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Effects of Earthworms (*Allolobophora longa*) on Potassium, Zinc and Manganese Availability Enhancement in a Clay Loam Soil

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EARTHWORMS contribute significantly to nutrients availability for plant growth. An incubation soil trial inoculated with adult individuals of earthworm species *Allolobophora Longa* (S^{+AL}) was conducted under controlled conditions to study the role of the earthworm on some nutrients availability in a clay loam soil. The control treatment was conducted without earthworms (S). The experiment period was contained for 90 days. The results showed that the earthworm-inoculated soil reduced soil bulk density by 6.9% and soil reaction (pH) by 0.16 unit. Soil salinity (EC) of the S^{+Al} treatment was higher than that of untreated soil. The treated soil with arthworms was superior in its total N and P content compared to non-treated soil. Considering the role of earthworms in nutrients availability enhancement, there was a significant correlation between the presence of the *A. longa* earthworms and the increase of extractable potassium by 56% compared to the untreated soil. In terms of nutrients availability, the S^{+Al} realized higher levels of the extractable zinc, iron and manganese, except copper, which decreased compared to untreated soil. The opptained results showed positive impact of A. long earthworm on realsing of the soil elements and decompating of soil which are criuial to build a healthy soils. In addition, applying of A. longa earthworm species into the soil could be an alternative approach for releasing nutrients from their pools especially under organic farming.

Keywords: Earthworms, micronutrients, soil characteristics extractable potassium, icubation, soil decompuction.

1. Introduction

Soil is the store of elements. It provides plants and various soil organisms with their nutritional needs. Poor soil characteristics, nutrients deficiency, deterioration of soil biodiversity and mismanagement are common problems that leed to soil degradation. In Egypt where the climate is arid, soil salinization (EC more than 4 $dS.cm^{-1}$), alkalization (pH more than 8.5), high concentration of carbonate, depletion of soil organic matter content and complexation reactions between clay minerals and soil elements are the dominant problems that causing soil degradation (Abdullahi *et al.*, 2023; Allam et al., 2024; El-ramady *et al.*, 2024). However, clay soil has high ability to retain large quantites of nutrients due to the high negative charges on the surfaces of clay paticles. But the plant available forms represents small portion from the total amount (e.g., 2% in case of potassium). Acorrdingly, various cultivated crops under such condtions are suffring from deficiency of maro and micronutrients (El-Fouly and Abou El-Nour, 2021; Tolba *et al.*, 2021). Maintaing biodiversity in the soil has been suggested as an approach for soil productivity and sustainability enrichment (Adem *et al.*, 2023). Soil flora and fauna are both vital components that contribute to soil health. Among soil fauna, earthworms are known as soil engineers which have a crucial role as soil characteristics improvers (chemical, physical, and biological) through their burrowing activities, gut microflora, cast and mucus secretion which results in an increase in nutrients availability (Xiao *et al.*, 2022; Iordache, 2023). In addition, they have the ability to degrade microplastic pollutants (Sharma *et al.*, 2024). Due to their beneficial effects on soil structure and nutrient cycling enhancement, earthworms are essential to ecosystem processes. They can increase water holding capacity, improving the activity of large number of beneficial soil microorganisms through their excretions (mucus and casting), acceleration the decomposition of soil organic matter, provides plant with N, P, K, and Ca through their fresh casting (Akhila and Entoori, 2022). Cast (the solid excreted part) consists of beneficial groups of

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microorganisms, microbial enzymes, as well as macro and micronutrients. It has the ability to modify soil bulk density by integrating organic matter into the soil (Sabrina *et al.*, 2009; Iordache, 2023). Like clay, earthworms cast shares the capacity to retain and transfer cation nutrients on negative exchange surface sites. In comparison to the parent organic material that the earthworms consumed, Card et al. (2004) found that the microbial activity in earthworm casts were 10–20 times higher. In many ways, earthworms improve soil fertility by promoting soil turnover and structural development. The concentration of extractable - soil Zn and Couper (Cu) was increased in the presence of earthworm (Eisenia fetida) in treated soil with sewage sludge (Karimi et al., 2020).

Earthworms can be classified into three ecophysiological categories (epigeic, anecic, and endogeic) according to their lifestyle and diet. Epigeic earthworms live primarily above ground and feed on decaying organic debris. Endogeic are present in soil and feed on organic compounds present in the soil. In comparison, anecic feed by digging vertical burrows into the soil and dragging organic material from the surface into underground tunnels (Richardson et al., 2015; Sizmur and Richardson, 2020; Xiao *et al.*, 2022). Nielson. (1965) extracted a plant-growth promoting substances from *A. caliginosa, L. rubellus, E. fetida, and A. long* earthworm's extracts. The effect of such substances are similar to indole-3-acetic acid (IAA).

The density of earthworms in soils depends on abundant food and suitable climatic conditions such as adequate soil moisture content (25-85%), moderate temperature (15-25^{o}C), and neutral soil pH (6.8-7.2) as well as the vegetation. In agricultural areas, there is a lower earthworm density than in grassland or forestry due to the insufficient soil organic matter content, food sources as well as genotoxicity by chemical fertilizers and pesticides (Singh *et al.*, 2018). The contribution of earthworm in soil fertility in the harsh climate areas has less intention. The most predominant and popular earthworm in the Egyptian soils is *Allolobophora longa (A. longa)* species which is classified as endogeic earthworm (Emam, Basma, 2018). Such worms beloved soil as a habitat and can not adapted in the condtions with high organic matter content such manure (Guild, 1951; El-Sayed *et al.*, 2023). Guild, (1955) estimated that the mature individuals of *A. longa* can ingest 35–40 g dry weight of dung per annum, while *A. caliginosa* and *L. rubellus* can ingest 20–24 g and 16–20 g, respectively.

According to Brown *et al.*, (1999)**,** the earthworm community in the soil can be managed to increase soil production. There is a little available information about the influance of the Egyptian earthworm (*A. longa*) on the availability of native macro and micronutrients. The main goal of the current experiment is to study the effect of *Alolobophora longa* specie on nutrients availability and some soil characteristics of a clay loam soil.

2. Materials and Methods

2.1. The experiment features

The tested soil was collected from 0-15 cm surface soil from the agricultural station farm, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt (27^{o}12′ 23″ E and 31^{o} 09` 51″ N), with general characteristics of clay loam in texture (sand = 25.5, silt = 38, clay = 37.5%), bulk density of 1.18 g.cm^{-3}, organic matter content of 13.42 g.kg^{-1}, soil pH 8.36, and EC 1.81 dS.m^{-1} (Table 1).

Table 1. General characteristics of the incubated bulk soil (S) and the soil inoculated with A. longa earthworm (S^{+AL}) after 90 days of incubation.

Analysis	Non-treated soil (S)	The soil with treated earthworm (S+^{AL})
BD (g.cm^{3})	1.60±0.63a	1.49±0.02b
pH	8.36±0.06a	8.20±0.21b
EC (dS.m^{-1})	1.81±0.06a	1.87±0.01a
Total macronutrients (g.kg^{-1})		
OC	7.80±0.12a	8.51±0.01a
N	0.49±0.20b	0.60±0.03a
P	2.54±0.31a	2.56±0.01a
K	7.43±0.21a	7.26±0.07a
C/N ratio	15.92±0.32a	14.17±0.21b

* values represent the average value of three replicates ± standard deviation; small different letters represent the significant difference based on Duncan's test at $p < 0.05$.

Adult individuals earthworms of *Alolobophora longa L.* (A. longa) were distinguished (according to the morphological characteristics of thier species) and collected manually from the agricultural farm, in autumn of 2022.

2.2. Expermintal setup

The collected soil was air dried and then passed through a stanless steel sieve with dimeter of 2 mm. Five kg of the sieved soil was placed in plastic box (30 cm width x 35 cm length x 20cm height). The soil was moist to 60% moisture level then inoculated with fivty individual adult *A. longa* earthworm (Fig. 1). The experiment was conducted during autumn season of 2022 (October, November, and Desember) at the laboratory of Agricultural Zoology and Nematology department. The boxes were incubated in dark place at 25^{o}C for 90 days and they watered ones in a while to keep the moisture content at the field capacity level. The soil without earthworm was considerd as a control treatment. Each treatment was repeated three times.

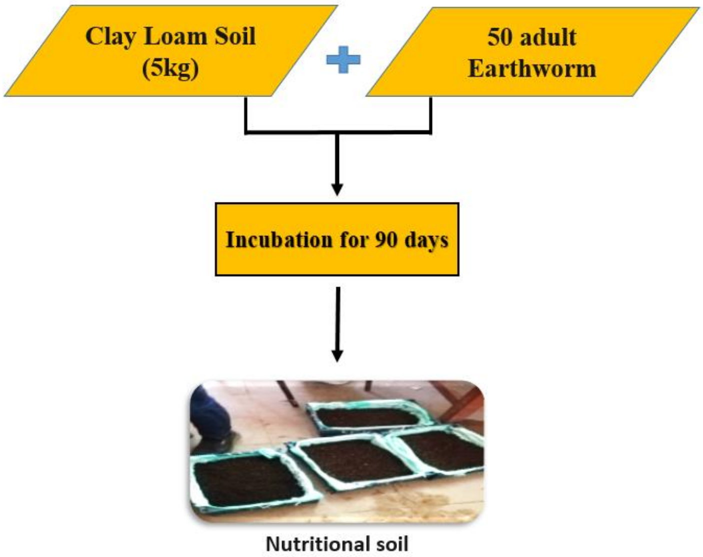

Fig. 1. An incupation experiment of tested soil inoculated with A. Longa earthworms.

2.3. Earthworm Surviving

The number of live earthworms was accounted after 15, 30, 45, 60, 75, and 90 days for the earthworm-treated soil. On the other side, the cocoons were accounted for only at the end of the trial.

2.4. Chemical analyses

The soil samples was taken after homogenized the whole soil in the box at the intial time and by the end of the trial (after 90 days). The collected samples were air dried, milled, and then sieved using a 2 mm stainless steel sieve. The fine samples were stored in glass gars for the subsequent analyses. The pH was measured using pH meter in a suspension of 1:10 (w/v) soil dry weight to deionized water volume ratio after shaking for 1 h, equilibrated for 1 h and then centrifuged at 3500 rpm for 15 minutes. The Electrical conductivity (EC) was measured in the solution of (1:1) soil/water ratio using an electrical conductivity meter according to (Falcon et al., 1987)**.** The total organic carbon (TOC) was determined using the loss ignition method in a muffle furnace at 550 ^{o}C for 4 h as described by (Nelson and Sommers, 1996)**.** For determining the total elements, the samples were digested with a concentrated sulfuric acid (H_2SO_4 98%), and hydrogen peroxide (H_2O_2) was added to weight the sample. Total kjeldahl Nitrogen (TN) was determined according to Stevenson. (1982) Carbon/nitrogen (C/N) ratio estimated from TN% and TOC%. Total potassium (TK) content was measured using flame photometer (BWB XP Flame Photometer, UK). Phosphorus concentration was determined by the molybdenum blue phosphorus method using a spectrophotometer (UV/Vis Spectrophotometer, US) (Olsen et al., 1954; Crouch and Malmstadt, 1967). Available macronutrients [nitrogen (N), phosphorus (P), and potassium (K)], were determined and analyzed according to the methods described by Subbiah and Asija. (1956), Olsen et al. (1954), Black. (1965), respectively. The DTPA (diethylene triamine petaceticacid) micronutrients extraction method was used for estimating the available content of Fe, Zn, Cu, and Mn in the samples according to (Lindsay and Norvell, 1978). The extractable content of Fe, Zn, Cu, and Mn were measured using atomic absorption technique (PerkinElmert. AAS 800, German). Two replicates from each sample were analyzed.

2.5. Statistical analyses

The recorded data were analysed using ANOVA to test the significant differences betwwen the treatments and completed by Tukey test at $p<0.05$ significance level. The statistical analysis was performed using the software Minitab, version 17.3.1 (Informer Technologies Inc.). For data calculation and drawing, Microsoft excel software and OriginPro, version 8.5 SR1 (Origin Lab Corporation, USA) were used, respectively (Stevenson, 2009).

3. Results

3.1 Earthworm surviva

The number of the survive earthworms was recorded after the days of 15, 30, 45, 60 and 90, while the cocoons were accounted at the end of the experiment (Table 2).

Table 2. Numbers of survive earthworms and cocoons.

Time (day)	No. of survive worms	No. of Cocoons
0	50.00±0.00	__
15	47.0±1.73	__
30	45.3±0.00	__
45	45.0±0.53	__
60	45.0±1.53	__
90	45.0±2.00	80.49±4.70

*± is the standard deviation

Following inoculation the soil with the mature indifedual *A. longa* earthworm, the worms moved into the subsurface soil layer, but they tended to grouped again, and then distributed in the soil. The number of survive worms decreased during the first month of the trail and then all survival worms continuous to be a live tell the end of the experiment (Table 2). Regarding to the cocoons (the worm's eggs), the number of cocoons was accounted at the end of the experiment which counted in average 80 cocoon (about 2 cocoons per a worm).

3.2. Soil characteristics as affected by the presence of A. longa earthworm

Data existed in table (1) shown that the general characteristics of the soil as affected by the presence of *A. longa* earthworm. After 90 day of incubation, the soil bulk density (BD) of the S^{+AL} treatment was decreased by 6.88% compared to the untreated soil (S). In addition, the S^{+AL} showed a reduction in the soil reaction (pH) value by 0.16 unit in comparing with the S treatment. On the contrast, the electrical conductivity (EC) of the soil-water extract (1:10) was relatively higher for the S^{+AL} treatment than that of the S by 0.07 EC unit (Table 1).

3.2. Effect of A. longa on the soil content of plant nutrients

3.2.1. Total soil content of Macronutrients

After 90 days of incubation,the total organic carbon (OC) content increased by 9% in the soil treated with *A. longa* earthworms higher than the control (Table 1). Regarding to the total nitrogen content (TN), the inoculation of the clay loam soil with *A. longa* increased the total soil nitrogen content by 22.2% in comparison with the non inoculated treatment (Table 1). Considering the total phosphorus (TP) content of the inoculated soil, it was relatively similar to the S treatment; while the total potassium (TK) decreased but not significantly in the earthworm-treated soil compared to the bulk soil. This decrease in the total K could be due to the feeding regime of the earthworm. The non-treated soil had a highest C/N ratio (15.9) than treated one (14.2).

3.2.2. Effect of A. long on the availability of plant Macronutrients

Fig. 2 shows the extractable macronutrient for both treatment after 90 day of incubation. Figure 2a explained that the soil inoculated with *A. longa* earthworms (S^{+Al}) has a low extractable N content compared to the non-treated one (S). The reduction in the extractable N content was accounted about 18.2% in the S^{+AL} treatment. Considering the extractable soil-P, it was higher in the S^{+AL} by 7% (Figure 2b, while the extractable – K was strongly increased by 56% compared to the bulk soil (Figure 2c).

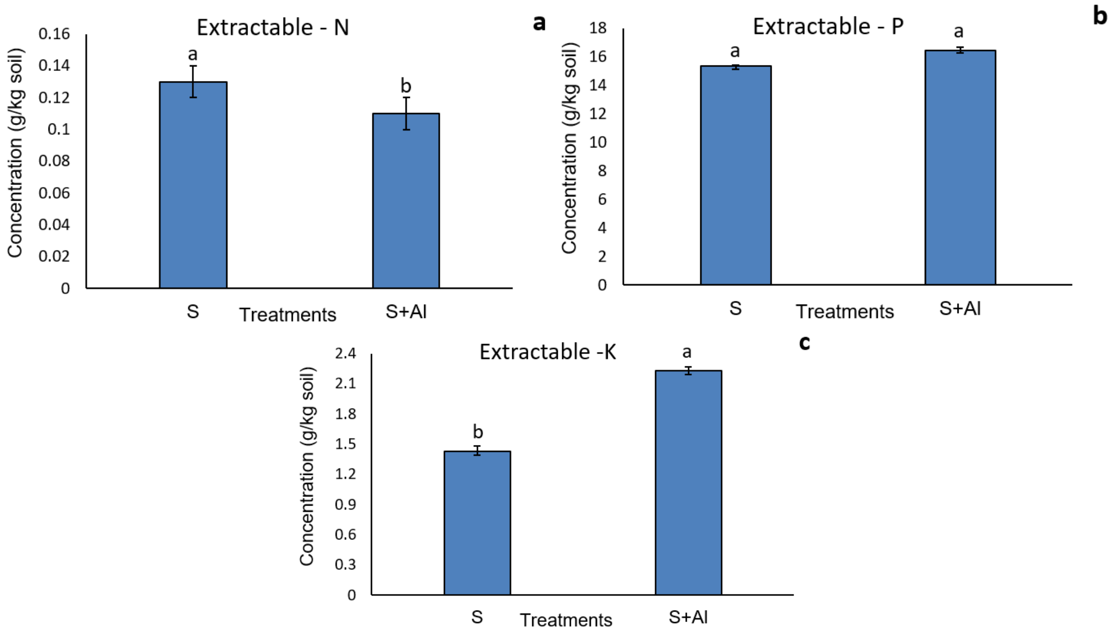

Fig. 2. Extractable a) nitrogen (N), b) phosphorus (P), and c) potassium (K) for the non-treated soil (S) and the soil inoculated with *A. long* earthworm (S^{+Al}), after 90 day of incubation. Different small letters refer to the significant difference after Duncan's test at $p < 0.05$.

3.3.4. Effect of A.longa on the DTPA-Extractable Micronutrients

The amount of plant nutrients extracted from soil using the method of DTPA (ethelendiaminpenta acitic acid) or EDTA (as chelating agents) correlates strongly with the amount of nutrients absorbed by plants (Bibiso *et al.*, 2016; Basak et al., 2021). Thus, the DTPA-extractable micronutrients are considered a direct test for the availability of plant micronutrients. As presented in Figure 3b, the presence of A. long species in the soil raised the extractable iron (Fe), manganese (Mn), and zinc (Zn) content by 22% (5.68 ppm), 14% (7.52ppm), and 127% (2.6 ppm), respectively, but it redused the DTPA-extractable cupper (Cu) content by 10% (0.56ppm) in comparing with the non-enoculated soil.

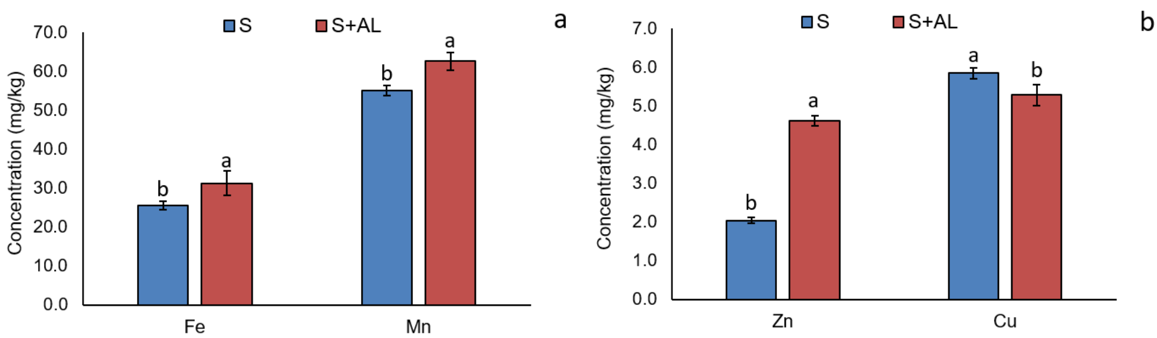

Fig. 3. DTPA-extractable a) iron (Fe) and manganese (Mn) and b) zinc (Zn) and copper (Cu) for the non-treated soil (S) and the soil inoculated with *A. long* earthworm (S^{+Al}), after 90 day of incubation. The small letters show the significant difference based on Duncan's test at $p < 0.05$.

4. Discussion

The increasing in the mortality of worms during the intial stage of the experiment could be due to the translocation of the earthworms to the new environment and changes in the habitate. Regarding to soil characteristics, the decrease in the bulk density (BD) of the soil inoculated with the A. long species earthworm is probably due to burrowing activity of the large number of such earthworm (one handred worm for each 5 kg of soil) which have the ability to move through the soil and create a poros matter (large numbers of holes). An increased in earthworm abundance reduces soil bulk density, and vice versa (Lang and Russell, 2023; Reiff *et al.*, 2024). The reduction in the soil pH confirmed the role of the the worm in reducing the soil pH, which it excreates buffering materials (mucus), enzymes and organic acids. In addition, when the level of CO_2 rise in the soil, the dissolved CO_2 also rises, leading to an increase in carbonic acid levels, ultimately resulting in a decrease in soil pH. Schrader, (1994) observied that epidermal earthworm mucus buffers the pH of the burrow wall. Moreover, the reduction of soil pH could has significant effect on the release of nutrients from the soil constituents. The rasing of the EC value of the S^{+AL} treatmend could be due to releasing of minerals due to the role of the earthwarm in the nutrients availability enhancement.

Regarding the soil organic carbon (OC) content, the raising of the OC in the S^{+AL} treatment could be due to the decomposition of soil organic matter (SOM) by the worms in the tested soil. Hoeffner et al. (2022) reported that *Aporrectodea anecic* species (AAS) consume mainly aged plant-derived materials already incorporated into the soil and only a small proportion of surface-available plant-derived materials. They also noticed that the casts of *Aporrectodea* did not seem to depend on the presence of litter at the soil surface. On the other way, (Alekseeva et al., 2006) observed that when no litter was provided to the soil surface, the carbon content of *A. giardi* casts was still higher than the bulk soil (5.3% for *A. giardi* casts, 3.8% for soil at 0-20 cm and 1.2% for soil at 40-60 cm). They also noted that *Aporrectodea longa* burrow into the soil to search for soil organic matter incorporated in the soil. While the increase in the total soil nitrogen content appears to simply reflect the increase of soil microbiota, which comes from the gut of worms during the excreation process. (Willem *et al.*, 2018) reported that the earthworm casts, especially fresh casts, contain higher amounts of nitrogen compared to bulk soil. The reduction in the soil C/N ratio is probaply due the raising in the total nitrogen content in the S^{+AL} treatment (Table 1).

Regarding to the availability of the macronutrients, the reduction in the extractable-N in the S^{+AL} treatment could be due to the consumption of easily extractable nitrogen by worms as a result of the lack of adequate supply of organic matter in the soil. On the other hand, earthworms can contribute to soil nitrogen pools by the excretion of urine, mainly in the form of ammonia and urea (Lang and Russell, 2022), or mucus (a mixture of carbohydrates and proteins) (Guhra *et al.*, 2020; Shutenko *et al.*, 2020)**.** Lang, et al. (2023) found that endogeic and epigeic earthworms significantly increased the amount of NO^{3-} (endogeic: + 140 %; epigeic: + 70 %) and mineral nitrogen (endogeic: + 83 %; epigeic: + 54 %).

The increases in the extractable phosphorus and potassium content in the *A- long* inoculated soil could be attributed to the secretions of these worms, which in turn release such nutrients in the treated soil. Therfore, the increase in the extractable plant nutrients in the soil treated with *A. longa* confarm its crucial role in the nutrients availability. (Basker and Kirkman, (1993) suggested that the increase in potassium content in the cast of *Aporectoda calegonaze* earthworm is mainly due to intestinal transit processes. Another study showed that the earthworms *Pheretima carnosathe* enhanced potassium feldspar weathering. They increased the water-soluble potassium and the nitric acid extractable-K by 23 and 17%, respectively (Liu *et al.*, 2011)**.** On the other hand, the enhancement of the plant growing in the in earthworm-treated soil is not due to their direct changes in nutrient availability (as a direct result of earthworm activity), but rather to changes in microbiome resulting from worm processing of the soil material (Hodson *et al.*, 2023)**.**

Regarding to the DTPA-extractable micronutrients, The increases in the extractable Fe, Mn, and Zn could be because of the action of the microbial comunity in the gut of these earthworms which may be essential in production of chelating agents and/or transforming metals into a more plant-available form. On the opposite way, the reduction in the extractable Cu might be due to the sensitivity of the worm to this metal because of its toxisity effect. (Bityutskii and Yakkonen, 2012) observed that the earthworm *Aporrectodea caliginosa* primed the release of mobile and available micronutrients in soil. They also reported that these earthworms can significantly impact the formation of mobile and available micronutrients in a soil. (Tomati and Galli, 1995) showed that the concentration of plant available form of ferrous and zinc in the casts of *Alolopophora caliginosa* had doubled compared to the soil content of these nutrients.

5. Conclusion

This research work has shown a crucial role of the Egyptian earthworm *A. long* in plant nutrient availability enhancement. Compared to the untreated soil, *A. longa* worms have a strong ability to release potassium ions from the soil constituents which could be due to both of their activity in the soil and the microorganisms in their cast content. Although earthworms feed on SOM, but it could decrease in the condition of insufficient SOM. In addition, the worms enhanced the availability of Fe, Zn, and Mn in the soil which develops our knowledge to understand the role of these worms in the climate–affected soils and improve the soil characteristics. Further future research towards the role of this adapted earhworm in the new reclimed soils are needed.

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