ORIGINAL RESEARCH
published: 27 April 2022
doi: 10.3389/fenvs.2022.884889

Growth and Pb Uptake of *Brassica campestris* Enhanced by Two Ecological Earthworm Species in Relation to Soil Physicochemical Properties

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A comprehensive understanding of the influence of earthworms on the growth and Pb accumulation of leafy vegetables is significant for soil management and human health. This study was aimed to evaluate the different influences of two ecological earthworm species on the growth and Pb accumulation of *Brassica campestris* in a Pb-contaminated soil and their relationship with soil physico-chemical properties. In a 30-day microcosm experiment, the anecic and native earthworm species *Amynthas aspergillum* and the epigeic species *Eisenia fetida* were inoculated in soil artificially contaminated with Pb at different levels (i.e., 0, 100, 500, and 1,000 mg kg^{-1}), and *B. campestris* was grown. With a survival rate of 81%–100%, *A. aspergillum* was more tolerant to Pb contamination than *E. fetida* with 46%–84%. At the same time, earthworm inoculation significantly increased soil Pb bioavailability ($p < 0.05$). At the 500 and 1,000 mg kg^{-1} Pb levels, the treatments with earthworm inoculation showed higher plant biomass, leaf area, and chlorophyll concentration than the treatments without earthworm. The principal component analysis (PCA) showed that earthworm inoculation exerted a stronger effect on soil properties than Pb contamination, but the latter had a stronger effect on plant growth and Pb accumulation. Compared with *A. aspergillum*, *E. fetida* had a greater effect on soil cation exchange capacity, available Pb, and plant growth and Pb accumulation. In contrast, *A. aspergillum* had a greater effect on soil C and N contents than *E. fetida*. The co-inertia analysis revealed that plant Pb accumulation was positively correlated with soil available Pb and CEC. The leaf chlorophyll concentration was closely related to soil Eh, pH, and Dissolved organic carbon. The findings of this study showed that in the Pb-contaminated soils, earthworm inoculation exerted a strong effect on soil physicochemical properties and the growth and Pb accumulation of the leafy vegetable *B. campestris*. Both the epigeic earthworm species *E. fetida* and the anecic species *A. aspergillum* were associated with higher Pb accumulation or concentration in *B. campestris*, which may bring a possible risk to food security.

Keywords: earthworm, Pb-contaminated soil, leafy vegetable, Pb bioavailability, plant growth

OPEN ACCESS

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Specialty section:
This article was submitted to Soil Processes, a section of the journal Frontiers in Environmental Science

Received: *27 February 2022*
Accepted: *04 April 2022*
Published: *27 April 2022*

Citation:
Tibihenda C, Zhang M, Zhong H, Xiao L, Wu L, Dai J, Liu K and Zhang C (2022) Growth and Pb Uptake of Brassica campestris Enhanced by Two Ecological Earthworm Species in Relation to Soil Physicochemical Properties. Front. Environ. Sci. 10:884889. doi: 10.3389/fenvs.2022.884889

1 INTRODUCTION

Soil Pb contamination has a detrimental impact on soil health and functionality, and it also poses a risk to humans and other living organisms due to biomagnification *via* the food chain (Zulfiqar et al., 2019; Kumar et al., 2020). *Brassica campestris* is one of the most widely consumed and grown leafy vegetables in Asia; meanwhile, it is a significant route for Pb to enter the food chain. When *B. campestris* is cultivated in Pb-contaminated soils, Pb is absorbed by the roots and translocated to the edible organs (Liu et al., 2020; Ali et al., 2022), which causes concern for vegetables by highlighting a potential food safety problem. Therefore, information regarding Pb accumulation and possible risks in leafy vegetables is urgently needed to raise public awareness.

When it comes to the bioavailability of Pb, the total concentration in the soil does not always indicate how Pb is readily accessible to plants. The bioavailable Pb is the fraction of Pb taken up by plants, useful in accessing potential metal bioaccumulation in plants. However, some studies have shown that Pb uptake by *B. campestris* is related to Pb bioavailability in soil (Yang et al., 2016; Liu et al., 2019). Many factors influence the transformation of Pb from unavailable to available forms. Soil attributes such as pH, redox potential (Eh), organic matter content, cation exchange capacity (CEC), and competing cations govern the bioavailability of Pb in soil (Romero-Freire et al., 2015; Xiao et al., 2017a; Xiao et al., 2017b). Xiao et al. (2020b) found that soil microbial properties (e.g., soil microbial biomass, soil respiration, soil enzyme activities) significantly affect the accumulation of Pb in brassica leaves. So far, few studies have focused on the effect of soil macrofauna on Pb accumulation in leafy vegetables, especially earthworms, which are keystone species in the soil ecosystem (Gluhar et al., 2021).

Pb bioavailable fraction in soil reflects Pb accumulation in plants. Earthworm activities such as feeding, digging, and metabolite excreting strongly influence soil properties and Pb availability (Porfido et al., 2022; Zhang et al., 2022), hence enhancing soil Pb participation in the food chain. The abilities of earthworms to regulate soil properties and Pb bioavailability are intimately connected to the diverse ecotypes of earthworms that differ in lifestyles, digestive systems, and feeding behaviors (Jeyanthi et al., 2016; Sinkakarimi et al., 2020). There are two distinct processes in which various ecological earthworms can mediate the fate of Pb. First, the intake of soil Pb incorporated in mineral particles or organic staff ingested by earthworms varies with the feeding habits of earthworms, resulting in varying Pb bioavailability and accumulation. Second, earthworm activities such as borrowing, casting, and composting influence soil physicochemical properties, leading to changes in Pb bioavailability (Wang et al., 2018; Richardson et al., 2020). Therefore, a new insight to be gained is how earthworm ecotypes affect the chemical behavior of Pb to involve the food chain of vegetable-soil systems. In previous studies, different ecological earthworm species have been investigated for their influences on metal accumulation in plant leaves, stems, and roots (Du et al., 2014; Wang et al., 2020; Guo et al., 2022). However, there is still a lack of knowledge on how earthworms of different ecotypes influence the soil physicochemical properties and Pb bioavailability in the vegetable-soil system.

Based upon the above argument, a microcosmic experiment was set up with two ecological earthworm species. The anecic species *Amynthas aspergillum* feeds on a mixture of soil and little-decomposed organic debris, showing an exceptional soil-ingesting ability. The epigeic species *Eisenia fetida*, which is a composting earthworm, lives on high quality organic matters (compost or manure heaps) (Zhang et al., 2020; Paul et al., 2022). Most previous studies relied on the impact of *E. fetida* on vermicompost. So far, few studies have focused on the effects of these two ecological earthworm species on Pb accumulation, the growth of leafy vegetables, and the underlying physico-chemical mechanism. In this study, firstly, we expected that Pb accumulation and the growth of *B. campestris* are sensitive to the presence of earthworms. Secondly, we hypothesized that the anecic species *Amynthas aspergillum* would be more efficient in Pb transformation and accumulation than *E. fetida* because Pb is bound to soil particles and has a high affinity to organic matter and different ecological earthworm species have different feeding habits. Last but not the least, we hypothesized that Pb transformation in the soil-plant system and the growth of *B. campestris* would be influenced by the changes in soil physico-chemical properties caused by earthworms and/or Pb input levels.

2 MATERIALS AND METHODS

2.1 Soil, Earthworm Species, and Plant

The soil was collected from the 0–20 cm layer of a fallow vegetable field located at 23°54′ N and 113°27′ E in Qingyuan City, Guangdong Province, China. After plant residues and stones were removed, the soil was air-dried and sieved to <2 mm. The soil pH, organic C, total N, and C: N ratio were 6.06, 16.6 $g \cdot kg^{-1}$, 1.30 $g \cdot kg^{-1}$, and 12.7, respectively, and Pb content was 20 $mg \cdot kg^{-1}$.

Earthworms of *A. aspergillum* and *E. fetida* were purchased from a biofertilizer company in Qingyuan, Guangdong Province, China. Before the experiment, the earthworms were cultured in uncontaminated soil with a moisture content of 40%–60% of field water capacity at 25°C, and organic matter was added for better growth.

Seeds of *B. campestris* were provided by the Guangdong Academy of Agricultural Sciences.

2.2 Experimental Design

A total of 96 pots (18.5 cm × 12 cm × 16 cm) were used in this experiment. Each pot was filled with 1 kg soil, which had been spiked with $PbCl_2$ to set up 4 Pb contamination levels of 0, 100, 500, and 1,000 $mg \cdot kg^{-1}$ (OECD, 2000) and had been aged for 10 months. Six treatments were further set up in four replicates for each Pb level: 1) no plant or earthworm (S), 2) *B. campestris* (SP), 3) *A. aspergillum* (SA), 4) *E. fetida* (SE), 5) *B. campestris* + *A. aspergillum* (SPA), and 6) *B. campestris* + *E. fetida* (SPE). In each pot of the SP, SPA, and SPE treatments, three seedlings of *B. campestris* were transplanted, and to each pot of SA, SE, SPA, and SPE, 23 ± 1 g of healthy clitellate adult earthworms were introduced. The transplanted seedlings were healthy and uniform in size. Similarly, the introduced earthworms were uniform in weight, 5.73 ± 0.4 g individual^{-1} for *A. aspergillum* and 0.4 ± 0.1 g individual^{-1} for *E. fetida*.

During the experiment, the temperature was maintained at 25°C, and the light intensity was 400–800 lx. The earthworms were prevented from escaping according to Zhou et al. (2016) and Chen et al. (2017). Soil moisture in each pot was maintained at field capacity. The day before harvesting, leaf chlorophyll concentration was measured on the newest fully expanded leaf using a Konica Minolta SPAD-502 chlorophyll meter (Alordzinu et al., 2021). After 30 days, plant height, shoot diameter, and the number of branches were recorded. The plants were harvested, washed, and oven-dried. The earthworms in each pot were collected, counted, and weighed before being cultured for 7 days at 25°C to empty the guts. The soil in each pot was air-dried and passed through 2-mm and 0.149-mm sieves for chemical analysis.

2.3 Laboratory Analyses

Soil pH and Eh were measured at 1:2.5 soil: water ratio. Soil total N was quantified by Kjeldahl digestion, organic C was determined by the dichromate digestion method, and CEC was estimated by the ammonium acetate (1 mol L^{-1}, pH 7.0) method (Sparks et al., 1996). Dissolved organic carbon (DOC) was quantified according to Dai et al. (2004). Available N was measured by the alkaline diffusion method (Cornfield, 1960).

Soil total Pb was determined by flame atomic absorption spectroscopy (FAAS) after soil samples were digested with $HCl–HF–HNO_3–HClO_4$ in an electrically heated block digester (Amacher, 1996). Soil bioavailable Pb was extracted with pH 7.3 diethylenetriaminepentaacetic acid (DTPA) extractant consisting of 0.005M DTPA, 0.1M triethanolamine (TEA), and 0.01M calcium chloride ($CaCl_2$) at a soil: solution ratio of 1:2 for 2 h, centrifuged at 4,000 g for 20 min, then filtered. Finally, Pb was quantified by FAAS (Lindsay and Norvell, 1978).

For Pb concentration in earthworms, 0.20 g of dried and crushed (0.2 mm) earthworm sample was homogenized with 8 ml concentrated HNO_3 and 2 ml concentrated $HClO_4$ for 12 h and digested at 250°C for 2 h. After cooling, the solution was diluted to a final volume of 50 ml using deionized water, and Pb concentration was quantified by FAAS. Using a microwave digestion system, 0.20 g of plant sample was wet-digested with aqua regia solution (4 ml HNO_3 and 1 ml HCl), and Pb concentration was quantified using FAAS (Moral et al., 1996).

2.4 Calculations

Leaf area was calculated according to the Montgomery equation (Shi et al., 2019; He et al., 2020):

$$\mathrm{LA}\ (\mathrm{cm}^2) = \mathrm{MP}\,(\mathrm{LL} \times \mathrm{LW})$$

Where LA is leaf area (cm^2), LL is leaf length (cm), LW is leaf width (cm), and MP is Montgomery coefficient, which is 0.68.

The transfer factor (TF) of Pb was calculated as (Gupta et al., 2008):

$$\mathrm{TF} = \frac{\text{Pb content in the aboveground parts}}{\text{Pb content in the belowground parts}}$$

The survival rate (SR) of earthworms (%) was calculated as (Wu et al., 2020a):

$$\mathrm{SR}\,(\%) = \frac{\text{Number of earthworms at the end of the experiment}}{\text{Number of earthworms at the start of the experiment}} \times 100$$

2.5 Statistical Analysis

Data were processed using the SPSS statistical software and analyzed using one-way ANOVA, followed by Duncan's multiple range test and *t*-test. Data are presented as mean ± standard deviation, and the significance level was set at $p < 0.05$. Principal component analysis (PCA) and co-inertia analysis were performed using the Ade-4 package in R to explore the influences of earthworms on soil properties, Pb bioavailability in soil, and Pb accumulation in the plant (Thioulouse et al., 1997).

3 RESULTS

3.1 Earthworm Biomass, Survival Rate, and Pb Accumulation

In all treatments, *A. aspergillum* tolerated Pb contamination better than *E. fetida* (**Table 1**). At the 0 and 100 mg kg^{-1} Pb contamination levels, the biomass of *A. aspergillum* was decreased by 16% and 5%, respectively, and at the 500 and 1,000 mg kg^{-1} Pb levels, it was increased by 11% and 2%, respectively, in SPA at the end of the experiment compared with at the beginning of the experiment ($p > 0.05$). At the 0 and 1,000 mg kg^{-1} Pb contamination levels, the biomass of *A. aspergillum* was reduced by 12% and 13%, respectively, and at the 100 and 500 mg kg^{-1} Pb levels, it was increased by 2% and 18% ($p < 0.05$), respectively, in SA at the end of the experiment compared with at the start of the experiment. The survival rate of *A. aspergillum* was high in all treatments, ranging from 81% to 100% (**Table 1**).

The biomass of *E. fetida* was markedly decreased at the end of the experiment compared with at the beginning of the experiment. At the 100, 500, and 1,000 mg kg^{-1} Pb contamination levels, the biomass of *E. fetida* was significantly decreased by 54% ($p < 0.05$), 47% ($p < 0.01$), and 38% ($p < 0.01$), respectively, in SPE at the end of the experiment compared with at the start of the experiment. It decreased significantly by 22% ($p < 0.05$), 21% ($p < 0.05$), 22% ($p < 0.05$), and 24% ($p < 0.05$) in SE at the 0, 100, 500, and 1,000 mg kg^{-1} Pb levels, respectively. Generally, *E. fetida* survived better in the SE treatments, with the survival rates ranging from 77% to 79% across the Pb contamination levels. It displayed lower survival rates in the SPE treatments at the 100, 500, and 1,000 mg kg^{-1} Pb levels, which were 46%, 53%, and 62%, respectively (**Table 1**). At the higher Pb contamination levels, the earthworms accumulated more Pb in their bodies ($p < 0.05$,

TABLE 1 | Biomass and survival rate of the two earthworm species, *Amynthas aspergillum* and *Eisenia fetida*, in the treatments with different soil Pb concentrations.

Earthworm species	Soil Pb	Treatment	Biomass		Survival rate
	mg·kg^{-1}		Day 0	Day 30	%
			g·pot^{-1}		
A. aspergillum	0	SA	22.8 ± 1.22A^{NS}	20.2 ± 3.81A	87.5 ± 14.4A
		SPA	22.8 ± 1.93A^{NS}	19.2 ± 4.62A	81.3 ± 12.5A
	100	SA	23.6 ± 1.61A	24.1 ± 2.46A^{NS}	93.8 ± 12.5A
		SPA	22.8 ± 1.84A^{NS}	21.7 ± 4.43A	87.5 ± 14.3A
	500	SA	22.6 ± 1.71A	26.6 ± 2.37A*	100 ± 0.00A
		SPA	22.3 ± 1.88A	24.7 ± 2.52A^{NS}	100 ± 0.00A
	1,000	SA	21.8 ± 1.81A^{NS}	19.1 ± 8.10A	81.3 ± 37.5A
		SPA	24.5 ± 1.07A	25.1 ± 4.47A^{NS}	87.5 ± 14.4A
E. fetida	0	SE	23.0 ± 0.00A	18.0 ± 2.38A*	78.2 ± 10.3A
		SPE	23.0 ± 0.00A^{NS}	19.3 ± 4.02A	84.0 ± 17.5A
	100	SE	23.0 ± 0.00A	18.1 ± 3.54A*	78.6 ± 15.4A
		SPE	23.0 ± 0.00A	10.6 ± 5.28B*	46.1 ± 23.0B
	500	SE	23.0 ± 0.00A	17.9 ± 3.16A*	78.0 ± 13.8A
		SPE	23.0 ± 0.00A	12.1 ± 3.35B**	52.6 ± 14.6B
	1,000	SE	23.0 ± 0.00A	17.6 ± 2.53A*	76.5 ± 11.0A
		SPE	23.0 ± 0.00A	14.3 ± 2.38AB**	62.3 ± 10.4AB

Abbreviations: SA, A. aspergillum *was inoculated; SPA,* Brassica campestris *was grown and* A. aspergillum *was inoculated; SE,* E. fetida *was inoculated; SPE,* B. campestris *was grown and* E. fetida *was inoculated.*

Different letters indicate significant difference between the different Pb levels for a same treatment at $p < 0.05$. *The t-test was used to compare the biomasses at days 0 and 30 for a same treatment and a same Pb level, with* ***$p < 0.001$: **$p < 0.01$: *$p < 0.05$: *ns* $p > 0.05$.

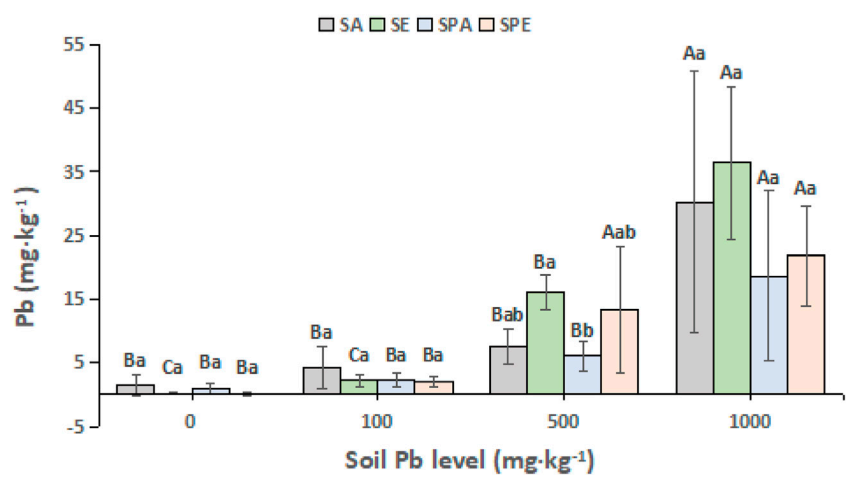

FIGURE 1 | Pb accumulation in the two earthworm species, *Amynthas aspergillum* and *Eisenia fetida*, under different Pb contamination conditions. *SA: *A. aspergillum* was inoculated, SE: *E. fetida* was inoculated, SPA: *Brassica campestris* was grown and *A. aspergillum* was inoculated, SPE: *B. campestris* was grown and *E. fetida* was inoculated. Different uppercase letters indicate significant differences for a same treatment between different soil Pb levels at $p < 0.05$; different lowercase letters indicate significant differences between treatments for a same soil Pb level at $p < 0.05$.

Figure 1). At the 500 mg kg^{-1} Pb level, *E. fetida* accumulated significantly more Pb in SE than *A. aspergillum* in SPA ($p < 0.05$).

3.2 Soil Properties and DTPA-Extractable Pb

3.2.1 Soil Properties

At the end of the experiment, soil pH ranged from 5.20 to 6.27, with the highest values in SP and the lowest in SE ($p < 0.001$, **Table 2**). At the 0, 100, 500, and 1,000 mg kg^{-1} Pb levels, the inoculation of *A. aspergillum* alone (i.e., the SA treatment) substantially decreased soil pH by 0.3, 0.2, 0.4, and 0.4 units, respectively, the inoculation of *E. fetida* alone (i.e., the SE treatment) significantly decreased soil pH by 0.5, 0.7, 0.8, and 0.7 units, respectively, while the growth of plant alone (i.e., the SP treatment) increased soil pH by 0.3, 0.3, 0.3, and 0.1 units, respectively, compared with the respective S treatments ($p < 0.05$).

TABLE 2 | Effects of earthworm inoculation and plant growth on soil chemical properties under different Pb contamination conditions.

Soil Pb	Treatment	pH	Eh	Organic C	DOC	Total N	Available N	CEC
mg·kg^{-1}			mV	g·kg^{-1}	mg·kg^{-1}	g·kg^{-1}	mg·kg^{-1}	cmol·kg^{-1}
0	S	6.01 ± 0.07Aab	56.5 ± 3.70Ac	13.8 ± 2.35Aa	65.8 ± 17.9Aab	0.65 ± 0.09Ac	64.8 ± 3.50Aa	3.80 ± 0.19Aa
	SP	6.29 ± 0.07Aa	40.8 ± 3.59Bd	14.0 ± 0.52Aa	61.7 ± 16.6Ab	0.69 ± 0.03Abc	61.3 ± 6.70Aa	3.59 ± 0.18Ba
	SA	5.71 ± 0.24Abc	73.5 ± 13.7Ab	15.6 ± 0.42Aa	75.1 ± 39.0Bab	0.78 ± 0.04Aa	70.0 ± 8.08Aa	3.63 ± 0.11Aa
	SE	5.56 ± 0.53Ac	95.8 ± 9.67Aa	14.2 ± 0.91Aa	80.5 ± 35.4Aab	0.79 ± 0.04Aa	64.8 ± 3.50Aa	3.65 ± 0.09Ba
	SPA	5.82 ± 0.24Abc	67.5 ± 13.9Abc	14.5 ± 1.38Ca	101 ± 7.48Aab	0.72 ± 0.05ABabc	68.3 ± 6.70ABa	3.66 ± 0.41Aa
	SPE	5.62 ± 0.13Bbc	78.8 ± 7.46Ab	14.6 ± 0.66Aa	121 ± 62.5Aa	0.74 ± 0.05Aab	63.0 ± 5.72Aa	4.01 ± 0.48Aa
100	S	6.04 ± 0.05Ab	54.8 ± 2.87Bc	12.1 ± 0.71Ac	54.3 ± 2.99Aa	0.67 ± 0.02Ab	63.0 ± 0.00Aab	3.78 ± 0.19Aa
	SP	6.37 ± 0.02Aa	36.3 ± 1.50Bd	12.5 ± 0.89Ac	46.6 ± 13.2Ba	0.53 ± 0.02Bc	61.3 ± 3.50Ab	3.77 ± 0.17ABa
	SA	5.71 ± 0.17Ac	73.3 ± 9.39Ab	15.0 ± 0.56Ab	61.0 ± 42.6ABa	0.73 ± 0.13Aa	59.5 ± 7.00Bb	3.94 ± 0.30Aa
	SE	5.37 ± 0.10Ad	92.8 ± 5.80Aa	14.4 ± 0.59Ab	81.2 ± 27.4Ba	0.73 ± 0.03Aa	64.8 ± 3.50Aab	3.67 ± 0.25Ba
	SPA	5.91 ± 0.13Ab	61.8 ± 7.41Ac	17.3 ± 1.11Aa	73.4 ± 14.1Aa	0.76 ± 0.03ABa	68.3 ± 3.50ABa	3.89 ± 0.21Aa
	SPE	5.66 ± 0.15ABc	76.5 ± 8.43ABb	14.3 ± 0.83Ab	80.7 ± 19.4Ba	0.52 ± 0.02Bc	59.5 ± 4.04Ab	3.97 ± 0.21Aa
500	S	5.95 ± 0.03Ab	59.5 ± 1.73Ac	11.7 ± 0.36Ac	58.4 ± 3.45Aa	0.67 ± 0.03Abcd	64.8 ± 3.50Aa	3.74 ± 0.49Aa
	SP	6.27 ± 0.03Aa	41.5 ± 1.91Bd	14.1 ± 1.11Ab	55.8 ± 9.25ABa	0.66 ± 0.02Acd	66.5 ± 7.00Aa	3.89 ± 0.05Aa
	SA	5.73 ± 0.06Ac	72.5 ± 3.79Ab	16.3 ± 0.89AAa	58.6 ± 22.8ABa	0.75 ± 0.02ABab	66.5 ± 4.04ABa	3.94 ± 0.19Aa
	SE	5.20 ± 0.14Ad	102 ± 8.10Aa	13.1 ± 1.27Abc	86.8 ± 5.89Ba	0.60 ± 0.11Bd	66.5 ± 4.04Aa	4.01 ± 0.09Aa
	SPA	6.05 ± 0.12Ab	54.0 ± 6.83Ac	16.4 ± 0.31ABa	83.5 ± 24.3Aa	0.79 ± 0.03Aa	70.0 ± 0.00Aa	3.98 ± 0.29Aa
	SPE	5.92 ± 0.24Ab	61.3 ± 13.8Bc	13.4 ± 2.11Abc	91.0 ± 51.5Ba	0.72 ± 0.03Aabc	66.5 ± 9.04Aa	3.88 ± 0.19Aa
1,000	S	5.99 ± 0.05Aab	57.5 ± 2.65Acd	13.2 ± 1.65Aa	47.9 ± 21.1Bbc	0.62 ± 0.06Ab	61.3 ± 3.50Aa	4.02 ± 0.11Aa
	SP	6.13 ± 0.10Ba	49.8 ± 5.50Ad	12.9 ± 1.77Aa	29.1 ± 24.3Bc	0.68 ± 0.07Aab	63.0 ± 0.00Aa	3.96 ± 0.06Aab
	SA	5.62 ± 0.11Ac	78.0 ± 6.06Ab	15.6 ± 1.91Aa	58.1 ± 10.4Aabc	0.74 ± 0.03Ba	64.8 ± 3.50ABa	3.76 ± 0.12Ab
	SE	5.31 ± 0.09Ad	96.3 ± 5.38Aa	13.3 ± 0.76Aa	96.5 ± 53.3Ba	0.69 ± 0.01Aab	64.8 ± 3.50Aa	4.00 ± 0.21Aab
	SPA	5.88 ± 0.16Ab	64.0 ± 9.06Ac	14.9 ± 0.92BCa	69.6 ± 16.3Aabc	0.69 ± 0.08Bab	63.0 ± 0.00Ba	3.81 ± 0.20Aab
	SPE	5.89 ± 0.12ABb	63.3 ± 6.95Bc	13.2 ± 0.27Aa	87.7 ± 27.6Bab	0.71 ± 0.04Aa	63.0 ± 5.72Aa	3.91 ± 0.16Aab

Abbreviations: DOC, dissolved organic carbon; CEC, cation exchange capacity; S, without earthworm or plant; SP, Brassica campestris *was grown; SA,* Amynthas aspergillum *was inoculated; SE,* Eisenia fetida *was inoculated; SPA,* B. campestris *was grown and* A. aspergillum *was inoculated; SPE,* B. campestris *was grown and* E. fetida *was inoculated. Different uppercase letters indicate significant differences between different soil Pb levels for a same treatment at* $p < 0.05$*; different lowercase letters indicate significant differences between treatments for a same soil Pb level at* $p < 0.05$.

In contrast, soil Eh (36.25–102.75 mV) showed its lowest values in SP and the highest in SE (**Table 2**). Earthworm inoculation alone (i.e., the SA and SE treatments) significantly increased soil Eh in comparison with the S and SP treatments. The growth of *B. campestris* alone (i.e., the SP treatment) substantially lowered soil Eh as compared with the S treatment except at the 1,000 mg kg^{-1} Pb level (**Table 2**). At the 0, 100, 500, and 1,000 mg kg^{-1} Pb levels, the inoculation of *E. fetida* alone (i.e., the SE treatment) significantly increased soil Eh by 39, 38, 43, and 39 mV, respectively, while the growth of *B. campestris* alone (i.e., the SP treatment) substantially decreased soil Eh by 16, 19, 18, and 8 mV, respectively, compared with the respective S treatments. Soil Eh was generally higher in the SPE treatment than in the SPA treatment except at the 1,000 mg kg^{-1} Pb level.

The treatments with earthworm inoculation generally increased soil organic C content compared with the earthworm-free treatments except at the 500 mg kg^{-1} Pb level, where soil organic C content in the SP treatment was higher than those in the *E. fetida* addition treatments (**Table 2**). The treatments with inoculation of *A. aspergillum* (i.e., SA and SPA) generally had higher contents of soil organic C than the treatments with inoculation of *E. fetida* (i.e., SE and SPE), with significant differences at the 100 and 500 mg kg^{-1} Pb levels ($p < 0.05$). At the 100 mg kg^{-1} Pb level, soil organic C was 24%, 19%, 43%, and 18% higher in SA, SE, SPA, and SPE, respectively, than in S. At the 500 mg kg^{-1} Pb level, soil organic C was 39%, 12%, 40%, and 15% higher in SA, SE, SPA, and SPE, respectively than in S (**Table 2**).

Soil DOC was higher in SA, SE, SPA, and SPE than in S and SP ($p > 0.05$) and had the lowest values in SP (**Table 2**). The SPE treatment had a higher DOC than the SPA treatment regardless of Pb contamination level ($p > 0.05$). At the 0 and 500 mg·kg^{-1} Pb levels, SPE exhibited the highest DOC among all treatments, which was 84% and 56%, respectively, higher than that in S, but a significant difference was only observed between SPE and SP at the 0 mg·kg^{-1} Pb level ($p < 0.05$). However, at the 100 and 1,000 mg·kg^{-1} Pb levels, the highest DOC was found in the SE treatment, which was 50% and 101%, respectively, higher than that in S, but significant differences were only observed between SE and S, SE and SP, and SPE and SP at the 1,000 mg kg^{-1} Pb level.

Earthworm activity generally increased soil total N content (**Table 2**). At the 0 mg kg^{-1} Pb level, soil total N was increased by 20% and 22% ($p < 0.05$) in SA and SE, respectively, compared with S. At the other Pb levels, soil total N was also increased in SA and SE except at the 500 mg kg^{-1} level where total N was considerably lower in the SE treatment than in the S treatment. The SPA and SPE treatments had close values of soil total N at all Pb contamination levels except at 100 mg kg^{-1} Pb levels where SPA had a much higher total N. At the 0, 100, 500, and 1,000 mg kg^{-1} Pb levels, soil total N in SPA was 11%, 13%, 18%, and 11%, respectively, higher than that in the respective S treatment. At the 0, 500, and 1,000 mg kg^{-1} Pb levels, soil total N

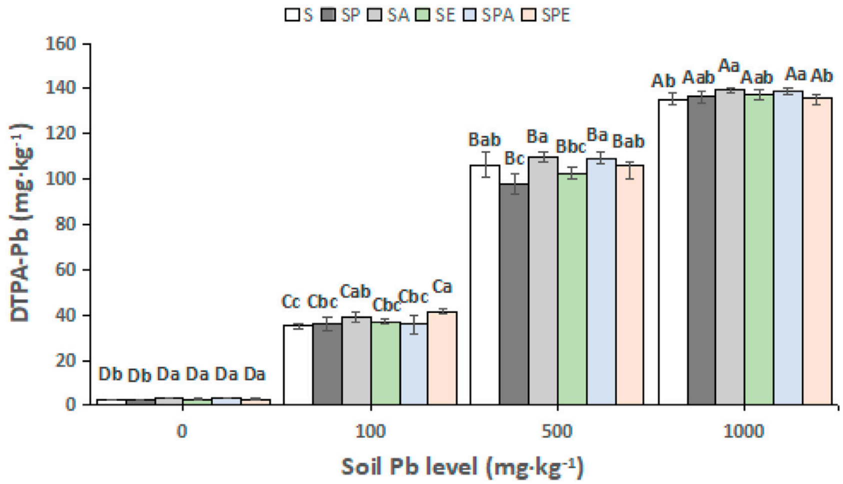

FIGURE 2 | Diethylenetriaminepentaacetic acid (DTPA)-extractable Pb contents in different treatments at 0, 100, 500, and 1,000 mg kg^{-1} Pb contamination levels. *S: without plant or earthworm, SP: *Brassica campestris* was grown, SA: *Amynthas aspergillum* was inoculated, SE: *Eisenia fetida* was inoculated, SPA: *B. campestris* was grown and *A. aspergillum* was inoculated, SPE: *B. campestris* was grown and *E. fetida* was inoculated. Different uppercase letters indicate significant differences between different Pb levels for a same treatment at $p < 0.05$; different lowercase letters indicate significant differences between treatments for a same soil Pb level at $p < 0.05$.

in SPE was increased by 14%, 7%, and 15%, respectively, compared with the respective S treatments (**Table 2**).

At both the 100 and 500 mg kg^{-1} Pb levels, the highest soil available N was found in SPA (**Table 2**). For the SA treatments, soil available N was 70.0 mg kg^{-1} at the 0 mg kg^{-1} Pb level but decreased significantly to 59.9 mg kg^{-1} at the 100 mg kg^{-1} Pb level. For the SPA treatments, soil available N was 70.0 mg kg^{-1} at the 500 mg kg^{-1} Pb level but decreased significantly to 63.0 mg kg^{-1} at the 1,000 mg kg^{-1} Pb level.

For the same Pb contamination level, there were no significant differences in soil CEC between the treatments except at the 1,000 mg kg^{-1} Pb level, where the CEC in S was significantly higher than that in SA.

3.2.2 DTPA-Extractable Pb

For a same treatment, there were significant differences ($p < 0.05$) in soil DTPA-extractable Pb content between the 4 Pb contamination levels (**Figure 2**). At the end of the experiment, soil DTPA-extractable Pb was generally higher in the treatments with inoculation of earthworms than in S and SP, and the differences were significant at the 0 mg kg^{-1} Pb level ($p < 0.05$). At the 100 mg kg^{-1} Pb level, soil DTPA-extractable Pb was significantly higher ($p < 0.05$) in SPE than in S, SP, SE, and SPA. At the 500 mg kg^{-1} Pb level, soil DTPA-extractable Pb was significantly higher ($p < 0.05$) in SA and SPA than in SP and SE. At the 1,000 mg kg^{-1} Pb level, soil DTPA-extractable Pb was significantly higher ($p < 0.05$) in SA and SPA than in S and SPE. Compared with SP, soil DTPA-extractable Pb in SPE was increased by 22% ($p < 0.05$), 13% ($p < 0.05$), and 8% ($p > 0.05$) at the 0, 100, and 500 mg kg^{-1} Pb levels, respectively, but was decreased by 0.8% at the 1,000 mg kg^{-1} level. Similarly, compared with SP, soil DTPA-extractable Pb in SPA was raised by 26% ($p < 0.05$), 12% ($p < 0.05$), and 2% ($p > 0.05$) at the 0, 500, and 1,000 mg kg^{-1} Pb levels, respectively, but was decreased by 2% at the 100 mg kg^{-1} Pb level.

3.2.3 PCA Analysis of the Soil Properties

PCA analysis revealed significant differences in soil properties between treatments at the same Pb pollution level (**Figures 3A,B**). The first factor extracted (36.3% of the variance explained) indicated the effects of earthworm inoculation and plant growth on soil pH, Eh, and C and N forms across all treatments (**Figure 3A**). The presence of earthworms and plants increased the contents of different soil C and N forms and soil Eh but decreased soil pH (**Figure 3B**, $p < 0.05$). The second factor extracted (23.3% of the variance explained) characterized the influence of Pb contamination level on soil CEC and available Pb content (**Figure 3C**). The high Pb contamination levels resulted in high soil CEC and available Pb content. Compared with *A. aspergillum*, the inoculation of *E. fetida* led to greater increases in soil CEC and DTPA-extractable Pb at the 1,000 mg kg^{-1} Pb level and smaller decreases in soil pH. The distance between SPA and SPE was longer than that between SA and SE, indicating greater interspecific differences in the plant + earthworm treatments than in the earthworm alone treatments.

3.3 Plant Growth, Pb Accumulation, and TF

3.3.1 Plant Growth

Generally, the plant growth was not much affected by the Pb contamination levels. Only at the 500 and 1,000 mg kg^{-1} Pb levels, yellow leaves of *B. campestris* were observed in the treatment without earthworms (i.e., SP), indicating that plant growth was impaired (**Table 3**).

The addition of earthworms mitigated Pb phytotoxicity and markedly improved plant growth, which was evident by the increases in plant biomass, leaf chlorophyll concentration, and

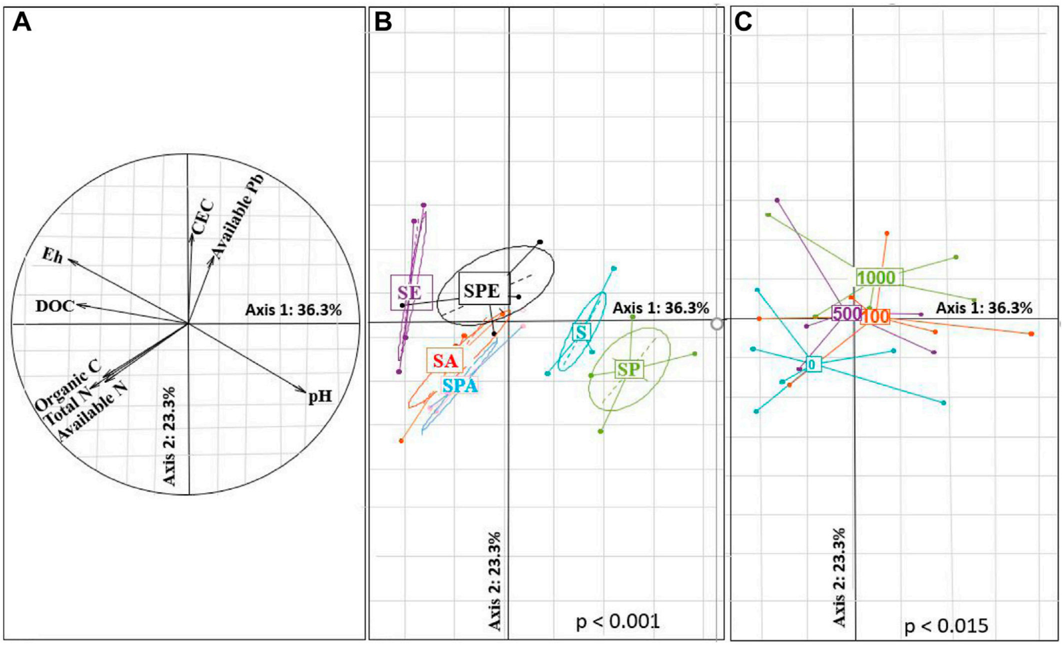

FIGURE 3 | Principal component analysis of soil characteristics in the different treatments. **(A)**: Correlation circle of the soil properties; projection of experimental points according to treatments; **(B)** Treatments with or without earthworms. **(C)** Treatments at different Pb contamination levels of 0, 100, 500, and 1,000 mg·kg^{-1} Pb. DOC: dissolved organic carbon; CEC: cation exchange capacity. S: without plant or earthworm, SP: *Brassica campestris* was grown, SA: *Amynthas aspergillum* was inoculated, SE: *Eisenia fetida* was inoculated, SPA: *B. campestris* was grown and *A. aspergillum* was inoculated, SPE: *B. campestris* was grown and *E. fetida* was inoculated.

TABLE 3 | Effects of the two earthworms species, *Amynthas aspergillum* and *Eisenia fetida*, on the growth of *Brassica campestris*.

Soil Pb	Treatment	Plant height	Shoot diameter	Number of branches	Leaf chlorophyll concentration	Leaf area	Aboveground fresh biomass
mg·kg^{-1}		cm	mm		SPAD Value	cm^2	g
0	SP	24.8 ± 8.60ABa	5.12 ± 0.67ABa	8.25 ± 2.22ABa	37.5 ± 3.47Aa	23.3 ± 10.9ABa	24.1 ± 10.1ABa
	SPA	11.7 ± 3.40Bb	5.04 ± 1.34ABa	7.25 ± 1.23Aa	38.1 ± 5.01Ba	21.5 ± 4.01Ba	15.2 ± 6.98Ca
	SPE	19.9 ± 8.24Aab	4.68 ± 1.13a	8.25 ± 0.96Ba	36.5 ± 5.14Ca	26.9 ± 15.8Aa	25.3 ± 13.2Ba
100	SP	18.2 ± 3.11Bb	5.41 ± 0.62ABa	9.00 ± 1.41ABa	37.1 ± 3.83Aa	31.5 ± 4.03Aa	20.8 ± 3.85ABb
	SPA	19.3 ± 3.65Ab	5.17 ± 1.09ABa	7.00 ± 1.41Aa	40.5 ± 7.31ABa	23.5 ± 7.82Ba	21.0 ± 4.06BCb
	SPE	29.8 ± 6.22Aa	4.96 ± 0.77Aa	9.00 ± 1.41ABa	43.7 ± 4.02Ba	34.2 ± 11.9Aa	32.1 ± 7.13ABa
500	SP	28.3 ± 9.62ABa	5.78 ± 0.26Aa	10.0 ± 1.41Aa	23.9 ± 4.11Ab	28.2 ± 1.68Ab	30.2 ± 1.42Aa
	SPA	25.9 ± 1.64Aa	6.14 ± 0.80Aa	8.50 ± 1.73Aa	46.6 ± 4.89Aa	34.1 ± 5.40Aab	35.2 ± 3.32Aa
	SPE	26.2 ± 1.57Aa	6.41 ± 0.94Aa	10.3 ± 1.26Aa	52.1 ± 5.24Aa	41.5 ± 12.3Aa	40.9 ± 11.1ABa
1,000	SP	28.8 ± 9.38Aa	4.84 ± 0.41Bab	7.00 ± 1.15Ba	15.7 ± 7.85Bb	17.4 ± 2.06Bc	19.3 ± 4.51Bb
	SPA	24.2 ± 7.08Aa	4.42 ± 0.44Bb	10.8 ± 5.74Aa	47.1 ± 2.08Aa	26.1 ± 2.00ABa	25.1 ± 4.40Bb
	SPE	29.3 ± 6.90Aa	6.36 ± 1.58Aa	10.3 ± 0.96Aa	51.6 ± 2.09Aa	40.4 ± 7.72Ab	45.8 ± 14.4Aa

Abbreviations: SP, B. campestris *was grown; SPA,* B. campestris *was grown and* A. aspergillum *was inoculated; SPE:* B. campestris *was grown and* E. fetida *was inoculated. Different uppercase letters indicate significant differences between different Pb contamination levels for a same treatment at* p < 0.05*; different lowercase letters indicate significant differences between treatments for a same Pb contamination level at* p < 0.05*.*

leaf area at the end of the experiment across all Pb contamination levels ($p < 0.05$, **Table 3**). Compared with plants alone (i.e., SP), the addition of *E. fetida* in SPE increased plant biomass by 55% ($p < 0.05$), 36% ($p > 0.05$), and 138% ($p < 0.05$) at the 100, 500, and 1,000 mg kg^{-1} Pb levels, respectively, and the addition of *A. aspergillum* in SPA increased plant biomass by 17% and 30% at the 500 and 1,000 mg kg^{-1} Pb levels, respectively. At the 500 and 1,000 mg kg^{-1} Pb levels, the introduction of *A. aspergillum* significantly increased leaf chlorophyll concentration by 95% ($p < 0.05$) and 199% ($p < 0.05$), respectively, and the introduction of *E. fetida* significantly increased leaf chlorophyll concentration by 118% ($p < 0.05$) and 228% ($p < 0.05$), respectively. Also, at the 500 and 1,000 mg kg^{-1} Pb levels, the introduction of *A. aspergillum* increased leaf area by 21% ($p > 0.05$) and 51% ($p <$

TABLE 4 | Accumulations and contents of Pb in the aboveground (AG) and belowground (BG) parts of *Brassica campestris*.

Soil Pb		Pb content				Pb accumulation				
	Treatment	AG-Pb	BG-Pb	% variation	% variation	AG-Pb	BG-Pb	% variation	% variation	TF
$mg{\cdot}kg^{-1}$		$mg{\cdot}kg^{-1}$	$mg{\cdot}kg^{-1}$	AG-Pb	BG-Pb	$mg{\cdot}plant^{-1}$	$mg{\cdot}plant^{-1}$	AG-Pb	BG-Pb	
0	SP	9.62 ± 6.47Bb	9.43 ± 5.27Ca	-	-	12.2 ± 4.08Ba	12.3 ± 3.29Ca	-	-	0.98 ± 0.12Aa
	SPA	34.8 ± 17.4Ba	19.9 ± 15.9Ca	262	111	16.8 ± 7.32Ca	9.44 ± 8.19Ba	37.4	−23.1	2.52 ± 1.44Aa
	SPE	7.28 ± 2.66Cb	3.97 ± 2.98Ba	−24.3	−57.9	9.43 ± 4.46Ba	6.45 ± 6.18Ba	−22.7	−47.5	2.65 ± 2.06Aa
100	SP	11.9 ± 4.70Bb	31.7 ± 19.7Ca	-	-	14.5 ± 2.74Bb	38.6 ± 21.3BCb	-	-	0.50 ± 0.33Aa
	SPA	24.0 ± 4.56Ba	37.8 ± 8.10Ca	102	19.4	20.0 ± 4.98Cb	33.5 ± 15.4Bb	38.7	−13.4	0.65 ± 0.16Ba
	SPE	22.2 ± 8.86BCa	41.3 ± 20.5Ba	86.8	30.5	51.6 ± 29.9Ba	84.1 ± 26.0Ba	257	117.6	0.62 ± 0.28Ba
500	SP	47.2 ± 26.9Ba	122 ± 38.4Ba	-	-	153 ± 56.9Ba	424 ± 157ABa	-	-	0.41 ± 0.21Aa
	SPA	82.0 ± 38.6Ba	175 ± 67.2Ba	73 8	42.8	130 ± 32.7Ba	289 ± 98.5Aa	−15.3	−32.0	0.47 ± 0.14Ba
	SPE	73.5 ± 57.1ABa	141 ± 54.4Aa	55.7	14.7	261 ± 222ABa	498 ± 227Aa	70.1	17.3	0.55 ± 0.35Ba
1,000	SP	121 ± 55.9Aa	230 ± 94.0Aab	-	-	404 ± 302Aa	729 ± 484Aa	-	-	0.70 ± 0.62Aa
	SPA	170 ± 92.2Aa	321 ± 86.4Aa	40.3	39.2	164 ± 20.7Aa	356 ± 137Aa	−59.4	−51.5	0.52 ± 0.20Ba
	SPE	112 ± 41.5Aa	182 ± 43.3Ab	−7.54	−21.1	456 ± 222Aa	733 ± 265Aa	12.9	0.53	0.60 ± 0.10Ba

Abbreviation: TF, transfer factor. The different uppercase letters indicate significant differences between the different Pb contamination levels for a same treatment at $p < 0.05$*; different lowercase letters indicate significant differences between treatments for a same Pb level at* $p < 0.05$.

0.05), respectively, and the introduction of *E. fetida* increased leaf area by 47% ($p < 0.05$) and 133% ($p < 0.05$), respectively (**Table 3**).

3.3.2 Pb Accumulation in the Plants

The plants treated with *E. fetida* addition (i.e., SPE) had higher Pb accumulation in both the belowground and aboveground parts than the plants in SP and SPA regardless of Pb contamination level (**Table 4**). Compared with SP, Pb accumulation in the aboveground plant parts increased by 257% ($p < 0.05$), 70%, and 13%, and that in the belowground plant parts increased by 118% ($p < 0.05$), 17%, and 0.5% in SPE at the 100, 500, and 1,000 mg kg^{-1} Pb levels, respectively. In contrast, at the 0 mg kg^{-1} Pb level, Pb accumulation in the aboveground and belowground plant parts was reduced by 22% and 48%, respectively, in SPE compared with SP. For the SPA treatments, Pb accumulation was decreased in the belowground plant part regardless of Pb contamination level but was decreased in the aboveground plant parts only at the 500 and 1,000 mg kg^{-1} Pb levels as compared with the SP treatments.

The concentration of Pb in aboveground plant parts was higher in SPA than in S and SPE regardless of Pb contamination level (**Table 4**). Compared with SP, the Pb accumulation in SPA was increased by 262% ($p < 0.05$), 102% ($p < 0.05$), 74% ($p > 0.05$), and 40% ($p > 0.05$) in the aboveground plant parts and by 111% ($p > 0.05$), 19% ($p > 0.05$), 43% ($p > 0.05$), and 39% ($p > 0.05$) in the belowground plant part at the 0, 100, 500, and 1,000 mg kg^{-1} Pb levels, respectively.

3.3.3 TF of Pb

The TF index reflects the distribution of Pb between the aboveground and belowground plant parts (**Table 4**). The TF ranged from 2.65 to 0.41, with significantly higher values at the 0 mg kg^{-1} Pb level as compared with at the other Pb levels. At the 0 mg kg^{-1} Pb level, high TF values of 0.98, 2.52, and 2.65 were obtained for SP, SPA, and SPE, respectively. In contrast, the TF values at 100, 500, and 1,000 mg kg^{-1} Pb were notably low, indicating that a larger proportion of Pb was kept in the roots and prevented from being transferred to the aboveground edible parts when *B. campestris* was grown in the Pb-contaminated soils.

3.3.4 PCA Analysis of the Plant Parameters

The results of PCA analysis indicated significant differences in the plant growth properties between treatments at the different Pb pollution levels (**Figures 4A–C**). The first factor extracted (44.4% of the variance explained) showed the influence of Pb addition on plant biomass, leaf area, shoot diameter, plant height, and Pb accumulation and translocation (**Figure 4A**). Higher Pb contamination levels showed higher plant biomass, leaf area, and Pb accumulation but lower TF values ($p < 0.05$, **Figure 4C**). The second factor extracted (20.8% of the variance explained) characterized the influence of earthworm introduction on leaf area, chlorophyll concentration, and Pb accumulation of plants (**Figure 4B**),

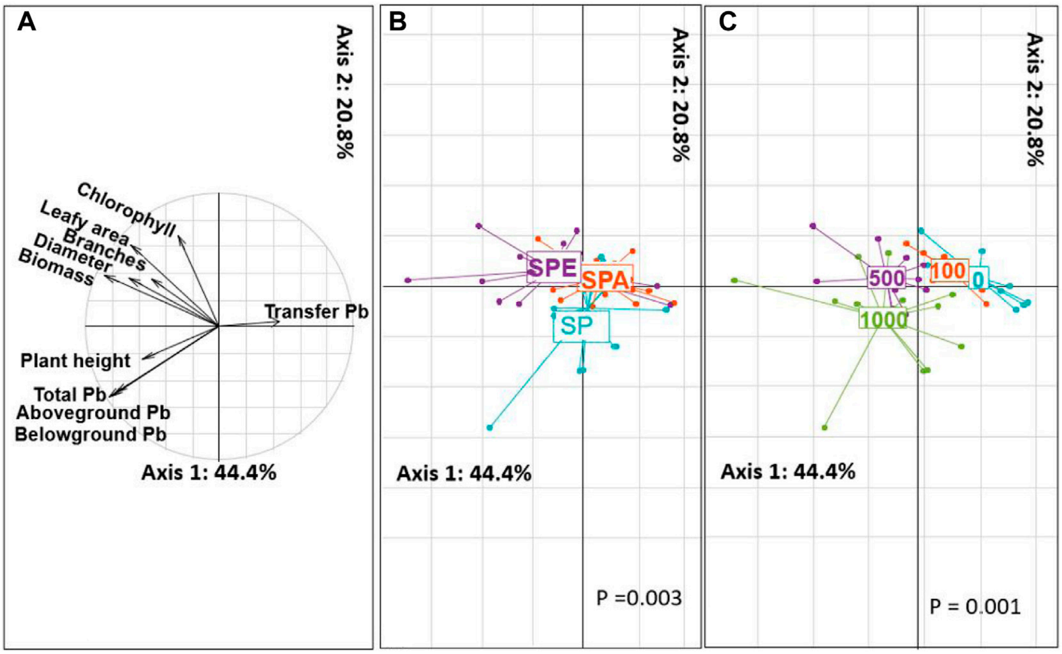

FIGURE 4 | Principal component analysis of the plant growth properties in the different treatments. **(A)** PCA projection of plant growth properties and Pb uptake; **(B)** Treatments with or without earthworms; **(C)** Treatments at different Pb contamination levels of 0, 100, 500, and 1,000 mg·kg^{-1} Pb; SP: *Brassica campestris* was grown, SPA: *B. campestris* was grown and *Amynthas aspergillum* was inoculated, SPE: *B. campestris* was grown and *Eisenia fetida* was inoculated.

showing that earthworm introduction increased leaf area and chlorophyll concentration compared with the respective earthworm-free treatments. The two earthworm species *E. fetida,* and *A. aspergillum* showed their interspecific difference in their influences on the growth and Pb accumulation of *B. campestris.*

3.4 Co-Inertia Analysis of the Soil and Plant Growth Properties

The co-inertia analysis showed that the first axis explained 34.2% of the total variation, and the second axis explained 18.6% of the total variation (**Figure 5**). Soil bioavailable Pb and CEC were significantly positively correlated with plant Pb accumulation, biomass, leaf area, height, and shoot diameter (**Figures 5A,B**), which clearly distinguished the treatments between high and low Pb contamination levels (**Figure 5C**). Compared with the treatments with low Pb contamination levels, those with high Pb contamination levels exhibited higher soil CEC, available Pb content, plant biomass, leaf area, and plant Pb accumulation. In addition, soil Eh was positively correlated with leaf chlorophyll concentration, while soil pH was negatively correlated with leaf chlorophyll concentration. The treatments with earthworm inoculation (SPA and SPE) were separated from those without earthworm inoculation (SP), with the former associated with higher projected values of soil Eh and leaf chlorophyll concentration but a lower projected value of soil pH.

4 DISCUSSION

4.1 Earthworm Survival and Pb Accumulation

Previous studies concern the effects of metals on earthworms in terms of loss of weight, mortality, and metal accumulation in tissue (Xiao et al., 2020a; Zhang et al., 2022). until yet, few studies have focused on the differential survival and Pb accumulation between the anecic species *A. aspergillum* and the epigeic species *E. fetida* in Pb-contaminated soils.

At all the tested Pb contamination levels in our study, the earthworm species *A. aspergillum* exhibited a higher survival rate than *E. fetida*. This earthworm species have been commonly found to survive well in stressful environments. According to Wu et al. (2020b), *A. aspergillum* survived against soil acidification and aluminum toxicity with a survival rate of 85.8% and an extremely low weight loss rate of 13.4%. In contrast, the biomass and survival rate of *E. fetida* were low at the tested Pb levels, especially the high Pb levels. Similar findings were reported by Nahmani et al. (2007a) and Sizmur et al. (2011) that the population, biomass, sexual development, and cocoon production of *E. fetida* were dramatically reduced when the concentrations of heavy metals (e.g., Zn, Cd, Cu, and Pb) in soil exceeded acceptable levels. However, *E. fetida* had a lower survival rate in treatments that contained plants than those without plants. Our findings could be explained by the possibility that soil Pb and certain compounds in plant root exudates interact to generate toxic molecules that impair *E. fetida*. For better survival of *E.*

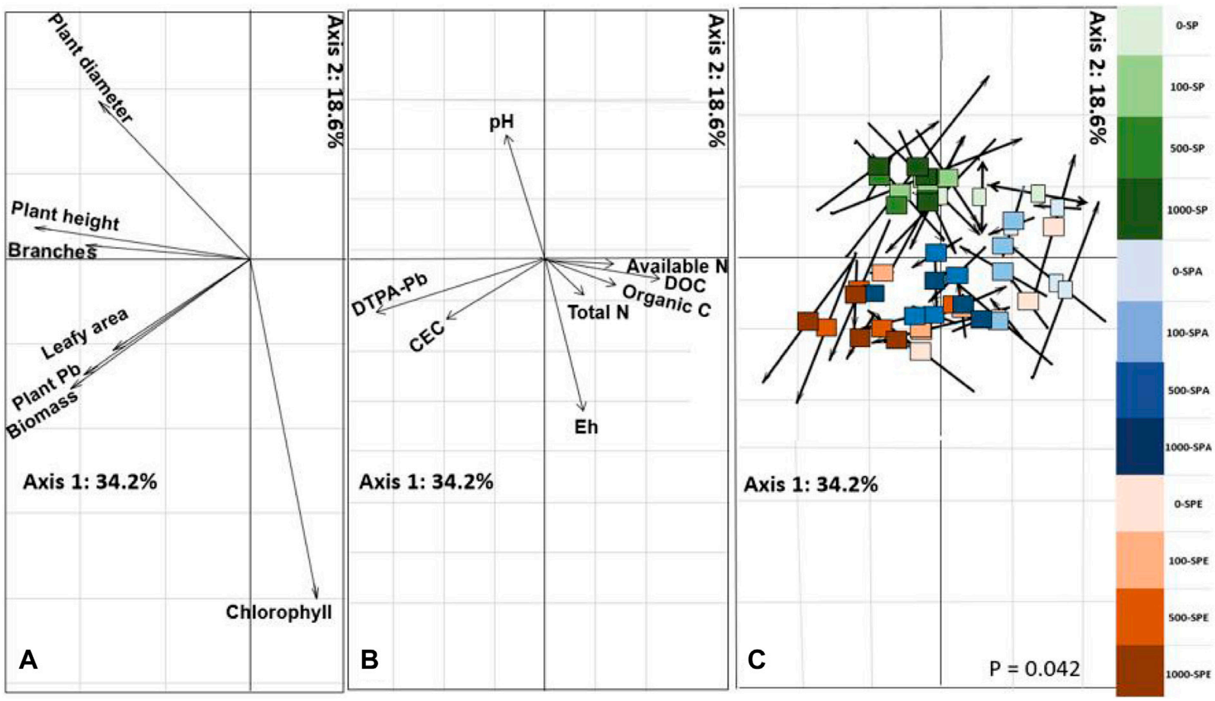

FIGURE 5 | Co-inertia analysis between soil properties and plant characteristics in the different treatments. **(A)** Co-inertia projection of plant growth and Pb uptake properties; **(B)** Co-inertia projection of soil properties; **(C)** Score plot of treatments; The squares and arrows represent the projected coordinates of soil physio-chemical properties and plant growth parameters and Pb accumulation of each pot. DOC: dissolved organic carbon; CEC: cation exchange capacity. SP: *Brassica campestris* was grown, SPA: *B. campestris* was grown and *A. aspergillum* was inoculated, SPE: *B. campestris* was grown and *E. fetida* was inoculated. Pb contamination levels include 0, 100, 500, and 1,000 mg kg^{-1} Pb.

fetida in highly polluted soils, organic matter is often added as a nutrient and energy source (Nahmani et al., 2007b; Zhang et al., 2016; Wu et al., 2020a; Raiesi et al., 2020).

In this study, both earthworm species accumulated Pb in their bodies and accumulated more Pb in the more heavily polluted soils. Studies show that earthworm species differ in feeding preference, behavioral characteristics, and metal bioaccumulation (Hickman and Reid, 2008; Butt and Lowe, 2011; Zhang et al., 2022). Compared with *A. aspergillum*, *E. fetida* accumulated more Pb at the 500 and 1,000 mg kg^{-1} Pb levels. The reason may be because Pb is readily bound to organic matter, and *E. fetida* requires a large amount of organic matter for food. Therefore, more Pb was accumulated by *E. fetida* with the ingestion of organic matter. Other researchers reported similar results that *E. Veneta*, an epigeic species, accumulated significantly more Pb than *Lumbricus Terrestris*, an anecic species, and *Allolobophora chlorotica* in 112 days (Sizmur et al., 2011). Earthworm bioaccumulation of Pb can be a useful indicator of soil bioavailable Pb for Pb risk assessment and remediation strategy development (Xiao et al., 2020a; Oorts et al., 2021).

4.2 Effect of Earthworm Introduction on Plant Growth

Pb deactivates critical enzymes, inhibits enzyme activities, detrimentally affects various physiological and biochemical processes, impairs photosynthesis, causes cell death, and leads to leaf chlorosis and stunted growth of plants (Zulfiqar et al., 2019). Earthworm introduction has improved plant growth in Pb-contaminated soils (Wang et al., 2006; Yu et al., 2005a). In a Pb/Zn mine tailings-polluted soil with Pb at 1,202 mg·kg^{-1}, the shoot biomass of *Leucaena leucocephala* was improved from 30.0 to 34 g after the addition of *Pheretima guillelmi* (Ma et al., 2006). Similarly, *E. fetida* was reported to considerably promote the growth of maize (*Zea mays*) and barley (*Hordeum vulgare*) in soil contaminated with Cu, Cd, Pb, and Zn (Ruiz et al., 2009). With the introduction of *Pontoscolex corethrurus*, an endogeic and geophagous earthworm species, to a Pb-contaminated soil (1,000 mg·kg^{-1} Pb), the shoot biomass of *Lantana camara* increased from 21.52 to 31.77 g, and its root biomass increased from 19.11 to 24.21 g (Jusselme et al., 2013; Jusselme et al., 2012). In this study, the introduction of earthworms significantly increased the biomass, leaf chlorophyll concentration, and leaf area of *B. campestris* at the high Pb contamination levels (**Table 3**; **Figure 4**). Chlorophyll, a primary pigment of green leafy vegetables, was most affected by earthworm inoculation (**Figure 4**). The significant relationships between leaf chlorophyll concentration and the soil properties of pH, Eh, and DOC (**Figure 5**) indicate that the earthworms influenced leaf chlorophyll concentration by increasing soil Eh and DOC content and decreasing soil pH. Soil Eh is an indicator of soil aeration status. Earthworm activity in soil improves soil aeration (higher Eh), which improves plant growth and quality (higher chlorophyll concentration) (Noh and Jeong, 2021). The digestion process of earthworms speeds up the decomposition of soil organic matter (higher DOC) (Zhang et al., 2016), which provides more nutrients for plant growth and results in better plant growth with a higher chlorophyll concentration. Soil microorganisms strongly influence the forming and decomposition of soil organic matter (Sokol et al., 2022). Further studies should be performed to elucidate the underlying mechanism of how earthworms affect plant growth at the molecular level (Jacquiod et al., 2020).

4.3 Effect of Earthworm Introduction on Plant Pb Uptake

The Pb uptake of *B. campestris* is affected by biotic and abiotic environmental factors (Kaur et al., 2018; Xu et al., 2018; Farahat et al., 2021).

In this study, Pb contamination was the deciding factor in Pb accumulation in the plants (**Figure 4**). When *B. campestris* was exposed to high levels of Pb, more Pb was accumulated in the roots, and less Pb was translocated to the aboveground organs (**Table 4**; **Figure 4**), consistent with the finding of Zhang Z. et al. (2020). The earthworms affected Pb bioavailability and plant accumulation as well by altering soil properties, increasing DOC, and reducing pH (**Figure 5**). Some studies indicated the association between the increased bioavailable Pb and the influences of earthworms on soil pH, organic carbon, and DOC (Lemtiri et al., 2016; Nannoni et al., 2014; Wen et al., 2004). The possible explanation is that earthworms may release soluble organic acids, which are responsible for soil pH decrease (Sizmur et al., 2011). Earthworm activities stimulate metal transformation. It was found that available Cd and Zn concentrations in *A. morrisi* casts were higher than those in the bulk soil, and *E. fetida* increased the organically bound Pb (Zhang C. et al., 2020). In this study, the inoculation of earthworms significantly affected soil CEC and available Pb content, resulting in higher Pb accumulation in *B. campestris* and higher biomass and leaf area (**Figure 5**). Wu et al. (2020b) also found more exchangeable base cations in earthworm casts than in the no-ingested soil. High soil CEC implies high concentrations of such exchangeable base cations as K, Ca, and Mg, which contribute to the increase of plant biomass (Cui et al., 2021). In addition, these cations compete with Pb for binding sites on soil organic matter and oxides, resulting in Pb release into the soil solution and absorption by the plants (Costa et al., 2017; Zhou et al., 2020).

E. fetida increased Pb accumulation in the aboveground and belowground parts of *B. campestris* by 12.9%–257% and 0.53%–118%, respectively (**Table 4**). This is mainly due to the increase in plant biomass (**Table 3**). Kaur et al. (2018) also showed that *E. fetida* addition mitigated the harmful effects of metals on plant growth, improved photosynthetic efficiency, and boosted metal uptake. Moreover, numerous studies have demonstrated that geophagous earthworms significantly increase Pb uptake of plants by improving Pb availability and plant growth (Ruiz et al., 2011; Ardestani et al., 2019; Zhang C. et al., 2020). In contrast, the introduction of *A. aspergillum* mainly increased the Pb concentration instead of its accumulation in plants (**Table 4**). This is due to the higher DTPA-extractable Pb concentration and lower plant biomass in SPA compared with SPE (**Figure 2**; **Table 3**). The higher DTPA-extractable Pb concentration in the soil led to higher Pb uptake by the plants, which resulted in the higher Pb concentration in the plants with lower plant biomass. The mechanisms of how different earthworm species influence metal accumulation in plants demand further studies.

5 CONCLUSION

In this study, the anecic and native earthworm species *A. aspergillum* presented a higher Pb tolerance than the epigeic species *E. fetida*, which is crucial for the operation of earthworm-assisted bioremediation techniques in the future. *E. fetida* had a greater effect on soil CEC and available Pb, while *A. aspergillum* had a greater effect on soil C and N contents. Inoculation of *A. aspergillum* mainly increased the Pb concentration rather than total accumulation in plants. These results revealed that different ecological earthworm species have different effects on the biogeochemical cycle of Pb and soil physico-chemical properties. Further studies are required to elucidate the mechanism of how different earthworm species influence soil properties and plant growth under heavy metal contamination conditions. Moreover, the high Pb concentration and accumulation in *B. campestris* in this short-term (30 days) experiment causes a concern. A hyperaccumulator plant may need to be introduced together with earthworms to heavy metal-contaminated leafy vegetable fields for safe production, which should focus on future studies.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

CT: writing-original draft, methodology, data curation. MZ: methodology, writing-review and editing; HZ: writing–review and editing; LX: writing–review and editing. LW: data curation, investigation. JD: investigation. KL: methodology, formal analysis, data curation, writing review and editing. CZ: conceptualization, methodology, formal analysis, data curation, writing–original draft, writing–review and editing, funding acquisition, project administration.

FUNDING

Guangdong Natural Science Foundation (Grant No. 2021A1515011543), National Natural Science Foundation of

China (Grant Nos. 41201305 and 41601575), National Science and Technology Fundamental Resources Investigation Program of China (2018FY100300) supported this work, in addition to support from the China Scholarship Council.

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