

The fetida Eisenia worms are poisoned by a combination of substance, pyrethroid pesticide, and cypermethrin.

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Abstract

The effects of a sub-lethal dose of a combination of cypermethrin and heavy metal on the growth, reproduction, and juvenile population of the epigeic earthworm *Eisenia fetida* were studied in laboratory settings using artificial soil glass containers with a pH of 5-6.5 and a temperature of $20 \pm 2^\circ\text{C}$. *E. fetida* subjected to 25+200 mg/L of ciprofloxacin plus copper, 25+200 mg/L of ciprofloxacin plus chromium, and 25+50 mg/L of ciprofloxacin plus lead. The findings showed that following 28, 49, and 70 days of exposure to *E. fetida*, all metals, including cypermethrin and heavy metals, had a mild-to-relatively-significant effect on body weight, cocoon production, and juvenile number. The utilisation of *E. fetida* as a biomarker for evaluating pesticide and metal pollution was also suggested by this innovative study.

KeyWords : *Soil invertebrate; Comparative toxicity; Sub-lethal dose; Metal interaction; Cocoon; Juveniles*

INTRODUCTION

The increased use of pesticides in the modern world has raised awareness of the potential for major environmental contaminations resulting from their use in soil. The use of these chemicals on soils reduces soil fertility, changes soil structures,

and upsets the equilibrium between the flora and fauna that live there, endangering living things. In order to identify the interaction behaviour of organic and metal toxins in the environment, which typically results from many environmental conditions leading to the opposite of their intended usage, laboratory and field experiments are conducted to examine the impacts of toxicity in both contexts.

1974 saw the first synthesis of it [1]. A synthetic substance called cyperkill is comparable to the pyrethrins found in pyrethrum extract, which is derived from the chrysanthemum plant. The purpose of pyrethroids, such as cyperkill, is to outlast pyrethrins in terms of effectiveness [1]. Because cyperkill is extremely poisonous to fish in addition to bees, cockroaches, and other insects, its impact on animals is clearly visible. In laboratory testing animals, cyperkill elicited a nervous system response that resulted in restlessness, impaired coordination, prostration, and paralysis.

Mice given 0.3 to 4.3 mg/g of cyperkill showed signs such as convulsions, salivation, and writhing [3]. Similar symptoms, such as tremors, convulsions, writhing, salivation, and burrowing behaviour, were displayed by rats exposed to cyperkill [4].

Rats who were newborns were more susceptible to cyperkill than adult rats because their livers lacked fully formed enzymes that break down cyperkill [5]. Individuals who handle or deal with pyrethroids and pyrethrins, such as cyperkill, can experience burning, tingling, itching, and dizziness. Increasing agricultural productivity to feed the growing population has necessitated the use of many insecticides to protect crops against insect pests. However, the careless use of these herbicides in agricultural areas has given rise to a serious environmental issue [6-8]. Numerous non-target organisms are known to be poisonous to insecticides, and they are also known to cause major sub [9-11] increases and decreases in growth rate and reproductive potential [12-14].

A pyrethroid insecticide called cypermethrin is used to suppress insect pests like Cox [15] by interfering with the nervous system's normal operation [16]. Pyrethroids are effective at low rates and

reasonably priced, despite evidence that they are detrimental to beneficial insects [17]. But the impact of pyrethroids on earthworms—a significant group—hasn't been thoroughly investigated. Usmani [18] studied the effects of cypermethrin, one of the main pyrethroid pesticides currently used globally to combat insect pests, on a non-fetida, an epigeic earthworm that is well-known for its effectiveness in recycling nutrients and is widely utilised to produce vermicompost worldwide. Although it is one of the main soil contaminants, little is known about how harmful chromium is to soil organisms.

Therefore, copper, chromium, and lead were selected to be employed in this study from among the several metals that pollute terrestrial ecosystems. There is not as much research on chromium's toxicity in soil organisms. Even less is affected by the insecticide and heavy metal combination on the soil fauna. A well-researched model for heavy metal toxicity is the earthworm. The uptake and buildup of metals in earthworms has been the subject of numerous literary works. Many of them measured the amount of metal present, growth, the density of worms (Pizal, 19), the rate of accumulation (Vijver, 20), and the rate of excretion (Lock, 21). Two species of earthworms, the widely distributed *Aporrectodea Caliginosa* and the European Species *E. fetida*, are well known for their abilities to test soil contaminants, such as pesticides and heavy metals. Nonetheless, a number of studies on these latter species were carried out in the zoology lab and published in a number of journals. However, *E. fetida* saving, 1826 was chosen for this study because it is a common test organism in terrestrial ecotoxicology and can be readily bred on a variety of organic wastes with a short generation time. As a result, various endpoints, including mortality, changes in body weight, the number of cocoons, and fecundity, can be observed quickly. *E. fetida* was also selected for this investigation due to its well-documented development and reproduction [22, 23] and its status as a suitable model species [24]. This study aims to further evaluate the effects of soil contaminants on the soil animals denoted by *E. fetida*, which attempts to evaluate the impacts of sublethal concentrations of soil contaminants at several endpoints. These evaluations include the effects of metals and pesticides on worm growth and reproduction as well as the toxicity of mixtures containing pesticides and metals.

MATERIALS AND METHODS

As a research sample, the European strain of *E. fetida* was

imported from the Czech Republic and raised in the zoology lab for over two years Wu [25]. Because *E. fetida* has a higher capacity for reproduction than the native species, it was selected for this study. The Organisation for Economic Cooperation and Development (OECD) [26] states that *E. fetida* was kept in glass aquariums with culture media at a temperature of $20 \pm 2^\circ\text{C}$. For the duration of the trial, the food was a synthetic soil supplemented once a week with powdered barley grains. The rearing soil's moisture content was initially set at about 60% of its water-holding capability.

After that, the soil was consistently kept moist by being sprayed with water. When fungal growth was found on the soil's surface, it was eliminated. Every eight weeks, the rearing soil was replaced until the worms needed for the experiment weighed an average of seven to nine grammes. In this study, adult worms were subjected to artificial soil contaminated at 50+25 ppm, 200+25 ppm, and 200+25 ppm concentrations of lead+Cyperkill, copper+Cyperkill, and chrome+Cyperkill, respectively. In order to achieve a pH of 5-6.5, the artificial soil utilised by OECD [26] was composed of 70% quartz sand, 20% kaolin clay, 10% sphagnum peat, and calcium carbonate. 250 grammes of soil were put into glass containers measuring 12 cm W, 15 cm L, and 20 cm H. 100 millilitres of lead+Cyperkill, copper+Cyperkill, and chromium+Cyperkill were added to each container and properly mixed. Every treatment was repeated three times, and ordinary water was used for the control treatment, which also had three repetitions. After their initial body weight as a whole replicate was taken, ten mature *E. fetida* were subsequently put into each test container by KERN and Sohn GmbH.

The doses that were employed were chosen based more on the discovery of long-lasting effects than a cute direct mortality, after a preliminary trail test. The body weight of the worms was measured once again 28, 49, and 70 days following treatment. Every test container had five grammes of barely grain powder put on top as food, supplement, and soil moisture content. Five millilitres of water were supplied as needed. These measurements were taken once a week. In this experiment, the following parameters were measured: Worm body weight fluctuation. Worms produce cocoons 28, and 70 days following treatment. and the quantity of young that emerged from the cocoon 70 days following treatment. All of the data were run through SPSS, and the significant differences were found using an ANOVA, and the mean differences were found using a T-test.

Results

Cypermethrin+Copper: The mean body weight of worms treated for ten weeks to the 200+25 ppm Cypermethrin+Copper mixture (Table 1). The findings indicate a noteworthy dissimilarity in body weight between the Cypermethrin+Copper treated group and the control group ($F=30.78$, $P<0.05$). Additionally, there is a substantial variation in the worms' body weight as a function of time ($F=16.79$, $P<0.05$). The body weight mean $\pm$ standard deviation shows that the worms' weight increased overtime; thus, the mean $\pm$ standard deviation for the Cypermethrin+Copper treated group was 7.50 ± 0.36 , 7.36 ± 0.41 , 8.53 ± 0.40 , and 9.20 ± 0.34 , while the control group's values were 8.66 ± 0.73 , 8.56 ± 0.32 , 9.80 ± 0.60 , and 10.93 ± 1.10 at zero, 28 days, 49 days, and 70 days. **Cypermethrin+Chromium:** The mean body weight of worms treated for ten weeks to the 200+25 ppm mixture of Cypermethrin and Chromium (Table 2). The findings indicate a noteworthy variation in body weight between the control group and the Cypermethrin+chromium treatment group ($F=18.54$, $P<0.05$). Additionally, there is a significant difference in the worms' body weight over time ($F=9.85$, $P<0.05$). The mean $\pm$ S.D. of the body weight shows that the worms' weight increases over time; thus, the mean $\pm$ S.D. for the Cypermethrin+chromium treated group was 7.56 ± 0.60 , 7.63 ± 0.51 , 8.63 ± 0.90 , and 9.06 ± 0.70 , while the control group at zero, 28 days, 49 days, and 70 days had 8.66 ± 0.73 , 8.56 ± 0.32 , 9.80 ± 0.60 , and 10.93 ± 1.10 , respectively.

DISCUSSION

The combined application of cypermethrin and heavy metal did not significantly affect earthworm mortality in comparison to their individual applications. None of the worms in the heavy metal mixture with cypermethrin showed any signs of death. The environmental parameters of temperature and moisture were consistently maintained at $20 \pm 2^\circ\text{C}$ and 60–70%, respectively. The food was supplied in accordance with the findings of [26,27], which documented the low toxicity of cypermethrin to earthworms and clarified that this non-toxicity may stem from the fact that pyrethroids are adsorbed onto the organic matter of soil particles, thereby rendering a portion of the dose inaccessible to the worms. According to Ingesfield [27], the LC50 figure was significantly higher than the compound's maximum advised agronomic dose, indicating that cypermethrin would not. This study shows that the combination application of copper

and cyperkill has clearly reduced the quantity of juveniles as well as their body weight. Because of its toxicity, earthworm populations may be negatively impacted, endangering the regular operation of soil ecosystems. The outcomes additionally demonstrated that the combination of pesticide and heavy metal may have an effect on long-term responses like development and reproduction. This result was consistent with that of Zhou [34], who found that the combination of pesticides was far more harmful to *E. Andrei* than any pesticide used alone. The findings also demonstrated that the combination of pesticides and heavy metals in earthworms can have a bigger effect than either substance alone.

Because of how pesticide-heavy metal mixtures affect earthworm reproduction, the consequences of contamination can persist for multiple generations, resulting in a marked drop and reduction in genetic diversity, which may in turn pose problems for the soil ecosystem's ability to operate. Therefore, before applying to sensitive areas, the ecological danger of combined pesticides or heavy metal-pesticide mixtures on soil organisms should be thoroughly investigated. The findings of this test corroborate the findings of Zhou [36] and the effect of cyperkill on the growth of the earthworm *E. fetida* reported by Mosleh [35]. These long-term toxicity tests' findings showed that cyperkill may have detrimental effects on both adult and paediatric juvenile earthworms, albeit in their developmental stage, juveniles are more sensitive. Zaltauskaite's discovery appears to be supported by the evaluation of the effects of lead metal on worm growth [29]. Although additional investigations [30,31] found a statistically negligible drop in body weight after 70 days of metal exposure, the link between lead and body weight remained negative. The earthworm *E. fetida* was used in an experiment to test the effects of Cd, Cu, Pb, Ni, and Zn on the growth of *E. fetida* in laboratory cultures. The results showed that copper at 200 ppm affected body weight relative to the control, whereas chrome at 200 ppm resulted in a reduction of body weight. Cadmium was found to be the most toxic metal, with significant. Our findings showed that the combination of copper, lead, and chromatic with pesticides had a somewhat less than additive effect on the growth, cocoon production, and juvenile population of *E. fetida*. The results, which are consistent with those published, showed unequivocally that the addition of heavy metal to artificial soil had a detrimental effect on the growth of *E. fetida*. The effects of pollutant mixtures in soil, such as pesticide-pesticide or pesticide-heavy metals, were either

underappreciated for a very long time or their effects were not thoroughly studied. More attention has, however, recently focused on potential connections between soil organisms and the soil itself, as well as the potential harm to the soil ecosystem that results from these interactions.

According to Zhou [34], the use of toxicity data from a single pesticide trial may understate the ecological risk of pesticides that are actually present in the soil due to the increase in toxicity mixture. Therefore, in order to assess the ecological danger that pollutants pose to the environment, it is even more crucial to research pesticide or pollutant mixtures. The hazardous effects of pesticides and/or heavy metals on a variety of soil creatures, including woodlice, *Porcellio scaber*, *Procladius laevis*, *Hemilipistis reumori*, *Armadillo officinalis*, and the earthworm *Aporrectodea caliginosa*, were studied in some detail in the Libyan instance.

Nevertheless, the consequences of this combination of contaminants have not yet been investigated. As such, it appears crucial to investigate the effects of single or combined pesticide-pesticide and pesticide-heavy combination of metals. In addition to focusing on the agrosystem of Libyan soil, particularly in its eastern portions, the aforementioned species are also being studied, along with the possibility of other soil animals and the inclusion of most pesticides that have not yet been tested in the study. Cypermethrin at concentrations starting at 15 µg soil, yet no dose-related reaction was observed within the studied concentration range. According to reports, growth can be used as a sensitive metric to assess an insecticide's toxicity to earthworms [37, 38]. Loss of weight could be a sign of feeding inhibition, which lowers consumption rate and influences growth rate. The results of the individual toxicity tests conducted on earthworms indicate that natural soil contains considerable amounts of Cd and Cr. The precise interactions between coexisting heavy metals in the environment are crucial to determine as these interactions have a substantial impact on the metals' bioaccumulation processes in living things as well as their toxicological consequences at various biological levels.

CONCLUSION

Therefore, the current study concludes that the test specimen's ability to reproduce is greatly impacted by sub-lethal doses of cypermethrin, and its widespread use in agro-ecosystems needs to be closely monitored. Additionally, the growth and reproductive parameters of earthworms exposed to agricultural

pesticides were shown in this study to be potentially helpful bio-indicators of soil contamination.

Compared to the individual pesticides or metals, the combination of pesticides and heavy metals has a higher impact on endpoints such as earthworm growth and reproduction (*E. fetida*). Therefore, even while each pesticide appears to be safe on its own, it should be crucial to test pesticide-heavy metal mixtures on soil animals prior to their application. Additionally, this study demonstrated the serious consequences that can arise from the improper and excessive use of hazardous metals and organic pesticides, either alone or in combination, for people and the environment, particularly the soil fauna.

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