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Combined Toxicity of Cadmium, Chlorpyifos (Salute) and Glyphosate (Round- up) on Earthworm Growth and Reproduction

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Abstract:

This study investigated the potential interaction and combined toxicity of the heavy metal Cadmium (Cd) with two common pesticides—the organophosphate insecticide Salute (Chlorpyrifos) and the herbicide Round-up (Glyphosate)—on the growth and reproduction of the earthworm species, Eisenia fetida, under laboratory conditions.

Eight adult to pre-adult worms were exposed for eight weeks to single treatments of Cd, Salute, or Round-up, as well as their metal-pesticide mixtures, using concentrations based on field use and typical unpolluted soil levels for Cd (5 mg/Kg).

Significant differences in worm body weight were reported after eight weeks among the different treatments compared to the control (F=6.19, P\textless0.05). The lowest body weight was recorded in the Cd-Salute mixture (7.24 \pm 1.02 g), indicating a synergistic interaction and a moderate to low effect on body weight. Conversely, the highest body weight was observed in the Cd-Round-up mixture (9.83 \pm 2.69 g), suggesting a relative antagonistic effect when combined, though the difference was not statistically significant.

For reproduction, measured by juvenile numbers, the Cd-Salute mixture did not show significant differences compared to the control. However, the Cd-Round-up mixture revealed a significant difference (F=29.809, P\textless0.05), with the Round-up treatment alone having the least mean juvenile number (133.3 \pm 89.04), followed by the Cd-Round-up mixture (169.6 \pm 51.08). This result indicated that Round-up seemed to have a greater negative impact on juvenile production, and the mixture with Cd reflected an additive effect.

Overall, the results confirm that the mixed pollutants can lead to impacts on chronic responses like growth and reproduction. The combination of the herbicide Round-up and Cadmium had an adverse effect on juvenile number, while the insecticide Salute and its metal combination did not show an obvious negative effect on juveniles.

ملخص باللغة العربية

هدفت هذه الدراسة إلى تقييم التفاعل المحتمل والسمية المشتركة للمعدن الثقيل الكادميوم مع مع اثنين من المبيدات الشائعة: المبيد الحشري الفسفوري العضوي سالوت (كلوربيريفوس) ومبيد الأعشاب راوند-أب (جلايفوسات)، وتأثيرها على نمو وتكاثر دودة الأرض من نوع Eisenia fetida تحت الظروف المختبرية.

تم تعريض ثماني ديدان بالغة إلى ما قبل البلوغ لمدة ثمانية أسابيع لمعالجات فردية من الكادميوم، سالوت، أو راوند-أب، بالإضافة إلى مخاليط المعدن والمبيدات. تم اختيار تركيزات المبيدات بناءً على الاستخدام الحقلي، بينما استخدم الكادميوم بتركيز يمثل مستواه في التربة الطبيعية غير الملوثة (5 ملجم/كجم).

أظهرت النتائج اختلافات معنوية في وزن جسم الديدان بعد ثمانية أسابيع بين المعالجات المختلفة مقارنة بالمجموعة الضابطة (F=6.19, P\text less0.05).تم تسجيل أقل وزن لجسم الدودة في خليط كادميوم-سالوت (7.24 ±1.02جرام)، مما يشير إلى

تفاعل تآزري وتأثير معتدل إلى منخفض على وزن الجسم. على النقيض، لوحظ أعلى وزن لجسم الدودة في خليط كادميوم-راوند-أب (2.69 ± 9.83 جرام)، مما يشير إلى تأثير تعاكسي نسبي عند دمجهما، على الرغم من أن الفرق لم يكن معنوياً إحصائياً.
بالنسبة للتكاثر، الذي تم قياسه بعدد اليرقات(juveniles) ، لم يُظهر خليط كادميوم-سالوت اختلافات معنوية مقارنة بالمجموعة الضابطة. ومع ذلك، كشف خليط كادميوم-راوند-أب عن اختلاف معنوي (F=29.809, P\text less0.05). كانت أقل قيمة لمتوسط عدد اليرقات في معالجة راوند-أب وحدها (133.3 ± 89.04)، تليها معالجة خليط كادميوم-راوند-أب (169.6 ± 51.08) أشارت هذه النتيجة إلى أن راوند-أب كان له التأثير السلبي الأكبر على إنتاج اليرقات، وعكس الخليط مع الكادميوم تأثيراً إضافياً.
بشكل عام، تؤكد النتائج أن مخاليط الملوثات يمكن أن تؤدي إلى تأثيرات على الاستجابات المزمنة مثل النمو والتكاثر. كان للجمع بين مبيد الأعشاب راوند-أب والكادميوم تأثير ضار على عدد اليرقات، في حين لم يُظهر المبيد الحشري سالوت وخليطه المعدني تأثيراً سلبياً واضحاً على اليرقات.

Introduction

Earthworm is considered as a domain soil organism and has been recommended as test species to evaluate soil contaminations in acute as well as chronic toxicity studies (Haeba*et al*., 2013).
Earthworms are the soil dwelling invertebrates, which have great agricultural importance, they influence the soil structure by ingestion , which leads to the break down of organic matter and its ejection as a surface or subsurface cast (Edwards and Lofty,1977).
Earthworms are known to play a major role in the development and maintenance of soil structure in the breakdown and incorporation of organic residues in the soil and as a source of food for terrestrial organism (Edwards and Bohlen, 1996).
The species *E. fetida* is most commonly used in ecotoxicology and is recogrized as a useful bio-monitor for testing the chemical toxicity of soil (Brulle*et al*., 2006).
The pesticide effect on earthworms depends on the used chemical substance, generally , herbicides manifest low toxicity on earthworms but indirectly can produce the reduction of populations by decreasing the organic matter input and weed coverage. The fungicides and fumigants are very toxic substance for earthworms (Booth *et al*.,2001and Rida and Bouché, 1997). and weed coverage. The fungicides and fumigants are very toxic substance for earthworms (Booth *et al*.,2001and Rida and Bouché, 1997).
Differences in bioaccumulation of various metals also appears to be related to differences in metal bioavailability (Li *et al*., 2010) as affected by environmental factors affecting solubility (pH and redox) and metal complexation with soil organic matter(Liu *et al*.,2017). A common method for estimating bioavailable concentrations of heavy metals uses soil extraction with the metal chelator, diethyl enetriamine penta acetic acid (DTPA) which has been developed into a formal test method to address the availability of heavy metals to earthworms(Dai *et al*., 2004).
In general, accumulation of metals by earthworm species occurs through two pathways which include absorption following dermal contact(Nannoni*et al*., 2014) or else by ingestion of organic matter and adsorption through the gut tissues (Morgan *et al*.,2004 and Hobbelen*et al*., 2006). Because of the diversity of ecological characteristics and feeding habits of different earthworm species, the relative importance of dermal contact versus gut adsorption may vary (Nannoni*et al*., 2011).
Heavy metals can enter the soil from different sources such as pesticides, fertilizers, organic and inorganic materials, as well as sewage sludge residues (Capri and Trevisan, 2002)
The bio-accumulative ability of earthworms enables them to be used as living organisms for the bio-monitoring of soil pollution ,where, elevated concentration of Cadmium (Cd), Copper (Cu),Lead (Pb) and Zinc (Zn) in soils can affect the density, viability, cocoon production, growth and sexual development of worms (Hirano and Tamae,2011).
Heavy metals cause mortality (Davies *et al*.,2003) and affect the population size, avoidance and species diversity of earthworms and reproduction is likely to be of particular importance in eco-toxicological assessment because of its influence on population dynamics(Spurgeon *et al*., 2005).
Cd is a major heavy metal toxic to the environment that causes harmful
events in many biological processes of humans, animals and plants [18]. Cd is a physically soft metal, not biodegradable, and with a half-life of 15–30 years in humans [13]. In the industry, it has a wide

range of applications owing to its outstanding characteristics against corrosion and resistance to oxidation: in fact, it is used in the construction of Ni-Cd batteries, in the production of many materials as color element, in the coating of mechanical parts and in photography [18]. Among the different compounds, only a small part (1–10%) of the orally ingested dose of cadmium chloride ($CdCl_2$) is absorbed through the intestine [19,20];

instead, cadmium oxide (CdO) is absorbed through respiration.

Pesticides pollution will inevitably become the major outcome of intensive pesticides, earthworms acts as suitable test animal as mentioned by OECD (Zhang *et al*., 2014)

The pesticide exposure induces detrimental effects on the physiology and metabolism of earthworm (Bansiwal and Rai, 2010).

Laboratory and field experiment concerning the impacts of both organic and metals toxicity is a continuous process in order to detect their interaction behavior in the environment which usually results due to several environmental factors leading to the reverse of their intended use(Aebeed and Amer, 2018).

Materials and Methods

- The Test Organism

The earthworm *Eiseniafetida* was chosen for this experimental work, because of their important contribution for their role in soil components, both soil structure and functions as well as their use as biological indicators in toxicity.

Eiseniafetida was obtained from a stock culture that maintained in the zoology department lab of the faculty of science for more than five years on an artificial universal pot ground soil (Egmond, spain,) which contains all required nutrients along with enough organic matter for earthworm growth, and PH of 5-6.5 and EC of 0.8- 1.5mm Sh/cm. This soil is the guidelines for testing set by the Organization for EconmicCoorperation and development (OECD, 1984).

- The Pesticides

Two different pesticides were chosen for the experiment including an OP insecticide Salute,and the herbicide Round up all are well known for their wide use in Benghazi open agroecosystem against different insect, weeds and plant pathogens, (fungal diseases).

- Chlorpyrifos (formulation Salute)

The insecticide salute is an organophosphours consisting of two compounds used at 0.05ml/Kg soil.

1- Chlorpyrifos acting against insects, Nematods and mites.

2- Dimethoate mostly works against insects.

Is is a contact and systemic organophosphours insecticide and acaricides effective against a large number of insect pests of different crops.

The compound is a potent cholinesterase inhibitor in both vertebrates and invertebrate even at slightly high dose (Whitehead, 1995).

- Glyphosate (formulation Round up)

Glyphosate (Round-up) herbicide is a non selective and systemic against broad leaf weeds used at 0.5ml/Kg.

A non- residual phosphonic acid herbicide effective againest both annual and perennial weeds in and around several crops. This compound is widely used for several years in most agroecosystems of Benghazi as well as many other regions of Libyan farms.

The compound can irritate eyes and skin if it come in direct contact, otherwise it is safe when used as recommended for each weed (Whitehead,1995).

- Worm Rearing

The worms were reared in medium size plastic containers approximately 41cm long, 35cm wide and 20cm deep heaving approximately 15-17cm of the sieved artificial soil which moistured up to 65-70% water holding capacity, frequent barley flour was added to the soil surface as food supplement.

All rearing containers were held under the laboratory conditions where, both temperature and humidity were monitored but not controlled. However, their range were about 18- 22± 2C° and 60±5 RH during the experiment.

Containers monitor of the culture indicated that the worms were growing and breeding normally and no mortality was observed.

- The Experiment

The experiment was based on finding the possible interaction of the heavy metals –cadmium with the insecticide Salute,and the herbicide Round-up when they come together in the soil habitat of the earthworm.

Consequently – one concentration of each pesticide was chosen on the bases of the used field use with the consideration of the remaining deposit of the pesticide and the limited free space of the treated worms.

Three replicats were set for each pesticide as well as for the one metal separately. An additional three replicats were also set for the pesticide- metal mixture for detecting the interaction behavior between the single and the combined mixture of all treatmentsthe three replicates of same soil quantity at 400ml of H_2o and without pesticide or metals were designed as control (Table1).

Table1. Pesticides- Metal Combination treatments in the earthworms *Eiseniafetida* – metals mg/kg and pesticide ml/liter and8 worms per replicate.

Treatments	mg/Kg	mL/Liter	R1	R2	R3
Cadmium	5		8	8	8
Salute		0.05	8	8	8
Roundup		0.5	8	8	8
Cd+ Sal			8	8	8
Cd+ Ru			8	8	8
Control			8	8	8

Eight adult to pre adult worms were used per replicate the heavy metal cadmium was used at 5 mg/Kg respectively these values were chosen on the bases of their presence in most natural unpolluted soil.

The metal was cadmium nitrate obtained from the chemistry department of the faculty of science.

The pesticides were original field use Emulsifiable concentration(EC) formulation obtained from a known farmer at Benghazi .

- Experiment Procedure

- The metal

1. The designated weight of each metal were measured and dissolved in 50ml H_2O and placed in shaker for 2 hours to ensure the complete dissolving, 5ml were then added to 395ml of H_2O to make the 400ml solution required for 125 grams of the oven dried test soil for the metal treatment.
2. The pesticides (EC- formulation) were calculated and prepared on the bases of the required 400ml solution to the 125 grams test soil per each container.
3. Metal- Pesticide mixture prepared by adding 5ml of metal solution to 395ml of pesticide solution per the 125 gram of test soil.
4. Each replicate soil was then mixed thoroughly by hand with the specified metal alone, pesticide alone and their mixtures. control replicate received plain water at 400ml per replicate 125 gram of soil.
5. Treated soil were allowed to stand for one day for settling.

6. Eight adult to pre adult *Eiseniafetida* were transferred into each test container, covered by mesh and a glass cover that which allowing relative gas exchange and low water vapore escape which may affect test soil moisture and consequently worm health and behavior, this cover also prevent flies from entering the test containers.

The test duration was set for 8 weeks Mortality, worm body weight, cocoon and juvenile were recorded at the first 4 weeks and at the end of the experiment 8 weeks.

The eight weeks were considered for two reasons

a) The juvenile worms can grow to the maturity and can give cocoon and juvenile.
b) This same period has been recommended as the soil condition likely will change in nutrition and structure which eventually affect the worm life and biology.

- Data Collection and Statistical Analysis

After the end of the test duration data of both period 4 and 8 weeks were collected sorted and subjected to statistical analysis.

All statistical analysis were done by SPSS software package, where two way analysis of variance for mean separation of the different treatments.

4. Results

- Body weight of treated adult worms

After eight weeks of the worm exposure to the metal alone and Metal- Pesticide mixtures a significant differences ($F=6.19$, $P<0.05$) were reported between the different combinations of metal- pesticides treatments as compared to that of control worm body weight. As can be seen that only Cadmium-Round up with mean of 9.83±2.69

All other metal-pesticides mixtures were less than that of control and that the least worm body weight were reported in Cd-Salute7.24±1.02

Table 1. The mean ± SD in grams of *E. fetida* body weight exposed to heavy metals and Pesticide treated soils after eight weeks of exposure.

Treatments	Mean ±SD
Cd	7.7±1.29
Cd+Ru	9.8±2.69
Cd+Sal	7.2±1.02
Control	8.5±2.65
Ru	9.7±2.22
Sal	8.7±1.69

The body worms weight in the metal, pesticide and their combination

1. **Cadmium- Salute Mixture** Cd, Sal and Cd+ Sal

Table 2 and Fig 1 showed the impact of the insecticide salute, the metal cadmium and their combination on the worm body weight.

The result revealed that no significant differences were reported in the worm body weight ($F=1.30$, $P> 0.05$)

This results indicated that Cd alone and Salute alone 7.78 and 8.7 respeclively came close to control whereas, Cd+ Salute 7.24 reported lower weight, yet not significantly different for control, this also reflect an synergistic interaction consequently moderate to low effect an the body weight

Table 2. The mean± SD in grams body weight for *E. fetida* in Control, Cd, Sal, Cd+ Sal) of treated soils.

Weight	Mean ±SD
Control	8.5±2.65
Sal	8.7±1.69
Cd	7.7±1.29
Cd+Sal	7.2±1.02

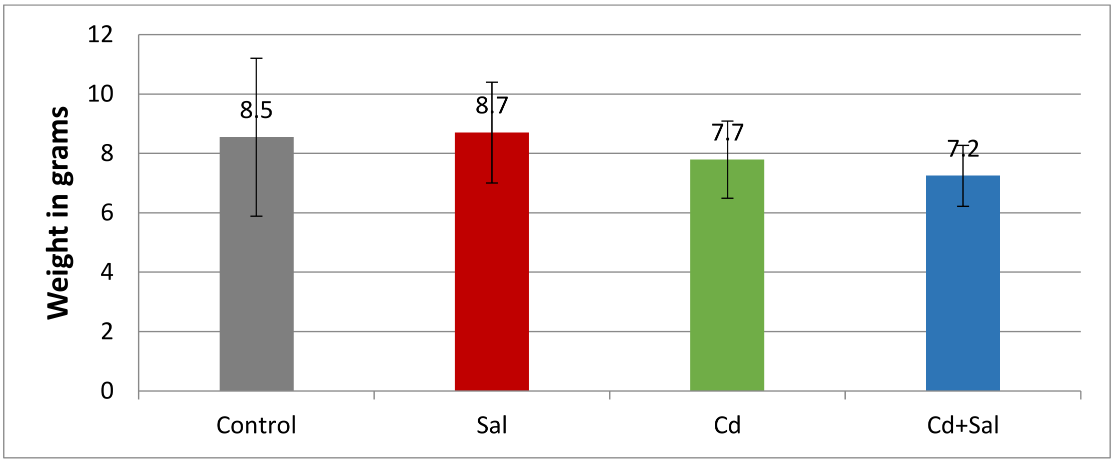

Figure 1. The mean± SD in grams of body weight of in Control, Cd, Sal and Cd+ Sal) of treated *E.fetida*.

1-Cadmium- Round up Mixture Cd, Ru and Cd+ Ru

Table 2 showed the mean± SD of the worm body weight after exposure to Cd, Ru, and Cd-Ru mixture over 8 weeks .

As can be seen no significant differences were reported (F=1.69,P >0.05), however, Cd treated worms showed the lowest mean 7.78±1.29, whereas, Cd+Ru treated worms showed the highest body weight 9.83±2.69 and Ru treated with 9.76±2.22.

Although the differences were not significant, however the results indicated relative antagonestic effect when Cd and Ru were used in mixture compared to their single use.

Table 3. The mean± SD in grams of body weight of Control, Cd, Ru, Cd+ Ru) of treated *E. fetida*.

Weight	Mean ±SD
Control	8.5±2.65
Ru	9.7±2.22
Cd	7.7±1.29
Cd+Ru	9.8±2.69

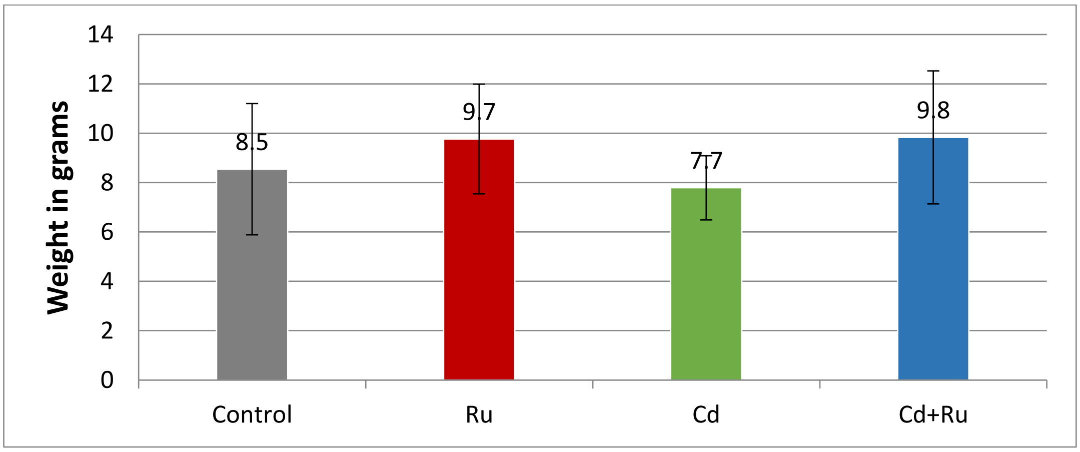

Figure 2. The mean± SD in grams of body weight of Control, Cd, Ru, Cd+ Ru) of treated *E. fetida*.

The juvenile numbers, from Metal, Pesticide and their combination

1. **Cadmium- Salute Mixture** Cd, Sal and Cd+Sal

The juvenile (F=1.2, P> 0.05) between the control, Cd ,Sal and Cd+ Sal treatment

This results indicated the number of juveniles produced by the worms after the 8 eight weeks did not reveal significant differences between the different treatments (F=1.21, P> 0.05) respectively similar case was also reported in the juvenile number 465.6 in the mixture compared to Sal and Cd with 450.3 and 608 respectively revealing an addative interaction

Table 4. The mean ± SD of juveniles number of control, Cd, Sal and Cd+Sal) of treated worm.

Juveniles	Mean ±SD
Control	436±54.83
Sal	450±192.5
Cd	608±83.35
Cd+Sal	465±124.91

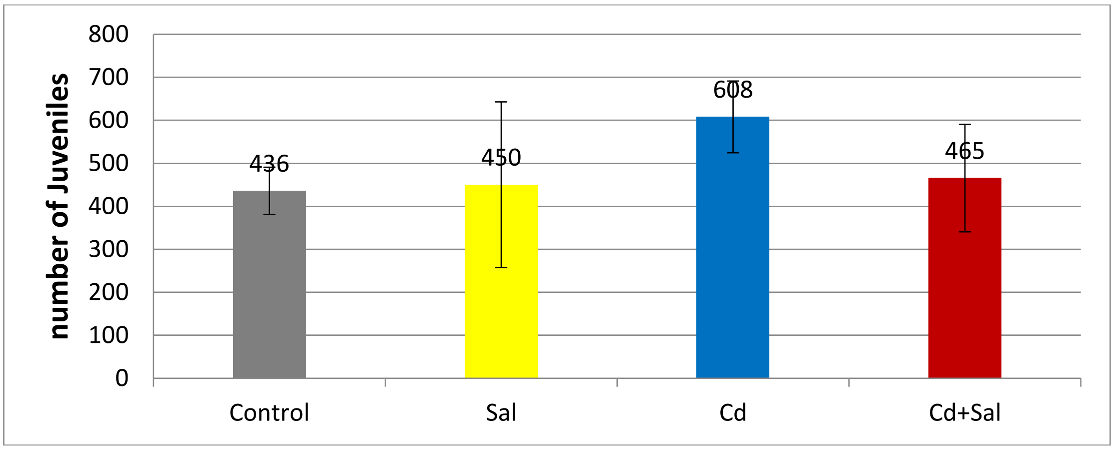

Figure 3. The mean ± SD of juveniles number of control, Cd, Sal and Cd+Sal) of treated worm.

1. **Cadmium- Round up Mixture** Cd, Ru and Cd – Ru

The Means± SD value of juveniles produced from Cd, Ru and Cd-Ru treated worms reveled significant difference ($F=29.809$, $P< 0.05$) (Table5-Fig4) Although Cd is considered a non essential heavy metal, however, revealed the highest mean number 608±83.35 followed by control with 436.±54.83, whereas, the least mean value was that of Ru alone with 133.3±89.04 and Cd-Ru169.6±51.08.

The Cd-Ru mixture then reflected an addative effect as it came less than that of Cd alone but greater than that of Ru alone Ru seems to have greater impact on the juvenile production whereas, Cd has low effect on the juvenile production.

Table 6. The mean ± SD of juveniles number of control, Cd, Ru and Cd+Ru) of treated worm.

Juveniles	Mean ±SD
Control	436±54.83
Ru	133±89.04
Cd	608±83.35
Cd+Ru	169±51.08

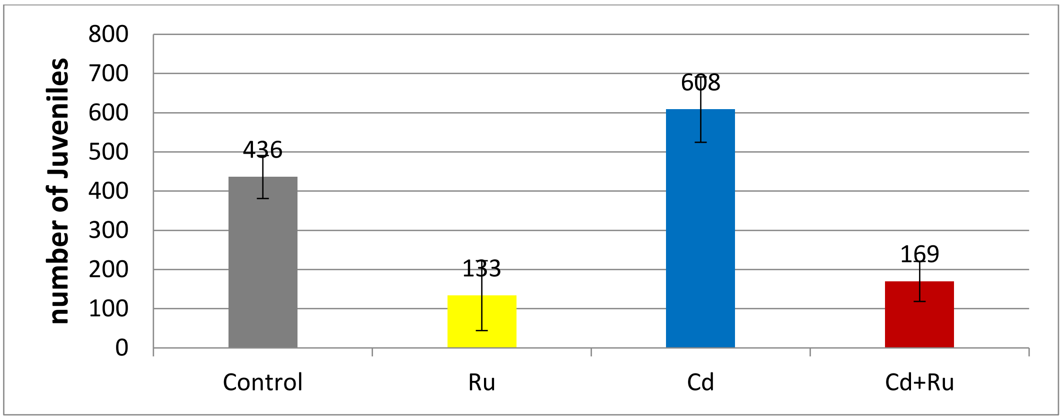

Figure 5. The mean ± SD of juveniles number of control, Cd, Ru and Cd+Ru) of treated worm.

Discussion

The present study focused on the toxicity of two pesticides the insecticide Salute, the herbicide Round up with heavy metals Cadmium on the growth and reproduction of the earthworm species *Eiseniafetida*under Lab conditions

There are many other reports on the toxic effects of pesticides on earthworms, where, Hans *et al*., (1993) found that three organochlorine insecticides (aldrin, endosulfan and lindane) have negative effects on the earthworm *Pheretimaposthuma*which varied according to the duration of exposure. On the other hand, chlorpyrifos, carbofuran and mancozeb have been found to be more toxic to *Perionyx excavates* than *Eiseniaandrei*under tropical condition (De Silva, 2009). In the present study, however, the insecticide Salute revealed miled effect on *E. fetida*.

Many investigators have reported a high toxicity of chlopyrifos and cypermethrin insecticides on many species of earthworms in different countries, the effects ranging between moderate mortality to *Perionyxexacavtus*(Chakravorty and Kaviraj, 2010) and decrease in body weight of *Eiseniafetida*(Yasmin and D'Souza, 2010).

Shahmansouri*et al*. (2005) revealed that though heavy metals like Cr, Cd, Pd, Cu and Zn were boiaccumulated by *E.fetida*there was a decrease in their body weight when exposed to higher concentrations.

A similar decrease in body weight of the earthworm *Pheretimaguillelmi*at high concentration of lead in soil has been recorded by Zheng and Canyang(2009).

However, the studies of Maleckie*t al*.,(1982) indicated that the body mass of earthworms decreases with the increase in the contamination of the substrate; among the metals they studied, cadmium had the strongest reducing effect on the body mass of earthworms.

Heavy metals impose negative impact on growth of earthworms which, considered as potential bio-indicators for contamination and pollution through their survivability, cocoon production and growth which noted in the present study which confirm the finding of (Beyer *et al* ,1980).

Helling*et al*., (2000) tested in laboratory the effect of copper oxychloride, while Yasmin and D'Souza, (2007) investigated the impact of carbendazim, glyphosate and dimethoate on *E. fetida*where both found a significant reduction in the earthworm growth in a dose- dependent manner.

The results have showed that the mixed pesticide with heavy metals can lead to impacts on chronic response such as growth and reproduction. This finding came in agreement with Zhou *et al*.,(2011) who concluded that pesticides mixture was significantly more toxic to *E. andrei*than either pesticide alone consequently, the study of pesticide or pollutants mixtures is even more important in evaluating the ecological risk of pollutants on the ecosystem.

The works conducted so far seems to suggest that if a mixture consists of a few metals, each present in a relatively high concentration, they may have antagonistic or synergistic effects; however, if the mixture consists of many metals, each present in a low concentration, additivity of concentrations dominates. Additive effects of metals should be taken into account when assessing the risk of metal-contaminated field sites (Van Straalen, 1993).

Significant body weight reduction was observed in *E. fetida*when reared on Cadmium, , Salute (OP) and Round up (herbicide) or their Metal- Pesticide combination as compared to control worm. However, the overall body weight reported greater reduction after 8 weeks compared to the first 4 weeks reflecting that the longer the exposure time the greater the impact of the metals and pesticides or their combinations on the body weight.

In an earlier study Spurgeon and Hopkin (1996) reported lower average weights of *E.fetida*after 5 weeks to Cd and Zn contaminated soil. Furthermore, Drobne and Hopkin (1995) has reported that a number of soil invertebrates have reported a reduction in growth in animals exposed to metal contaminated diets.

The exposure of earthworms and other organisms to any pollutants- combination is known as interaction of pollutants and it is seldom that any living organism exposed to a single compound, furthermore exposure to combinations of pollutants will no doubt lead to manifestations of effects different from those that would expected from exposure to each pollutant separately Aebeed and Mohamed (2013), this was also noted the present study.

The combined effects will be synergistic, antagonistic or potentiative, depending on the chemicals and physiological conditions of the organism exposed.

This results approved that the herbicide Round up and Cd combination had an adverse effect on juvenile number of *E. fetida*, whereas the insecticide Salute and its metal combination did not show obvious negative effect on juvenile.

This result slightly contradicted with the finding of Awgie and Mohamed (2012) who reported juvenile reduction with Salute and Round up and an increasing number with the fungicide Rubajan.

In an early study, Alshawish and Mohamed(2002) have reported that several pesticides including chloropyrifos, methomyl, cymermethrin and dicofol (Insecticides) and Mancozeb a fungicide and Galant herbicide had resulted in moderate to high mortality to the earthworm *A. caliginosa*, whereas the long term toxicity of 10 weeks revealed that only the herbicide Galant and the carbamatemethomyl has resulted in body weight reduction.

A given pollutant can alter growth by both direct and indirect effect. Growth can be impaired by direct toxic effect on physiology of the exposed worms or by change in the energy budget as an individual attamps to prevent accumulation in sensitive tissues.

On the other hand Drobne and Hopkin (1995) have attributed that growth reduction in soil invertebrate exposed to metal or pesticide contaminated diets can be due to reduction in consumption or avoidance and have elaborated that the mechanism of metal elimination have metabolic costs for maintenance and repair and this increased requirement for repair mean requirement for energy and ultimately result in reduction in the available energy for growth.

The present results revealed that the herbicide Round-up in combination with cadmium had an effect on E. fetida body weight, in fact in the three mentioned treatments a greater body weight than that of control was reported which agree with(Awgie and Mohamed, 2012).

Earthworms can detoxify metals such as Cd and Zn by binding in granules or through metallothionine- like proteins in some tissues, however, the later case can have negative impact to the worm due to the possible inhibition to the metallthionine which considered as a vital scavenger for metals and other radicals.

The heavy metals essentially become contaminants in the soil environments because, (i) their rates of generation via manmade cycles are more rapid relative to natural ones,(ii) they become transferred from mines to random environmental locations where higher potentials of direct exposure occur, (iii) the concentrations of the metals in discarded products are relatively high compared to those in the receiving environment and (iv) the chemical form (species) in which a metal is found in the receiving environmental system may render it more bioavailable (D'amoreet al., 2005).

Testing of soil organisms like earthworm to mixtures of toxic substances not an easy task especially when this conducted in the laboratory that is because of different factors including the behavior of the toxic agent the deposition of toxic agent on the soil and the behavior and the retrial movement of the earthworm in addition to soil character and weather conditions However, it seems that above all of these uncertainties, laboratory studies of Toxic mixture on soil organism including earthworm could provide vital information on the possible effects of the pollutant to the soil environment regardless of the prevailing.

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