ECOTOXICOLOGICAL EFFECTS OF POTASSIUM NITRATE FERTILIZER ON MORTALITY, GROWTH RATE AND MORPHO-HISTOLOGICAL LEVELS OF *Aporrectodea trapezoides* (Dugès, 1828) EARTHWORM

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Abstract. The present study was conducted to assess the potential hazards of potassium nitrate (NK) fertilizer on the adult earthworm *Aporrectodea trapezoides* (Dugès, 1828) as a bioindicator animal test of soil pollution. The research was based mainly on two complementary toxicity tests in order to point out the 50 LC values then the adverse effect on growth rate and morpho-histological alterations in worms. Four increasing concentrations for the lethality test ranged from 2500 to 5500 mg/kg of dry soil weight (d.w.) of potassium nitrate fertilizer. Therefore, the sub-lethal's concentrations were determined based on 10%, 30%, 40%, and 50% of 14 days' median lethal concentration (LC50), with a control set for both tests. The 50 LC values of NK fertilizer were recorded relatively higher at 7 days than at 14 days of exposure, with 5530.43 and 4955.70 mg/kg d.w., respectively. Significant inhibitory effects on the growth rate of earthworms were found under exposure to various concentrations and time exposures. Morphological deformations were also accentuated with high concentrations, namely: clitellar swelling, bloody lesions, yellowish fluid, body strangulation, and fragmentation, as major morpho-pathological changes in worms. While the histopathological manifestations included epidermal layer injuries, vacuolated body walls, and circular and longitudinal muscles were disintegrated completely with increasing concentrations of fertilizer. The bioindicator *A. trapezoides* earthworm displayed a worthwhile biological control agent against the toxic salinity of potassium nitrate fertilizer with a strong concentration and exposure time-response relationship. The present work is noteworthy for being used in the biological control of environmental risk assessment of chemical fertilizers and then as biomarkers to evaluate soil toxicity.

Key words: earthworm; biomonitoring; potassium nitrate; chemical fertilizer, *Aporrectodea trapezoides,* ecotoxicology

INTRODUCTION

The usage of agrochemical products in excessive amounts, such as pesticides and chemical fertilizers, on agricultural land in order to increase crop yields thereafter meets the growing global food demand [3, 12]. However, the excessive usage of such agrochemicals adversely affects the soil ecosystem [14, 25] by impairing the physical, chemical, and biological components, especially the non-target beneficial microorganisms and earthworms [9]. Due to their valuable role in agroecosystems and the assessment of soil quality [18], earthworms are the major soil macro-fauna and suitable indicator species for monitoring the impact of pollutants, changes in soil structure, and agricultural practices [11]. They act as aerators, grinders, crushers, chemical degraders, and biological stimulators [10]. As a result to the surrounding environmental stressors of soil (such as pesticides, heavy metals, and organic/ inorganic fertilizers) the earthworms' sensitivity are not limited only to their strong direct or indirect interactions with soil. However, since the existence of chemoreceptors and sensory tubercles on earthworms' bodies confers them the ability to detect a wide range of soil contaminants [30]. In light of the importance of earthworms in the dynamics of soil structure, little data is known associating earthworms with inorganic fertilizers. In the literature, some researchers have investigated the positive effect of fertilizers on earthworms and their populations [7]. On the other side, others emphasized the harmful effect of chemical fertilizers on soil organisms [4, 36]. Simplicio *et al.* (2017) [34] investigated that the terrestrial snail (*B. glabrata*) was more sensitive to potassium nitrate fertilizer than zebrafish (*D. rerio*) based on their 96-hour 50 LC values. In a recent study, Halaimia *et al.* (2021) [13] pointed out the severe impact of NPK fertilizer on juvenile earthworms of *A. caliginosa*. The mortality was scored at four weeks, with high inhibitory effects on the growth rate. Simultaneously, Abbiramy and Ross (2013a, b), Bhattacharya and Sahu (2016) [1, 2, 5] performed the same study unless the paper contact method was applied. Therefore, an understanding of the influence of inorganic fertilizers on earthworms' endpoints is extremely required to clarify these discrepancies for advancing sustainable agriculture and, after that, predicting soil pollution. The present study aimed fundamentally to (1) determine the median lethal concentrations (CL50) of NK fertilizer and (2) examine the ecotoxicological sublethal effects of this fertilizer on the endogeic earthworm *Aporrectodea trapezoides* endpoints, such as growth rate inhibition and morpho-histological deteriorations. To the best of our knowledge, this study constitutes the first baseline data to be used in ecotoxicology biocontrol and risk assessment of potassium nitrate fertilizer on the widely dominant earthworm in Algerian soil, *A. trapezoides*, filling up some gaps in this subject's knowledge.

MATERIALS AND METHODS

Soil and earthworm collection

Soil and adult earthworm sampling were collected in an upland non-irrigated paddy field that had no record of input of agrochemicals (since five years at least) from the Ain Ben Beida region, located in the northeast of Guelma province (36°28'11.05" N, 7°25'42.89" E) of Algeria. Based on its predominance in agricultural land, *Aporrectodea trapezoides* earthworm was adopted as a test specimen with an average weight between 0.700 g and 1.800 g. All earthworms were kept in their native soil for two weeks of adaptation under laboratory conditions, with uncontaminated cow dung added to provide food before the tests started. The soil sample was air-dried for five days, homogenized, and sieved through a 2 mm mesh to remove stones, roots, and other large particles prior to its use in the test. The main physico-chemical characteristics of the soil were carried out in the laboratory of pedological analysis of the Algerian Fertilizer Company (Annaba's FERTIAL), as shown in Table 1.

Chemical product

The tested inorganic fertilizer of the experiment was largely commercialized in Algeria and is potassium nitrate (13-0-46) fertilizer, which was purchased from Guelma's regular fertilizer distributors. The fertilizer solutions' were prepared in deionized water on the day of the experiments and used immediately.

Experimental set-up

Lethal test

The acute toxicity experiments were carried out according to OECD Guideline-207 (Organization for Economic Co-operation and Development; OECD, (1984)) [27]. The acute bioassay consisted of three replicates for each concentration, with 10 adult earthworms in each replicate. 1 kg of natural soil is placed into 5 L containers with a perforated cover to maintain aeration. Potassium nitrate fertilizer was added as the following concentrations: 2500, 3500, 4500, and 5500 mg/kg of dry soil weight (d.w). Parallelly, a control set was applied only with distilled water. The fertilizer solutions were spiked by simple pulverization and gently mixed, allowing a homogeneous distribution of the product. Then 250 mg (only on the first day of the experiment) of cow dung was added to each container in order to avoid their starvation. The test was performed for a 14-day treatment period, and the number of dead earthworms per group was recorded on days 7 and 14 after the start of the experiment. Earthworms were considered dead when they did not react to any mechanical stimulus in the anterior region.

Sub-lethal test

The sub-lethal toxicity test was performed according to OECD (2004) [28] guideline 222 in triplicate with 10 adult earthworms in each replicate and 1 kg of natural soil substrate introduced into 5 L plastic containers. The sub-lethal concentrations of potassium nitrate fertilizer were determined based on 10%, 30%, 40%, and 50% of 14 days' median lethal concentration (LC50) value. In parallel, control series were also run with only distilled water. The natural soil substrate was spiked with the fertilizers' solutions by simple pulverization. Throughout the exposures, earthworms were fed weekly 7 g of fine cow dung (dried and rewetted to 80% moisture content) during the fourth week of the exposure period.

Growth inhibition rate

The biomass of tested earthworms was recorded on the 1st day, then at the 2nd and 4th weeks of the incubation period. The earthworms were first removed, washed with distilled water, dried, and weighed using an electro-balance (Ohaus), and after that returned to the soil. In order to determine the worm's growth inhibition rate (%) from various exposure periods, the following equation of Shi *et al.* (2007) [33] was adopted:

$$GIn = (W0 - Wt) / W0 \times 100\%$$

where: GIn (%) represents the growth inhibition rate, W0 represents the average weight of earthworms at the initial day, and Wt represents the average weight after n days of exposure.

Morphological study

Qualitative evaluation of morphological alterations and skin damage in earthworms was recorded only at the end of the sub-lethal toxicity test.

Histological study

Control and treated earthworms were collected randomly over the 28-day exposure period. Therefore, transverse sections from post-clitellar regions were performed. The histological study was carried out according to Hould and de Shawinigan's (1984) [15] method. The samples were examined by an optical microscope (X 100) of the OPTICA type (Italy) equipped with a digital camera (Optika ISview).

Statistical Analysis

The normality of the data was verified using the Kolmogorov-Smirnov test, and the homogeneity of variances was checked by Levene's test. The 7- and 14-days 50 LC values were estimated by probit analysis through the concentration-response relationship of *A. trapezoides* earthworm under exposure to potassium nitrate fertilizer. Earthworms' growth rates were determined in triplicate, and the data were analyzed using a two-way ANOVA, followed by a post hoc analysis using Fisher's least significant difference (LSD) test, where $p < 0.05$ indicates a statistically significant difference, using SPSS (version 20.0; SPSS, Chicago, IL, USA).

RESULTS

Effects on the mortality rate

The present investigation found that there was a notable variation in mortality (%) of adult *A. trapezoides* earthworms with respect to the tested inorganic fertilizer and at different exposure times. The mortality (%) of earthworms increased gradually with increasing concentrations and exposure times. It was started at 3500, 4500, and 5500 mg/kg of soil d.w. with 30, 53, and 93% at 7 days, parallel to 50, 60, and 100% at 14 days, respectively. With exception to the low concentration of 2500 mg/kg of soil d.w., where no mortality was recorded at both 7 and 14 days (Fig. 1). On the other side, the concentration-response test recorded no mortality in the control series during all exposure times. The 50 LC values of potassium nitrate fertilizer were recorded relatively higher at 7 days than at 14 days of exposure, with 5530.43 and 4955.70 mg/kg d.w., respectively (Tables 2 and 3).

Table 1. The physico-chemical characteristics of the soil

Properties	Results
pH	7.34 ± 0.01
K	0.17 ± 0.2 (meq/100 g)
Na	1.57 ± 0.05 (meq/100 g)
Mg	2.68 ± 0.34 (meq/100 g)
Ca	34.93 ± 0.15 (meq/100 g)
P	1.80 ± 0.23 ppm
N	0.150 ± 0.65 %
Total Carbone	7.5 ± 0.04 %
Electrical Conductivity	0.16 ± 0.54 mS/cm
organic matter	2.060 ± 0.08 %
Texture	Sand 12%; silt 76%; Clay 12%

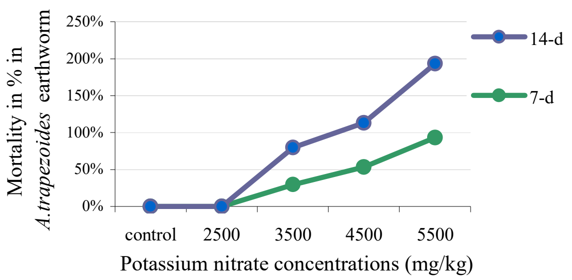

Figure 1. Mortality percentages of *Aporrectodea trapezoides* earthworm under exposure to potassium nitrate fertilizer in the acute toxicity test

Effects of potassium nitrate (NK) fertilizer on the growth of worms

In our study, a two-way ANOVA test was conducted to examine the effects of potassium nitrate concentrations on earthworms' growth rates at different exposure times. There was a significant main effect of NK concentrations on earthworms' lowering growth rate (F4,15 = 4.89; p = 0.027). The main effect of time was also significant (F2,15 = 5.15; p = 0.036).

The growth rates decreased highly significantly with the increasing concentrations of potassium nitrate at the following concentrations: concentration 10% versus control (p = 0.006), concentration 30% versus control (p = 0.025), concentration 40% versus control (p = 0.007), and concentration 50% versus control (p = 0.008), with no significant concentration relationship. In addition, the fertilizer showed an inhibitory effect on the gain of weight, with a significant time-relationship at the 1st day versus the 14th day (p = 0.051) and a high level of significant growth inhibition between the 1st day and the 28th day (p = 0.015). However, the interaction between potassium nitrate concentrations and time exposure had highly significant effects on earthworms' growth rate decrease, F7,15 = 102.45 (p = 0.001), indicating that the effect of potassium nitrate concentrations on earthworms' growth rate was significantly greater at the 28th day than at the 14th day compared to the 1st day and control series.

According to Shi *et al.*'s (2007) [33] equation, the growth inhibition of earthworms was positive for all concentrations and exposure weeks, which means that the earthworms were losing weight compared to the negative growth inhibition of the control group. The growth inhibition rate of treated groups for all concentrations at the 2nd week showed an upward trend compared to the 4th week, which means that the earthworms lost more weight at the 4th week than at the 2nd week (Fig. 2). Indicating that the longer the exposure time, the more self-repairing function and anti-damage mechanisms of *Aporrectodea trapezoides* decreased.

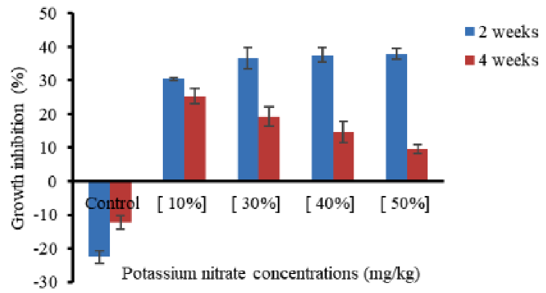

Figure 2. Growth inhibition rates (%) of *Aporrectodea trapezoides* after exposure to different concentrations of potassium nitrate fertilizer (mean± SD, n = 3 repeats, with 10 individuals for each one).

Morphologic alteration

Morphological changes were assessed on the 28th day. Intact bodies in the control series and at the low concentration of 10% of 14 days' 50 LC value were clearly noticed. *A. trapezoides* earthworm displayed many abnormal symptoms, including secretion of mucous, bleeding with yellowish fluid evacuation, and whole body coiling, in addition to body fragmentation, more tissue damage, and body wall dissolved in the form of pulp when exposed to different concentrations of potassium nitrate fertilizer. Moreover, the worms in these containers remained at the bottom, and a lower number of castings was observed compared to the control series and low treatment groups (10%). These anomalies are highlighted by the increasing concentrations (Fig. 3).

Histology

The histopathological changes of earthworms' body wall are illustrated in (Fig. 4). Transverse sections of untreated earthworms showing normal intact architecture with well-arranged circular and longitudinal muscles and epidermal layer. At 10 % of 14 days' 50 LC value of potassium nitrate, *A. trapezoides* showed an occasional erosion of epidermis and circular muscle layer. The earthworm *A. trapezoides*, under exposure to 30% of 14 days' 50 LC of potassium nitrate, displayed severe breakdown of the body wall with the appearance of blood vessels in the internal side of the longitudinal muscle layer. While 40% of 14 days' 50 LC of potassium nitrate showed necrosis and cleavage of the longitudinal muscle layer with huge disarrangements. The damage caused by fertilizer was much greater in the case of higher concentration (50% of 14 days' 50 LC), which indicated a consistent decline in the amount of connective tissue with huge exfoliation of circular and longitudinal muscle layers from each other, leading to large vacuoles along the body wall.

Table 2. Lethal concentrations (LC 10, LC25, LC50, LC90) of potassium nitrate fertilizer on earthworms after 7-days of exposure

Exposure conc. mg/kg	Total test worms	No. of dead worms replicate			Total No. of dead worms	% Mortality	LC_{10} (mg/kg)	LC_{25} (mg/kg)	LC_{50} (mg/kg)	LC_{90} (mg/kg)
		1	2	3						
0	30	0	0	0	0	0				
2500	30	0	0	0	0	0				
3500	30	1	5	4	10	30	3633.44	4483.67	5530.43	8414.13
4500	30	4	6	6	16	53.33				
5500	30	9	10	9	28	93.33				

During the Probit analysis, Chi-Square value, χ^2, was 7.655 and no heterogeneity factor was observed at 95% confidence limits. The Coefficient of determination value, R^2 was 0.743.

Table 3. Lethal concentrations (LC 10, LC25, LC50, LC90) of potassium nitrate fertilizer on earthworms after 14-days of exposure

Exposure conc. mg/kg	Total test worms	No. of dead worms replicate			Total No. of dead worms	% Mortality	LC_{10} (mg/kg)	LC_{25} (mg/kg)	LC_{50} (mg/kg)	LC_{90} (mg/kg)
		1	2	3						
0	30	0	0	0	0	0				
2500	30	0	0	0	0	0				
3500	30	2	7	6	15	50	3215.97	3992.17	4955.70	7636.58
4500	30	5	7	6	18	60				
5500	30	10	10	10	30	100				

During the Probit analysis, Chi-Square value, χ^2, was 13.789 and no heterogeneity factor was observed at 95% confidence limits. The Coefficient of determination value, R^2 was 0.643.

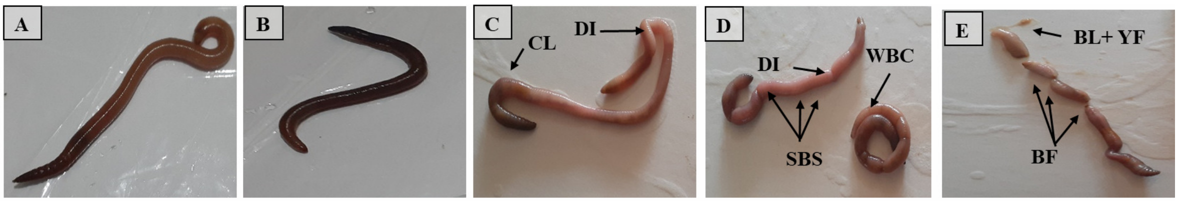

Figure 3. Morphological abnormalities in earthworm *Aporrectodea trapezoides* exposed to different concentrations of potassium nitrate (NK) fertilizer (A-E). (A) Control and (B) 10% of 14 days'50 LC of NK, (C) 30% of 14 days'50 LC of NK, (D) 40% of 14 days'50 LC of NK, (E) 50% of 14 days'50 LC of NK. CL: clitellar swelling, SBS: successive body strangulation, DI: discoloration of the integument, BL: bloody lesions, YF: yellowish fluid, WBC: whole body coiling, BF: body fragmentation.

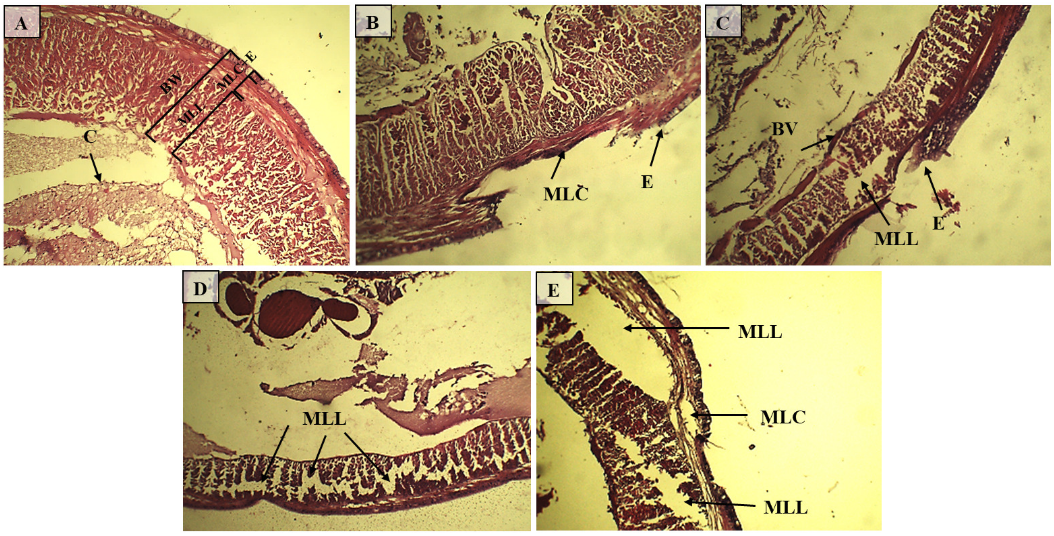

Figure 4. Histopathological changes in the skin of postclitellar region of *A. trapezoides* (Magnification: ×100) under different concentrations of potassium nitrate (NK) fertilizer (A-E). (A) Control and (B) 10% of 14 days'50 LC of NK, (C) 30% of 14 days'50 LC of NK, (D) 40% of 14 days'50 LC of NK, (E) 50% of 14 days'50 LC of NK. E, epidermis; MLC, circular muscle layer; MLL, longitudinal muscle layer; BW, body wall; C, coelom (body cavity); BV, Blood vessel respectively.

DISCUSSION

Agro-ecosystems were heavily polluted due to anthropogenic activities through modern agricultural practices that was emphasized by excessive and continuous usage of fertilizers, namely inorganic fertilizers and pesticides. Soil toxicity tests using different concentrations of potassium nitrate fertilizer were undertaken in order to better understand its reactions on the endogeic earthworm with the natural soil instead of the artificial one, as well as to be closer to real conditions. The concentration-response test found no mortality at the control series during all exposure times for the tested fertilizer, indicating that the natural soil was adequate and considered valid to perform the toxicity tests according to the OECD (1984) [27] guidelines. At first, there were distinct variations in earthworm mortality, which increased steadily with increasing concentrations of potassium nitrate fertilizer and exposure times. Which started from the second concentration on. The toxicological effects of potassium are attributed mainly to the high value of salinity (high pH level with high electrical conductivity), which is accentuated by the interaction of the nitrate (NO_3) compound. The salinization process that emerged from potassium compounds can change the composition of local fauna, which acts indirectly on toxicity [31]. The first low concentration, 2500 mg/kg d.w., was clearly recorded with no mortality at both 7 and 14 days.

Overall, our data indicates that *Aporrectodea trapezoides* has an eco-toxicological tolerance to low salinity (NK), which was emphasized by Jun *et al.*'s (2012) [20] findings that highlighted that in the presence of low soil salinity, *A. trapezoides* could retain reproducing.

Contrary to expectations, some studies demonstrated that the applications of chemical fertilizers with nitrogen and phosphorous caused significant increases in earthworm abundance and biomass in oxisoil [37] and in calcic luvisol [16]. The most likely clarification of this positive correlation between earthworm abundance, biomass, and total nitrogen content in soil is attributed to the strong relationship that exists between inorganic fertilizer with nitrogen, crop growth, and the feeding behavior of earthworms. Turning now to the most interesting aspects of the current study that emerged from Finney's *Probit analysis*. The 50 LC values of potassium nitrate fertilizer were scored relatively higher at 7 days than at 14 days of exposure, with 5530.43 and 4955.70 mg/kg d.w., respectively, indicating that potassium nitrate fertilizer was found to be highly toxic to *Aporrectodea trapezoides* at 14 days than at 7 days of exposure. Our findings on potassium nitrate fertilizer are in line with the results obtained by Dowden and Bennet (1965) [8], as cited in Simplício *et al.* (2017) [34], where the 50 LC value for the aquatic organism *Lepomis macrochirus* was 5500 mg/l. Moreover, the 50 LC of *Daphnia magna* was 490 mg/l [8] and that of *Poecilia reticulate* was 1380 mg/l [32]. Based on the newest findings until now of Simplício *et al.* (2017) [34], who mentioned that the terrestrial snail (*B. glabrata*) was more sensitive to potassium nitrate fertilizer based on the 96-hour 50-LC values that were 102.73 mg/L than the zebrafish (*D. rerio*) with 993.65 mg/L. This discrepancy in sensitivity is attributed mainly to the ecological differences of the various species and their food habitats (ecological groups) in addition to the specific physiological characteristics of each species [26]. Sub-lethal effects such as earthworms' growth inhibition rate are typical indicators for assessing the toxic effects of chemicals [33, 40]. The two-way ANOVA test displayed greater significant inhibitory effects on the gain of weight at 28^{th} days than at 14^{th} day, compared to the 1^{st} day and control series. Moreover, the interaction between fertilizers' concentrations and exposure times was found to be highly significant for earthworms' growth rate decrease. The noticeable earthworms' biomass loss observed in the current study could be due to individual behavioral changes such as feeding rate, which means the worms' inability to directly ingest salinized soil that resulted under exposure to potassium nitrate fertilizer. According to Curry (1994) [6], the biological adaptations and niche problems incite earthworms to starve rather than feed in contaminated soils when the pollutant's concentration exceeds the tolerable limits. Other previous studies revealed accurately that the biomass and size of earthworm populations in agricultural soil could be affected by soil salinity [17, 20]. Inhibited growth and decreased weight have also been reported in earthworms exposed to toxic chemicals by many other researchers [19, 23, 41]. A variety of chemicals show different levels of toxicity potential in different animal models, indicating that no single chemical shows the same toxicity potential.

Morphological changes were assessed only on the 28^{th} day. Morphological alterations such as mucous secretion and bleeding, in addition to successive body strangulation and fragmentation, were observed when exposed to different concentrations of potassium nitrate fertilizer. Surprisingly, only at the low concentration of 10% potassium nitrate were undamaged and healthier worms clearly displayed. Intact bodies were also observed in the control series, revalidating the adaptability of soil samples to perform the toxicity tests. The behavior of earthworms under exposure to chemical substances or xenobiotics represents the indirect effects that will have consequences for soil ecosystem functions. The morphological alterations of *A. trapezoides* earthworms under exposure to fertilizer were accentuated with increasing concentration. These results are in keeping with previous studies by Singh *et al.* (2019) [35], that found almost the same morphological changes in worms exposed to the pesticide triazophos, which are most prominent in mixtures with deltamethrin. Similarly, *Perionyx excavatus* and *Eisenia andrei* displayed abnormal swelling, secretion of mucous, bleeding, and fragmentation when exposed to different xenobiotics such as lead acetate, tetra ethyl lead (TEL), and méthyl-tert-butyl éther (MTBE) [38, 39].

The histopathological changes of earthworms are strongly used to monitor soil pollutants in the environment. The epidermis is the first barrier for earthworms to defend against environmental pollutants. Once it is damaged, soil contaminants may easily enter the organism. The normal skin of earthworms is smooth and contains a certain number of secretory cells, which can secrete mucus conducive to earthworm movement. Various histopathological changes in the earthworm's body wall (epidermis, circular, and longitudinal muscles) have been accentuated in *A. trapezoides* earthworms along with increasing concentrations of potassium nitrate fertilizer. Erosion of the epidermis, injuries to structural muscle integrity, and a vacuolated body wall appeared with huge exfoliation of both circular and longitudinal muscles from each other. Parallel to the control groups, which maintained their normal architecture for the whole body wall. Our findings demonstrated the highly significant toxicity of potassium nitrate fertilizer, which was highlighted with increasing concentrations in terrestrial invertebrates. Qi *et al.* (2018) [29] found that the insecticide cycloxaprid atrophied the longitudinal muscle layer and induced more damage to the earthworm epidermis. Injurious effects on circular and longitudinal muscles in earthworms were also reported after exposure to metals [22]. The histopathological effects may retard the normal functioning of these tissues and, potentially, the homeostasis of the earthworms. Additionally, a study reported that damaged tissues affect earthworms' bioenergetics, which ultimately disturb the overall energy budget, disfunctioning physiological processes like growth and reproduction [21] and eventually leading to death [24].

Overall consequences revealed that there was a wide variation in the toxicity of potassium nitrate fertilizer on *A. trapezoides* earthworms compared to the control series. These results provide new insights in the toxicological context and reveal that the endogeic earthworm *A. trapezoides* is sensitive to soil salinization caused by NK. Moreover, the 50 LC values decreased during exposure time (14 d) for the same fertilizer and became more toxic than before (7 d). Thereby, it displayed a strong concentration and exposure time-response relationship. To our knowledge, this is the first study in which the effects of potassium nitrate fertilizer on the earthworm *A. trapezoides*' mortality, biomass, morphology, and histology endpoints were investigated.

Given the results presented here, it can be concluded that the toxicity of potassium nitrate fertilizer on the endogeic earthworm *Aporrectodea trapezoides* produced evidently strong toxicological effects in the ecotoxicological biocontrol and risk assessment context. Fundamentally, the negative lethal effects on survival, as well as sub-lethal ones such as drastic reductions in biomass and severe morphological and histological injuries, are concentration-dependent. Therefore, they could be used as potential markers to evaluate and predict the degree of residual soil contamination. Our study should encourage future research to investigate other toxicity effects, i.e., biomarkers at the biochemical or bimolecular level, innate immunity, and the eco-physiological differences between different earthworm species.

Abbreviations. NK: Potassium nitrate fertilizer; d.w.: dry weight of soil; LC: Lethal Concentration; ANOVA: Analysis of variance; P: P-Value; SD: Standard error.

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

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