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Development of a site-specific system for the rehabilitation of a former copper mine, Sto. Niño, Philippines

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Abstract

Sto. Niño is a legacy mine site located in the Tublay municipality of Benguet District in northern Luzon, Philippines that was closed and abandoned in 1982. The site comprises a former open pit and block caving operation together with rock waste dumps and a tailings storage facility that have had no formal rehabilitation but where local people are now living and farming. The GCBC DEFRA funded Bio+Mine project commenced in 2022 with the aims of providing an in-depth audit of the abandoned site in terms of geological, hydrological, ecological, and social parameters and to co-design nature and people positive interventions for the regeneration of the mine site together with local indigenous communities.

Remote sensing, using historic and current satellite data, and new drone deployments using multispectral and lidar cameras were used to develop a baseline assessment of the site. A range of geological and surface water samples were also collected across the legacy mine area, tailings storage facility and the former underground block-caving area where artisanal and small-scale mining operations remain active. Samples were analysed for both their physico-chemical and metagenomic characteristics. Plants on the site were also studied and proved to be an ideal 'natural laboratory' for the audit of endemic heavy metal hyperaccumulator plants and invertebrate bioindicators like earthworms. DNA metagenomic sequencing of microbiomes using water, soil and plant root samples was undertaken as well as water from streams, waste dumps and seepages.

Initial findings suggest that Cu and Zn are the only highly elevated trace elements in ground water; levels of As, Cd, Cr and Mo are generally low. However, only two sites yielded water quality potential for domestic use, therefore active biological treatment options are being scoped using metabolic activity of locally naturally occurring bacteria to concurrently (i) increase the water pH, (ii) remove metals, such as Cu, Al, Zn and Mn and (iii) reduce sulphate concentrations. Several endemic plant species were found to exhibit phytostabilization affinities toward Ni, Zn and Mn and onsite use of these will be investigated. Earthworms were found to be more abundant and diverse in a site where locals had engineered an agricultural plot so use of these as indicators of soil health will be further explored.

Microbiome DNA sequencing will continue to be used to evaluate the impact of mine waste on soil biodiversity in land used for agriculture. These data will also be used for the identification of microorganisms contributing to acid mine drainage but also identify new and native bacteria and fungi that have biotechnological potential and application in biological treatment of water. The soil microbiome sequencing data will help to assess the impact of hazardous metals on soil biodiversity, health and agriculture but also identify potentially beneficial microorganisms to enhance the efficacy of hyperaccumulating plants to remove contaminants from soils.

Keywords: *mine rehabilitation, site-specific system, monitoring ecosystem, water quality, social engagement*

1 Introduction

The legacy of historic mining has often resulted in areas that have physico-chemical properties that are unable to support sustainable post-mining activities (Bradshaw 2000). It is estimated that the USA alone has more than 600,000 closed mine sites, many with environmental legacy issues (Worrall et al. 2009). Ecosystem services in post mining landscapes should be reconstructed and designed to be 'net-nature-positive' to deliver beneficial outcomes for all stakeholders. Ideally closure planning would have included the collaborative involvement of all stakeholders from the start (socially embedded rather than socially engaged) which would then help deliver an inherently reconstructive 'cradle-to-cradle' approach (Herrington & Tibbett, 2022) to the operation transferring the site back from the mining company to government or a third party for future use. There are at least 27 abandoned, and problematic former mines in the Philippines (Aggangan et al., 2019). The Sto. Niño copper mine is one the mines that closed in 1982, without a closure plan and the local community rapidly reoccupied the site despite the many challenges it presented.

The Sto. Niño mine lies in the Municipality of Tublay, Benguet, Philippines. Mining claims in the area dated back to 1907. Mining operations were carried out from 1972 to 1981 in two zones: open pit operations working the Southwest orebody at Sto. Niño and an underground block caving operation working the Northeast orebody in the Ullmann district. The tailings were pumped underground from surface thickeners to an adjacent valley to the north where the tailings storage facility was constructed. The site was completely abandoned in 1982. The mine site was left unreconstructed but local people have moved back, with farming being their main occupation. Underground small-scale mining for gold and base metals is still being carried out in the Ullman region, above the former underground block-caving operation.

The Biodiversity Positive Mining For The Net Zero Challenge (Bio+Mine) project (https://bioplusmine.earth), focuses on the Sto. Niño site as this legacy mine constitutes a prime example i) to understand what happens when sites are left abandoned, on geological, environmental, and socio-economic levels, and ii) to explore remediation and intervention options that would deliver the most positive impact by working closely with the local community and exploiting the biodiversity of the local environment. Bio+Mine is funded by the UK's Department of Environment, Food and Rural Affairs (DEFRA) under the Global Centre on Biodiversity for Climate programme (UK Government 2023)

Phase 1 of the two-phase project started in August 2022, and began with an intensive programme of engagement with the local communities, politicians, and other stakeholders prior to factually assessing the legacy issues on the ground. The methodologies and results from this engagement programme have been documented in Alonzo et al. (2023a, b). Following agreements resulting from extensive consultation with local stakeholders at all levels of administration, an on-site surveying and sampling programme was developed. A suite of measurements was made with the goal of providing an up-to-date audit of the site. The data collected are currently being used to prioritize, inform and design possible interventions to improve site conditions. These interventions will constitute the core objectives of Phase 2 that commenced in April 2023.

Phase 2 interventions being considered include development of biodiversity-reliant agri-ecosystems that can also recover valuable metals from waste materials whilst neutralising problematic components at the site, using local farming practices that have a positive impact on biodiversity. The ambition of Bio+Mine is to upcycle the agricultural expertise of the local population to help decontaminate soils to permit third party use of these soils for further agricultural benefits and other purposes. Proposed interventions will look for ways to encourage natural carbon sequestration and biodiversity gains that could also be incorporated into a full-scale system of intervention for future years. Site-specific strategies are underpinned by the knowledge, skills, and practices of the local communities. We aim for Bio+Mine to be a model for wider implementation in other legacy and active mines worldwide.

The key objectives for the Bio+Mine project are:

1. *Phase 1: Engage with the community, and then make an assessment of the physical and ecological issues of the site*: How can a legacy mining site such as Sto. Niño mine be used as a natural laboratory to help reconstruct post-mining ecosystems? What are the priority issues for Sto. Niño?
2. *Phase 2: Local participation and knowledge transfer*: What data, technologies and level of stakeholder participation are needed to guide the development of sustainable and effective nature-positive bioremediation strategies that support ongoing agricultural activities undertaken by the local community?
3. *Phase 3: Programme sustainability, evaluation and transferability*: How do we ensure that interventions lead to positive outcomes from a systemic point of view (including net positive social and environmental results whilst aiming to promote carbon capture), and how do we quantify the impacts of the identified solutions? What requirements (social, technical, and economic) are needed to ensure the development of successful solutions in legacy mines more generally?

The goal of Phase 1 initially focused on delivering site-specific audit of what were the most impacted areas of the abandoned mine site, tailings storage facility and an undisturbed control site located nearby, and to use that audit to identify the critical issues that need to be addressed and to document the resources available at the site, including people, their expertise, and local microbial and plant biodiversity. Informed by these baseline data, a programme of interventions will be commissioned during Phase 2.

This study documents the scope of measurement performed during Phase 1 and present interim results from the field sampling and analytical programme which included remote sensing (satellite and drone systems), sampling of rocks, soils, water and vegetation.

2 Site Audit Methodologies

Note that methodologies are described very superficially in the text and raw data is omitted on space grounds. These are available from the authors upon request.

2.1 Remote sensing

Various remote sensing products were used, ranging from satellite data to drones, to monitor and assess the state and evolution of the Sto. Niño site. Objectives were to provide spatio-temporal context to support fieldwork and aid the interpretation of in-situ measurements, to collect very high-resolution data to help interpretation of coarse resolution historical satellite imagery products, which are the only data available covering the full period, and to evaluate how modern drone technology and machine learning can be used to assess and manage natural resources efficiently and measure biodiversity.

Satellite images from Landsat 5 and 7 were used to produce quarterly median composite images from 1989 to 2022. The Normalized Difference Vegetation Index (NDVI), a measure of pixel 'greenness' which is often used as a metric for live vegetation, was computed from these quarterly averaged data. Time series of mean NDVI values in the mining site and in a nearby control area were used as a proxy to inform on the rate of vegetation recovery in the area affected by mining since 1989.

Analysis of the satellite data from 1989 indicates that the Sto. Niño site has not yet recovered to pre-mining conditions (**Figure 1**). A comparison of quarterly averaged NDVI shows that while vegetation levels have likely improved through time, the level and type of vegetation in the mining site never recovered to pre-mining (control) conditions, as confirmed by field observations. The data show improvements since 1989 when compared with a reference area nearby. Comparison of the average conditions for the two areas shows that seasonal and interannual variability seems to affect both sites with similar pacing but with a different magnitude. The resolution of the Landsat 5 and 7 satellite images is too coarse and not suitable to make any further assessment about how local habitats and biodiversity have evolved since the site was abandoned.

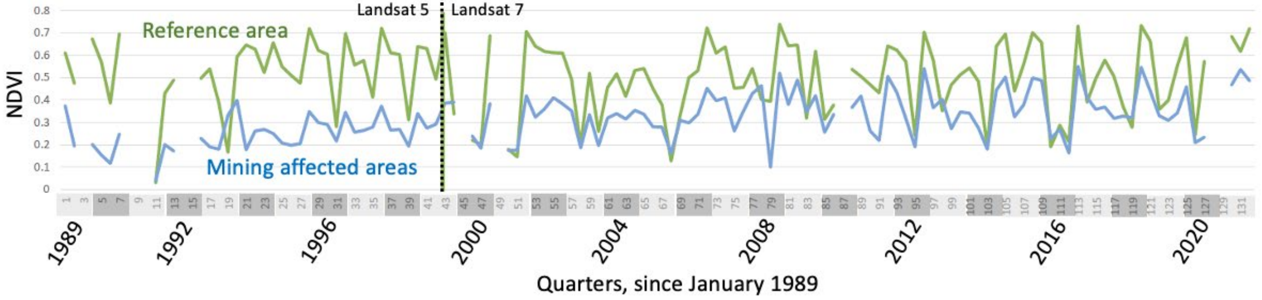
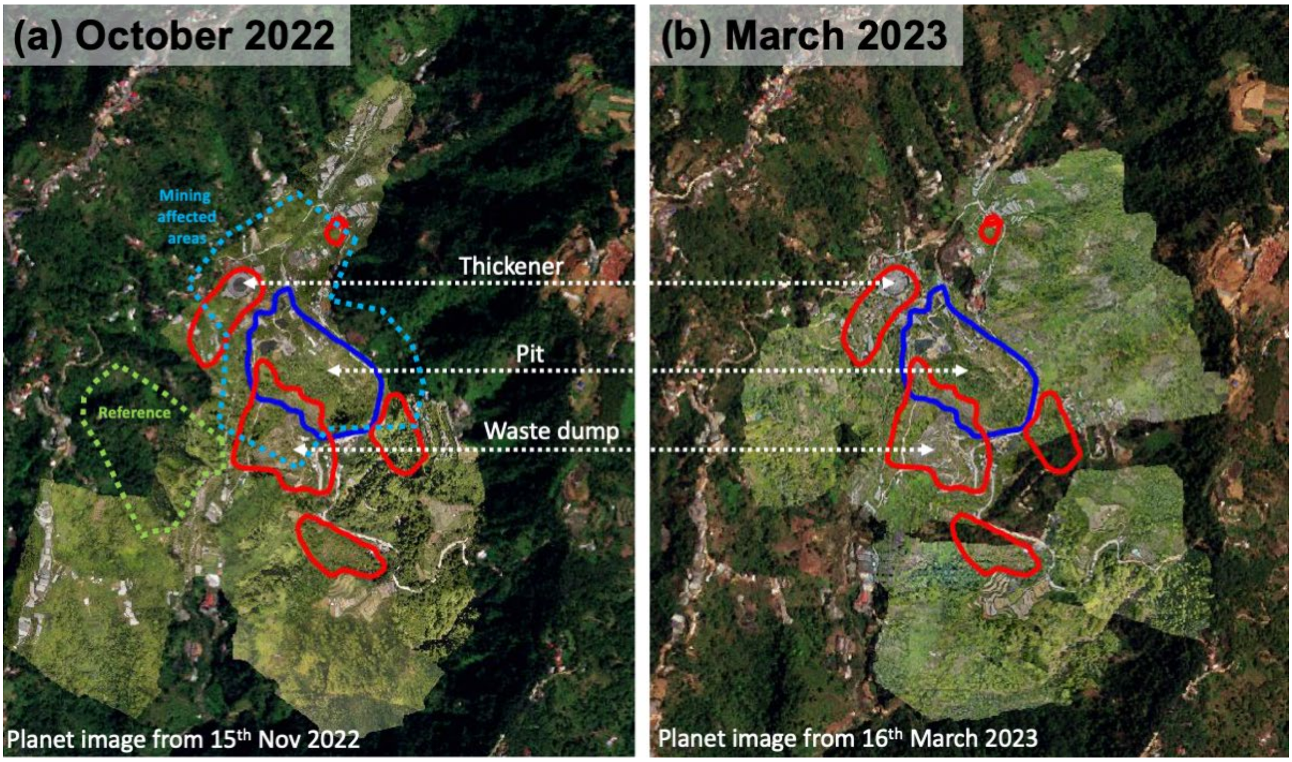


Figure 1 **Quarterly and spatially averaged Normalized Difference Vegetation Index (NDVI) in the mining affected areas and in a nearby reference area. The vertical dashed bar indicates the change from Landsat 5 to 7. Polygons marking the reference and mining affected areas are shown in Figure 2a. The median of all images available is taken for each quarter to reduce the impact of clouds; missing data are due to lack of cloud-free images for that quarter**

To complement satellite data, multiple very high-resolution surveys of the site were also conducted with drones, using a Zenumuse L1 Lidar instrument with a 20 Mp RGB camera mounted on a DJI Matrice 300 drone, a DJI Phantom 4 multispectral (6 x 2 Mp resolution) and a DJI Mavic 3 Multispectral (1 x 20 Mp + 4 x 5 Mp resolution). Repeat surveys were carried out of the entire study site, covering roughly two square kilometers, between October 31 and November 9, 2022, during the wet season (immediately after typhoon Nalgae/Paeng), and between March 7-11, 2023, during the dry season. The DJI Matrice 300 and the DJI

Phantom 4M were flown at constant altitudes, typically 100 m, relative to the takeoff altitude, in 2022, leading to a variable ground resolution distance of 2-30 centimeters, depending on location. During the 2023 survey, the DJI Matrice/L1 and the DJI Mavic 3M instruments were flown in terrain following mode at an altitude of 90 and 50 meters, respectively, delivering a consistent ground resolution distance better than 2.5 cm through the site. Illustrative orthomosaic reconstructions from the hundreds of RGB images collected during both field campaigns are shown in **Figure 2**.



(a) (b)

Figure 2 Orthomosaic reconstructions of the Sto. Niño site using (a) a DJI Phantom 4 Multispectral flown at constant altitude relative to various take off points in October/November 2022 and (b) a DJI Mavic 3 Multispectral flown in 'terrain follow mode' at an altitude of 50 m above ground in March 2023. Drone data is embedded in a three meters resolution PlanetScope image taken near the time of field data collection to highlight improvement in resolution between drone and available satellite imagery

Development of machine learning algorithms capable of identifying and mapping specific species or habitats across the study areas is underway. Given the information provided by the high-resolution images, the multispectral data and information from Lidar instrument, one can envision evaluating how plant health varies spatially within each species or habitat group mapped by machine learning, map and reveal the impact of mining features, including acid drainage or metal leaching, on the ecosystem, as well as measuring plant growth and carbon socks across the site. A quantitative analysis of temporal seasonal variability at the pixel level is also underway.

2.2 Geological study

Sto. Niño is a Miocene-age porphyry copper deposit, similar to others in the Luzon arc like the currently operating Sto. Tomas II deposit, where mineralisation is hosted in andesitic/dioritic and dacitic porphyry sub-volcanic intrusive rocks (Cooke et al. 2011). At Sto. Niño, a resource of 286 million tonnes of ore at a grade of 0.35% Cu, 0.008% Mo, 1.7g/t Ag and 0.2g/t Au was defined prior to mining but it is not known how much of that was mined before it closed. The ore mineralogical assemblage recorded at the site comprised: hypogene biotite, bornite, calcite, chalcopyrite, chlorite, epidote, gold, kaolinite, magnetite, molybdenite, muscovite/sericite, pyrite and secondary minerals chalcocite, azurite and malachite (Hammarstrom et al., 2014).

A total of 109 outcrop and loose waste samples were collected from different parts of the Sto Niño site in November 2022, representing un-mineralised bedrock (control), mineralised bedrock (open pit, artisanal mining site, Ullman adit), tailings storage facility, waste dumps and engineered site (Figure 3).

(a) (b)

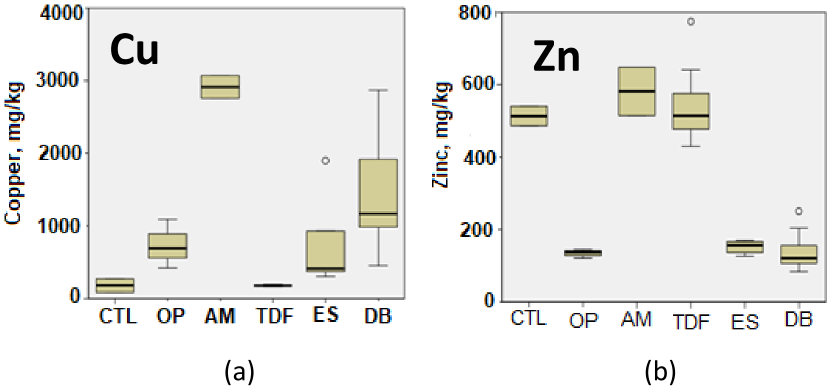
Figure 3 **Legacy mine site (a), tailings storage facility (b) and sampling locations. Geological, water and ecological sampling points are represented by red circles, blue squares and green triangles, respectively (engineered site is the cluster of five red samples shown lower centre on image (a))**

From these, 12 rock samples, 40 waste material samples and 15 soils were selected for detailed chemical and mineralogical characterisation to investigate the mineralogical variability and dynamics across the site and to identify which elements are elevated with respect to the control site and in which mineral phases these are deported. Bulk chemistry was assessed with ICP-AES and ICP-MS, and mineralogy with XRD and SEM coupled with Energy Dispersive X-ray spectroscopy (EDX). Modal mineralogy of the samples was measured using a TESCAN-TIMA Automated Mineral Analyser (AMA)

Bulk chemistry of the waste samples revealed the Si rich nature of all the samples, with SiO_2 present up to 60 wt%. Other abundant elements were Al, Fe, Na, K, Mg and Ca. Sulphur levels were low in all samples except for those from the artisanal mining site, which reported up to three wt% S. Amongst trace elements, Cu was most highly elevated, particularly in waste dump B, the engineered field and the artisanal mining area, followed by Zn, particularly in the tailings storage facility and in Ulmann. Pb was highly elevated in only one sample (from Ulmann) and slightly elevated in some tailings samples. Levels of As, Cd, Mo, Se, Te, Th, Tl, U, W were generally low.

Bulk mineralogy was broadly similar between waste samples from across the site. It was dominated by quartz, plagioclase, chlorite, vermiculite and muscovite/illite. Some differences were observed in the tailings samples and actinolite/Mg hornblende was additionally identified. Primary sulphide minerals were not identified with XRD except for pyrite in the samples from the artisanal mining area. Primary Cu, Zn and Pb minerals (chalcopyrite, malachite, sphalerite and galena) were identified in the rock samples with SEM-EDX and AMA. The abundance of these minerals differed significantly between the rock samples with malachite only detected in some samples from the artisanal mining site, galena and sphalerite only in one sample from Ulmann and chalcopyrite in one of the open pit samples. Very low abundance of primary sulphide minerals (pyrite, chalcopyrite, sphalerite, galena) was observed in the waste samples (<2 mass%). Fe oxides and oxyhydroxides (hematite and goethite), Mn oxides/oxyhydroxides, fluoroapatite and ilmenite were identified with SEM-EDX and AMA in the waste samples and plumbojarosite in one sample from the Ullmann adit.

The Cu rich grains identified with SEM-EDX analysis were particularly abundant in the samples from waste dump B and the engineered field, in agreement with the highest bulk Cu concentrations reported in these samples (**Figure 4**). Chlorite, illite, goethite and Mn oxyhydroxides were identified as major hosts of Cu in the waste samples. Zinc enriched grains were identified only in the samples from the tailings storage facility, which also had the highest bulk Zn concentrations (**Figure 4**). Zinc was found associated with chlorite and Mn oxyhydroxides, in addition to discrete particles of Zn silicate identified in the tailings storage facility samples. There were no trace elements associated with pyrite in any of the samples tested.



(a) (b)

Figure 4 **Box plots showing variation in the (a) Cu and (b) Zn concentrations in the waste samples from the Sto. Niño site. CTL- Control, OP- Open Pit, AM – artisanal mining site, TDF- tailings storage facility, ES – engineered field site, DB - waste dump B**

2.3 Surface water studies

A total of 13 water samples were collected (**Figure 3**) across Sto. Nino, Ullman adit and the tailings storage facility. Water samples were characterized for various physico-chemical properties (Total Suspended Solids (TSS), flowrates), cations (Cu, Mn, Al, Zn, Ni, Se, As, Na, Ca, Sb, B, Fe, Co, Cr, Pb, Cd, K, Mg, Ba and Hg) and anions (sulphate, phosphate, chloride, and nitrate). Some water samples were filtered for microbial analysis through metagenomics. Measured water pH ranged from 3.32 to 7.38.

TSS were analysed using the gravimetric oven drying method at 103-105°C. Metals were analysed using ICP-OES while anions were detected using ion chromatography (IC). Although the total iron was analysed using ICP, its speciation as ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions was further determined, where ferrous iron was measured with the 1,10-phenanthroline method and ferric iron was determined by difference. Alkalinity (as mg $CaCO_3$/L) was determined by titration with 0.02 N sulfuric acid.

Microplanktonic communities were assessed in 10 of the water sites. The planktonic biomass was determined by filtering water through a sterile 0.22 µm pore size Sterivex-GP® filter units (Merck Millipore, Darmstadt, Germany), and stored at -20°C until DNA was extracted using a DNAeasy Power Water kit. Taxonomic assignment was performed using the SILVA database, version 138 (Quast et al., 2013).

Table 1 shows how samples collected during Phase 1 compare with Philippines national standards. 'Critical' samples are all found in the downstream portion of the Sto. Niño river, downstream south from the mine site. These sites can be classified as acidic, all containing elevated concentrations of sulphate, Cu, Mn and Al. This suggests that the dissolution of sulphide minerals influences water chemistry. It was additionally observed that samples SN5 and SN10, directly draining the old workings and waste dumps have significantly higher concentrations of Cu.

Samples upstream (SN7) and downstream (SN3) of the Sto. Niño river as well as sample (SN6) in a stream draining the waste dump were found to be pH neutral even though high concentration of sulphates were determined. This may be due to the high buffering capacity due to the presence of carbonate in the bedrock, supported by the presence of elevated Ca and Mg in the same samples. Limestone boulders were found in the streams which may indicate that a degree of natural rock buffering occurs at the site.

Among the water bodies, two possible sources of drinking water were found: Sto. Niño river upstream of the minesite (SN7), and a stream from a nearby local village (SN11).

Table 1 **Summary of findings based on chemical analyses of surface water samples**

Assessment	Description	Sample Site
Critical	Low pH (4.21-5.67); High metal concentration Beyond Philippine standards in multiple aspects[1]	SN4, SN5, SN6, SN10
Normal	pH within Philippine standards[1] Metal contents within Philippine standards[1]	SN0, SN1, SN2, SN3, SN8, SN9, SN12
Potential Drinking Water Source	pH within Philippine standards[2] Metals within Philippine standards[2]	SN7, SN11

[1] Department of Environment and Natural Resources, Department Administrative Order 2016-08 and 2021-19, [2] Department of Health Philippine National Standards for Drinking Water of 2017.

Microbial diversity was investigated using 16S rRNA gene high throughput Illumina sequencing in 10 water samples. Sequences belonging to the Proteobacteria represented the most abundant phylum in all the samples (Figure 5). The phylum of Proteobacteria was dominated by the class Gammaproteobacteria and Alphaproteobacteria. Additionally, sequences belonging to the phyla Firmicutes and Bacteroides were detected, with different relative abundances, in all the samples. The microbial communities in all sampled locations comprised diverse assemblages of bacteria and archaea. At the SN5 and SN10 sites, Acidobacteria were among the most abundant phyla. The most abundant archaeal sequences detected belong to the phylum Crenarchaeota and showed higher abundance (SN4, SN5 and SN9). SN9 also had high abundance of sequences belonging to the bacterial phylum Verrucomicrobia, whereas SN8 had a higher proportion of Cyanobacteria.

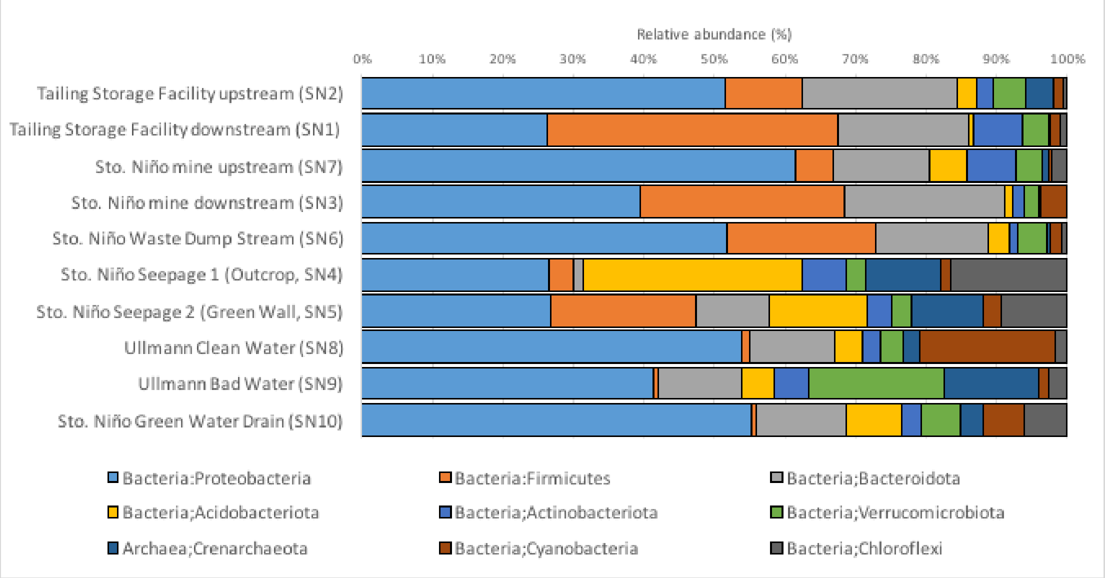


Figure 5 **Relative abundance of the nine most abundant bacteria and archaea phyla in the water samples**

2.4 Ecological study

Mine sites pose serious threats to biodiversity due to both physical and chemical effects of the operations and their legacies (Sonter et al., 2018). Similarly, the presence of vegetation at the former mining site may have played a significant role in mitigating pollution as it has the ability to stabilise soils and prevent the spread of contaminants through processes such as phytostabilization (e.g. Kimmerer 1984; Sheoran et al., 2010). These effects can be expressed in the soil microbiome where bacterial functions are changed by the presence of mine waste materials (e.g. Xiao et al. 2021) and that has knock-on effects on the invertebrate and plant communities as well. In addition to soil, plants and roots, earthworms were also collected, as these organisms are often used as indicators of soil functional health (Figure 3).

Subsurface samples were collected using a simplified boring core to a depth of about 30 cm to evaluate the vertical distribution of contaminants with depth. Five focus areas were sampled (control, tailing storage facility, the engineered site [Bugtong Farm], waste dump B and waste dump A). At most sites, soils had developed although on waste dumps they were poorly developed.). At each location, three quadrants measuring 100 m2 and 10 m apart were established. Soil samples were sieved, air-dried, and chemically analysed by ME-MS41L at ALS, an aqua regia digestion followed by ICP-MS and ICP-AES. This is a partial extraction method (yielding pseudo-total concentrations) that is suitable for solubilisation of sulphide minerals, carbonates, phosphates, to release elements adsorbed on clay particles; or trapped in manganese and iron oxides and oxyhydroxides and organically bound metals. Some silicates and aluminosilicate minerals are therefore not dissolved. A 'geoaccumulation index' was calculated from the data using the formula described by Muller (1969).

Representative plant samples were taken, making sure to take samples that represent the abundant species in the area. Shoot and root parts were thoroughly washed with tap water and distilled water (Bose et al., 2008; Hussain et al., 2019; Santos et al., 2021) before freeze-drying, and rhizosphere soil was air-dried. The rhizosphere soil (~ one mm thin layer of soil around plant roots) was collected by gently shaking the plants until all loose soil was completely removed, and the soil adhering to the roots was then collected (Li et al., 2021; Chen et al., 2023). Around 0.5g of rhizosphere samples were stored at 4°C prior to processing of bacterial DNA extraction. Samples were separately placed in a collection bag, and sent to NASAT Labs (Cabuyao City, 4025 Laguna, Philippines) for XRF analysis. Some plant species were identified morphologically whereas the identity of others was verified by DNA barcoding using ITS rDNA or plastid regions according to Ghorbani & de Boer (2017).

Earthworms were taken opportunistically inside the established 100 m2 quadrants. Genomic DNA (gDNA) was extracted from the earthworms using the Qiagen Blood and Tissue Kit. Microbial gDNA was extracted from 0.5 g soil samples (per sample with three replicates) and rhizosphere soils using the DNeasy PowerSoil Pro Kit (QIAGEN, LLC.). All gDNA samples underwent PCR amplification and Illumina sequencing using MiSeq PE300. Taxonomic gene markers were amplified using PCR with primers containing MiSeq sequencing adapters with dual indexing for the 16S rRNA and 18S rRNA gene (Caporaso et al., 2012) as well as fungi ITS (Schoch et al. 2012) and COI (Hebert et al., 2003).

(A) (B) (C) (D)

Figure 6 Morphologically identified plant species: Pennisetum sp. (A), Ageratina riparia (B), Vaccinium sp. (C), and Nephrolepis sp. (D)

A translocation factor (TF) was determined from the analyses of the plants and roots, defined as the ratio of metal concentration in the shoots to the roots: TF = Cshoot / Croot, as well as a 'bioconcentration factor' (BCF) defined as the ratio of metal concentration in the shoot or roots to that in soil: BCF = Cshoot / Csoil, using the pseudo-total concentration of metal in the soil. Plants with both bioconcentration factors and translocation factors greater than one (TF and BCF>1) have the potential to be used in phytoextraction. Plants with bioconcentration factor greater than one and translocation factor less than one (BCF>1 and TF<1) have the potential for phytostabilization (Yoon et al., 2006).

Chemical analysis of the soil samples showed elevated Cu in waste dump A (WDA) and waste dump B in relation to the Cu concentration of the reference site. The geoaccumulation index displayed uncontaminated to moderately contaminated pollution levels of Cu in WDA and WDB while other sites were uncontaminated. Plant species from the area (Figure 6) such as Pennisetum sp., Ageratina riparia, Vaccinium sp. and Nephrolepis sp. exhibited phytostabilization affinities towards Ni, Vaccinium sp. and Nephrolepis sp. towards Zn, and Vaccinium sp. for Mn. Despite having elevated amounts of Cu in the soil, the tissue samples of these representative plant species showed that they can only accumulate a small amount of Cu in their roots.

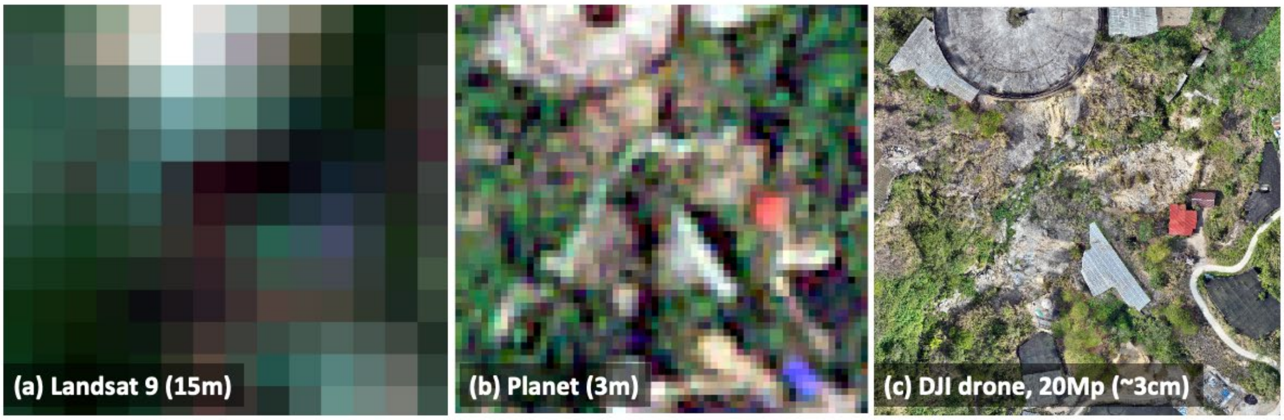
Bacterial key species found in the soil and the rhizosphere were identified using DNA 16S rRNA gene sequencing. The analysis of the soil from three different sites, including the control site, showed that Proteobacteria, Planctomycetota, Acidobacteriotota, and Verrucomicrobiota were the most abundant phyla present in all three sites.

It was found that earthworms were more abundant and diverse where locals had engineered an agricultural plot as compared to other sampling areas. Use of earthworms as indicators of soil health will be further explored through mesocosm experiments planned in the Phase 2 programme in late 2023. Mesocosm experiments will also be used to test if earthworms can enhance the efficacy of hyperaccumulating plants in removing contaminants from soils and to assess the impact of metals on soil biodiversity, health, and agriculture.

3 Discussion

3.1 Remote sensing as a tool for legacy site assessment, monitoring and remediation.

Passively collected satellite data over several decades shows that the vegetation in the area affected by mining has not recovered to levels seen in adjacent control areas, but the relatively coarse resolution of these satellite images in relation to the features of relevance at the site limits the interpretative value of these data (Figure 7).



(a) (b) (c)

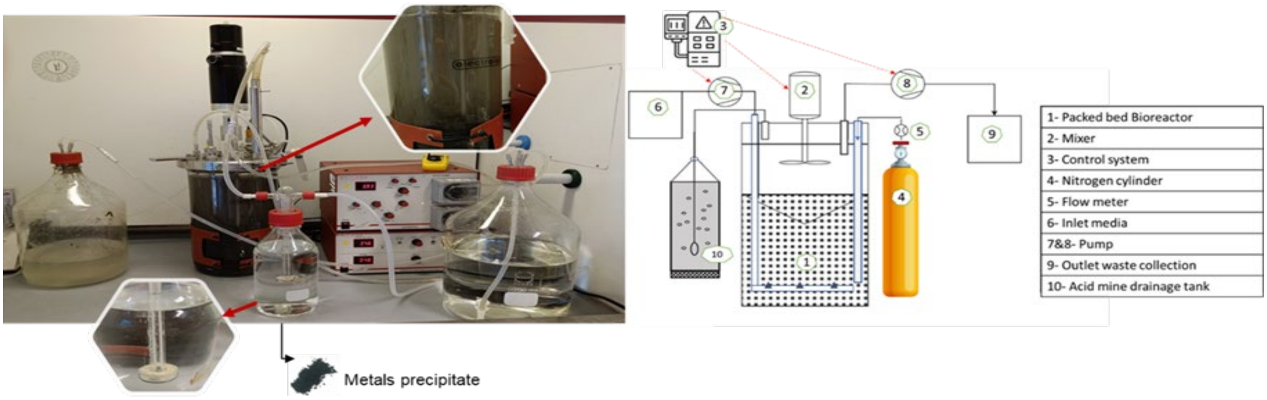
Figure 7 Illustrative example showing how the pixel resolution evolves as a function of imagery products. All three images cover the same area in Sto. Niño, with the thickener clearly visible in (b) and (c) but completely pixelated and unidentifiable in (a)

High resolution drone data are proving to be very useful as they can be used to fill the resolution gap between satellite data and field observations and therefore can be used to map and monitor the site with a resolution suitable to identify individual plants, leaves or flowers and even to measure how the health of these plants change in space and time. High resolution drone images, coupled with generative adversarial neural networks, could also lead to resolution-enhanced satellite imagery archives and a re-interpretation of these data.

Future priorities involve collection of additional data to better constrain the influence of temporal variability on ecosystem health in the study area, and the development of bespoke algorithms to identify important species or habitats, map their distribution and quantify change in the area. Lidar provides very accurate 3D information about the physical characteristics of the site and of the vegetation, while photogrammetry and multispectral imaging provides a mechanism to map and evaluate plant health. Going forward, the addition of thermal infrared imagery would also permit detection and quantification of warm-blooded animals in the site, such as birds and mammals. Deploying a Lidar – multispectral imagery - thermal infrared triplet of instruments in conjunction with the geological, chemical and ecological programmes on the ground would provide unprecedented information able to map the various habitats in the site. These data will provide a means to evaluate the state of health of these habitats spatially and their evolution through time with unique resolution allowing for very targeted and therefore more effective interventions.

3.2 Bio+Mine Intervention: bio-based solution for treatment of Sto. Niño mine water

Early on, it was recognised that any bio-based water treatment proposed for Sto. Niño would involve the use of a well-known, widely distributed specific group of bacteria, the sulphate-reducing bacteria (SRB), for the generation of hydrogen sulphide (H2S) in bioreactors for bio-sulphido-genesis. Biological hydrogen sulphide production requires the consumption of sulphate and acidity present in acid mine drainage (AMD), tackling two of the three main issues related to these types of waters. The third issue is related to the presence of heavy metal ions such as Cu in the mine water, which is easily removed by precipitation methods due to the interaction between these metal ions and the biologically generated H_2S.



(a) (b)

Figure 8 Image of sulfidogenic bioreactor in the laboratory (a) and schematic representation of the bioreactor set-up (b)

Preliminary results are presented here that show how a bioreactor can be developed to treat water and potentially extract copper at the same time (Figure 8). Water sample SN5 was selected for the bioremediation experiments due to its elevated metal content and low pH. The remediation process was performed in a laboratory-scale bioreactor using a synthetic version of the SN5 mine water (same elemental composition and pH value) targeting initially the removal of copper (Stage 1). Bioreactor pH was set at 4.5 (optimum pH for the SRB consortium) while the feed liquor, containing basal salts and glycerol as substrate, was set at 2.1 to counterbalance proton consumption. To maintain anoxic conditions, oxygen-free nitrogen was sparged continuously through the bioreactor. The nitrogen stream also acted as a carrier gas for the biologically generated H_2S to the precipitation tank containing synthetic SN5 mine water.

The next stage of treatment (Phase 2) will utilise the Cu-free mine water as the reactor feed liquor to the bioreactor, where concentrations of sulphate will be reduced, other metals (Zn, Al, Mn) removed and effluent water pH increased. Several analyses were performed to investigate bacterial activity and content of metals in the mine water. Changes in concentration of Cu were analysed by colorimetric assay and other metals by ICP-OES. Concentration of glycerol was analysed by a colorimetric assay while sulphate and acetate were analysed by IC. XRD was used to identify the main mineral phases in the solid product generated.

In initial tests, over 99% of dissolved Cu was removed in 40 minutes (average rate of 0.28 mg/l/min). The pH of the AMD after copper removal (stage 1) was 3.5, lower than it is initial value (pH 4.6). This was expected as copper sulphide precipitation generates acidity. Solid product was analysed to confirm the presence of copper sulphides. XRD analysis identified covellite as the main sulphide phase.

The ICP-OES results showed that during stage one of the treatment process there was no change in Zn, Mn and Al concentration in the SN5 water. To overcome that, together with reducing sulphate content and acidity, the next stage of treatment will utilise the Cu-free mine water as the reactor feed liquor to the bioreactor. Table 2 shows characteristics of the SN5 water before and after treatment.

Table 2 Composition of the mine water before and after treatment. Metals concentrations are shown in mg/l

Stage	Cu	Zn	Al	Mn	Sulfate	pH
Before treatment	10.48	0.97	2.39	2.06	315	4.5
After stage one treatment	<0.01	0.97	2.39	2.06	315	3.5
After stage two treatment	<0.01	0.06	0.09	2.06	290	5.5

As part of the Phase 2 programme in 2024, a small pilot scale integrated bioremediation plant will be implemented at Sto. Niño for the treatment of the impacted water at site SN5. The intervention will involve a similar biological approach to that tested in the laboratory aiming to reduce water acidity and sulphate content and remove high concentrations of Cu, Al. Further experiments will be carried out to see how Mn might be removed. The biological component, i.e., the microorganisms, will in this case be harvested from the local microbiome, informed by the metagenomic work carried out across the site eliminating the introduction of external microbial species to the local area.

4 Conclusion

Copper appears to be the only highly elevated trace element in samples from the Sto Niño site with high variations in concentrations observed between different parts of the site and between samples from the same areas (particularly the engineered field and waste dump B). Concentrations of S are low in most of the waste samples in agreement with a very low abundance of primary sulphide minerals found in these samples thus indicating a relatively low potential for generation of acid mine drainage (e.g., from waste dump B). Cu is seen associated with secondary and oxidised mineral phases which include goethite, Mn oxyhydroxide, illite and chlorite. Remobilisation of Cu from these phases is likely if acidic or reducing conditions develop and solubilise the Cu host phases.

The water bodies at Sto. Niño were identified and assessed, and both impacted (critical) water bodies and potential sites as safe sources of drinking water were found. Contaminated water sources will be remediated using a pilot biological treatment in Phase 2 using local, naturally occurring bacteria strains to reduce water acidity and high concentration of Cu, Al, Mn and sulphate. A complete analysis will be conducted to determine what further conventional chemical treatment would be required before using the potential sources of drinking water as a potable source. Temporal variability and total flow capacity of these potential sources of clean water will also need to be considered to estimate how much these sources could contribute to the well-being and development of the community.

Microbial communities were characterized from the same water bodies as for the water chemistry and this is the first sequencing assessment to date of microplanktonic communities at the site. The bacteria and archaea assemblages comprised diverse communities with significant differences in taxonomic groups between sites. Many bacteria groups such as Proteobacteria are common for freshwater water, however an assessment of microbial composition at higher taxonomic resolution is still required to screen the water microplankton data for the presence of bacteria that might be an indicator of sewage runoff. The synthesis of results of microbial communities and chemical water parameters will permit conclusions to be drawn on the water quality. For assessment of soil from waste dumps and other sites, DNA 16S rRNA gene sequencing analysis of a variety of soil samples has provided a description of the microbial community composition and diversity within the studied ecosystem and highlighted the prominent bacterial taxa, some of which may be investigated for their bioremediation capabilities, particularly their tolerance of heavy metals.

The presence and distribution of earthworm species across the five different sites was found to be variable. Earthworm abundance and diversity are recognized to be influenced by key soil quality factors, such as soil texture, nutrient availability, and the quantity and quality of plant residue inputs within the agroecosystem (Hendrix et al. 1992). Further in-depth investigation is warranted to comprehensively explore the abundance, diversity, and potential of the identified earthworms as indicators of soil quality across the studied sites.

Statistical analysis will be applied to evaluate the relationship between microbial communities and environmental variables, in particular pH and metal concentrations, and to determine abiotic parameters such as water chemistry that influence the microplankton community composition. The findings will help to determine if and how metals from the waste dumps affect the stream biodiversity and inform potential future treatment approaches. The results will also help to better understand microbial-driven biogeochemical processes linked to metal leaching, potential for development localised AMD and transport of metals into the water bodies. The 16S rRNA gene communities will also allow the screening for bacteria and archaea of potential in biomining applications.

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References

Aggangan, N. S., Anarna, J. A., & Cadiz, N. M., (2019) Tree Legume – Microbial Symbiosis and Other Soil Amendments as Rehabilitation Strategies in Mine Tailings in the Philippines, Philippine Journal of Science, 148, 481-491

Alonzo, D., Tabelin, C. B., Dalona, I. M., Beltran, A., Orbecido, A., Villacorte-Tabelin, M., Resabal, V. J., Promentilla, M. A., Brito-Parada, P., Plancherel, Y., Jungblut, A. D., Armstrong, R., Santos, A., Schofield, P. F., & Herrington, R. (2023a). Bio+Mine project: Empowering the community to develop a site-specific system for the rehabilitation of a legacy mine. International Journal of Qualitative Methods, 22, 160940692311763. https://doi.org/10.1177/16094069231176340

Alonzo, D., Dalona, I. M., Armstrong, R., Villacorte-Tabelin, M., Tabelin, C. B., Beltran, A., Orbecido, A., Brito-Parada, P., Plancherel, Y., Santos, A., Herrington, R., Jungblut, A. D., Schofield, P. F., Promentilla, M. A., Resabal, V. J., Suelto, M. (2023b), Development of a site-specific system for the rehabilitation of legacy mines: The intersections of social technical, and environmental data, this volume

Bradshaw A. (2000). The use of natural processes in reclamation-advantages and difficulties. Landsc Urban Plan 51:89–100

Bose, S., Chandrayan, S., Rai, V., Bhattacharyya, A. K., & Ramanathan, A. L. (2008). Translocation of metals in pea plants grown on various amendment of electroplating industrial sludge. Bioresource Technology, 99(10), 4467-4475.

Caporaso, J., Lauber, C., Walters, W., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G. & Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME Journal, 6, 1621–1624. https://doi.org/10.1038/ismej.2012.8

Chen, W., Wu, Z., Liu, C., Zhang, Z., & Liu, X. (2023). Biochar combined with Bacillus subtilis SL-44 as an eco-friendly strategy to improve soil fertility, reduce Fusarium wilt, and promote radish growth. Ecotoxicology and Environmental Safety, 251, 114509.

Christian, J, Ladd, C & Baecher, G 1993, ‘Reliability applied to slope stability analysis’, Journal of Geotechnical Engineering, vol. 120, no. 12, pp. 2180-2207.

Cooke, D. R., Deyell, C. L., Waters, P. J., Gonzales, R. I., & Zaw, K. (2011). Evidence for magmatic-hydrothermal fluids and ore-forming processes in epithermal and porphyry deposits of the baguio district, Philippines. Economic Geology, 106(8), 1399-1424. https://doi.org/10.2113/econgeo.106.8.1399.

de la Vergne, J 2003, Hard Rock Miner’s Handbook, McIntosh Engineering, North Bay, Ontario, viewed 3 March 2014, http://www.altomines.com/pdfs/HardRockMinersHandbook.pdf

Department of Environment and Natural Resources. (2016). Water Quality Guidelines and General Effluent Standards of 2016. Environmental Management Bureau | Initially established as a supporting body for the Department of Environment and Natural Resources in 1987. https://emb.gov.ph/wp-content/uploads/2019/04/DAO-2016-08_WATER-QUALITY-GUIDELINES-AND-GENERAL-EFFLUENT-STANDARDS.pdf

Department of Environment and Natural Resources. (2021). Updated Water Quality Guidelines (WQG) and General Effluent Standards for Selected Parameters. https://ncr.denr.gov.ph/ .https://ncr.denr.gov.ph/images//dao-2021-19-updated-water-quality-guidelines-wqg-and-general-ef_p21757.pdf

Department of Health. (2017). Philippine National Standards for Drinking Water of 2017. Food and Drug Administration. https://www.fda.gov.ph/wp-content/uploads/2021/08/Administrative-Order-No.-2017-0010.pdf

Ghorbani, A., Saeedi, Y., & de Boer, H.J., (2017). Unidentifiable by morphology: DNA barcoding of plant material in local markets in Iran. PLoS One, 12. https://doi.org/10.1371/journal.pone.0175722

Hammarstrom, J. M., Ludington, S., Robinson, G. R., Bookstrom, A. A., Zientek, M. L., Mihalasky, M., Zürcher, L., Berger, B. B., Dicken, C. L., & Gray, F. (2014). Undiscovered Phanerozoic porphyry copper Deposits-A global assessment. Acta Geologica Sinica - English Edition, 88(s2), 532-534. https://doi.org/10.1111/1755-6724.12374_18

Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. Proceedings of the Royal Society B: Biological Sciences, 270(1512), 313–321. https://doi.org/10.1098/rspb.2002.2218

Herrington, R. & Tibbett, M., (2022). Cradle-to-cradle mining: a future concept for inherently reconstructive mine systems?, in AB Fourie, M Tibbett & G Boggs (eds), Mine Closure 2022: 15th International Conference on Mine Closure, Australian Centre for Geomechanics, Perth, pp. 19-28, https://doi.org/10.36487/ACG_repo/2215_0.02

Hendrix, P., Mueller, B., Bruce, R., Langdale, G., & Parmelee, R. (1992). Abundance and distribution of earthworms in relation to landscape factors on the Georgia Piedmont, U.S.A. Soil Biology and Biochemistry, 24(12), 1357-1361. https://doi.org/10.1016/0038-0717(92)90118-h

Hussain, A., Priyadarshi, M., Dubey, S., 2019. Experimental study on accumulation of heavy metals in vegetables irrigated with treated wastewater. Applied water Science, 9:12

Kimmerer, R. W. (1984). Vegetation development on a dated series of abandoned lead and zinc mines in southwestern Wisconsin. American Midland Naturalist, 111(2), 332. https://doi.org/10.2307/2425328

Kuganathan, K 2005, ‘Geomechanics of Mine Fill’, in Y Potvin, E Thomas & A Fourie (eds), Handbook on Mine Fill, Australian Centre for Geomechanics, Perth, Western Australia.

Li, K. S., Zeghbroeck J, V., Liu, Q., & Zhang, S. (2021). Isolating and characterizing phosphorus solubilizing bacteria from rhizospheres of native plants grown in calcareous soils. Frontiers in Environmental Science, 9, 802563.

Muller, G. (1969). Index of geoaccumulation in sediments of the Rhine River. Geo Journal, 2:109–118.

Potvin, YH & Wesseloo, J 2013, ‘Towards an understanding of dynamic demand on ground support’, in Y Potvin & B Brady (eds), Proceedings of the Seventh International Symposium on Ground Support in Mining and Underground Construction, Australian Centre for Geomechanics, Perth, pp. 287-304.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, 590–596. doi: 10.1093/nar/gks1219.

Santos, A. J. C., Divina, C. C., & Monserate, J. J. (2021). Heavy Metal Contamination in Soil and Phytoremediation Potential of Naturally Growing Plants in Bagong Silang Dumpsite, Talavera, Nueva Ecija, Philippines. Mindanao Journal of Science and Technology, 19(1).

Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A. & Chen, W. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci;109(16):6241-6. doi: 10.1073/pnas.1117018109.

Sheoran, V., Sheoran AS., and Poonia P., (2010). Soil reclamation of abandoned mine land by revegetation: A review, International Journal of Soil, Sediment and Water, Vol 3(2), Article 13.

Sonter, L. J., Ali, S. H., & Watson, J. E. (2018). Mining and biodiversity: Key issues and research needs in conservation science. Proceedings of the Royal Society B: Biological Sciences, 285(1892). https://doi.org/10.1098/rspb.2018.1926

UK Government (2023) https://www.gov.uk/government/publications/global-centre-on-biodiversity-for-climate/global-centre-on-biodiversity-for-climate-policy-information, accessed 26/06/2023.

World Health Organization (2011). Guidelines for Drinking-Water Quality, vol. 4.

Worrall, R., Neil, D., Brereton, D. & Mulligan, D., (2009). Towards a Sustainability Criteria and Indicators Framework for Legacy Mine Land. Journal of Cleaner Production 17 (16): 1426–1434. doi:10.1016/j.jclepro.2009.04.013.

Xiao, E., Ning, Z., Xiao, T., Sun, W., & Jiang, S. (2021). Soil bacterial community functions and distribution after mining disturbance. Soil Biology and Biochemistry, 157, 108232. https://doi.org/10.1016/j.soilbio.2021.108232

Yoon, J., Cao, X., Zhou, Q., & Ma, L. Q. (2006). Accumulation of PB, CU, and Zn in native plants growing on a contaminated Florida site. Science of The Total Environment, 368(2-3), 456-464. https://doi.org/10.1016/j.scitotenv.2006.01.016