

Mitigation of earthworm behavior against lithium pollution using biochar

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Abstract

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Application of lithium has been increased in recent years due to its use in various modern gazettes and forced to find new reserves and extraction through mining. The mining process and improper disposal of lithium containing gazettes significantly added this element to the surrounding areas, especially to the terrestrial and soil ecosystems. The increasing concentration of lithium affected the soil biodiversity and altered behavior was expected for macro-organisms. Present study aimed to investigate the different concentrations of lithium salt (Li_2CO_3) on the behavior of the species of earthworm (*Eisenia fetida*), according to ISO 17512-1:2008 standards. In recent years, researches on biochars are drastically increased due to its unique role in soil health improvement. Thus, the biochar has been included in this work as a conditioning material to study the mitigation effects of lithium on earthworm (*E. fetida*) behaviour. The findings suggested that lithium promoted the earthworm avoidance on dose dependent manner while 1% (w/w) addition of biochar in soil mitigated the avoidance behaviour. These mitigating effects were correlated to certain soil physio-chemical properties change, better soil's buffering capacity against stress by lithium in presence of biochar. The findings of present study may force new investigation to restore the soil health and earthworm behaviour near the mining areas.

Keywords: Biochar, Earthworm behaviour, Emerging contaminant, Lithium, Mitigation.

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Introduction

The intensive lithium mining contributed to higher accumulation of this emerging contaminant in soil and linked to soil degradation, erosion, loss of biodiversity, and adverse impacts on local ecosystems (Bolan et al., 2021). Various chemicals are involved in lithium extraction which contribute contaminants to soil and water, with potential risks to human health and the environment (Aral and Vecchio-Sadus, 2011; Gao et al., 2021; Konstantinova et al., 2023). Compared to other soil cations, lithium is more mobile and prone to leaching, which can contaminate aquifers and be absorbed by plants, potentially causing toxicity at concentrations between 50 and 170 $\mu\text{g L}^{-1}$ (Kszos and Stewart, 2003). Pollution resulting from lithium mining activities is not limited to extraction sites but also occurs during processing and improper disposal of waste, such as from lithium batteries (Gao et al., 2021).

To mitigate negative environmental impacts, effective remediation or mitigation strategies are necessary. Growing concerns on terrestrial ecosystems and human health caused by increasing lithium concentrations

in soil at potentially toxic levels (Bolan et al., 2021; Shakoor et al., 2023). Among various strategies, the application of pyrogenic materials to soil, such as biochar, has been widely used (Myers et al., 2003). Biochar is a solid carbonaceous product obtained from biomass feedstock, such as agricultural, forestry, and urban waste etc (Yaashikaa et al., 2020). Biochar has garnered significant interest due to its ability to mitigate climate change effects, remove pollutants, and improve soil quality and health (Pathak et al., 2024; Rao et al., 2024), largely due to its unique properties, including high specific surface area, high porosity, functional groups, stability, and high cation exchange capacity (Burachevskaya et al., 2023). Biochar production is not only environmentally beneficial but also an efficient technology for waste reuse with low energy consumption (< 700 °C) (Myers et al., 2003), and can be used as a conditioning agent (Brar et al., 2024; Jha et al., 2023; Rajput et al., 2024). Bulk biochar, with particle sizes between 0.04 to 20 mm, is commonly used to improve soil quality and sustainability, both in agricultural and environmental practices (Brar et al., 2024).

The main objective of terrestrial ecotoxicology is to obtain information for contaminated matrices, such as soil, through laboratory tests using standardized species and conditions to assess associated risks (Loureiro et al., 2005). Such assays can help determine if lithium concentrations in soil are high enough to cause adverse effects in organisms used as biological models or bioindicators (Macedo et al., 2024). Soil plays a fundamental role by providing a range of ecosystem services critical for promoting environmental sustainability and food production (Telo da Gama, 2023). This assessment may follow a preliminary or prospective strategy, aiming to evaluate the potential risk of toxic elements in organisms through controlled laboratory tests using artificial or natural soil matrices at different concentrations (Albert and Bloem, 2023; de Santo et al., 2019). Biological indicators, due to their high sensitivity and notable soil biodiversity, allow the use of various organisms in these tests.

Earthworms are widely used as bioindicators due to their response to different soil uses and management and their high sensitivity to disturbances and contamination (Paoletti et al., 1998). The species *Eisenia fetida* is used as a standard reference according to OECD (1984) guidelines, due to its high sensitivity, easy laboratory adaptation, and short generation time (OECD, 1984; Lowe and Butt, 2007; Paoletti et al., 1998). Considering the previously mentioned aspects regarding the potential effects of lithium on the soil's ability to provide ecosystem services and the potential of conditioning materials such as biochar. The main objectives of the current study are to evaluate: (i) the effect of different lithium concentrations (Li^+) in the form of lithium carbonate (Li_2CO_3) on the behavior of the species *E. fetida*, according to the standards required for such studies (ISO 17512-1:2008); and (ii) the effect of biochar application as a strategy to mitigate the potential effects of the studied lithium concentrations.

Material and Methods

An artificial soil (AS) was prepared according to the standards established for investigations based on the effect of chemical substances (OECD, 1984), serving as an alternative to natural soils. The artificial matrix consists in a mixture of 70 % fine sand, with particle sizes between 50 and 200 μm , 20 % kaolin, with a minimum of 30% kaolinite, and 10 % dry peat sieved and free of plant residues. A composite sample was collected, and the main physicochemical parameters were analyzed. The results for AS revealed a KCl 1M pH value of 4.7, which was corrected to a final value of 6.1 by adding CaCO_3 , an electrical conductivity (EC) of 0.15 dS m^{-1} , organic matter content of 57.6 g kg^{-1} , cation exchange capacity of 6.2 $\text{cmol}_c \text{kg}^{-1}$ and a sandy loam texture class.

A commercial biochar, obtained from the pyrolysis of forest residues, at temperatures between 400-600 °C, at limited oxygen availability, was used as a soil conditioner. Like the AS, a composite sample of biochar was collected for initial physicochemical characterization, with some parameters analyzed presented in Table 1.

Table 1. Physico-Chemical parameters of commercial biochar used in the present study.

parameter	d_{ap}^1 (g cm^{-3})	diameter (mm)	SS ² ($\text{m}^2 \text{g}^{-1}$)	H_2O^3 (g kg^{-1})	pH H_2O -	EC ⁴ (dS m^{-1})	VC ⁵ (g kg^{-1})
value	0.35	≤ 10	≥ 500	< 300	9.0	1.8	≤ 50

¹ Bulk density; ² Specific surface; ³ Water content; ⁴ Electric conductivity; ⁵ Volatiles content.

For experimental design, five lithium treatments were considered, based on concentrations of 0, 10, 20, 40, and 80 mg Li kg^{-1} , in the form of lithium carbonate (Li_2CO_3). The selection of the lithium concentration range in the soil was based on values observed under edaphic stress conditions, resulting from lithium levels in soils of Portugal or pollution events, such as waste deposition sites from mining activities (Quina and Pinheiro, 2020). Based on the mitigation effects of lithium in the soil a second modality, with the same lithium concentrations, was considered with the biochar addition, at a dose of 1 % (w/w). The dose of

applied biochar was selected as per previous studies where the dose of biochar was standardized for pollution toxicity (Li et al., 2023; Liberati et al., 2023; Rajput et al., 2023). For each treatment, a control treatment without lithium addition and with biochar addition was also included. Three repetitions were conducted for each treatment.

To study the effect of lithium concentration and biochar mitigation, an avoidance test was conducted following the guidelines for chemical substance studies (ISO 17512-1:2008), used the behavior of *E. fetida* biological model as the main endpoint. A total of 30 transparent PVC test-containers, with 19 cm*14 cm*14 cm dimensions, with similar perforated lids, were used according to the treatments and repetitions tested. Each test container was divided into two equal sections, each filled with 500 g of dried artificial soil (AS), adjusted to 60% of its maximum water-holding capacity (WHC). One section was treated with lithium (test-section) at the specified concentrations (Li10, Li20, Li40, and Li80) and was marked with a (+) sign. The other section, which served as the control soil, with no added lithium (Li0), was marked with a (-) sign. In accordance with the guidelines (ISO 17512-1:2008) (CF), a dual test was also conducted for validation purposes, where the respective sections of the control treatment were compared with each other. This procedure was also applied to treatments without lithium and with biochar application. A total of 10 adult *E. fetida* earthworms, acclimated to the artificial soil conditions and with developed clitellum and a fresh weight between 300 and 600 mg, were placed at the interface between the two soil sections in each test container (Figure 1).

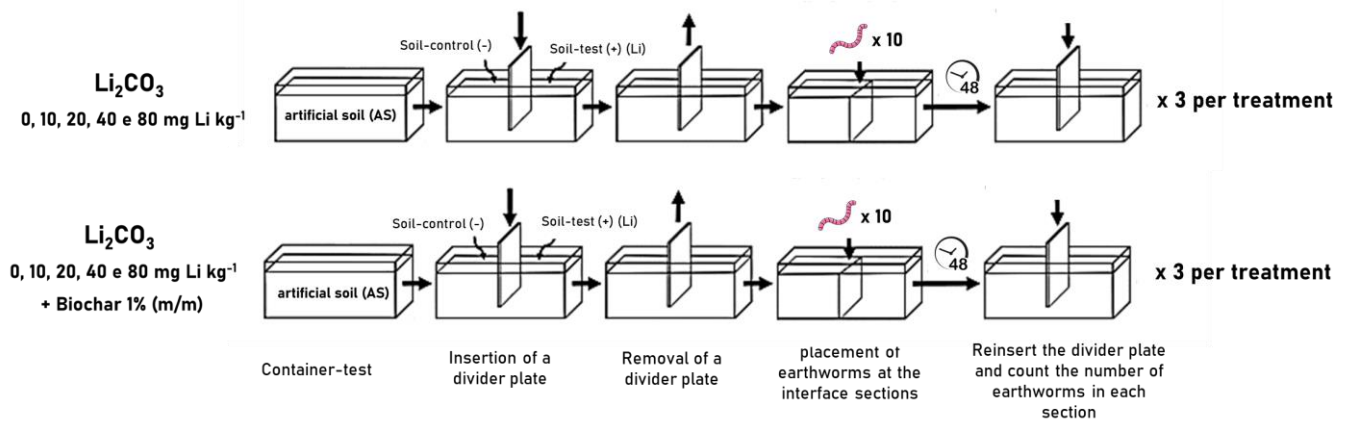


Figure 1. Avoidance test procedure applied to study lithium concentrations (0, 10, 20, 40, and 80 mg kg⁻¹) in artificial soil (AS), with the addition of biochar at 1% (w/w).

The containers were sealed and placed under controlled laboratory conditions, with a temperature of 20 ± 2 °C and a photoperiod of 16 hours of light and 8 hours of darkness, for a 48-hour exposure period, during which the earthworms were not fed. After the exposure period, the partition plate was repositioned at the interface between the compared sections in each test container, and the number of individuals in each section, control-soil (-) and test-soil (+), was counted for the different treatments and repetitions studied. Based on the number of individuals in each test section, the percentage of avoidance (%A) for each treatment was determined using the model described by Busch et al. (2012) ($\% A = [(n_c - n_t) / N] * 100$), where n_c , n_t , and N refer to the number of earthworms in the control-soil, test-soil, and total number of earthworms per test-container, respectively. According to the results, positive values (+) indicate avoidance, while negative values (-) suggest no response or attraction to the presence of lithium (ISO 17512-1:2008). After the exposure period, more dynamic physicochemical parameters of the soil, such as pH (KCl 1 M), EC and water content, were also determined to better understand the effect of lithium concentration and biochar presence on earthworm behavior.

The data were subjected to normality and homogeneity tests using Shapiro-Wilk and Bartlett tests, respectively, followed by a one-way analysis of variance (ANOVA) and a Least Significant Difference (LSD) test, with a 5% probability level.

Results and Discussion

For the dual or double control test (Figure 2), where the respective test-sections, correspond to the same treatment, are compared the earthworms do not show a significant preference, resulting in an average distribution between 40 and 60%, fulfilling the normative range for this type of treatment (OECD, 1984).

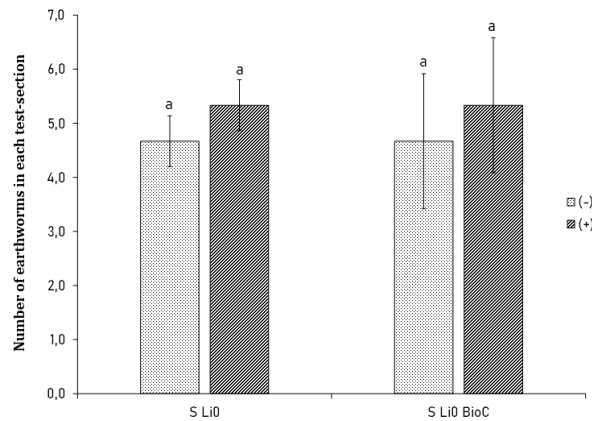


Figure 2. Mean number ($n=3$) of earthworms observed in the avoidance assay, for the respective sections, for the treatments without lithium (S Li0), and with biochar (S Li0 BioC), in the dual control test. (Bars relative to each treatment, followed by the same letter, do not differ significantly from each other, by the Student's t-test, for 5% probability; vertical bars indicate standard error (SE) ($n=3$).

In addition, the absence of mortality in all studied treatments reveal null interference of soil artificial matrix in earthworm's behavior, allowing the validation of the experimental test in accordance with the standards for this type of test (ISO 17512-1:2008). These results are supported by other authors (Loureiro et al., 2005), where observed a similar trend, demonstrating the innocuity of the artificial matrix on the behaviour of *E. fetida*. This is partly explained by a balance among the main soil factors, such as texture, organic matter, cation exchange capacity, and pH, according to the proportions of the constituent materials used (OECD, 1984). The results also demonstrate the absence of negative effects from the type and dose (1 %) of biochar used, with the results falling within the normative values, with the distribution of individuals between 40 to 60% observed between the test-sections for the control treatment with biochar (S Li0 BioC) (Figure 2). These results are related to the physicochemical characteristics of this type of material (Table 1), influenced by the source biomass (forest residues) and the pyrolysis conditions ($T < 700$ °C), which result in low levels of heavy metals and hydrocarbons (results not shown), thus not affecting the behaviour and distribution of the earthworms.

For lithium exposure, the avoidance results are presented in Figure 3. The negative avoidance results observed in the control treatment (Li0) (-6.7%) indicate a lack of response, suggesting an attraction or preference of the *E. fetida* for conditions in absence lithium in the soil.

In contrast, in the presence of lithium in the soil, the biological model exhibited avoidance behavior, which was concentration-dependent ($p < 0.001$), with values ranging between 17% and 40% (figure 3). In an artificial matrix prepared according to OECD guidelines (OECD, 1984), using *E. fetida* and *E. andrei* as biological models, respectively, observed a similar trend, with avoidance behaviour dependent on the lithium presence and concentration in soil. Other studies, using different bioindicators or endpoints from those used in the current study, suggests a similar trend with a significant loss of soil habitability or an increase in oxidative stress (Xu et al., 2021), with increasing lithium concentration in the soil. Despite the avoidance behaviour observed in the presence of lithium in the soil, the recorded values for the highest concentration studied (80 mg kg^{-1}) remained below the soil habitability threshold of 60% avoidance, as defined by Loureiro et al. (2005).

In turn, in the treatments with the addition of biochar, negative avoidance values were observed at all tested lithium concentrations (Figure 3). In the case of the control with no lithium (Li0) and with biochar addition (Solo+BioC), the results showed a similar trend to the treatment without biochar (Solo), transcribe by a no response, reflected by negative avoidance values (-6.7%). These results indicate that the biochar applied not influence the behaviour of the earthworms, unlike other studies with *E. fetida*, where the application of biochar led to avoidance behaviour (Namoi et al., 2019; Wang et al., 2024). The type of biochar used, namely its characteristics related to the concentration of compounds such as heavy metals or polycyclic aromatic hydrocarbons, which are strongly influenced by both the source (de Resende et al., 2018) and the pyrolysis conditions (Burachevskaya et al., 2021), may can explain these differences. In the treatments with lithium, the addition of biochar promotes a non-response, indicating an attraction of the earthworms to the test-section containing the conditioning material. This trend became more pronounced with the increase of soil lithium concentration, with the avoidance values ranging between (-) 13% and (-) 33% (Figure 3).

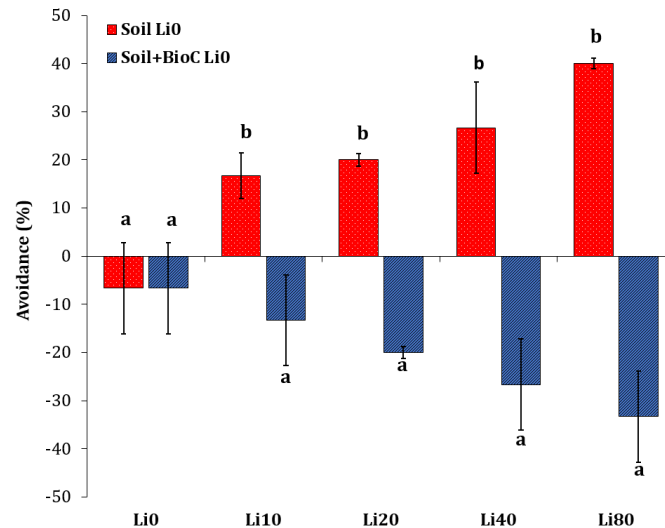


Figure 3. Percentage (%) of avoidance or preference of *E. fetida* in the lithium treatments with (Solo+BioC) and without (Solo) biochar application, for the different concentrations under studied. (for each concentration, bars relative to each treatment, followed by the same letter, do not differ significantly from each other, by the Student's t-test, for 5% probability; vertical bars indicate standard error (n=3)).

The differences observed between treatments with and without biochar may be attributed to the influence of these conditioning materials on soil properties (Figure 4). These changes could impact soil habitability and subsequently alter the behaviour of *E. fetida*. The high adsorption capacity due to particle size reduction, increased surface area and electrical charges from the pyrolysis process, enhanced lithium reactivity through adsorption phenomena. This reduces its bioavailability, mitigates potential toxic effects on soil biology and improves soil habitability. At the level of soil physico-chemical properties, the removal of acidic functional groups during the pyrolysis process allows the carbonaceous material to exhibit alkaline characteristics. Biochar can be applied to the soil to raise pH levels due to its alkalizing effect, creating more favourable conditions for *E. fetida* (Wang et al., 2024). With the increase in lithium concentration in the soil, due to the alkalizing nature of the lithium salt used (LiCO_3), there is a corresponding rise in both soil pH and salinity in the treatments with lithium (figure 4). The application of biochar mitigates these effects creating less stressful conditions, explaining the higher preferences observed in treatments with higher lithium concentrations, with more negative avoidance values (Figure 3). The high adsorption capacity of the tested biochar material also contributes to increased water retention (Hu et al., 2023), creating more favourable conditions comparatively to treatments with null application (Figure 4). Unlike the treatments with biochar, the water content measured at the end of the exposure period are significantly below the optimal values, between 60% and 70% of the CMRA, influencing the behaviour of *E. fetida*, as observed by Wever et al. (2001) with juveniles of *Aporrectodea tuberculata*.

Other factors related to the fertilizing value of the biochar may also explain the observed results. These materials are rich in carbon, with some portions potentially being in more soluble forms, depending on the type of biomass and pyrolysis conditions (Burachevskaya et al., 2023), and can serve as a substrate for earthworms to obtain energy (Garbuz et al., 2020). In addition to high carbon content, these materials also contain significant levels of nutrients such as calcium and potassium, as well as nitrogen, phosphorus, and micronutrients (Garbuz et al., 2020). The improvement of soil chemical fertility resulting from biochar application (Singh Yadav et al., 2023), along with increased water retention and soil aeration, creates more favourable conditions for soil fauna growth and activity. This allows earthworms to develop resistance mechanisms against toxicity processes caused by the presence of elements such as lithium in the soil (Sun et al., 2022; Gudeta et al., 2023).

There are several mechanisms that can mitigate the toxic effects of lithium, involving physical, chemical, and biological processes. Biochar as a carbonaceous material promote efficient adsorption and mitigation of metals, including lithium (Kamran and Park, 2020). Lithium can be adsorbed through van der Waals forces or electrostatic interactions, which promote rapid adsorption of lithium on the surface and within the pores of biochar (Raji et al., 2023). The effect of this mechanism is quick, although its capacity is limited, depending on the lithium concentration and the type of biochar, particularly its surface area and micro- and mesoporous structure (Murtaza et al., 2022). A second mechanism of chemical origin is associated with the presence of hydroxyl (-OH), carboxylic (-COOH), and phenolic functional groups, which promote the

complexation of lithium with oxygen or other atoms present in the biochar. This mechanism is stronger than physical adsorption, however, it has limitations related to its specificity and the availability of active functional groups. It was related by ion exchange phenomena, in which lithium ions present in the soil solution are exchanged with other cations in the biochar, such as sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), or calcium (Ca^{2+}) (Kavanagh et al., 2018). This mechanism is strongly dependent on the initial composition of the biochar (Jagadeesh and Sundaram, 2023), which in turn is influenced by the feedstock materials and pyrolysis conditions (Qiu et al., 2022). In more stable carbonaceous materials, mitigation processes can occur through the reduction of lithium mobility via encapsulation, where lithium ions are trapped within the internal structure of the biochar, reducing their mobility and availability in solution (Murtaza et al., 2022).

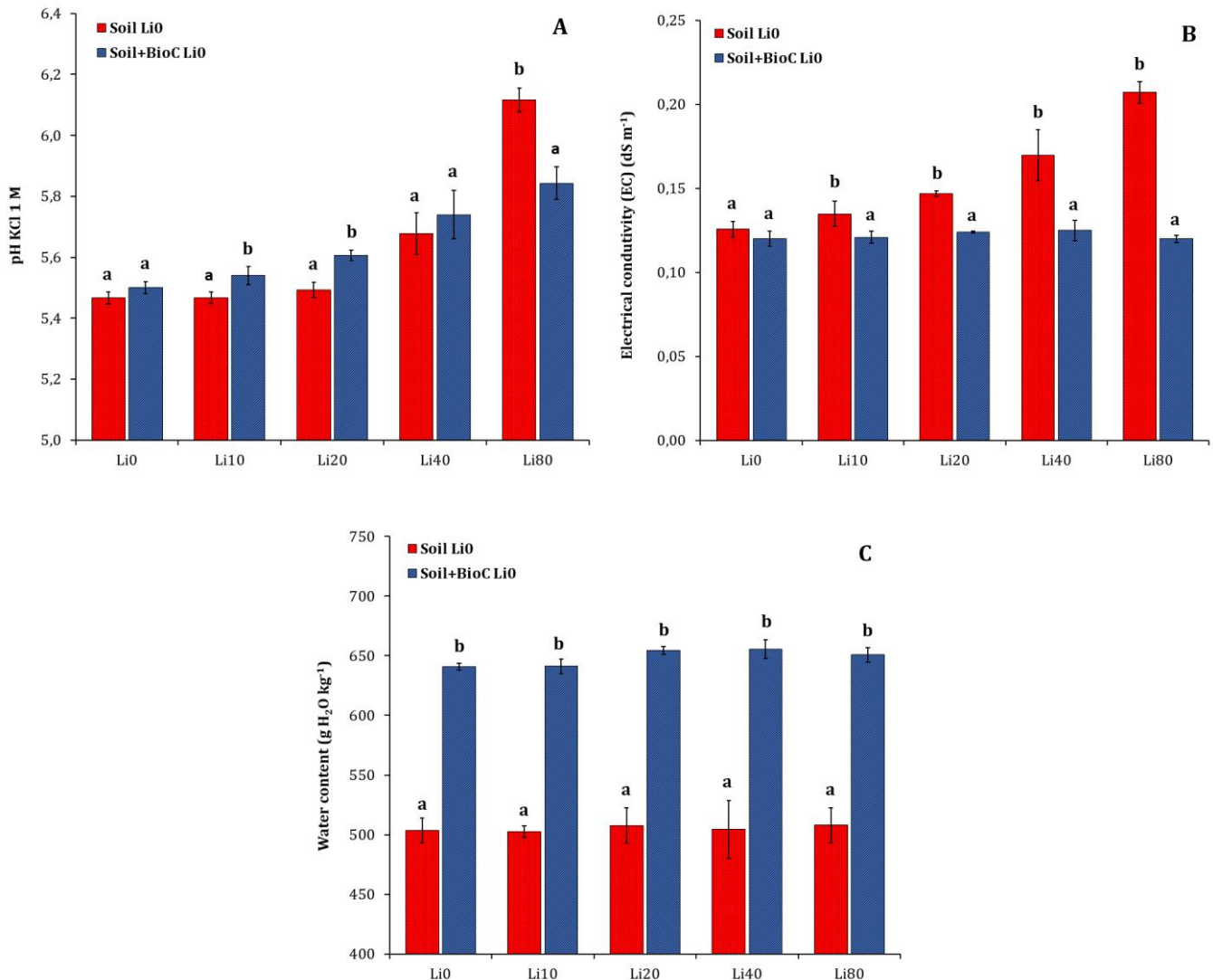


Figure 4. Mean values (n=3) of (A) pH 1M KCl, (B) electric conductivity (EC) (dS m⁻¹), and (C) water content (H₂O) (g kg⁻¹), for the lithium treatments under study, with (solo+BioC) and without biochar (Solo) application. (for each concentration, bars relative to each treatment, followed by the same letter, do not differ significantly from each other, by the Student's t-test, for 5% probability; vertical bars indicate standard error (n=3).

Conclusion

Based on the results obtained, it is possible to conclude that the presence of lithium in the soil promote earthworm's avoidance, with this behaviour depending on the concentration. The avoidance behaviour is mitigated by the application of biochar at doses equivalent to 1% (w/w), with the effects of this application becoming more pronounced for lithium soil highest concentration. These mitigating effects are related to the improvement of certain soil physico-chemical properties, which enhance the soil's buffering capacity against stress caused by the presence of lithium.

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