Evaluation of Acid Mine Drainage Sludge As Soil Substitute For The Reclamation of Mine Solid Wastes

Yihan Chi

Sun Yat-Sen University

Qinru Lin

Sun Yat-Sen University

Rongchuan Zhuang

Sun Yat-Sen University

Mingyu Xiong

Sun Yat-Sen University

Zhihong Ye (✉ Issyzhh@mail.sysu.edu.cn)

Sun Yat-Sen University

Research Article

Keywords: acid mine drainage sludge, soil substitute, mine reclamation, heavy metal(loid), soil fertility, soil microbe

Posted Date: August 24th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-770572/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Environmental Science and Pollution Research on November 9th, 2021. See the published version at <https://doi.org/10.1007/s11356-021-17290-z>.

Abstract

The reclamation of mine waste deposits is imperative but often hindered by the scarcity of natural topsoil. Studies have used various materials to reconstruct topsoil but mainly in coal mines, with scant information for metalliferous mines. Acid mine drainage sludge (AMDS), as a mass-produced waste in metalliferous mines, is a potential topsoil substitute because of its capacity to buffer acidification and support plant growth. In this study, a pot experiment with three plant species was conducted to evaluate the environmental safety and sustainable fertility of AMDS with the amelioration of fertilizers. Results showed that all the plants could survive in the AMDS even without fertilizers. The contents of heavy metal(loid)s were high but remained stable over the experimental period. Improving the AMDS with fertilizers enhanced its nutrient supply by increasing macronutrient contents and activities of soil enzymes, leading to significant increases in plant biomass. The AMDS also sustained its nutrient status throughout the experiment. Nevertheless, the extremely low NH_4^+ -N and bioavailable phosphorus contents made these nutrients hardly accessible to plants, owing to the composting of the organic manure and the low richness and diversity of the bacterial community in the AMDS. Present results suggest that AMDS can be used as a viable and safe substitute for natural soil in reclaiming mine solid wastes but also needs proper improvement, especially for enhancing the microbial richness and diversity.

Introduction

Tailings and waste rocks are produced in large quantities in mining process (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). In China, there were 9656 tailing ponds and 57791 solid waste dumps, and the existing tailings and solid wastes amounted to more than 20 and 60 billion tons, respectively, according to a survey in 2015 (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 (Paster 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 310 thousand hectares, respectively (Wang and Xue, 2017). Reclamation and vegetation of these waste deposits have been regarded as one of the most practical and sustainable ways to solve the aforementioned problems (Karaca et al. 2018). However, the establishment of initial plant cover is often constraint by the scarcity of topsoil in mine areas (Chu and Bradshaw, 1996; Wang et al. 2021). Taking soil from other sites is difficult to implement because of its high cost as well as the restrictions and policies of local governments (Jin and Wu, 2020). Besides, borrowed topsoil varied in quality and usually required further amendment (Chu and Bradshaw, 1996). In this context, materials which possessed similar edaphic properties to natural soil began to be used as topsoil substitutes in the reclamation of mine waste deposits (Gorman et al. 2000; Dayton and Basta, 2001).

Various substances, including organic manure (Larney and Angers, 2012), sedimentary rocks (Wilson-Cokes et al. 2013), soil stripping material (Hu et al. 2020) and solid wastes (Gorman et al. 2000), have been tested and used to reconstruct topsoil in mine areas. Recent studies also evaluated the combinations of multiple materials mixed in different proportions and proposed the optimal proportions for plant growth (Kuang et al. 2019; Wang et al. 2021). Among these materials, industrial solid wastes produced in mines had gained popularity due to lower transportation cost. For instance, fly ash and coal gangue are commonly used in the reclamation of open-pit coal mines (Gorman et al. 2000; Kuang et al. 2019). However, few studies had focused on assessing and selecting suitable mine wastes as soil substitutes for the reclamation and revegetation of metalliferous mines.

In metalliferous mines, acid mine drainage (AMD) is commonly generated when sulfur-rich material is exposed to water and oxygen (Akcil and Koldas, 2006). To minimize its environmental hazards, AMD needs to receive active or passive treatments before it can be discharged (Viadero et al. 2006). The active treatment method has been more commonly adopted, in which the pH of AMD is elevated by alkaline agents such as limestone, hydrated lime and ammonia (McDonald et al. 2006; Mbonimpa et al. 2016). In this process, metal ions in AMD are precipitated as hydroxides and large quantities of sludge (i.e., acid mine drainage sludge, AMDS) were produced, which are usually deposited in tailing ponds and hence cause problems similar to other mine wastes (Viadero et al. 2006; Demers et al. 2017). To avoid its excessive stockpiling, different methods for the disposal and recycle of AMDS had been developed in the past two decades. These included recovering the valuable elements and compounds, such as metals and ochre, from AMDS and reusing it in wastewater treatment, preparing building materials, and inhibiting AMD generation (Sibrell et al. 2009; Demers et al. 2015a; Liu et al. 2019). However, most of these methods have not been widely accepted due to the uncertainty of their future returns, high cost or possibility of secondary pollution (Rakotonimaro et al. 2017). According to previous findings, AMDS can maintain alkaline to neutral pH in tailing ponds for decades and could support plant growth (Demers et al. 2015a,b; Mbonimpa et al. 2016). Although studies had repeatedly suggested the utilization of AMDS in mine reclamation (Demers et al. 2015a,b and 2017), information is still scant about whether it can be used as topsoil substitute in the revegetation of mine waste deposits. Little is known about the leaching behavior of the toxic metal(loid)s in AMDS during reclamation, especially when it is enriched with organic amendments, which is almost necessary in mine reclamation (Pardo et al. 2014). The validation of this strategy can fill the gap and provide viable solutions for both the reclamation of mine waste deposits and the disposal of AMDS.

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

2.1. 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 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 wettsteinii* Hack.), were planted in nylon rhizo-bags and placed in plastic pots. All the pots containing 5-cm layer of waste rocks at the bottom were filled with 3 kg of dewatered AMDS collected from Zijinshan Copper Mine, China (Table 1). Compost chicken manure (as organic fertilizer; Table 1) was mixed with the AMDS at rates of 0 (CK), 2.5% (M1), 5.0% (M2) and 10.0% (M3) in different treatments, respectively, before planting. Chemical fertilizer (N:P₂O₅:K₂O = 15:15:15) was added to the pots at doses of 0 (CK), 1.0 (M1), 2.0 (M2) and 4.0 (M3) g pot⁻¹, respectively, in different treatments every three months. All the treatments were performed in four replicates. Plants were watered daily using an automated sprinkler system.

Plant and AMDS samples were collected every three months. Plant samples were divided into aerial parts and roots, oven-dried at 60°C for 72 h and milled into fine powder. Rhizosphere and bulk AMDS samples were collected from rhizobags and outside bags, respectively. A portion of each AMDS sample was directly stored at -20°C for microbial analyses. The rest of these samples were air-dried and ground to pass a 2-mm sieves for determining the physicochemical properties and contents of elements of AMDS.

2.2. 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 HSO₄-HClO₄ (Zheng, 2012). Bioavailable P and potassium (K) were extracted using the Mehlich 3 extractant (Mehlich, 1984). Exchangeable cadmium (Cd), iron (Fe), manganese (Mn), 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, 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%.

2.3. Microbial analyses

The activities of β -glucosidase, acid and alkaline phosphatases in AMDS were determined following the method of Tabatabai (1994). The activity of urease was measured following the method of Guan (1986). To investigate the bacterial community characteristics of AMDS, the total DNA in bulk and rhizosphere AMDS of Bermuda grass 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 sequenced 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. 2013). 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).

2.4. 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 3.6.0) and figures were created using the ggplot2 package (Version 3.3.5) in R software (R Core Team, 2020).

Results

3.1. Plant biomass

In this study, the aerial and root biomass of alfalfa, Bermuda grass and broad-leaved paspalum increased significantly by the applications of chemical and organic fertilizers ($p < 0.05$; Fig. 1). Higher fertilizer application rates generally had better increasing effects on plant biomass, but the effects were less significant in root biomass. At the 12-month sampling, the aerial biomass of alfalfa, Bermuda grass and broad-leaved paspalum in the M3 treatment (with 4.0g of chemical fertilizer applied every three months and 10.0% manure application) were 4.10-, 1.16- and 65.91-fold higher than in the control, respectively, and the respective root biomass in the M3 treatment were 0.76-, 1.21- and 95.05-fold higher than in the control. Fertilizers had apparently greater increasing effects on the biomass of broad-leaved paspalum at the last sampling, despite that they had greater increasing effects in Bermuda grass at the first two samplings. Plant aerial and root biomass in all the treatments increased significantly with time ($p < 0.05$; Fig. 1). The biomass of broad-leaved paspalum showed a continuous increasing trend over the

experimental period, whilst the biomass of alfalfa peaked at six months and the biomass of Bermuda grass peaked at nine months.

3.2. Heavy metal(loid)s in plants

The applications of fertilizers significantly decreased the concentrations of heavy metal(loid)s in the aerial parts and root in alfalfa and broad-leaved paspalum ($p < 0.05$; Figs. 2 and S2). At the last sampling in the M3 treatment, the As, Cd, Cu, Fe, Mn and Zn concentrations in the aerial parts of alfalfa were 50.8%-75.9% lower than in the control, and the concentrations of these elements in its root were 78.0%-82.2% lower than in the control. Meanwhile, the M3 treatment decreased the As, Cd, Cu, Fe, Mn and Zn concentrations of broad-leaved paspalum by 35.1%-55.2% and 54.0%-73.2% in the aerial parts and roots, respectively. By contrast, the fertilizers had significant reducing effects on the heavy metal(loid) concentrations of Bermuda grass only at the first sampling ($p < 0.05$; Figs. 2 and S2). Plant heavy metal(loid) concentrations declined significantly with time in alfalfa and broad-leaved paspalum ($p < 0.05$) but remained stable in Bermuda grass (Figs. 2 and S2). From the first to the last sampling, the aerial heavy metal(loid) concentrations of alfalfa and broad-leaved paspalum decreased averagely by 57.1% and 71.8%, respectively, whilst their root heavy metal(loid)s decreased averagely by 16.7% and 24.1%, respectively. Over the experimental period, the heavy metal(loid) concentrations in broad-leaved paspalum exhibited a gradual falloff, while those in alfalfa dropped sharply from the 3-month to the 6-month sampling and fluctuated afterwards.

3.3. Chemical properties of the AMDS

In this study, the pH of AMDS was slightly but significantly reduced by the applications of chemical and organic fertilizers ($p < 0.05$; Fig. 3). 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. The pH of AMDS also declined significantly with time over the experimental period ($p < 0.05$). Compared with that at the first sampling, the pH of the AMDS in all the treatment was 0.09- to 0.25-unit lower at the last sampling. Similarly, there were also minor but significant decreases over time in the EC of AMDS ($p < 0.05$; Fig. 3). An average reduction of 7.8% in the EC in all the treatments could be observed from the first to the last sampling. Fertilizers generally had no significant influence on the EC of AMDS.

The applications of fertilizers significantly increased the TOC, total N, P and K and extractable K contents in AMDS (Fig. 3; $p < 0.05$). Compared with the control, the M3 treatment raised the TOC, total N, P and K and extractable K in rhizosphere and bulk AMDS by 6.27-, 2.14-, 2.34-, 9.11- and 1.45-fold on average, respectively. NH_4^+ -N and extractable P contents were undetectable in AMDS across all the treatments. Over the experimental period, most of these nutrient indices remained steady. However, compared with the first sampling, rhizosphere TOC in the M2 (with 2.0g of chemical fertilizer applied every three months and 5.0% manure application) and M3 treatments was significantly higher at the 12-month sampling in broad-leaved paspalum. Meanwhile, total N content increased by an average of 101.5% from the 6-month

to the 12-month sampling in the rhizosphere of alfalfa. Over the same period, total P in the rhizosphere of broad-leaved paspalum increased by an average of 32.1% and extractable K in the rhizosphere of Bermuda rose by an average of 158.5%.

The total concentrations of As, Cd, Cu, Fe, Mn and Zn in AMDS were not significantly affected by fertilizer application, compared with the control treatment (Fig. 4). The concentrations of the extractable Cd, Cu, Fe, Mn and Zn were decreased by fertilizer applications, but the effects appeared to be significant only at the first two samplings ($p < 0.05$; Fig. 4). The total concentrations of all the heavy metal(loid)s tested in this study remained stable in the rhizosphere but fluctuated in bulk AMDS over the experimental period. Meanwhile, there were minor increases in the extractable concentrations of As, Cd, Cu and Zn with time ($p < 0.05$). 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. By contrast, the extractable Fe and Mn declined by 34.6% and 40.0%, respectively, from the first to the last sampling.

3.4. Microbiological properties of the AMDS

In this study, the activities of β -glucosidase, urease, acid and alkaline phosphatases in AMDS were significantly raised by the applications of chemical and organic fertilizers ($p < 0.05$, Fig. 5). Compared with those in the control, the activities of β -glucosidase, urease, acid and alkaline phosphatases were averagely 2.69-, 4.90-, 1.31- and 1.18-fold higher in the M3 treatment. In most treatments, the activities of these enzymes stayed steady from the 6-month to the 12-month sampling. However, the activities of β -glucosidase and phosphatases increased significantly in the control treatment over the experimental period ($p < 0.05$). The bacterial community in AMDS exhibited very low richness and diversity (Table 2). The application of fertilizers did not have significant effects on the species richness or the diversity of the bacterial community, which also did not change with time during the experimental period.

Discussion

The present study clearly indicated that AMDS could be used as a viable substitute for natural soil in the revegetation and reclamation of mine waste deposits. All the plants, including alfalfa, Bermuda grass and broad-leaved paspalum survived in the AMDS, especially when fertilizers were applied (Fig. 1). Studies have validated the potential of various solid waste materials as soil substitutes, including fly ash (Ram et al. 2006), slate powder (Paradela et al. 2008) and weathered brown sandstone (Wilson-Kokes et al. 2013), in mine reclamation, but most of them focused on improving the originally insufficient or barren topsoil. In these cases, solid wastes were combined with other substrates and typically made up 10%-50% of the reconstructed topsoil (Zhu et al. 2021). Compared with previous research, present results proposed a more effective disposal method of AMDS and a feasible way to establish vegetation on mine tailings and waste rocks with no original soil. More importantly, the total concentrations of heavy metal(loid)s in AMDS did not exhibit significant decreases in the present study (Fig. 4). The toxic elements showed a low tendency to be released from AMDS at least in the short term (e.g., one year) and were hence unlikely to pose threats to neighboring environment. Higher pH and solid content of AMDS played vital roles in

maintaining a low leachability of its toxic elements (Zinck et al. 1997; Rakotonimaro et al. 2017). Therefore, the stability of the heavy metal(loid)s in the AMDS used in the present study could be attributed to its slightly alkaline pH and early dewatering. Besides, significant decreases in the EC of the AMDS were detected in this study (Fig. 3). This was because salt was subjected to strong downward leaching in reconstructed topsoil, which would clearly facilitate plant growth (Olatuyi and Leskiw, 2014). However, it should be noted that the pH of AMDS displayed minor declines with time over the experimental period (Fig. 3), whilst the concentrations of bioavailable As, Cd, Cu and Zn in AMDS increased slightly over the same period (Fig. 4). The pH decrease could result from the local acid precipitation with an average pH of 4.38 (Qin et al. 2006). Local abundant rainfall might have also promoted sulfide oxidation and acidic-leachate generation in the waste rocks at the bottom (Kossoff et al. 2014). The pH of AMDS was negatively correlated with the extractable As, Cd and Cu concentrations in the present study (Fig. 5). These together implied possible remobilization of heavy metal(loid)s if the acidification of AMDS would continue. According to McDonald et al. (2006), the neutralization potential of AMDS against acidification was dominated by its mineral composition, and its toxic elements started leaching when pH fell below 6.5. Therefore, long-term monitoring of the pH was imperative for mine reclamation using AMDS.

In the present study, the application of chemical and organic fertilizers clearly improved the nutrient conditions of AMDS and thus enhanced plant growth (Figs. 1 and 3). Plant aerial and root biomass were positively correlated with the contents of TOC, total N, P and K and extractable K in AMDS (Fig. 6). 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). Diverse organic amendments, such as biosolids, livestock manure and crop residues were used in soil rehabilitation to improve soil nutrient status (especially the organic carbon), microbial biomass and activity, and ameliorate soil physicochemical properties (Larney and Angers, 2012). However, the contents of NH_4^+ -N and bioavailable P in AMDS were extremely low, albeit with the application of fertilizers. This could be partly ascribed to the limited mineralization of organic N and P in compost manure in which labile organic compounds had been transformed into stable humus-like substances (Dere et al. 2011, 2012). 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 (Capek et al. 2021). In this study, the bacterial community of AMDS possessed very low richness and diversity across all the treatments (Table 2), which should be largely responsible for the scarcity of NH_4^+ -N and bioavailable P in AMDS. This also explained the almost unchanged total N and P contents in AMDS (Fig. 3) since the added inorganic N and P in chemical fertilizer was difficult to be immobilized but readily leached out. Microbes were crucial for soil nutrient cycling and plant growth but were 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, although it increased the activities of soil enzymes related to nutrient cycling (Fig. 5). In this case, microbial agent needed to be applied to improve soil microbial biomass and sustainable nutrient conditions (Hu et al. 2020; Zhu et al. 2021).

Organic amendments, depending upon their nature, might have positive or negative effects on metal(loid) bioavailability by changing soil pH and redox potential as well as forming chelates with metal(loid)s (Walker et al. 2004; Pardo et al. 2014). In our present study, the pH of AMDS decreased mildly with increasing application rates of organic manure (Fig. 3), owing to the slightly lower pH of the organic manure than AMDS (Table 1). The nitrification of the ammonium in the chemical fertilizer might also contribute to the pH decrease (Francioli et al. 2016). However, the bioavailability of As, Cd, Cu and Zn was reduced, rather than raised by higher doses of fertilizers (Fig. 4). Since the extractable concentrations of As, Cd, Cu and Zn were significantly positively correlated with their total concentrations in AMDS ($p < 0.05$; Fig. 6), which were much lower in the organic manure (Table 1), it could be inferred that organic manure application diluted the metal(loid) pool of AMDS and reduced bioavailable metal(loid) contents. Furthermore, the application of chemical and organic fertilizers mitigated the accumulation of heavy metal(loid)s in plant aerial parts and root in this study, especially in alfalfa and broad-leaved paspalum (Fig. 2). This was likely to be caused not only by the immobilization of toxic metal(loid)s, but also by the “dilution effect” of plant biomass increases (Reeve et al. 2016). In our present study, significant negative correlations were observed between plant aerial biomass and aerial metal(loid) concentrations ($p < 0.05$; Fig. 6). Reductions in the concentrations of toxic elements in plant tissues meant lower phytotoxicity and less growth inhibition (Franzaring et al. 2018).

In the present study, different plants showed distinct growth responses to the environmental conditions of AMDS (Fig. 1). Alfalfa was the first to reach its maximum aerial and root biomass at the 6-month sampling, which were much lower than the maximum biomass of the other plants. The aerial biomass of broad-leaved paspalum outcompeted the other plants at the last sampling in the M3 treatment. However, its growth relied heavily on fertilizer supplement, which exhibited the largest disparity between the M3 treatment and the control. By contrast, the biomass of Bermuda grass differed by only 1.24-fold at most among different treatments, which was superior or at least comparable to that of broad-leaved paspalum at their peaks. These implied that Bermuda grass could be proposed as a more suitable pioneer species in the reclamation of mine waste deposits using AMDS. Previous studies revealed that pioneer species in mine reclamation commonly possessed co-tolerance to heavy metals (Li et al. 2013; Rola et al. 2015). In our present study, Bermuda grass had the lowest root-to-shoot transfer rates of heavy metal(loid)s among the three plants (Fig. 2). This signified an effective tolerance mechanism to these toxic elements (Adrees et al. 2015). Legumes (e.g., alfalfa) were often suggested in mine reclamation due to their N fixation ability (Ye et al. 2001; Zhu et al. 2021). However, the total N content in the rhizosphere of alfalfa was not significantly higher than those in the rhizosphere of the other plants in the present study, although it increased from the 6-month to the 12-month sampling (Fig. 3).

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 revegetation as it was more tolerant to toxic heavy metal(loid)s and less dependent on fertilizer application. The total concentrations of the toxic heavy

metal(loid)s remained stable 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 required long-term monitoring. The total 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 $\text{NH}_4^+\text{-N}$ 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 using cost-effective agents and employing this strategy on a field scale.

Declarations

- **Ethics approval and consent to participate**

Not applicable

- **Consent for publication**

Not applicable

- **Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

- **Competing interests**

The authors declare that they have no competing interests.

- **Funding**

This work was supported by the open fund of the State Key Laboratory of Comprehensive Utilization of Low-Grade Refractory Gold Ores.

- **Authors' contributions**

YC designed the research protocol, determined the contents of metal(loid)s 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.

Acknowledgements

Funding: This work was supported by the open fund of the State Key Laboratory of Comprehensive Utilization of Low-Grade Refractory Gold Ores.

References

1. Adrees M, Ali S, Rizwan M, Zia-ur-Rehman M, Ibrahim M, Abbas F, Farid M, Qayyum MF et al (2015) Mechanisms of Silicon-Mediated Alleviation of Heavy Metal Toxicity in Plants: A Review. *Ecotoxicol Environ Saf* 119:186–197
2. Akcil A, Koldas S (2006) Acid Mine Drainage (AMD): Causes, Treatment and Case Studies. *J Clean Prod* 14:1139–1145
3. Čapek P, Choma M, Tahovská K, Kaňa J, Kopáček J, Šantrůčková H (2021) Coupling the Resource Stoichiometry and Microbial Biomass Turnover to Predict Nutrient Mineralization and Immobilization in Soil. *Geoderma* 385:114884
4. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG et al (2010) QIIME Allows Analysis of High-Throughput Community Sequencing Data. *Nat Methods* 7:335–336
5. Chu LM, Bradshaw AD (1996) The Value of Pulverized Refuse Fines (PRF) as a Substitute for Topsoil in Land Reclamation. I. Field Studies. *The Journal of Applied Ecology* 33:851
6. Dayton EA, Basta NT (2001) Characterization of Drinking Water Treatment Residuals for Use as a Soil Substitute. *Water Environ Res* 73:52–57
7. Demers I, Bouda M, Mbonimpa M, Benzaazoua M, Bois D, Gagnon M (2015) Valorization of Acid Mine Drainage Treatment Sludge as Remediation Component to Control Acid Generation from Mine Wastes, Part 2: Field Experimentation. *Miner Eng* 76:117–125
8. Demers I, Benzaazoua M, Mbonimpa M, Bouda M, Bois D, Gagnon M (2015) Valorisation of Acid Mine Drainage Treatment Sludge as Remediation Component to Control Acid Generation from Mine Wastes, Part 1: Material Characterization and Laboratory Kinetic Testing. *Miner Eng* 76:109–116
9. Demers I, Mbonimpa M, Benzaazoua M, Bouda M, Awoh S, Lortie S, Gagnon M (2017) Use of Acid Mine Drainage Treatment Sludge by Combination with a Natural Soil as an Oxygen Barrier Cover for Mine Waste Reclamation: Laboratory Column Tests and Intermediate Scale Field Tests. *Miner Eng* 107:43–52
10. Dere AL, Stehouwer RC, McDonald KE (2011) Nutrient Leaching and Switchgrass Growth in Mine Soil Columns Amended with Poultry Manure. *Soil Sci* 176:84–90
11. Dere AL, Stehouwer RC, Aboukila E, McDonald KE (2012) Nutrient Leaching and Soil Retention in Mined Land Reclaimed with Stabilized Manure. *J Environ Qual* 41:2001–2008
12. Edgar RC (2013) UPARSE: Highly Accurate OTU Sequences from Microbial Amplicon Reads. *Nat Methods* 10:996–998

13. Francioli D, Schulz E, Lentendu G, Wubet T, Buscot F, Reitz T (2016) Mineral vs. Organic Amendments: Microbial Community Structure, Activity and Abundance of Agriculturally Relevant Microbes Are Driven by Long-Term Fertilization Strategies. *Front Microbiol* 7:1–16
14. Franzaring J, Ancora S, Paoli L, Fongoh AH, Büttner P, Fangmeier A, Schlosser S, Monaci F (2018) Phytotoxicity of Polymetallic Mine Wastes from Southern Tuscany and Saxony. *Ecotoxicol Environ Saf* 162:505–513
15. Gorman JM, Sencindiver JC, Horvath DJ, Singh RN, Keefer RF (2000) Erodibility of Fly Ash Used as a Topsoil Substitute in Mineland Reclamation. *J Environ Qual* 29:805–811
16. Guan SY (1986) *Soil Enzymes and Their Research Methodology*. Agriculture Press, Beijing, pp 274–339 (in Chinese)
17. Heshmati GA, Pessarakli M (2011) Threshold Model in Studies of Ecological Recovery in Bermudagrass (*Cynodon dactylon* L.) under Nutrient Stress Conditions. *J Plant Nutr* 34:2183–2192
18. Hu Z, Zhu Q, Liu X, Li Y (2020) Preparation of Topsoil Alternatives for Open-Pit Coal Mines in the Hulunbuir Grassland Area, China. *Appl Soil Ecol* 147:103431
19. Jin XT, Wu TT (2020) Application of Free-pass Soil Spraying Technology in Mine Dump. *World Nonferrous Metals* 20:191–193 (in Chinese)
20. Karaca O, Cameselle C, Reddy KR (2018) Mine Tailing Disposal Sites: Contamination Problems, Remedial Options and Phytocaps for Sustainable Remediation. *Rev Environ Sci Biotechnol* 17:205–228
21. Keeney DR, Nelson DW (1982) Nitrogen–inorganic forms. In: Page AL, Miller RH, Keeney DR (Eds.), *Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties*, 2nd ed., Agronomy, No. 9. ASA, SSSA, Madison, pp 643–698
22. Kossoff D, Dubbin WE, Alfredsson M, Edwards SJ, Macklin MG, Hudson-Edwards KA (2014) Mine Tailings Dams: Characteristics, Failure, Environmental Impacts, and Remediation. *Appl Geochem* 51:229–245
23. Kuang X, Cao Y, Luo G, Huang Y (2019) Responses of *Melilotus Officinalis* Growth to the Composition of Different Topsoil Substitute Materials in the Reclamation of Open-Pit Mining Grassland Area in Inner Mongolia. *Materials* 12:3888
24. Larney FJ, Angers DA (2012) The Role of Organic Amendments in Soil Reclamation: A Review. *Can J Soil Sci* 92:19–38
25. Li S, Di X, Wu D, Zhang J (2013) Effects of Sewage Sludge and Nitrogen Fertilizer on Herbage Growth and Soil Fertility Improvement in Restoration of the Abandoned Opencast Mining Areas in Shanxi, China. *Environ Earth Sci* 70:3323–3333
26. Liu M, Iizuka A, Shibata E (2019) Acid Mine Drainage Sludge as an Alternative Raw Material for M-Type Hexaferrite Preparation. *J Clean Prod* 224:284–291
27. Mbonimpa M, Bouda M, Demers I, Benzaazoua M, Bois D, Gagnon M (2016) Preliminary Geotechnical Assessment of the Potential Use of Mixtures of Soil and Acid Mine Drainage

- Neutralization Sludge as Materials for the Moisture Retention Layer of Covers with Capillary Barrier Effects. *Can Geotech J* 53:828–838
28. McDonald DM, Webb JA, Taylor J (2006) Chemical Stability of Acid Rock Drainage Treatment Sludge and Implications for Sludge Management, 40. *Environmental Science & Technology*, pp 1984–1990
 29. Mehlich A (1984) Mehlich 3 Soil Test Extractant: A Modification of Mehlich 2 Extractant. *Commun Soil Sci Plant Anal* 15:1409–1416
 30. Olatuyi SO, Leskiw LA (2014) Long-Term Changes in Soil Salinity as Influenced by Subsoil Thickness in a Reclaimed Coal Mine in East-Central Alberta. *Can J Soil Sci* 94:605–620
 31. Owen JR, Kemp D, Lèbre É, Svobodova K, Pérez Murillo G (2020) Catastrophic Tailings Dam Failures and Disaster Risk Disclosure. *International Journal of Disaster Risk Reduction* 42:101361
 32. Palansooriya KN, Shaheen SM, Chen SS, Tsang DCW, Hashimoto Y, Hou D, Bolan NS, Rinklebe J et al (2020) Soil Amendments for Immobilization of Potentially Toxic Elements in Contaminated Soils: A Critical Review. *Environ Int* 134:105046
 33. Paradelo R, Moldes AB, Barral MT (2008) Characterization of Slate Processing Fines According to Parameters of Relevance for Mine Spoil Reclamation. *Appl Clay Sci* 41:172–180
 34. Pardo T, Bernal MP, Clemente R (2014) Efficiency of Soil Organic and Inorganic Amendments on the Remediation of a Contaminated Mine Soil: I. Effects on Trace Elements and Nutrients Solubility and Leaching Risk. *Chemosphere* 107:121–128
 35. Park I, Tabelin CB, Jeon S, Li X, Seno K, Ito M, Hiroyoshi N (2019) A Review of Recent Strategies for Acid Mine Drainage Prevention and Mine Tailings Recycling. *Chemosphere*, 219:588–606
 36. Pastor M, Quecedo M, Fernández Merodo JA, Herreros MI, González E, Mira P (2002) Modelling Tailings Dams and Mine Waste Dumps Failures. *Géotechnique*, 52:579–591
 37. Qin P, Du YD, Liu JL, Song LL, Liu AJ, Wang QQ (2006) Distribution Pattern and Influencing factors of the acid rainfall in Guangdong Province, China. *Journal of Tropical Meteorology* 22:297–300 (in Chinese)
 38. Quast C, Priesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2012) The SILVA Ribosomal RNA Gene Database Project: Improved Data Processing and Web-Based Tools. *Nucleic Acids Res* 41:590–596
 39. R Core Team (2020) R: A Language and Environment for statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>
 40. Rakotonimaro TV, Neculita CM, Bussière B, Benzaazoua M, Zagury GJ (2017) Recovery and Reuse of Sludge from Active and Passive Treatment of Mine Drainage-Impacted Waters: A Review. *Environ Sci Pollut Res* 24:73–91
 41. Ram LC, Srivastava NK, Tripathi RC, Jha SK, Sinha AK, Singh G, Manoharan V (2006) Management of Mine Spoil for Crop Productivity with Lignite Fly Ash and Biological Amendments. *J Environ Manage* 79:173–187

42. Reeve JR, Hoagland LA, Villalba JJ, Carr PM, Atucha A, Cambardella C, Davis DR, Delate K (2016) Organic Farming, Soil Health, and Food Quality: Considering Possible Links, in: Advances in Agronomy. Elsevier Inc., pp 319–367
43. Rola K, Osyczka P, Nobis M, Drozd P (2015) How Do Soil Factors Determine Vegetation Structure and Species Richness in Post-Smelting Dumps? *Ecol Eng* 75:332–342
44. Sibrell PL, Montgomery GA, Ritenour KL, Tucker TW (2009) Removal of Phosphorus from Agricultural Wastewaters Using Adsorption Media Prepared from Acid Mine Drainage Sludge. *Water Res* 43:2240–2250
45. Tabatabai MA (1994) Soil enzymes. In: Weaver RW, Angel JS, Bottomley PS (eds) *Methods of Soil Analysis. Part 2, Book Series 5*. Soil Science Society of America, Madison, pp 775–833
46. Viadero RC, Wei X, Buzby KM (2006) Characterization and Dewatering Evaluation of Acid Mine Drainage Sludge from Ammonia Neutralization. *Environ Eng Sci* 23:734–743
47. Walker DJ, Clemente R, Bernal MP (2004) Contrasting Effects of Manure and Compost on Soil pH, Heavy Metal Availability and Growth of *Chenopodium Album* L. in a Soil Contaminated by Pyritic Mine Waste. *Chemosphere*, 57:215–224
48. Walkley A, Black IA (1934) An Examination of the Degtjareff Method for Determining Soil Organic Matter and A Proposed Modification of The Chromic Acid Titration Method. *Soil Sci* 37:29–38
49. Wang HJ, Xue YZ (2017) Situation Analysis on Conservation and Comprehensive Utilization of Mineral Resources in China. *Conservation Utilization of Mineral Resources* 2:1–12 (in Chinese)
50. Wang L, Wang F, Wang S, Huang Y, Zhang Z, Bai Z, Cao Y (2021) Analysis of Differences in Chemical Properties of Reconstructed Soil under Different Proportions of Topsoil Substitute Materials. *Environ Sci Pollut Res* 28:31230–31245
51. Wilson-Kokes L, Emerson P, DeLong C, Thomas C, Skousen J (2013) Hardwood Tree Growth after Eight Years on Brown and Gray Mine Soils in West Virginia. *J Environ Qual* 42:1353–1362
52. Ye ZH, Wong JWC, Wong MH, Baker AJM, Shu WS, Lan CY (2000) Revegetation of Pb/Zn Mine Tailings, Guangdong Province, China. *Restor Ecol* 8:87–92
53. Ye ZH, Yang Z, Chan GY, Wong MH (2001) Growth Response of *Sesbania Rostrata* and *S. Cannabina* to Sludge-Amended Lead/Zinc Mine Tailings. *Environ Int* 26:449–455
54. Ye ZH, Shu WS, Zhang ZQ, Lan CY, Wong MH (2002) Evaluation of Major Constraints to Revegetation of Lead/Zinc Mine Tailings Using Bioassay Techniques. *Chemosphere* 47:1103–1111
55. Zheng R-L, Cai C, Liang J-H, Huang Q, Chen Z, Huang Y-Z, Arp HPH, Sun G-X (2012) The Effects of Biochars from Rice Residue on the Formation of Iron Plaque and the Accumulation of Cd, Zn, Pb, As in Rice (*Oryza sativa* L.) Seedlings. *Chemosphere* 89:856–862
56. Zhu Q, Hu Z, Liu X, Wu Y (2021) Topsoil Alternatives Selection for Surface Coal-Mined Land Reclamation in Inner Mongolia, China: An Experimental Study. *Int J Min Reclam Environ* 35:421–434
57. Zinck JM, Wilson LJ, Chen TT, Griffith W, Mikhail S, Turcotte S (1997) Characterization and Stability of Acid Mine Drainage Sludges. MEND Report, 3.42.2a, 319. <http://mend-nedem.org/mend->

58. Zornoza R, Acosta JA, Faz A, Bååth E (2016) Microbial Growth and Community Structure in Acid Mine Soils after Addition of Different Amendments for Soil Reclamation. *Geoderma* 272:64–72

Tables

Table 1 The physicochemical properties of the organic manure and AMDS used in this study (mean $\pm$ SE, n = 6)

	AMDS	Organic manure
Bulk density (g cm ⁻³)	0.88 $\pm$ 0.02	
Field capacity (g kg ⁻¹)	446 $\pm$ 20	
EC (mS cm ⁻¹)	3.03 $\pm$ 0.02	
pH	8.23 $\pm$ 0.02	8.01 $\pm$ 0.02
TOC (g kg ⁻¹)	3.18 $\pm$ 0.34	
N (g kg ⁻¹)	0.32 $\pm$ 0.02	6.44 $\pm$ 0.17
P (g kg ⁻¹)	0.28 $\pm$ 0.01	4.13 $\pm$ 0.05
K (g kg ⁻¹)	0.17 $\pm$ 0.01	5.91 $\pm$ 0.62
As (mg kg ⁻¹)	416 $\pm$ 9	14 $\pm$ 5
Cd (mg kg ⁻¹)	26.3 $\pm$ 0.2	1.8 $\pm$ 0.0
Cu (mg kg ⁻¹)	1172 $\pm$ 19	44 $\pm$ 1
Fe (g kg ⁻¹)	81.7 $\pm$ 0.6	25.0 $\pm$ 0.6
Mn (mg kg ⁻¹)	561 $\pm$ 4	518 $\pm$ 7

Note: EC, electrical conductivity; TOC, total organic carbon.

Table 2 The α -diversity indices of the microbial community in AMDS (mean $\pm$ 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.

Note: 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.

Figures

Figure 1

Plant aerial and root biomass in different fertilizer treatments at different sampling times. 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 lower-case letters indicate significant differences at $p < 0.05$ level between different fertilizer treatments at each sampling time. Different upper-case letters indicate significant differences at $p < 0.05$ level between different sampling times in each fertilizer treatment.

Figure 2

Concentrations of As, Fe and Zn in aerial parts and root of plants with different fertilizer treatments at different sampling times. 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 lower-case letters indicate significant differences at p < 0.05 level between

different fertilizer treatments at each sampling time. Different upper-case 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.

Figure 3

The pH, EC, contents of TOC, extractable K and total N, P and K of AMDS in different fertilizer treatments at different sampling times. 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 lower-case letters indicate significant differences at $p < 0.05$ level between different fertilizer treatments at each sampling time. Different upper-case letters indicate significant differences at $p < 0.05$ level between different sampling times in each fertilizer treatment. TOC, total organic carbon; EC, electrical conductivity.

Figure 4

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 lower-case letters indicate significant differences at $p < 0.05$ level between different fertilizer treatments at each sampling time. Different upper-case letters indicate significant differences at $p < 0.05$ level between different sampling times in each fertilizer treatment. Concentrations of total and extractable Mn is presented in Fig. S2.

Figure 5

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

Figure 6

Pearson's correlation matrix between the edaphic characteristics of AMDS and plant characteristics. Except for the correlations marked with "x", which indicates that these correlations were not significant, all other correlations were significant at $p < 0.05$ level. The color and size of the circles are proportional to the correlation coefficients. The prefix letters "t" and "ex" denote the total and extractable concentration of elements in soil, respectively. The prefix letters "ar" and "rt" denote the aerial and root of plant, respectively. GLU, URE, ACP and ALP stand for the activities of β -glucosidase, urease, acid phosphatase and alkaline phosphatase, respectively. BM, biomass; EC, electrical conductivity; TOC, total organic carbon.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterial.docx](#)