RESEARCH ARTICLE

Bioindicator nematodes in relation to an abiotic stress gradient in soils influenced by mining

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Abstract

The attributes of nematodes are presented as valuable tools for determining the quality of soil, especially that of mining companies. The aim of this study was to evaluate the behavior of nematodes against a stress gradient in a rainy season and a dry season in soils influenced by mining. Thus, field sampling was carried out over 100 m² in triplicate for four types of soils classified according to their uses (pasture, maize cultivation, fig cultivation, and eucalyptus cultivation), and these areas were located on the periphery (500 to 1500 m) of the Ollachea mining community of Puno in Peru; subsequently, the samples were processed in the laboratory to determine edaphic, agrochemical, heavy metal, and microbiological parameters and identify the nematodes. The abiotic stress gradient was determined by a principal component analysis; and through a canonical correlation analysis, the relationships between the abiotic stress gradient and the nematodes were determined. Canonical correlation analysis showed that there were significant correlations: in the rainy season, *Helicotylenchus* and vanadium (r = 0.99), *Globodera* and titanium (r = 0.97), and *Tylenchus* and lead (r = 0.96); in the dry season: *Meloidogyne* and vanadium (r = 0.99), and *Hemicycliophora* and lead (r = 0.91). In conclusion, the abiotic stress gradient had a high correlation with bacterivorous, fungivorous, and phytoparasitic nematodes and a low correlation with omnivorous and predatory nematodes, showing the bioindicator capacity of nematodes in relation to stress parameters that impact soil quality.

Keywords: edaphic ecosystems; edaphic nematodes; functional groups; physicochemical parameters; soil health.

1. Introduction

Mining is one of the most destructive anthropogenic practices that occur in soil (Ezenne et al., 2019), producing alterations in soil's physical, chemical, and biological properties (Kaya et al., 2015). These alterations are represented by an abiotic stress gradient (Rosli et al., 2018).

Abiotic stress gradient is understood as the factors that exert fundamental restrictions on the distribution, form, and function of sessile soil organisms (Puglielli et al., 2023). It also consists of all those foreign elements of the soil system or the existence of an unusual level that generates a harmful effect on soil organisms and their consumers (Egea et al., 2022).

Abiotic stress gradients are determined by analysis with physicochemical parameters (Kaya et al., 2015).

The main drawback of this method is that it provides ephemeral data (Campos-Herrera et al., 2016) "at a given point and time" (Ezenne et al., 2019) and is expensive (Ouyang et al., 2021). However, in recent years, bioindicators (Murphy et al., 2023) that provide data by season (Šalamún et al., 2018) have been used since organisms have different life cycles (Darré et al., 2019), address the issues with using ephemeral data (Bongers & Esquivel, 2015).

Organisms such as protozoa, algae and fungi present some bioindicator characteristics (Fournier et al., 2022); however, they have some disadvantages such as being at low levels of the food chain, which is why they do not integrate the chemical, physical and biological properties of food resources (Gautam et al., 2022); these organisms also have

short life cycles, which is why their populations are less stable and are subject to temporary fluctuations due to ephemeral releases of nutrients (Kooch & Ghaderi, 2023).

Solving these drawbacks are edaphic nematodes, which present suitable characteristics compared to other organisms (Gu et al., 2018) since nematodes are found in all types of soil, as long as there is moisture (Calvão et al., 2019), are abundant (Bal et al., 2017) and easy to identify (Aujoulat et al., 2019), and represent up to 5 trophic groups (Campos-Herrera et al., 2016).

Although there are studies of nematodes in agricultural soils that indicate soil quality, it is necessary to further study the bioindicator capacity of nematodes in other types of soil (Kabir et al., 2018) to provide an adequate determination of soil quality. Therefore, this research aims to evaluate the bioindicator behavior of edaphic nematodes in soils influenced by mining.

2. Materials and methods
2.1 Study area
The study area was located on the periphery (500 to 1500 m) of the artisanal mining community of Ollachea, located in the Minapampa area of the mining company Kuri Kullu SA, in the Puno region, Carabaya Province and Ollachea district (Figure 1), at 13°47′41″S, 70°28′17″ W and at an altitude of 3041 masl. This area has a temperate and warm climate with an average annual temperature ranging between 6 and 18 °C, with a total average annual rainfall between 790 and 1000 mm. The soils have a texture ranging from moderately coarse to fine with a pH that is slightly acidic with calcareous lithological material present in shallow and steep soils.
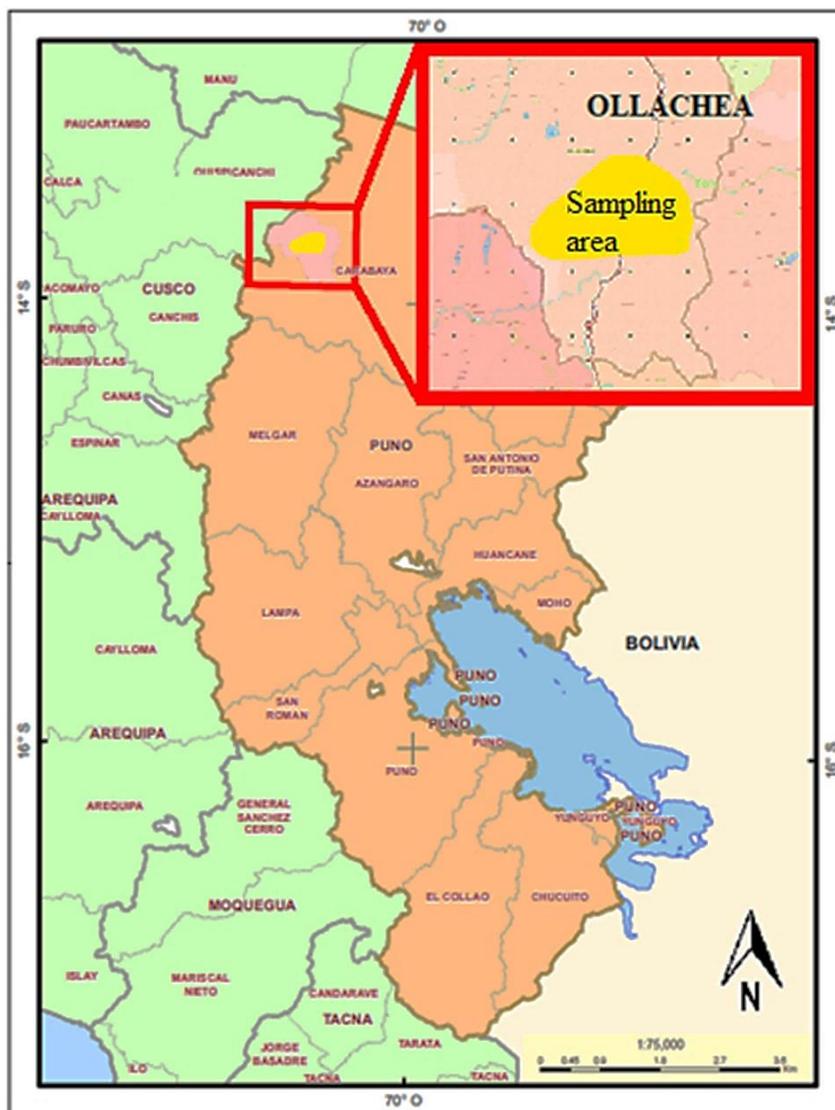

Figure 1. Location of the sampling area on the periphery of the mining community of Ollachea, Carabaya, Puno (Authors developed map in QGIS 3.24).

This study area has gold mineralization, which occurs free, disseminated and in micro veins with sulfide dissemination, the extraction activity that the area presents produces different events of remobilization of gold and sulfides as a result of metamorphism and tectonism, which enrich the areas. with contaminants that have detection limits in ppm and some in ppb (Mciver et al., 2022).

This study area was chosen based on the following criteria: presence of several types of edaphic eco-systems around the mining community and current land use suitable for agricultural and livestock activities that have a high influence of mining residues.

2.2 Sampling points and temporality

Four types of soil classified according to their use capacity were sampled: grassland (13° 47' 19" S, 70° 28' 00" W), clean crop - corn crop (13° 48' 39" S, 70° 27' 07" W), permanent crop - fig crop (13° 48' 15" S, 70° 28' 30" W) and forestry - eucalyptus (13° 47' 01" S, 70° 28' 49" W); in 100 m^2 for each type of soil, between 500 and 1500 m away from the mine. The sampling was carried out in 1 rainy season and 1 dry season of 2021 to ensure a good representativeness of sampled nematodes (Achicanoy et al., 2012).

2.3 Sample collection

In the pasture and maize crop soils, zigzag sampling was used (Sechi et al., 2018); in the fig and eucalyptus crop soils, star-shaped sampling was used (Kaya et al., 2015). The extraction of the samples was performed with a cannula auger with a 4 cm diameter at a depth of 20 cm (Piedra, 2015). The sampling consisted of 20 subsamples and 3 repetitions for each type of soil (Coyne et al., 2007); the samples were placed and labeled in black polyethylene bags.

2.4 Measurement of edaphic, agrochemical, and heavy metal parameters

For each of the sampling points, 7 edaphic parameters were analyzed: cation exchange capacity (saturation method with ammonium acetate), calcium carbonate (NOM-021-SEMARNAT-2000 Item 7.1.1 method AS-07), conductivity (ISO 11265: 1994 Cor1: 1996), soil moisture (gravimetric method), organic matter (NOM-021-SEMARNAT-2000 Item 7.1.1 method AS-07), pH (EPA SW 846 method 9045 D, revision 4), and texture (sand, silt, and clay) (Robinson's pipette method); 9 nutritional parameters were analyzed (EPA method 200.7 Rev. 4.4 EMMC version/1994): boron (ppm), phosphorus (ppm), manganese (ppm), potassium (ppm), zinc (ppm), sulfur (meq/ 100 g), Al^{+3} + H^+ (meq/100 g), calcium (meq/100 g), and magnesium (meq/ 100 g); 26 heavy metals were analyzed (EPA method 3050B/method 200.7 Rev. 4.4 EMMC version/1994):

antimony (mg/kg), arsenic (mg/kg), barium (mg/kg), beryllium (mg/kg), bismuth (mg/kg), cadmium (mg/kg), cerium (mg/kg), cobalt (mg/kg), chromium (mg/kg), cesium (mg/kg), copper (mg/kg), tin (mg/kg), strontium (mg/kg), iron (mg/kg), lithium (mg/kg), mercury (mg/kg), molybdenum (mg/kg), nickel (mg/kg), silver (mg/kg), lead (mg/kg), selenium (mg/kg), silicon (mg/kg), thallium (mg/kg), titanium (mg/kg), uranium (mg/kg), and vanadium (mg/kg); and finally, 2 microbiological parameters were analyzed: microbial biomass carbon (Anderson & Domsch, 1978; Beck et al., 1995; Höper, 2006) and edaphic respiration (alkali trap).

2.5 Determination of edaphic nematodes

To obtain representativeness of active nematodes, the modified Whitehead tray method was used, and to obtain representativeness of inactive forms and slow nematodes, the modified centrifugation-flotation method was used (Hernández-Ochandia et al., 2016). With an Olympus stereoscope of 0.8 to 4.5 X magnification, nematodes were identified at the taxonomic level of genus. The identification of the specimens was performed based on taxonomic keys (Guerrero, 2017).

Determination of the abiotic stress gradient

The abiotic stress gradient was determined through a modification of the methodology proposed by Touron-Poncet et al. (2014), in which all the physicochemical variables (edaphic parameters, nutritional parameters, agrochemicals, and heavy metals) measured at all sampling points were standardized so that the data were comparable, and then, a principal component analysis (PCA) was performed. Exclusion of uncorrelated variables was determined by the variance inflation factor (VIF ≤ 10). The first axis was selected as the abiotic stress gradient, with this axis being the component that explained the highest percentage of variance of the PCA.

2.6 Determination and analysis of the relationship between nematodes and the abiotic stress gradient

The values of the abiotic stress gradient parameters were standardized between 0 and 1. Next, canonical correlation analysis (CCA) was performed to determine the correlation of nematodes and the abiotic stress gradient.

2.7 Data processing

All statistical analyses (PCA, CCA, and significance verification analysis) were performed using the statistical program RStudio (version 4.0.2), an active version of the R community, which is an open source data analysis software that is currently one

of the most efficient and innovative programs being used for scientific studies (R Development Core Team, 2016).

3. Results and discussion

3.1 Determination of the abiotic stress gradient

For the rainy season, the 44 variables obtained a VIF ≥ 10; therefore, they were considered for the PCA (full data in Supplementary Material).

Table 1
Percentage of the variance accumulated by components 1, 2 and 3 for the selection of the abiotic stress gradient in the rainy season

PC	Eigenvalue	% Variance
1	55073	94.348
2	2559.8	4.3853
3	739.416	1.2667

Component 1 of the PCA explained 94.35% of the cumulative variance (Table 1). Therefore, it was selected as the abiotic stress gradient for this season and was represented by the abiotic variables potassium (r = 0.10), silt (r = 0.01), clay (r = -0.01), arsenic (r = -0.01), barium (r = 0.12), and cerium (r = 0.03.), cobalt (r = 0.01), chromium (r = 0.03), cesium

(r = 0.02), copper (r = 0.01), lithium (r = 0.08), nickel (r = 0.04), lead (r = 0.02), silicon (r = 0.94), strontium (r = 0.02), titanium (r = 0.28), and vanadium (r = 0.03) (Figure 2).

For the dry season, the 44 variables obtained a VIF ≥ 10; therefore, they were considered for the PCA (full data in Supplementary Material). Component 1 explained 97.77% of the cumulative variance (Table 2). Therefore, it was selected as the abiotic stress gradient for this season and was represented by the abiotic variables potassium (r = 0.04), arsenic (r = 0.02), barium (r = 0.11), cerium (r = 0.02), cobalt (r = 0.01), chromium (r = 0.02), cesium (r = 0.02), copper (r = 0.01), lithium (r = 0.07), nickel (r = 0.03), lead (r = 0.01), silicon (r = 0.95), strontium (r = 0.01), titanium (r = 0.26), and vanadium (r = 0.03) (Figure 3).

Table 2
Percentage of the variance accumulated by components 1, 2 and 3 for the selection of the abiotic stress gradient in the dry season

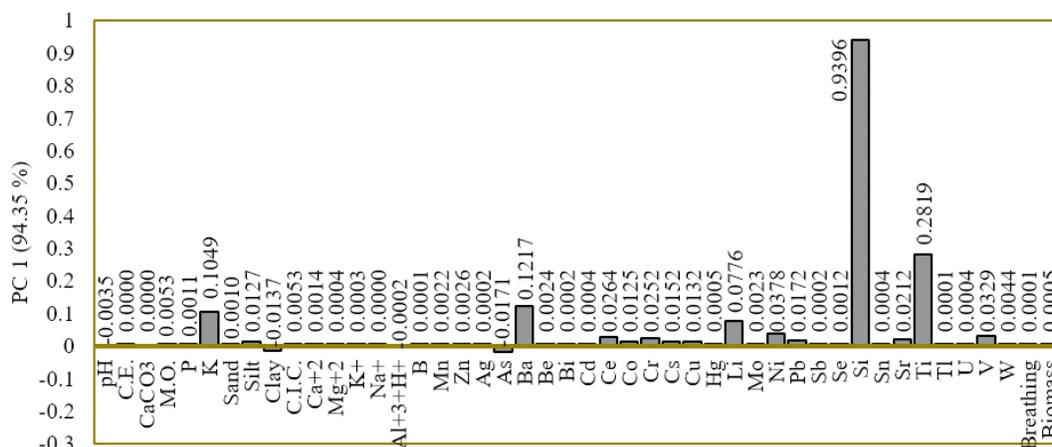| PC	Eigenvalue	% Variance
1	129802	97.773
2	2227.92	1.6782
3	728.39	0.54866

Figure 2. Relationship of the abiotic variables with component 1 in the rainy season.
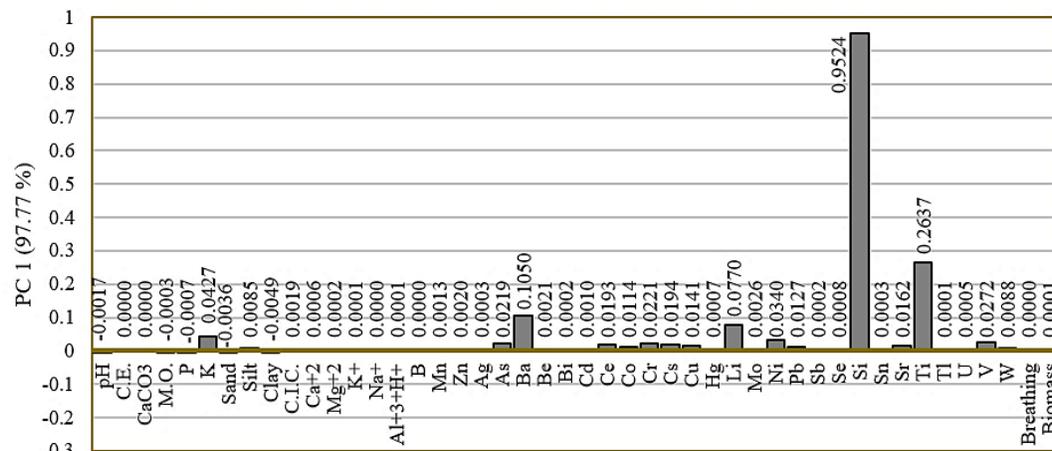

Figure 3. Relationship of abiotic variables with component 1 in the dry season.
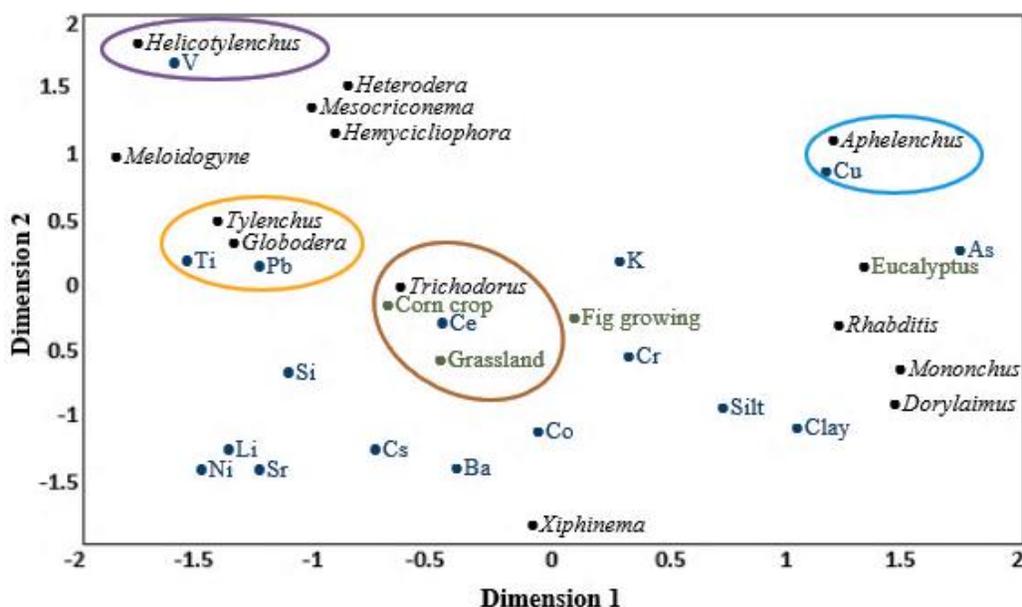
Figure 4. Relationship of nematodes and the abiotic stress gradient variables in the rainy season.

3.2 Determination and analysis of nematodes in relation to the abiotic stress gradient

For the rainy season, in the CCA (**Figure 4**), dimension 1 explained 67.73% and dimension 2 explained 18.44% of the data variability, and together, they explained 86.17% of the variance (**Table 3**); therefore, they were the 2 dimensions considered for the analysis. The CCA showed that there were significant correlations between *Globodera* and lead and titanium (r = 0.96 and 0.97, respectively); *Tylenchus* and lead and titanium (r = 0.96 and 0.94, respectively); *Helicotylenchus* and vanadium (r = 0.99); *Trichodorus* and cesium (r = 0.84); and *Aphelenchus* and copper (r = 0.93).

Table 3
Percentage of the variance accumulated by dimensions 1, 2 and 3 for the canonical correspondence analysis in the rainy season

Dimension	Eigenvalue	%
1	0.31032	67.73
2	0.084486	18.44
3	0.06339	13.83

For the dry season, in the CCA (**Figure 5**), dimension 1 explained 64.93% and dimension 2 explained 19.98% of the data variability, and together, they explained 84.91% of the variance (**Table 4**); therefore, they were the 2 dimensions considered for the analysis. The CCA showed that there were significant correlations between *Globodera* and vanadium, nickel, and titanium (r = 0.87, 0.76 and 0.70, respectively); *Tylenchus* and vanadium, nickel and titanium (r = 0.91, 0.86, and 0.78, respectively); *Meloidogyne* and vanadium, nickel and titanium (r = 0.99, 0.98, and 0.95, respectively); *Hemycicliophora* and lead and strontium (r = 0.91

and 0.88, respectively); and *Aphelenchus* and *Rhabditis* and arsenic (r = 0.40 and 0.60, respectively).

Table 4
Percentage of the variance accumulated by dimensions 1, 2 and 3 for the canonical correspondence analysis in the dry season

Dimension	Eigenvalue	%
1	0.54988	64.93
2	0.16922	19.98
3	0.12782	15.09

In this study, there were both general and specific interactions regarding soil type. Regarding the general interactions, nematodes and abiotic stress gradient variables were indifferent to soil type since the correlations with soil types were not significant, which allowed us to generalize the interactions regardless of the type of soil. Regarding the specific interactions, there were significant correlations in the rainy season between *Trichodorus* and cerium in the pasture and corn soils and in the dry season between *Aphelenchus* and *Rhabditis* and arsenic in the eucalyptus soil. According to Papa et al. (2020) and Petrovskaia et al. (2021), observing both types of interactions allowed us to infer that the number of samples and time periods used were optimal.
In this study, the interactions between the nematodes and the abiotic stress gradient variables had a higher frequency of significant positive correlations (tolerance); in contrast, there were no significant negative correlations (sensitivity). This result agrees with those of Ekschmitt and Korthals (2006), who note that the bioindication of specific soil toxins should preferably be based on tolerant nematodes and not on sensitive ones.
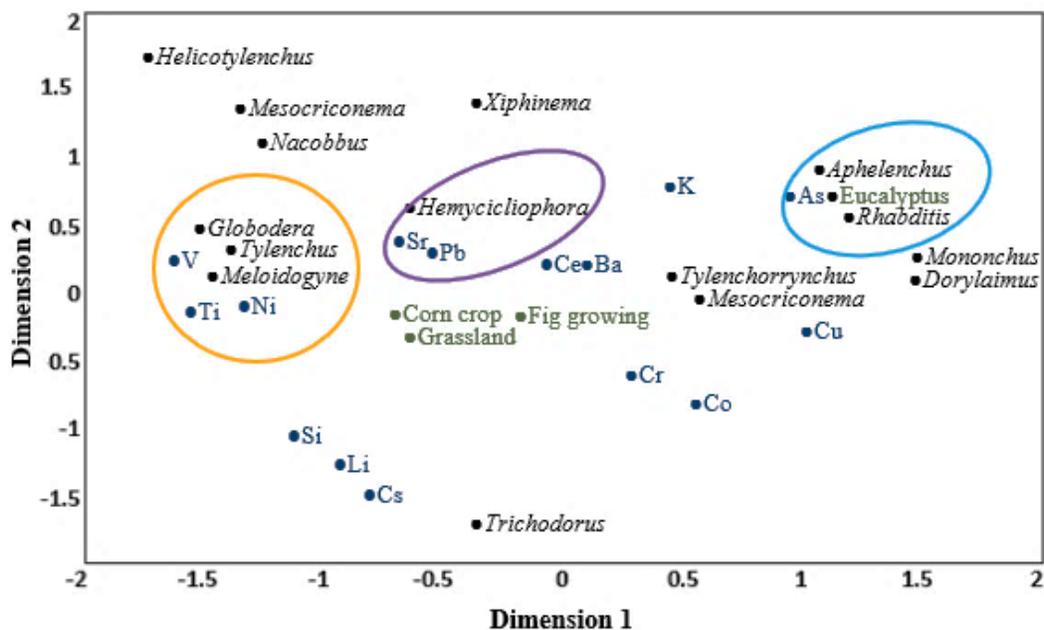
Figure 5. Relationship of nematodes and the abiotic stress gradient variables in the dry season.

The effects of temporality on the structure and function of a nematode food web can influence the interactions of nematodes and impact variables (da Silva et al., 2021), as observed in this research with the interactions of nematodes and the abiotic stress gradient variables in the rainy and dry seasons; this scenario occurred in this study as these interactions were different, mainly because rainfall is considered one of the environmental variables that most affects the diversity and metabolic activity of nematodes (Song et al., 2017) in that nematodes depend on soil water films to move and capture their food (Nielsen et al., 2014). While fungivorous nematodes are sensitive to disturbances (Sánchez-Moreno & Talavera, 2013), some nematodes of this functional group can be tolerant and therefore indicators of disturbances related to mining (Martinez et al., 2018), as observed in this study with *Aphelenchus* and copper in the rainy season and arsenic in the dry season. This result was obtained because these nematodes feed on fungi that infect the leaf sheaths and cortex of some roots with concentrations of heavy metals (Seijas, 2004).

Nematodes with survival strategy "r" are resistant to specific disturbances in soil (Gutiérrez et al., 2016), as was observed in this study with *Rhabditis* and arsenic; thus, this nematode presents a survival strategy "r" and has a high capacity for tolerance to contaminants (Park et al., 2011); having short life cycles (Losi et al., 2021) and being opportunists of soil enrichment (da Silva et al., 2021) and the first colonizers in ecological succession (du Preez et al.,

2018), these nematodes are excellent indicators of soil quality in terms of heavy metals (Han et al., 2009).

The presence of organisms of different trophic groups decreases significantly in radioactive soils (Garnier-Laplace et al., 2013); bacterivorous and fungivorous nematodes may be more resistant to soils with this type of element (Lecomte-Pradines et al., 2014). However, in this study, it was observed that *Trichodorus*, which is an herbivorous nematode, had a high association with the radioactive element cerium, which demonstrates that this type of nematode could present structural and functional characteristics making it tolerant to stressful conditions (Kabir et al., 2018).

Some nematodes may have different tolerance levels toward some heavy metals (Gutiérrez et al., 2016), as in the cases of *Tylenchus* and *Globodera* and titanium and lead in the rainy season and *Tylenchus*, *Globodera*, and *Meloidogyne* and nickel, titanium, and vanadium in the dry season. It seems likely that the tolerance of these nematodes to heavy metals provides them the potential for selective adaptation since this capacity is not observed in all herbivorous nematodes (Ekschmitt & Korthals, 2006).

Certain nematodes can be tolerant to soil stressors (Hou et al., 2022), as observed in this research with *Helicotylenchus* and vanadium in the rainy season, because the exchangeable forms of heavy metals favor the absorption rates of plants that feed on this type of nematode (Park et al., 2011).

4. Conclusions

The bioindicator behavior of the nematodes showed specific interactions between some genera and specific abiotic stress gradient variables; additionally, generalist interactions occurred. However, both in the rainy season and in the dry season, the abiotic stress gradient was highly correlated with bacterivorous, fungivorous, and phytoparasitic nematodes and had a low correlation with omnivorous and predatory nematodes, showing the bioindicator capacity of nematodes for stress parameters that impact soil quality.

In this research, specific and general relationships of nematodes with heavy metals were found, for this reason, the validation of these relationships in other types of soil with mining influence is recommended, this with the purpose of increasing the robustness and effectiveness in the evaluation of soil quality.

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References

Achicanoy, J., Navia, J., & Betancourth, C. (2012). Dinámica poblacional de nematodos de vida libre en diferentes usos y manejos del suelo. *Revistas de Ciencias Agrícolas, 29,* 26-38.

Aujoulat, F., Pagès, S. Masnou, A., Emboulé, L., Teyssier, C., Marchandin, H., & Jumas-Bilak, E. (2019). The population structure of Ochrobactrum isolated from entomopathogenic nematodes indicates interactions with the symbiotic system. *Infection, Genetics & Evolution, 70,* 131-139.

Bal, H., Acosta, N., Chen, Z., Grewal, P., & Hoy, C. (2017). Effect of habitat and soil management on dispersal and distribution patterns of entomopathogenic nematodes. *Applied Soil Ecology, 121,* 48-59.

Bongers, T., & Esquivel, A. (2015). Manual morfología de los nematodos. Universidad Nacional de Costa Rica.

Calvão, T., Duarte, C., & Pimentel, C. (2019). Climate and landscape patterns of pine forest decline after invasion by the pinewood nematode. *Forest Ecology and Management, 433,* 43-51.

Campos-Herrera, R., El-Borai, F., Rodríguez, J. & Duncan, L. (2016). Entomopathogenic nematode food web assemblages in Florida natural areas. *Soil Biology and Biochemistry, 93,* 105-114.

Coyne, D., Nicol, J., & Claudius-Cole, B. (2007). Practical plant nematology: a field and laboratory guide. SP-IPM Secretariat, International Institute of Tropical Agriculture (IITA), Cotonou, Benin. Existe.

da Silva, J., Ferris, H., Cares, J., & Esteves, A. (2021). Effect of land use and seasonality on nematode faunal structure and ecosystem functions in the Caatinga dry forest. *European Journal of Soil Biology, 103(2021).*

Darré, E., Cadenazzi, M., Mazzilli, S. R., Rosas, J. F., & Picasso, V. D. (2019). Environmental impacts on water resources from summer crops in rainfed and irrigated systems. *Journal of environmental management, 232,* 514-522.

du Preez, G., Daneel, M., Wepener, V., & Fourie, H. (2018). Beneficial nematodes as bioindicators of ecosystem health in irrigated soils. *Applied Soil Ecology, 132(2018),* 155-168.

Egea, I., Estrada, Y., Flores, F., & Bolarín, M. (2022). Improving production and fruit quality of tomato under abiotic stress: Genes for the future of tomato breeding for a sustainable agriculture. *Environmental and Experimental Botany, 204,* 105086.

Ekschmitt, K., & Korthals, G. (2006). Nematodes as sentinels of heavy metals and organic toxicants in the soil. *Journal of Nematology, 38(1),* 13-19.

Ezenne, G., Jupp, L., Mantel, S., & Tanner, J. (2019). Current and potential capabilities of UAS for crop water productivity in precision agriculture. *Agricultural Water Management, 218,* 158-164.

Fournier, B., Steiner, M., Brochet, X., Degrune, F., Mammeri, J., et al. (2022). Toward the use of protists as bioindicators of multiple stresses in agricultural soils: A case study in vineyard ecosystems. *Ecological Indicators, 139,* 108955.

Garnier-Laplace, J., Geras'kin, S., Della-Vedova, C., Beaugelin-Seiller, K., Hinton, T., Real, A., & Oudalova, A. (2013). Are radiosensitivity data derived from natural field conditions consistent with data from controlled exposures? A case study of Chernobyl wildlife chronically exposed to low dose rates. *Journal of Environmental Radioactivity, 121,* 12-21.

Gautam, M., Mishra, S., & Agrawal, M. (2022). Bioindicators of soil contaminated with organic and inorganic pollutants. *New Paradigms in Environmental Biomonitoring Using Plants,* 271-298.

Guerrero, R. (2017). Manual de nematodos fitoparásitos identificados de especies cuarentanarias. *Agrocalidad, 136(1),* 1-42.

Gu, Y., Han, C., Fan, J., Shi, X., Kong, M., & Li, F. (2018). Alfalfa forage yield, soil water and P availability in response to plastic film mulch and P fertilization in a semiarid environment. *Field Crops Research, 215,* 94-103.

Gutiérrez, C., Fernández, C., Escuer, M., Campos-Herrera, R., Beltrán, M., Carbonell, G., & Rodríguez, J. (2016). Effect of soil properties, heavy metals and emerging contaminants in the soil nematodes diversity. *Environmental Pollution, 213,* 184-194.

Han, D., Zhang, X., Tomar, V., Li, Q., Wen, D., & Liang, W. (2009). Effects of heavy metal pollution of highway origin on soil nematode guilds in North Shenyang, China. Journal of *Environmental Sciences, 21(2),* 193-198.

Hernández-Ochandia, D., Rodríguez-Hernández, M., Miranda-Cabrera, I., & Holgado, R. (2016). Métodos para la extracción de nematodos presentes en suelos del agrupamiento Ferralítico en Cuba. *Revista Protección Vegetal, 31,* 228-232.

Hou, J., Hu, C., Li, P., & Lin, D. (2022). Multidimensional bioresponses in nematodes contribute to the antagonistic toxic interaction between pentachlorophenol and TiO$_2$ nanoparticles in soil. *Journal of Hazardous Materials, 424(2022).*

Kabir, M., Mwamula, A., Lee, K., Jeong, M., Lee, D., & Park, J. (2018). Spatial distribution of Heterodera trifolii in Chinese cabbage fields. *Journal of Asia-Pacific Entomology, 21(2),* 688-694.

Kaya, Ç., Yazar, A., & Sezen, S. (2015). SALTMED model performance on simulation of soil moisture and crop yield for quinoa irrigated using different irrigation systems, irrigation strategies and water qualities in turkey. *Agriculture and Agricultural Science Procedia, 4,* 108-118.

Kooch, Y., & Ghaderi, E. (2023). The effect of Crataegus and Berberis canopy types on bioindicators of soil quality in a semi-arid climate. *Journal of Arid Environments, 208,* 104862.

Lecomte-Pradines, C., Bonzom, J., Della-Vedova, C., Beaugelin-Seiller, K., Villenave, C., Gaschak, S., & Garnier-Laplace, J. (2014). Soil nematode assemblages as bioindicators of radiation impact in the Chernobyl Exclusion Zone. *Science of the Total Environment, 490,* 161-170.

Losi, V., Grassi, E., Balsamo, M., Rocchi, M., Gaozza, L., & Semprucci, F. (2021). Changes in taxonomic structure and functional traits of nematodes as tools in the assessment of port impact. *Estuarine, Coastal and Shelf Science, 260(2021)*.

Martinez, J., Torres, M., dos Santos, G., & Moens, T. (2018). Influence of heavy metals on nematode community structure in deteriorated soil by gold mining activities in Sibutad, southern Philippines. *Ecological Indicators, 91(2017)*, 712-721.

Mciver, D., Valdivieso, Y., & Rojas, J. (2022). Caracterización geoquímica de La mineralización aurífera del yacimiento orogenico Ollachea cordillera oriental-sureste del Perú. Minera Irl.

Murphy, S., Hathcock, C., Espinoza, T., Fresquez, P., Berryhill, J., et al. (2023). Comparative spatially explicit approach for testing effects of soil chemicals on terrestrial wildlife bioindicator demographics. *Environmental Pollution*, 316, 120541.

Nielsen, U., Ayres, E., Wall, D., Li, G., Bardgett, R., Wu, T., & Garey, J. (2014). Global-scale patterns of assemblage structure of soil nematodes in relation to climate and ecosystem properties. *Global Ecology and Biogeography, 23(9)*, 968-978.

Ouyang, Y., Chen, D., Fu, Y., Shi, W., Provin, T., Han, A., & Shaik, E. (2021). Direct cell extraction from fresh and stored soil samples: impact on microbial viability and community compositions. *Soil Biology and Biochemistry, 155(2021)*.

Papa, D., Almeida, D., Silva, C., Figueiredo, E., Stark, S., Valbuena, R., & d'Oliveira, M. (2020). Evaluating tropical forest classification and field sampling stratification from lidar to reduce effort and enable landscape monitoring. *Forest Ecology and Management, 457(2020)*.

Park, B., Lee, J., Ro, H., & Kim, Y. (2011). Effects of heavy metal contamination from an abandoned mine on nematode community structure as an indicator of soil ecosystem health. *Applied Soil Ecology, 51(1)*, 17-24.

Petrovskaia, A., Ryzhakov, G., & Oseledets, I. (2021). Optimal soil sampling design based on the maxvol algorithm. *Geoderma, 402(2021)*.

Piedra, R. (2015). Guía de muestreo de nematodos fitoparásitos en cultivos agrícolas. Instituto Nacional de Innovación y Transferencia en Tecnología Agropecuaria. Costa Rica.

Puglielli, G., Laanisto, L., Gori, A., & Cardoso, A. (2023). Woody plant adaptations to multiple abiotic stressors: where are we? *Flora*, 152221.

R Development Core Team. (2016). Introducción a R Notas sobre R: Un entorno de programación para Análisis de Datos y Gráficos Versión 1.0.1.

Rosli, N., Leduc, D., Rowden, A., Probert, P., & Clark, M. (2018). Regional and sediment depth differences in nematode community structure greater than between habitats on the New Zealand margin: Implications for vulnerability to anthropogenic disturbance. *Progress in Oceanography, 160*, 26-52.

Šalamún, P., Hanzelová, V., & Miklisová, D. (2018). Variability in responses of soil nematodes to trace element contamination. *Chemosphere, 210*, 166-174.

Sánchez-Moreno, S., & Talavera, M. (2013). Los nematodos como indicadores ambientales en agroecosistemas. *Ecología y Medio Ambiente, 22(1)*, 50-55.

Sechi, V., De Goede, R., Rutgers, M., Brussaard, L., & Mulder, C. (2018). Functional diversity in nematode communities across terrestrial ecosystems. *Basic and Applied Ecology, 30*, 76-86.

Seijas, I. (2004). Estudio de la nematofauna edáfica asociada a cultivos frutícolas de manzano y a biotopos forestales de roble en Galicia como base previa al control biológico de plagas de babosas con nematodos zooparásitos. Departamento de Biología Animal, Universidad de Santiago de Compostela, España.

Song, D., Pan, K., Tariq, A., Li, Z., Sun, X., & Wu, X. (2017). Large-scale patterns of distribution and diversity of terrestrial nematodes. *Applied Soil Ecology, 114*, 161-169.

Touron-Poncet, H., Bernadet, C., Compin, A., Bargier, N., & Céréghino, R. (2014). Implementing the Water Framework Directive in overseas Europe: A multimetric macroinvertebrate index for river bioassessment in Caribbean islands. *Limnologica, 47*, 34-43.