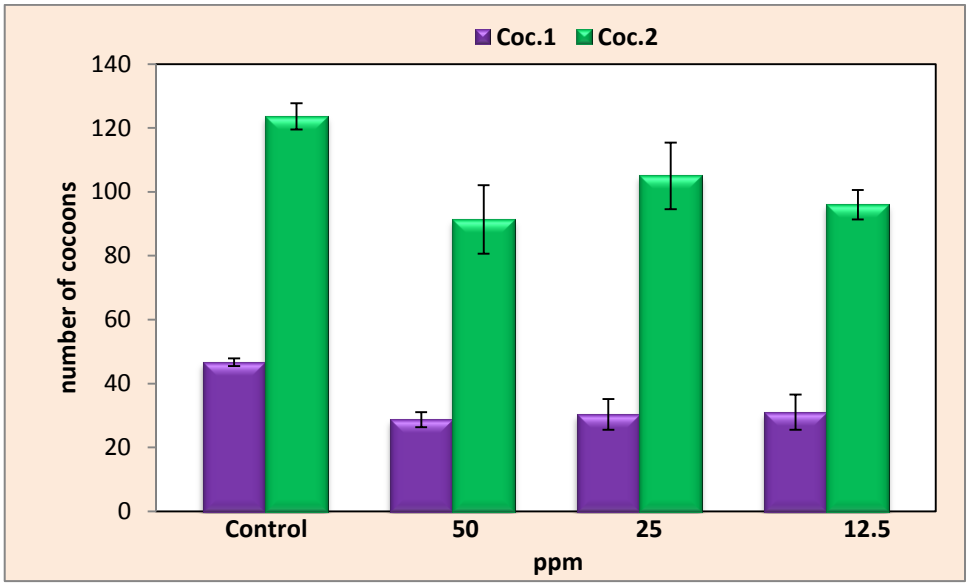
Cadmium:

Cadmium effect on worm cocoon production was evaluated 28 days post treatment and 70 days post treatment. The results reveled significant difference in cocoon number delivered by control worms as compared to that derived by all cadmium treated worms at the70 days, but not at the28 days post treatment ($F=2.65$, $P > 0.05$), for 28 days and ($F=11.00$, $P < 0.05$) at 70 days post treatment .

The mean ± SD of cocoon of control and cadmium treated worms were 46.67±2.08 for control and 29.67±1.5, 28.0±13.11 and 34.67±11.9 for 50, 25 and 12.5 respectively after 28 days post treatment .Whereas, the values of cocoon at 70 days post treatment were 123.67±7.09 for control and 84.33±2.08, 108±14.7 and 99.33±4.61 for 50, 25 and 12.5ppm respectively Table (9), fig (9).

Table (9): The mean ± SD of number of cocoon per worms in control and three concentration of the Cadmium treated soils, after 28 and 70 days post treatment.

Time	**Concentration**			
	control	**50ppm**	**25ppm**	**12.5ppm**
After 28 days	46.67±2.082	29.67±1.528	28.00±13.115	34.67±11.930
After 70 days	123.67±7.095	84.33±2.082	108.00±14.799	99.33±4.619



Coc.1=Days 28, Coc.2=Days 70.

Figure (9): The mean ± S.D of number of cocoon per worms in control and three concentration of the Cadmium treated soils, after 28 and 70 days post treatment.

Combination experiment:-

Cadmium- Cyperkill mixture:

The effect of the Cadmium -Cyperkill mixture at (25 +25ppm) each on the worm cocoon production was evaluated at 28 days post treatment and 70 days post treatment table (10). When the number of cocoon produced by Cadmium-Cyperkill mixture, the results clearly showed no significant difference ($F= 6.08$, $P > 0.05$), at 28 days post treatment, the mean ± SD for Cadmium Cyperkill treatment were 34.0 ± 7.0 and 46.67 ± 2.08 for control treatment.

The cocoon production after 70 days has also shown no significant difference (F=0.28, P > 0.05) compared to control. The mean ± SD for Cadmium -Cyperkill mixture 101.00 ± 6.55 and 123.67 ± 7.0 for control table (10).

Cadmium - Remiltine mixture:

The number of cocoons produced by control worms were comparable to the cocoon number produced by Cadmium-Remiltine mixture (25+500ppm), treated worms. Clearly, showed insignificant difference (F= 6.08, P > 0.05), at after 28 days period and further no significant difference (F=0.31, P > 0.05), after 70 days period. The mean ± SD of cocoon produced by control worms were 46.67 ± 2.08 as compared to 27.00 ± 7.00 for Cadmium-Remiltine mixture after 28 days post treatment.

The mean ± SD of cocoon produced by control worms were123.67 ± 7.09. Compared to 85.67 ± 7.37 for Cadmium-Remleltin mixture treated worms after 70 days table (10).

Remiltine - Cyperkill mixture:

The effect of the Remiltine-Cyperkill on the worm cocoon production was evaluated at 28 days post treatment and 70 days post treatment table (10). When the number of cocoon produced by control worms were compared with the cocoon numbers produced by Remiltine-Cyperkill concentration (25 + 500ppm), treated worms clearly showed a significant (F=8.64, P < 0.05), lower cocoon numbers than control worms at 28 days.

The mean ± SD of cocoons produced by control worms were 46.67±2.08 compared to 26.33± 13.79 for Remiltine-Cyperkill mixture. Even after 70 days still significant difference (F=1.98, P > 0.05), in cocoon number between control and treated worms the mean ± SD Were 123.67± 7.09 for control and 72.33± 18.14, for the Remiltine-Cyperkill mixture table (10).

Table (10): The mean ± S.D of number of cocoon per worms in control and one concentration of the Cadmium + Cyperkill , Cadmium+ Remiltine and Remiltine +Cyperkill treated soils.

Time	After 28 days	After 70 days
Control	46.67±2.082	123.67±7.095
Cadmium + Cyperkill (25 + 25ppm)	34.00±7.000	101.00±6.557
Cadmium+ Remiltine (25 + 500ppm)	27.00±7.00	85.67±7.371
Cyperkill + Remiltine (25 + 500ppm)	26.33±13.796	72.33±18.148

The Juvenile numbers, from treated worms:-

Cyperkill:

The Juvenile numbers produced by cocoons of the control worms and The Cyperkill treaded worms were further counted. The analysis of variance revealed significant difference in juvenile number between the control and treated worms (F= 9.72, P < 0.05). t-test revealed that the number of Juvenile produced by control worms were greater than that produced by The 50ppm and 12.5ppm Cyperkill treated worms Table (11), Fig (10).

Table (11): The mean ± SD of number of juveniles per worms in control and Cyperkill treated soils.

Time	Concentration			
	control	50ppm	25ppm	12.5ppm
After 70 days	93.67±5.508	68.00±6.245	81.67±10.408	68.33±2.887

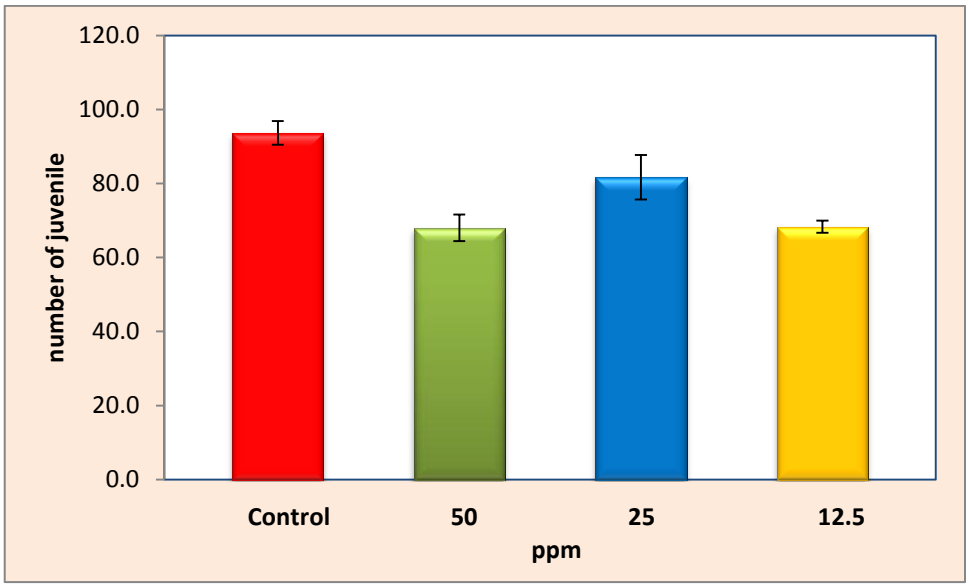


Figure (10): the mean ± SD of number of juveniles per worms in control and three concentration of the Cyperkill treated soils.

Remiltine:

The juvenile numbers produced by the different concentrations 1000, 500 and 250ppm of Remiltine treated worms were further evaluated. The analysis

of variance revealed that no significant different (F=3.0, P > 0.05), were reported between the mean number of juvenile in control and that of all Remiltine concentrations.

The mean ± S.D of juveniles were 93.67±5.50 for control and 60.67±14.36, 82.33±13.05 and 87.67±20.40 for 1000, 500 and 250ppm treated worms .It seems evident that more yet not significant number of juvenile produced by control and that the highest Remiltine concentration 1000ppm resulted in the smallest number of juvenile Table (12) and Fig (11) .

Table (12): The mean ± S.D of number of juvenile per worms in control and Remiltine treated soils.

Time	**Concentration**			
	control	**1000ppm**	**500ppm**	**250ppm**
After 70 days	93.67±5.508	60.67±14.364	82.33±13.051	87.67±20.404

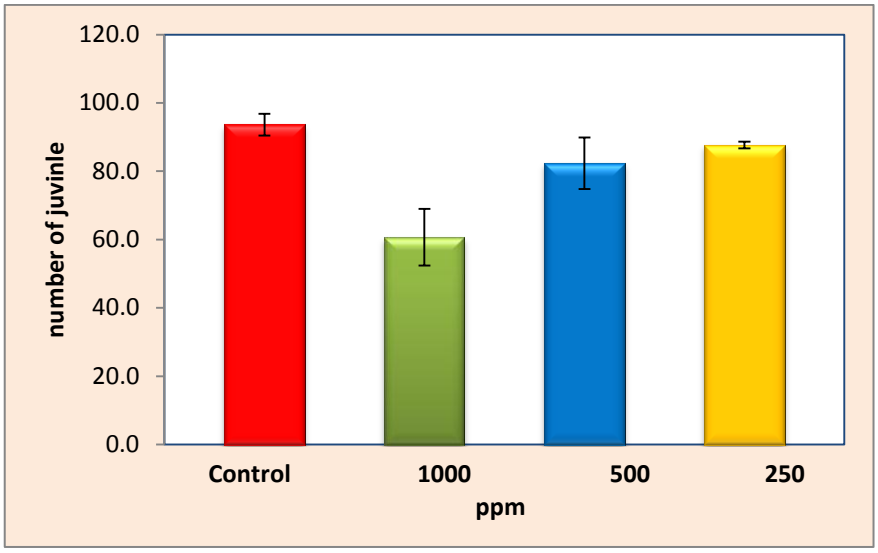


Figure (11): The mean ± SD of number of juvenile per worms in control and three concentration of the Remiltine treated soils.

Cadmium:

The number of juveniles produced from cocoon delivered by cadmium treated *E.fetida*, showed significant reduction at all concentrations compared with those produced from control worms ($F=34.67$, $P < 0.05$). The mean ± SD of juveniles were 93.67±5.50 from control and 61.33±1.52, 82.33±2.08 and 80.00±5.00 for 50, 25 and 12.5ppm respectively. The result revealed greater reduction of juveniles on the highest concentration 50ppm compared to that of the other two concentrations 25 and 12.5ppm, Table (13), Fig (12).

Table (13): The mean ± S.D of number of juvenile per worms in control and three concentration of the Cadmium treated soils.

Time	Concentration			
	control	50ppm	25ppm	12.5ppm
After 70 days	93.67±5.508	61.33±1.528	82.33±2.082	80.00±5.000

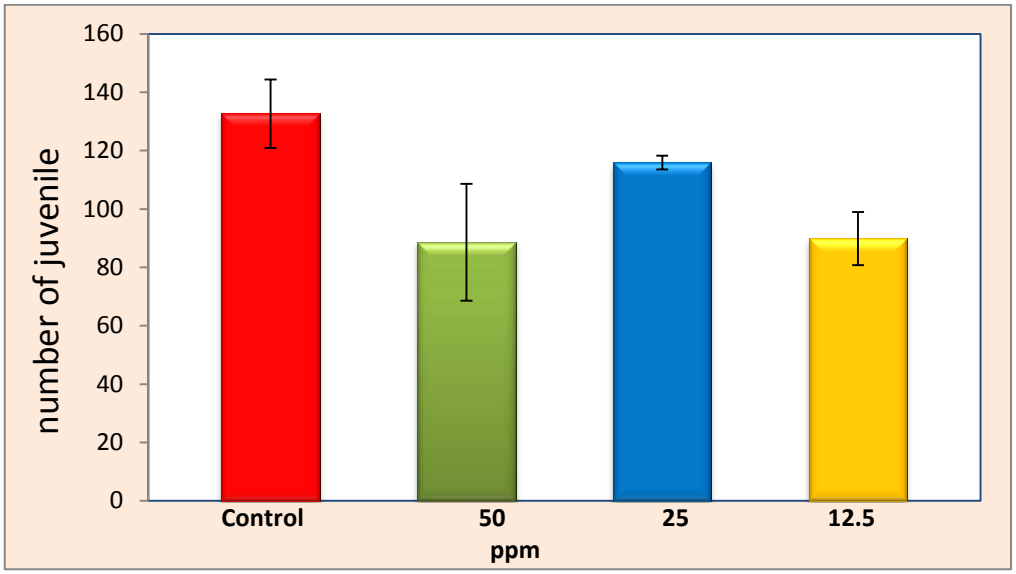


Figure (12): The mean ± SD of number of juvenile per worms in control and three concentration of the Cadmium treated soils.

Combination experiment:-

Cadmium -Cyperkill mixture:

The juvenile numbers from the control worms and the Cadmium-Cyperkill mixture (25+25ppm), treated worms were insignificantly difference ($F=1.77$, $P > 0.05$). The mean ± SD for treatment cadmium - Cyperkill were

95.67 ± 9.29 compared 93.67 ± 5.50 for control after 70 days was post treatment Table (14).

Cadmium and Remiltine mixture:

The juvenile numbers produced by cocoons of the control worms and Cadmium -Remiltine mixture treated worms were further counted. The t- test revealed significant difference ($F=0.39$, $P < 0.05$) the mean ± SD of juvenile produced by control 93.67 ± 5.50 compared to 54.33 ± 4.50. For Cadmium -Remiltine mixture 25+500ppm Table (14).

Cyperkill- Remiltine mixture:

The juvenile numbers produced by cocoons of the control worms and Cyperkill- Remiltine treated worms were further counted, the mean ± SD of these juvenile is presented in Table (14). Significant difference in juvenile number between the treatment ($F=3.356$, $P < 0.05$), the mean ± SD Was 93.67 ± 5.50 for control and 49.97 ± 13.61, for the Cyperkill-Remiltine mixture at (25+500ppm) after 70 days post treatment.

Table (14): The mean ± S.D of number of juvenile per worms in control and Cadmium +Cyperkill, Cadmium + Remiltine and Remiltine +Cyperkill treated soils.

Time	**After 70 days**
Control	93.67±5.508
Cadmium +cyperkill (25 + 25ppm)	95.67±9.292
Cadmium + Remiltine (25 + 500ppm)	54.33±4.509
Cyperkill + Remiltine (25 + 500ppm)	49.67±13.614

The cocoon hatchability:-

When the pesticides cadmium metal Cyperkill and Remleltin were tested at different concentrations for cocoon hatchability, juvenile number and juvenile weight the effects were variable.

Cadmium:

The results revealed that Cadmium, has no significant effects on cocoon hatching ($F=1.78$, $P > 0.05$), at the concentrations tested after 28 days post treatment. The mean ± SD were 4.00±0.00 for control compared to 4.67±0.57, 3.33±1.15, 4.33±1.15, 4.33±0.57 and 5.00±0.00 for cadmium 100, 40, 20, 10 and 5ppm respectively table (15).

No significant effects on number of worms, were also observed in cocoon hatching after 70 days post treatment. Where, The mean ± SD of number of worms were 22.67 ± 3.21 for control compared to 16.00 ± 6.08, 15.00 ± 7.00, 21.00±1.00, 19.67±4.72 and 20.67±6.65 for cadmium 100, 40, 20, 10 and 5ppm respectively table (15).

No significant effects were observed on weight of worms as ANOVA test showed ($F=2.55$, $P > 0.05$), at the 70 days. The mean ± SD of weight of worms were 132.65±20.32 for control compared to 44.88±26.31, 104.11±15.49, 132.17±15.44, 121.40±29.00 and 136.57±78.52 for cadmium 100, 40, 20, 10 and 5ppm, respectively.

The results revealed that only the highest cadmium concentration has resulted in the lowest worm weight compared to control and the other cadmium concentrations table (15).

Table (15): The mean ± SD of number of hatching cocoon, number of worms and weight of worms in milligrams in control and five concentrations of cadmium treated soil.

Concentration	Hatching cocoon	Number of worms	Weight of worms
control	4.00±0.00	22.67± 3.215	132.653±20.323
100ppm	4.67±0.577	16.00±6.083	44.880±26.319
40ppm	3.33±1.155	15.00±7.000	104.113±15.497
20ppm	4.33±1.155	21.00±1.000	132.173±15.447
10ppm	4.33±0.577	19.67±4.726	121.406±29.008
5ppm	5.00±0.00	20.67±6.658	136.570±78.526

Cyperkill:

Cyperkill, no significant effects on cocoon hatching ($F=1.80$, $P > 0.05$), after the 28 days post treatment. The mean ± SD were 4.0 ± 0.0 for control compared to 4.33 ± 1.15, 4.00 ± 1.00, 4.33 ± 0.57, 3.33 ± 0.57 and 5.00±0.00 for Cyperkill 100, 50, 25, 12.5 and 6.25ppm, respectively table (17) .

Insignificant different in the number of worms, hatched from the different concentration as ANOVA test showed ($F=1.41$, $P > 0.05$), at the 70 days post treatment. The mean ± SD of number of worms were 22.67±3.21 for control compared to 20.00±3.60, 18.00±7.55, 20.00±4.58, 13.33±3.51and 20.67±4.04 for Cyperkill 100, 50, 25, 12.5 and 6.25ppm, respectively table (16).

Furthermore, no significant effects on the body weight of the worms, where The ANOVA test showed ($F=1.98$, $P > 0.05$), at the 70 days. The mean ± SD of weight of worms were 132.65±20.32 for control compared to 88.64±30.43, 115.92±4.00, 89.91±15.78, 140.82±25.46 and 102.94 ± 45.66 for Cyperkill 100, 50, 25, 12.5 and 6.25ppm, respectively table (16).

Table (16): The mean ± SD of number of hatching cocoon, number of worms and weight of worms in milligrams in control and five concentrations of Cyperkill treated soil.

Concentration	Hatching cocoon	Number of worms	Weight of worms
control	4.00±0.00	22.67± 3.215	132.653±20.323
100ppm	4.33±1.155	20.00±3606	88.640±30.433
50ppm	4.00±1.000	18.00±7.550	115.926±4.002
25ppm	4.33±0.577	20.00±4.583	89.916±15.787
12.5ppm	3.33±0.577	13.33±3.512	140.826±25.462
6.25ppm	5.00±0.00	20.67±4.041	102.940±45.661

Remiltine:

The fungicide Remiltine, revealed no significant effects on cocoon hatching ($F=0.34$, $P > 0.05$), after the 28 days post treatment. The mean ±SD were 4.00±0.00 for control compared to 4.33±0.75, 4.33±0.57, 4.00±1.00, 4.33±1.15 and 4.67±0.57 for Remiltine 2000, 1000, 500, 250 and 125ppm respectively table (17).

The number of worms hatched from cocoons were also non significant as the ANOVA test showed ($F=2.32$, $P > 0.05$), at the 70 days post treatment. The mean ± SD of number of worms were 22.67±3.21 for control compared to 11.67±1.52, 16.00±5.56, 17.33±3.51, 21.00±6.92 and 17.33±3.51for Remiltine 2000, 1000, 500, 250 and 125ppm respectively table (17).

However, a significant effects on weight of worms. The ANOVA test showed ($F=4.78$ $P < 0.05$) at the 70 days. The mean ± SD of weight of worms were 132.65±20.32 for control compared to 32.13±8.52, 73.68±15.47, 74.92 ± 38.51, 92.10±4.00 and 124.31±53.99 for Remiltine 2000, 1000, 500, 250 and 125ppm, respectively. Whereas, the 2000ppm, reported lower body weight compared to that of control and other concentration.

Table (17): The mean ± SD of number of hatching cocoon, number of worms and weight of worms in milligrams in control and five concentrations of Remiltine treated soil.

Concentration	Hatching cocoon	Number of worms	Weight of worms
control	4.00±0.00	22.67± 3.215	132.653±20.323
2000ppm	4.33±0.577	11.67±1.528	32.133±8.528
1000ppm	4.33±0.577	16.00±5.568	73.683±15.470
500ppm	4.00±1.000	17.33±3.512	74.923±38.514
250ppm	4.33±1.155	21.00±6.928	92.100±4.003
125ppm	4.67±0.577	17.33±3.512	124.316±53.996

DISSCUSION

The present result revealed that all three compounds, the insecticide Cyperkill, the fungicide Remiltine and heavy metal Cadmium separately or their paired combinations did not resulted in an acute mortality to *E. fetida* at any of their concentrations used and that was due to two reasons. First, acute mortality is no longer considered as the main endpoint in toxicity evaluation and second, The growth and reproduction which can be measured for much longer time are considered an important endpoints dealing with the ecological surveillance not only for population but also for the earthworms population dynamics.

The proposed objective in this study agree with at that of (Zaltanskaite and Sodiene, 2010) who stated that, mortality is generally accepted to be a rather insensitive parameter. Therefore sub lethal effect such as change in body weight and reproduction were more important to assess.

The correlation between cadmium concentrations and body weight was relatively negative when compared with control, although slight increase in body weight was observed at long time intervals. These results came in support of (Zaltanskaite & Sodiene, 2010).

However, *E.fetida* body weight reduction due to heavy metal was observed in some studies, (Berthelot *et al*., 2008). Whereas, no impact on body weight or even body weight increase was observed in other studies (Van Gestal *et al.*, 1993). This relationship between metals and body weight has

explained by (Spurgeo and Hopkin, 1996), who stated that the worms living in metal-contaminated soils reach the lower weight or need more time to reach the maximum weight than in non-polluted sites.

On other hand cadmium treated worms have shown reduction in reproduction rate in terms of cocoon production and that the greater the cadmium concentration the greater the cocoon reduction. These results came in support of (van Gestal *et al*., 1989). However, (Burgos *et al*., 2005), have stated that no clear relationship between cadmium concentrations in soil and cocoon production rate. Furthermore, reduction in cocoon production of *E.fetida* exposed to metals including cadmium was reported by several authors (Spurgeon *et al*., 1994 and Savared *et al*., 2007).

The present results also showed that mortality was the least sensitive endpoint than growth, whereas, cocoon production was sensitive to cadmium metal than both mortality and growth. These results came similar to (Van Gestel, 1992), who confirmed a higher sensitivity of the earthworms reproduction test in comparison with the acute earthworm mortality test.

Body weight change is less clearly defined in testing protocols and may be interpreted in different ways. Ring tests have shown that reproducibility of body weight change is sufficient, but an inverse relationship between reproduction and body weight change was found: animals that rapidly gain weight do not reproduce at the same time and the mechanisms influencing this process are not yet fully understood negative impact of pesticides on earthworms growth has been reported by various researchers. (Zhou *et al*., 2008).

The sensitivity tests on pesticides toxicity on earthworm showed that both Cyperkill and Remiltine have toxicity to earthworms, and that is in agreement with other studies (Booth and O`Halloran, 2001; Alshawish *et al*., 2004).

Alshawish *et al*., (2004) tested the effect of Cyperkill, chorpyrifos, dicofol, mancozeb and haloxyfopetotyl on their chronic toxicity on *Aporrectodea caliginosa* in laboratory cultures. They concluded that Cyperkill at 50mg kg was least toxic compared the pesticides. Their study then confirm the present results as the Cyperkill revealed insignificant change in *E. fetida* body weight after 70 days.

From this study, it indicates that the mixed use of Cyperkill and Remleltin has obviously increased not only the cocoon hatching, but also the growth and reproduction toxicity. This increased toxicity can lead to adverse impacts on earthworm populations, threatening the normal functioning of soil ecosystems.

The results also showed that the mixed pesticide can lead to the greater impacts on chronic response such as growth and reproduction than of cocoon hatching response in earthworms. This finding came in agreement with (Zhou *et al*., 2011) who concluded that pesticides mixtures was significantly more toxic to *E. Andrei* than either pesticide alone.

As in the findings of this study, the effective dose of the mixed pesticides of Cyperkill – Remiltine mixture that disrupted the normal processes of growth and reproduction is higher than that of cadmium- Cyperkill mixture and cadmium- Remiltine mixture.

The results also showed that the mixed pesticides can lead to a greater impact than the individual pesticides in earthworms.

The impacts of mixed pesticide on earthworm reproduction means that the effects of contamination can last for more than one generation, leading to significant decline and reductions of genetic diversity, which may subsequently cause disruption of the functioning of the soil ecosystem. Therefore, the ecological risk of mixed pesticides on soil organisms should be studied in detail before applying to sensitive environments.

The results observed here came in support of effect on growth of the earthworm *E.fetida* from soils treated by Cyperkill reported by (Mosleh *et al*., 2003), this test also support the finding of (Zhou *et al*., 2008) the results of these chronic toxicity tests demonstrated that Cyperkill could lead adverse impacts on both the growth and reproduction of adult and juvenile earthworms, while juveniles are more sensitive during the development stage.

On the other hand the effect of the fungicide mancozeb on growth and reproduction was exhibited a mean loss in body mass and reduced cocoon production at 1000 and 500ppm, this results seems to confirm the finding of (Vermeulen *et al*., 2000), who stated the worms exhibited a mean loss in body mass with increasing concentrations of mancozeb.

The impacts of the metal cadmium on the worm growth were also evaluated and the result seems to confirm the finding of (Zaltauskaite and Sodien., 2010) the relationship between Cadmium concentrations and body weight was negative, though 70 days and statistically insignificant body weight reduction due to metal exposure was observed in other studies (Berthelot *et al*., 2008; Van Gestel *et al*., 2009).

But no significant impacts on body weight, or even an increase in weight, were also reported (Van Gestel *et al*., 1993; Spurgeon *et al*., 1994).

Cadmium also inhibited the reproductive rate of worms, where, the cocoon production rate under exposure to cadmium was reduced in comparison with the control group. Similar results were obtained by (Burgos *et al*., 2005). Reduction in cocoon production due to cadmium exposure was reported in several studies (Van Gestel *et al*., 1993; Spurgeon *et al*., 1994; Savard *et al*., 2007). Our findings indicate that growth is least sensitive endpoint than cocoon production being more sensitive.

The number of cocoon produced by worms exposed to Cyperkill concentration were fewer than that of control worms throughout the experiment duration, this results came to support the finding of (Zhou *et al*., 2008), who reported that *E.fetida* exposed to the insecticide Cyperkill at 5, 10, 20, 40 and 60 mg kg showed similar results.

For a very long time the impact of pollutants mixture in soil, such as pesticide – pesticide or pesticide-heavy metals were either underestimated or simply not much works were undertaken to determine their impacts. Lately, however, greater emphases were directed on the possible interactions in the soil or in the organisms inhabiting the soil and the consequent negative effect on the soil ecosystem. For this (Zhou *et al*., 2011), elaborated that the increase in toxicity mixture means that the use of toxicity data from a single pesticide experiments may underestimate the ecological risk of pesticides that are actually present in the soil.

Consequently, the study of pesticide or pollutants mixtures in even more important in evaluating the ecological risk of pollutants on the ecosystem.

For Libyan case several works were conducted concerning the toxic effects of pesticides and / or heavy metals separately on several soil animals mainly woodlice, *porcellio scaper*, *porcellio laevis*, *Hemilipestis reumori*, *Armadello officinalis* and the earthworm *Aporrectodea caliginosa*.

However, the effects of the mixture of these pollutants were not studied so far. Consequently it seems very important to undertake the impact of paired or multiple pesticide-pesticide and pesticide – heavy metal mixture not only on the mentioned above species do meaning the agro system of Libyan soil specially in the eastern regions, but also to extend the study to include other soil animals that might be present and to include the majority of pesticides that have not so far involved into the test.

CONCLUSION

1. Growth and reproductive parameters of earthworms exposed to agro pesticides seems to be useful bioindicators of soil pollution.
2. All of the observations in this test indicated negative impact of pesticides on earthworm growth and reproduction at high concentration but no significant change at lower concentration.
3. The cocoon hatchability test for cocoon exposed to five concentrations of these pesticides and cadmium revealed no marked effect on the rate of hatching, but there was an effect on the number and weight of juveniles.
4. The mixture of the two pesticides, Cyperkill and Remiltine causes greater effects on endpoints such as growth and reproduction on earthworms *E. fetida* than the individual pesticides.
5. Consequently, it should be very important to evaluate pesticide mixtures on soil animals before their use even is each pesticide alone seem to be safe.

SUMMARY

1. *E.fetida* (savigny, 1826) was found to be a valiable model animal for the evaluation of pesticide metals or their mixtures impact on soil animals.
2. This laboratory study was designed to investigate the growth and reproduction toxicity and cocoon hatchability toxicity.
3. *E.fetida* exposed to two commonly used pesticides in Benghazi agro ecosystem and metal cadmium at different concentration.
4. Adult- sub adult worms as well as cocoons were reared in the lab for more than two years.
5. Worms was maintained in plastic pens on a culture media as described by OECD (2004), at room temperature of 20 ± 2 C° the food consisted of artificial soil mixed with barley grains powder as a food supplement every week.
6. The moisture conditions of the rearing soil was started at approximately 60% water holding capacity, rearing continued throughout the experiments, change in body weight after zero, 28, 49 and 70 days, cocoon production after 28 and 70 days, number of juveniles after 70 days were observed.
7. The parameters measured for cocoon hatchability test were Cocoon hatching after 28 days, Juvenile numbers after 70 days and Young worms body weight after 70 days.

8. Pesticides in general are widely used in Benghazi farming system with very usual misuse in dose and interval applications. The two compound

selected in this study were insecticide (Cyperkill), fungicide Remiltine with two active intergradient (mancozeb + Cymoxinal) are used for both agriculture and public health.

9. Cadmium, the third compound selected for study is cadmium chloride as a source of the heavy metal cadmium.
10. Three concentrations of each pesticides and cadmium were listed for the growth and reproduction toxicity study.
11. Five concentrations of each pesticides and cadmium were listed for the cocoon hatchability toxicity study.
12. The artificial soil used OECD (1984) with pH to 5- 6.5. A weight of 250 grams of soil was transferred into glass container (12cmW, 15cmL, 20cmH) to which 100 ml of each of pesticide, cadmium and their mixtures except control, were added and mixed thorough, ten worm, were added and 5 grams barley grains powder were added on surface for growth and reproduction test.
13. Five cocoons were then transferred into each of five concentrations of Cyperkill, Remiltine and Cadmium. Treated 40 grams with 33ml of each of Cyperkill, Remiltine and Cadmium except control.
14. The results of growth and reproduction test all pesticides have shown negative effect on body weight at high concentration and no significant difference on the body mass at lower concentration compared to control.
15. No significant difference on body weight between the cadmium treated worms compared to control.
16. Juvenile number was more sensitivity from growth, cocoon production all treatment.

17. Growth and reproduction test demonstrated that the Cyperkill and Remiltine was the most toxic to *E.fetida*, whereas, the metal cadmium was less toxic.
18. The mixture of the two pesticides, Cyperkill and Remiltine causes greater effects on endpoints such as growth and reproduction on earthworms *E.fetida* than the individual pesticides.
19. The cocoon hatchability of all treatments (pesticides and cadmium) revealed no marked effect was observe on the rate of hatching, but affected the number and weight of juveniles.
20. The earthworms have different sensitivity to Cyperkill at different aspects and stages of life. Thus, eco-toxicological risk assessments of Cyperkill and may be of other contaminants should involve multiple test methods with both juvenile and adult test animals.
21. In summary, the environmental risk of toxicants, however, is judged on the effects of individual pesticides. Consequently, the increase in toxicity of the pesticide mixture means that the use of toxicity data obtained exclusively, from single-pesticide experiments may underestimate the ecological risk of pesticides that actually present in the field.

REFERENCE

Alshawish, S A, Mohamed A I, Nair G. 2004. Prolonged toxicity of sub- lethal dosages of chemical pesticides on the body mass and cocoons of *Aporrectodea caliginosa* (Savigny 1826) (*Oligochaeta Lumbricidae*) inhabiting Benghazi, Libya. Proceedings of the National Academy of Sciences India Section B (Biological Sciences), 74 (part2) 123-133.

Barnes, T., Verlangier, H., and Wilson, M. 1983. Reproductive toxicity of methyl-l-butyl carbamoyl -2-benzimidazole carbamate benomyl in male wistar rats. Toxicology 28: 103-115.

Beeby, A. 2001. "wath do sentinels stand for?" Environmental pollution, View at publisher. View at Google Scholar. View at Scopus, vol. 112, no. 2, pp.285-295.

Berthelot, Y., Valton E., Auroy A., Trottier B., Robidoux P. Y. 2008. Integration of toxicological and chemical tools to assess the bioavailability of metals and energetic compounds in contaminated soils. Chemosphere. Vol. 74. P. 166–177.

Booth, L. H, O`Halloran K. 2001. A Comparison of biomarker responses in the earthworm *Aporrectodea Caliginosa* to the organophosphorus insecticides diazinon and chlorpyrifors. Environmental Toxicology and chemistry, 20: 2494-2502

Bouche, M. B. 1977. strategies lombriciennes .In: U. Lohm, T. Persson (Eds) Soil organisms as components of ecosystems. Ecol Bull (Stockholm) 25: 122-132.

Boyle, T. B., Fairchild J. F .1997. The role of mesocosm studies in ecological risk analysis. Appl Ecol 7: 1099–1102.

Bronschein, R., Reiter. L. and Pearson, D. 1980. Behavioral effects of moderate lead exposure in children and animal. models. crit .rev. toxicology 8 : issue 1 and 2.

Burgos, M. G., Winters C., Sturzenbaum S. R., Rander son P. F., Kille P., Morgan A. J. 2005. Cu and Cd eff ects on the earthworm *Lumbricus rubellus* in the laboratory: multivariate statistical analysis of relationships between exposure, biomarkers,and ecologically relevant parameters. EnvironmentalScience and Technology. Vol. 39. P. 1757–1763.

Butt, K .R. 1997. Reproduction and growth of the earthworm Allolobophora chlorotica (savigny, 1826) in controlled environment .Pedobiologia 41 . P.369.

Callahan, C. A., Shirazi, M. A and Neuhauser, E. G. 1994. Comparative toxicity of chemicals to earthworms Environmental toxicology and Chemistry, 13 (2): 291-298.

Cantalamessa, F. 1993. Acute toxicity of two pyrethroids, permethrin and cypermethrin, in neonatal and adult rats. Archives of Toxicology, 67, 510-513.

Capri, E. and Trevisan, M. I. 2002. Met alli pesanti di origine Agricola nei suolice nelle acque sotterranee, Pitagora Editcriee, ISBN 9788837112622, Bologha, Italy.

Chisolm, J. J. Jr. 1970. Treatment of acute lead intoxication-choice of chelating agents and supportive therapeuticmeasures. Clin.Toxicol., 3: 527-40.

Churchfied, S., Hollier, J.and Brown , V.K. 1991. The effects of small mammal predators on grassland invertebrates, investigated by field enclosures experiment. oikos, 60 : 283 – 290.

Chuttani, H.K., Gupti, p1965. S. Acute copper sulfate poisoning .Am. J. med., 39: 849-54.

Cluzeau, D., Lagarde, R., Texier, C and fagolle, L. 1992. Relevance of life-history parameters in Earthworms"(P. W. Greiy-smith, H. Becker, P. J. Edwards and F. Heimbachoeds) PP.225-229. intercept, London.

CEC. 1978. Criteria (dose/effect Relationships) for cadmium. Pergamon press, oxford, England.

Cremyln, R. 1978. Pesticide Preparation and Mode of Action. Wlley and Sons. Chichester, Brisbane, Toranto.

Culy, M. D. and Berry, E. C. 1995. Toxicity of soil- applied granular insecticides to earthworm populations in cornfields, Dowh to Earth, vol.50, pp.20-25.

Darwin, C. 1809–1882. The formation of vegetable mould through the action of worms, with observations on their habits. Release date 2000–10–01.

Dell'Omo, G. A. Turk and R. F. Shore, 1999. Secondary poisoning in the common shrew (*Sorex araneus*) fed earthworms exposed to an organophosphate pesticide. Environmental Toxicology and Chemistry, vol. 18, no. 2, pp. 237–240. View at Publisher · View at Google Scholar.

Depledge, M. H., Weeks, J.M and Bjerregaard, p. 1994. Heavy metals . In : Calow P (ed) Handbook of ecotoxicology , Blackwell , Oxford ., 2 : 79-105.

Edwards, C. A., and Bohlen, P. J. 1992. The effects of toxic chemicals on earthworms .Rev .Environ.Contam.Toxicol.125, 23-99.

Edwards C. A (Ed.). 1998, 2004. Earthworm Ecology (1st Ed. 1998; 2nd Ed. 2004) CRC, BocaRaton FL.

Farm Chemicals Handbook, 1997. Meister Publishing Co. Willoughby, OH 44094.

Fleckenstein, T., and Graff,O.1983. Heavy metal uptake from municipal waste compost by the earthworm *Eisenia Foetida* (Savigny 1826). Anim. Res. Devel.18, 62-69.

Fragoso, C. Brown G. feijoo, A. 2004. The influence of Gilberto Righi on tropical earthworm taxonomy: the value of a full-time taxonomist .Pedobiologia 47: 400-404.

Fraser, P. M., Haynes. R. J. and Williams, P. H. 1994. Effects of pasture improvement and intensive cultivation on microbial biomass, enzyme – worm populations. Biol. Fertil. Soils, 17: 185 -190.

Friber, L. 1948. Proteinuria and emphysema. among workers exposed to cadmium and nickel dust in a storage battery plant . proc . Int . cong .Ind . med., 9: 641-4.

Gammon, D. W. 1981. Two classes of pyrethroid action in the cockroach. Pestic. Biochem. Physiol. 15:181-191.

Georgescu, B. Doina Iozon, A. Aelgiu, D. Mierlita. 2002. The fertilizing value of the ecological manure obtained as a.vesult of the womecm posting.

Gunadi, B. and Edwards, C. A(2003). The effect of multiple applications of different orgainic wastes on the growth, fecundity and survival of Eisenia fetida (savigny) (Lumbricdac)- pedobioloyia. 47 (4). 321-330.

Hess, R. H., moore, B, J., Linder, R. E., and Abuel -Atta, A .A. 1991. The fungicide benomyl (methyl 1-butyl carbamate2- benzimidazole carbamate) causes testicular dysfunction by inducing the sloughing of germ cells and occlusion of efferent ductules.fundam.Appl.toxicol.17.733-745.

Hoese, B. 1981. morphologic undfunktion des wasserlcitungstems der terrestrischen isopodan (crustacean, isopoda, oniscidae).

Hogger, C.H., and Ammon, H. U. 1994. Testing the toxicity of pesticides to Earthworms Laboratory and field Tests.`IOBC/WPRS Bulletin.

Jain, K and Singh, J. 2004. Modulating of fly ash induced genotoxicity in vica faba by vermicomposting. Ecotoxicology and Environ safety, 59, pp.89-94.

Jayer, T. Fleuren RHLJ, Hogendoorn EA, De Korte G. 2003. Elucidating the routes of exposure for organic chemicals in the earthworm, *Eisenia andrei* (Oligochaeta). Environ Sci Technol 37: 3399–3404.

Kammenga, J.E., Dallinger, R., Donker, M.H., Köhler, H.R., Simonsen, V., Triebskorn, R. &Weeks, J.M. (2000). Biomarkers in terrestrial invertebrates for ecotoxicological soilrisk assessment. Review of Environmental Contamination and Toxicology, Vol. 164, (June 2000), pp. 93-147, ISSN 0179-5953.

Klaassen, C. D.1996. Amdur, M. O., & Doull, J. (Eds.). *Casarett & Doull's Toxicology. The Basic Science of Poisons.*(5th ed.). Toronto: McGraw-Hill Companies, Inc.

Knisel, W.G. (Ed.). 1993. Groundwater Loading Effects of Agricultural Management Systems. (Version 2.10). [Online].

Lanno, R. P. McCarty, L. S. 1997. Earthworm bioassays: Adopting techniques From Aquatic toxicity testing. soil Biol Biochem 29: 693-697.

Lawrence, J. L. and Casida, J. E. 1982. Pyrethroid toxicology:mouse intracerebral structure-toxicity relationships.

Lee, k. E .1959. the earthworm Fauna of new Zealand. New Zeal Depart Sci ind Res Bull 130 :486.

Lee, K. E .1985. Earthworm –Their Ecology and Relationships with Soils and Land Use .Academic, New York, NY, P.411.

Lofs –Holmin, A. 1985. Vermiculture: present Knowledge of the art of earthworm farming –a summary of recent literature. Report 20. Swedish University of Agricultural Sciences Department of Ecology and Environment. Uppsala.

Lofs, A. 1992. measuring effects of pesticides on earthworm in the field :effect criteria and endpoints. In: P. W. Greig–Smith et-al. (Editors), Ecotoxicology of Earthworms. Intercept, Hants., PP. 85 -89.

Loh, T.C., Lee, Y.C., Liang, J.B. & Tan, D. 2004. Vermicomposting of cattle and goat manures by Eisenia foetida and their growth and reproduction performance.– Biores. Technol. 96: 11–114.

Lokke, H, Van Gestel CAM .1998. Handbook of Soil Invertebrate Toxicity Tests. Wiley.

Mohamed, A. L., Nair, G. A. Kassem, H. H and Nuruzzaman. 1995. Impacts of pesticides on the survival and body mass of the earthworm Aporrectodea caliginosa trapezoids (Annelida: Oligochaeta). Acta.zool.fennica, 196: 334- 337.

Morgan, R. k. and Bowden. R. 1993.copper accumulation in soils from two different aged apricot orchards in central otago, New Zealand .In t. J. Environ. stud., 43: 161-167.

Morgan, R. K. and Johnson , H. 1991.the accumulation of copper in a New Zealand orchard soil., J. R. Soc .N.21:323-327.

Moriarty, F. 1983. Ecotoxicology, The study of pollutants in Ecosystems .Academic press, London, Uk. National Research Council: Lead: Airborne lead in perspective .National Academy of sciences, washing-ton, D. C., 1972.

Mosleh, Y.Y, Paris-Palacios S, Couderchet M, Vernet G. 2003 "Acute and Sublethal effects of two insecticides on earthworms (*Lumbricus terrestris*

L) under Laboratory Conditions", Environmental Toxicology, Vol. 18, No.1, PP.1-8.

Neehay, B. R. 1978. Williams, B. J. Sleinsland, O. S and Hall, C. E: Increased vascular response to adrenergic stimulation in vats exposed to cadmium. J. Toxicol. Environ. Health, 4: 559-567.

OECD, 1984.Guidelines for testing chemicals, No, 207. Earthworms, Acute Toxicity Tests, Organization for Econmic Cooperation and Development, paris, france.

OECD, 2004. Guidelines for testing chemicals, No, 222. Earthworms, Reproduction Tests (*Eisenia Fetida /Andrei*), Organization for Econmic Cooperation and Development, paris, france.

Perry, H. M. Jr and Erlanger, M. W. (1947). metal- induced hypertension following chronic feeding of low doses of cadmium and mercury. J. Lab. Clin. Med. 83: 541-547.

Potter, D. A. ,Buxton, M. C. ,Redmond, C. T., Patterson ,C.G., & powell,A. J. 1990. Toxicity of pesticides to earthworms (Oligochaeta :Lumbricidae) and effect on thatch degradation in Kentucky bluegrass turf.J.Econ.Entomol.,83(6):2362-2369.

Potter, D. A., Spicer, P.G., Redmond, C. T., and powell, A. J. 1994. Toxicity of pesticides to earthworms in Kentucky bluegrass turf. Bulletin of Environmental contamination and toxicology, 52(2): 176-181.

Presley, M. L., MCElroy, T. C and Diehl, W. J. 1996. soil moisture and temperature interact to alect growth,survivorship, fecundity and tness in the earthworm *Eisenia fetida*.Comp. Biochem. phgsiol. 114: 319-326.

Reinecke, A. J & Vilioen, S.A.1990. The influence of worm density on growth and cocoon production of the compost worm *Eisenia Foetida* (Oligochaeta).Rev .Ecol.Biol.sol., 27(2):221-230.

Review, by DuPont Agricultural Products. June 30, 1997.

Roark, J. H. and Dale, J. L. 1979. The effect of turf fungicides on earthworms. Ark. Acad. Sci. proc. 33, 71-74.

Sanchez-Hernandez, J. C. 2006. Earthworm biomarkers in ecological risk assessment, Rev Environ Contam Toxicol , 188, pp. 85-126.

Savared, K. Berthelot, Y. Auroy, A. Spear, P. A. Trottier, B. Robidoux P. Y. 2007. Effects of HMX-lead mixtures on reproduction of the earthworm *Eisenia andrei*. Archives of Environmental Contamination and Toxicology. Vol. 53.P. 351–358.

Schroeder, 1962b. Abnormal trace metals in man: Chronic dis., 15: 941 -64.

Schroeder, H.; frost, D.v.; and balassa ,j.j. ,1970b. Essential trace metals in man: Selenium .j. chronic Dis., 23: 227-43.

Sims, R.W., Gerard B. M .1999.synopses of the British fauna (31)-Earthworms Linnean society. London and the Estuarine and Brackish-water sciences Association.

Singh, N. B., Khare, A. K., Bhargava, D. S. & Bhattacharya, S. 2004. Optimum moisture requirement during vermicomposting using *Perionyx excavatus*. – App. Ecol. Environ. Res. 2(1): 53–62.

Sollmann, T. 1957, manual of pharmacology–w .B ,saunders .co., Philadelphia, pp.1191-1354.

Spurgeon, D. J .and Hopkin, S.P. 1996. Effects of metal-contaminated soils on the growth, Sexual development ,and early cocoon production of the earthworm *Eisenia Fetida* with particular reference to zinc. Ecotox. Environ. Safety 35:86-95.

Spurgeon, D. J. and Hopkin S. P. 1996. Eff ects of metal-contaminated soils 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.

Spurgeon**,** D. J. Hopkin S. P. Jones D. T. 1994. Effects of cadmium, copper, lead and zinc on growth, reproduction and survival of the earthworm *Eisenia fetida* (Savigny): assessing the environmental impact of point-source metal contamination in terrestrial ecosystems. Environmental Pollution. Vol. 84. P. 123–130.

Thomson, W. T. 1997. Agricultural Chemicals. Book IV: Fungicides. 12th edition. Thomson Publications, Fresno, CA 93791.

Tomlin, C. (Ed.). 1994. A World Compendium. The Pesticide Manual. Incorporating the agrochemicals handbook. (10th ed.).

U.K, 1997. Crop Protection Publications. U.S. Environmental Protection Agency. Cymoxanil; Pesticide Tolerances for Emergency Exemptions. Federal Register Document 97-12475. May 13, Bungay, Suffolk.

USEPA, 1989. Cypermethrin Pesticide Fact Sheet. Washington, D.C.

USEPA, 1997. Cymoxanil. Pesticide Tolerances for Emergency Exemptions. Federal Register Document. 97-12475.

van Gestel C. A. M., Koolhaas J. E., Hamers T., van Hopper M., van Roovert M., Korsman C., Reinecke S. A. 2009. Effects of metal pollution on earthworm communities in a contaminated floodplain area: linking biomarker, community and functional responses. Environmental Pollution. Vol. 157. P. 895–903.

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.

Van Gestel, C. A. M., Dirven-van Breemen E. M., Baerselman R., Emans H. J. B., Janssen J. A. M., Postuma R.,van Vliet P. J. M. 1992. Comparison of sublethal and lethalcriteria for nine diff erent chemicals in standardized toxicity tests using the earthworm *Eisenia andrei*. Ecotoxicology and Environmental Safety. Vol. 23. P. 206–220.

Van Gestel, C. A. M., van Dis W. A., van Breemen E. M., Sparenburg P. M. 1989. Development of a standardized reproduction toxicity test with the earthworm species *Eisenia fetida andrei* using copper, pentachlorphenol, and 2,4-dichloroaniline. Ecotoxicology and Environmental Safety.Vol. 18. P. 305–312.

Venter, J. M and Reinecke, A. J. 1988.the life-cycle of the compost worm Eisenia fetida (oligochaeta). S Afr J Zool, 23:161-165.

Vermeulen, L. A, Reinecke A .J, Reinecke S.A. 2000. Evalaution of the fungicide manganese-zinc Ethylene bis (dithio carbamate) (mancozeb) for sub lethal and A cute toxicity to *Eisenia fetida* (*oligochaeta*) Journal of Ecotoxicology and Environmental Safety 48, 183-189.

Vijver, M. G, Vink j. P. M, Miermans C. J. H ,Van Gestel C. A. M .2003.Oral sealing using glue :A new method to distinguish between intestinal and dermal uptake of metals in earthworms. Siol Biol Biochem 35: 125 -132.

Walker, C. H., Hopkin, S. P., Sibly, R. M., Peakall, D. B., 1996. Principles of Ecotoxicology, Taylor and Francis, London.

WHO, 1976. Task Group on Environmental Health: Environmental Health criteria .I . Mercary, world Health organization, Geneva.

WHO, 1989. *Environmental Health Criteria. Cypermethrin* (Vol. 82). Geneva: United Nations Environmental Programme, the International Lab our Organization, and the World Health Organization.zoomorphologic, 98, 135-167.

WHO, 1993. Reproduction, embryo toxicity and teratogenicity. In "Environmental health, criteria 149, carbendazin "(World Health organization, Ed.), pp. 59-69. WHO, Geneva.

Zaltauskaite, J. and Sodiene, I. 2010. Effects of total cadmium and lead concentrations in soil on the growth, reproduction and survival of earthworm *Eisenia fetida*. Department of Environmental Sciences,Vytautas Magnus University, Ekologija. Vol. 56. No. 1–2. P. 10–16.

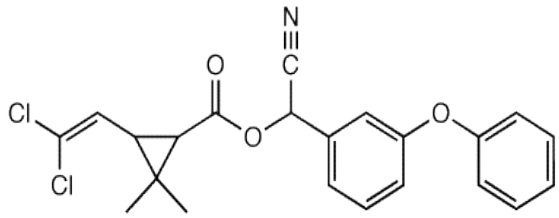
Zhou, S. P, Duan C. Q, Michelle W. H, Yang F. Z, Wang X. H, 2011. Individual and combined toxic effects of cypermethrin and chlorpyrifos on earthworm. Journal of Environmental Sciences, 23 (4): 676-680.

Zhou, S. P, Duan C. Q, Wang X. H, Wong H. G, 2008. Assessing Cypermethrin-contaminated soil with three different, earthworm test methods, Journal of Environmental Seiences, 20 (11): 1381-1385

APPENDIXES

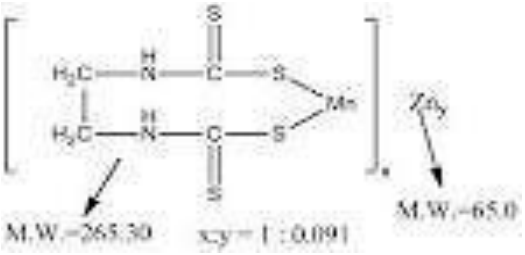
APPENDIX (1)

Appendix (1.1). Cypermethrin (Cyperkill ® EC 25).



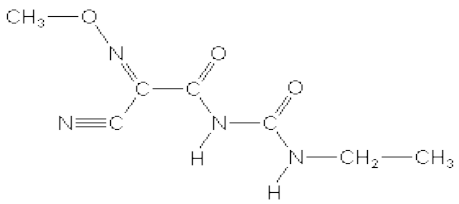
(RS)-α-cyano-3 phenoxybenzyl-(1RS)-cis, trans-3-(2, 2-dichlorovinyl)-2, 2-dimethylcyclopropane carboxyl ate.

Appendix (1.2). Mancozeb (Remiltine ® wp 68).



Ethylin di thio charbamate add zn, and mn

Appendix (1.3). Cymoxanil (Remiltine ® wp 68).



1-[(*EZ*)-2-cyano-2-methoxyiminoacetyl]-3-ethylurea

APPENDIX (2)

Appendix (2.1): Showing the Adult worm**.**

Appendix (2.2): Showing the Plastic pens for worms culture**.**

Appendix (2.3): Showing the Cocoons of *Eisenia fetida*.

APPENDIX (3)

Appendix (3.1): The common name, trade name, intended use and manufacturing company for each pesticide.

Common name	Trade name	Intended use	Manufacturing company
Cypermethrin	cyperkill®EC 25	Pyrethriods insecticide	CHimac – AGRIPHAR S.A. made in Begium
Mancozeb +cymoxanil	Remiltine®WP 68	Fungicide	Made in China

Appendix (3.2): The common name, formula, and manufacturing company for Cadmium chloride.

Common name	Formula	Manufacturing company
Cadmium chloride	$CdCl_2.3H_2o$	Merck E. Merck .Darmstadt Made in Germany

APPENDIX (4).

Abbreviation.

W = width

L = length

H = hight

Const. 1= first concentration.

Const. 2= second concentration.

Const. 3= third concentration.

Coc. 1 = cocoon number after 28 days.

Coc. 2 = cocoon number after 70 days.

OECD = Organization for Economic Co-Operation and Development.

USEPA = U. S. Environmental Protection Agency.

WHO = World Health Organization.

CEC = Commission of the European Communities

جامعة بنغازي

كلية العلوم

قسم علم الحيوان

التأثير المشترك للسيبر كيل و الريملتين و الكاديوم علي بالغات وشرانق وصغار دودة الأرض *Eisenia fetida*

أطروحة مقدمة كجزء من متطلبات الإجازة العليا الماجستير في العلوم

مقدمه من الطالب

أعبيد سالم أعبيد

إشراف

أ.د. عبد الله ابراهيم محمد

أغسطس - 2013

التأثير المشترك للريملتين وسيبر كيل و الكادميوم علي بالغات وشرانق وصغار دودة الأرض *Eisenia fetida*

ملخص البحث

1. تم اختيار دودة الأرض الأوربية *Eisenia fetida* لهذه الدراسة كونها تعتبر النماذج الحيوية الجيدة لدراسة تلوث التربة ولما تتمتع به من كفائه في التربة المعملية والإنتاجية للمواليد.
2. تم تصميم هذه الدراسة ألمختبريه لغرض التحقيق في التأثير السام علي نمو وتكاثر دودة الأرض عند استخدام هذه المبيدات أو المعادن الثقيلة أو خلائيطها.
3. تعرضت دودة الأرض *Eisenia fetida* لإثنين من المبيدات الزراعية التي تستخدم عادة في البيئة الزراعية في بنغازي كذالك معدن الكادميوم عند تركيزات مختلفة.
4. تم تربية الديدان البالغة وغير البالغة وشرانق في المختبر لأكثر من سنتين.
5. استمرت تربية الديدان في أحواض من البلاستيك مع وسط التربية كما هو موضح من قبل منظمة التعاون والتنمية (2004) ,عند درجة حرارة °2C ± 20, يتألف الغذاء من التربة الاصطناعية مع مسحوق من دقيق الشعير كمكمل غذائي عند كل أسبوع.
6. نسبة الرطوبة كانت حوالي 60% استمرت طول التجربة,ملاحظة التغير في الوزن بعد 28 و 49 و 70 يوم وإنتاج الشرانق بعد 28 و70 يوم وعدد الصغار بعد حوالي 70يوم.
7. بالنسبة للاختبار معدل الفقس لوحظ بعد 28 يوم أما عدد الصغار ووزنها فكان بعد 70 يوم .
8. تستخدم المبيدات بشكل عام علي نطاق واسع في النظام البيئي الزراعي ببنغازي مع الجرعات المعتادة تم اختبار اثنين من هذه المبيدات للدراسة وهما المبيد الحشري سيبر كيل والمبيد الفطري الريملتين مع اثنين من المادة الفعالة وهما Mancozeb Cymoxinal, حيث تستخدم كل من المبيدين في الزراعة والصحة العامة.

9. الكادميوم يعتبر المركب الثالث الذي اختير لهذه الدراسة حيث يعتبر كلوريد الكادميوم كمصدر للكادميوم .
10. تم اختيار ثلاث تركيزات لكل من المبيدات و الكادميوم وتركيز واحد للمخاليط لدراسة التأثير علي كل من النمو و التكاثر لدودة الأرض *Eisenia fetid*a .
11. تم اختيار خمس تركيزات لكل من المبيدات و الكادميوم لدراسة تأثير علي معدل الفقس .
12. التربة الاصطناعية المستخدمة كما هو موضح في (OECD, 1984) مع رقم هيدروجيني من 5 إلي 6.5 حيث تم اخذ مقدار وزنه 250جرام من التربة المعالجة مع 100مل من كل من المبيدات و الكادميوم و مخالطها باستثناء مجموعة السيطرة إلي أوعية زجاجية ,(H 20cm و L 15cm ,W 12cm) و تم أضافة 10ديدان لكل من أوعية الاختبار بعد الانتهاء من وضع الديدان يتم إضافة الغذاء من خلال رش 5 جرام من دقيق الشعير علي السطح.
13. يتم وضع خمس من الشرانق في أوعية تحتوي علي 40 جرام من التربة ألاصطناعية مع محلول يحتوي علي 33 مل من كل من المبيد الحشري سيبر كيل و المبيد الفطري الريملتين ومعدن الكادميوم باستثناء مجموعة السيطرة.
14. أظهرت الدراسة أن كل من المبيد الحشري سيبر كيل والمبيد الفطري الريملتين ومعدن الكادميوم لها تأثير سلبي علي وزن الجسم مقارنة بمجوعة السيطرة كما لوحظ أنه لا يوجد فرق عند التركيز المنخفض .
15. لا يوجد فرق يذكر في وزن الجسم ما بين الديدان المعالجة بالكادميوم مقارنة بمجموعة السيطرة.
16. كان التأثير علي عدد الصغار أكثر حساسية من النمو وإنتاج الشرانق في كل من المعاملات مقارنة بمجموعة السيطرة.
17. كان تأثير الكادميوم اقل علي وزن الجسم مقارنة بالمبيد الحشري سيبر كيل والمبيد الفطري الريملتين .
18. تظهر الدراسة أيضا أن خليط كلا من المبيدين الحشري سيبر كيل و الفطري الريملتين يتسببان في خطر اكبر علي نقطة نهاية الاختبار مثل معدل النمو والتكاثر علي ديدان الأرض *Eisenia fetid*a من المبيدات الفردية.

19. كشف اختبار معدل الفقس لكل من المبيد الحشري سيبر كيل والمبيد الفطري الريملتين ومعدن الكادميوم لا يوجد تأثير يذكر علي معدل الفقس مقارنة بمجموعة السيطرة. ولكن لوحظ تأثير واضح على عدد و وزن الصغار مقارنة بمجموعة السيطرة.
20. تمتلك ديدان الأرض *Eisenia fetid*a حساسية مختلفة لسيبر كيل في جوانب ومراحل الحياة المختلفة , ينبغي تقييم المخاطر البيئية لسمية مبيد السيبر كيل وربما غيرها من الملوثات علي طرق تشمل اختبارات متعددة مع حيوانات الاختبار علي حد سواء لكل من الصغار و البالغين .
21. يتضح من النتائج التي تم الحصول عليها من هذه الدراسة أن الآثار السلبية ارتبطت بخلائط المبيدات بخلاف ما هو ملاحظ لكل مبيد على حده, عليه فأن الدراسة الملاحظ تكون مفيدة وضرورية لتقييم الآثار السلبية علي البيئة.