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Growth and Reproduction of the Earthworm After Exposure to Eisenia fetida Sub Lethal Concentration from Remilitine and Lead Mixture

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الملخص

تلعب ديدان الأرض دورًا مهمًا في مراقبة التلوث المعدني ومعترف بها على نطاق واسع في النظم البيئية. في هذه الدراسة دودة الأرض البالغة ايسيا فاتيدا تعرض إلى تربة ملوثة بالرصاص المعدني ومبيد الفطريات ريمالتين بتركيز شبه مميت (50 + 500 جزء في المليون) لمدة عشرة أسابيع لتقويم نمو جسم الدودة بعد 28 و49 و70 يوماً من التعرض، كانت معاملات التكاثر (إنتاج الشرنقة وعدد الأحداث لكل شرنقة) بعد 49 و70 يومًا من التعرض. أظهرت النتائج أن القيم المتوسطة لوزن جسم الدودة التي تعرضت لخليط الرصاص-ريميلين خلال 70 يومًا تقدر فرقًا معنويًا مقارنة بموقع التحكم حيث سجلت الديدان الضابطة وزنًا أعلى. علاوة على ذلك، أظهر اختبار تاء زيادة معنوية في عدد الأحداث في المجموعة الضابطة مقارنة بالديدان المعالجة .ومع ذلك، في نهاية التجربة التي استمرت 70 يومًا، لم يُبَلَّغْ عن اختلاف كبير في إنتاج الشرنقة يمكن أن يرجع ذلك إلى قدرتها على التحمل والتأقلم.

الكلمات المفتاحية: *ايسيا فاتيدا* ، تربة اصطناعية، رصاص، ريميلين، نمو ديدان الأرض.

Abstract

Earthworms play an important role in metal pollution monitoring and are widely recognized in terrestrial ecosystems. In the present study, adult *Eisenia fetida* (Saving) were exposed to soils contaminated with the metal lead and the fungicide Remiltine combination at a sub-lethal concentration (50+500 ppm) for ten weeks to evaluate the worm body growth after 28, 49 and 70 days of exposure, the reproductive parameters (cocoon production and the juvenile number per cocoon) were after 49 and 70 days of exposure. Results showed that the mean values of the worm's body weight exposed to the lead-Remiltine mixture over 70 days estimate a significant difference as compared to the control site ($F=6.06$ $P<0.05$) as the control worms reported higher weights. Furthermore, the t-test revealed a significantly higher juvenile number in the control compared to the treated worms ($F=13.43$ $P<0.05$). However, at the end of the experiment of 70 days, no significant difference was reported in cocoon production ($F = 0.06$ $P>0.05$).

Keywords: eisenia fetida, artificial soil, lead, remiltine, growth and annelida.

1. INTRODUCTION

Intensification of agricultural practices and especially the use of pesticides and metals often result in a loss of biodiversity[1]. The present study focuses on earthworms because they represent a large fraction of soil-living biomass in many temperate ecosystems and play an important role in soil, functioning as ecosystem engineers[2]. They influence organic matter dynamics, soil structure, and microbial community[3,4,5]. They actively participate in soil aeration, water infiltration and the mixture of soil horizons, representing an important source of food for many other organisms like birds[3,6]. Earthworms have sometimes been used as bioindicators for soil quality and the environmental impacts of cropping systems and pollutants[7,8]. Many studies for growth, mortality and/or reproduction of *Eisenia fetida* are currently used to assess the effects of pesticides under laboratory conditions before marketing authorization[9,10,11].

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Pesticides are no longer subject to any further evaluation by the national agencies that authorized their use. Yet in cultivated fields, non-target organisms, such as earthworms are exposed to frequent and different insecticides, fungicides and herbicide applications and because of the major role they play in soil functioning, the effects of pesticides on these soil organisms should be investigated further. Most published ecotoxicological research on earthworms has targeted metals[12] while the results of insecticides have been much less studied. To date, almost 400 materials of plant protection merchandise, additionally referred to as pesticides, are authorized in Europe and are inclusive of natural compounds and metals. In medical literature, maximum studies on the effects of insecticides on earthworms were carried out in the 1980s. Some more recently, however, focus on compounds that are now not authorized in Europe. This is the case with many studies on carbofuran[13,14], benomyl[15,16,17], carbaryl[18,19], dieldrin and n-dichlorodiphenyltrichloroethane[20]. Recently, reporters confirmed that 'older pesticides had more inhibitory results on earthworms than the more modern ones' and reported that 'newer pesticides are commonly less toxic to non-target organisms (e.g. earthworms) due to their exceptionally better selectiveness'[19, 21]. Others reported that the mixture of pesticide-heavy metals causes greater effects on endpoints such as growth and reproduction of earthworms *E.*

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fetida than the individual pesticides or metals alone. Consequently, it should be very important to evaluate pesticide-heavy metal mixtures on soil animals before their use even if they seem to be safe[22,23]. The objective of this study was to assess the effects of the heavy metal lead and the fungicide Remiltine on the adult growth and developmental stages of cocoons and juveniles of the earthworm *Eisenia fetida* as a model species.

2. MATERIALS AND METHODS:

European strain *E. fetida* was previously brought from the Czech Republic and reared in the zoology lab of the Faculty of Science, University of Benghazi, for several years since then. *E. fetida* was chosen for this study because of its reproductive potential as compared to the local species. Mature *E. fetida* were retained in a glass aquarium on a culture media as defined by the Organization for Economic Cooperation and Development[11] at room temperature of 20 ± 2°C. Food comprised of a synthetic soil was mixed with barley grains powder as a food supplement every seven days throughout the rearing period. Humidity settings of the soil were started at around 60% water-holding capacity. The humidity, then, was maintained by regularly sprinkling water on the soil. Mycological growing was detached when detected on the surface of the soil. Rearing soil was renewed every two months until the worms required for research were at a stable weight of 7 to 9 g/worm. Adult worms were exposed to the artificial soil that was contaminated with one concentration of lead + Remilitine (50 + 500 ppm). The artificial soil used OECD consisting 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 (12 cm W, 15 cm L, 20 cm H) with 100 mL of each of the lead+ Remilitine mixture. Each treatment was replicated three times and the control treatment with three replicates was set using plain water. Ten adult *E. fetida* were transferred into each test container after their initial body weight as a whole replicate was taken. The used dosages were designated on the bases of a primary trial test with the aim of finding prolonged effects rather than acute direct mortality. The extra body weight of *E. fetida* was again taken after 28, 49 and 70 days post-treatment**.** The factors measured in this research were: change in body weight, cocoon creation by *E. fetida* after 28 and 70 days post-treatment, and the number of youths produced from the cocoon after seventy days post-treatment**.** All data were subjected to SPSS, whereas a T-test was used for the mean differences.

3. RESULTS:

The change in body weight:

The mean values of body weight of worms exposed to the lead-Remiltine mixture (50+500 ppm) over 10 weeks revealed significant differences in body weight between the lead-Remiltine treated and control (F=6.06 P<0.05). Furthermore, there is a significant difference in the worm's body weight as pass (F=10.75 P<0.05). The mean ± S.D of the body weight revealed an increase in worms' weights over a long time, thus the mean ± S.D was 8.26g ± 0.40 8.13g ± 0.76 8.80g ± 0.65 and 9.90g ± 0.81 for the lead-Remiltine treated compared to 8.66g ± 0.73 8.56g ± 0.32 9.80g ± 0.60 and 10.93g ± 1.10 for the control at zero, 28 days, 49 and 70 days (Fig. 1).

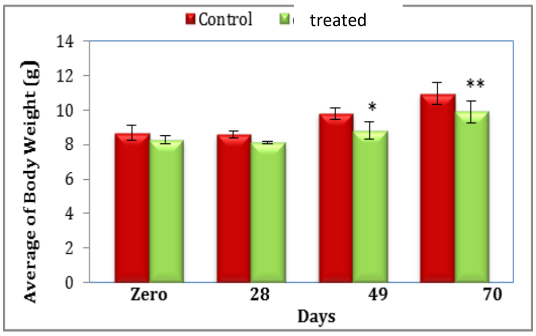

Fig. 1. The mean ± S.D. of the worms' body weight in grams in control and lead-Remiltine treatments (50 + 500 ppm). *Significantly different from controls at p<0.05, ** p<0.01.

Cocoon production:

The results showed a significant difference (F=10.32 P<0.05) after the first part of the experiment, the 28-day period, as the mean ± SD of cocoon produced by control worms was 37.00 ± 2.00 compared to 45.33 ± 18.71 of lead-Remilitine treated worms**.** On the other hand, at the end of the experiment after the cocoons were treated for 70 days, no significant difference was reported (F= 0.06 P>0.05) the mean ± SD of cocoons was 114.67 ± 15.88 for control compared to 128.00 ± 20.07 for lead + Remilitine treated which could be due to adaptability as shown in (fig. 2).

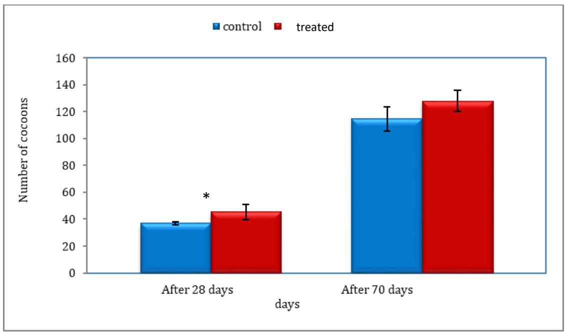

Fig. 2. The mean ±S.D. of the number of cocoons per worms in control and lead-Remiltine treated (50 + 500 ppm) soils, after 28 and 70 days' post-treatment. *Significantly different from controls at p<0.05.

Juvenile number:

The juvenile number produced by cocoons of the control worms and lead-Remilitine treated worms were further counted. The mean ± S.D of these juveniles is presented in (Fig. 3). The t-test revealed a high significant difference in juvenile numbers between treatments (F=13.43 P<0.01). The mean ± S.D of these mean ± S.D were 94.00 ± 35.53 for control juveniles and 84.00 ± 2.64 for lead-Remilitine at 70 days post-treatment.

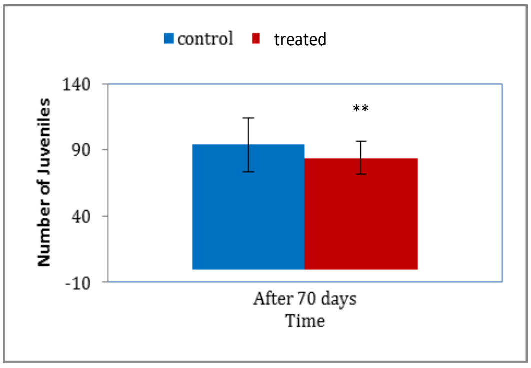

Fig. 3. The mean ±S.D. of number of juveniles per worms in control and lead- Remiltine treated 50 + 500 ppm) soils. ** Significantly different from controls at $p < 0.01$.

4. DISCUSSION:

Soil contamination is considered a global issue and an effort has been made to compare, and reduce pollution from the environment by chemical analyses. However, these are expensive and do not give a good picture of the toxicity of pollutants, showing approximately the toxicity of pollutants. These days, many scientists encourage using biota for assessing chemical compounds' availability in soil. The principal sorts of contaminants are especially pesticides and heavy metals in particular in agriculture, which arise as a single chemical in addition to in combinations. The growth in soil pollution levels in particular via heavy metals and pesticides has endangered human existence. Therefore, efforts had been made to reveal, reduce and get rid of pollution from soil. To obtain this objective, the present results clearly demonstrated that the lead nitrate mixed with the fungicide Remilitine in the soil had a moderating effect at sub-lethal concentration on the growth of *Eisenia fetida*. Some studies have been carried out on the effects of metals on the growth of earthworms, the results of which are comparable to those reported here. Scientists tested the effect of Cd, Cu, Pb, Ni and Zn on the growth of *Eisenia fetida* in laboratory cultures[24]. They concluded that cadmium was the most toxic metal, with significant decreases in growth at 50 µg/kg in dry soil. The lowest concentrations of other metals that have significantly retarded growth were 100 µg/kg for Cu and 2000 µg/kg for Zn, both as nitrate. Other researchers estimated an EC50 value for the effect of cadmium on the growth of *Eisenia andrei* of 96 µg/kg dry soil; the EC50 for copper was greater than 100 µg/kg[25]. The EC50 values that we found for the single metals are thus similar to data in the literature. Similar decreases in body weight of earthworm *Pheretima guillelmi* at high concentrations of lead in soil has been recorded by recent investigators[26]. Studies reported that the weight of the earthworms was a more sensitive index compared to the mortality indicating toxic effects of acetochlor and methamidophos[27]. Weight loss has also been reported for Organochlorine pesticide intoxication[28,29,30] and for the effects of fungicides and herbicides in *Eisenia fetida* and *Lumbricus terrestris*[30,31]. Researchers confirmed that sulfate significantly reduced the weight of juvenile *Aporrectodea trapezoides* within 5 weeks when applied to soil fenamiphos and cocoon and hatchling production32 viability of the worms produced[28,33,34,35] and their sexual maturation[36]. Cocoon production was found to be the most sensitive parameter for paraquat fentin, benomyl, phenmedipham, carbaryl, copper chloride, dieldrin[31,] while cocoon hatchability was the most sensitive for pentachlorophenol, parathion and carbendazim, copper chloride[31,37]. The numbers of juveniles per cocoon can be regarded as sensitive parameters to evaluate the toxicity of acetochlor on earthworms as reported by[38,32] and have also found that cocoon production in *Aporrectodea trapezoides* was inhibited by endosulfan and fenamiphos at normal application rates and methiocarb at 10x normal rate. The results of earthworm ecotoxicological tests may be confounded with different properties of soils such as organic matter, water holding capacity, pH, cation exchange capacity, carbon/nitrogen ratio, and clay content and its interaction with chemical substances and different species of earthworm chosen as test species[39,40]. A more recent study revealed that the survival of juvenile *Eisenia fetida* is more sensitive to copper-Remilitine contaminated soils than that of the mature worms[41]. This is in agreement with our current results, where the interaction of chromium-Remilitine mixture is antagonistic, whereas the toxicity of Remilitine alone is more severe on the body weight than chromium-Remilitine mixture[42].

5. CONCLUSIONS:

The mixture of lead-fungicide causes greater effects on the growth and reproduction of the earthworms *E. fetida* than the individual fungicide or lead. Consequently, it should be very important to evaluate pesticide heavy metal mixture on soil animals before their use even when the pesticide alone seems to be safe. In addition, the greater sensitivity of juvenile worms indicates the importance of considering effects on different life stages which requires conducting a risk assessment of the effects of pollutants in soils.

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