Revista Mexicana de Ingeniería Química

Ingeniería Ambiental

Role of *Eisenia foetida* in the degradation of profenofos in presence of native bacterial communities

Rol de *Eisenia foetida* en la degradación de profenofós en presencia de comunidades bacterianas nativas

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Abstract

The use of profenofos (PFF) increased due to its effectiveness against pests resistant to other organophosphates (OP). Its presence in the environment could produce acute or chronic poisoning for people who use it for daily agricultural activities as well as for people who get in contact with polluted soil, water, air or food. In this context, proposing alternatives to accelerate OP degradation processes is important. Therefore, the objective of the present study was to evaluate for 28 days the degradation process of PFF in soils with 50 mg PFF/kg (pH = 7.20 ± 0.31) using *Eisenia foetida* (EF) and native bacteria (NB). The "control group" showed a PFF degradation of 52.95 ± 1.69% in sterilized soils. NB achieved a degradation of 63.60 ± 3.27%, EF degraded PFF by 72.65 ± 1.92% and the combination of EF and NB, 79.21 ± 1.79%. Bacteria with potential PFF degradation capacity were isolated and identified as *Klebsiella oxytoca* and *Pseudomonas aeruginosa*. It was shown that EF play an important role by accelerating the degradation of PFF in soils, presenting a possible synergy with *Klebsiella oxytoca* and *Pseudomonas aeruginosa*.
Keywords: *Eisenia foetida*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, degradation, profenofos.

Resumen

El uso de profenofós (PFF) se incrementó por su eficacia frente a plagas resistentes a otros organofosforados (OP) y su presencia en el medio ambiente podría producir intoxicaciones agudas o crónicas ya sean ocupacionales o por contacto con suelos, aguas, aire o alimentos contaminados. El plantear alternativas para acelerar el proceso de degradación de los OP es importante, por lo cual, el objetivo del presente estudio fue evaluar durante 28 días el proceso de degradación del PFF en suelos con 50 mg/kg de PFF (pH=7.20 ± 0.31) usando *Eisenia foetida* (EF) y bacterias nativas (NB). El "grupo control" con suelos esterilizados mostró degradación de PFF en un 52.95 ± 1.69%. Las NB lograron una degradación del 63.60 ± 3.27%. "EF" en suelos esterilizados degradaron un 72.65 ± 1.92% y la combinación de EF y NB 79.21 ± 1.79%. Se aislaron bacterias con capacidad de degradación de PFF y se identificaron como *Klebsiella oxytoca* y *Pseudomonas aeruginosa*. Se demostró que EF desempeña un papel importante al acelerar la degradación de PFF en suelos existiendo un probable sinergismo con *Klebsiella oxytoca* y *Pseudomonas aeruginosa*.
Palabras clave: *Eisenia foetida*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, degradación, profenofós.

1 Introduction

Today, the use of agrochemicals (AC) has become a necessary activity for farmers in many countries. Pesticides, among the most used ACs, can be classified into insecticides, herbicides and fungicides (Liu *et al*., 2009) and are used for pest control and to guarantee agricultural production. Some pesticides have high persistence in the environment (Silva *et al*., 2019) and can be transported from agricultural fields by runoff or leaching (Le *et al*., 2017) or by air, making contact with nearby ecosystems, causing damage to animals and humans. Pesticides are said to have carcinogenic and cytotoxic effects, and to cause infertility, immunological and respiratory diseases (Chawla *et al*., 2018). Damage to health has been recently deduced based on hypothetical mechanisms such as oxidative stress which appear as a consequence of a work-related or environmental exposure (Teodoro *et al*., 2019). These health problems are generally due to a weak regulation of the use of pesticides by institutions (Shattuck, 2019).

Likewise, pesticide's packaging labels do not often provide toxicity and risks information in an appropriate language understandable for the user (Rother, 2018) leading to "improper use" without environmental liability.

Among the most widely used pesticides in agriculture we find organophosphates (OP) (Zou *et al*., 2018), whose main effect is acute neurotoxicity due to the inhibition of acetylcholinesterase (AChE) activity in the neuro-muscular junctions and the central nervous system (Marutescu and Chifiriuc, 2017). This is known as the cholinergic syndrome, which causes millions of cases of poisoning with more than 15% fatality rate each year. In addition, OPs are associated with neurodegenerative diseases, dementia, attention deficit disorder with hyperactivity and Parkinson's disease (Jokanović, 2018).

One of the most used OPs is profenofos (PFF), whose chemical name is O-(4-bromo-2-chlorophenyl)-O-ethyl-S-propyl phosphorothioate. PFF is a direct-action OP capable of inhibiting β-esterases, AChE, butyrylcholinesterase and carboxylesterase of its target organism (Dadson *et al*., 2013). PFFs are commercially known as Curacron® or Selecron® (Unger, 1996). These products are used in field crops, vegetables and fruit trees for its effectiveness against insects resistant to other OP pesticides (Khalifa *et al*., 2017). It is used as well in the control of lepidoptera in cotton and tobacco crops (Malghani, Chatterjee, Hu *et al*., 2009). Users justify its application because of its selective toxicity: it affects insects instead of mammals (Gotoh *et al*., 2001), however, its potential genotoxic effect in freshwater fish *Labeo rohita* has been reported in sublethal concentrations (Nataraj *et al*., 2017), as well as its neurotoxicity for adults at prolonged periods of exposure in low doses (Chu *et al*., 2018), which would mean PFFs represent a danger to aquatic ecosystems and humans.

Taking into account the residuality and the toxicity of pesticides, minimizing the risks to human health and the environment is a major challenge for agricultural sustainability that needs to be addressed (Möhring *et al*., 2019). Therefore, research aimed at soil remediation or care must be carried out using an environmentally friendly and profitable approach (Jabeen *et al*., 2015), so that its application can be feasible. This is the reason why, today, the aim is to isolate and identify microorganisms from soils contaminated with OP by means of morphological,

biochemical, 16S rRNA characterization (Blanco *et al*., 2020), among others, and to take advantage of their pesticide degradation capacity. Current studies have isolated strains with pesticide degradation capacity such as *Alcaligenes* sp. JAS1, *Ochrobactrum* sp. JAS2, *Sphingobacterium* sp. JAS3 (Abraham *et al*., 2014), *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Bacteroidetes, Firmicutes* (Wei *et al*., 2018), *Aspergillus, Pseudomonas, Chlorella, Arthrobacter* (Kumar *et al*., 2018), *Burkholderia gladioli* (Malghani, Chatterjee, Hu *et al*., 2009), *Pseudoxanthomonas suwonensis* strains (Talwar and Ninnekar, 2015), *Brevibacterium frigoritolerans* and *Bacillus aerophilus* (Jariyal *et al*., 2018).

In addition, there are existing records of the use of earthworms to optimize or accelerate the degradation of pesticides such as chlorpyrifos, tebuconazole (Svobodová *et al*., 2018), and DDT (1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane) (Xu *et al*., 2019), giving favorable results. Specifically, *Eisenia foetida* significantly accelerated the degradation of pesticides such as fipronil (Qu *et al*., 2014), atrazine (Lin *et al*., 2018) and metolachlor (Sun *et al*., 2019), in soils. In addition, they contributed to degradation rates faster than indigenous degraders such as *Sphingomonas* and *Microascales, Mortierella and Escherichia coli, Rhodococcus, Pseudomonas fulva* and *Methylobacillus* (Hao *et al*., 2018).

Therefore, the present research aimed to determine the role of *Eisenia foetida* in the degradation of PFF in soils in the presence of native microbial communities, as well as to isolate and identify bacteria with PFF-degrading capacity.

2 Materials and methods

2.1 Standards, reagents and equipment

A Pestanal® profenofos analytical standard was purchased from Sigma Aldrich. The acetonitrile for the High Performance Liquid Chromatography (HPLC) was obtained from Merck. Ultrapure water was obtained using a Merck Simplicity System Millipore water purifier. A Chromolith RP-18e 4.6 x 100 mm column, a High-Performance Liquid Chromatograph with a HPLC-DAD Hitachi Chromaster diode array, and a Branson sonicator were used. All the other reagents were of analytical grade.
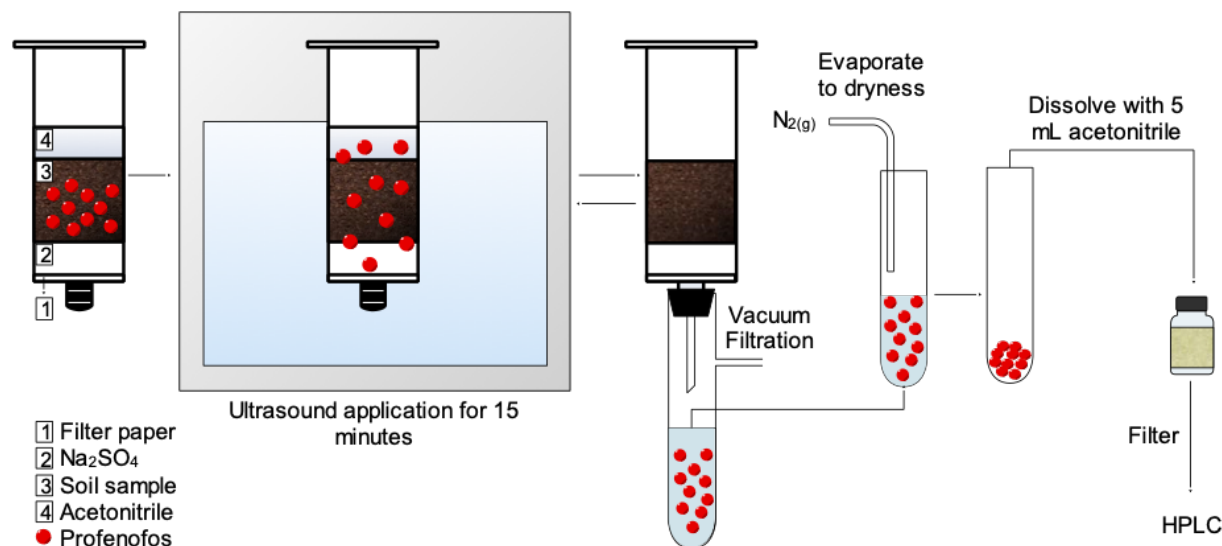
Fig. 1. Schematic diagram of the procedure of extraction of PFF from contaminated soils.

2.2 Determination of PFF through High Performance Liquid Chromatography (HPLC)

The quantification of PFF was performed through HPLC under isocratic conditions. The mobile phase was made up of acetonitrile: ultra-pure water (60:40), a flow of 2 mL/min, a wavelength of 205 nm and a run time of 5 minutes. The method was validated through the determination of linearity, sensitivity, precision and accuracy parameters (Quattrocchi *et al.*, 1992).

2.3 Eisenia foetida earthworms

Eisenia foetida was obtained from worm farming companies in the city of Arequipa, Peru, and transferred to the "Proyecto Mercurio" research laboratory located at the Catholic University of Santa María, where they underwent acclimatization in the soil during a week before the laboratory tests. Adult earthworms (with well-developed clitheliums) were used for PFF degradation tests (Wang *et al.*, 2012; Xu *et al.*, 2019).

2.4 Extraction of PFF from contaminated soils

PFF were extracted from the soil as shown in the schematic diagram in Figure 1. The process consisted of placing two 2 cm diameter filter paper discs in glass syringes, a sample of 5 g of soil, 2 grams of anhydrous sodium sulfate and 10 mL of HPLC grade acetonitrile, then submitted to ultrasounds for 15 minutes, and vacuum filtered in a test tube. A second extraction was carried out with 5 mL of acetonitrile using the same process. The result of the filtration process was evaporated using N_2 (g). It was reconstituted with 5 mL of acetonitrile, filtered using a WHATMAN 45 μm NYL filter and analyzed through HPLC under established conditions.

2.5 Soil preparation

Soil samples were collected from the Inclán district, located in the department of Tacna, Peru (-17.795041°, -70.495038°) where Selecron® has been applied in recent years on garlic and onion production lands. Soils were sieved at ambient temperature on an ASTM (American Society for Testing and Materials) 90 mesh screen to remove stones and other debris and then stored at 4 °C until later use (Malghani, Chatterjee, Hu *et al.*, 2009). In order to perform the degradation tests, the methodology established by the Organization for Economic Cooperation and Development (OECD, 1984) for toxicity analysis tests in soils was followed. The soil samples were contaminated with PFF at a concentration of 50 mg/kg, by dissolving the organophosphate in 5 mL of petroleum ether, putting it into contact with 150 g of fine sand and then homogeneously dispersing it in the studied soils. Finally, the samples were enriched with 10% organic matter and the humidity was adjusted to approximately 40%. Soil pH was controlled and set at approximately 7 to facilitate the acclimatization and survival of *Eisenia foetida*. This, due to the fact that some studies performed for three OPs (chlorpyrifos, triazophos, and

dimethoate) with *E. fetida* for 14 days in acidified soils at pH 5.5 , 4.3 and 3.1 have shown that OP toxicity increases slightly with decreasing soil pH (Zou *et al.*, 2018).

2.6 Study of PFF degradation in contaminated soils

Degradation tests were carried out in glass containers with 0.5 kg of soil with a PFF concentration of 50 mg/kg. The containers were divided in the four groups listed in Table 1. In the "EF" group, the degradation of PFF was evaluated in the presence of *Eisenia foetida*. The soils were previously sterilized in an autoclave at 121 °C for 15 minutes, then they were contaminated with PFF and 10 adult earthworms were added (Hao *et al.*, 2018). In the "NB" group, the degradation of PFF in the presence of only native bacteria was evaluated. On the other hand, in the "EF-NB" group the degradation of PFF in the presence of *Eisenia foetida* (10 earthworms) and native bacteria was evaluated. Finally, a "Control group" was set up, where the degradation of PFF in sterile soils in the absence of earthworms was evaluated. Samples were taken in a zig-zag pattern and with the quartering method on days 0, 7, 14, 21 and 28 (Lin *et al.*, 2018) to be analyzed by HPLC. The degradation percentage was calculated using the following formula:

$$Degradation(\%) = \frac{C_0 - C_t}{C_0} \times 100 \qquad (1)$$

where, "C_t" is the PFF concentration in soils at a time t, and "C_0" is the initial PFF concentration.

2.7 Isolation of PFF-degrading bacteria by enrichment culture

2.7.1 Enrichment and isolation of PFF-degrading cultures

The Basal Salt Medium (BSM) formulation carrying PFF included KH_2PO_4 3.0 g/L, NH_4Cl 1 g/L, NaCl 0.5 g/L, $MgSO_4$ 0.25 g/L, and PFF 100 mg/L (in a 10mM phosphate buffer solution, pH 7.0). All chemicals except PFF were sterilized in an autoclave and then, PFF was added in the sterile medium. For the preparation of the agar medium, 2.0% (w/v) agar was added (Siripattanakul-Ratpukdi *et al.*, 2015).

Table 1. Study groups arrangement for the evaluation of PFF degradation in contaminated soils.

Group	*Eisenia foetida*	Native bacteria
EF	Yes	No
NB	No	Yes
EF-NB	Yes	Yes
Control group	No	No

For bacterial isolation, 1 g of each soil sample was weighed in falcon tubes with 9 mL of saline and centrifuged at 5000 rpm. The supernatant was put into Eppendorf tubes, which were centrifuged at 10 000 rpm. Each pellet obtained was seeded in plates with sterilized fresh Agar BSM containing 100 mg PFF/L and then, the plates were incubated at 30 °C for 7 days. After this time, they were sub-cultured repeatedly in the same medium (BSM enriched with 100 mg PFF/L) and glucose was added to promote growth. The plates were incubated at 30 °C for 7 more days.

For the isolation and obtainment of discrete or pure colonies, each colony was taken and shaken in a vortex for 5 min with 1 mL of 0.01% triton dissolved in physiological serum. These solutions were seeded in enriched Agar BSM and incubated at 30 °C. The process was repeated until isolated colonies were obtained. Two pure colonies were obtained and coded as EVc and EPEB.

2.7.2 Isolation of genomic DNA and sequencing of the 16S rRNA gene

In an Eppendorf tube, 100 μL of 100 μm glass beads, 500 μL of sterile water, 1 μL of proteinase K and a bacterial colony were added. The mix was vortexed at full speed for 5 minutes. After this, 500 μL of a phenol-chloroform-isoamyl alcohol solution was added, then the tube was shaken vigorously 30 times and centrifuged at 15000 rpm for 10 minutes. The supernatant was collected in a new tube, 600 μL of isopropyl alcohol was added and the mix was centrifuged at 15000 rpm for ten minutes. The supernatant was removed, and the pellet was washed with ethanol, which was then evaporated. Finally, it was re-suspended with 50 μL sterile water. DNA fragments were visualized by electrophoresis using 5 μL of isolated DNA. For the PCR amplification of the gene encoding 16S rRNA, the methodology used by (Malghani, Chatterjee, Hu *et al.*, 2009; Rani *et al.*, 2019) was taken as a

reference. Finally, nucleotide sequence similarities were determined using BLAST (National Center for Biotechnology Information Database). The sequences of the isolated subjects obtained were presented in GenBank (Guerrero-Barajas *et al*., 2019; Jabeen *et al*., 2015).

2.8 Statistical analysis

Results were expressed as the mean ± standard deviation (SD). The data was processed using OriginPro 9.0 and GraphPad Prism 6.0, and the difference between treatments was determined through a one-way analysis of variance (ANOVA)(Haq *et al*., 2019) and a Tukey post hoc test. The most optimal interaction was determined using Statgraphics Centurion XVIII software. Probabilities less than 0.05 (p <0.05) were considered as a significant difference.

3 Results and discussion

3.1 Quantification method for PFF in soils by HPLC

Figure 2 shows that the retention time of PFF at the established chromatographic conditions was 2.9 minutes. The calibration graph prepared for the proposed determination method for PFF in soils at concentrations from 0.5 to 3.0 mg/L showed a coefficient of determination (R^2) of 0.9994, with limits of detection (LOD) and quantification (LOQ) of 0.0477 and 0.1450 mg/L, respectively. The relative standard deviation (RSD) was 2.27% and the accuracy calculated by the percentage recovery method (% R) was 99.06%. With these results it is verified that the method is linear ($R^2 > 0.995$), precise (RSD <2.7%) and exact with a % R between 90 and 110% (Quattrocchi *et al*., 1992).
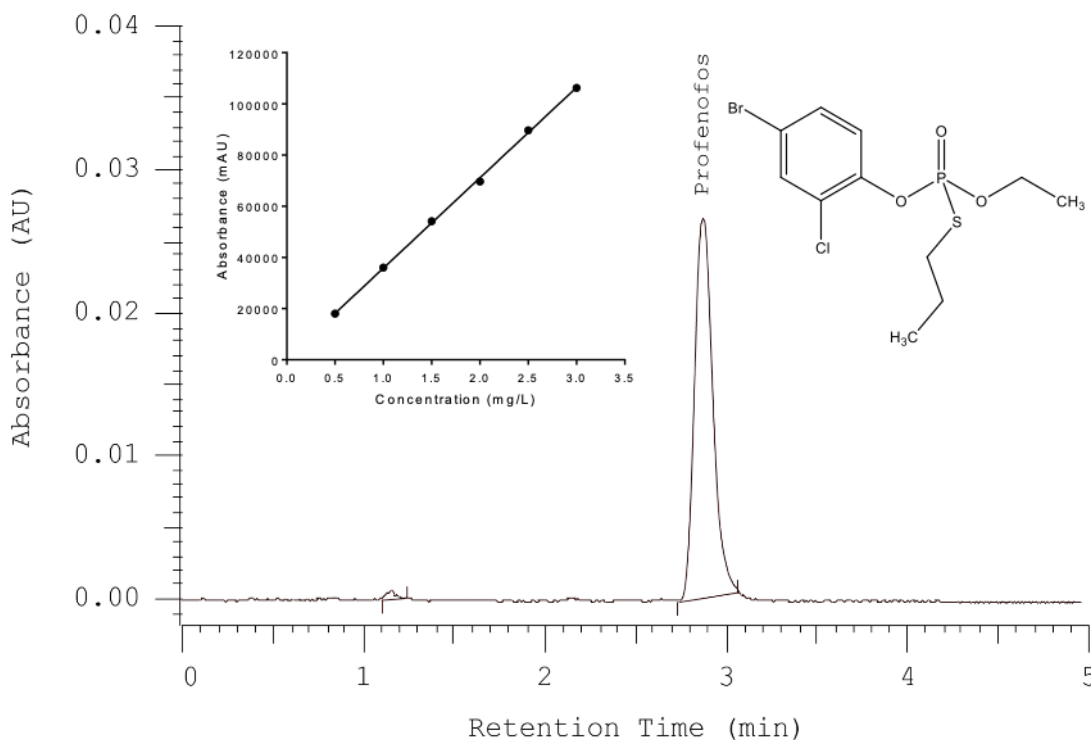

Fig. 2. Chromatogram at 205 nm corresponding to PFF with a retention time of 2.9 minutes and a linear method response.

Table 2. PFF degradation percentage on days 0, 7, 14, 21 y 28 expressed in average ± standard deviation (SD)

Time (Days)	EF-NB	EF	NB	Control Group
0	0	0	0	0
7	42.41 ± 2.50	20.58 ± 3.22	20.24 ± 3.52	14.79 ± 2.74
14	50.71 ± 1.98	33.42 ± 2.81	31.20 ± 2.73	28.35 ± 3.01
21	64.74 ± 3.05	62.59 ± 3.59	52.04 ± 3.09	38.39 ± 1.98
28	79.21 ± 1.79	72.65 ± 1.92	63.60 ± 3.27	52.95 ± 1.69

*EF: PFF degradation in sterile soils in presence of *Eisenia foetida*, NB: PFF degradation in soils in presence of only native bacteria, EF-NB: PFF degradation in soils in presence of *Eisenia foetida* and native bacteria. Control group: PFF degradation in sterile soils in the absence of earthworms.

The equation of the line was the following:

$$y = 512.05 + 35281x \tag{2}$$

where: "*y*" corresponds to the area of the peak (mAU) and "*x*" to the PFF concentration (mg/L). This equation was used to calculate the PFF concentration in soils every 7 days.

3.2 Profenofos degradation in soils

Table 2 shows the results of PFF degradation in percentages ± standard deviation (SD) on days 0, 7, 14, 21 and 28. Table 2 and Figure 3 show that on day 28, the PFF degradation in the "control group" (with sterilized soils and in the absence of earthworms) attained 52.95 ± 1.69%. In relation to this result, a study carried out in buffer solutions at pH 5.0, 7.0 and 9.0 indicated that when pH is low the OP are relatively stable, and as pH increases the molecule is hydrolyzed easily (Yang *et al*., 2008). In the present study, pH was 7.20 ± 0.31, which indicates that the PFF molecule was being hydrolyzed as time passed.

On the other hand, the soils of the "NB" group that presented only native bacteria achieved a degradation of 63.60 ± 3.27%. In the "EF" group, where the activity of 10 earthworms (*Eisenia foetida*) in sterilized soils was evaluated, a PFF degradation of 72.65 ± 1.92% was obtained. On the other hand, for the group "EF-NB" in which the activity of native bacteria in the presence of *Eisenia foetida* was studied, a degradation of 79.21 ± 1.79% occurred. Some studies showed that *Eisenia foetida* accelerates the degradation rate of fipronil after 7 days, attaining 76% for R-(-)-fipronil and 85% for S-(+)-fipronil from an initial concentration of 25 mg/kg (Qu *et al*., 2014). Acetochlor degradation was faster in soils with earthworms than in soils without earthworms, with degradation rates increased by 62.3±15.2% and 9.7±1.7%. In addition, earthworms stimulated

indigenous degraders such as *Sphingomonas* and *Microascales* and took suspicious intestinal degraders such as *Mortierella* and *Escherichia coli* into the degradation process. The study also presented some possible anaerobic degradation species such as *Rhodococcus, Pseudomonas fulva* and *Methylobacillus* (Hao *et al*., 2018). Earthworms (*Eisenia foetida*) were found to increase degradation of metolachlor up to 30 and 63% from initial concentrations of 5 and 20 mg/kg, respectively, on day 15. The study indicates as well that fungi are the main responsible for the degradation of metolachlor in the soil, instead of bacteria. Possible fungal degraders would be *Sordariales, Microascales, Hypocreales* and *Mortierellales* (Sun *et al*., 2019). In the present research study, greater interest was given to bacteria, since other pesticides present in the soil had deteriorated the fungal community in the long term (Qu *et al*., 2014). Furthermore, epigenic *Eisenia foetida* and endogenous *Amynthas robustus* accelerated the degradation of atrazine from 39.0% in sterile soils to 94.9%-95.7%. The most probable mechanism is the neutralization of the soil's pH, favoring the native deg raders of atrazine and excreting the intestinal bacteria that degrade atrazine (Lin *et al*., 2018).

The results obtained for PFF degradation would present advantages since this OP is highly toxic for target organisms and relatively safe for non-target species such as *Eisenia foetida*. A 14-day PFF toxicity study in earthworm using the method of contact by paper shows that *E. foetida* was not only affected by direct toxicity, but also exhibited significant histological and morphological changes in its body walls. The earthworms showed body ruptures, bloody lesions, excessive formation of internal glandular cell masses and disintegration of its circular and longitudinal muscles, which were unable to regulate the internal coelomic pressure, leading

to fragmentation (Chakra Reddy and Venkateswara Rao, 2008). The study carried out by Chakra and Venkateswara could explain the behavior of *E. foetida* in the present investigation, which was carried out in soils, since up to day 14 (Figure 3) it is observed that the degradation of PFF is slow and/or similar to the one of the group with native bacteria or the control group, however, from day 14 to 28 it was noted that the PFF degradation increased, which would be due to the adaptation of *E. foetida* to the contaminated medium or to the decrease in PFF in the soil.

Regarding the role of *Eisenia foetida* in PFF degradation, the one-way analysis of variance (ANOVA) resulted in a lower than 0.05 probability ($p > 0.05$) and Tukey's post hoc test indicates that all groups differ from each other with a 95% confidence. This explains that the application of earthworms in sterile soils and soils with native bacteria significantly increases the degradation of PFF (Figure 4) at the studied concentration.

Probably, the PFF would be bioacumulating in *Eisenia foetida*, a similar study found that, the organophosphate pesticide chlorpyrifos and the triazole fungicide tebuconazole can bioaccumulate in worms, this was determined when the bioaccumulation factors (BAF) were found finding values between 4.5 - 6.3 for chlorpyrifos and 2.2 - 13.1 for tebuconazole and this difference could be due to the higher hydrophobicity of chlorpyrifos compared to Tebuconazole which probably caused an increase in availability by additional absorption by ingestion into earthworms. Also, the same study indicates that higher clay content may contribute to the degradation of chlorpyrifos due to hydrolysis catalyzed by clay (Svobodová *et al.*, 2018). Another study evaluated the degradation of metolachlor in soils by finding degradation metabolites such as oxanilic acid (MOXA) and metolachlor ethane sulfonic acid (MESA). In the same study, it was shown that the addition of worms to soils achieved significantly higher MOXA concentrations compared to when no worms were applied, likewise, MESA was more prevalent in soils when worms were applied before 7 days, but then its proportion decreased below the non-worm treatment, which could be due to the bioaccumulation capacity of earthworms since MOXA and MESA were detected in the worms. However, bioaccumulation factors (BAF) suggest that metolachlor and MOXA do not accumulate in earthworms reflected by their BAF of less than one.
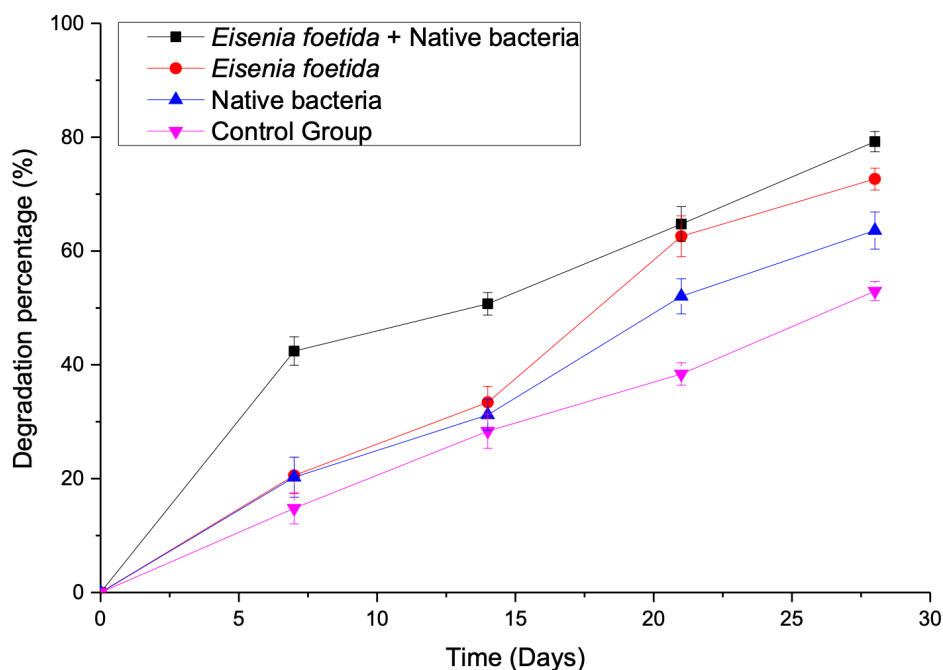

Fig. 3. Percentage of PFF degradation at 0, 7, 14, 21 and 28 days. On day 28, it is shown that in the "Control Group" there is a degradation of 52.95 ± 1.69%. Soils with only native bacteria degrade PFF by 63.60 ± 3.27%. PFF in sterile soils with *Eisenia foetida* (ten earthworms) are degraded by 72.65 ± 1.92% and soils with native bacteria and earthworms achieve a degradation of 79.21 ± 1.79%.

Instead, MESA of BAF values at most sampling points were greater than one, indicating that MESA has some bioaccumulation capacity (Sun *et al.*, 2019). Likewise, in an experiment of fipronil accumulation in earthworms, the bio-concentration factors were calculated, finding values between 1.24 to 1.33 in natural soil, demonstrating the capacity of fipronil absorption in earthworms (Qu *et al.*, 2014). The degradation of PFF in soils in the presence of *Eisenia foetida* has not been previously reported, so attention should be paid to the bioaccumulation of PFF or other organophosphates and their possible metabolites in earthworms.

In another study, acetochlor degradation was evaluated in contaminated soils, where it was shown that acetochlor would have some biotoxicity for soil microorganisms, however, this inhibition could be repaired by the earthworm, this would be due to the influence of biotic and abiotic effects, as the improvement of the physicochemical properties of the soil that would involve the improvement of the pH and organic carbon content through activities such as secretion and excretion, promoting the indirect degradation of acetochlor by stimulating enzymatic activities and soil microbes. In addition, the earthworm's accelerating effect on dehydrogenase implied the highest microbiological activity, which was related to the highest degradation efficiencies. Likewise, the increased activities of urease and alkaline phosphatase in acetochlore-enriched soil indicated that the nitrogen and phosphorus cycle may be involved in the degradation process, on the other hand, in the sterile groups, the effects of earthworms on soil enzymes were more evident due to the limited status of indigenous microorganisms where earthworms had direct interaction with microorganisms through digging, urination, defecation and other activities that would result in an increase in indigenous microbes in the soil that could be associated with faster degradation rates (Hao *et al.*, 2018). On the other hand, one research indicates that mucus produced by earthworms could stimulate microbial activity, therefore enhancing the degradation of organic contaminants, demonstrating that DDT degradation can occur in the gut of earthworms and would be dominated by the composition of the microbial community more than the diversity of the intestinal microbial community (Xu *et al.*, 2019).
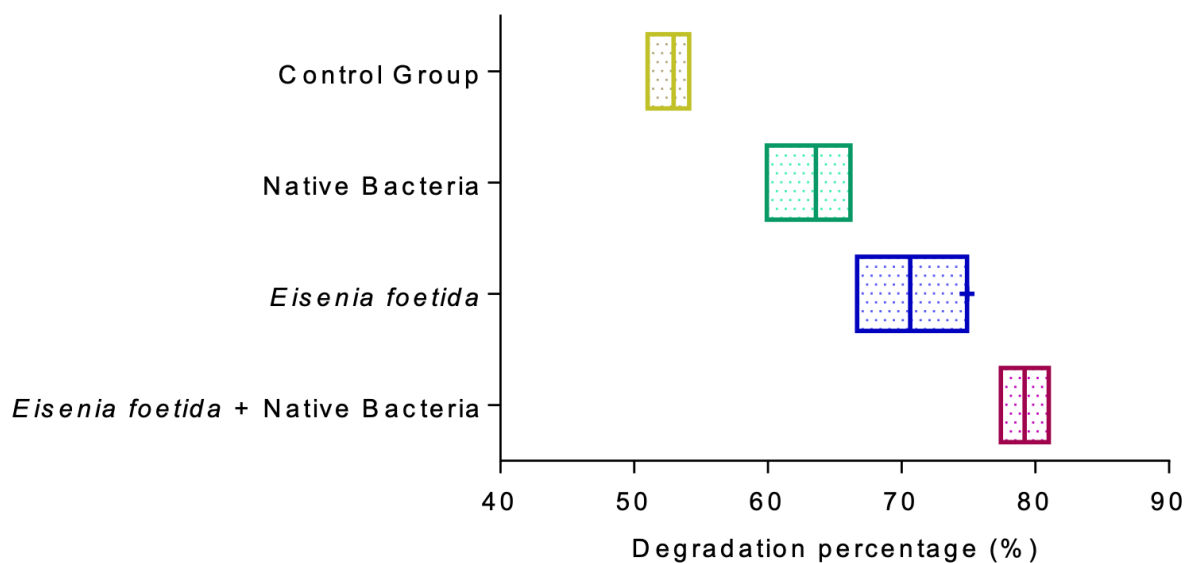

Fig. 4. Comparison of the percentage of degradation obtained on day 28. The one-way analysis of variance (ANOVA) indicates that there is a significant difference in at least one group (p <0.05). Tukey's test indicates that all groups differ in terms of PFF degradation percentage at 95% confidence.
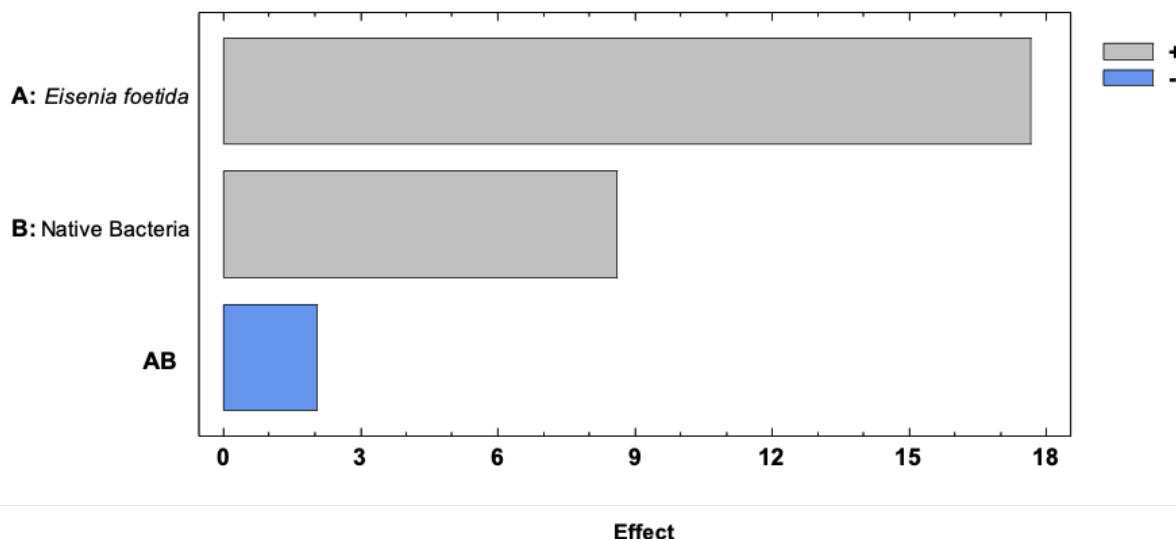
Fig. 5. Pareto diagram. *Eisenia foetida* shows a higher influence in PFF degradation in contaminated soils.

The Pareto diagram in Figure 5 shows that the factor with the greatest influence on PFF degradation in soils was *Eisenia foetida*, since a higher percentage of degradation was achieved in the groups where it was present than in those when only native bacteria acted.

For this degradation process, a mathematical model is proposed, which includes the three components that are defined in the following equation. This equation helps to interpret the results obtained in the present research study.

$$Degradation(\%) = 67.1025 + 8.8275EF + 4.3025NB - 1.0225EF \cdot NB$$

(3)

where "EF" is *Eisenia foetida* and "NB" is "native bacteria". These factors take values of -1 for the low level "No" and $+1$ for the high level "Yes".

Identification of PFF-degrading bacterial consortiums

In the present research study, two PFF-degrading bacterial strains encoded as EPEB and Evc were isolated and subsequently identified as *Klebsiella oxytoca* and *Pseudomonas aeruginosa*. The NCBI assigned access numbers for EPEB and Evc are KX670811 and KX670812, respectively.

A research study performed in PFF-contaminated soils in central China's Hubei province isolated and characterized PFF-degrading bacteria such as *Pseudomonas putida* and *Burkholderia gladioli* (Malghani, Chatterjee, Hu *et al.*, 2009; Malghani, Chatterjee, Yu, *et al.*, 2009). Also, *Achromobacter xylosoxidans, P. aeruginosa, Bacillus* sp. and *Citrobacter koseri* were identified in cotton field soils that had been historically treated with PFF (Jabeen *et al.*, 2015). Another study isolated *Pseudoxanthomonas suwonensis* (Talwar and Ninnekar, 2015), as well. *Pseudomonas aeruginosa* and *Pseudomonas plecoglossicida* were characterized in chilli cultivated soils in Ratchathani, Thailand (Siripattanakul-Ratpukdi *et al.*, 2015; Subsanguan *et al.*, 2020). The degradation mechanism of *P. aeruginosa* could be the breakdown of the ester linkage of the original compound leading to the formation of 4-bromo-2-chlorophenol, which suggests that the bacterium contains enzymes that have esterase activity (Malghani, Chatterjee, Hu *et al.*, 2009). Likewise, in another study, molybdenum reducing bacteria were isolated and identified from soils periodically sprayed for several years with the herbicide glyphosate (Roundup®) through cultural testing, morphological and biochemical studies using ABIS online software identifying the *Klebsiella oxytoca* Saw-5 strain that was optimal for molybdenum reduction using glucose as an electron donor and evaluated whether glyphosate and other pesticides could support molybdenum reduction with negative results, however, it is indicated that the bacteria could grow in glyphosate (Sabullah *et al.*, 2016), as well as, in the presence of profenofos found in this investigation.

Conclusions

After 28 days of treatment, PFF undergo hydrolysis processes: degradation of 52.95 ± 1.69% was observed in sterile soils with 40% humidity at a pH of 7.20 ± 0.31. The presence of native bacteria shows a positive effect, it increases the degradation of PFF up to 63.60 ± 3.27%. It was shown that *Eisenia foetida* accelerates the PFF degradation process up to 72.65 ± 1.92% in sterile soils. *Eisenia foetida* and native soil bacteria have a synergistic effect on PFF degradation attaining values of 79.21 ± 1.79%. Two strains of bacteria with potential PFF degradation capacity were isolated from garlic and onion production soils, which were identified as *Klebsiella oxytoca* and *Pseudomonas aeruginosa*. The results indicated that *Eisenia foetida* plays an important role in accelerating the degradation of PFF in soils. Furthermore, there could be synergy with *Klebsiella oxytoca* and *Pseudomonas aeruginosa*, which could increase the degradation of PFF until day 28. Thus, this study shows that *Klebsiella oxytoca* and *Pseudomonas aeruginosa* could be related to the degradation of PFF, however, more studies are required to understand the mechanism that both microorganisms use to degrade PFF and the products of the degradation. In addition, the literature indicates that other possible mechanisms of degradation could be the cutaneous or intestinal bioaccumulation of pesticide or its possible metabolites in the earthworm, as well as, the possibility that degradation occurs at the intestinal level of the earthworm.

Acknowledgments

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