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A new approach to evaluate the functional role of earthworms as bioremediators of certain pesticides in soil

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Running title: A new approach to assess the vermiremediation process

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Abstract

Vermiremediation is a promising technology for restoring soil functioning. Unfortunately, assays used to assess the efficacy of this process fail to evaluate the biological quality of soils. So, this study aimed to determine the functional role of earthworms (*Eisenia fetida* and *Aporrectodea caliginosa*) as bioremediators. The soil used for the experiment was collected from an agricultural field in Egypt. After collection, the soil was divided into two parts. Soil microarthropods were extracted from the first part. Sub-lethal concentrations

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of aldicarb, chlorpyrifos, and carbofuran were added to different sets in addition to distilled water as control, and earthworms were introduced. Thereafter, alternative units from the two parts were mixed. The obtained results revealed that while pesticides had a detrimental effect on decreasing the abundance and diversity of soil oribatid mites even when introduced at sub-lethal doses, the presence of *Eisenia fetida* has increased oribatid mite abundance. Furthermore, some species tended to the presence of earthworms, whereas others showed a positive correlation with the presence of *Eisenia fetida*. In conclusion, vermiremediation using epigeic species such as *Eisenia fetida* had a positive effect on the abundance of oribatid mites, which could increase soil health, therefore enhancing crop production. Consequently, we suggest that assessing oribatid mite abundance is a way to detect the efficacy of earthworms in the vermiremediation process.

Keywords: *Aporrectodea caliginosa*; *Eisenia fetida;* Oribatid mite; Pesticides; Vermiremediation.

Introduction

Over the past years, global hunger has become a threatening problem. In 2017, about 800 million people were estimated to suffer from hunger. Consequently, eradication of hunger and feeding the growing population are major global societal challenges (1,2). In the fight against hunger, food and nutrition security must be regarded as central policy priorities (3). To maximize agricultural production and fulfill food demands, tonnes of agrochemicals, including pesticides are used every year. For instance, global imports of pesticide formulations exceeded 4.5 million tonnes in 2019 (4,5).

Briefly, pesticides are chemical compounds; used to protect crops against pests for increasing agricultural output (6,7). Nonetheless, indiscriminate application and overuse of pesticides caused deleterious impacts on individuals and ecosystems. Acute and chronic issues, including neurological disorders, abnormality, asthma, and cancer are reported in humans (8,9,10). Concerning soil ecosystems, pesticides can affect non-target organisms and kill beneficial microbes. Any pesticide lasts for a time in the soil to decrease its amount by half, known as the half-life. Half-lives of pesticides, in laboratory conditions, range from 16 to more than 60 days in the case of non-persistent and persistent pesticides, respectively (11,12). In the environment, pesticide concentrations decline through breakdown or dissipation (12). Accordingly, persistent residuals of these chemicals cause biodiversity loss, soil contamination, and environmental pollution (4,8,13). Therefore, to restore soil functioning and safely meet human needs, it is urgent to biodegrade and remove soil contaminants (4,14). Currently, different strategies are tested for their efficacy in cleaning up contaminated soil (15,16).

Vermiremediation is the utilization of earthworms to remove soil pollutants (17). It has been recommended as a promising eco-friendly technology for soil sanitation, which positively affects the health of agricultural soil and increases crop production (18,19). Earthworms are easily cultured, commercially available, have cosmopolitan distributions, and have short life spans (20). They consume organic materials, which are inaccessible to other animals. Additionally, they can absorb poisonous chemicals within organic matter through ingestion or absorption through body walls. Therefore, earthworms are mediators in all biodegradation and bioconversion processes (4,19,21). Different studies have investigated the potential of earthworms in the vermiremediation of pesticide-contaminated soils e.g., (20,22,23,24).

To ensure the efficacy of the vermiremediation process, biochemical assays are conducted including quantification of certain enzymes in soil and earthworm species (25). However, chemical analyses have many problems in terms of accuracy

and high cost and usually fail to assess the biological quality of soils, thereby using different target species is crucial (26). Among soil fauna, oribatid mites are characterized by great diversity (27,28,29), and large densities (30). They are potential indicators of soil quality (31,32). To date, there are no studies, to our knowledge, that have focused on using oribatids as bioindicators or bio evidence to test the performance of earthworms in the remediation of pesticide-contaminated soil. Accordingly, the present study aimed to test the null hypothesis that the soil oribatid mite population could be used as a bioevidence that may confirm the functional role of earthworms (*Eisenia fetida* and *Aporrectodea caliginosa*) as bioremediators.

Materials and methods

Study site

This study was conducted at the Laboratory of the Department of Zoology, Tanta University, Tanta, Egypt in 2022.

Experimental animals and their maintenance

Mature adult earthworms of the species *Aporrectodea caliginosa* and *Eisenia fetida* were obtained from different garden biotopes near Tanta City, Al-Gharbiya Governorate, Egypt, and an Agricultural Research Institute in Cairo, respectively. Sampling of *Aporrectodea caliginosa* individuals was performed by hand sorting. Average weights of 0.85 g and 0.56 g for *Aporrectodea caliginosa* and *Eisenia fetida*, respectively, were used in this study. Then, earthworms were cultured in plastic pots (10 cm height and 25 cm diameter) and maintained in our laboratory with cow dung as a substrate and food. The worms were acclimatized on soil type recommended by OECD (Organisation for Economic Co-Operation and Development; (33); (70% (w/w) sand, and 30% (w/w) kaolinite clay). In addition, 1% (w/w) calcium carbonate was used for setting pH 6.5. The earthworm culture was maintained at 20 ± 2℃ with normal daylight hours and 70-85% relative humidity. After three weeks, they were removed from the culture, rinsed with tap water, and stored in Petri dishes on damp filter paper for 48h (in the dark at 20 ℃) to devoid gut contents. Adult earthworms with well-developed clitella were then chosen for toxicity tests. Subsequently, they were washed with distilled water, manually dried with moist paper, and placed in the test units.

Toxicity tests

Adult earthworms were exposed to different concentrations of the following three pesticides; Aldicarb (formulated as aldicarb®; granular mix, 10% active ingredient; CAS number: 116-06-3): 2-methyl-2- (methylthio)propionaldehyde-*O*-(methyl carbamoyl) oxime, Chlorpyrifos (formulated as Chlorpyrifos®; granular; 48% active ingredient; CAS number: 2921-88-2): *O*, *O*-Diethyl *O*-(3,5,6-trichloropyridin-2-yl) phosphorothioate and Carbofuran (formulated as carbofuran®; granular; 25% active ingredient; CAS number: 1563-66-2): 2.2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate. Filter-paper contact toxicity method was used to calculate lethal concentrations (LC); selected pesticides were suspended in distilled water and loaded onto the filter paper in flat-bottom glass vials. Controls were also run in parallel with distilled water only. For each treatment, ten replicates were used, each consisting of one earthworm per vial, and each vial was closed with a cap having a ventilation hole to avoid the escaping of earthworms and placed in the dark. From the number of earthworms that died after 12, 24, and 48h$_s$ of exposure to pesticides, the LC_{10}, and LC_{50} values were calculated (33). Finally, LC_{10} for each pesticide was chosen for the experiment (i.e. 7.19 mg/kg and 3.59 mg/kg; 8.09 mg/kg and 4.49 mg/kg; 2.69 mg/kg and 1.79 mg/kg for chlorpyrifos, carbofuran, aldicarb in case of *Eisenia fetida* and *Aporrectodea caliginosa*, respectively).

Experimental design

The soil used for the experiment was collected from a field at Shubra AL-Namla (30°48′19″N 30°55′11″E), Al-Gharbiya Governorate, Egypt. After collection, the soil was divided into 2 parts; 1

and 2. Soil microarthropods were extracted from Part 1 using modified Berlese-Tullgren funnels. After that, part 1 was further divided into 5 sets; $Ap._1$, $Ap._2$, $Es._1$, $Es._2$, and WOE_1, whereas Part 2 was subdivided into 3 sets; M_1, M_2, and WOE_2. Each set consisted of 24 experimental units. Each unit consisted of a plastic pot of 25 cm diameter x 10 cm height filled to its half with soil. Exceptionally, units within $Ap._1$ and $Es._1$ sets were filled with soil. Ten earthworms of *Aporrectodea caliginosa* were introduced to each unit within $Ap._1$ and $Ap._2$ sets and similarly, ten earthworms of *Eisenia fetida* were introduced to each unit within $Es._1$ and $Es._2$ sets. On the contrary, no earthworms were added to other sets. Sub-lethal concentrations (LC_{10}) for aldicarb, chlorpyrifos, and carbofuran in addition to distilled water as control were applied as treatments to different sets within part 1. Each treatment consisted of 6 experimental units as replicates. After two months, to allow selected pesticides to nearly reach their half-lives (34,35,36,37), experimental units within $Ap._2$, $Es._2$ and WOE_1 sets were mixed with alternative units within M_1, M_2, and WOE_2 sets, respectively.

The final sets from now on are termed $Ap._1$; experimental units filled with soil containing *Aporrectodea caliginosa* without microarthropods. $Ap._2$; experimental units filled to its half with soil containing *Aporrectodea caliginosa* without microarthropods. $Es._1$; experimental units filled with soil containing *Eisenia fetida* without microarthropods, Es.2; experimental units filled to its half with soil containing *Eisenia fetida* without microarthropods. And WOE_1; experimental units without earthworms filled to its half with soil without microarthropods. M_1, M_2, and WOE_2 sets are experimental units filled to their halves with soil without extraction of soil microarthropods. A representative diagram for experimental design is shown in Figure (1). After an additional two months, the number of earthworms within each set was counted. In addition, soil microarthropods were extracted from $Ap._2$, $Es._2$, and WOE sets using modified Berlese-Tullgren funnels. Under the microscope, oribatid mites were separated from other microarthropods, preserved, and identified at the species level (28).

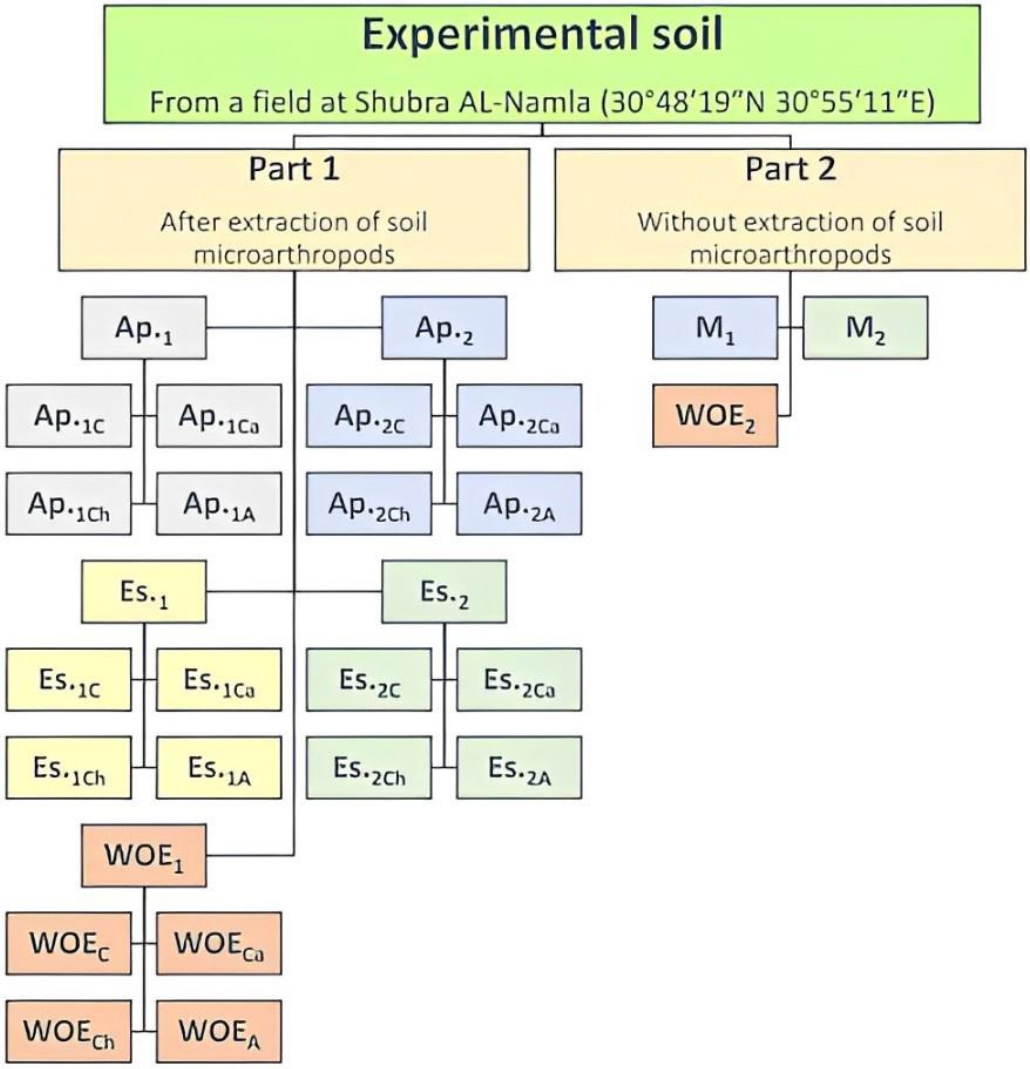

Figure (1) Shows the layout of treatments used in this experiment design. Ap.$_1$; experimental units filled with soil containing *Aporrectodea caliginosa* without microarthropods. Ap.$_2$; experimental units filled to its half with soil containing *Aporrectodea caliginosa* without microarthropods. Es.$_1$; experimental units filled with soil containing *Eisenia fetida* without microarthropods, Es.2; experimental units filled to its half with soil containing *Eisenia fetida* without microarthropods. And WOE$_1$; experimental units without earthworms filled to its half with soil without microarthropods. M$_1$, M$_2$, and WOE$_2$ sets are experimental units filled to their halves with soil without extraction of soil microarthropods. Alternative experimental units within sets that share the same colors are mixed after the first 2 months of the experiment. Treatments are indicated by capital letters: A = Aldicarb, Ch = Chlorpyrifos, Ca = Carbofuran, and C = control (water only).

Statistical analysis

To minimize the occurrence of zeroes, the data for the six experimental units within the same set (treatment) were added together. To detect the impact of different pesticides on earthworms' abundance at day 60, differences in the number of earthworms per treatment were tested using a Kruskal–Wallis test. If significant differences were detected between treatments, (38) a procedure for multiple comparisons was performed to detect pairwise differences.

To meet the assumptions of normality, the numbers of individual oribatid mites/treatment (x) were transformed to log (x+1). Two-way analysis of variance (ANOVA) was done to test the impact of earthworm and pesticide application on total oribatid abundance. If significant differences were detected, Tukey's method for multiple comparisons was used to detect all pairwise differences between different treatments. Principal component analysis (PCA) was performed to assess the relationship between the oribatid community composition, the presence, and type of earthworms, in addition to different pesticides applied in an ordination plot, using PAST, V4.08 (39).

Oribatids diversity was measured using Simpson's index (D), equitability (J), an index sensitive to changes in dominance structure in addition to Shannon-Wiener diversity index (H), and Shannon equitability (E), an index sensitive to changes in rare species (29). Qualitative and quantitative Sorensen's similarity indexes between different treatments were assessed (40), and the scheme concerning the dominance classification denoted by (41) was followed.

Results

1. Total abundance of earthworms

At the beginning of the experiment, 60 individuals of *Aporrectodea caliginosa* and *Eisenia fetida* were introduced/treatment; i.e. 10 individuals/experimental unit. At the end of the experiment (day 60), $Ap._1$ ranged from 49±0.6 to 56±0.4 and $Ap._2$ ranged from 49±0.7 to 55±0.5 in aldicarb and control treatments, respectively. Whereas, the total abundance of $Es._1$ ranged from 50±0.5 to 58 ±0.3 and $Es._2$ ranged from 51±0.6 to 57±0.3 in aldicarb and control treatments, respectively Table (1). Kruskal–Wallis test indicated a statistically significant difference in the total abundance of $Es._1$ between treatments Table (1). Pairwise comparisons revealed a statistically significant difference between $Es._{1C}$ and $Es._{1A}$ treatments ($p<0.017$). Statistically significant differences were not detected between treatments in groups $Ap._1$, $Es._2$, and $Ap._2$ Table (1).

2. Total abundance and species composition of oribatid mites

Nine species of oribatid mites belonging to nine genera and eight families were extracted from the investigated treatments in Table (2). Total abundance at the start of the experiment was 65±0.95. At the end of the experiment, total abundance in the control plots ranged from 33±0.76 to 55±0.60 individuals/treatment in $Ap._{2C}$ and $Es._{2C}$, respectively Figure (2). Total abundances of oribatid mites in different treatments were generally lower than the controls. Two-way analysis of variance (ANOVA) indicated that earthworm and pesticide application significantly impacted total oribatid abundances. Post-hoc analysis revealed that pesticide application decreased total abundance, on the contrary, total abundances were significantly higher in Es. than Ap. and WOE groups.

Regarding species composition, the most abundant species were *Scheloribates laevigatus, Zygoribatula undulata,* and *Lamellobatus h. aegypticus, Rhysotritia a. ardua,* and *Lohmania hispaniola*. *Scheloribates laevigatus* was eudominant in control plots in nearly all pots, regardless of the treatment. *Zygoribatula undulata* and *Lamellobatus h.aegypticus* were dominant in the majority of plots. *Rhysotritia a. ardua* and *Lohmania hispaniola* showed different dominances across treatments. However, they were subdominant in about half of the treatments.

Principal component analysis (PCA) Figure (3) clearly showed that the two dimensions explained 70.27% of the total variation in species abundances,

and indicated clear partitioning of control and aldicarb treatments from other treatments. Two main groups could be distinguished from the PCA biplot; the first group includes *Scheloribates laevigatus*, *Lohmania hispaniola*, *Zygoribatula undulata*, *Cillioppia magnus*, and *Anachipteria aegyptiaca*. They were positively correlated with control groups. ie., Ap.$_{C}$ and WOE$_{C}$. However, this group showed a negative response to Es.$_{Al}$, WOE$_{Al}$, WOE$_{ca}$, and WOE$_{Ch}$ treatments. The second group includes *Lamellobatus h. aegypticus*, *Rhysotritia a. ardua*, and *Xylobatus capucinus*. this group positively responded to Ap.$_{Ch}$, Es.$_{Ca}$, Es.$_{Ch}$, and Es.$_{C}$ treatments. However, these species were negatively correlated with Ap.$_{Ca}$ and Ap.$_{Al}$. *Tectocepheus sarekensis* was strongly correlated with Ap.$_{Ca}$ and Ap. $_{Al}$ treatments. Moreover, *Scheloribates laevigatus*, *Lamellobatus h. aegypticus*, *Zygoribatula undulata*, *Lohmania hispaniola*, and *Rhysotritia a. ardua* had moderate to strong relationships with each other.

3. Species diversity of oribatid mites

Two diversity indices (Simpson's D and Shannon's H′) were used to detect the impact of pesticide application and the presence of earthworms on the oribatid community Table (3). The number of species decreased from 9 at the beginning of the experiment to range from 5 to 6 in the aldicarb treatment. Simpson's D and Shannon's H′ in the majority of treatments tended to decrease in comparison to the controls. At the start of the experiment Shannon's diversity index (H') reached 1.91 and evenness (E) was 0.86, whereas Simpson's index (D) reached 6.25 and equitability (J) was 0.69. At the end of the experiment. The highest H' values were recorded in WOE$_{C}$, Es.$_{C,}$ and Ap.$_{Ch}$ treatments (1.81, 1.68, and 1.57, respectively), whereas the lowest values were recorded in aldicarb treatment (1.34, 1.34, and 1.28) in WOE$_{Al}$, Es.$_{Al}$ and Ap.$_{Al}$, respectively. Similarly, the highest D values were detected in control treatments (5.91, 5.26, and 5.15) in WOE$_{C}$, Ap.$_{C,}$ and Es.$_{C}$, respectively. On the other hand, the lowest D values were recorded in WOE$_{Al}$, Es.$_{ca}$, and Ap.$_{Al}$; 3.34, 3.33, and 2.85, respectively. Equitability (J) and evenness (E) values showed differences across different treatments Table (3).

4. Effects on community similarity

Generally, qualitative similarities between treatments were higher than quantitative similarities. The similarity between oribatid communities across different pesticide treatments was higher between Es. treatments than other treatments Table (4). Qualitative similarities between Es.$_{C}$ & Es.$_{Ch}$ treatments, Es.$_{C}$ & Es.$_{Ca}$ and Es.$_{Ca}$ & Es.$_{Ch}$ were the highest of all (Qs= 1). Additionally, the highest quantitative similarity was detected between Es.$_{Ca}$ & Es.$_{Ch}$ (CN= 0.87). On the other hand, the lowest qualitative similarity was recorded between Ap.$_{Ch}$& Ap.$_{Al}$ (Qs= 0.61), whereas the lowest quantitative similarity was detected between WOE$_{C}$ & WOE$_{Ca}$ (CN=0.54).

Table (1): Abundance of earthworms/treatment at the end of the experiment (Abundance±SE)

	Control	Aldicarb	Carbofuran	Chlorpyrifos	$\chi2_{(3)}$	p
Ap.$_{1}$	56±0.4	49±0.6	50±0.7	50±0.6	6.59	0.09 n.s.
Ap.$_{2}$	55±0.5	49±0.7	50±0.7	50±0.5	3.12	0.37 n.s.
Es.$_{1}$	58±0.3	50±0.5	52±0.5	53±0.5	10.19	0.017*
Es.$_{2}$	57±0.3	51±0.6	53±0.6	54±0.6	5.53	0.14 n.s.

$\chi2_{(3)}$: Kruskal–Wallis test statistic (3 degrees of freedom), p: significance level. **Ap.$_{1}$:** *Aporrectodea caliginosa* After extraction of soil microarthropods**, Es.$_{1}$**: *Eisenia fetida* After extraction of soil microarthropods. **AP.$_{2}$**: *Aporrectodea caliginosa* without extraction of soil microarthropods, **Es.$_{2}$**: *Eisenia fetida* without extraction of soil microarthropods. n.s., non-significant, *p < 0.05, significant.

Table (2): List of species of oribatid mites, their relative contribution, and dominance classification at different treatments

Species	IC	Day 60											
		Control			Chloropyfiros			Carbofuran			Aldicarb		
		WOE	Es.	Ap.	WOE	Es.	Ap.	WOE	Es.	Ap.	WOE	Es.	Ap.
Rhysotritia ardua	7.69 C	9.52 C	12.72 B	3.03 D	14.28 B	9.09 C	4 D	11.76 B	6.45 C	-	5 C	7.14 C	8.69 C
Lohmannia hispaniola	10.76 B	7.14 C	7.27 C	9.09 C	4.76 D	3.03 D	4 D	5.88 C	3.22 D	5.55 C	10 B	7.14 C	-
Tectocepheus sarekensis	6.15 C	2.38 D	-	6.06 C	-	-	-	-	-	11.11 B	-	-	4.34 D
Cillioppia magnus	3.07 D	4.76 D	-	6.06 C	-	-	-	-	-	-	-	-	4.34 D
Scheloribates laevigatus	27.69 B	26.19 B	30.9 A	36.36 A	47.61 A	42.42 A	44 A	29.41 B	48.38 A	50 A	45 A	42.85 A	56.52 A
Zygoribatula undulata	16.92 B	21.42 B	20 B	15.15 B	9.52 C	21.21 B	20 B	17.64 B	9.67 C	16.66 B	15 B	14.28 B	13.04 B
Xylobates capucinus	3.07 D	2.38 D	5.45 C	-	4.76 D	3.03 D	8 C	11.76 B	6.45 C	-	-	-	-
Lamellobates hauseri aegypticus	16.92 B	19.04 B	20 B	15.15 B	19.04 B	18.18 B	12 B	17.64 B	22.58 B	11.11 B	25 B	28.57 B	13.04 B
Anachipteria aegyptiaca	7.69 C	7.14 C	3.63 D	9.09 C	-	3.03 D	8 C	5.88 C	3.22 D	5.55 C	-	-	-

WOE: without earthworms, Es.: *Eisenia fetida*, Ap.: *Aporrectodea caliginos*, IC: Initial control (Day 0), %; relative dominance in community; dominance class is indicated by capital letters, A eudominant: over30% of individuals, B dominant; 10-30% of individuals, C sub-dominant;5-10%of individuals, and D minor;1-5% of individuals (The relative dominance of each species was classified according to Engelmann 1978).

Table (3): Species diversity and equitability values of soil oribatid mites in different treatments

Diversity index	IC	Day 60											
		Control			Chlorpyfiros			Carbofuran			Aldicarb		
		WOE	Es.	Ap.	WOE	Es.	Ap.	WOE	Es.	Ap.	WOE	Es.	Ap.
Species richness	9	9	7	8	6	7	7	7	7	6	5	5	6
D	6.25	5.91	5.15	5.26	3.47	3.81	3.84	5.88	3.33	3.28	3.34	3.54	2.85
J	0.69	0.65	0.73	0.65	0.57	0.54	0.54	0.84	0.47	0.54	0.66	0.708	0.47
H	1.91	1.81	1.68	1.44	1.38	1.51	1.57	1.71	1.42	1.39	1.34	1.34	1.28
E	0.86	0.82	0.86	0.69	0.77	0.77	0.8	0.87	0.72	0.77	0.83	0.83	0.71

IC: Initial control (Day 0), D: Simpson's index, J: Equitability, H: Shannon- wiener index, and E: Evenness, WOE: without earthworms, Es.: *Eisenia fetida*, Ap.: *Aporrectodea caliginosa*.

Table (4): Qualitative and quantitative similarities between different treatments within WOE, Es. And Ap. Groups

Comparison	Qs	CN	Comparison	Qs	CN	Comparison	Qs	CN
WOE_C & WOE_{Ch}	0.8	0.6	$Es._C$ & $Es._{Ch}$	1	0.75	$Ap._C$ & $Ap._{Ch}$	0.8	0.79
WOE_C & WOE_{Ca}	0.87	0.54	$Es._C$ & $Es._{Ca}$	1	0.72	$Ap._C$ & $Ap._{Ca}$	0.85	0.7
WOE_C & WOE_{Al}	0.71	0.64	$Es._C$ & $Es._{Al}$	0.83	0.67	$Ap._C$ & $Ap._{Al}$	0.85	0.75
WOE_{Ca} & WOE_{Ch}	0.92	0.73	$Es._{Ca}$ & $Es._{Ch}$	1	0.87	$Ap._{Ca}$ & $Ap._{Ch}$	0.76	0.74
WOE_{Al} & WOE_{Ca}	0.83	0.7	$Es._{Al}$ & $Es._{Ca}$	0.83	0.84	$Ap._{Al}$ & $Ap._{Ca}$	0.66	0.73
WOE_{Ch} & WOE_{Al}	0.9	0.82	$Es._{Ch}$ & $Es._{Al}$	0.83	0.81	$Ap._{Ch}$ & $Ap._{Al}$	0.61	0.75

WOE: without earthworms, Es.: *Eisenia fetida*, Ap.: *Aporrectodea caliginos*a, and different pesticides applied (C: control, Ca: carbofuran, Ch: chloropifyros and Al: aldicarb).

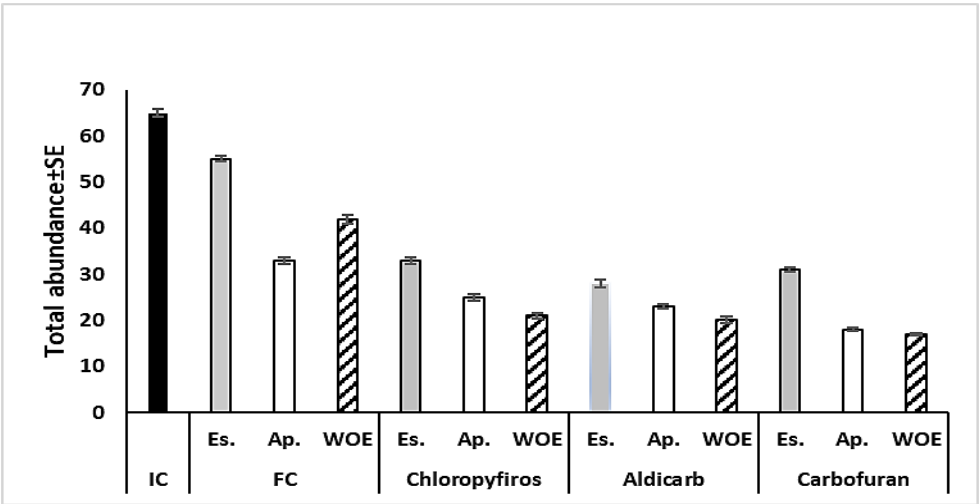

Figure (2): Total abundance of soil oribatid mites at the beginning and end of the experiment across different treatments, IC: initial control Day 0 (at the beginning of the experiment), FC: final control (at the end of the experiment), Es.: *Eisenia fetida*, Ap.: *Aporrectodea caliginosa* and WOE: without earthworms.

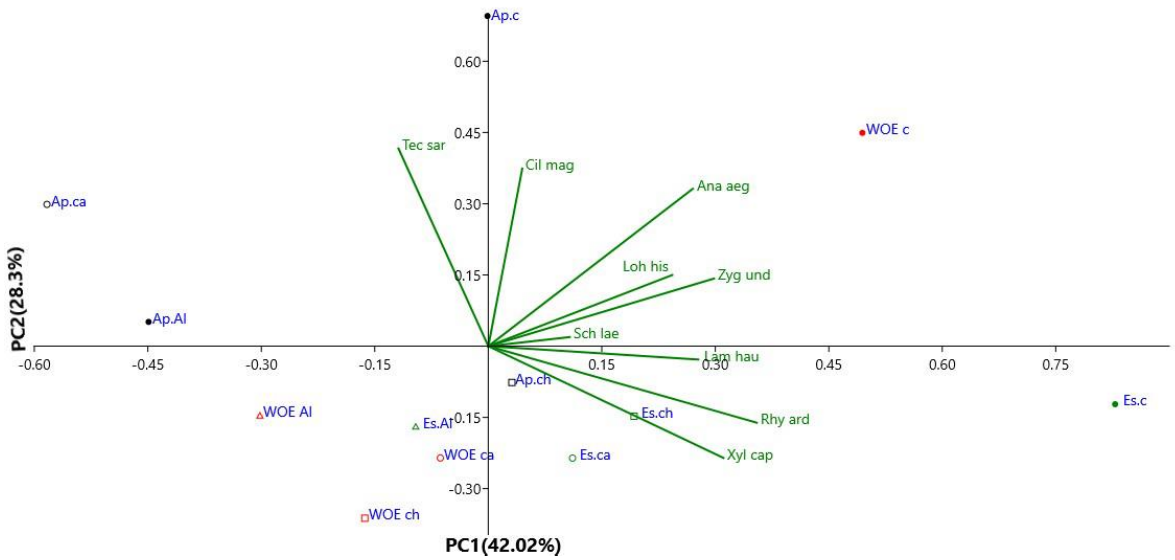

Figure (3): Biplot of the principal component analysis (PCA) for oribatid community composition (Sch lae: *Scheloribates laevigatus*, Zyg und: *Zygoribatula undulata*, Lam hau: *Lamellobatus h. aegypticus*, Rhy ard: *Rhysotritia ardua*, Loh his: *Lohmania Hispaniola*, Cil mag: *Cillioppia magnus*, Ana aeg: *Anachipteria aegyptiaca*, Xyl cap: *Xylobates capucinus* and Tec sar: *Tectocepheus sarekensis*), the presence and species of earthworms (WOE: without earthworms, Es.: *Eisenia fetida*, Ap.: *Aporrectodea caliginos*), and different pesticides applied (C: control, Ca: carbofuran, Ch: chloropifyros and Al: aldicarb).

Discussion

Over the past years, the unsystematic use of pesticides has negatively impacted the soil ecosystem. Alongside this, vermiremediation is a promising eco-friendly strategy to restore soil quality. From this perspective, the present study aimed to evaluate the potential of two earthworm species in the vermiremediation process, using soil oribatid mites as a bioevidence to investigate soil quality.

The present study revealed that, despite the application of sub-lethal concentrations of chosen pesticides, aldicarb induced significant declines in *E. fetida* individuals. Additionally, significant decreases were only detected in Es.$_1$, which may indicate that the presence of mites in Es.$_2$ may buffer such declines. Similar findings were reported by (42) who found that the carbamate insecticide aldicarb is a big threat to soil organisms.

The present study showed that the highest abundances of oribatid mites were observed in control treatments. The effect of pesticides on the abundance of oribatid mites may be due to the direct or indirect toxicity of pesticides to the mites. Negative effects of pesticides on mites as non-target organisms were also reported (29,43,44). Concerning different pesticides, no significant differences were detected between them, which may be due to the use of sub-lethal concentrations and differences in the response of individual species. On the contrary, total abundances were significantly higher in Es. groups than in Ap. and WOE groups. Many oribatid mites are fungivorous. Consequently, they might compete with edaphic earthworm species such as *Aporrectodea caliginosa*, thus decreasing oribatids' abundance (45,46,47). On the other hand, epigeic earthworms such as *Eisenia fetida* feed on plant litter and humus materials at the soil surface, therefore oribatid mite abundance tends to increase instead (46,48).

Responses of oribatid mites to soil pollution vary greatly according to the species (49,50,51,52). Besides, species-specific responses were reported in pesticide studies. For instance, some oribatid species were detected as pesticide-responsive such as *Scheloribates laevigatus* and *Zygoribatula exarata*, while others were found to be non-responsive to pesticides such as *Rhysotritia a. ardua* (29,53). In this study, *Scheloribates laevigatus*, *Lohmania hispaniola*, *Zygoribatula undulata*, *Cillioppia magnus*, and *Anachipteria aegyptiaca* showed a positive correlation with control groups, as a result, they could be described as pesticide-responsive. In addition, the same species showed a negative correlation to WOE groups, which may indicate their tendency toward treatments containing earthworms. (54) found that *Scheloribates laevigatus* and *Zygoribatula undulata* are positively affected by the presence of earthworms. Other species, such as *Lamellobatus h.aegypticus*, *Rhysotritia a. ardua*, and *Xylobatus capucinus* positively responded to Es. treatments, which may reflect a tendency toward the presence of *Eisenia fetida*. (55) indicated that soil fauna are affected by biotic and abiotic changes in soil induced by earthworm activities. However, this may increase or decrease the abundance of certain species depending on their capability to adapt to the structures created by different earthworms.

The present study showed that species richness and diversity indices were higher in control treatments than in different pesticide treatments. Pesticide use negatively affects soil diversity, even when applied at recommended rates, which may indicate species-specific responses that lead to decreases in the abundance of sensitive species from pesticide treatments (51,56,57). Under pesticide application, the community structure of oribatid mites became more biased toward specific species (29). However, it is impossible to separate the responses of different species to diverse pesticides (58). Furthermore, we found that quantitative similarities between treatments were lower than qualitative similarities. Similar results were obtained by (59), as she demonstrated that pollution had a greater effect on the number of individuals than on the number of species, which may reflect differences in species composition and community structure.

Conclusion

pesticides especially aldicarb had a negative effect on the abundance and diversity of soil oribatid mites even when applied at sub-lethal concentrations. Responses of oribatid mites to pesticide application varied according to the species. *Scheloribates laevigatus*, *Lohmania hispaniola*, *Zygoribatula undulata*, *Cillioppia magnus*, and *Anachipteria aegyptiaca* were pesticide responsive. In addition, they preferred the presence of earthworms. *Lamellobatus h. aegypticus*, *Rhysotritia a. ardua*, and *Xylobatus capucinus* tended the presence of *Eisenia fetida*. In addition, the presence of *Eisenia fetida* has increased the abundance of oribatid mites, which could increase soil health, therefore enhancing crop production. Thus, increasing abundance may be accepted as a parameter in assessing the efficacy of *Eisenia fetida* in the vermiremediation process. Accordingly, the present study confirmed the potential use of the soil oribatid mite population as a bioevidence of the functional role of the earthworm *Eisenia fetida* as a biodegradator of pesticides in soil. However, *Aporrectodea caliginosa* did not show significant activity as a bioremediator.

Ethics Approval

All protocols used in this study were approved by the Faculty of Science Ethics Committee, Tanta University, Egypt (Code: IACUC-SCI-TU-0429).

Conflict of Interest

The authors declare that they have no conflict of interest.

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