Evaluation of acid mine drainage sludge as soil substitute for the reclamation of mine solid wastes

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Abstract
The reclamation of mine waste deposits is often hindered by the scarcity of natural topsoil. Acid mine drainage sludge (AMDS), as a mass-produced waste in metalliferous mines, is a potential topsoil substitute but had not been validated. In this study, a pot experiment with three plant species was conducted to evaluate the capacity of AMDS to support plant growth, buffer acidification, and immobilize heavy metal(loid)s when reclaiming mine waste rocks. Chemical fertilizer and compost chicken manure were applied to AMDS at different rates to explore their effects on plant growth and the physicochemical properties of AMDS. Results showed that all the plants could survive in AMDS even without fertilization. The contents of heavy metal(loid)s in rhizosphere remained almost unchanged over the experimental period, indicating low leachability of revegetated AMDS. Fertilizers enhanced macronutrients and soil enzyme activities, leading to significant increases in plant biomass. However, owing to manure composting and low richness and diversity of the bacterial community in AMDS, the NH4+-N and bioavailable phosphorus contents were extremely low. Bermuda grass was a suitable pioneer species for reclamation for its better adaptability to nutrient deficiency and heavy metal(loid) stress. Overall, AMDS is a viable soil substitute for mine reclamation due to its capability to support plant growth and environmental safety.

Keywords Acid mine drainage sludge · Soil substitute · Mine reclamation · Heavy metal(loid) · Soil fertility · Soil microbe

Introduction
Tailings and waste rocks are produced in large quantities in mining processes (Park et al. 2019). The global mining industry was estimated to produce about 14 billion tons of mine tailings in 2010 (Owen et al. 2020). According to a survey in 2015, there were nearly 10,000 tailing ponds and 60,000 solid waste dumps in China. Existing tailings amounted to more than 20 billion tons and solid wastes exceeded 60 billion tons, respectively (Wang and Xue 2017). The stockpiling of these potentially hazardous wastes poses serious threats to mining safety as well as the neighboring environment (Kossoff et al. 2014). The weathering, leaching, and spreading of toxic heavy metal(loid)s such as arsenic (As), cadmium (Cd), and lead (Pb) in mine wastes can result in the contamination of water, soil, plants, and even crops in downstream regions (Karaca et al. 2018). Meanwhile, heavy precipitation may cause severe landslide or debris flow in poorly managed waste piles (Pastor et al. 2002). The storage of these solid wastes also takes up plenty of space and causes severe damages to natural landscape. In China, the tailing ponds and solid waste dumps occupied about 67 and
valuable metals (e.g., rare earth metals), sulfuric acid, and iron.

These mainly include recovering resources, such as the excessive stockpiling, different methods for the disposal of mine wastes (Demers et al. 2017; Kefeni et al. 2017). To avoid acid mine drainage sludge, AMDS), which are usually deposited in tailing ponds, and hence cause problems similar to other acid drainage (Zinck and Griffith 2013; Rakotonimaro et al. 2017). Some studies reported the capabilities of AMDS as a barrier to prevent the oxidation of the underlaying sulfides and as a capillary barrier to inhibit the upward movement of acid and toxic metal(loid) s (Demers et al. 2015a, b; Mbonimpa et al. 2016). Nevertheless, this strategy had been questioned for its efficiency due to the preferential channeling and crusting of AMDS which could facilitate the diffusion of oxygen; hence, a plant cover on AMDS was suggested to fix this problem (Rakotonimaro et al. 2017). Based on its physical properties, AMDS could provide a foundation for root growth but might hinder plant growth due to nutrient deficiency and heavy metal(loid) toxicity. Although there were evidence suggesting that AMDS could support plant growth (Demers et al. 2015b), the interaction between the vegetative cover and AMDS in reclaming the solid wastes in metalliferous mines is poorly understood, which deserves attention. To date, little is known about the leaching behavior of the toxic metal(loid)s and the changes in the physicochemical properties in AMDS during revegetation, especially when it is enriched with organic amendments, which is almost necessary in mine reclamation (Pardo et al. 2014). The growth behavior of plants and its environmental restriction factor in AMDS also requires elaboration. As the ponds and piles of AMDS must be reclaimed at the end of mining activities, the knowledge can provide important theoretical and practical basis.

Therefore, the aims of the present study were to (1) evaluate the feasibility of using AMDS as soil substitute in mine restoration, analyze the growth of different plants, and the temporal change of the nutrient elements and heavy metal(loid)s in AMDS; (2) elucidate the effects of organic manure and chemical fertilizer applications on plant growth and the physicochemical properties of AMDS; (3) explore the relationships between plants, AMDS, and the microbes in AMDS as well as their impacts on the ultimate reclamation. Results of this study will provide valuable reference for improving the environment of mine and neighboring area and promoting safe production.

Materials and methods

Experimental design

A pot experiment was conducted in the Sun Yat-sen University in Guangzhou, Guangdong Province, China (23° 4′
N, 113° 24' E). The trial was initiated in late August 2018 and ran over 12 months. Two tolerant plants commonly used in mine reclamation, i.e., Bermuda grass (Cynodon dactylon L.) and alfalfa (Medicago sativa L.) (Ye et al. 2000; Heshmati and Pessarakli 2011; Hu et al. 2020), and a native grass broad-leaved paspalum (Paspalum wettsteini Hack.), were planted in nylon rhizobags and placed in plastic pots. All the pots containing a 5-cm layer of waste rocks at the bottom were filled with 3 kg of AMDS (with a 15-cm thickness) collected from Zijinshan Copper Mine, China (Table 1). The AMDS was produced by adding quicklime to neutralize AMD and subsequently dewatering the loose sludge. This tan-colored sludge was mainly composed of Ca, Fe, and Al (hydr)oxides, sulfides, and sulfates (Table S1). The waste rocks, with uneven grain sizes, were less than 4 cm in diameter (Table S2). The pots were placed in the open air and plants were irrigated with rainwater and an automatic irrigation system in not rainy days. Compost chicken manure (as organic fertilizer; Table 1) was mixed with the AMDS at rates of 0 (CK), 1.0 (M1), 2.0 (M2), and 4.0 (M3) g pot⁻¹, respectively. A portion of each AMDS sample was directly stored at −20 °C for microbial analyses. The rest of these samples were air-dried to constant weight and ground to pass a 2-mm sieves for determining the physicochemical properties and contents of elements of AMDS.

### Physicochemical analyses

The pH and electrical conductivity (EC) of AMDS were determined using a pH/EC meter (SG78-FK-CN, Mettler Toledo, USA) at a soil-to-water ratio of 1:5 (w/v). Total organic carbon (TOC) content was determined following the wet oxidation method (Walkley and Black 1934). Total nitrogen (N) was quantified using the Kjeldahl method and NH₄⁺-N was measured using the indophenol-blue method after extraction by 2 M KCl (Keeney and Nelson 1982). To determine total phosphorus (P), AMDS samples were digested with H₂SO₄-HClO₄ (Bai et al. 2013). Bioavailable P and potassium (K) were extracted using the Mehlich 3 extractant (Mehlich 1984). Exchangeable cadmium (Cd), iron (Fe), copper (Cu), and zinc (Zn) in AMDS were extracted using the diethylenetriaminepentaacetic acid (DTPA) (Palansooriya et al. 2020). To determine the total concentrations of these elements in AMDS and plant tissues, samples were digested with aqua regia and concentrated HNO₃, respectively. The P contents in the digestions and extracts were measured using the molybdenum-blue method (Francioli et al. 2016). The concentrations of K, Cd, Pb, Fe, manganese (Mn), Cu, and Zn were measured using the atomic absorption spectroscopy (ZA-3000, HITACHI, Japan), and the concentration of As was measured using the atomic fluorescence spectrometry (AFS-8220, Beijing Titan Instruments, China). Plant (GBW-07435) and soil (GBW-07603) reference materials were used for quality control in determining the element concentrations in plant tissues and soils, respectively. The recoveries of all the elements were 90 ± 10%.

### Microbial analyses

To determine the activities of β-glucosidase, acid, and alkaline phosphatases in AMDS, samples were incubated at 37 °C for 1 h with buffered p-nitrophenyl β-D-glucoside (tris(hydroxymethyl)aminomethane buffer, pH 12) and p-nitrophenyl phosphate (modified universal buffer, pH 6.5 for acid phosphatase and pH 11.0 for alkaline phosphatase) solutions, respectively (Tabatabai 1994). To determine the activity of urease, AMDS samples were incubated at 37 °C for 24 h with urea solution (Guan 1986). The activities of these enzymes were determined by colorimetrically measuring the products (i.e., p-nitrophenol and NH₃-N) released using UV spectrophotometry (T6, Persee, China). To investigate the bacterial community characteristics of AMDS, the total DNA in bulk and rhizosphere AMDS of Bermuda

| Table 1 | The physicochemical properties of the organic manure and AMDS used in this study (mean ± SE, n = 6) |
|---------|------------------------------------------------------------------------------------------------|
| AMDS    | Organic manure                                                                                 |
| Bulk density (g cm⁻³) | 0.88 ± 0.02                                       |
| Field capacity (kg g⁻¹) | 446 ± 20                                        |
| EC (mS cm⁻¹) | 3.03 ± 0.02                                      |
| pH      | 8.23 ± 0.02                                      |
| TOC (g kg⁻¹) | 3.18 ± 0.34                                      |
| N (g kg⁻¹) | 0.32 ± 0.02                                      |
| P (g kg⁻¹) | 0.28 ± 0.01                                      |
| K (g kg⁻¹) | 0.17 ± 0.01                                      |
| As (mg kg⁻¹) | 416 ± 9                                        |
| Cd (mg kg⁻¹) | 26.3 ± 2                                         |
| Cu (mg kg⁻¹) | 1172 ± 19                                        |
| Fe (g kg⁻¹) | 81.7 ± 0.6                                       |
| Mn (mg kg⁻¹) | 561 ± 4                                         |
| Zn (g kg⁻¹) | 1.68 ± 0.17                                      |

EC, electrical conductivity; TOC, total organic carbon
grasses was extracted using a PowerSoil® DNA Isolation Kit (MO BIO Laboratories, USA) following the manufacturer’s instructions. Extracted DNA was evaluated using electrophoresis and its concentration and purity were verified using spectrophotometry (NanoDrop, USA). The V4–V5 region of the bacterial 16S rDNA was amplified using a primer set 515F/907R. The PCR reactions were carried out in triplicate and then pooled. The PCR products were mixed at equimolar concentrations and purified before sequencing on the Illumina MiSeq platform (Illumina, USA). The raw sequence reads were trimmed, and low-quality reads were discarded. The clean reads were clustered into operational taxonomic units (OTUs) at 97% similarity using UPARSE (version 10.0.240) (Edgar 2013). The RDP classifier was used to assign taxonomical classifications to OTUs against the SILVA database at a confidence threshold of 70% (Quast et al. 2012). The metrics of α-diversity including species richness, Chao1, dominance, Shannon and Simpson diversity indices, were calculated in QIIME (version 1.9.1) (Caporaso et al. 2010).

Statistical analyses

The significance of differences between different manure treatments, plants, and sampling times was determined by analysis of variance. Least significant difference was calculated as the post hoc test to compare the means of different treatments. The correlations between different soil and plant characteristics were assessed using the Pearson’s correlation analysis. All statistical analyses were performed using R software (Version 4.1.0) and figures were created using the ggplot2 package (Version 3.3.5) in R software (R Core Team 2020).

Results and discussion

Temporal changes of the heavy metal(loid)s in AMDS

The total concentrations of all the heavy metal(loid)s tested in this study generally remained unchanged in the rhizosphere but fluctuated in bulk AMDS over the experimental period (Fig. 1). This indicated that after revegetation, the toxic elements were unlikely to be released from AMDS at least in the short term (e.g., 1 year) and thus undesirable impacts to surrounding environment could be largely prevented. Previous studies found that increasing the pH and solid content of AMDS was crucial for limiting the leachability of the toxic elements in it (Zinck et al. 1997; Rakotonimaro et al. 2017). Accordingly, the low mobility of the heavy metal(loid)s in the AMDS used in the present study could be attributed to its slightly alkaline pH and effective dewatering pretreatment.

It is also important to note that the metal(loid) concentrations in bulk AMDS exhibited apparent rises and falls around the 9-month sampling (Fig. 1). A possible explanation for this might be that without a vegetation cover to retain its moisture, the bulk AMDS and its underlaying waste rocks underwent active leaching and re-precipitation processes, resulting in the migration of the heavy metal(loid)s between the bulk AMDS and waste rocks. According to previous studies, prolonged drought and temperature rise could intensify the leaching of the heavy metals in mine solid wastes due to enhanced oxidation of sulfide minerals (Bang et al. 2020), while continuous wetting facilitated the immobilization of these elements (Kim et al. 2021). In this way, our finding highlighted the importance of revegetating mine solid wastes.

However, in terms of the bioavailability of the heavy metal(loid)s, minor increases in the extractable As, Cd, Cu, and Zn concentrations in AMDS were observed over the experimental period ($p < 0.05$; Fig. 1). Compared with those at the first sampling, the extractable As, Cd, Cu, and Zn at the 12-month sampling increased averagely by 33.6%, 16.4%, 20.9%, and 15.1%, respectively. It was highly likely that these increases were triggered by the gradual decrease in the pH of AMDS. As shown in Figs. 2 and 3, the pH was 0.09- to 0.25-unit lower at the last sampling than at the first sampling and was significantly negatively correlated with the extractable As, Cd, and Cu concentrations ($p < 0.05$). Local climate might have played a key role in the acidification process. The high temperature and abundant rainfall in Guangdong Province created cycles of alternate wetting and drying condition in the experiment pots, which led to gradual oxidation of the sulfide minerals and subsequent generation of acidic leachates in the waste rocks, which moved upward (Kossoff et al. 2014). Local rainwater was also reported to have a low pH of around 4.4, leading to the acidification of local soil (Qin et al. 2006). These results implied possible remobilization of heavy metal(loid)s if the observed acidification of AMDS would continue. According to McDonald et al. (2006), the toxic elements in AMDS started leaching when its pH fell below 6.5. Therefore, long-term monitoring of the pH was imperative for mine reclamation using AMDS.

An average reduction of 7.8% in the EC in all the treatments could be observed from the first to the last sampling (Fig. 2). This was because salt was subjected to strong downward leaching in reconstructed topsoil (Olatuyi and Leskiw 2014). As shown in Table S1, the AMDS contained considerable amount of Na. The decrease of EC would clearly facilitate plant growth.

Effects of fertilizers on the physicochemical and microbial properties of AMDS

The application of chemical and organic fertilizers in this study had no significant influence on the total concentrations
Fig. 1 Concentrations of total and extractable heavy metal(loid)s in rhizosphere and bulk AMDS in different fertilizer treatments at different sampling times. Total concentrations of these elements are presented in dot and line charts and extractable concentrations are presented in bar charts. M1, 2.5% manure application and 1 g of chemical fertilizer applied every three months; M2, 5.0% manure application and 2 g of chemical fertilizer applied every three months; M3, 10.0% manure application and 4 g of chemical fertilizer applied every three months. CK, control, no fertilizer application. Different lowercase letters indicate significant differences at $p < 0.05$ level between different fertilizer treatments at each sampling time. Different uppercase letters indicate significant differences at $p < 0.05$ level between different sampling times in each fertilizer treatment. The names of experimental treatments are the same hereinafter.
Likewise, the bioavailability of As, Cd, Cu, and Zn was not consistently affected by the application of fertilizers, except for some minor reductions of extractable Cd, Cu, and Zn at the first two samplings ($p < 0.05$; Fig. 1). This seemed contradictory to the pH declines of AMDS with increasing application rates of fertilizers in this study (Fig. 2), which were supposed to increase metal(loid) mobility. At the last sampling, the pH of the AMDS in the M3 treatment was 0.05-, 0.08-, 0.21-, and 0.04-unit lower than in the control in the rhizosphere of alfalfa, Bermuda grass, and broad-leaved paspalum and bulk AMDS, respectively. This could mainly be attributed to the slightly lower pH of the organic manure than AMDS (Table 1) and the nitrification of the ammonium in the chemical fertilizer (Francioli et al. 2016). Although the extractable heavy metal(loid) concentrations were negatively...
correlated with soil pH, they were strongly positively correlated with their total concentrations in AMDS \((p < 0.05; \text{Fig. } 3)\), which were much higher than those in the manure (Table 1). Therefore, the effect of the pH declines could be offset by the dilution of the metal(loid) pool in AMDS caused by manure addition, leading to the independence between the bioavailability of heavy metal(loid)s and fertilizer application.

Unlike other metal(loid)s, the extractable Fe concentrations in AMDS was significantly reduced by the application of fertilizers \((p < 0.05; \text{Fig. } 1)\). Compared with the control, the extractable Fe concentration was 5.6–28.9% lower in the M3 treatment at the last sampling. Iron was mainly precipitated as amorphous oxides/hydroxides in AMDS, and thus could readily adsorbed onto organic matter, which had strong binding ability to Fe (hydr)oxides (Demers et al. 2017; Bao et al. 2021). The organic manure thus played a key role in mitigating the Fe mobility in AMDS. For similar reasons, the extractable Fe decreased significantly with time in this study \((p < 0.05; \text{Fig. } 1)\), owing to the decreases in the EC of AMDS. This was because the adsorption capacity of organic matter could be impaired by high ionic strength (Bao et al. 2021).

The nutrient conditions of AMDS, including the contents of TOC; total N, P, and K; and extractable K contents, were significantly improved by the applications of chemical and organic fertilizers \((p < 0.05; \text{Fig. } 2)\). However, the contents of \(\text{NH}_4^+\)-N and extractable P in AMDS were extremely low even with the maximum rates of fertilizers in this study. This might be explained by the limited mineralization of the organic N and P in the compost chicken manure used in the present study. According to Dere et al. (2011, 2012), the composting of manure transforms its labile organic compounds into stable humus-like substances, which are less susceptible to rapid mineralization (Dere et al. 2011, 2012).

Another possible explanation for the scarcity of \(\text{NH}_4^+\)-N and bioavailable P was that the bacterial community of AMDS in the present study possessed very low richness

![Pearson's correlation matrix between the edaphic characteristics of AMDS and plant characteristics.](image)
and diversity across all the treatments (Table 2). The mineralization and immobilization of organic N and P is largely driven by soil microbes and is closely related to microbial biomass and community composition (Čapek et al. 2021). In this way, the total N and P contents in AMDS remained almost unchanged (Fig. 2) since the added inorganic N and P in chemical fertilizer was difficult to be immobilized but readily leached out. Microbes are crucial for soil nutrient cycling and plant growth but are usually insufficient in derelict wasteland (Li et al. 2013). Different organic materials had diverse effects on the activity and diversity of soil microbes (Zornoza et al. 2016). In the present study, compost chicken manure obviously did not exert significant effect on the bacterial community in AMDS (Fig. 5). In this post chicken manure showed relatively lower microbial biomass and sustainable nutrient conditions (Hu et al. 2020; Zhu et al. 2021). It might take years to establish a well-functioned microbial community in reclaimed mine soil (Li et al. 2021). Therefore, further study requires a much longer duration than the present one to fully reveal the changes and effects of soil microbes in reclamation.

The activities of all the soil enzymes measured in this study were significantly raised by the applications of chemical and organic fertilizers (p < 0.05; Fig. 4). At the end of the trial, the activities of β-glucosidase, urease, acid, and alkaline phosphatases were averagely 2.7-, 4.9-, 1.3-, and 1.2-fold higher in the M3 treatment than in the control, respectively. The enzyme activities were significantly positively correlated with the total N, P, K and TOC of AMDS (p < 0.05; Fig. 3). These results were in line with Yin et al. (2016) that the enzyme activity of reconstructed soil could be enhanced by improving soil nutrient condition. Furthermore, Li et al. (2018) reported that the enzyme activities in reclaimed mine soils correlated more strongly to microbial abundance than diversity. This might explain the disparate effects of fertilizer application on enzyme activities and bacterial diversity in the present study.

### Plant growth and heavy metal(loid) contents

All the plants in this study, i.e., alfalfa, Bermuda grass, and broad-leaved paspalum survived in the AMDS even without fertilization (Fig. 5). This clearly demonstrated that AMDS could be used as a viable substitute for natural soil in the revegetation of mine waste deposits. The applications of chemical and organic fertilizers further promoted plant growth by increasing their aerial and root biomass by 1- to 66-fold and 1- to 95-fold, respectively, compared with the control. Figure 3 illustrates significant positive correlations between plant biomass and the nutrient contents (i.e., TOC; total N, P, and K; and extractable K) in AMDS, but no correlation could be observed between plant biomass and the total/bioavailable contents of metal(loids). These results provided solid evidence for the view that nutrient deficiency was the main restriction in reclaiming mine waste deposits with reconstructed soil (Chu and Bradshaw 1996; Ye et al. 2002; Wang et al. 2021).

It is noteworthy that plants species showed different growth responses to the treatments of this study (Fig. 5). The biomass of broad-leaved paspalum exhibited a continuous increasing trend over the experimental period, while the biomass of alfalfa peaked at 6 months and the biomass of Bermuda grass peaked at 9 months. The maximum aerial and root biomass of Bermuda grass and broad-leaved paspalum were comparable, which were much higher than those of alfalfa. Broad-leaved paspalum showed rapid growth between the 9-month and 12-month sampling and ultimately possessed the highest aerial biomass among the three plants in the M3 treatment. By contrast, the aerial and root biomass of Bermuda grass declined significantly over the same period (p < 0.05). This discrepancy might be stemmed from their different drought resistance because this period, i.e., from late May to late August, is generally the hottest period of the year (Guangzhou Meteorological Bureau 2020). The different drought resistance between the two plants might

### Table 2 The α-diversity indices of the microbial community in AMDS (mean ± SE, n = 4*)

| No | Time    | Fertilizer | Richness | Chao1  | Dominance | Shannon | Simpson |
|----|---------|------------|----------|--------|-----------|---------|---------|
| 1  | 6 months| CK         | 73.0     | 75.9   | 0.81      | 2.76    | 0.19    |
| 2  | 6 months| M1         | 75.5±3.0 | 78.1±3.3| 0.44±0.11 | 1.59±0.31| 0.56±0.11|
| 3  | 6 months| M2         | 70.7±2.3 | 74.8±1.6| 0.45±0.20 | 1.45±0.56| 0.55±0.20|
| 4  | 6 months| M3         | 73.5±1.6 | 75.9±1.5| 0.42±0.13 | 1.32±0.39 | 0.58±0.13|
| 5  | 12 months| CK         | 73.7±1.7 | 77.1±2.2| 0.45±0.19 | 1.39±0.47 | 0.55±0.19|
| 6  | 12 months| M1         | 74.8±3.1 | 78.3±3.1| 0.35±0.11 | 1.25±0.25 | 0.65±0.11|
| 7  | 12 months| M2         | 72.0±2.0 | 75.1±2.4| 0.69±0.09 | 2.65±0.76 | 0.31±0.09|
| 8  | 12 months| M3         | 72.8±1.8 | 76.6±3.0| 0.75±0.10 | 2.99±0.76 | 0.25±0.10|

*Only one sample in the control met the minimum required DNA concentration for sequencing at the 6-month sampling.
relate to their root morphological traits that broad-leaved paspalum tended to develop thicker roots (Zhou et al. 2014; Li et al. 2017). According to Riaz et al. (2010), drought stress could significantly reduce the aerial biomass of Bermuda grass and hinder its root growth. Nevertheless, broad-leaved paspalum showed the largest disparity in its biomass between the M3 treatment and the control. This indicated that the growth of broad-leaved paspalum relied heavily on fertilizer supplement. By contrast, Bermuda grass, with an only 1.2-fold difference in biomass at most among fertilizer treatments, better adapted to the nutrient-limited condition of AMDS.

The heavy metal(loid) concentrations in the aerial parts and root of alfalfa and broad-leaved paspalum declined significantly with time ($p < 0.05$) but hardly changed in those of Bermuda grass (Figs. 6 and S2). Over the experimental period, the concentrations of As, Cd, Fe, Mn, Zn, and Cu in broad-leaved paspalum decreased by averages of 71.8% and 24.1% in its aerial parts and root, respectively, while the respective decreases in alfalfa averaged 57.1% and 16.7% with a sharp fall from the 3-month to the 6-month sampling. The concentrations of these elements were also mitigated by the applications of chemical and organic fertilizers in the two plants. The “dilution effect” derived from plant biomass

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**Fig. 4** The activities of β-glucosidase, urease, acid, and alkaline phosphatases in rhizosphere and bulk AMDS in different treatments at different sampling times. Different lowercase letters indicate significant differences at $p < 0.05$ level between different fertilizer treatments at each sampling time. Different uppercase letters indicate significant differences at $p < 0.05$ level between different sampling times in each fertilizer treatment.

|          | CK | M1 | M2 | M3 |
|----------|----|----|----|----|
| Alfalfa  |    |    |    |    |
| Bermuda grass |    |    |    |    |
| Broad-leaved paspalum |    |    |    |    |
| Bulk AMDS|    |    |    |    |

|          | 6 month | 12 month |
|----------|---------|----------|
| β-glucosidase |        |          |
| Urease     |        |          |
| Acid phosphatase |    |          |
| Alkaline phosphatase |    |          |

The activities of β-glucosidase, urease, acid, and alkaline phosphatases in rhizosphere and bulk AMDS in different treatments at different sampling times. Different lowercase letters indicate significant differences at $p < 0.05$ level between different fertilizer treatments at each sampling time. Different uppercase letters indicate significant differences at $p < 0.05$ level between different sampling times in each fertilizer treatment.
increases were supposed to be responsible for such decreases in plant metal(loid) concentrations (Reeve et al. 2016). As shown in Fig. 3, the concentrations of these elements in plant aerial parts were significantly negatively correlated with aerial biomass \( (p < 0.05) \), and those in the root of alfalfa and broad-leaved paspalum were also negatively correlated with root biomass \( (p < 0.05; \text{data not shown}) \). Reductions in the concentrations of toxic elements in plant tissues implied lower phytotoxicity and less growth inhibition (Franzaring et al. 2018).

Among the plant species adopted in this study, Bermuda grass generally had the highest metal(loid) concentrations in root but the lowest concentrations in aerial parts, hence possessed the lowest root-to-shoot transfer rates of heavy metal(loid)s (Fig. 6). This signified an effective tolerance mechanism to these toxic elements (Adrees et al. 2015). Such co-tolerance to heavy metal(loid)s was commonly considered as a prerequisite for pioneer plant species used in mine reclamation (Li et al. 2013; Rola et al. 2015). This, along with its superior adaptability to nutrient deficiency mentioned above, suggested that Bermuda grass was a suitable pioneer species for the reclamation of mine solid wastes using AMDS.

**Conclusion**

All the three plants, i.e., alfalfa, Bermuda grass, and broad-leaved paspalum survived in the AMDS, and the application of chemical and organic fertilizers further enhanced their growth. Bermuda grass could be considered as a suitable pioneer species in reclamation because it was more tolerant to toxic heavy metal(loid)s, owing to its lower root-to-shoot transport efficiency of these elements, and less dependent on fertilizer application. The total concentrations of the toxic heavy metal(loid)s remained almost unchanged over the experimental period and was not affected by fertilizer applications. The concentrations of extractable heavy metal(loid)s increased slightly due to minor increases in the pH of AMDS, which was likely to be induced by acid precipitation and related upward movement of heavy metal(loid)s in waste rocks. The total
Fig. 6 Concentrations of As, Fe, and Zn in aerial parts and root of plants with different fertilizer treatments at different sampling times. Different lowercase letters indicate significant differences at $p < 0.05$ level between different fertilizer treatments at each sampling time. Different uppercase letters indicate significant differences at $p < 0.05$ level between different sampling times in each fertilizer treatment. Concentrations of other heavy metals are presented in Fig. S2.
contents of N, P, and K; extractable K; and the activities of related soil enzymes in AMDS were significantly increased by the application of fertilizers and did not vary with time over the experimental period. In this study, the exceptionally low richness and diversity of the bacterial community, together with the poor leachability of the organic manure used limited the mineralization of the organic N and P, leading to undetectable levels of NH₄⁺-N and bioavailable P in AMDS. This should be a crucial factor limiting plant growth in AMDS. Overall, the present study indicated that AMDS with proper amelioration by fertilizers was a viable substitute to natural soil for the reclamation and revegetation of mine solid wastes. Future research should focus on improving the microbial biomass and diversity of AMDS and looking at their changes over time. The necessity of continuously adding fertilizers and the effects of plant litter on the reclamation should also be evaluated in the long term on a field scale.

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**Author contribution** YC designed the research protocol, determined the contents of metal(loids), and wrote the first draft of the manuscript. QL performed sample collection and most of the determination in this study. RZ and MX revised the research protocol and collected experiment materials (including acid mine drainage sludge and waste rocks) for this study. ZY supervised the entire experiment and preparation of the manuscript. All authors commented on the previous version and approved the final version of this manuscript.

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**Data availability** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Declarations**

**Competing interests** The authors declare no competing interests.

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