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Ecotoxicological impact of three pesticides on *Eisenia fetida* (californian red worm) on *Allium cepa* (onion) crop in the district of Lurín, Lima, Peru

Impacto Ecotoxicológico de tres plaguicidas sobre *Eisenia fetida* (lombriz roja californiana) en el cultivo de *Allium cepa* (cebolla) en el distrito de Lurín, Lima, Perú

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Abstract

The research evaluated the impact of three pesticides on *Eisenia fetida* in the cultivation of *Allium cepa* (onion) in the district of Lurín, Lima, Peru. The mean lethal dose values (LD_{50}) at 14 days, evasion percentage and food activity were evaluated as well as the exposure ratio of the toxic (TER) and the environmental risk coefficient (RQ) on pesticides zeta-cypermethrin, pendimethalin and profenofos based on ecotoxicological tests with *E. fetida*. The LD_{50} values at an exposure time at 14 d for the three pesticides were: zeta-cypermethrin>profenofos>pendimethalin. A relationship with the dose of pesticides close to the LD_{50} was calculated for the evasion and for food activity. TER value for the three pesticides was calculated which indicated no ecotoxicological impact for *E. fetida*. However, in the CR value, there were high risk values in the total, where zeta-cypermethrin indicated 92.77% of the environmental risk, while pendimethalin presented the lowest environmental risk with 0.09%. Within Peruvian legislation, these pesticides are still allowed to be used; hence, based on this research, it is suggested that zeta-cypermethrin be evaluated in more detail, because it was the only pesticide that presented a significant CR. In conclusion, zeta-cypermethrin causes an environmental impact on the onion crop in Lurin.

Keywords: Environmental risk, *Eisenia fetida*, DL_{50}, TER, RQ, Zeta-cipermetrin.

Resumen

La investigación evaluó el impacto de tres plaguicidas sobre *Eisenia fetida* en el cultivo de *Allium cepa* (cebolla) en el distrito de Lurín, Lima, Perú. Se calcularon los valores de dosis letal media (DL_{50}) a los 14 días, porcentaje de evasión y actividad alimentaria, y posteriormente la proporción de exposición del tóxico (TER) y el coeficiente de riesgo ambiental (CR) para los plaguicidas zeta-cipermetrina, pendimetalina y profenofos en base a ensayos ecotoxicológicos con *E. fetida*. Los valores de DL_{50} a un tiempo de exposición a los 14 días para los tres plaguicidas fueron: zeta-cipermetrina >profenofos >pendimetalina. Se calculó una relación con la dosis de los plaguicidas cercanos a la DL_{50} para la evasión y para la actividad alimentaria. El valor TER para los tres plaguicidas fue calculado e indicó que no hay impacto ecotoxicológico para *E. fetida*. Sin embargo, en el valor de CR se presentaron valores de riesgo alto en el total, donde la zeta-cipermetrina indicó el 92,77 % del riesgo ambiental, mientras que la pendimetalina presentó el menor riesgo ambiental con 0,09 %. Dentro de la legislación peruana, estos plaguicidas siguen siendo permitidos, por lo cual, en base a esta investigación, se recomienda que se evalúe a más detalle la zeta-cipermetrina, debido a que fue el único plaguicida que presentó un CR significativo. En conclusión, la zeta-cipermetrina ocasiona impacto ambiental en el cultivo de cebolla del distrito de Lurín.

Palabras clave: Riesgo ambiental, *Eisenia fetida*, DL_{50}, TER, CR, Zeta-cipermetrina.

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1 Introduction

There is interest in controlling insects and other disease vectors, as happens with the threat to food and other agricultural products (Ferrer, 2003). FAO (2003) states that "pesticides are any element or combination intended to prevent, suppress or manage any pest, including vectors that affect humans or animals". In Peru, until 2017, 428 active ingredients were reported to the National Agricultural Health Service (SENASA) (Cruz, 2017). The Law N° 0020-2013-AG-SENASA-DIAIA, presents a list of pesticides registered, canceled and prohibited in the country, including those studied in this work.

The pyrethroid group has been used since the 70s for agricultural activities, including zeta-cypermethrin, and are used for agricultural activities (Lao et al., 2012). Pendimethalin is an herbicide from the dinitroanilines family used in pre-planting, pre-emergence of grass and early post-emergence for crops (Ponz, 2010). Profenofos is a pesticide belonging to the group of phosphorus that operates with the contact (Reddy and Rao, 2008). The species *Eisenia fetida* (known as California red worms) is one of the organisms that plays a beneficial role to the soil. Giménez et al. (2004) highlight the importance of *E. fetida* in the integration and putrefaction of organic matter, the recycling of nutrients and soil conservation, being beneficial indicators for assessing soil pollution (Wen et al., 2020).

The district of Lurín, Lima, Peru, has very fertile soils that provide food to the markets of Metropolitan Lima. Moreno and Huerse (2010), mention that onion, from the point of view of its gross economic value contributed to the basin, is an important crop and it must be considered when conducting a phytosanitary evaluation. To determine the impact of the three pesticides, it is important to link *E. fetida* responses, using relevant toxicity parameters such as biological and behavioral activity. The bioassays employ laboratory-acclimatized, guidance organisms, and these include an initial view for pesticide impact assessment (Piola, 2011; Tian et al., 2018; Sotelo-Vásquez and Iannacone, 2019; Aparecida-Giordani et al., 2020).

For this reason, the aim of this research is to evaluate the impact of the three most commonly used pesticides on *A. cepa* culture, and ecotoxicological tests were conducted to find DL_{50} (mean lethal dose), CR (risk coefficient) and TER (individual toxic exposure) used in *E. fetida*.

2 Materials and methods

2.1 *Eisenia fetida*

Adult earthworms (*E. fetida*) were obtained from the National Agrarian University La Molina (UNALM) and transferred to the Laboratory of Animal Ecology and Biodiversity (LEBA) of the National University Federico Villarreal (UNFV), Lima, Peru. The adaptation was carried out in a 15 L container at 19 $\pm$ 2$^{\circ}C$ and a period of 16 h of light and 8 h of darkness. For the worm tests, a weight range of 200-600 mg was considered (Organization for Economic Co-operation and Development, 1984).

2.2 Artificial Soil

Artificial soil was prepared according to Organization for Economic Co-operation and Development (2004) standard with some modifications described in Romero and Cantú (2008). The components of artificial soil were: 70% fine sand, 20% clay and 10% moss. Prior to the beginning of the tests, the moisture content was adjusted to 50% using the Avalos-Ruiz and Iannacone (2020) method.

2.3 Pesticides

Pesticides used were zeta-cypermethrin from Furia brand with a formulation of 180 $g \cdot L^{-1}$ with concentrations of 30 $mg \cdot kg^{-1}$, 60 $mg \cdot kg^{-1}$, 120 $mg \cdot kg^{-1}$, 240 $mg \cdot kg^{-1}$ and 480 $mg \cdot kg^{-1}$; profenofos from the Selecrom brand with a formulation of 500 $g \cdot L^{-1}$ with concentrations of 625 $mg \cdot kg^{-1}$, 1 250 $mg \cdot kg^{-1}$, 2 500 $mg \cdot kg^{-1}$, 5 000 $mg \cdot kg^{-1}$ and 10 000 $mg \cdot kg^{-1}$; and pendimethalin from the Arrow brand with a formulation of 400 $g \cdot L^{-1}$ with concentrations of 2 666, 67 $mg \cdot kg^{-1}$, 5 333.33 $mg \cdot kg^{-1}$,10 667.67 $mg \cdot kg^{-1}$, 21 333.33 $mg \cdot kg^{-1}$ and 42 667.67 $mg \cdot kg^{-1}$.

2.4 Ecotoxicity tests

2.4.1 Mortality and other parameters

All tests were performed with four replications and adjusted to 50% of humidity for the tests. To validate the test, it was considered as a principle that the control mortality does not exceed 10 %. The protocol of Avalos-Ruiz and Iannacone (2020) was used for this test, which consisted of measuring physical and chemical indicators such as temperature, humidity, pH and organic matter, as well as mortality for the determination of DL_{50} at 14 days of exposure, and average weights at the beginning and end of the test at 14 days (Hulbert et al., 2020; Avalos-Ruiz and Iannacone, 2020). All worms that were not found at the moment were recorded as dead; it was decided to measure their progeny (eggs and young offspring) present in artificial soil, but none of them was evident in the treatments of the three pesticides.

2.4.2 Evasion

This test used the protocol of García-Santos and Keller-Forrer (2011). Transparent, disposable rectangular 1000 mL flexo-lid containers were used where the treated floor was placed on one side and the control was placed on the other. Later, adult worms were placed on the dividing line of both soils and allowed to penetrate them (McGuirk et al., 2020). After three days, the partitions were reinserted and the number of worms on each side was counted. Zeta-cypermethrin doses of a formulation of 180 $g \cdot L^{-1}$ were 15 $mg \cdot kg^{-1}$ and 30 $mg \cdot kg^{-1}$, for profenofos of a formulation of 50 $g \cdot L^{-1}$ were 312.5 $mg \cdot kg^{-1}$ and 625 $mg \cdot kg^{-1}$, and pendimethalin of a formulation of 400 $g \cdot L^{-1}$ with doses of 1 333.33 $mg \cdot kg^{-1}$ and 2 666.67 $mg \cdot kg^{-1}$. The Equation 1 from De Silva and Van Gestel (2009) was used to determine evasion.

$$NR(E) = \frac{C - T}{N} \times 100 \tag{1}$$

NR represents evasion (percentage), *C* represents total *E. fetida* in the control soil, *T* represents total *E. fetida* in the impacted soil, and *N* represents total *E. fetida* at the beginning of the test (Alves et al., 2013).

2.4.3 Food Activity (AA)

Acrylic sheets of 100 mm long, 10 mm wide and 0.1 cm thick were used to evaluate food activity. These sheets had 16 holes of 0.1 cm of diameter and were filled with a bait substance using a combination consisting of cellulose carbohydrate (69 %), wheat bran (30 %) and active carbon (1 %) (Van Gestel and Weeks, 2004). The trial was performed for three days, then leaves were removed, and the number of holes without bait in the leaves of each treatment were counted. AA was determined on the basis of the average proportion of holes that were completely or partially empty per concentration (Piola, 2011).

2.5 Statistical Data Management

DL_{50s}, DL_{50s}-inferior and DL_{50s}-superior were determined with Excel-Probit-2016 calculator (Raj Mekapogu, 2016). SPSS 25.0 statistical program was used to define the homocesticity of the variances, the normality of the data and the type of tests, like ANOVA or non-parametric tests, depending on the case, at a significance level of 0.05. A Chi-square test (χ^2). was performed for the evasion and food activity tests.

2.6 Environmental Impact Assessment (EIA)

To determine environmental impact assessment (EIA), parameters were identified to find potential threats to *E. fetida* and the agroecosystem. Two approaches to environmental impact assessment were used; the first was based on a calculation of TER for *E. fetida*, while the second CR for each local sample (Pivato et al., 2017; Wee and Aris, 2017; Avalos-Ruiz and Iannacone, 2020).

2.6.1 Exhibition

The data collected from the literature was analyzed. The controlled environmental dose (MECs) of pesticide residue in agricultural soil was used to represent the PECs (projected environmental dose in soil) (Vašíčková et al., 2019).

2.6.2 Impact

Environmental impact was evaluated using data found from DL_{50} and PNEC (dose without ecological effect). The PNEC value was found using a factor of 1 000 for short-term assays. For pesticides with a Kow greater than 2, the value of its DL_{50} was divided by 2 as proposed in (European Commission, 2019) based on the Equation 2.

$$PNEC = \frac{DL_{50}}{1000} \tag{2}$$

2.6.3 Risk

The environmental risk for each of the three pesticides was initially found using the criteria based on TER values. According to the European Commission mentioned by Hartnik et al. (2008), if the value of acute TER is less than 10, no authorization should be given for the use of pesticides. The acute TER value was found with the Equation 3.

$$TER_{species} = \frac{DL_{50}/PNEC_{\text{species}}}{MEC_{\text{max or mean}}} \tag{3}$$

The CR was classified using the equation 4 into four levels: 1. Zero risk ($CR < 0,01$), 2. Low risk ($0,01 \geq 0,1$) 3. Medium risk ($0,1 \leq CR < 1$) and high risk ($CR \geq 1$) Sánchez-Bayo et al. (2002). The sum of the CR of the three pesticides allowed to determine the total environmental risk from the application of zeta-cypermethrin, profenofos and pendimethalin; finally, the contribution of each of the three pesticides was quantified.

$$CR = \frac{MEC_{soil}}{PNEC_{mss}} \tag{4}$$

3 Results

The acute toxicity of zeta-cypermethrin on *E. fetida* was known at 14 days of exposure (Table 1). The DL_{50} at 14 d of exposure was 48.26 $mg \cdot kg^{-1}$. Regarding the average of weights, a marked decrease was observed when the dose was increased. Acute toxicity of profenofos on *E. fetida* was established at 14 days of exposure (Table 2). DL_{50} at 14 d of exposure was 1250 $mg \cdot kg^{-1}$. Regarding the average of weights, a significant decrease was observed.

Acute toxicity of pendimethalin on *E. fetida* was found at 14 d of exposure (Table 3). The DL_{50} at 14 d of exposure was 3 771.23 $mg \cdot kg^{-1}$. Regarding the average of weights, a significant decrease was observed. Table 4 shows the evasion percentage of the three pesticides at 60 %, 40 % and 80 % in the doses of 30 $mg \cdot kg^{-1}$, 625 $mg \cdot kg^{-1}$ and 2 666.67 $mg \cdot kg^{-1}$, respectively. The Chi-square analysis (χ^2) indicates that a connection was found between evasion and the presence of the pesticide for both doses of zeta-cypermethrin, profenofos and pendimethalin, excepting the dose 312.5 $mg \cdot kg^{-1}$ of profenofos and 1 333.33 mg of pendimethalin.

The data found in this test (Table 5) show that the percentage of pesticide food activity at 3 d of exposure has values of 33.33 %; 29.12 % and 32.66 % in dose concentrations of 30 $mg \cdot kg^{-1}$, 625 $mg \cdot kg^{-1}$ and 2 666.67 $mg \cdot kg^{-1}$, respectively. The Chi-square analysis (χ^2) indicates that there is a connection between food activity and the presence of the pesticide for zeta-cypermethrin, profenofos and pendimethalin at the doses already mentioned. TER values (Table 6) were obtained, which were 482.6, 12 500 and 377 123 $mg \cdot kg^{-1}$. On the other hand, CRs of each pesticide were 1.04, 0.08 and 0.001. It is shown that CR t is higher than 1, representing a high environmental impact. The contribution of zeta-cypermethrin was 92.77 %, followed by profenofos with 7.14 % and pendimethalin with 0.09 %.

4 Discussion

4.1 Acute Ecotoxicity and Other Important Parameters

E. fetida study showed effects on mortality and weight reduction for zeta-cypermethrin (DL_{50} = 48.26 $mg \cdot kg^{-1}$). Similar results were found in the Czech Pesticide Database (PPDB, 2019) showing a DL_{50} value of 37.50 $mg \cdot kg^{-1}$. Junquera (2011) mentions that cypermethrin is also known as zeta-cypermethrin. It allows to be contrasted with other studies such as Hartnik et al. (2008) who obtained an alpha-cypermethrin value of 762 $mg \cdot kg^{-1}$. Zhou et al. (2008, 2011) showed values of 84.14 $mg \cdot kg^{-1}$ and 86.04 $mg \cdot kg^{-1}$, respectively.

Organic matter (OM) has the characteristic of accumulating zeta-cypermethrin, and earthworms make it more available through its cuticle or during feeding (Styrishave et al., 2010). Based on this, the amount of organic matter in the study substrate was initially found at 5.42 %, so there is not much absorption of the contaminant. This fact also occurs in the study of Hartnik et al. (2008), since its soil presented 2.2 % of organic matter where the compound was available in its mineral particles and/or water; therefore, there is a higher risk of the pesticide to be present in worms. Pesticides can be retained by organic matter of the soil and may be degraded by organisms found in the organic matter, which will depend not only on the properties of pesticides, but also on the nature and concentration of pesticides in the organic matter (Araneda et al., 2016).

Table 1. Mortality and average weights of *Eisenia fetida* exposed to zeta-cypermethrin at 14 d of exposure.

Eisenia fetida			
Doses (mg·kg^{-1})	**Acute ecotoxicological effect of zeta-cypermethrin (% M)** 14 d (± DE)	**Average Weights (g)**	
		0 d ± DE)	14 d (± DE)
Control	0.00 (± 0.00)a	2.89 (± 0.06)a	3.18 (± 0.05)a
30	20.00 (± 0.71)b	2.55 (± 0.07)b	1.72 (± 0.15)b
60	65.00 (± 1.25)c	2.42 (± 0.06)b	1.10 (± 0.17)c
120	100.00 (± 0.00)d	2.51 (± 0.07)b	0.00 (± 0.00)d
240	100.00 (± 0.00)d	2.40 (± 0.04)b	0.00 (± 0.00)d
480	100.00 (± 0.00)d	2.31 (± 0.04)b	0.00 (± 0.00)d
DL_{50}	48.26	N.C	N.C
DL_{50}-low	35.70	N.C	N.C
DL_{50}-upper	65.24	N.C	N.C
PNEC	0.04826	N.C	N.C
KW	22.78	14.6	22.34
Sig	0.00	0.01	0.00
Levene test	6.4	0.41	8.07
Sig	0.00	0.84	0.00
SW	0.76	0.94	0.79
Sig	0.00	0.14	0.00

% M: % mortality. DE: Standard Deviation. NC: Not applicable. DL_{50}: Mean lethal dose. DL_{50}-Low: DL_{50} (Low Limit). DL_{50}-Upper: DL_{50} (Upper Limit).
PNEC: Dose without ecological effect. KW: Kruskall–Wallis statistician. Levene test: Statistical to evaluate homocesticity of variances.
SW: Shapiro–Wilks Statistician: Test to evaluate normality.
Equal lowercase letters in the same column indicate that mortality (%) is statistically similar according to Tukey's multiple comparison test.

Other variables that determine the presence of zeta-cypermethrin are water solubility and octanol/water distribution coefficient (Kow). Both variables correlate significantly with the mobility of insecticides in the soil (Somasundaram et al., 1991). Zeta-cypermethrin has a Kow log of 5.5, which indicates that it is relatively lipophilic, and therefore its infiltration potential is low in agricultural soils due to its low solubility in water and adsorption to the soil (Sakata et al., 1986). This was evidenced in the work of Hulbert et al. (2020), where a higher number of alive *E. fetida* was found on the surface compared to a greater depth.

According to Wang et al. (2012), the reduced toxicity of alpha-cypermethrin is due to its accelerated metabolic process, because it can be metabolized prior to reaching the central nervous system. There was clear evasion so that this dose increases as in the food activity. This behavior is observed by the presence of chemoreceptors of *E. fetida* on its body surface (Zhou et al., 2007). On the other hand, profenofos obtained a DL_{50} mortality of 1 250 $mg \cdot kg^{-1}$ and weight reduction 14 days after the test. Other investigations such as Bart et al. (2018) showed values such as 127 $mg \cdot kg^{-1}$. Harnpicharnchai et al. (2013) reports that the average value of profenofos in soil is 0.041 $mg \cdot kg^{-1}$ in summer, while it is 0.016 $mg \cdot kg^{-1}$ in winter.

Regarding soil evasion with profenofos, it was observed that there was only a relation with the dose of 625 $mg \cdot kg^{-1}$. Chakra and Rao (2008) mention that it may be because earthworms are affected by pesticides through skin contact or through contaminated soil residues. Mainly because these toxins pass through the skin and reach the celomic fluid to be transported by the body.

Organic matter is essential for the feeding of worms. In the study conducted by Gómez et al. (1999), the influence of profenofos was investigated in *Azospirillum brasilense* cells, and they found that it significantly reduced nitrogen fixation in the soil, which may be related to the amount of organic matter available and which may affect food activity as observed at 625 $mg \cdot kg^{-1}$, where food activity was dose-related. A decrease in weight was observed with pendimethalin with respect to the increase in pesticide concentrations and a DL_{50} of 3 771.23

$mg \cdot kg^{-1}$. In the same database (PPDB, 2019) a value of $DL_{50} > 1000\ mg \cdot kg^{-1}$ was found. In the study by Mosqueda et al. (2019) it is mentioned that the permitted range of worms is 3 545.96 $mg \cdot kg^{-1}$ for the pesticide Prowl H_2O, being close to the value obtained in this study as well as the one of Traoré et al. (2018) with a value of 3 555.96 $mg \cdot kg^{-1}$.

Table 2. Mortality and average weights of Eisenia fetida exposed to profenofos at 14 d exposure.

Eisenia fetida			
Doses (mg·kg^{-1})	**Acute ecotoxicological effect of profenophos (% M)** 14 d (± DE)	**Average Weights (g)**	
		0 d ± DE)	14 d (± DE)
Control	0.00 (± 0.00)a	2.89 (± 0.06)a	3.18 (± 0.05)a
625	25.00 (± 1.56)b	2.22 (± 0.09)b	1.61 (± 0.39)ab
1250	50.00 (± 1.83)c	2.23 (± 0.04)b	1.17 (± 0.47)b
2500	100.00 (± 0.00)d	2.19 (± 0.08)b	0.00 (± 0.00)c
5000	100.00 (± 0.00)d	2.27 (± 0.08)b	0.00 (± 0.00)c
10000	100.00 (± 0.00)d	2.13 (± 0.05)b	0.00 (± 0.00)c
DL_{50}	1250	N.C	N.C
DL_{50}-low	810.30	N.C	N.C
DL_{50}-upper	1928.30	N.C	N.C
PNEC	0.8103	N.C	N.C
KW	21.94	11.39	22.03
Sig	0.00	0.04	0.00
Levene test	10.69	0.54	6.65
Sig	0.00	0.75	0.00
SW	0.73	0.84	0.76
Sig	0.00	0.14	0.00

% M: % mortality. DE: Standard Deviation. NC: Not applicable. DL_{50}: Mean lethal dose. DL_{50}-Low: DL_{50} (Low Limit). DL_{50}-Upper: DL_{50} (Upper Limit).
PNEC: Dose without ecological effect. KW: Kruskall–Wallis statistician. Levene test: Statistical to evaluate homocesticity of variances.
SW: Shapiro–Wilks Statistician: Test to evaluate normality.
Equal lowercase letters in the same column indicate that mortality (%) is statistically similar according to Tukey's multiple comparison test.

In relation to the evasion and food activity, it would be related to their availability as they could be degrading with temperature increase and humidity decrease, and therefore would be less available. These results are according to the study conducted by Zimdahl et al. (1984), where the degradation of pendimethalin was higher than 30°C, decreasing as well as soil moisture. The same would happen in the ecotoxicological test where the average temperature ranged from 20.6 to 20.7°C and the humidity decreased from 75% to 25%, being more available in the soil and affecting *E. fetida*.

4.2 Environmental Impact Assessment

Regarding zeta-cypermethrin, TER was 482.6 which indicated low toxicity with respect to *E. fetida*. Considering the European Community legislation as a reference, it would not have an impact on the environment since it is less than 10, thus it does not represent a risk to *E. fetida*. A TER of 1 500 was determined for alpha-cypermethrin, as cited by Hartnik et al. (2008); while the CR obtained a value of 1.04, which is considered a high environmental risk. Contrary to TER, the CR approach is used in this study to identify the most sensitive organism and to assess the toxicity of the combination of the three pesticides used in onion cultivation; however, the environmental impact of earthworms in soil may be similar in other crops where zeta-cypermethrin, profenofos and pendimethalin are used.

Table 3. Mortality and average weights of *Eisenia fetida* exposed to pendimethalin at 14 d of exposure.

Eisenia fetida			
Doses (mg·kg^{-1})	**Acute ecotoxicological effect of pendimethalin (% M)** 14 d (± DE)	**Average Weights (g)**	
		0 d ± DE)	14 d (± DE)
Control	0.00 (± 0.00)a	2.89 (± 0.06)a	3.18 (± 0.05)a
2 666.67	42.50 (± 0.85)b	2.17 (± 0.09)b	1.25 (± 0.15)b
5 333.33	57.50 (± 1.10)c	2.15 (± 0.07)b	0.92 (± 0.22)b
10 666.67	100.00 (± 0.00)d	2.32 (± 0.12)b	0.00 (± 0.00)c
21 333.33	100.00 (± 0.00)d	2.22 (± 0.09)b	0.00 (± 0.00)c
42 666.67	100.00 (± 0.00)d	2.16 (± 0.06)b	0.00 (± 0.00)c
DL_{50}	3771.23	N.C	N.C
DL_{50}-low	1866.24	N.C	N.C
DL_{50}-upper	7620.78	N.C	N.C
PNEC	3.77123	N.C	N.C
KW	22.19	10.66	22.21
Sig	0.00	0.06	0.00
Levene test	9.00	0.42	8.82
Sig	0.00	0.83	0.00
SW	0.78	mn1c|0.84	0.75
Sig	0.00	0.14	0.00

% M: % mortality. DE: Standard Deviation. NC: Not applicable. DL_{50}: Mean lethal dose. DL_{50}-Low: DL_{50} (Low Limit). DL_{50}-Upper: DL_{50} (Upper Limit).
PNEC: Dose without ecological effect. KW: Kruskall–Wallis statistician. Levene test: Statistical to evaluate homocesticity of variances.
SW: Shapiro–Wilks Statistician: Test to evaluate normality.
Equal lowercase letters in the same column indicate that mortality (%) is statistically similar according to Tukey's multiple comparison test.

Table 4. Evasion effect of zeta-cypermethrin, profenofos and pendimethalin on *Eisenia fetida* at 3 d of exposure.

Pesticides	*Eisenia fetida*				
	Evasion Effect	**Parameters**			
	Doses (mg· kg^{-1})	Without Toxic (%)	With Toxic (%)	χ^2	Significance
Zeta-Cypermethrin	15	45	55	4.27	0.04
	30	60	40	7.91	0.01
Profenofos	312.5	5	95	0.05	0.82
	625	60	40	7.91	0.01
Pendimethalin	1 333.33	40	60	3.33	0.07
	2 666.67	80	20	15.24	0.00

Statistical χ^2: Chi Square.

TER in profenofos was 12 500, higher than that of zeta-cypermethrin. Vašíčková et al. (2019) define TER as an approximation that characterizes the risk of an independent compound and gives a general estimate of the environmental impact for each species in the soil. CR is applied since its exposure is divided depending on its toxicity, as in the case of profenofos, which represented a low risk with a value of 0.08. Residues of profenofos in the soil indicate environmental concerns, such as adverse effects on crops and migration to groundwater (He et al., 2010; Bedi et al., 2015; Gonzales-Condori et al., 2020).

Finally, pendimethalin proved to have lower TER and CR values in the range of environmental impact in the different classifications with values of 377 123 and 0.001, yielding zero risk values in both cases. Goto and Sudo (2018) suggest that the simple lipid content of pendimethalin and trifluralin was not the only factor affecting bioaccumulation potential, but differences in the composition of lipids could generate variability in bioconcentration in biological organisms (Van der Heijden and Jonker, 2011).

Table 5. Effect of food activity of zeta-cypermethrin, profenofos and pendimethalin on *Eisenia fetida* at 3 d of exposure.

Pesticides	***Eisenia fetida***			
	Evasion Effect	**Parameters**		
	Doses ($mg \cdot kg^{-1}$)	Food Activity (%)	χ^2	Significance
Zeta-Cypermethrin	15	54.19	0.76	0.39
	30	33.33	10.97	0.00
Profenofos	312.5	39.65	3.63	0.06
	625	29.12	16.28	0.00
Pendimethalin	1 333.33	49.28	0.05	0.83
	2 666.67	32.66	10.86	0.00

Statistical χ^2: Chi Square.

Table 6. Environmental impact values of zeta-cypermethrin, profenofos and pendimethalin on *Eisenia fetida*.

Parameters	**Zeta-Cypermethrin**	**Profenofos**	**Pendimethalin**
MEC or PEC ($mg \cdot kg^{-1}$)	0,05 (BASF, 2014)	0,1 (Van den Brink et al., 2003)	0,005 (Vašíčková et al., 2019)
(DL_{50})	48.26	1 250	3 771.23
PNEC (DL_{50})	0.04826	1.25	3.77123
TER (DL_{50})	482.6	12 500	377 123
CR (DL_{50})	1.04	0.08	1
Contribution (%)	92.77	7.14	0.09
CR t (DL_{50})		1121	

MED: Controlled environmental dose of pesticide. PEC: Predicted environmental dose of the soil. PNEC: Dose without ecological effect. TER: Toxicity exposure ratio. CR: Environmental risk coefficient. CR t: The total environmental risk coefficient of a studied pesticide area. Contribution: contribution % to the environmental risk per pesticide.

5 Conclusions

The data found from DL_{50}-14d show that the ecotoxicity level based on mortality and reduction in *E. fetida* weight from the most used pesticides in onion cultivation in the district of Lurín, Lima, Peru, was below the recommended doses for pest control by manufacturers, except zeta-cypermethrin. Evidence of evasion and food activity with *E. fetida* was associated with the dose closest to DL_{50}. TER value for the three zeta-cypermethrin, profenofos and pendimethalin was calculated, and it did not have any environmental impact on *E. fetida*. However, high-risk values were presented in the total CR, where zeta-cypermethrin contributed with the highest value, profenofos showed low risk and pendimethalin did not show risk for onion cultivation in Lurín. In Peru, RD N° 0020-2013-AG-SENASA-DIAIA shows that these three pesticides are still registered and can be used; so, based on this research, it is recommended that the impact on other soil organisms be evaluated more deeply, especially zeta-cypermethrin in chronic reproductive trials on *E. fetida*, because it was the only pesticide that presented an environmental risk.

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