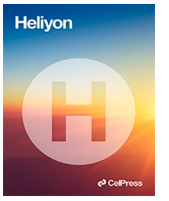
Heliyon 10 (2024) e37734

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Responses of biomarkers, joint effect and drilosphere bacterial communities to antimony (III and/or V) contamination

Jing Bai [a,b,1], Linyu Chen [a,1], Xiaoqi Yang [a], Yuyang Deng [a], Juan Wan [a], Yu Zheng [a,b], Ying Song [a,b], Zeliang Yang [a,b], Guohong Xiang [a,b], Renyan Duan [a,b,*]

[a] College of Agriculture and Biotechnology, Hunan University of Humanities, Science and Technology, Loudi, 417000, China
[b] Development and Utilization and Quality and Safety Control of Characteristic Agricultural Resources in Central Hunan, Loudi, 417000, China

ARTICLE INFO

Keywords:
Sb(III)
Sb(V)
drilosphere
multiple biomarker responses
microbial diversity

ABSTRACT

Contamination of soils with antimony (Sb) is becoming increasingly severe and widespread, and the associated ecological risks cannot be ignored. To evaluate how different Sb forms affected the earthworm *Eisenia fetida* in soil, the biomarker response index (BRI), effect addition index (EAI), and microbial diversity were characterized after single and joint application of Sb(III) and Sb(V). The results showed that Sb(III) was better enriched by earthworms than Sb(V). The metallothionein (MT) content in earthworms increased under Sb stress, and the superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) activities also showed an increasing trend, suggesting waken-up antioxidant capacity. Severe alterations for health status were observed under combined treatment. Additionally, the EAI indicated that Sb(III) and Sb(V) had synergistic and antagonistic effects at low and high concentrations, respectively. The bacterial populations in the drilosphere (gut and burrow lining) appeared to be more susceptible to Sb contamination than in the non-drilosphere, their specific microecology may be an important factor in soil Sb migration and transformation. The abundance of Actinobacteria exhibited a significant decrease with increasing concentrations of single Sb(III) and Sb(V), while the abundance of Bacteroidia increased. The correlation heatmap showed that *Sphingobacterium faecium* was highly tolerant to Sb. These results provide not only an important basis for the ecological risk assessment of Sb in the soil environment but also new insights into the altered drilosphere bacterial communities under Sb stress.

Abbreviations: Sb, Antimony; *Eisenia fetida*, *E. fetida*; BRI, Biomarker response index; BAF, Bioaccumulation factor; MT, Metallothionein; SOD, Superoxide dismutase; CAT, Catalase; GST, glutathione S-transferase; AL, Alteration level; EAI, Effect addition index; Treatment CE, control; Treatment TL, 50 mg/kg Sb(III); Treatment TH, 100 mg/kg Sb(III); Treatment FL, 100 mg/kg Sb(V); Treatment FH, 200 mg/kg Sb(V); Treatment ML, 50 mg/kg Sb(III) + 100 mg/kg Sb(V); Treatment MH, 100 mg/kg Sb(III) + 200 mg/kg Sb(V); Group T1, Low concentration treatments group; Group T2, High concentration treatments group.

* Corresponding author. College of Agriculture and Biotechnology, Hunan University of Humanities, Science and Technology, Loudi, 417000, China.
E-mail address: duanrenyan78@163.com (R. Duan).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e37734
Received 16 March 2024; Received in revised form 6 September 2024; Accepted 9 September 2024
Available online 10 September 2024

1. Introduction

Antimony (Sb), a hazardous and carcinogenic metalloid, may harm the heart, lungs, liver, kidneys, and nervous system by inhibiting enzymatic activity, disturbing the cellular ionic balance, and interfering with protein and sugar metabolism [1]. China has the largest reserves and production of Sb in the world, accounting for 78 % of global output [2]. However, unregulated mining and smelting have led to increasingly severe pollution in mining areas and their surrounding environments [3]. Previous studies have found that the maximum Sb concentration in agricultural soil samples from "world's antimony capital" Xikuangshan mine, reached 2124.00 mg/kg, posing a significant ecological risk factor [4]. Sb primarily exists in trivalent and pentavalent forms [Sb(III) and Sb(V)] in the environment. The Sb(III) has stronger toxicity and mobility in anaerobic conditions. Moreover, Sb(III) is the major form in the earthworm gut, whereas Sb(V) is the predominant ionic species in the surrounding soil [5].

Earthworms are sensitive to soils contaminated by heavy metals and are therefore used as model organisms for standard toxicity tests in terrestrial ecosystems as International Standards Organization (ISO) [6]. It has been reported that earthworms promote sulfamethoxazole degradation by stimulating both intestinal and soil degraders [7]. Furthermore, as soil ecosystem engineers, earthworms can alter the structural, chemical, and physical properties of soil and the bioavailability and distribution of soil pollutants [8].

The drilosphere formed by the activities of earthworms such as burrowing, ingestion, digestion, secretion, and excretion is a hot spot for microbial reactions. Drilosphere offer favorable habitats and ample bioavailable nutrients for soil microbial communities [9]. Studies showed that the earthworm gut is the key to maintaining its metabolism and transformation of nutrients and environmental pollutants [10]. In addition, indigenous microorganisms could affect the speciation, mobility, bioavailability, and fate of Sb by promoting the release of Sb from the deposit to a wider environment and oxidizing the more toxic antimonate [Sb(III)] to less toxic antimonate [Sb(V)] [11]. Some investigators found that the accumulation of arsenic in the soil can affect microbial biomass and the structure of microbial communities [12]. Furthermore, some studies have shown that Sb may inhibit the growth of microorganisms in the gut of earthworms, thus adversely affecting the surface-casting activity of earthworms [13]. According to Huang et al., due to the unique intestinal microenvironment of earthworms, the gut bacterial community network of earthworms is less stable and more sensitive to Sb species than that of the soil [5]. However, there is currently no research on how different forms of Sb affect the microbial communities within microscale drilosphere (gut and burrow lining). Therefore, it is of great significance to understand the oxidation and transformation of Sb and the microbe-mediated interactions between microorganisms and various forms of Sb.

Biomarkers are biological parameters that measure the exposure to and effects of environmental pollutants. The contents of malondialdehyde and reactive oxygen species, the activities of superoxide dismutase (SOD), catalase (CAT), peroxidase, and acetylcholinesterase, along with DNA damage are often used as biomarkers for earthworms. Studies have shown that the levels of metallothionein (MT) as well as the activities of SOD, CAT, and glutathione S-transferase (GST) in *E. fetida* were increased under Sb and Cd combined pollution [14]. Additionally, recent studies have found through biomarker response index (BRI) analysis that Sb $>$ 60 mg/kg often seriously impacted the health of earthworms [15]. However, in-depth studies are rare, with very few focusing on the single and combined effects of Sb (III) and Sb(V) on the biomarker responses of *E. fetida*.

In this study, *E. fetida* was exposed to soil contaminated with Sb(III) and/or Sb(V). Multiple biomarkers, including CAT, SOD, GST, and MT, were analyzed to describe the health of earthworms by biomarker response index (BRI) and effect addition index (EAI). Furthermore, the non-drilosphere and drilosphere microbial diversity was measured using high-throughput 16S rRNA sequencing to reveal the ecotoxicological effects of different forms of Sb. Therefore, this study provided a comprehensive basis for the ecological risk assessment and bioremediation of Sb-polluted soils.

2. Materials and methods

2.1. Experimental soil, earthworms, and chemicals

The experimental soil was collected from the top layer (0–20 cm) at the Jiu Er Base (27° 46′ 27″ N, 112° 1′ 30″ E) of the Hunan University of Humanities, Science, and Technology in Loudi, China. Table S1 lists the main physical and chemical characteristics of the tested field soil. Briefly, soil had the characteristics of pH 7.28, sand 20.12 %, clay 44.08 %, silt 35.80 %., and organic matter 30.34 g/kg. The collected fresh soil was sieved using a 2-mm mesh and stored in the dark at 4 °C until further use.

E. fetida, with no previous exposure to Sb, was purchased from Wangjun Earthworm Base, Jurong, Jiangsu Province, China. Healthy mature adult earthworms (400–600 mg weight each), with well-developed clitellae, were randomly selected before the experiment began. Earthworms were incubated for 1 month in the non-contaminated experimental soil before use.

The Sb (III) and Sb(V) test reagents used were potassium antimony tartrate ($KSbOC_4H_4O_6{\cdot}½H_2O$, CAS Number: 28300-74-5) and potassium hexahydroxoantimonate (H_6KO_6Sb, CAS Number: 12208-13-8), which were purchased from Xilong Scientific Co., Ltd., Shantou, Guangdong Province, China and Shanghai Maclin Biochemical Technology Co., Ltd, respectively. The stock solutions were prepared in ultrapure water (18.2 MΩ * cm). Ultrapure water was used to obtain a range of Sb (III) and Sb(V) treatment concentrations. All chemicals were of analytical grade and were cused without further purification.

2.2. Earthworm exposure assay design

Soil-containing polyvinyl chloride (PVC) columns (height 12.5 cm, upper diameter 14 cm, bottom diameter 10 cm) were supplemented with *E. fetida* at a density of 20 individuals/kg soil and Sb(III) and/or Sb(V). The Sb(III) and Sb(V) treatment concentrations are listed in Table S2. The approximate concentration range was selected according to the results of chronic toxicity tests for *E. fetida*

after 14 days of exposure in soil aged for 10 d [16]. Sb(III) treatment level was 50 mg/kg (TL) and 100 mg/kg (TH), respectively, which was 1/5–1/2 of the median lethal concentration. Sb(V) treatment level was 100 mg/kg (FL) and 200 mg/kg (FH), respectively, which was 1/20–1/10 of the median lethal concentration. Soil without Sb(III) and Sb(V) addition was used as control (CE). Treatment ML (soil with combined Sb(III) 50 mg/kg + Sb(V) 100 mg/kg); treatment MH (soil with combined Sb(III) 100 mg/kg + Sb(V) 200 mg/kg). The T1 (TL, FL, and ML) and T2 (TH, FH, and MH) groups represent the low- and high-concentration treatments, respectively.

First, 1000 g of soil was spiked with different amounts of Sb(III) and/or Sb(V) stock solution for 0.5 h, and then a soil water content of ~25 % of its dry weight was achieved by adding ultrapure water. Earthworms were rinsed in deionized water, allowed to defecate for 24 h on wet filter paper in the dark at 25 °C. All freshly spiked soils were equilibrated and aged for 10 d at 25 °C in an incubator before 20 earthworms were placed in the soil. During the entire experiment, the dead earthworms were promptly removed, and soil organic matter is abundant no additional addition. The entire experiment was performed in an incubation environment where the temperature was maintained at 25 °C with the 12:12 h light/dark cycle and the initial moisture was maintained. After incubation for 14 days, four earthworms were taken from each treatment and randomly divided into two groups to determine the total Sb concentration and biomarkers.

2.3. Microbial diversity assessment

Drilosphere and non-drilosphere matrices were extracted as described in our previous study [17]. The soil without earthworm disturbance (S) was collected as non-drilosphere soil, while avoiding the inclusion of any intestinal contents, cast, or burrow lining after cultivation ended. The drilosphere soil, including gut content (G) and burrow lining (B), was also collected. The burrow lining was collected using a sterilized knife to scrape the surface cave wall soil. The gut contents were obtained by dissection. There were three matrices for each treatment level (CE, TL, TH, FL, FH, ML, and MH), with 21 samples altogether. The sample information is presented in Table S3,with a sample size of n = 4 for each group.

2.4. Determination of total Sb, Sb (III), and Sb(V) concentration

Soil samples were air-dried and sieved (100-mesh). After expelling the gut contents, earthworm tissues were powdered using liquid nitrogen in an agate mortar before determining the total Sb, Sb(III), and Sb(V) concentrations. A concentrated acid mixture was used to completely digest both the soil and earthworm samples [18]. The 0.5 g soil samples were treated with a mixture of 5 mL of HNO_3 and 1 mL of HF, whereas the 0.2 g freeze-dried earthworm samples were treated with 2 mL of HNO_3. A dual-channel atomic fluorescence photometer was used to calculate the total Sb (AFS-2100, Beijing Haiguang Instrument Co. Ltd., Beijing, China). The Sb detection limit was 0.010 mg/kg. According to previous reports [19], the matrix (0.5 g) was extracted with 10 mL of 100 mM citric acid (pH 2.08) for 1 h in a 25 mL centrifuge tube. The supernatant was centrifuged at 8000×*g* for 10 min at 4 °C, and then filtered through a 0.45 μm filter membrane to a constant volume of 10 mL, then the Sb(III) and Sb(V) contents were determined using LC-AFS.

Sb enrichment of *E. fetida* was quantified using the Sb bioaccumulation factor (BAF) as follows using Eq. (1)(2)(3):

$$\mathrm{BAF}_{\text{Total Sb}} = \frac{\text{Total Sb concentration in } \textit{E. fetida}}{\text{Total Sb concentration in soil}} \tag{1}$$

$$\mathrm{BAF}_{\mathrm{Sb(III)}} = \frac{\text{Sb(III)concentration in } \textit{E. fetida}}{\text{Sb(III)concentration in soil}} \tag{2}$$

$$\mathrm{BAF}_{\mathrm{Sb(V)}} = \frac{\text{Sb(V)concentration in } \textit{E. fetida}}{\text{Sb(V)concentration in soil}} \tag{3}$$

2.5. Determination of biomarkers

E. fetida in each replicate was homogenized for the examination of biomarkers, including the activities of SOD, CAT, and GST, along with MT concentration. The enzymatic activities of biomarkers were recorded using ELISA kits obtained from Nanjing Jiancheng Bioengineering Institute as described in our previous study [20].

2.6. Characterization of toxicity interactions

BRI and EAI were used to quantitatively characterize the individual and joint toxicity differences of Sb(III) and Sb(V) on *E. fetida* in natural soils.

BRI was mainly represented by the score (S) of the alteration level (AL) and weighting (W) of the biomarker [21]. All biomarker responses were divided into four levels according to their ALs and BRI using Eq. (4) and (5) (Table S4).

$$AL = \frac{|BRt - BRc|}{BRc} \times 100\% \tag{4}$$

$$BRI = \frac{\sum (S_n \times W_n)}{\sum W_n} \tag{5}$$

where *BRt* and *BRc* refer to biomarker responses (averages of four replicates) of the contamination treatments and CE, respectively, *Sn* and *Wn* correspond to the score and weighting, respectively, of biomarker *n*. Biomarkers are assigned a weighting based on the relevance of biological levels [22]. SOD, CAT, MT, and GST were assigned weights of 1, 1, 1, and 1.5, respectively.

The total effect (*E*) was calculated using Eq. (6). The joint effect of Sb(III) and Sb(V) on the integrated responses of *E. fetida* was characterized by EAI [23,24], which was calculated as follows using Eq. (7):

$$E = \frac{BRIc - BRIt}{BRIc} \tag{6}$$

$$EAI = 1 - \frac{1 - \left(1 - E_{Sb(III)-single}\right) \times \left(1 - E_{Sb(V)-single}\right)}{E_{Sb(III),Sb(V)-mix}} \tag{7}$$

where *BRIt* and *BRIc* refer to the *BRI* of the contamination treatment and CE, respectively. $E_{Sb(III)\text{-}single}$ and $E_{Sb(V)\text{-}single}$ refer to the total effect of Sb(III) and Sb(V) when they are applied individually; $E_{Sb(III),\ Sb(V)\text{-}mix}$ refer to the total effect of Sb(III) and Sb(V) mixture. The *EAI* equaling 0, >0, or <0 at a specific effect level denotes addition, synergism, or antagonism, respectively.

2.7. 16S rRNA gene sequencing

First, an OMEGA Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA) kit was used for DNA extraction according to the manufacturer's instructions. The ~468 bp highly variable V3-V4 region of the bacterial 16S rRNA gene was used for sequencing. The specific primers were selected for PCR amplification of the V3-V4 region of 16S rDNA of bacteria, 338F (5′-ACTCCTACGGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). A Quant IT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific Inc., MA, USA) was used to analyze the PCR products in a microplate reader (FLx800, Bio-Tek Instruments, USA), and the samples were mixed according to the amount of data required for each sample. The library was constructed using an Illumina TruSeq Nano DNA LT Library Prep Kit. Finally, the library was inspected and a NovaSeq 6000 SP Agent Kit (500 cycles) 2 × 250 bp double-ended sequencing was performed.

For quality control, the Quantitative Insights Into Microbial Ecology (QIIME, v1.8.0) pipeline was employed to process the sequencing data, as previously described [25]. After chimera detection, the remaining high-quality sequences were clustered into operational taxonomic units (OTUs) at 97 % sequence identity by UCLUST. OTUs containing less than 0.001 % of total sequences across all samples were discarded.

2.8. Statistical analysis

Data were expressed as the means ± standard deviation (SD), and processed using Microsoft Excel. One-way ANOVA was performed with SPSS 16.0 software at the $p < 0.05$ confidence level using the Duncan's multiple range test. Sequence data analyses were performed using the QIIME and R packages (v 3.2.0). Figures were prepared using Origin 2019.

3. Results

3.1. Bioaccumulation of Sb in earthworms

After 14 days of exposure, compared with the low-concentration group (TL, FL, ML), the earthworm mortality in the high-concentration treatment group (TH, FH, MH) increased significantly ($p < 0.05$) (Table 1). The combined high concentration treatment (MH) showed the highest mortality rate of 55 %. Significant changes in earthworm morphology were observed under Sb exposure, including multiple segmentation, partial atrophy, pyogenic tissue fluid, and abnormal swelling (Fig. S1). To understand the toxic effects of the two forms of Sb and their bioavailability for earthworms, the contents of Sb (III) and Sb(V) in the earthworms and soil were determined. Overall, the Sb forms in the soil and earthworms varied greatly. With increasing treatment concentration, the Sb concentration in the soil and earthworms also showed a gradual upward trend. ($p < 0.05$). In the same treatment group, Sb(III) was

Table 1
The mortality (%) of *Eisenia fetida*, contents (mg/kg) and bioaccumulation characteristics of different Sb species.

Group	Mortality	Sb(III)			Sb(V)		
		Sb(III) in soil	Sb(III) in earthworm	$BAF_{Sb(III)}$	Sb(V) in soil	Sb(V) in earthworm	$BAF_{Sb(V)}$
CE	11.25 ± 2.5c	0.12 ± 0.01g	0.12 ± 0.01g	1.05 ± 0.14f	1.96 ± 0.09f	1.79 ± 0.14e	0.91 ± 0.1a
TL	16.25 ± 2.5c	0.37 ± 0.05f	0.57 ± 0.07f	1.55 ± 0.14e	41.36 ± 0.68e	23.59 ± 0.54d	0.57 ± 0.02b
TH	38.75 ± 4.79b	2.23 ± 0.16c	4.26 ± 0.07d	1.92 ± 0.13d	78.94 ± 1.47d	42.94 ± 3.17c	0.54 ± 0.04b
FL	12.5 ± 2.89c	0.56 ± 0.04e	1.52 ± 0.08e	2.71 ± 0.14c	83.81 ± 0.74d	47.22 ± 2.48c	0.56 ± 0.03b
FH	38.75 ± 6.29b	1.78 ± 0.08d	5.54 ± 0.11c	3.12 ± 0.12b	144.19 ± 2.66b	78.17 ± 5.26a	0.54 ± 0.03b
ML	38.75 ± 7.5b	2.6 ± 0.11b	7.15 ± 0.38b	2.75 ± 0.12c	111.97 ± 1.21c	63.31 ± 2.32b	0.57 ± 0.03b
MH	55 ± 4.08a	5.06 ± 0.14a	17.19 ± 0.48a	3.4 ± 0.15a	239.77 ± 12.87a	67.91 ± 8.66b	0.28 ± 0.04c

Note: Values are the mean and standard deviation of the four replicates. Different letters in each column represent the significant differences between different treatments (ANOVA, $p < 0.05$).

higher in earthworms than in soil, whereas Sb(V) was the opposite. Besides, Sb(III) was lower than Sb(V) in earthworms and soil, which indicated a substantial conversion of Sb(III) to Sb(V). In the treatment and CE groups, the BAF of earthworms for Sb(III) was >1, whereas that of Sb(V) was <1, thus indicating that *E. fetida* was better at enriching Sb(III) than Sb(V) (Table 1).

Additionally, with the increasing Sb(III) and Sb(V) concentrations in the soil, the Sb concentration in earthworms increased significantly ($p < 0.05$) and was higher in earthworms than soil in each treatment group. As shown in Table S5, the $BAF_{total\ Sb}$ of earthworms was >1 in Sb-contaminated soil, which indicated that earthworms could be enriched in Sb.

3.2. Biomarkers

3.2.1. Multiple biomarker responses

As shown in Fig. 1a, SOD activity showed an increasing trend with increasing single Sb(III) and Sb(V) concentrations. For example, at TH [100 mg/kg Sb(III)] treatment, the SOD activity was significantly higher (1.79-fold) than the CE ($p < 0.05$). Additionally, the FL and FH treatments were, respectively, 1.54-fold and 2.17-fold higher than the CE ($p < 0.05$), respectively. Furthermore, the ML and MH treatment groups increased SOD activity by 185 % and 173 %, respectively, as compared with the CE.

Similarly, the CAT activity increased with increasing Sb(III) concentration (Fig. 1b). The TL and TH treatments showed, respectively, 1.52- and 1.99-times higher CAT activity than the CE, respectively. After earthworms were exposed to the individual Sb(V) treatment, the CAT activity in the earthworms remained stable. After the earthworms were exposed to the combination of Sb(III) and Sb(V), the CAT activity of each treatment group was ~2.79 times higher than that of the CE, maximizing at MH.

In Fig. 1c, the GST activity first showed an upward trend and then decreased with the increasing Sb(III) concentration. GST activities of 15.31 U/g FW and 13.24 U/g FW were found in the TL and TH treatments, respectively, being 1.40-fold and 1.22-fold of the CE. GST activity showed an increasing trend with increasing Sb(V) concentration, with the maximal value at Sb(V) 200 (155 % higher than the CE, $p < 0.05$). The ML and MH treatment groups had 1.56- and 1.77-times higher GST activity than that of the CE ($p < 0.05$), respectively, showing a significantly increasing trend.

After 14 days of poisoning, the MT concentration in earthworms gradually increased with the increasing single and combined Sb (III) and Sb(V) concentrations in soil (Fig. 1d). There was no significant difference ($p > 0.05$) between the T1 group (low-concentration treatment) and the CE. The maximum MT concentration was observed in MH (130 % higher than the CE), with a significant difference ($p < 0.05$).

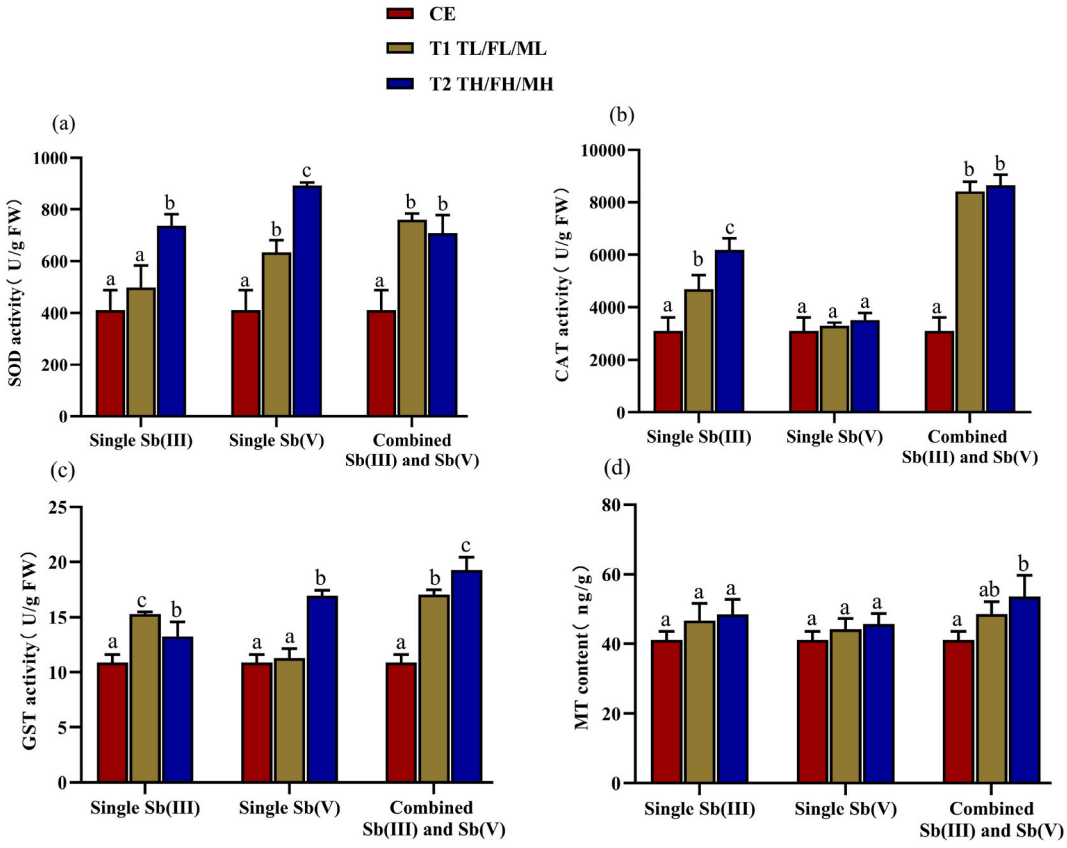


Fig. 1. Individual and joint effects of Sb(III) and Sb(V) on multiple earthworm biomarkers. (a) SOD, (b) CAT, (c) GST, and (d) MT. Results are expressed as mean ± SD (n = 3). Different letters indicate significant differences among treatments according to ANOVA ($p < 0.05$).

Based on the magnitude of the response of the above biomarkers, we concluded that the MT concentration and the SOD, CAT, and GST activities in earthworms increased under Sb pollution.

3.2.2. Integration of multiple responses

ALs indicate the relative magnitude of change between the magnitude of the biomarker responses in the treatment and CE groups. As seen in Fig. S2, with an increase in the treatment level, the greater the AL (range: 0–20 %, 20–50 %, 50–100 %, and >100 %), the more noticeable the degree of response; moreover, a greater magnitude of alteration (slight, medium, large, and severe) was associated with a smaller corresponding category (4, 3, 2, and 1, respectively). In the 200 mg/kg Sb(V) treatment (FH), the SOD response showed the most severe alteration (AL of 117.31 %) (category 1, Fig. S2a). In Fig. S2b, in the combined treatment of Sb(III) and Sb(V), the AL of CAT showed the most significant response compared with other biomarkers, reaching 171.63 % and 179.21 %, respectively. Both were assigned to Category 1.

3.2.3. Assessment of the interaction in the mixed Sb treatments

The BRI can be obtained by multiplying the weight of biomarker and the score of corresponding coefficients of variation. Table S4 presents the biological health status of earthworms based on BRI. In general, as the treatment level increased, the BRI decreased significantly, thereby indicating that the higher the stress intensity, the poorer the health status of the earthworms. Specifically, the BRI values of the Sb(III) and Sb(V) treatments and the combined treatment were 2.78–3.00, 2.67–3.56 and 2.00–2.22, respectively, showing a dose-response relationship within the treatment (Fig. 2). The highest BRI value of 3.56 was found in the FL treatment, which showed a negligible or slight alteration in health status. Additionally, the TH and FH treatments resulted in moderate and major alterations in health status, respectively. However, the BRI values for the ML and MH treatments were <2.5. Therefore, severe alterations for health status were found under combined treatments.

As can be seen in Table S6, in T1, the EAI value was 0.25, indicating a synergistic effect between Sb(III) and Sb(V) at low concentrations. However, with an increase in treatment concentration, the EAI value in T2 reached −0.07, thus proving that the relationship between Sb(III) and Sb(V) was antagonistic at high-concentration treatments.

3.3. Diversity of bacteria

3.3.1. Abundance of bacterial communities

There were three types of matrices: 1) bulk soil without earthworm disturbance (S), 2) soil lining earthworm burrows (B), and 3) earthworm gut contents (G). Each matrix contained seven treatments (CE, TL, TH, FL, FH, ML, and MH), with the specific treatment concentrations of Sb shown in Table S2. CES, TLS, THS, FLS, FHS, MLS, and MHS represent control, Sb(III) 50 mg/kg, Sb(III) 100 mg/kg, Sb(V) 100 mg/kg, Sb(V) 200 mg/kg, Sb(III) 50 + Sb(V) 100 mg/kg, and Sb(III) 100 + Sb(V) 200 mg/kg in S, respectively. Similarly, CEB, TLB, THB, FLB, FHB, MLB, and MHB represent treatments with seven different Sb concentrations in B. CEG, TLG, THG, FLG, FHG, MLG, and MHG represent treatments with seven different Sb concentrations in G.

Seven taxonomic levels (domain, phylum, class, order, family, genus, and species) were represented. From Fig. 3a, it can be seen that all seven microbial taxa in the burrow lining and gut were more affected by Sb contamination than the bulk soil. This indicated that the B and G matrices were worthy of particular attention. Sb pollution increased the number of bacterial taxonomic units in the gut and burrow matrices of TL treatment. As the concentration of soil Sb pollution increased, the total number of taxonomic units in B and G matrices showed a decreasing trend, with the numbers at the genus decreasing from 343 (CEB) and 290 (CEG) to 115 (MHB) and 44

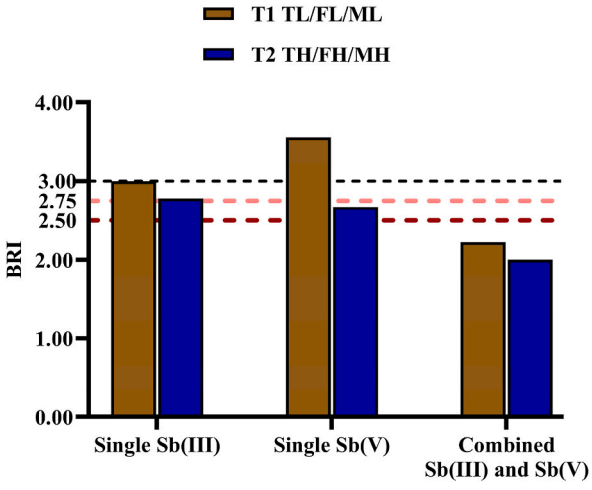


Fig. 2. Earthworm health status under individual and joint stress of Sb(III) and Sb(V) after 14 days of exposure. Horizontal lines refer to the first (BRI = 3.00), second (BRI = 2.75), and third (BRI = 2.50) thresholds for impairment categories of health status.

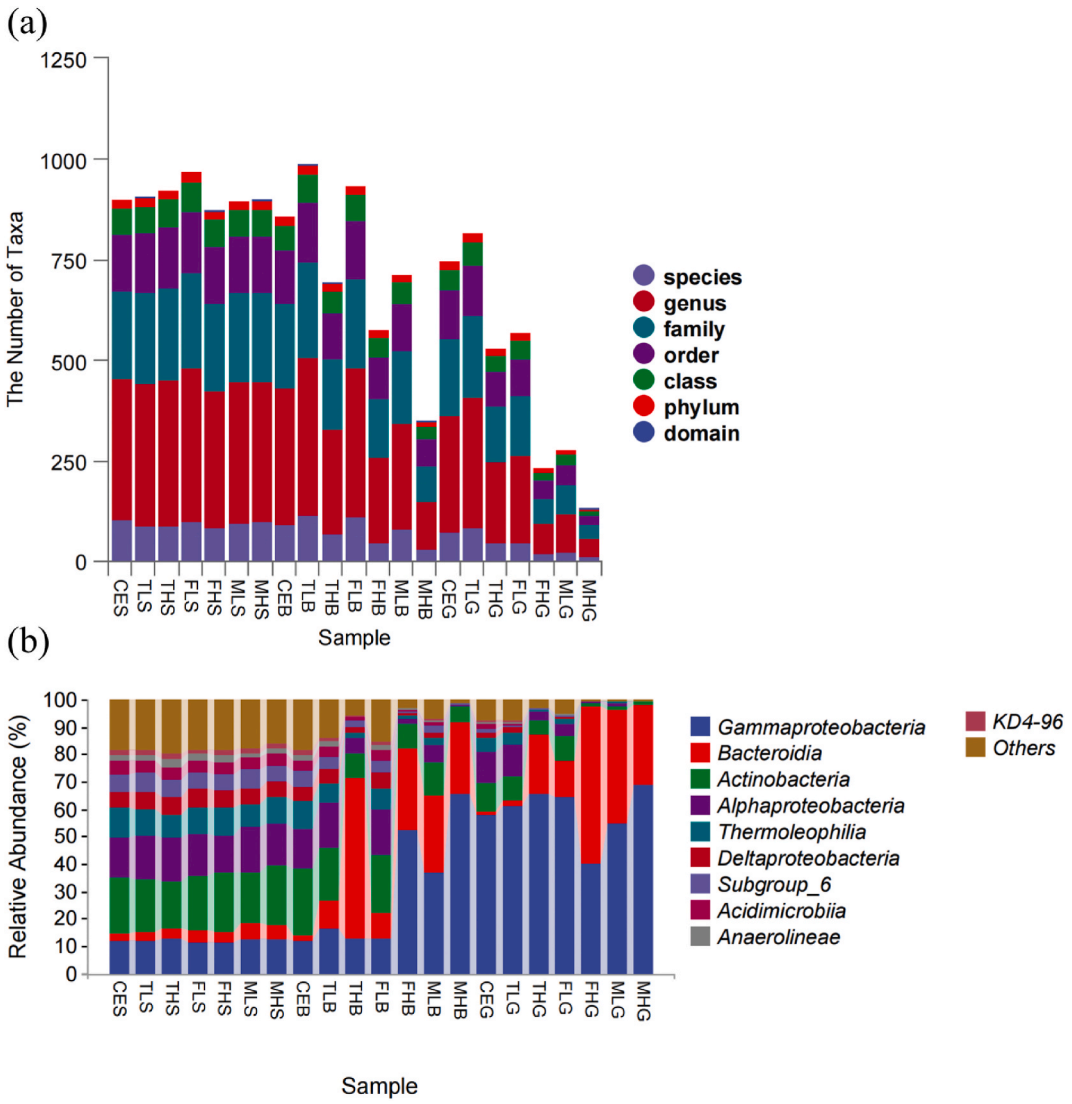


Fig. 3. (a) The number of seven taxonomic levels and (b) relative abundance of different bacterial taxa in the soil treated with individual Sb(III) or Sb(V), or their combination at the class levels in the bulk soil without earthworm disturbance, soil lining earthworm burrows, and the earthworm intestinal contents. Values are the mean of the four replicates.

(MHG), respectively.

Fig. 3b illustrated a complex trend in the relative abundance of Actinobacteria and Bacteroidia at the class level. The abundance of Actinobacteria exhibited a significant decrease with increasing concentrations of single Sb(III) and Sb(V), while the abundance of Bacteroidia increased. For instance, compared to CEB, the abundance of Actinobacteria in the THB group decreased from 40 % to 13 %, while that of Bacteroides increased from 10 % to 59 %. However, the abundance of Bacteroides displayed opposite trends in the composite treatment. Furthermore, the abundance of Gammaproteobacteria was the highest in the gut (40–65 %). There were also some differences in the effects of Sb (III) and Sb (V) on the Gammaproteobacteria. Therefore, the results showed that different Sb forms affected the abundance of specific bacteria in drilosphere, with little effect on the non-drilosphere.

3.3.2. Alpha diversity

Alpha diversity refers to the richness, diversity, evenness, and other indicators of species in a locally uniform habitat, also known as the habitat biodiversity. To comprehensively evaluate the α-diversity of the microbial communities, the Chao1, Shannon, and Pielou indices were used to represent richness, diversity, and evenness, respectively. Rarefaction curves were drawn to assess the effectiveness of coverage of bacterial community in all samples (Fig. S3).

Fig. S4 showed that Sb-contaminated soil reduced population richness. However, the Chao1 indices of TLB and TLG samples were 1.42 and 1.24 times higher than those of CEB and CEG samples, respectively. This indicated that the lower individual Sb(III)

concentration improved the microbial abundance of the soil lining the earthworm burrows and earthworm gut contents. Furthermore, the Pielou indices in the individual Sb(III), Sb(V) or the combined treatments were 0.90–0.91, 0.47–0.90, and 0.36–0.69 in S, B, and G, respectively, indicating that the population evenness decreased due to earthworm activity. After single and combined Sb contamination, the Pielou and Shannon indices of the soil lining earthworm burrows and in the earthworm gut contents decreased significantly, thereby indicating that although population evenness and diversity decreased, the impact on the soil was not severe. Therefore, Sb pollution and earthworm activity generally affected microbial diversity and population evenness, respectively.

3.3.3. Differences in bacterial species composition

The differences between species among several substrate treatments are evident from Fig. 4a. LEfSe (LDA effect size) analysis was performed to identify microbial species that demonstrate inter-group differences. Fig. 4b showed that there were significant differences between the Proteobacteria phylum and Gammaproteobacteria class at the inter-group level, with the highest abundance in MLG and MHG treatments. At the same time, the LDA (Linear discriminant analysis) scores of Proteobacteria phylum and Gammaproteobacteria class were higher than other taxonomic units, indicating their greater impact on inter-group differences.

To further compare the differences in species composition among the samples and display the distribution trends of species abundance for each sample, a heat map was used for the species composition analysis. Fig. 4c indicated that the microbial community structures of CEB, FLB, TLB, and all S were similar at the genus levels. Moreover, B and G showed similarities in their community structure. Hence, all the samples were divided into bulk soil treatment without earthworm disturbance and earthworm gut contents, whereas the soil lining the earthworm burrows was divided into two types based on different treatment concentrations.

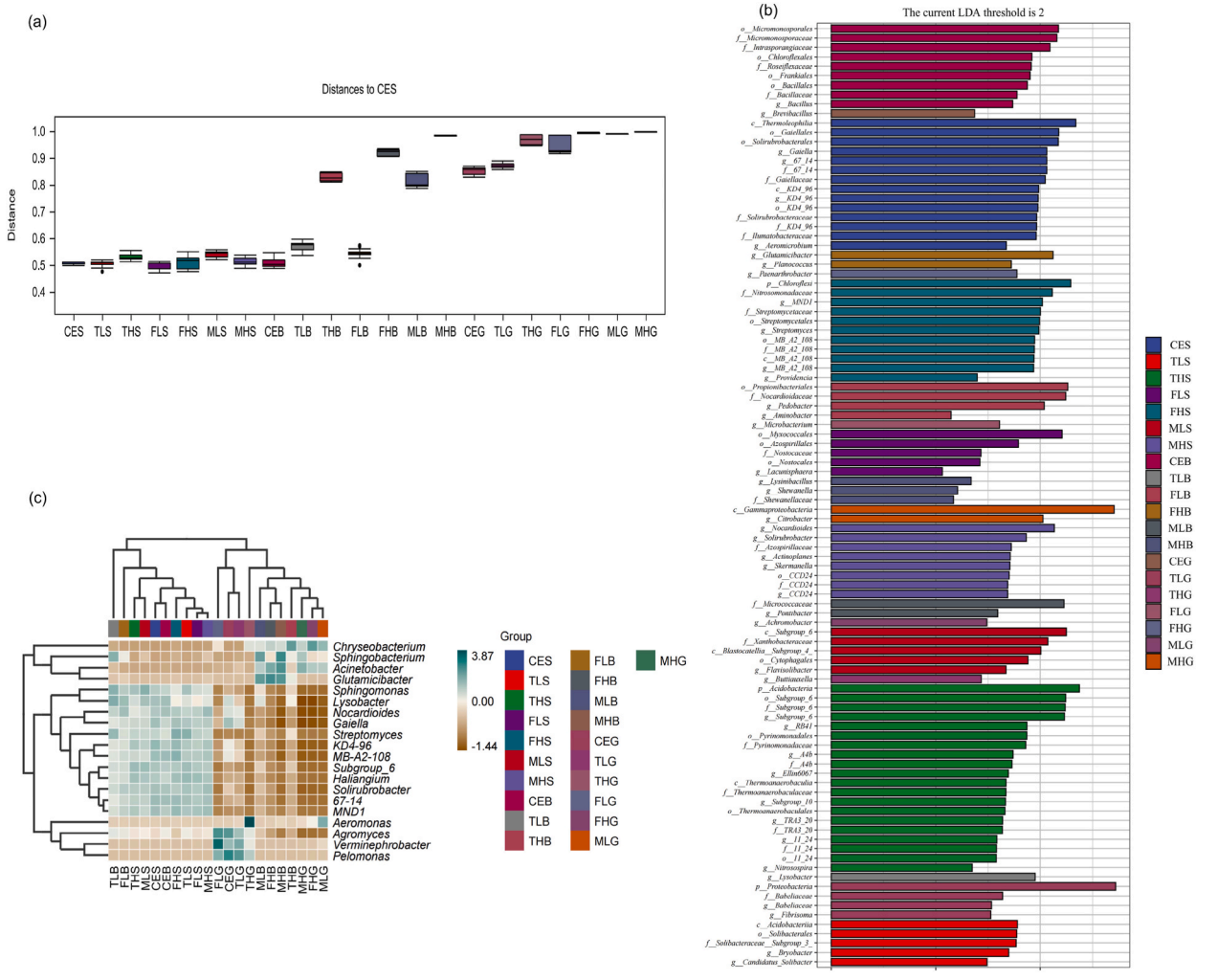


Fig. 4. (a) Box plot. (b) Bar chart of LDA (Linear discriminant analysis) values distribution for significantly different species. The y-axis represents the classification units with significant differences between groups, and the x-axis represents the logarithmic LDA scores for each classification unit. (c) Heat maps at the genus levels. The tree plots on the left represent the cluster analysis of the top 20 bacteria at genus levels, whereas the tree plots on the top represent the clustering analysis of all samples.

Clustering of bacterial taxa based on similarity of distribution was detected in 21 samples. Fig. 4c shows that the genera *Chryseobacterium*, *Sphingobacterium*, *Acinetobacter*, and *Glutamicibacter* were dominant in the burrow-lining soil containing high Sb concentration, whereas *Sphingomonas*, *Lysobacter*, *Nocardioides*, *Gaiella*, *Streptomyces*, *KD4-96*, *MB-A2-108*, *Subgroup-6*, *Haliangium*, *Solirubrobacter*, *67-14*, *and MND1* were dominant in the bulk soil without earthworm disturbance and the burrow-lining soil polluted by individual low Sb concentration. The remaining four bacterial taxa (*Aeromonas*, *Agromyces*, *Verminephrobacter*, *and Pelomonas*) were dominant in the gut contents of earthworms that grew in soil polluted with a single low-concentration of Sb. Overall, Sb pollution affected the abundance of some bacterial taxa in the soil and earthworms, thereby changing the bacterial community structure.

3.3.4. Bacterial community changes under Sb stress

Fig. 5 indicated that the bacterial communities in gut was affected by Sb contamination. The first two axes explained 64.64 % and 18.49 % of the total variance, respectively. GST was the strongest determinant of the bacterial community, followed by SOD, SbE (Sb in earthworms), Sb(III), Sb(V), mortality, and BRI. ML, MH, and FH samples clustered together and were positively correlated with GST, SOD, Sb, and mortality. Thus, it could be seen that Sb had a significant effect on bacterial abundance. CE, TL, and FL samples clustered together, positively with BRI but negatively with other environmental factors. We also found that GST and SOD were more strongly associated with changes in microbial communities than CAT and MT. The results suggested that GST and SOD were more suitable as biomarkers for exploring chemical indicators of microbial responses under Sb stress.

As seen from the Pearson correlation between the microbial and environmental factor, the Sb(III) and Sb(V) content in soil (Sb(III)S and Sb(V)S) showed a significant positive correlation with the four biomarkers (CAT, MT, GST, and SOD), and were negatively associated with BRI and diversity indices (Chao1, Pielou, and Shannon) (Fig. 6). This finding was consistent with previous research results. Additionally, BRI, the abundance of *Marmoricola* sp., *Subgroup 6*, and *Lysobacter dokdonensis* was significantly positively correlated with the diversity index ($p < 0.05$), and negatively correlated with Sb content, four biomarkers, and mortality. Notably, the effect of environmental factors on the abundance of *Sphingobacterium faecium* was opposite to that of these three bacteria. The results suggested that *Sphingobacterium faecium* was highly tolerant to Sb.

4. Discussion

4.1. Accumulation

In this experiment, the soil Sb concentration significantly affected the Sb accumulation in earthworms (Table S5). Earthworm accumulation of toxic heavy metals is affected by many factors, including soil's physical and chemical properties, pollution status, earthworm genus, and environmental conditions (temperature and soil moisture) [26]. Earthworms can accumulate metals from the soil either by direct dermal contact with heavy metals in the soil or by ingesting bulk soil. Before contaminants enter their tissues, the gut can be metabolized, fixed, excreted, or isolated in the tissue or vacuole through a series of processes. Researchers have found that earthworms accumulate metals by forming organo-metallic complexes in the chloragogenous tissue surrounding the posterior alimentary canal [27]. These processes have an impact on Sb mobility and availability throughout the ecosystem.

Sb forms in soil are closely related to their mobility, bioavailability, and toxicity in soil. Some scholars have found that Sb is mainly present in the iron oxide and sulfide phases and exists in the pentavalent form, which is consistent with our research results [28]. After 14 days of exposure, Sb(III) was higher in earthworms than in the soil, whereas Sb(V) showed the opposite trend (Table 1). It was speculated that Sb(V) was rapidly reduced to Sb(III) under anaerobic conditions in the intestine. Alternatively, this may be due to the

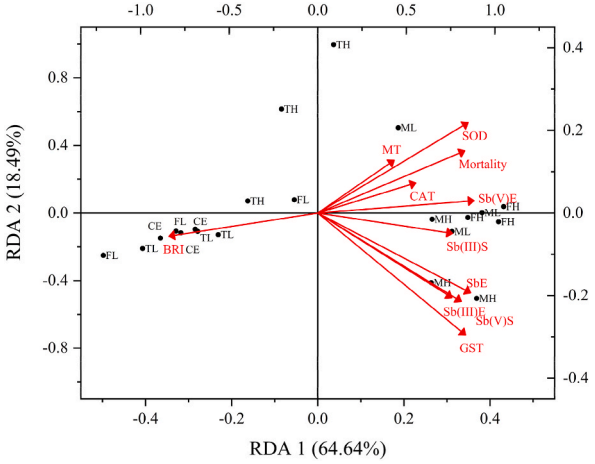


Fig. 5. Redundancy discriminant analysis (RDA) of bacterial community structure, BRI, Sb, and enzyme activity in earthworms under different Sb concentrations. SbE, Total Sb concentration in earthworms; Sb(III)S, Sb(III) in soil; Sb(V)S, Sb(V) in soil; Sb(III)E, Sb(III) in earthworms; Sb(V)E, Sb (V) in earthworms. The same below.

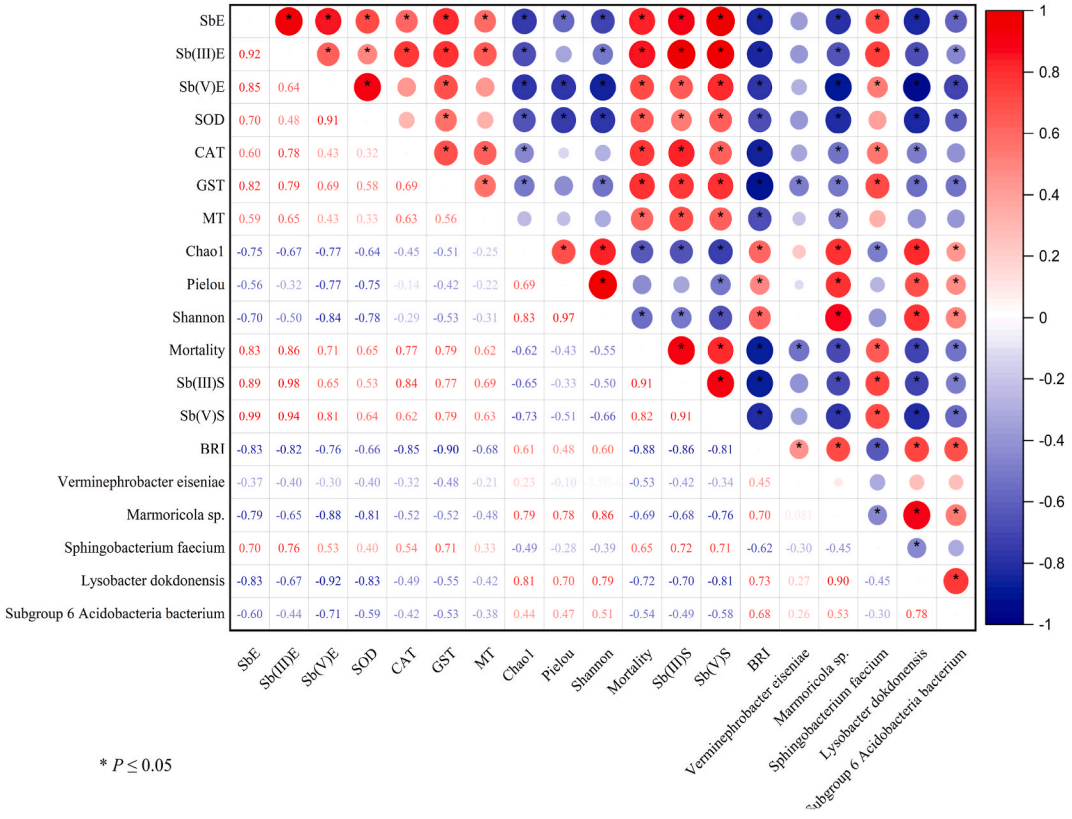


Fig. 6. The correlation heatmap of bacterial community structure, BRI, Sb, and enzyme activity in earthworms under different Sb concentrations. * number marks the "microbial - environmental factor" with significant association ($p < 0.05$).

lower pH in the intestine, which is conducive to the formation of Sb(III) [29]. Multiple studies have reported that pH affects Sb adsorption and may alter microbial community structure [30,31]. Previous studies have shown that Sb(III) is not very stable under aerobic conditions, whereas Sb(V) is highly stable under oxidizing conditions [32]. Moreover, Sb (III) is more readily absorbed by various compounds such as hydroxides of Fe, Mn, and Al, humic acid, and clay minerals in soil, which can induce catalytic oxidation of Sb (III) [31]. According to the previous reports, the abundance of reductive Sb(V) genes in the gut of earthworm was higher than that in the soil. It was further demonstrated that Sb(V) entered the gut of earthworms and was reduced to Sb(III), which was eventually stored or transformed in the gut [5]. Our results showed that Sb(V) was higher in the soil than in earthworms. This is because Sb(III) is widely oxidized in the soil, and under anaerobic conditions, soil can act as a catalyst to promote oxidation of Sb(III) [33].

With a deeper understanding of the microbiome in earthworm gut, microorganisms are also an important factor affecting the species of Sb in the soil. Reductive precipitation of Sb (III) under oxic-anoxic conditions appears to be mainly microbially mediated [34]. To our knowledge, a variety of typical Sb-oxidizing bacteria are widely present in Sb-contaminated soils, such as *Pseudomonas*, *Comamonas*, *Acinetobacter*, and *Sphingomonadaceae* [35]. In this study, *Acinetobacter* was dominant in the burrow-lining and gut of high concentration Sb(V) treatment and compound treatment (Fig. 4c). Therefore, more toxic Sb(III) could be transformed into low toxicity Sb(V) by functional bacteria. Gu et al. studied the response of the bacterium *Acinetobacter johnsonii* JH7 to Sb(III) stress and found that Sb(III) induced the production of reactive oxygen species, leading to oxidative stress and upregulation of antioxidant enzyme activity, as revealed by genomic and proteomic analysis [36]. Furthermore, the ars proteins functioned cooperatively to expel Sb(III) thereby reducing Sb toxicity. Downregulation of the phosphate-specific transporter might reduce the uptake of Sb(V). In a word, the interaction of microbial community with Sb oxidation, reduction, bioaccumulation and migration plays an important role in the formation, migration and bioavailability of Sb.

4.2. Biomarkers

Multiple biomarker responses can only quantitatively describe biochemical processes at the cellular level, and therefore should be normalized systematically. Therefore, an integration index is necessary as it integrates and simplifies complex and diverse response results into quality categories with visual characteristics [21].

To combat these adverse reactions, the body has a sophisticated defense system that reduces its own toxicity through detoxification and internal antioxidant systems. CAT, SOD, and GST are three key enzymes in the antioxidant enzyme system, which are widely distributed in organisms. CAT catalyzes H_2O_2 decomposition, whereas SOD detoxifies toxic superoxide radicals [37]. CAT and SOD

have a good synergistic effect [38]. Studies have reported that SOD, CAT, and POD activities in earthworms (*Eisenia andrei*) increased in the presence of soil Sb [15]. This is consistent with our study results, indicating that the antioxidant system of earthworms is enhanced under Sb stress. MT is a class of cysteine-rich, low-molecular metal-binding proteins that neutralizes toxic heavy metals and acts as an antioxidant [39]. Therefore, earthworms absorb heavy metals, which bind to MT, thus regulating the dynamic balance of intracellular metals to achieve detoxification [40]. Studies have shown that MT has a role in Cd detoxification [41]. However, severe exposure conditions may inhibit the activities of antioxidant enzymes, thereby altering the oxidoreductase balance and reducing oxidative stress tolerance. It has been shown that species, timing, and metal concentration greatly influence the expression of the earthworm MT genes [42]. Although GST has diverse isoenzymes, it cannot decompose H_2O_2; instead, it has the dual functions of scavenging and detoxifying peroxides. It plays a crucial role in self-protection against exogenous pollutants. This study found that soil Sb pollution stimulated GST activity by 4–80 % in most cases. However, the highest dose of Sb(III) (100 mg/kg) inhibited GST activity, which could be explained by the attenuated detoxification ability of earthworms after exposure to high concentrations of Sb. This is consistent with work related to changes in GST content in earthworms reported by Jiang et al. [43].

In this study, Sb(III) and Sb(V) exhibited synergistic and antagonistic effects at low and high concentrations, respectively. We speculated that exposure to different Sb forms may act on different enzymes or proteins, causing increased damage to the organism and thus producing a synergistic effect. In terms of the mechanism of the antagonistic effect, different Sb forms affected the binding site on the receptor through different mechanisms, and they mutually inhibited or hindered each other's actions, thereby decreasing toxicity. Antagonistic effect is very common, such as soil Sb and Hg [44]. Furthermore, it was found that the combined effect of soil Sb and cadmium on earthworms is always antagonistic [14]. Gu et al. discovered that the expression of the *pst* gene, which encodes a transmembrane transporter responsible for Sb(V) uptake, is reduced under Sb(III) stress conditions [36]. However, the mechanism behind this result that we observed has not been well explained. Hence, further studies using advanced methods are needed to evaluate the mechanism of Sb toxicity in earthworms at the cellular, molecular, and genetic levels. In addition, different experimental designs could be tried to determine the relationship between enzyme activity and gene expression under different forms of Sb stress. A series of Sb concentrations could also be set to verify the combined effects of different forms of Sb.

4.3. Effects of Sb species on the bacterial community structure

In this study, the LEfSe analysis revealed significant differences at the inter-group level between the Proteobacteria phylum and Gammaproteobacteria class, both of which were significantly enriched in MLG and MHG treatments with the highest abundance (Fig. 4b). The increase in abundance of Proteobacteria is considered as a sign of imbalance of bacterial community in earthworm gut [45]. The result indicated that the bacterial community in the gut remained in an unbalanced state after Sb compound treatment. Research has shown that Pseudomonas and Thermomonas in Proteobacteria were identified as transforming bacteria of Sb(III) [46]. Gammaproteobacteria were an indicator taxon in the guts of the soil invertebrates responding to environmental concentrations of soil pollutants [47]. Our study showed that the abundance of Gammaproteobacteria was the highest in gut (40–65 %).

Compared to the bulk soil without earthworm disturbance community, the bacterial populations in gut and burrow lining appeared to be more vulnerable to Sb pollution (Fig. 3b). We hypothesized that it was mainly due to the unique environment of the different substrates. The neutral pH and high organic matter content in gut provided a specific place for the soil bacteria. Previous studies have found that higher oxidation rates were observed under neutral and weakly alkaline conditions [48]. The neutralized soil pH promoted the development of a diverse soil microbial community [49]. In addition, the earthworm gut filters and stimulates the intake of a large number of soil microorganisms, resulting in differences in microbial communities and diversity between the earthworm gut and its surrounding environment [50,51]. In this study, the abundance of *Sphingomonas*, *Lysobacter*, *Nocardioides* were dominant in the bulk soil without earthworm disturbance, which may be due to the filtration of soil bacteria through earthworm gut. A study reported that the soil bacterial community structure at the genus level is altered by Sb(III) and Sb(V), whereby the abundance of some functional microbes increases under Sb pollution [52]. The aerobic conditions of the soil versus the anaerobic environment in the earthworm gut might affect the soil microbial community diversity [50]. According to Huang et al., the environmental adaptation potential and network vulnerability of generalists in the earthworm gut are generally higher than those in soil under Sb stress [53].

Meanwhile, the environment of the soil-lined earthworm burrows between the aerobic and anaerobic conditions. Kuzyakov et al. believes that the earthworms can increase soil aggregation and porosity through the process of excretion and burrowing, creating a relatively well-ventilated environment that affects the mobility of Sb and the microecosystem [54]. In addition, the soil lining earthworm burrows contain rich easily decomposable organic materials, including epidermal mucus, leading to accelerated carbon and nitrogen transformation. Earthworms altered the soil pH in the burrow line, likely as a result of their acidic epidermal mucus [55]. The earthworm feeds on soil rich in microorganisms, promoting the formation of intestinal bacterial communities by decomposing organic matter and mineralizing nutrients. At the same time, earthworm feces alter the soil microecological environment. This process potentially impacts soil protist diversity through trophic chain interactions.

4.4. Relationship between bacterial community structure and environmental factors in the earthworm gut

Studies have shown that the increased content or activity of GST and CAT in microorganisms under Sb(III) stress promotes the transformation of toxic Sb(III) into less toxic Sb(V), thus improving the microbial tolerance to Sb [56]. This aligns with our findings. According to the correlation heatmap, *Sphingobacterium faecium* abundance was positively correlated with the Sb content and enzyme activity (Fig. 6). This indicated that Sb promoted the growth of microorganisms with a certain tolerance under Sb-polluted conditions. *Sphingobacterium faecium* belongs to the genus *Sphingobacterium* and phylum Bacteroidota. Previous research has shown that fluorene

biodegradation by *Sphingobacterium* sp. KM-02 occurs in the presence of heavy metals [57]. An et al. also found that *Sphingobacterium_multivorum* can synergistically interact with other microorganisms, promoting the degradation and hexaconazole in the soil [58]. Proteobacteria, Bacteroidetes, Actinomycetota, and Gemmatimonadota were the most abundant phyla detected in the Xikuangshang Sb mine in China [59], which validated our trial. Other enriched genera have also been found in some iron ore environments. In addition, the abundance of *Lysobacter dokdonensis* was significantly positively correlated with the diversity index ($p < 0.05$). Some researchers have isolated a strain of the arsenic(III)-resistant bacterium *Lysobacter arseniciresistens* from iron-mined soil, belonging to the genus *Lysobacter* [60]. Some researchers have screened *Klebsiella aerogenes* X with high Sb tolerance and oxidative capacity from farmland soil exposed to Sb. The resistance mechanism of *Klebsiella aerogenes* X is mediated by oxidative stress, extracellular polymeric material limitation and cell damage [61]. However, the resistance mechanism of Sb oxidizing bacteria to Sb(III) is a complex system [62], involving a variety of biochemical reactions such as Sb transformation, methylation, oxidation–reduction, and chelation mechanisms [63]. Currently, there is a lack of sufficient evidence to indicate the correlation between microorganisms and Sb valence state transformation. Further research is needed to investigate the mechanism of Sb transformation in drilosphere.

5. Conclusions

The study found that Sb(III) was more enriched by earthworms than Sb(V), and the BRI decreased with the increasing Sb treatment levels; the combined treatments of Sb(III) and Sb(V) severely altered the health status of earthworms. Additionally, the synergistic effect was observed at low concentrations, and the antagonistic effect was seen at high concentrations. Sb contamination altered the drilosphere (burrow-lining soil and gut) bacterial community structure at class levels. The Proteobacteria phylum and Gammaproteobacteria class are significantly enriched in gut under combined treatment, suggesting that the gut bacterial community is in an imbalance. Overall, the toxic effects of Sb on earthworms were mainly related to the total Sb concentration in *E. fetida*, biomarkers, and abundance of *Sphingobacterium faecium*. Therefore, this study filled in a data gap regarding the responses of multiple biomarkers to soil Sb(III) and Sb(V) at different contents and explored the bacterial resistance or tolerance to Sb, with potentially significant implications for biodiversity and bioremediation of Sb-polluted soils. In the future, it is possible to consider multiple types of soil and various test species.

Data availability statement

Data will be made available on request.

Founding

This work was supported by the National Natural Science Foundation of China (grant number 41907037, 32371589); the Research Foundation of Education Bureau of Hunan Province, China (grant number 22A0608); and Natural Science Foundation of Hunan (grant number 2024JJ7244, 2023JJ50086, 2023JJ50475).

Compliance with ethical standards

No human trials were involved in this study. Thank earthworms for their sacrifice. All applicable international, national, and institutional guidelines for the care and use of animals were followed.

CRediT authorship contribution statement

Jing Bai: Writing – review & editing, Writing – original draft, Project administration, Funding acquisition. **Linyu Chen:** Writing – review & editing, Writing – original draft, Methodology. **Xiaoqi Yang:** Investigation. **Yuyang Deng:** Data curation. **Juan Wan:** Resources. **Yu Zheng:** Validation. **Ying Song:** Visualization. **Zeliang Yang:** Investigation. **Guohong Xiang:** Conceptualization. **Renyan Duan:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to express their gratitude to EditSprings (https://www.editsprings.cn) for the expert linguistic services provided. We would like to thank the editors and the anonymous reviewers for their helpful comments and suggestions on our paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37734.

References

[1] B. Zheng, Y. He, et al., Antimony contamination, consequences and removal techniques: a review, Ecotoxicol. Environ. Saf. 156 (2018) 125–134, https://doi.org/10.1016/j.ecoenv.2018.03.024.

[2] M. He, X. Wang, F. Wu, Z. Fu, Antimony pollution in China, Sci. Total Environ. 421–422 (2012) 41–50, https://doi.org/10.1016/j.scitotenv.2011.06.009.

[3] M. He, N. Wang, X. Long, et al., Antimony speciation in the environment: recent advances in understanding the biogeochemical processes and ecological effects, J. Environ. Sci. (China) 75 (2019) 14–39, https://doi.org/10.1016/j.jes.2018.05.023.

[4] J. Bai, W. Zhang, W. Liu, et al., Implications of soil potentially toxic elements contamination, distribution and health risk at Hunan's Xikuangshan mine, Processes 9 (2021) 1532, https://doi.org/10.3390/pr9091532, 1532.

[5] B. Huang, J. Long, J. Li, et al., Effects of antimony contamination on bioaccumulation and gut bacterial community of earthworm *Eisenia fetida*, J. Hazard Mater. 416 (2021) 126110, https://doi.org/10.1016/j.jhazmat.2021.126110.

[6] L. Xiao, M. Li, J. Dai, et al., Assessment of earthworm activity on Cu, Cd, Pb and Zn bioavailability in contaminated soils using biota to soil accumulation factor and DTPA extraction, Ecotoxicol. Environ. Saf. 195 (2020) 110513, https://doi.org/10.1016/j.ecoenv.2020.110513.

[7] Y. Zhang, K. Song, J. Zhang, et al., Removal of sulfamethoxazole and antibiotic resistance genes in paddy soil by earthworms (*Pheretima guillelmi*): intestinal detoxification and stimulation of indigenous soil bacteria, Sci. Total Environ. 851 (Pt 1) (2022) 158075, https://doi.org/10.1016/j.scitotenv.2022.158075.

[8] J. Curry, O. Schmidt, The feeding ecology of earthworms – a review, Pedobiologia 50 (6) (2007) 463–477, https://doi.org/10.1016/j.pedobi.2006.09.001.

[9] J. Lipiec, M. Brzezinska, M. Turski, et al., Wettability and biogeochemical properties of the drilosphere and casts of endogeic earthworms in pear orchard, Soil Res. 145 (2015) 55–61, https://doi.org/10.1016/j.still.2014.08.010.

[10] M. Sun, H. Chao, X. Zheng, et al., Ecological role of earthworm intestinal bacteria in terrestrial environments: a review, Sci. Total Environ. 740 (2020) 140008, https://doi.org/10.1016/j.scitotenv.2020.140008.

[11] R. Deng, Y. Chen, X. Deng, et al., A critical review of resistance and oxidation mechanisms of Sb-oxidizing bacteria for the bioremediation of Sb(III) pollution, Front. Microbiol. 12 (2021) 738596, https://doi.org/10.3389/fmicb.2021.738596. Published 2021 Sep. 7.

[12] A. Ghosh, P. Bhattacharyya, R. Pal, Effect of arsenic contamination on microbial biomass and its activities in arsenic contaminated soils of Gangetic West Bengal, India, Environ. Int. 30 (4) (2004) 491–499, https://doi.org/10.1016/j.envint.2003.10.002.

[13] Y. Baek, W. Lee, S. Jeong, et al., Ecological effects of soil antimony on the crop plant growth and earthworm activity, Environ. Earth Sci. 71 (2) (2014) 895–900, https://doi.org/10.1007/s12665-013-2492-y.

[14] Z. Xu, Z. Yang, W. Shu, et al., Combined toxicity of soil antimony and cadmium on earthworm *Eisenia fetida*: accumulation, biomarker responses and joint effect, J. Hazard Mater. 2 (2021) 100018, https://doi.org/10.1016/j.hazl.2021.100018.

[15] Z. Yang, Z. Xu, W. Shu, et al., Evaluation of soil antimony stress on the biological health status of earthworm *Eisenia andrei* using Biomarker Response Index, J. Soils Sediments 22 (7) (2022) 1999–2008, https://doi.org/10.1007/s11368-022-03153-8.

[16] J. Bai, D. Lu, L. Chen, et al., Ecotoxicological differences of antimony (III) and antimony (V) on earthworms *Eisenia fetida* (Savingy), Toxics 11 (3) (2023) 230, https://doi.org/10.3390/toxics11030230.

[17] H. Xu, J. Bai, W. Li, et al., Removal of persistent DDT residues from soils by earthworms: a mechanistic study, J. Hazard Mater. 365 (2019) 622–631, https://doi.org/10.1016/j.jhazmat.2018.11.043.

[18] W. Sun, E. Xiao, M. Häggblom, et al., Bacterial survival strategies in an alkaline tailing site and the physiological mechanisms of dominant phylotypes as revealed by metagenomic analyses, Environ. Sci. Technol. 52 (22) (2018) 13370–13380, https://doi.org/10.1021/acs.est.8b03853.

[19] M. Zhang, M. Kolton, Z. Li, et al., Bacteria responsible for antimonite oxidation in antimony-contaminated soil revealed by DNA-SIP coupled to metagenomics, FEMS Microbiol. Ecol. 97 (5) (2021) fiab057, https://doi.org/10.1093/femsec/fiab057.

[20] Y. Zheng, K. Zhou, J. Tang, et al., Impacts of di-(2-ethylhexyl) phthalate on *Folsomia candida* (Collembola) assessed with a multi-biomarker approach, Ecotoxicol. Environ. Saf. 232 (2022) 113251, https://doi.org/10.1016/j.ecoenv.2022.113251.

[21] J. Hagger, M. Jones, D. Lowe, et al., Application of biomarkers for improving risk assessments of chemicals under the Water Framework Directive: a case study, Mar. Pollut. Bull. 56 (6) (2008) 1111–1118, https://doi.org/10.1016/j.marpolbul.2008.03.040.

[22] F. Piva, F. Ciaprini, F. Onorati, et al., Assessing sediment hazard through a weight of evidence approach with bioindicator organisms: a practical model to elaborate data from sediment chemistry, bioavailability, biomarkers and ecotoxicological bioassays, Chemosphere 83 (4) (2011) 475–485, https://doi.org/10.1016/j.chemosphere.2010.12.064.

[23] J. Zhang, S. Liu, J. Zhang, et al., Two novel indices for quantitatively characterizing the toxicity interaction between ionic liquid and carbamate pesticides, J. Hazard Mater. 239–240 (2012) 102–109, https://doi.org/10.1016/j.jhazmat.2012.07.063.

[24] X. Li, M. Wang, W. Chen, et al., Evaluation of combined toxicity of Siduron and cadmium on earthworm (*Eisenia fetida*) using Biomarker Response Index, Sci. Total Environ. 646 (2019) 893–901, https://doi.org/10.1016/j.scitotenv.2018.07.380.

[25] J. Caporaso, J. Kuczynski, J. Stombaugh, K. Bittinger, et al., QIIME allows analysis of high-throughput community sequencing data, Nat. Methods 7 (5) (2010) 335–336, https://doi.org/10.1038/nmeth.f.303.

[26] R. Xiao, A. Ali, Y. Xu, et al., Earthworms as candidates for remediation of potentially toxic elements contaminated soils and mitigating the environmental and human health risks: a review, Environ. Int. 158 (2022) 106924, https://doi.org/10.1016/j.envint.2021.106924.

[27] A. Morgan, S. Stürzenbaum, C. Winters, et al., Differential metallothionein expression in earthworm (*Lumbricus rubellus*) tissues, Ecotoxicol. Environ. Saf. 57 (1) (2004) 11–19, https://doi.org/10.1016/j.ecoenv.2003.08.022.

[28] S. Denys, K. Tack, J. Caboche, et al., Bioaccessibility, solid phase distribution, and speciation of Sb in soils and in digestive fluids, Chemosphere 74 (5) (2009) 711–716, https://doi.org/10.1016/j.chemosphere.2008.09.088.

[29] K. Hockmann, B. Planer-Friedrich, S. Johnston, et al., Antimony mobility in sulfidic systems: coupling with sulfide-induced iron oxide transformations, Geochem. Cosmochim. Acta 282 (2020) 276–296, https://doi.org/10.1016/j.gca.2020.05.024.

[30] Y. Cai, L. Li, H. Zhang, Kinetic modeling of pH-dependent antimony (V) sorption and transport in iron oxide-coated sand, Chemosphere 138 (2015) 758–764, https://doi.org/10.1016/j.chemosphere.2015.07.067.

[31] A. Leuz, H. Mönch, C. Johnson, Sorption of Sb(III) and Sb(V) to goethite: influence on Sb(III) oxidation and mobilization, Environ. Sci. Technol. 40 (23) (2006) 7277–7282, https://doi.org/10.1021/es061284b.

[32] R. Cidu, R. Biddau, E. Dore, et al., Antimony in the soil-water-plant system at the Su Suergiu abandoned mine (Sardinia, Italy): strategies to mitigate contamination, Sci. Total Environ. 497–498 (2014) 319–331, https://doi.org/10.1016/j.scitotenv.2014.07.117.

[33] Y. Cai, Y. Mi, H. Zhang, Kinetic modeling of antimony(III) oxidation and sorption in soils, J. Hazard Mater. 316 (2016) 102–109, https://doi.org/10.1016/j.jhazmat.2016.05.027.

[34] E. Markelova, R. Couture, C. Parsons, et al., Speciation dynamics of oxyanion contaminants (As, Sb, Cr) in argillaceous suspensions during oxic-anoxic cycles, Appl. Geochem. 91 (2018) 75–88, https://doi.org/10.1016/j.apgeochem.2017.12.012.

[35] Y. Nakamaru, F. Martín Peinado, Effect of soil organic matter on antimony bioavailability after the remediation process, Environ. Pollut. 228 (2017) 425–432, https://doi.org/10.1016/j.envpol.2017.05.042.

[36] J. Gu, G. Sunahara, R. Duran, et al., Sb(III)-resistance mechanisms of a novel bacterium from non-ferrous metal tailings, Ecotoxicol. Environ. Saf. 186 (2019) 109773, https://doi.org/10.1016/j.ecoenv.2019.109773.

[37] H. Najmuldeen, R. Alghamdi, F. Alghofaili, et al., Functional assessment of microbial superoxide dismutase isozymes suggests a differential role for each isozyme, Free Radic. Biol. Med. 134 (2019) 215–228, https://doi.org/10.1016/j.freeradbiomed.2019.01.018.

[38] P. Vasantha-Srinivasan, M. Chellappandian, S. Senthil-Nathanet, et al., A novel herbal product based on Piper betle and Sphaeranthus indicus essential oils: toxicity, repellent activity and impact on detoxifying enzymes GST and CYP450 of *Aedes aegypti* Liston (Diptera: Culicidae), J. Asia Pac. Entomol. 21 (4) (2018) 1466–1472, https://doi.org/10.1016/j.aspen.2018.10.008.

[39] N. Hussain, S. Chatterjee, T. Maiti, et al., Metal induced non-metallothionein protein in earthworm: a new pathway for cadmium detoxification in chloragogenous tissue, J. Hazard Mater. 401 (2021) 123357, https://doi.org/10.1016/j.jhazmat.2020.123357.
[40] Z. Usmani, V. Kumar, Earthworms: 'the unheralded soldiers' standing steadfast against metal contamination, J. Environ. Sci. 9 (2) (2015) 48–65, https://doi.org/10.3923/rjes.2015.48.65.
[41] S. Stürzenbaum, C. Winters, M. Galay, et al., Metal ion trafficking in earthworms. Identification of a cadmium-specific metallothionein, J. Biol. Chem. 276 (36) (2001) 34013–34018, https://doi.org/10.1074/jbc.M103605200.
[42] E. Swart, E. Martell, C. Svendsen, et al., Soil ecotoxicology needs robust biomarkers: a meta-analysis approach to test the robustness of gene expression-based biomarkers for measuring chemical exposure effects in soil invertebrates, Environ. Toxicol. Chem. 41 (9) (2022) 2124–2138, https://doi.org/10.1002/etc.5402.
[43] L. Jiang, S. Ling, M. Fu, et al., Bioaccumulation, elimination and metabolism in earthworms and microbial indices responses after exposure to decabromodiphenyl ethane in a soil-earthworm-microbe system, Environ. Pollut. 289 (2021) 117965, https://doi.org/10.1016/j.envpol.2021.117965.
[44] Z. Fu, F. Wu, C. Mo, et al., Bioaccumulation of antimony, arsenic, and mercury in the vicinities of a large antimony mine, China, Microchem. J. 97 (1) (2011) 12–19, https://doi.org/10.1016/j.microc.2010.06.004.
[45] N. Shin, T. Whon, J. Bae, Proteobacteria: microbial signature of dysbiosis in gut microbiota, Trends Biotechnol. 33 (9) (2015) 496–503, https://doi.org/10.1016/j.tibtech.2015.06.011.
[46] P. Costa, L. Scholte, M. Reis, et al., Bacteria and genes involved in arsenic speciation in sediment impacted by long-term gold mining, PLoS One 9 (4) (2014) e95655, https://doi.org/10.1371/journal.pone.0095655.
[47] Q. Zhang, Z. Zhang, T. Lu, et al., Gammaproteobacteria, a core taxon in the guts of soil fauna, are potential responders to environmental concentrations of soil pollutants, Microbiome 9 (1) (2021) 196, https://doi.org/10.1186/s40168-021-01150-6.
[48] K. Wang, D. Min, G. Chen, et al., Oxidation of Sb(III) by Shewanella species with the assistance of extracellular organic matter, Environ. Res. 236 (Pt 2) (2023) 116834, https://doi.org/10.1016/j.envres.2023.116834.
[49] B. Tripathi, J. Stegen, M. Kim, et al., Soil pH mediates the balance between stochastic and deterministic assembly of bacteria, ISME J. 12 (4) (2018) 1072–1083, https://doi.org/10.1038/s41396-018-0082-4.
[50] H. Drake, M. Horn, As the worm turns: the earthworm gut as a transient habitat for soil microbial biomes, Annu. Rev. Microbiol. 61 (2007) 169–189, https://doi.org/10.1146/annurev.micro.61.080706.093139.
[51] D. Pass, A. Morgan, D. Read, et al., The effect of anthropogenic arsenic contamination on the earthworm microbiome, Environ. Microbiol. 17 (6) (2015) 1884–1896, https://doi.org/10.1111/1462-2920.12712.
[52] A. Wang, M. He, W. Ouyang, et al., Effects of antimony (III/V) on microbial activities and bacterial community structure in soil, Sci. Total Environ. 789 (2021) 148073, https://doi.org/10.1016/j.scitotenv.2021.148073.
[53] B. Huang, J. Long, J. Li, et al., Bacterial generalists in earthworm gut had stronger environmental adaptation potential and higher network vulnerability under antimony stress, J. Clean. Prod. 380 (2022) 134992, https://doi.org/10.1016/j.jclepro.2022.134992.
[54] Y. Kuzyakov, E. Blagodatskaya, Microbial hotspots and hot moments in soil: concept & review, Soil Biol. Biochem. 83 (2015) 184–199, https://doi.org/10.1016/j.soilbio.2015.01.025.
[55] S. Zhang, F. Hu, H. Li, et al., Influence of earthworm mucus and amino acids on tomato seedling growth and cadmium accumulation, Environ. Pollut. 157 (10) (2009) 2737–2742, https://doi.org/10.1016/j.envpol.2009.04.027.
[56] Q. Wang, T. Warelow, Y. Kang, et al., Arsenite oxidase also functions as an antimonite oxidase, Appl. Environ. Microbiol. 81 (6) (2015) 1959–1965, https://doi.org/10.1128/AEM.02981-14.
[57] I. Nam, Y. Kim, D. Cho, et al., Effects of heavy metals on biodegradation of fluorene by a *Sphingobacterium* sp. strain (KM-02) isolated from polycyclic aromatic hydrocarbon-contaminated mine soil, Environ. Eng. Sci. 32 (10) (2015) 891–898, https://doi.org/10.1089/ees.2015.0037.
[58] X. An, C. Tian, J. Xu, et al., Characterization of hexaconazole-degrading strain *Sphingobacterium multivorum* and analysis of transcriptome for biodegradation mechanism, Sci. Total Environ. 722 (2020) 137171, https://doi.org/10.1016/j.scitotenv.2020.137171.
[59] X. Sun, T. Kong, F. Li, et al., *Desulfurivibrio* spp. mediate sulfur-oxidation coupled to Sb(V) reduction, a novel biogeochemical process, ISME J. 16 (6) (2022) 1547–1556, https://doi.org/10.1038/s41396-022-01201-2.
[60] G. Luo, Z. Shi, G. Wang, *Lysobacter arsenicresistens* sp. nov., an arsenite-resistant bacterium isolated from iron-mined soil, Int. J. Syst. Evol. Microbiol. 62 (Pt 7) (2012) 1659–1665, https://doi.org/10.1099/ijs.0.034405-0.
[61] Q. Rong, C. Ling, D. Lu, et al., Sb(III) resistance mechanism and oxidation characteristics of *Klebsiella aerogenes* X, Chemosphere 293 (2022) 133453, https://doi.org/10.1016/j.chemosphere.2021.133453.
[62] J. Gu, J. Yao, R. Duran, et al., Comprehensive genomic and proteomic profiling reveal *Acinetobacter johnsonii* JH7 responses to Sb(III) toxicity, Sci. Total Environ. 748 (2020) 141174, https://doi.org/10.1016/j.scitotenv.2020.141174.
[63] M. Filella, N. Belzile, Y. Chen, Antimony in the environment: a review focused on natural waters: I. Occurrence, Earth Sci. Rev. 57 (1) (2002) 125–176, https://doi.org/10.1016/S0012-8252(01)00070-8.