

Biotechnology & Metals 2026

ALGAJAS s.r.o., Košice
Institute of Geotechnics, Slovak Academy of Sciences, Košice
Slovak Mining Society



7th International Scientific Conference



Editors:
Jana Sedláková-Kaduková, Alena Luptáková



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8–9 October 2026, Stará Lesná, High Tatras, Slovakia



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Proceedings

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Preface

Dear colleagues and friends,
it is our honour to welcome you to the 7th International Scientific Conference on Biotechnology and Metals in Stará Lesná, High Tatras.

The effects of climate change, biodiversity loss and environmental pollution are no longer distant predictions but part of our everyday lives. Science and technology have given us extraordinary power to reshape landscapes, extract valuable metals from almost any waste and engineer living organisms. Yet we too often ask only “can we”, and not “should we”. Every technology we develop has consequences for soil, water and the generations that follow, and therefore it always carries a value judgement.

Environmental biotechnology shows that real industrial and human needs can be met by working with nature rather than against it, using microorganisms and plants to recover resources, treat waste and restore damaged ecosystems. The goal of the Biotechnology and Metals conference is to bring together researchers, students, industry and practitioners, so that their combined expertise can turn this potential into practice.

The conference is organised by three institutions: ALGAJAS s.r.o., Košice; the Institute of Geotechnics, Slovak Academy of Sciences, Košice; and the Slovak Mining Society.

We wish you an inspiring time at the conference and in Stará Lesná. We hope that these two days will bring you new ideas, new collaborations and the resolve to ask in your own work not only “can we”, but also “should we, and how can we do it better”.

Jana Sedláková-Kaduková and Alena Luptáková

CONTENT

Magdaléna Bálintová, Lenka Demková, Adriana Eštoková, Alena Luptáková FTIR AND XRD CHARACTERIZATION OF SILICATE SOILS FROM THE ZLATÁ BAŇA MINING LOCALITY (SLOVAKIA).....	7
Zuzana Bártová BIOLOGICAL IRON OXIDATION OF ACID MINE DRAINAGE FOR MINERAL PIGMENT PRECURSOR RECOVERY	14
Daniel Benek, Sofia Gyorióvá, Martin Repček, Natália Zatlková, Adam Zeman MICROPLASTICS AND SYNTHETIC FIBRES IN AQUATIC ECOSYSTEMS	18
Claudia Čičáková, Daniel Kupka, Zuzana Bártová, Lenka Hagarová, Tomáš Faragó, Viktória Krajanová, Bronislava Lalinská, Slavomír Hredzák, Marek Matík, Vladimír Malý SECONDARY MINERAL FORMATION IN MINING-INFLUENCED WATER AFTER BIOLOGICAL PRE-TREATMENT	21
Bence Farkas, Rebeka Kósaová, Martin Urik BIOEXTRACTION OF MANGANESE FROM WASTE MATERIAL USING AN ADAPTED STRAIN OF THE MICROSCOPIC FILAMENTOUS FUNGUS ASPERGILLUS NIGER	25
Rebeka Frueholz, Clemens Habermaier, Lena Heibl, Jiří Kučera, Christian Zafiu, Vladimír Brummer, Marianne Haberbauer PHOSPHORUS RECOVERY FROM SEWAGE SLUDGE ASH USING BIOGENIC SULPHURIC ACID	28
Eudmila Galuščáková, Libuša Lengyelová, Lucia Sporinová, Ildikó Matušíková, Radoslava Matúšová, Beáta Piršelová ASSESSMENT OF CADMIUM TOLERANCE IN OAT (AVENA SATIVA) AND ITS POTENTIAL FOR REMEDIATION OF CONTAMINATED SOILS	32
Alexandra Hlavatá, Peter Nemeček, Viktória Košíková, Richard Hančinský, Vladimír Dvonka, Pavol Hauptvogel, René Hauptvogel, Laura Židek Jozefíková, Ildikó Matušíková ZINC AND ARBUSCULAR MYCORRHIZAL FUNGI IN DURUM WHEAT BIOFORTIFICATION: EFFECTS ON PLANT MORPHOMETRY, PHYSIOLOGY AND SOIL MICROBIOME	36
David Hrečin, Iva Janáková, Lenka Říhová Spěvákova USING ADAPTATION PERIOD TO ENHANCE BIOLEACHING OF INDIUM FROM LCD SCREENS.....	40
Simona Ilavská, Mária Pavlovičová, Peter Nemeček, Ildikó Matušíková, Anirban Debnath, Alexandra Hlavatá FLAX CULTIVAR VARIABILITY UNDER METAL STRESS: RELEVANCE TO PHYTOREMEDIATION OF CONTAMINATED ENVIRONMENTS	44
Jana Konečná, Helena Švarcová, Robo Švarc, Martin Frič THE REMEDIATION SCULPTURE	48
Rebeka Kósaová, Bence Farkas, Marek Bujdoš, Martin Urik EVALUATION OF FUNGAL POTENTIAL IN CADMIUM BIOLEACHING FROM SOLID MINING WASTES AND ITS REMOVAL USING CHEMICALLY MODIFIED MANGANESE OXIDES	52
Tereza Kupka Chowaniecová, Lukáš Kupka, Hana Vojtková, Barbara Stalmachová EFFECTS OF TEMPERATURE AND PH ON THE FUNCTIONAL DIVERSITY OF MICROSCOPIC FUNGI IN A THERMALLY ACTIVE SPOIL HEAP	56
Alena Luptáková, Magdaléna Bálintová, Adriana Eštoková, Eva Mačingová, Daniela Guglietta, Stefano Ubaladini, Miloslav Lupták POSSIBILITIES OF MINE WASTES BIOLEACHING FROM OLD MINING BURDENS.....	61

Valeria Mádainé Üveges, Shabnam Jameshourani, Ljudmilla Bokányi EVALUATION OF ACIDITHIOBACILLUS FERRIDURANS GROWTH AND BIOLEACHING PERFORMANCE ON ARSENIC-BEARING MINE TAILINGS	68
Mohsen Monirialamdari, Daniel Kupka, Eva Mačingová, Dávid Jáger, Claudia Čičáková CHARACTERIZATION OF DISSOLVED ORGANIC MATTER IN NIŽNÁ SLANÁ MINE WATER BY HPLC-FLD, HPLC-HRMS AND GC-MS.....	72
Lenka Říhová Spěvácová, Iva Janáková, David Hrečin BIODEGRADATION OF PET MICROPLASTICS IN A TEST MEDIUM	76
Jana Sedláková-Kaduková, Joanna Willner INDIUM REMOVAL FROM SOLUTION BY CHLORELLA VULGARIS BIOMASS	80
Miroslava Sinčák, Katarína Benediktová, Jana Sedláková-Kaduková GROWTH AND FERROUS-IRON OXIDATION KINETICS OF ACIDITHIOBACILLUS FERROOXIDANS UNDER A 5 MT STATIC MAGNETIC FIELD	84
Abidemi Sunmola, Daniel Kupka, Claudia Čičáková, Lenka Hagarová, Zuzana Bártová ACTIVATED CARBON-BASED FLUIDIZED-BED REACTOR FOR IRON OXIDATION BY ACIDOPHILIC AUTOTROPHS: IMPLICATIONS FOR BIOLEACHING AND AMD TREATMENT	88
Martin Urík, Marcel B. Miglierini IRON OXIDE NANOPOWDER TRANSFORMATIONS TRIGGERED BY ASPERGILLI.....	92
Joanna Willner, Agnieszka Fornalczyk, Magdalena Jabłońska-Czapla, George Yandem PHOTOVOLTAIC PANELS IN THE ENVIRONMENTAL CONTEXT – MATERIAL DEGRADATION AND RECYCLING CHALLENGES	95
George Yandem, Katarzyna Grygoyć, Małgorzata Wawer-Liszka, Marcin Szuszkiewicz, Joanna Willner, Agnieszka Fornalczyk, Magdalena Jabłońska-Czapla PHOTOVOLTAIC PANELS AS A POTENTIAL SOURCE OF TECHNOLOGY-CRITICAL ELEMENTS IN THE ENVIRONMENT	99
Zeinab Zamani, Eva Duborská EFFECT OF IODINE BIOFORTIFICATION ON PHOTOSYNTHETIC PIGMENTS IN MINT PLANTS	103
ALGAJAS s.r.o.	107
Slovak Academy of Sciences Institute of Geotechnics.....	108
Sponsors	109
IMWA 2027	111
Photos	112

FTIR AND XRD CHARACTERIZATION OF SILICATE SOILS FROM THE ZLATÁ BAŇA MINING LOCALITY (SLOVAKIA)

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Abstract

Mineralogical and chemical characterization of silicate-rich soils is essential for understanding their composition, origin and relationship to the geological conditions of a given area. Silicate soils from the Zlatá Baňa mining locality in eastern Slovakia were characterized using X-ray Fluorescence (XRF) analyses, Fourier Transform Infrared Spectroscopy (FTIR), and X-ray Diffraction (XRD). FTIR and XRD analyses identified characteristic silicate and aluminosilicate phases, with quartz occurring as the predominant mineral, accompanied by feldspars and clay minerals. XRF revealed significant variations in major-element composition, particularly in SiO₂ and Al₂O₃ contents, reflecting differences in the mineral composition of the investigated samples. The combined results demonstrate distinct mineralogical and chemical variability among the soils, related to the heterogeneous geological and hydrothermal conditions of the Zlatá Baňa mining area.

Keywords: Zlatá Baňa; silicate soils; XRD; FTIR; XRF; clay minerals

1 Introduction

Soil mineralogy is a fundamental factor controlling the physical, chemical and environmental properties of soils. The mineral composition affects important soil characteristics such as texture, water retention, surface reactivity, ion exchange capacity and the mobility of major and trace elements. These relationships are particularly important in soils developed in areas affected by volcanic activity, hydrothermal alteration and historical mining, where the mineralogical composition may reflect both the geological substrate and subsequent weathering and anthropogenic processes. Detailed characterization of mineral phases is therefore essential for understanding the formation, transformation and environmental behaviour of soils developed in mineralized areas [1,2].

The Zlatá Baňa locality is situated in the Slanské Vrchy Mountains in eastern Slovakia, within the Neogene Eastern Slovak Volcanic Field. The Zlatá Baňa deposit is classified as an intermediate-sulphidation epithermal Pb-Zn-Cu-Au-Ag-Sb deposit associated with the Neogene volcanic environment of the Western Carpathians. The mineralization is hosted by the Zlatá Baňa volcanic centre and is accompanied by hydrothermal alteration. In particular, illite, illite/smectite and kaolinite have been documented in association with stibnite-rich mineralization, indicating an important role of hydrothermal alteration in the mineralogical evolution of the locality [3]. The combination of volcanic parent material, hydrothermal alteration and historical mining activity makes the Zlatá Baňa area a suitable natural laboratory for investigating variations in soil mineralogy and geochemistry.

The mineralogical composition of soils can be investigated using complementary analytical techniques because individual methods provide different types of information. X-ray diffraction (XRD) is a principal method for the identification of crystalline mineral phases and provides information based on the characteristic diffractions of individual minerals. It is particularly valuable for the identification of quartz, feldspars and clay minerals. However, the interpretation of soil XRD patterns may be affected by low crystallinity, mixed-layer structures, preferred orientation of clay particles and overlapping reflections. Consequently, reliable mineralogical interpretation benefits from complementary analytical methods and appropriate sample preparation [4,5].

Fourier Transform Infrared Spectroscopy (FTIR) provides complementary information on the structural and chemical characteristics of minerals through their characteristic vibrational modes. Infrared absorption bands associated with Si–O, Al–O and hydroxyl groups can be used to identify and characterize silicate and aluminosilicate minerals. FTIR spectroscopy has been successfully applied to the identification of clay minerals and to the investigation of their structural and compositional variations [6]. The combined

application of XRD and FTIR is therefore advantageous because XRD primarily provides information on long-range crystalline order, whereas FTIR is sensitive to molecular vibrations and specific structural groups within minerals.

In addition to mineralogical characterization, determination of the elemental composition is important for understanding the chemical variability of soils. X-ray fluorescence (XRF) spectroscopy is a widely established technique for the qualitative and quantitative determination of major and trace elements in geological and environmental materials. It provides rapid multi-elemental information and is widely used for the geochemical characterization of rocks, sediments and soils [7,8]. In the present study, XRF data provide information on the major oxide composition of the investigated samples, including SiO_2 , Al_2O_3 , Fe_2O_3 , MgO , K_2O , CaO and SO_3 . These data can be used to complement the mineralogical information obtained by XRD and FTIR and to evaluate differences in the relative contribution of silicate, aluminosilicate and other mineral components among the samples.

The application of several complementary analytical techniques is particularly appropriate for the characterization of heterogeneous soils from a mineralized and historically mined locality. XRD allows the identification of crystalline mineral phases, FTIR provides information on characteristic structural groups, and XRF characterizes the major-element composition. The integration of these datasets can consequently provide a more comprehensive understanding of relationships between mineralogical composition and chemical characteristics of the investigated soils [9].

The objective of the present study was therefore to characterize silicate soils collected from selected sites within the Zlatá Baňa mining locality using XRD, FTIR and XRF analyses. Particular attention was given to the identification of quartz, feldspars and clay minerals, the characterization of silicate and aluminosilicate structural groups, and the evaluation of variations in the major-element composition of the soils. By combining mineralogical, spectroscopic and chemical data, the study aims to provide an integrated characterization of the investigated soils and to contribute to a better understanding of the influence of geological conditions, hydrothermal alteration and historical mining activity on their mineralogical and geochemical properties.

2 Material and methods

2.1 Sampling points

The locations of the sampling points are shown in Figure 1. Sampling points 1–5 are situated within the protected area of the historical Dubník Mines. Samples 1–4 were collected from the former mining area, whereas sample 5 was collected from a mine waste heap. The sampling point K represents the control site, located approximately 4 km from sampling point 5.

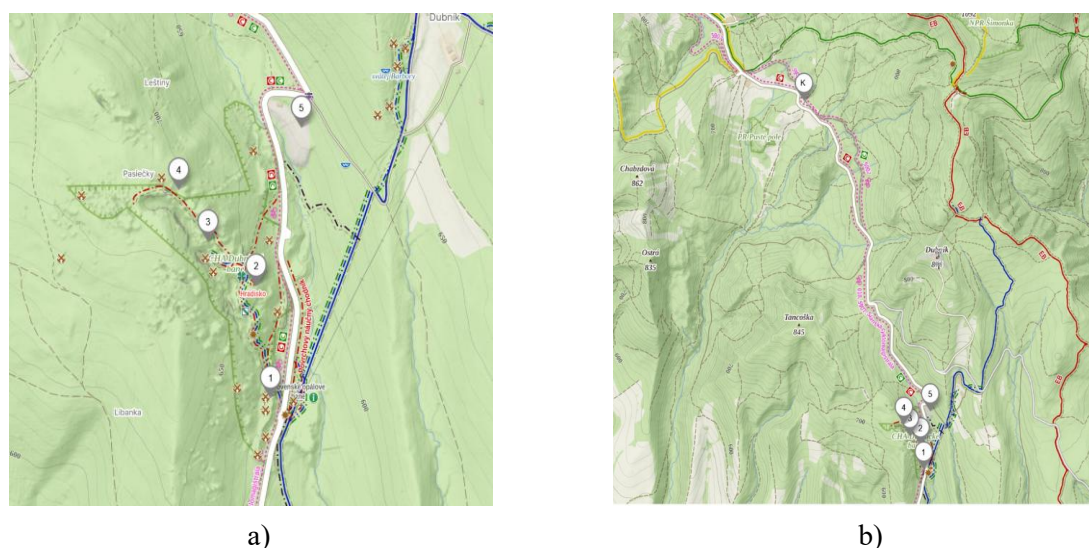


Fig. 1. Sampling points a) 1–5 in Protected Area – Dubník Mines, b) Location of control sampling point K, approx. 4 km from sampling point 5

2.2 Sample preparation and analytical methods

The soil samples were dried, homogenized, and sieved to a particle size of <0.063 mm prior to analysis. The chemical composition of the soil samples was determined by X-ray fluorescence (XRF) spectrometry using a SPECTRO iQ II spectrometer (AMETEK, Germany). The samples were analysed in powder form without fusion. The XRF analysis was used to determine the concentrations of the major elements expressed as their respective oxides.

Fourier Transform Infrared (FTIR) spectroscopy was performed using an ALPHA FT-IR spectrometer (Bruker Optics, Germany) equipped with an attenuated total reflectance (ATR) accessory. The spectra were recorded in the spectral range of 4000–400 cm⁻¹. The ATR-FTIR measurements were used to identify characteristic absorption bands associated with the mineralogical and structural components of the soil samples.

The crystalline mineral phases of the soil samples were identified using a Bruker D2 PHASER X-ray diffractometer (Bruker AXS GmbH, Germany) operating in Bragg–Brentano geometry (θ – 2θ configuration). Cu K α radiation ($\lambda = 1.54060$ Å) was used with a Ni K β filter and a scintillation detector. The instrument was operated at 30 kV and 10 mA. Identical scanning conditions were applied to all samples. The diffraction patterns were recorded over a total measurement time of approximately 5 h, using a step size of 0.05° (2θ) and a counting time of 15 s per step.

The XRD patterns were processed using DIFFRAC.EVA software, version 2.1. Mineral phase identification was performed using the ICDD PDF-2 database (Release 2009).

3 Results and discussion

Results of XRF analysis in Table 1 reveal differences in the major-element composition between the control soil (K) and the soils collected from the mining environment (samples 1–5).

Table 1. The XRF analysis of soils

Sample	MgO	Al ₂ O ₃	SiO ₂	SO ₃	K ₂ O	CaO	Fe ₂ O ₃
	%						
K	1.22	13.10	49.74	0.11	1.34	1.76	4.16
1	0.25	11.37	45.11	0.32	0.84	0.42	5.23
2	0.26	10.68	41.47	0.43	0.80	0.43	5.92
3	0.25	10.56	34.52	0.33	0.58	0.48	3.07
4	0.17	7.95	27.72	0.39	0.46	0.22	3.15
5	0.07	6.25	17.69	0.89	0.33	0.15	3.82

The control sample is characterized by the highest SiO₂ content (49.74 wt.%), followed by Al₂O₃ (13.10 wt.%). In comparison, the mining-area samples generally show lower concentrations of both oxides, although their contents vary considerably among the sampling sites. SiO₂ decreases from 45.11 wt.% in sample 1 to 17.69 wt.% in sample 5, while Al₂O₃ decreases from 11.37 wt.% to 6.25 wt.%. This pronounced variability indicates substantial differences in the relative contribution of Si- and Al-bearing mineral phases within the investigated materials. The relatively high SiO₂ content of the control sample is consistent with a greater contribution of quartz and other silicate minerals, in agreement with the mineralogical results. The lower SiO₂ and Al₂O₃ contents observed in several mining-area samples may reflect variations in the abundance of quartz, feldspars and clay minerals as well as the heterogeneous character of the mineralized environment.

Fe₂O₃ concentrations range from 3.07 to 5.92 wt.% in samples 1–5, compared with 4.16 wt.% in the control sample. The highest Fe₂O₃ content was recorded in sample 2 (5.92 wt.%), whereas samples 3 and 4 contain approximately 3.1 wt.%. These variations indicate differences in the distribution of Fe-bearing components among the sampling sites and may be related to local mineralogical differences within the mining area. The concentrations of K₂O and CaO are generally low in all samples. The control soil shows the highest K₂O (1.34 wt.%) and CaO (1.76 wt.%) contents, while lower concentrations are generally observed in the mining-area samples, suggesting differences in the abundance of K- and Ca-bearing mineral phases.

SO₃ displays a distinct pattern, particularly in sample 5, where its concentration reaches 0.89 wt.%, compared with only 0.11 wt.% in the control sample. Sample 5 is also characterized by the lowest SiO₂ content (17.69 wt.%) among all investigated samples. Importantly, sample 5 was collected from a mine

waste heap, and its distinct chemical composition may therefore reflect the mineralogical and geochemical characteristics of the deposited waste material rather than those of a naturally developed soil. The relatively elevated SO_3 concentration may indicate a greater contribution of sulphur-bearing mineral phases associated with the mineralized material.

Overall, the XRF data demonstrate chemical heterogeneity among the investigated materials. Compared with the control soil, the mining-area samples generally exhibit lower SiO_2 and Al_2O_3 contents, variable Fe_2O_3 concentrations and locally elevated SO_3 contents. The particularly distinct composition of sample 5 highlights the influence of the mine waste material and demonstrates that the geochemical characteristics of the sampling sites are strongly dependent on their specific geological and mining context. The observed variations are consistent with the heterogeneous geological and hydrothermal conditions of the Zlatá Baňa mining area and may also reflect the effects of historical mining activity and the redistribution of mineral components within the mining environment [3].

Figure 2 shows that the FTIR spectra of the soil samples from the Zlatá Baňa area exhibit broadly similar spectral features, indicating a comparable mineralogical composition of the investigated soils. The observed absorption bands occur in the $4000\text{--}3000\text{ cm}^{-1}$ area, around 1630 cm^{-1} , in the $1000\text{--}1100\text{ cm}^{-1}$, and below 600 cm^{-1} .

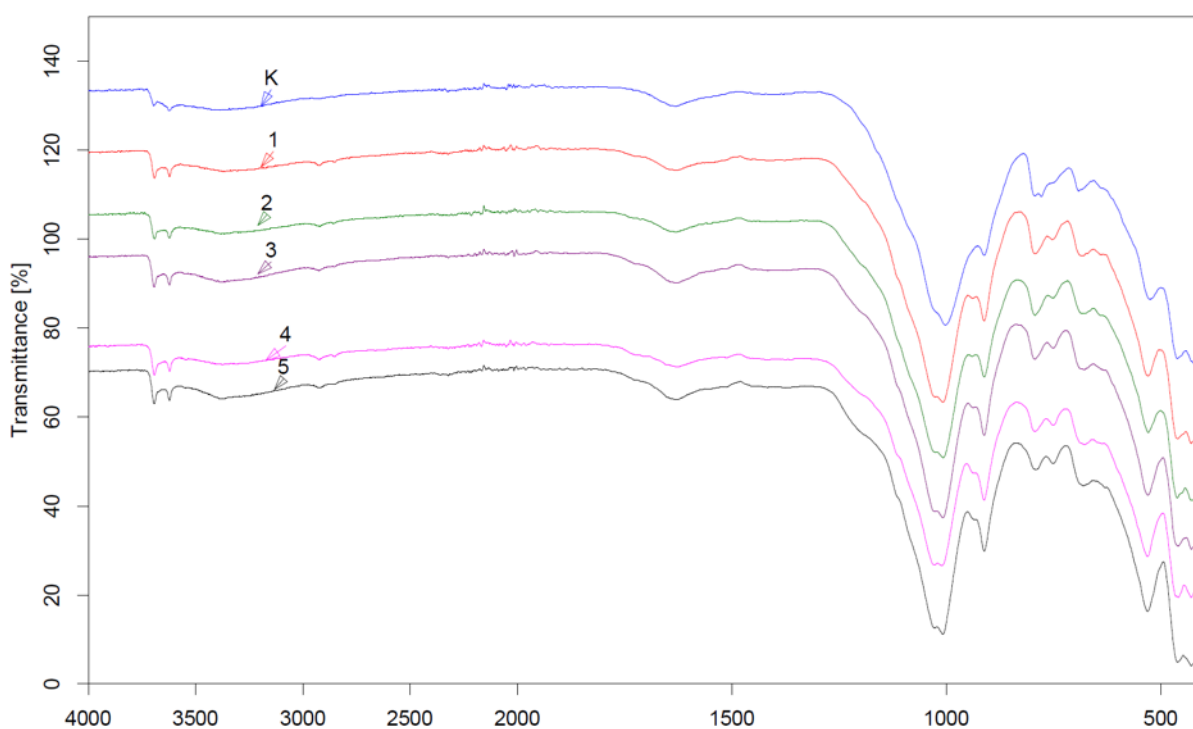


Fig. 2. FTIR spectra of soil samples from Zlatá Baňa

A particularly important feature is the strong absorption band in the area of approximately $1000\text{--}1100\text{ cm}^{-1}$, which is characteristic of Si–O stretching vibrations in silicate minerals. Together with the bands in the lower-wavenumber area, this suggests that the soils are dominated by silicate and aluminosilicate minerals.

The bands in the $\sim 3600\text{--}3700\text{ cm}^{-1}$ area can be associated with structural hydroxyl groups of clay minerals, while the broad band around $\sim 3400\text{ cm}^{-1}$ and the band near $\sim 1630\text{ cm}^{-1}$ indicate the presence of adsorbed or structurally associated water. The bands around $\sim 900\text{--}920\text{ cm}^{-1}$ may be related to Al–OH vibrations and therefore supports the presence of Al-bearing clay minerals [3].

The spectra also show the absorption bands in the $\sim 790\text{--}700\text{ cm}^{-1}$ area, which are consistent with quartz and other silicate phases. Therefore, the FTIR results indicate that the investigated soils contain a substantial proportion of quartz, clay minerals and other aluminosilicate phases [10].

The control sample K exhibits essentially the same major absorption peaks as samples 1–5. This indicates that the soils from the investigated area and the control soil have a similar basic mineralogical composition. However, differences in the relative intensity and shape of the absorption bands suggest variations in the abundance of individual mineral phases.

The mineralogical composition of the soils was investigated by X-ray diffraction (XRD). The XRD pattern of the control sample K (Figure 3) indicates a mineral composition dominated by quartz, with diffraction peaks corresponding to cristobalite, plagioclase, kaolinite and muscovite. The most intense quartz peak occurs at approximately $26.6^\circ 2\theta$, confirming quartz as the dominant crystalline silicate phase in the control soil.

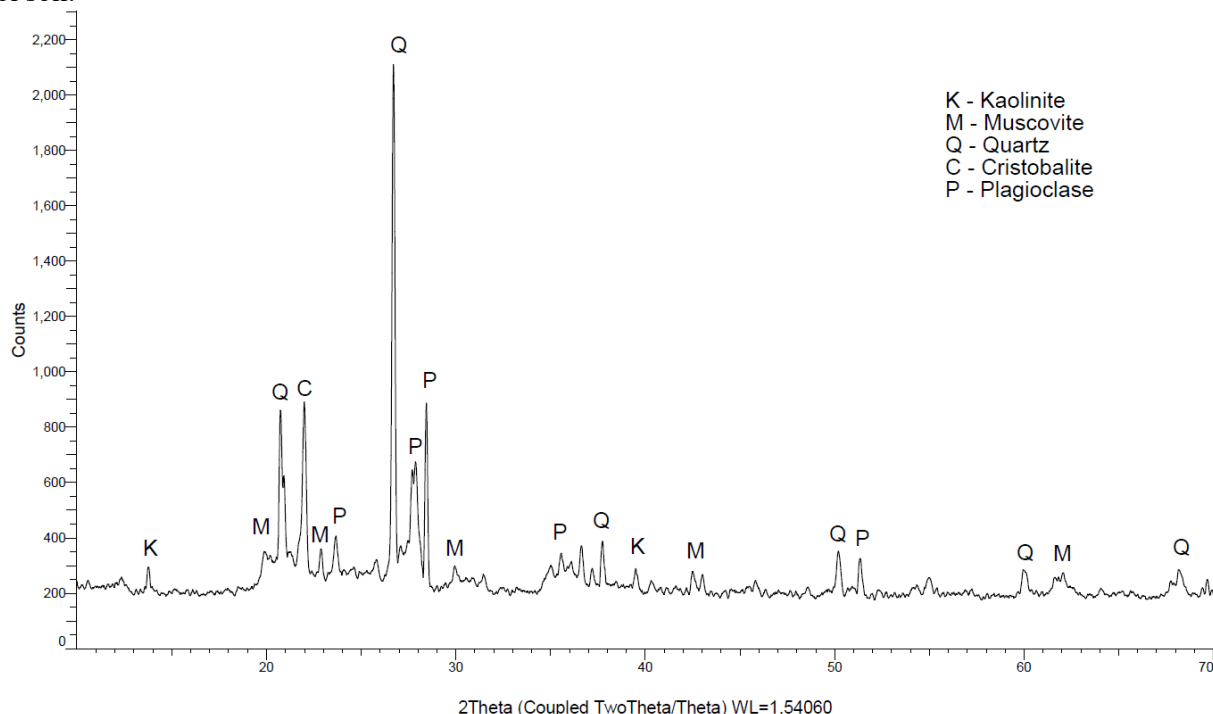


Fig. 3. XRD pattern of the control sample K

The presence of quartz is also supported by additional peaks at higher diffraction angles. Cristobalite is identified by characteristic diffraction peaks in the area around $21\text{--}22^\circ 2\theta$, while plagioclase is represented by several peaks, particularly in the approximate $22\text{--}30^\circ 2\theta$ area. Kaolinite occurs as a minor clay mineral phase, while muscovite is additionally identified in the control sample.

The XRD patterns of samples 1–5 (Figure 4) show a generally similar mineralogical composition. Quartz, cristobalite, kaolinite and plagioclase were identified in the investigated samples. Compared with the control, however, the relative intensity of the principal quartz peak changes considerably between the samples. In particular, the relative contribution of quartz is lower in samples 4 and 5 [11].

Sample 1 remains relatively similar to the control in terms of its mineralogical composition. The strong quartz-related reflection and the presence of cristobalite and plagioclase indicate a considerable contribution from crystalline silicate phases. This observation is in agreement with the relatively high SiO_2 concentration of 45.11 wt.% measured by XRF.

Sample 2 also contains the same major mineral phases, although the relative contribution of quartz is somewhat reduced compared with the control. The XRF data show a further decrease in SiO_2 to 41.47 wt.%, while Fe_2O_3 reaches its highest value of 5.92 wt.%. Thus, sample 2 is characterized by both a significant silicate component and an increased contribution of Fe-bearing material.

Sample 3 shows a further decrease in SiO_2 to 34.52 wt.%. Quartz, cristobalite, kaolinite and plagioclase remain identifiable in the XRD pattern, indicating that the basic mineralogical composition is retained despite the changing relative proportions of the phases.

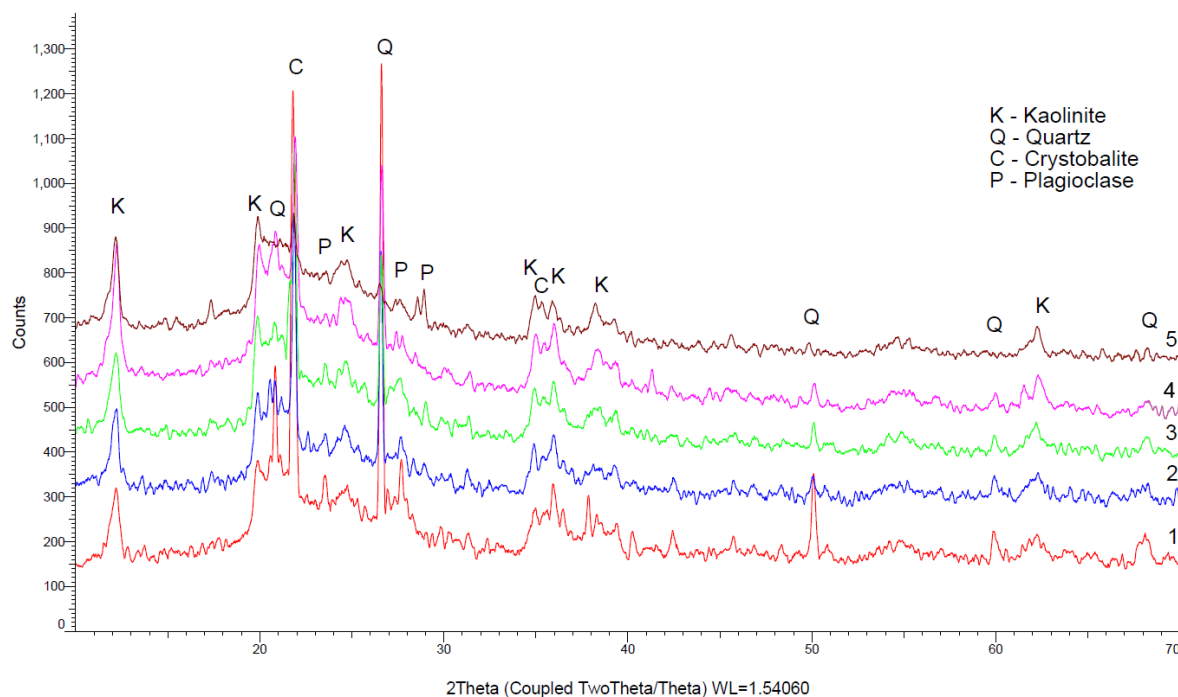


Fig. 4. XRD pattern of samples 1–5

Samples 4 and 5 exhibit the greatest mineralogical and chemical differences compared with the control. The quartz contribution becomes less significant, while diffraction peaks attributed to kaolinite remain clearly visible. This suggests a relative increase in the contribution of clay minerals compared with the quartz-dominated control material. The interpretation is consistent with the FTIR spectra, in which the characteristic bands associated with Si–O and O–H vibrations remain clearly observable.

Sample 5 represents the most distinct sample in the investigated set. Its SiO₂ concentration is only 17.69 wt.%, approximately 64% lower than that of the control. At the same time, the XRD pattern indicates a relatively strong contribution from kaolinite compared with the quartz-rich control. The mineralogical characteristics therefore suggest a transition from a predominantly quartz-rich silicate soil towards a soil with a greater relative contribution of clay/aluminosilicate phases.

4 Conclusions

The combined XRF, FTIR and XRD results show that the investigated materials from the Zlatá Baňa area are predominantly silicate–aluminosilicate materials containing quartz, cristobalite, plagioclase and kaolinite. Compared with the control sample K, the samples collected from the mining environment (1–5) show a progressive decrease in SiO₂ content and a corresponding reduction in the relative quartz contribution, particularly in samples 4 and 5.

The significant geochemical difference was observed in sample 5, collected from a mine waste heap, which has the lowest SiO₂ content (17.69 wt.%) and the highest SO₃ concentration (0.89 wt.%). This distinct composition may reflect the specific mineralogical and geochemical characteristics of the deposited mine waste material. Samples 1 and 2 are characterized by relatively higher Fe₂O₃ contents, with the maximum occurring in sample 2.

Overall, the results indicate significant chemical and mineralogical variability among the investigated materials from the mining environment, which is consistent with differences in the proportions of silicate and clay minerals and with the heterogeneous geological and hydrothermal conditions of the Zlatá Baňa area. The control sample K, collected outside the mining environment, provides a reference for comparison with the mining-affected samples, whereas the distinct composition of sample 5 reflects its origin from a mine waste heap. Further trace-element analyses would be useful for assessing possible relationships between the observed geochemical variations and the mineralization.

Acknowledgements

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BIOLOGICAL IRON OXIDATION OF ACID MINE DRAINAGE FOR MINERAL PIGMENT PRECURSOR RECOVERY

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Abstract

Acid mine drainage from the abandoned Smolník mine in Slovakia contains approximately 230 mg/L Fe at a pH close to 4, where abiotic Fe^{2+} oxidation proceeds slowly. We evaluated whether a pre-cultivated indigenous microbial consortium could accelerate Fe^{2+} oxidation and generate an Fe-rich precursor for mineral pigments. After 30 h of initial aeration, the mine water was inoculated with 50 mL of an exponentially growing consortium. Fe^{2+} decreased from approximately 230 to 1.4 mg/L within 29.5 h, corresponding to 99.4% oxidation. By comparison, 206 mg/L Fe^{2+} remained in the non-enriched control, equivalent to 10.7% oxidation. During the inoculated treatment, pH decreased from 4.1 to 2.6. Initial aeration reduced dissolved total Fe from 238.8 to 196.5 mg/L, and biological oxidation followed by filtration lowered it further to 15.7 mg/L without deliberate pH adjustment. Cu, Co, Mn and Mg remained predominantly dissolved, indicating selective Fe removal under the tested conditions. The first Fe-rich fraction scavenged arsenic before collection of the main precipitate. This orange-brown solid was amorphous to poorly crystalline and consistent with a schwertmannite-like phase. Pre-cultivation of microorganisms already present in the mine water therefore enabled rapid Fe^{2+} oxidation and produced a separable Fe-rich material suitable for evaluation as a mineral pigment precursor.

Keywords: acid mine drainage, biological iron oxidation, indigenous microbial consortium, Smolník, mineral pigment precursor

1 Introduction

Acid mine drainage and other mining influenced waters form when sulphide minerals oxidise, releasing acidity, sulphate, iron and associated metals [1]. Treatment commonly relies on neutralisation followed by precipitation of dissolved metals and acidity-generating constituents [2]. Although effective, this approach can produce large volumes of chemically heterogeneous Fe- and Al-rich sludge that are difficult to handle, dispose of or reuse. Controlled biological oxidation offers another route: dissolved Fe^{2+} is converted to Fe^{3+} before precipitation, allowing closer control over iron removal and the formation of Fe-rich secondary minerals [1, 3].

This oxidation step is especially relevant under acidic conditions. Below approximately pH 4, oxidation of Fe^{2+} by dissolved oxygen is slow, whereas acidophilic microorganisms can accelerate the reaction by several orders of magnitude [4]. The resulting Fe^{3+} hydrolyses and precipitates as minerals such as schwertmannite, ferrihydrite, jarosite or goethite; the products depend mainly on pH, sulphate concentration and solution composition [5].

The principal mine water discharge from the Pech shaft at Smolník typically has a pH of approximately 3.95–4.12 and a flow rate of about 6.5–10 L/s. Recent total Fe concentrations generally range from 190 to 260 mg/L, while sulphate exceeds 2 g/L in many samples. The water also contains Al, Mn, Mg, Zn, Cu, Co and As [6].

Previous 16S rRNA analysis found a diverse microbial community in Smolník mine water. *Acidithiobacillus* was not abundant in the Pech shaft water, whereas the iron-oxidising genus *Gallionella* was prominent, and more than 40% of sequences remained unclassified at genus level. When Pech shaft sediment was transferred to fresh 9K medium, the community shifted and *Acidithiobacillus* became dominant [7]. Selective cultivation in Fe^{2+} -rich medium can therefore enrich iron oxidisers that occur at low abundance in the original mine water.

The present study examined Fe^{2+} oxidation in real Smolník mine water using a pre-cultivated indigenous microbial consortium and assessed whether the resulting Fe precipitate could serve as a precursor for mineral pigments.

2 Methods

An indigenous microbial consortium was pre-cultivated in mine water collected from the main Pech shaft discharge. The water was supplemented with 9K salts to final concentrations of 1.6 mmol/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.76 mmol/L $(\text{NH}_4)_2\text{SO}_4$, 0.23 mmol/L K_2HPO_4 and 120 mmol/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. Cultures were grown in aerated, magnetically stirred baffled reactors with a 0.5 L working volume at 25 °C and 240 rpm. For the oxidation experiment, the mine water was first aerated for 30 h. The inoculated treatment received 50 mL of exponentially growing culture; a non-enriched control received no pre-cultivated inoculum.

pH was measured with an InLab Micro combined glass electrode, and oxidation-reduction potential was monitored with an InLab Redox Micro Pt-Ag/AgCl electrode. Both electrodes were connected to a Seven2Go S2 meter (Mettler Toledo).

Ferric iron was measured by UV spectrophotometry at 300 nm [8]. Ferrous iron was determined by a modified o-phenanthroline spectrophotometric method that is insensitive to Fe^{3+} interference [9].

Elemental concentrations in the water were determined by atomic absorption spectrometry (Varian AA240Z and AA240FS) and inductively coupled plasma mass spectrometry (Agilent 7700). Sulphate was analysed by ion chromatography (Dionex ICS 5000).

3 Results and Discussion

The initial Fe^{2+} concentration was approximately 230 mg/L. In the inoculated treatment, it decreased to approximately 123 mg/L after 6.5 h. The steepest decline occurred between 6.5 and 21.5 h, when the concentration fell to approximately 3.5 mg/L. At 29.5 h, only 1.4 mg/L Fe^{2+} remained, corresponding to an oxidation efficiency of 99.4% (Fig. 1).

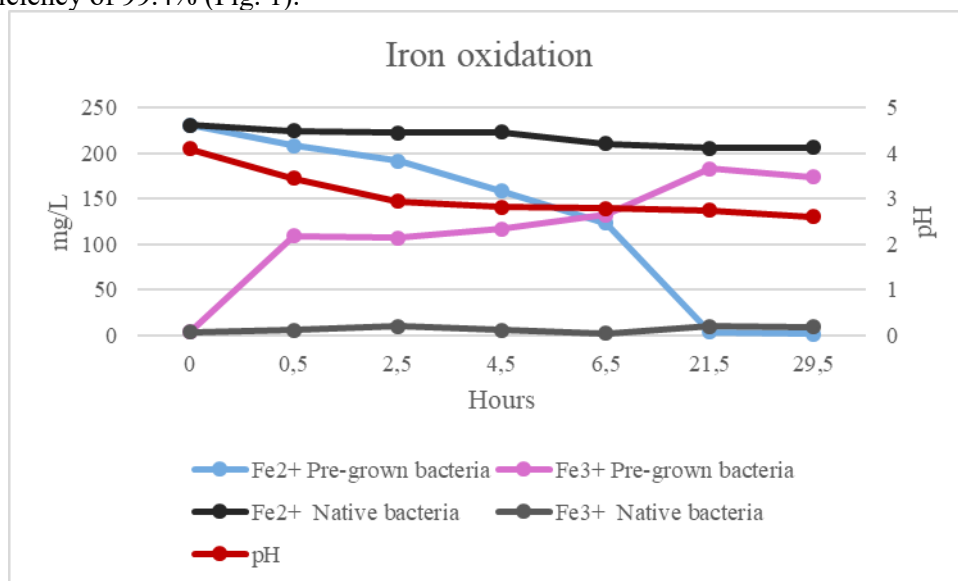


Fig. 1. Changes in Fe^{2+} and Fe^{3+} concentrations and pH during the 29.5 h oxidation experiment

In the non-enriched control, approximately 206 mg/L Fe^{2+} remained after 29.5 h, corresponding to only 10.7% oxidation (Fig. 2). Pre-cultivation therefore shortened the time required for near-complete Fe^{2+} oxidation under the tested conditions.

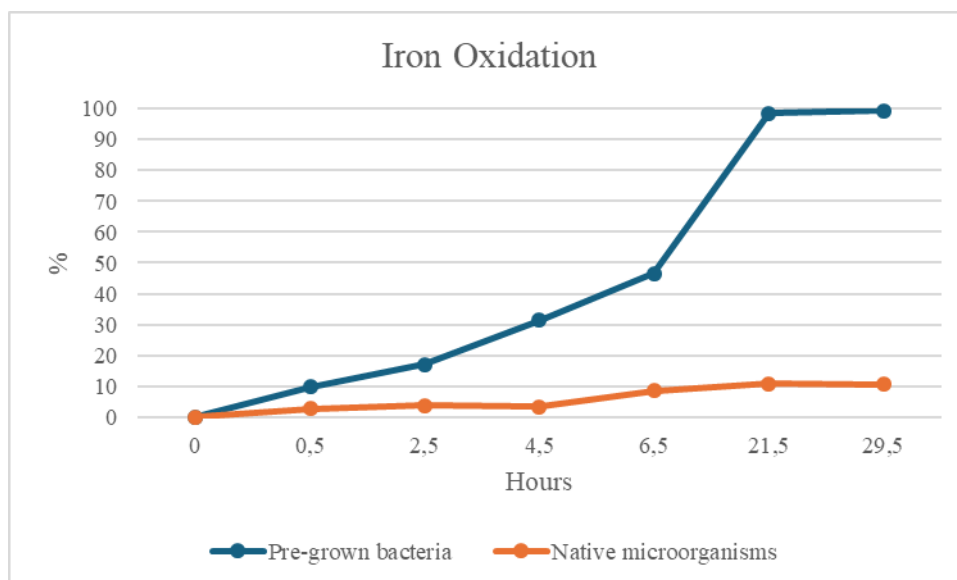


Fig. 2. Fe²⁺ oxidation efficiency in the inoculated and non-enriched treatments

The pH decreased from approximately 4.1 to 2.6 during oxidation. This decrease is consistent with proton release during Fe³⁺ hydrolysis and precipitation [5].

Initial aeration reduced dissolved total Fe from 238.8 to 196.5 mg/L. After biological oxidation and filtration, dissolved total Fe decreased further to 15.7 mg/L without deliberate pH adjustment. Most Fe removal therefore occurred during the microbial stage through the linked processes of Fe²⁺ oxidation, Fe³⁺ hydrolysis and precipitation.

Cu, Co, Mn and Mg remained predominantly dissolved during low-pH oxidation, showing that Fe was removed selectively under the tested conditions. The first Fe-rich fraction scavenged arsenic before the main pigment material was collected. The main precipitate had an orange-brown ochre colour and was amorphous to poorly crystalline, with characteristics consistent with a schwertmannite-like phase.

4 Conclusions

Pre-cultivation enriched an efficient Fe²⁺-oxidising consortium from the indigenous Smolník community. The inoculated treatment achieved 99.4% oxidation within 29.5 h, compared with 10.7% in the non-enriched control, despite the decrease in pH to 2.6.

Under these acidic conditions, the process selectively transferred most of the dissolved Fe into a separable, schwertmannite-like solid, while Cu, Co, Mn and Mg remained mainly in solution. Separation of an early arsenic-bearing Fe fraction from the principal precipitate may also improve the suitability of the recovered material for pigment production.

The process is best regarded as a biological oxidation and resource-recovery step rather than complete water treatment: 15.7 mg/L dissolved total Fe remained after filtration, so further polishing would be required where lower discharge limits apply. The approach recovered a large proportion of the dissolved iron as a mineral pigment precursor while leaving other dissolved elements available for subsequent separation.

Acknowledgements

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MICROPLASTICS AND SYNTHETIC FIBRES IN AQUATIC ECOSYSTEMS

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Abstract

Microplastics are a growing environmental problem. They are plastic particles smaller than 5 mm that can form from larger plastic waste or enter the environment as synthetic fibres. They can be found in rivers, lakes and mountain waters. This study focuses on monitoring microplastics and man-made fibres in selected aquatic ecosystems in Slovakia. Water samples were filtered through membrane filters and the captured particles were examined under a microscope. The aim was to identify the particles and compare their occurrence at different sites. The results confirmed the presence of microplastics in both areas affected by human activity and relatively protected mountain areas. The study shows the importance of regular monitoring of water and reducing plastic pollution.

Keywords: microplastic, man-made fibre, cellulose fibre, water sample, microscope

1 Introduction

Water is an important part of the environment and is essential for all living organisms. It can also transport various pollutants over long distances. Pollution can spread through rivers, lakes, and other water systems, affecting aquatic organisms and the surrounding environment. Some pollutants can also enter the food chain and ultimately affect humans.

One of the growing environmental problems is plastic pollution. Plastic products are widely used because they are cheap, lightweight and durable. However, plastic does not break down easily in nature. Larger pieces of plastic can gradually break down due to sunlight, temperature changes and mechanical abrasion. These particles, smaller than 5 mm, are called microplastics.

Microplastics can enter water from various sources. They can form through the breakdown of larger plastic waste or be released from synthetic textiles, especially during washing and use. Another source is the abrasion of plastic materials during everyday human activities. Due to their small size, microplastics can easily spread through water. They are found in rivers, lakes and mountain lakes, including relatively protected areas.

Microplastics are a concern not only for the environment but also for human health. They can enter the human body through food and water, by inhalation, or through skin contact. Their effects on human health are still being studied, but research suggests that they may affect the digestive and respiratory systems and trigger inflammatory responses.

For this reason, monitoring microplastics in the environment is important. This study focuses on the occurrence of microplastics and man-made fibres in selected aquatic ecosystems in Slovakia. The study has been carried out since 2021 at various locations. Water samples were filtered and the captured particles were examined under a microscope and compared with a database to determine their type.

2 Material and methods

2.1 Sampling and Sampling Sites

Water and snow samples were collected at different locations in Slovakia over a multi-year period. The sampling sites included both flowing and standing waters and represented different levels of anthropogenic impact.

In 2021, samples were collected from the Biela voda stream in Lendak, the Levočský stream, and the Orava Reservoir. In 2022, snow samples were obtained from Štrbské Pleso and Jamské Pleso. In 2023, samples were gathered from the Poprad River in Svit, with sampling carried out in May and November. In 2024, samples were collected from Popradské Pleso and Štrbské Pleso. In 2025, samples were collected from the Biely Váh River and Štrbské Pleso.

For the detailed analysis presented in this study, the results from 2023 and 2024 were used.

2.2 Sample Processing and Microscopic Analysis

The analysis of microplastics was based on the Microplastics Monitoring Protocol Trial (MMPT) methodology, which was developed and tested in collaboration with GLOBE Italia, LABTER-CREA Mantova and the Institute for Frontier Materials (IFM) at Deakin University, Australia.

After collection, 500 cm³ of each water sample was filtered in the laboratory under reduced pressure using a cellulose nitrate membrane filter with a diameter of 47 mm and a pore size of 0.45 µm. After filtration, the filter was transferred to a Petri dish and dried.

The dried filters were examined using a Proscope EDU-100 USB digital microscope at 300× magnification, equipped with a 5-megapixel image sensor. Each filter was divided into sections, which were photographed individually. Six photographs were obtained from each filter.

The photographs were subsequently analysed using Fiji software. The identified particles were classified into three groups: natural particles (cellulose fibres), synthetic fibres (man-made fibres), and microplastic fragments. The length of the fibres was measured, while the width and length of the microplastic fragments were recorded.

3 Results and discussion

All photographs were compared with the database, which was used to identify and classify the observed particles.

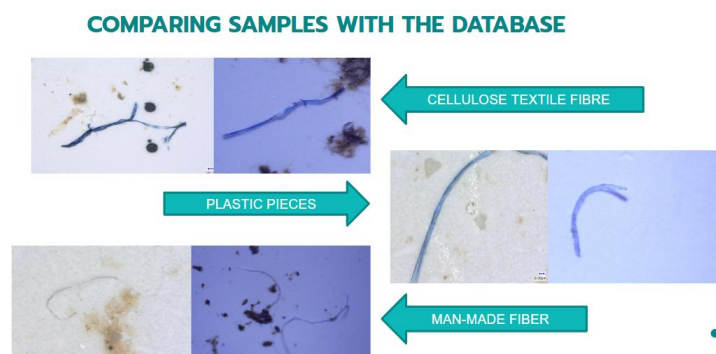


Figure 1 Comparison of obtained sample with the database

During the 2023/2024 school year, the study focused on detecting microplastics in the Poprad River upstream and downstream of the town of Svit. The aim of the study was to identify individual particles and determine their quantity in the samples. Sampling was carried out in November 2023 and May 2024. The obtained results are presented in Table 1.

Table 1. Number of particles in samples from the Poprad River

Particle type	November – upstream of Svit	November – downstream of Svit	May – upstream of Svit	May – downstream of Svit
Microplastic fragments	2	3	0	0
Man-made fibres	5	12	4	7
Cellulose fibres	5	0	0	0

The results show that man-made fibres were the most common particles, while microplastic fragments were found in smaller amounts. More particles were detected in November than in May. During the May sampling period, the weather was rainy, which may have contributed to the lower quantity of particles. A smaller number of particles was found upstream of Svit, which was in line with our expectations.

During the 2024/2025 school year, the study focused on detecting microplastics in the Popradské and Štrbské mountain lake in November 2024.

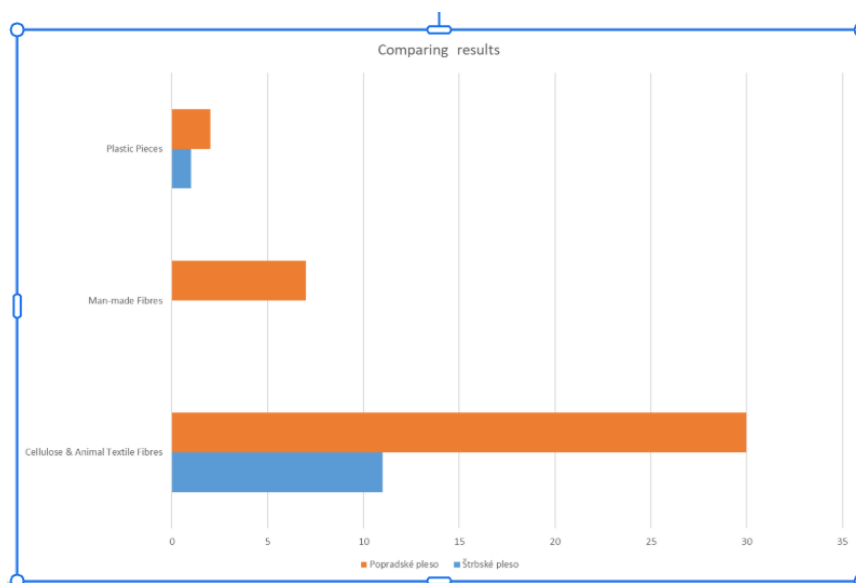


Fig. 2. Plastic pieces (up), man-made fibres (middle) and cellulose and animal textile fibres (bottom) in Popradské pleso (orange) and Štrbské pleso (blue) lakes.

The results show that cellulose and animal fibre were the most common particles in both mountain lakes. No man-made fibres were detected in the sample from Štrbské mountain lake, while some were found in the sample from Popradské mountain lake. The number of microplastic fragments was approximately the same in both samples.

4 Conclusions

The study confirmed the presence of microplastic particles and man-made fibres in the investigated water samples. Man-made fibres were the most frequently detected particles, while microplastic fragments occurred in smaller numbers.

In the Poprad River, more particles were detected in November than in May. A higher number of particles was also found downstream of Svit compared with the upstream sampling site. The rainy weather during the May sampling period may have influenced the smaller number of detected particles.

The results from Štrbské Pleso and Popradské Pleso showed that microplastic pollution can also occur in mountain areas. Although no man-made fibres were found in the Štrbské Pleso sample, some were detected in Popradské Pleso, while the number of microplastic fragments was similar in both samples.

The results show that microplastic pollution is not limited to areas with strong human activity. Regular monitoring of water is therefore important for understanding the occurrence and spread of microplastics in the environment.

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SECONDARY MINERAL FORMATION IN MINING-INFLUENCED WATER AFTER BIOLOGICAL PRE-TREATMENT

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Abstract

Acid mine water is characterised by high concentrations of dissolved metals and sulphates, creating challenging conditions for subsequent metal recovery. In this study, biologically mediated oxidation of ferrous iron was applied as a selective pretreatment step to remove ferric iron while maintaining divalent metals such as Mn, Co, and Ni in solution. Indigenous iron-oxidising bacteria continuously converted dissolved Fe^{2+} to Fe^{3+} , which simultaneously precipitated as secondary Fe(III) minerals as Fe(III) supersaturation increased. Subsequent solution amendment with NaOH or KOH for pH increase, or with K_2SO_4 for potassium enrichment, followed by equilibration enhanced the precipitation of ferric iron in the form of secondary minerals, with the resulting mineral phases determined by the applied amendment. XRD analysis confirmed the formation of jarosite after increasing potassium concentration via K_2SO_4 addition. Concentrations of Mn, Co, Ni, and Zn remained relatively stable throughout the experiment, demonstrating that biological pretreatment allowed their availability for downstream recovery processes. In contrast, arsenic concentrations decreased notably, likely due to coprecipitation with ferric iron phases. The study highlights the potential of combining biological oxidation with controlled secondary mineral formation to enhance iron removal while preserving critical raw materials in solution. Ongoing SEM-EDX analyses will further clarify mineral morphology and metal associations.

Keywords: secondary iron minerals, metal recovery, mining-influenced water, jarosite

1 Introduction

Mining-influenced water pose environmental legacy. Main source of acidity in acid mine drainage (AMD) is from oxidation of pyrite and other sulphides. AMD are typically characterised by low pH and elevated sulphates, metals and metalloids, as well. Typical high concentrations of Fe and Al ($>100 \text{ mg.L}^{-1}$), sulphates ($>1000 \text{ mg.L}^{-1}$) and Cu, Cr, Ni, Pb, and Zn ($>10 \text{ mg.L}^{-1}$). Dominant dissolved metals are Fe and Al, however cations such as Ca, Mg, Na, and K may also occur at elevated concentrations [1,2].

Stream acidity may also arise from naturally occurring organic acids leached from soils. However, in most instances the acidity of mining-influenced waters is primarily generated by sulphide oxidation [1].

Oxidation and hydrolysis of Fe in AMD generate hydrous ferric oxide precipitates, including schwertmannite and ferrihydrite, whose formation is strongly pH dependent. These phases form characteristic ochre precipitates and have high surface areas, promoting the adsorption and coprecipitation of trace metals. Consequently, iron precipitates strongly influence the mobility, fate, and transport of metals in AMD. In addition, Fe^{2+} oxidation and subsequent Fe^{3+} hydrolysis generate acidity and release dissolved metals into mining-influenced water [1].

The mining-influenced water investigated in this study originates from a flooded siderite mine located in the Spiš–Gemer Ore Mountains [3]. The biological process promotes the selective precipitation of ferric iron [4,5], while maintaining divalent metals such as Mn^{2+} , Ni^{2+} , Co^{2+} , and Mg^{2+} in solution [6]. This study focuses on the formation of secondary minerals after biological pretreatment of iron removal and characterisation of the secondary minerals.

2 Material and methods

2.1 Characteristics of bacterial oxidation experiment

Laboratory tests were conducted in quadruplicate way. Four 0.5 L reactors under stable thermostatic conditions (30°C) were magnetically stirred and air supplied to reactors was sterilised by filtration through

0.2 µm membrane filters. Detailed description of bacterial oxidation was already discussed in Čičáková et al. [6].

Concentration of metals in mining-influenced water was determined using atomic absorption spectrometer VARIAN AA240FS. Sulphate, magnesium, sodium, calcium and potassium concentrations were analysed by ion chromatography Dionex ICS 5000. Ferric iron was determined by a UV-spectrophotometric method at 300 nm [7]. Ferrous iron concentrations were determined using a modified o-phenanthroline spectrophotometric method insensitive to Fe^{3+} interference [8]. Physical-chemical parameters, such as pH and ORP were measured using probes Radiometer Analytical/HACH Lange for pH and Mettler Toledo InLab Redox-L type for ORP. Gas analysis, in term of inlet and outlet of O_2 and CO_2 measurements, were carried out using a paramagnetic oxygen analyser and an infrared carbon dioxide analyser (Sable Systems, USA), respectively. The analysers were calibrated using high-purity nitrogen (99.99% N_2) for zero point and ambient outdoor air for span calibration of O_2 (20.95%) and CO_2 (0.042%) concentrations. Air of the same quality was used to aerate the reactors. The data acquisition was performed by a software application developed in LabVIEW® (National Instruments).

The sludge was characterised by X-ray diffraction (XRD) using a D8 Advance diffractometer (Bruker AXS, Germany). The diffraction data were interpreted using X'Pert HighScore Plus 2.0a (version 2.0.1) software. The measured diffractograms were further processed using Diffracplus Basic and Microsoft Excel. Mineral abbreviation was supported by Warr [9].

Figure 1 depicts four reactors intended for biological oxidation of ferrous into ferric iron a) before experiment (left), b) after the experiment (right).



Fig. 1. Experiment set-up consisting of four 0.5 L reactors connected to online gas analysis, operated under stable temperature conditions, followed by pH adjustment and subsequent equilibration.

3 Results and discussion

Sample of mining-influenced water originated from flooded siderite deposit mine in Slovak Ore Mountains. The biological oxidation step converting ferrous to ferric iron has been described in detail in Čičáková et al. [6]. This study focuses on the formation of secondary minerals during and after biological treatment of iron oxidation followed by pH adjustment and subsequent equilibration of the solution. Initial concentration of selected observed species is summarised in Tab. 1.

Table 1. The characterisation of initial sample

name	concentration (mg.L ⁻¹)	name	concentration (mg.L ⁻¹)
Na ⁺	54.3	As	20.23
K ⁺	105	Fe	1.2
Ca ²⁺	475	Mn	69.1
Mg ²⁺	5703	Ni	2.6
SO ₄ ²⁻	29328	Zn	0.37
Cl ⁻	82.6	ORP (mV)	182
NH ₄ ⁺	38.5	pH	5.62

Biological oxidation carried out by indigenous bacteria oxidised 98.3 % of ferrous iron into ferric iron. The residual fraction of dissolved ferrous iron persisted in solution and should be eliminated prior to any subsequent recovery of critical raw materials, including Mn, Co, Ni, and Zn.

Initial water sample had pH values 5.62, which decreased to pH 2.5 after biological oxidation. During biological oxidation simultaneously precipitated Fe³⁺ as secondary Fe(III) minerals due to supersaturation of Fe³⁺ ions in the solution. Therefore, pH adjustment was performed by adding the required amounts of NaOH and KOH to raise the pH to 4 and promote the precipitation of residual of ferric iron in the form of secondary minerals (jarosite, schwertmannite, goethite, ferrihydrite, etc.), with the resulting mineral phases determined by the applied amendment.

The addition of K₂SO₄ was carried out for potassium enrichment to improve jarosite formation while maintaining constant pH conditions. The advantage of the biological oxidation step lies in the remaining of divalent cations Mn²⁺, Co²⁺, and Ni²⁺ in solution. Concentrations of selected metals and metalloids during the experiment are presented in Tab. 2. The relatively stable concentrations of Mn, Co, and Ni throughout the process indicate their suitability for subsequent recovery, for example through selective sorbents [10] or electrochemical methods [11]. The notable decrease in As concentration is likely attributable to its coprecipitation with ferric iron into the secondary sludge. Laboratory experiments are ongoing, and additional SEM-EDX analyses will be performed to further elucidate mineral morphology and elemental associations.

Table 2. Concentration of selected metals in the MIW during experiment

(mg.L ⁻¹)	<i>Before experiment</i>	<i>After biological oxidation</i>	<i>After equilibration</i>
Mn	69.15	67.5	56.6 ±3.15
Ni	2.6	2.43	2.18 ±0.31
Co	0.3016	0.3756	0.3 ±0.013
Zn	0.37	0.32	0.3 ±0.009
As	20.23	1.08	0.11 ±0.15

Based on the XRD analysis, jarosite formation was confirmed following the increase in K⁺ ions achieved by the addition of K₂SO₄ to the solution. In contrast, the predominant formation of schwertmannite was observed when KOH was used as the amendment. In the control sample, schwertmannite, ferrihydrite, and various iron oxide-hydroxide phases were detected, however, these mineral identifications require further verification using complementary techniques such as Raman spectromicroscopy. Additional morphological characterisation will be conducted in subsequent stages of the study to refine the interpretation of mineral phase development.

4 Conclusions

Series of experiments were carried out to study recovery of metals and critical raw materials in mining-influenced water. The conducted experiments demonstrated that biological oxidation is an effective pretreatment step for selective removal of ferrous iron from mining-influenced water. Approximately 98.3 % of Fe²⁺ was oxidised and subsequently precipitated as secondary ferric minerals, including jarosite formed after targeted K⁺ addition. Concentrations of Mn, Co, Ni, and Zn remained notably unaffected, confirming that the process preserves critical raw materials for further recovery. A significant decrease in As concentration indicates its coprecipitation with ferric phases, which may contribute to improved water

quality. Overall, the combined biological and mineral-forming approach shows strong potential for integration into multi-step metal recovery strategies. Further mineralogical characterisation will help to understanding of metal binding mechanisms in the produced sludge.

Acknowledgements

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BIOEXTRACTION OF MANGANESE FROM WASTE MATERIAL USING AN ADAPTED STRAIN OF THE MICROSCOPIC FILAMENTOUS FUNGUS *ASPERGILLUS NIGER*

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Abstract

The aim of this study was to evaluate manganese bioextraction from a manganese-rich waste generated during metallurgical production using an adapted strain of the filamentous fungus *Aspergillus niger*. Bioextraction experiments were performed for 9 days under static and dynamic cultivation conditions. Dynamic cultivation at 150 rpm promoted pellet formation, stronger medium acidification and approximately 27% higher biomass production than static cultivation, however static cultivation resulted in slightly higher manganese extraction. The final manganese concentrations reached approximately 500 mg.L⁻¹ under static conditions and 400 mg.L⁻¹ under dynamic conditions, corresponding to extraction efficiencies of $8.7 \pm 2.42\%$ and $6.9 \pm 0.9\%$, respectively. The difference between the cultivation systems was not statistically significant. These results demonstrate that the adapted *A. niger* strain can mobilize manganese from metallurgical waste, although biomass production and medium acidification do not directly determine the extraction efficiency.

Keywords: *Aspergillus niger*; bioextraction of manganese; metallurgical waste; fungal adaptation

1 Introduction

Manganese is the second most abundant transition metal in the Earth's crust. It is an essential element for living organisms and participates in various cellular functions associated with protein, carbohydrate and lipid metabolism [1]. At the same time, manganese-bearing waste produced during mining and metallurgical processing may represent both an environmental burden and a potential secondary source of manganese.

Microscopic filamentous fungi belong to a group of microorganisms whose metabolic activities significantly contribute to transformations of the chemical and mineralogical forms of different elements including manganese [2,3]. Fungi can promote mineral dissolution through several complementary mechanisms, including medium acidification, proton-promoted dissolution, complex formation and redox reactions involving extracellular metabolites.

Manganese oxides, hydroxides and oxyhydroxides are reactive mineral phases that play an important role in the biogeochemical cycling of numerous elements. Naturally occurring manganese minerals are susceptible to microbially induced transformations that may lead to mineral destabilization, dissolution or formation of new mineral phases [4,5].

The aim of the present study was to examine manganese bioextraction from a manganese-rich waste material originating from metallurgical production by an adapted strain of microscopic filamentous fungus *Aspergillus niger*. Manganese mobilization, fungal biomass production and medium acidification were compared under static and dynamic cultivation conditions.

2 Material and methods

2.1 Metallurgical waste material

The material used in the bioextraction experiments was a manganese-rich flue-dust fraction generated during metallurgical processing. Before the bioextraction experiments, the material was characterized by X-ray fluorescence spectrometry and powder X-ray diffraction to determine its elemental and mineralogical composition.

The elemental composition of metallurgical waste was determined using a SPECTRO XEPOS HE, XEP03 energy-dispersive X-ray fluorescence spectrometer. Powder X-ray diffraction patterns of the waste material were recorded using a Philips PW 3020 X'Pert diffractometer (PANalytical, Almelo, The Netherlands) equipped with a curved-crystal graphite monochromator and Cu radiation. Diffraction patterns were recorded over a range of 3° to 73° 2θ using a step of 0.05° 2θ.

The crystalline phases were identified using XRAYAN software, version 4.0.5 (Rigaku Corporation, Tokyo, Japan), and the International Centre for Diffraction Data powder diffraction database.

2.2 Fungal strain and adaptation procedure

An adapted strain of the microscopic filamentous fungus *Aspergillus niger* was used in the experiments. Adaptation was performed by repeated subcultivation over an extended period, during which the fungus was progressively exposed to increasing quantities of the manganese-rich waste material. The amount of waste added to 25 mL of Sabouraud dextrose agar increased stepwise from 1 to 20 g.

2.3 Bioextraction experiments

Bioextraction experiments were conducted in two cultivation systems: a static system and a dynamic system agitated on an orbital shaker at 150 rpm. For each experiment, 100 mL of sterile Sabouraud dextrose broth, SDB (HiMedia, Mumbai, India) was supplemented with 1 g of the metallurgical waste material. After homogeneous dispersion of the solid substrate, the suspensions were inoculated under sterile conditions with spores of the adapted *A. niger* strain. The fungal cultures were incubated for 9 days at 25 °C. Samples of the cultivation medium were collected at defined time intervals. Following filtration, the pH of the filtrates was measured and dissolved manganese concentrations were determined. The fungal biomass was recovered after cultivation and used to compare biomass production under static and dynamic conditions.

2.4 Determination of manganese concentration

The culture media filtrates were analysed for the total manganese content by flame atomic absorption spectroscopy (F AAS) at line Mn 279.5 nm using an AAS spectrometer Perkin-Elmer Model 1100 (Perkin-Elmer, Uberlingen, Germany).

3 Results and discussion

3.1 Chemical and mineralogical characteristics of the waste material

XRF analysis confirmed that the investigated flue-dust material was rich in manganese. Manganese was the dominant component (56.62 wt.% of elemental Mn). XRD analysis identified birnessite as the principal detected manganese-bearing crystalline phase.

3.2 Effect of cultivation conditions on fungal morphology and biomass

The cultivation conditions markedly affected fungal morphology. Static cultivation resulted in the formation of a surface mycelium. Following vegetative growth, aerial hyphae, conidiophores and fungal sporulation were observed. By contrast, agitation at 150 rpm promoted the formation of approximately spherical fungal pellets. Reproductive structures were not observed in the dynamic system.

Pellet formation during dynamic cultivation can decrease the apparent viscosity of the culture suspension, stabilize hydrodynamic conditions and improve nutrient and oxygen transfer compared with a highly dispersed filamentous mycelium. Consistent with these conditions, biomass production in the dynamic system was approximately 27% higher than under static cultivation.

3.3 Acidification of the culture medium

The initial pH of the culture medium was 5.65. During the 9-day cultivation, substantial acidification occurred in both cultivation systems. Under static conditions, the measured pH values ranged from 3.70 to 4.93. A more pronounced decrease was recorded in the dynamic system, in which the pH reached values between 2.12 and 2.37.

Nevertheless, the lower pH recorded under dynamic conditions did not result in higher manganese extraction. This finding suggests that bulk-medium pH was not the only factor controlling manganese dissolution. Other relevant factors may include the type and concentration of fungal metabolites, sorption of released Mn^{2+} by fungal biomass or precipitation of secondary manganese-bearing phases.

3.4 Manganese bioextraction

After 9 days, the concentration of manganese mobilized into the filtrate reached approximately 500 mg.L⁻¹ under static cultivation and 400 mg.L⁻¹ under dynamic cultivation. These concentrations corresponded to bioextraction efficiencies of $8.7 \pm 2.42\%$ and $6.9 \pm 0.9\%$, respectively. Although static cultivation produced a numerically higher manganese extraction efficiency, no statistically significant difference was

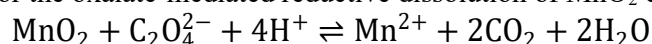
detected between the two cultivation systems. The variability associated with the static system was also greater, as indicated by the standard deviation of $\pm 2.42\%$.

Previous experiments with a non-adapted *A. niger* strain indicated that maximum manganese mobilization under static cultivation could occur around day 14. Therefore, the 9-day incubation used in the present study may have ended before maximum manganese extraction was achieved.

3.5 Proposed manganese dissolution mechanism

The manganese-bearing waste material contained MnO_2 mineral phase of birnessite. The dissolution of Mn(IV)-bearing phase may be enhanced by oxalate, which is a secondary extracellular metabolite commonly associated with fungal mineral transformations. Oxalate may act as a reducing and complexing agent, while protons participate in the acid-promoted destabilization of the mineral structure.

A simplified reaction for the oxalate-mediated reductive dissolution of MnO_2 can be expressed as:



In this reaction, Mn(IV) is reduced to soluble Mn^{2+} , whereas oxalate is oxidized to carbon dioxide. Oxalate concentrations were not determined in the present preliminary experiments. Consequently, the contribution of oxalate-mediated reductive dissolution cannot yet be distinguished from proton-promoted dissolution, complexolysis by other organic acids or other biologically induced processes. Future experiments should therefore include quantitative analysis of oxalate and other low-molecular-weight organic acids throughout cultivation.

4 Conclusions

The adapted strain of *Aspergillus niger* was able to grow in the presence of manganese-rich metallurgical waste and mobilize manganese into the liquid phase under both static and dynamic cultivation conditions.

It should be noted that dissolved manganese concentrations alone cannot distinguish between the total amount released through mineral dissolution and the net amount remaining in solution. A proportion of the mobilized manganese may have been removed from the liquid phase by biosorption onto fungal cell walls, intracellular accumulation or incorporation into newly formed biogenic mineral phases. Further analysis of manganese associated with the harvested fungal biomass and solid residues would therefore be required to establish a more comprehensive mass balance and better characterize the fate of mobilized manganese.

Acknowledgements

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PHOSPHORUS RECOVERY FROM SEWAGE SLUDGE ASH USING BIOGENIC SULPHURIC ACID

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Abstract

Phosphorus (P), derived from phosphate rock, is a critical resource for fertilizer production and food security; however, both P and phosphate rock are classified as EU critical raw materials, with supply highly dependent on non-EU imports. Incinerated sewage sludge ash (ISSA) represents a promising secondary P source, with approximately 7,000 tons of P lost annually due to sewage sludge disposal in Austria. Microorganisms such as *Acidithiobacillus thiooxidans* can metabolize elemental sulphur to produce biogenic sulphuric acid, thereby generating a biolixiviant that can be used to extract P from SSA. To lower process costs, waste sulphur sources can replace commercial sulphur. Within the study, different sulphur concentrations were investigated to maximize acid strength. For leaching, the optimization of ash composition, acid strength, pH, leaching duration, and pulp density plays a major role in determining the most effective conditions. P recovery is achieved through precipitation reactions that form compounds such as struvite, which can be directly used as an agricultural fertilizer, thereby closing the phosphorus cycle.

Keywords: Biogenic acid, *Acidithiobacillus thiooxidans*, phosphorus recycling, incinerated sewage sludge ash

1 Introduction

Phosphorus (P) is an essential element for all known forms of life and is a key component of fundamental biomolecules, including adenosine triphosphate (ATP), deoxyribonucleic acid (DNA), and ribonucleic acid (RNA). In plants, P plays a crucial role in photosynthesis, energy transfer, and numerous metabolic processes, making it an indispensable nutrient in agricultural fertilizers (Vance et al., 2003). P is primarily obtained from phosphate rock, the global reserves of which are highly concentrated in a few regions, particularly Morocco and Western Sahara, as well as China, resulting in significant geopolitical dependencies and supply risks (SCRREEN2, 2023). Approximately 80–90% of mined phosphate rock is used for fertilizer production, while the remainder is utilized in industrial applications, including food additives, detergents, flame retardants, and other specialty chemicals (European Sustainable Phosphorus Platform, 2022). The European Union has classified both phosphate rock and elemental P as Critical Raw Materials (CRMs), highlighting the strategic importance of securing alternative and secondary P resources. Mono-incineration of sewage sludge (SS) yields incinerated sewage sludge ash (ISSA), which has been identified as a promising secondary P resource, typically containing 5–10 wt.% P (Donatello & Cheeseman, 2013). At the same time, the direct agricultural application of sewage sludge is becoming increasingly restricted due to concerns regarding contaminants such as microplastics, pathogenic organisms, pharmaceutical residues, heavy metals, and per- and polyfluoroalkyl substances (PFAS) (Arvaniti et al., 2024; Christodoulou & Stamatelatou, 2016). Consequently, the development of efficient P recovery technologies from SS and ISSA has gained considerable attention as a pathway toward a more circular phosphorus economy.

Chemolithotrophic microorganisms such as *Acidithiobacillus* spp. obtain energy by oxidizing reduced inorganic sulphur compounds (RISCs) and/or ferrous iron. More specifically, *A. thiooxidans* gains energy through the oxidation of RISCs, including elemental sulphur, thereby acidifying the surrounding medium and producing biogenic sulphuric acid, which can subsequently be used in hydrometallurgical applications, such as the extraction of P from ISSA. To reduce costs, elemental sulphur can be replaced by alternative sulphur-rich waste streams, and cultivation conditions, such as level of sulphur dosage, must be optimized to increase the acid production capacity of the culture.

P extraction efficiency during the leaching process depends on the ash matrix, which may be affected by the incineration process itself. Addition of sulphates, carbonates, or chlorides prior to incineration has been shown to influence the mobility of heavy metals and potentially improve P plant bioavailability (Jeon & Kim, 2018; Luyckx & Caneghem, 2022). Upon solubilization of P from ISSA, recovery of P through precipitation as struvite ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$) by the addition of magnesium and ammonium salts is a promising method to produce a slow-release fertilizer. Struvite has been shown to be especially effective for plant species that release organic acids from their roots, thereby enhancing struvite dissolution and P uptake (Talboys et al., 2016).

2 Materials and methods

2.1 Biogenic acid production using *A. thiooxidans*

A. thiooxidans was cultivated in a basal salt medium according to cultivation conditions described by Frueholz et al. (2026). Initial experiments were conducted in 500 mL shake flasks with an inoculum volume of 10% and supplemented with different levels (1, 3, and 6% w/v) of biological sulphur obtained from a pulp manufacturing facility. Following these experiments, a series of cultivations was conducted in a double-jacketed 5 L reactor (Schmizo AG, Oftringen, Switzerland) using 6% w/v sulphur supplementation and a cultivation duration of 14 days. Sulphate concentration, OD600, and pH were measured as described in Frueholz et al. (2026) while protein concentration was determined following the Bradford assay. This work was conducted within the scope of a master thesis (Heibl, 2026).

2.2 ISSA leaching using biogenic sulphuric acid

For the leaching experiments, the biogenic sulphuric acid was used as a lixiviant and filtered through a sterile filter prior to use. An untreated ISSA (ISSA 1) was compared with an ISSA (ISSA 2) that had undergone addition using MgCl_2 . A pulp density of 10% was applied at a total volume of 1 L and a leaching duration of 2 h. After 2 h, the leachate was sterile filtered, and selected metals in solution were analysed by ICP-MS.

2.3 Struvite precipitation

Preliminary precipitation experiments were conducted using a synthetic leachate with the following concentrations (listed as g L^{-1}): P = 8.18, Mg = 1.57, Al = 0.86, Ca = 0.34, Fe = 2.91 in 0.5 M sulphuric acid. To reduce the concentration of interfering ions during the actual struvite precipitation process, a pre-precipitation step was conducted using $13 \text{ g L}^{-1} \text{C}_2\text{H}_2\text{O}_4$ and by adjusting the pH to 5. Struvite precipitation was performed by adding $18.5 \text{ g L}^{-1} (\text{NH}_4)_2\text{SO}_4$ and $53.7 \text{ g L}^{-1} \text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ and slowly raising the pH to 8.5 using 10 M NaOH. The precipitate was left to settle prior to sampling for liquid ICP-MS analysis.

3 Results and discussion

In Figure 1 the results of the shake flask experiments with different levels of sulphur are displayed. Sulphate concentration increases with increasing sulphur dosage, and there is a continuous increase in sulphate concentration over time.

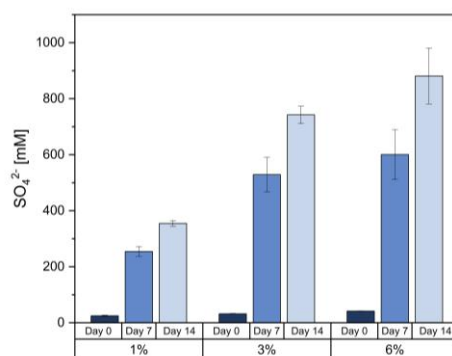


Fig. 1. Cultivation of *A. thiooxidans* using different levels of sulphur. Results are displayed as a mean of $n=4$ and error bars indicate standard deviation.

In Figure 2, the growth parameters of *A. thiooxidans* cultivated in a 5 L reactor supplemented with 6% sulphur are shown. Protein concentration and OD600 values fall after 9 days, whereas sulphate concentration

continues to increase until the end of the cultivation period. The pH is lowered until approximately 0.3. At the end of the experiment, a biogenic acid with an approximate concentration of 720 mM was produced.

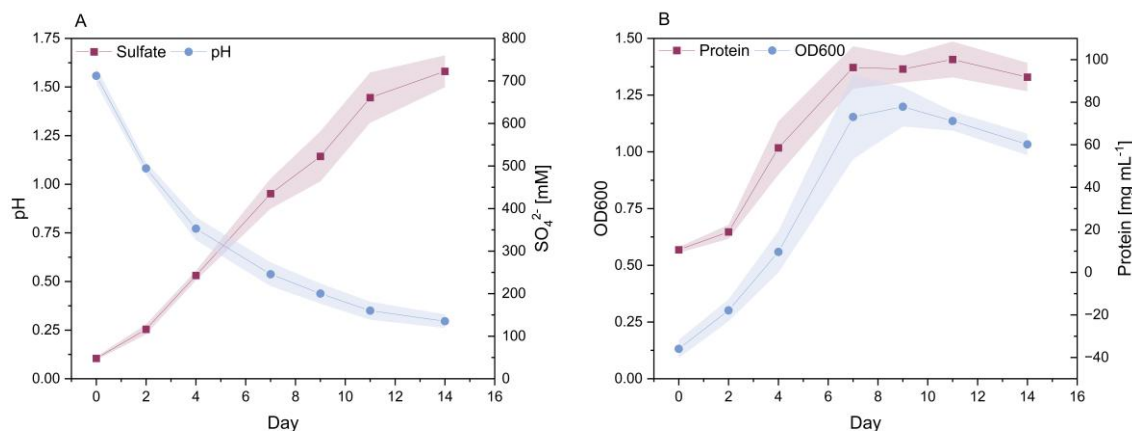


Fig. 2. Cultivation of *A. thiooxidans* in a 5 L reactor with 6% biological sulphur. Values represent the mean of $n=5$ and shaded areas show standard deviation. A) Course of sulphate and pH over time. B) Course of OD600 and protein over time.

In Figure 3A, the results of the leaching experiments regarding the control ISSA and the ISSA 1 (treated with additives) are presented. P solubilization corresponded to around 80% leaching efficiency for ISSA 1 and 90% leaching efficiency for ISSA 2. In terms of concentration, similar amounts of P and Ca were solubilized; however, ISSA 1 contained three times more Fe in the leachate compared with ISSA 2. This effect may be due to differences in Mg^{2+} concentration, requiring more acid for the dissolution of Mg-containing phases and resulting in a higher pH, ultimately leading to lower Fe solubilization. Further experiments at varying pH levels are required to further maximize P leaching while minimizing Fe solubilization. Regarding the preliminary precipitation experiment displayed in Figure 3B, the oxalate precipitation was effective in removing Fe and Ca, although only partially removing Al. Some P was lost; however, the majority of P could subsequently be precipitated as struvite. Additional experiments with the actual leachate are planned to determine the exact concentration of heavy metals in the final struvite product and to conduct plant growth experiments.

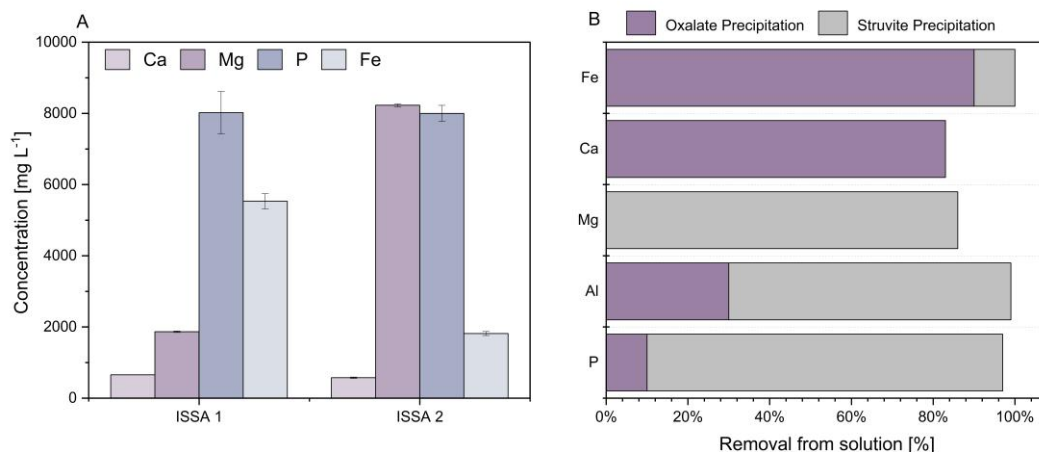


Fig. 3. A) Results of leaching experiments using biogenic acid, 10% pulp density, and 2 h leaching duration ($n=2$, error bars depict standard deviation). B) Accumulative removal from solution: Precipitation experiments consisting of a pre-precipitation step prior to struvite precipitation ($n=1$).

4 Conclusions

This series of experiments demonstrated the feasibility of producing a 0.7 M biogenic sulphuric acid from a biological waste sulphur source and subsequently using this biogenic acid for P extraction from ISSA. Using a pulp density of 10% and a leaching duration of 2 h, the majority of P was extracted, with ISSA 2

demonstrating improved results. Struvite was precipitated successfully, following an oxalate precipitation step to remove interfering ions such as Fe and Ca. Additional experiments are required to improve overall P selectivity and extraction efficiency, alongside an extensive characterization, including a determination of the heavy metal content, of the recovered struvite product.

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ASSESSMENT OF CADMIUM TOLERANCE IN OAT (*AVENA SATIVA*) AND ITS POTENTIAL FOR REMEDIATION OF CONTAMINATED SOILS

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Abstract

The aim of this study was to evaluate cadmium (Cd) tolerance in eight oat (*Avena sativa* L.) varieties and assess their remediation potential. Cadmium at 50 mg/kg soil had only a minimal effect on seed emergence, growth, and photosynthetic pigment content. A decrease in sulphur content was observed in all varieties, suggesting a possible role in Cd tolerance mechanisms. Cadmium accumulated mainly in roots rather than in shoots, indicating limited translocation to aboveground tissues. The varieties Pushinskij and V2 6/19 showed the highest Cd accumulation. Low translocation factor values suggest that oat is more suitable for phytostabilization than for phytoextraction of Cd-contaminated soils. These findings may contribute to the breeding of oat varieties for phytoremediation purposes.

Keywords: heavy metals, oat, remediation potential, tolerance

1 Introduction

Heavy metal contamination of soils represents a serious environmental problem caused mainly by industrial activities, urbanization, and intensive agriculture. Conventional physicochemical remediation methods are often expensive and unable to completely remove toxic elements from contaminated soils. Phytoremediation, based on the use of plants capable of accumulating and tolerating heavy metals, represents an environmentally friendly alternative [1].

Cadmium (Cd) is one of the most toxic heavy metals due to its high mobility and harmful effects on plant physiological processes, including photosynthesis, nutrient uptake, and enzyme activity. Agricultural soils are frequently contaminated with Cd due to the long-term application of phosphate fertilizers and organic amendments [2]. Since metal accumulation and tolerance vary among plant species and genotypes, the selection of suitable crops is essential for successful phytoremediation [3].

Oat (*Avena sativa* L.) is an important cereal crop with high nutritional and agronomic value. Previous studies have shown its ability to accumulate Cd from contaminated soils [4], although information about the Cd remediation potential of individual oat varieties remains limited. Therefore, the aim of this study was to evaluate Cd tolerance, accumulation capacity, and the phytoremediation potential of selected oat varieties grown under Cd stress.

2 Material and methods

2.1 Establishment of a pot experiment

Seeds of eight oat varieties (Pushinskij, Vok, Valentin, Vaclav, V2 6/19, Prokop, Racoon, and Aragon) were sown in pots (15 seeds/pot, pot diameter 15 cm) containing a white peat potting substrate (Klasmann KTS 2, Klasmann-Deilmann GmbH, Germany, pH KCl 6.7, gravimetric soil water content max. 70%, EC in μS 400, total Cd content 1.2 mg/kg, nitrogen content 1.65 g/kg, carbon content (C organic) 81.59 g/kg, phosphorus content 0.38 g/kg, potassium content 0.43 g/kg). The plants were grown in the Fitotron II plant growth chamber under controlled conditions: temperature 24 °C, humidity of 60%, with a 12/12 hour photoperiod and a light intensity of 20,000 lux. The plants were treated with tap water (control) or a Cd solution corresponding to 50 mg Cd/kg substrate in three biological replicates. Cadmium was used in the form of $\text{CdCl}_2 \cdot 2\text{H}_2\text{O}$. Plants were watered regularly throughout the experiment to maintain the maximum

sorption capacity of the soil. After three weeks, the seedlings were removed from the substrate and carefully washed free of substrate.

2.2 Determination of biomass and the tolerance index

The fresh weight (FW) of the roots and shoots of each plant was determined. The samples were then dried at 60 °C for two days to determine the dry weight (DW). The shoot tolerance index was calculated as follows: $TI (\%) = (\text{average biomass of Cd treated plants} / \text{average biomass of control plants}) \times 100$.

2.3 Measurement of photosynthetic pigment contents in leaves

The content of assimilation pigments (chlorophyll a – *Chla*, chlorophyll b – *Chlb*, total chlorophylls and carotenoids – *Car*) was determined in acetone extracts according to Lichtenthaler and Wellburn [5].

2.4 Measurement of cadmium and sulphur content in plant tissue and soil

Plant material and soil samples (0.5 g each) were digested in a mixture of 5 mL of water, 5 mL of HNO₃, and 1.5 mL of H₂O₂ (30%) using a Mars Xpress microwave oven. Cd content was measured by inductively coupled plasma optical emission spectroscopy (ICP-OES 725) according to Kováčik et al. [6]. Sulphur content was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES).

2.5 Evaluation of the efficiency of plant uptake

The bioaccumulation factor (BAF) and translocation factor (TF) were determined according to Usman et al. [7].

2.6 Statistical analyses

The obtained results were statistically analysed using XLSTAT software. The differences between the experimental variants were analysed using the t-test or the Kruskal-Wallis test followed by a Dunn's post hoc test ($p < 0.05$).

3 Results and discussion

The tested oat varieties exhibited high tolerance to Cd, with the TI ranging from 95.7 to 112.5 for shoot dry weight. The highest tolerance was found in the Aragon variety (TI = 112.5%) and the lowest in the Racoon variety (TI = 95.7).

The tested oat varieties accumulated higher concentrations of Cd in the roots (28.4–50.45 mg/kg DW) than in the shoots (17.27–18.30 mg/kg DW) (Fig. 1). The V2 6/19 variety showed the highest Cd accumulation in plant tissues, reaching 17.27 mg/kg DW in the shoots and 50.45 mg/kg DW in the roots (Fig. 1). In contrast, the Vok and Valentin varieties accumulated the lowest Cd concentrations in both roots and shoots.

The sulphur (S) content in the shoots either remained largely unchanged or decreased (Fig. 1). A statistically significant decrease was observed in the varieties Pushinskij, Valentin, and Racoon. The decrease in S content under Cd stress may indicate an increased demand for S-containing compounds involved in Cd detoxification, particularly in the synthesis of glutathione and phytochelatins responsible for Cd chelation and sequestration in vacuoles [8].

The high tolerance of the tested varieties was further supported by increased contents of photosynthetic pigments, with the most pronounced increases observed in the varieties exhibiting the lowest Cd content in the shoots (Fig. 2). Compared with the control, the *Chla* content increased by 1.27- to 2.04-fold, *Chlb* content by 1.08- to 1.33-fold, and *Car* content by 1.21- to 1.94-fold.

High tolerance to Cd at soil concentrations up to 20 mg/kg has previously been reported by Uraguchi et al. [9]. In contrast, growth inhibition, reduced photosynthetic pigment content, and lower yields have been reported at soil Cd concentrations as low as 10 mg/kg soil [4].

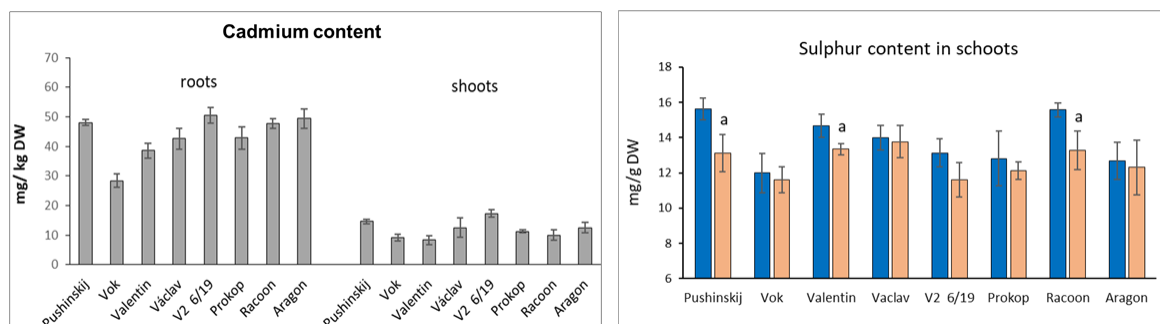


Fig. 1. Cadmium and sulphur content in roots and shoots of tested oat genotypes.

Data are presented as the arithmetic mean $\pm$ standard deviation. Statistically significant differences compared with the control were considered at $p < 0.05$.

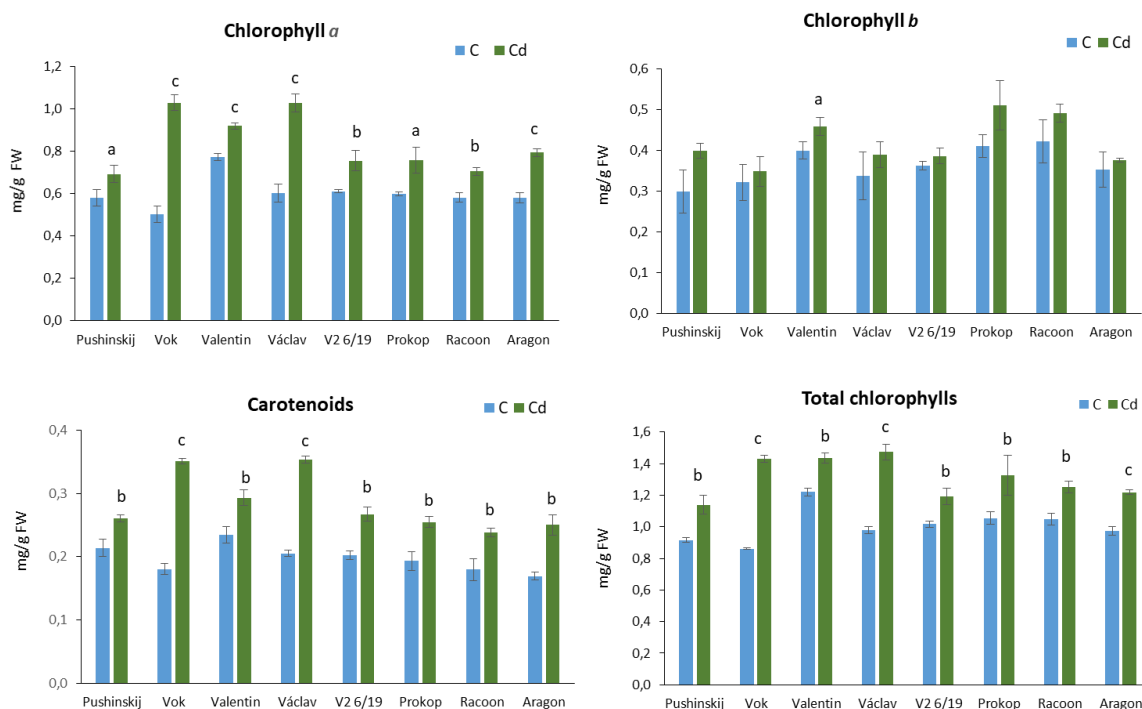


Fig. 2. Photosynthetic pigment content in oat shoots.

Data are presented as the arithmetic mean $\pm$ standard deviation. Significant differences compared with the control are indicated by different letters: a ($p < 0.05$), b ($p < 0.01$), and c ($p < 0.001$).

To evaluate the remediation potential of oat, the bioaccumulation factor (BAF) and translocation factor (TF) were determined (Table 1).

Table 1 Bioaccumulation factor (BAF) and translocation factor (TF) for the tested oat varieties

Variety	BAF for roots	BAF for shoots	TF shoots/roots
Pushinskij	0.96 ± 0.01 ab	0.29 ± 0.02 a	0.30 ± 0.02 abc
Vok	0.57 ± 0.03 d	0.18 ± 0.02 b	0.32 ± 0.06 ab
Valentin	0.77 ± 0.03 cd	0.19 ± 0.02 b	0.22 ± 0.05 cd
Vaclav	0.85 ± 0.05 bcd	0.25 ± 0.07 ab	0.29 ± 0.06 acd
V2 6/19	1.00 ± 0.04 a	0.22 ± 0.09 ab	0.35 ± 0.01 a
Prokop	0.86 ± 0.06 bcd	0.22 ± 0.01 ab	0.26 ± 0.01 bcd
Racoon	0.96 ± 0.02 abc	0.21 ± 0.06 b	0.21 ± 0.03 d
Aragon	0.99 ± 0.05 ab	0.25 ± 0.03 ab	0.25 ± 0.04 bcd

Data are given as means $\pm$ standard deviation of three biological replicates. Different letters indicate a significant difference ($p < 0.05$) between varieties for each parameter tested.

BAF values for roots were higher (0.57–1.00) than those for shoots (0.18–0.29). Relatively low TF values (0.21–0.35) also indicate low metal transport from roots to shoots (Table 2). The most efficient Cd transport to the shoot was shown by the varieties V2 6/19 (0.35), Vok (0.32) and Pushinskij (0.30).

4 Conclusions

The present study demonstrated that the tested oat varieties exhibited high tolerance to Cd and preferentially accumulated Cd in their roots. This high tolerance was associated with an increased content of photosynthetic pigments in the shoots, as well as a reduced sulphur content, which is most likely involved in the Cd detoxification process. The bioaccumulation factor values were higher in the roots (0.57–1.00) than in the shoots (0.18–0.29). The translocation factor ranged from 0.22 to 0.35. The high tolerance of oat to Cd, together with its considerable Cd accumulation in plant tissues, indicates its potential for the phytoremediation of Cd-contaminated soils, with the greatest potential observed for the V2 6/19 variety.

Acknowledgements

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ZINC AND ARBUSCULAR MYCORRHIZAL FUNGI IN DURUM WHEAT BIOFORTIFICATION: EFFECTS ON PLANT MORPHOMETRY, PHYSIOLOGY AND SOIL MICROBIOME

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Abstract

Zinc deficiency remains a major cause of micronutrient malnutrition and combining genetic and agronomic biofortification strategies is essential to enhance grain Zn accumulation in staple crops such as wheat. This study evaluated the effects of zinc fertilization (Zn), arbuscular mycorrhizal fungi inoculation (AMF), and their combination (AMF+Zn) on durum wheat (*Triticum durum* L., cv. IS Durablank) under controlled conditions, in comparison with an untreated control. Plant morphometric parameters (plant area, perimeter, compactness) were assessed using an automated RGB-based phenotyping platform, photosynthetic performance was evaluated via chlorophyll fluorescence (Fv/Fm, quantum yield), grain Zn concentration was determined by atomic absorption spectrometry, and soil microbiome composition was characterized by 16S rRNA amplicon sequencing. While morphometric and photosynthetic parameters showed only non-significant tendencies among treatments, grain Zn concentration was markedly higher in the Zn and AMF+Zn treatments compared with the control, and AMF inoculation induced a pronounced restructuring of the soil bacterial community, most notably in combination with Zn. These preliminary findings suggest a potential synergistic role of AMF in supporting Zn biofortification through soil-microbiome-mediated mechanisms and highlight the need for further studies integrating agronomic, physiological and metagenomic data across additional wheat cultivars.

Keywords: arbuscular mycorrhizal fungi, durum wheat, grain, soil microbiome, zinc biofortification

1 Introduction

Zinc deficiency represents one of the most widespread micronutrient disorders worldwide, affecting a large proportion of the global population [1]. Wheat grown on alkaline, calcareous soils with low organic matter is particularly prone to Zn deficiency, with consequences on crop productivity and Zn malnutrition in human populations [2]. Fertilizer-based zinc enrichment of cereal grains has therefore been proposed as a cost-effective and rapidly deployable strategy to address this problem on a large scale [3].

Genetic biofortification, through breeding of high-Zn wheat genotypes, has achieved considerable success and is now complemented by agronomic biofortification approaches, including soil and foliar Zn fertilization [4]. However, genetic gains in grain Zn content alone are often insufficient due to several soil-related constraints — including low moisture, low organic matter, and reduced root colonization by arbuscular mycorrhizal fungi — limit the phytoavailability and root uptake of zinc [3]. This underlines the need to combine mineral fertilization with biological strategies, such as inoculation with AMF, which can enhance nutrient acquisition through extraradical hyphal networks [5].

Long-term intensive cropping and selection for high-yielding cultivars have further contributed to a decline in grain mineral density across cereal crops [6], while a strong positive correlation between grain Zn and other essential micronutrients, such as Fe, suggests shared physiological and genetic mechanisms of mineral accumulation in the grain [7]. Within the broader context of sustainable crop production, integrating agronomic, biological, and genetic biofortification strategies is increasingly recognized as essential for improving both crop nutritional quality and long-term soil health [8].

The present study evaluates the combined and individual effects of Zn application into soil and AMF inoculation on grain Zn accumulation, plant morphometric and physiological parameters, and rhizosphere

microbiome composition in durum wheat (cv. IS Durablank). The aim of this study was to assess whether biological (AMF) and mineral (Zn) biofortification strategies act synergistically in enhancing grain zinc concentration while maintaining plant growth, photosynthetic performance and a favourable soil microbiome composition.

2 Material and methods

2.1 Experimental setup and plant growth conditions

The experiment was conducted on spring durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) cultivar 'IS Durablank' (National Registry Code: SK 115), grown in four biological replicates under four variants: control (untreated universal Klasmann substrate), AMF (Symbivit; arbuscular mycorrhizal fungi inoculation), Zn (ZnSO₄, 5 mg Zn/kg soil), and AMF+Zn (combined treatment). Plant growth and morphology were monitored non-invasively using an automated RGB-based phenotyping platform, extracting leaf area (mm²), plant perimeter (mm), and plant compactness from digital images (Photon Systems Instruments, spol. s.r.o., Drasov, Czechia).

2.2 Zn content determination

Grain mineral content was determined by atomic absorption spectrometry. Samples were homogenized (TissueLyser LT, Qiagen, Germany) and digested in concentrated HNO₃ (Multiwave 3000, Anton Paar, Austria), diluted to 25 mL with ultrapure water, and analysed for Zn on an iCE 3000 instrument (Thermo Scientific, USA; Solaar software), with accuracy verified against certified reference material.

2.3 Soil metagenomics

Soil microbiome composition was assessed by commercial 16S rRNA amplicon sequencing (Novogene), with bioinformatic processing following the QIIME2 pipeline.

2.4 Data analysis

Data are presented as treatment means $\pm$ SD and SE (n = 4). Treatment effects were evaluated by one-way ANOVA, followed by pairwise Welch's t-tests against the control; significance was set at $p < 0.05$.

3 Results and discussion

Morphometric evaluation of the plants using the automated phenotyping platform revealed variable, though statistically non-significant ($p > 0.05$; n = 4), treatment trends (Fig. 1). Total area of visible plant surface increased under AMF (+12%) and especially AMF+Zn (+14%) relative to the control, while Zn alone reduced it (−12%) (Fig. 1A). A similar, weaker trend was seen for norm length of plant perimeter, with AMF+Zn showing the highest value (+8%) and Zn and AMF alone differing minimally from control ($\leq 1.5\%$) (Fig. 1B). The most pronounced effect was observed for the compactness index, which decreased by 17% under Zn alone, suggesting a less compact, more elongated/irregular plant shape (Fig. 1C). In contrast, AMF and AMF+Zn deviated from control by no more than 2.3%.

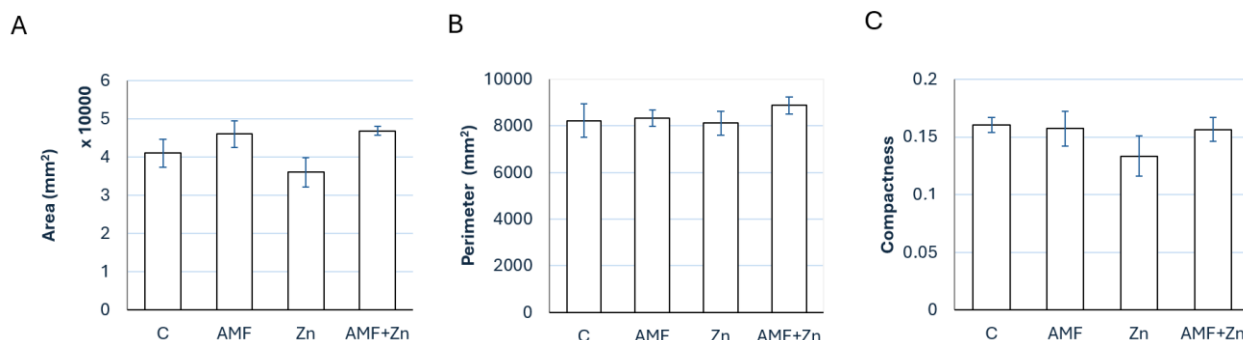


Fig. 1. Effect of arbuscular mycorrhizal fungi (AMF) and zinc (Zn) treatment on leaf morphometric and physiological parameters of durum wheat (cv. IS Durablank): (A) leaf area (mm²), (B) perimeter (mm) and (C) compactness. C – control; AMF – mycorrhizal inoculation; Zn – zinc fertilization; AMF+Zn – combined treatment. Data are presented as mean $\pm$ SE (n = 4).

Given the limited replication ($n = 4$) and high within-group variability, none of the observed differences reached statistical significance (ANOVA, $p > 0.05$ for all three parameters); these results should therefore be regarded as preliminary trends requiring confirmation with larger sample sizes.

Grain Zn concentration was highest in the Zn (156 mg/kg) and AMF+Zn (149 mg/kg) treatments, compared with the control (124 mg/kg), while the AMF-only treatment showed a slightly lower concentration (112 mg/kg, -10%). As grain Zn was determined from a single composite sample per treatment, these values are descriptive only and no statistical comparison could be performed.

Preliminary 16S rRNA analysis revealed distinct shifts in soil bacterial community composition among treatments (Fig. 2). The control was characterized by higher abundance of taxa such as Dependitiae, Elusimicrobiota, Acidobacteriota and Cyanobacteriota, while Zn+AMF showed marked enrichment of Actinomycetota, Bacillota, Chloroflexota and Gemmatimonadota (Fig. 2A), indicating a more pronounced community restructuring under the combined treatment than either factor alone. The Zn-only treatment showed a distinct profile, notably selective enrichment of Dadabacteria.

At the order level, the ternary plot (Fig. 2B) confirmed this pattern: Polyangiales, Chitinophagales and Vicinamibacterales clustered near the Control vertex, whereas Gemmatimonadales, Pseudomonadales and Hyphomicrobiales were disproportionately associated with AMF and Zn+AMF, with Gemmatimonadales showing the strongest shift. These preliminary, qualitative observations suggest that AMF — particularly combined with Zn — drives greater reorganization of the rhizosphere microbiome than Zn alone. Quantitative diversity and differential abundance analyses will be needed to confirm statistical significance.

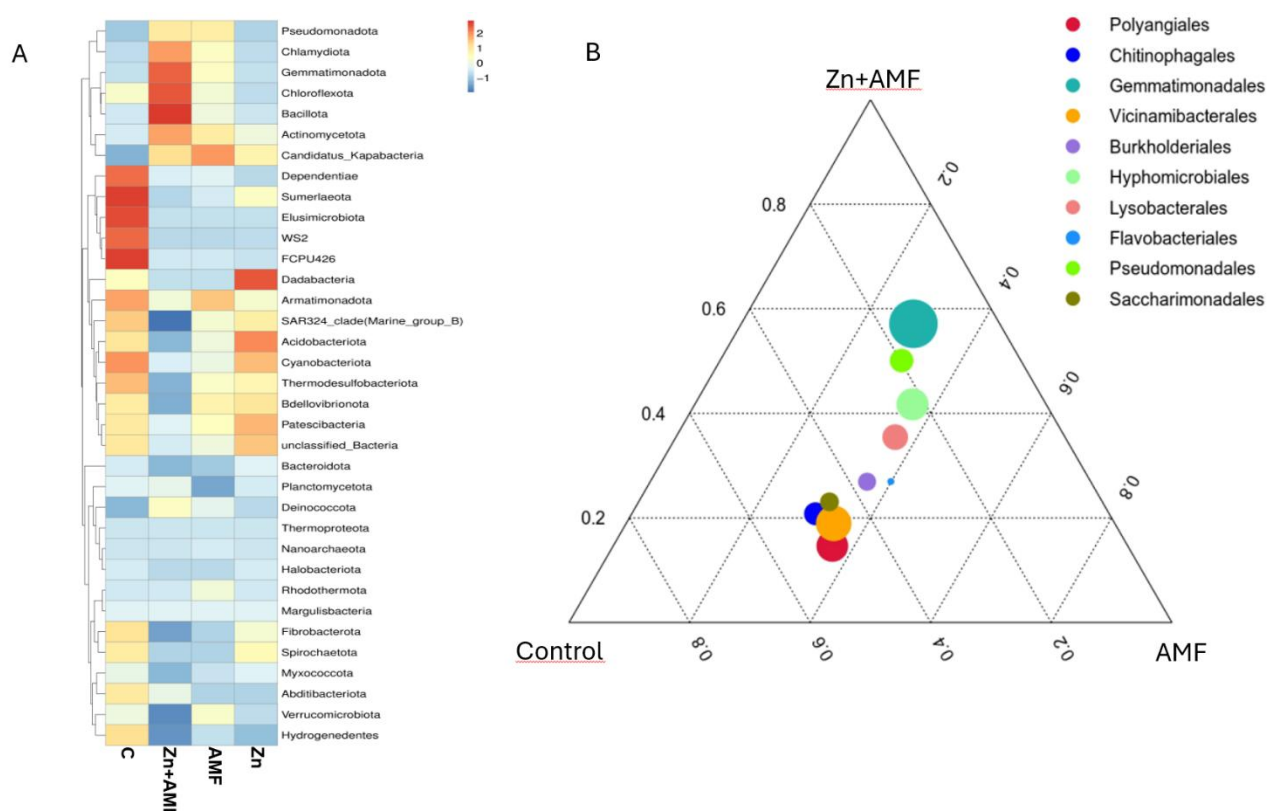


Fig. 2. Soil microbiome composition across treatments based on 16S rRNA amplicon sequencing. (A) Heatmap of relative abundance (row Z-score) of bacterial phyla across treatments (C – control, Zn, AMF, Zn+AMF), with hierarchical clustering of taxa. (B) Ternary plot of the top 10 most abundant bacterial orders across Control, AMF and Zn+AMF treatments, generated following Bulgarelli et al. [9]; bubble position reflects the relative distribution of each taxon among the three treatments, and bubble size reflects its overall relative abundance.

Unlike the morphometric and photosynthetic parameters, which showed only marginal, non-significant tendencies among treatments, grain Zn concentration displayed the most pronounced treatment-related difference, with both Zn-containing treatments (Zn and AMF+Zn) markedly exceeding the control. This suggests that Zn fertilization — with or without AMF — was effective in enhancing grain Zn accumulation even though this was not clearly reflected in visible growth parameters (leaf area, perimeter, compactness), consistent with reports that Zn biofortification does not necessarily compromise plant growth or yield [3, 5].

Notably, the AMF-only treatment resulted in slightly lower grain Zn than the control, which may indicate that, in the absence of additional Zn supply, AMF colonization alone was insufficient to enhance Zn uptake under the tested conditions — in contrast to the more favourable morphometric trends observed for AMF+Zn. This apparent discrepancy highlights the complexity of AMF–Zn interactions and reinforces the importance of combining biological and mineral biofortification strategies rather than relying on AMF inoculation alone [2, 3]. Because grain Zn data represent a single composite sample per treatment, these findings should be interpreted with caution and confirmed using biological replicates in future work.

4 Conclusions

This preliminary study shows that Zn fertilization, alone or combined with AMF, effectively increased grain Zn concentration in durum wheat (cv. IS Durablank), while morphometric parameters remained largely unaffected. AMF inoculation, particularly combined with Zn, additionally induced a more pronounced restructuring of the soil bacterial community than Zn fertilization alone, suggesting a potential role of AMF in mediating soil-plant interactions relevant to biofortification. Further studies comparing additional cultivars, with proper biological replication, will be needed to confirm the efficiency and cultivar-specificity of this approach.

Acknowledgements

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USING ADAPTATION PERIOD TO ENHANCE BIOLEACHING OF INDIUM FROM LCD SCREENS

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Abstract

This study focuses on the bioleaching of indium from ITO layers of waste LCD mobile phone screens using an adaptation period of the bacterial culture *Acidithiobacillus ferrooxidans*. The aim of the study is to evaluate whether the adaptation period plays a significant role in enhancing indium recovery. The experimental results of bioleaching performed with adapted and non-adapted bacterial cultures were compared to determine the importance of this step in the bioleaching process. The study further aims to provide a basis for future research and to contribute to the development of an optimized methodology not only for the recovery of indium from electronic waste but also for the recovery of other elements from different materials and waste. The material used for bioleaching consisted of LCD panel fractions with a particle size below 1 mm, which were subsequently subjected to bioleaching for a period of four weeks. ICP-OES analysis showed an increase in In concentration during bioleaching with both adapted and non-adapted *A. ferrooxidans*. After 28 days, the In concentration reached 4.252 mg/L for the adapted culture and 3.977 mg/L for the non-adapted culture. A comparison of the mean values showed a 6.9% higher In concentration for the adapted culture. These results suggest that bacterial adaptation may have a positive effect on In bioleaching from waste LCD material.

Keywords: bioleaching, bacterial culture, adaptation, LCD, indium

1 Introduction

With population growth the production and usage of electrical and electronic devices, and the amount of waste from them is growing in the same trend. This type of waste represents not just burden for the environment but a secondary source of raw materials, specifically metals [1]. Nearly all of them contain indium that is highly used in a lot of industries [2]. Indium is mostly used in form of indium tin oxide (ITO). It is used due to its important abilities such as electrical conductivity, transparency, mechanical stability and chemical stability and thus it is harder to replace it with alternatives. The most well-known application of ITO is in LCDs touch panels, OLED and LED screens, solar panels and more. It is made of indium oxide and tin oxide and typically produced in powder form; in LCDs there are two layers of it [3]. These layers can be separated, and the indium can be recycled. As raw material indium does not occur in economical deposits and is primarily recovered as a by-product of mining other materials. That is why is the recyculation of it quite important.

The recovery of ITO from waste can be performed using chemical leaching, especially chemical leaching. Despite their effectiveness, these methods can produce environmentally harmful emissions and chemical waste, emphasizing the importance of developing greener alternatives. Biotechnologies, particularly bioleaching methods, may provide new possibilities for the processing of waste containing ITO [4, 5]. These work in the same base as chemical processes, but they are using bacteria. They have an ability to make acidic environment without using large amounts of chemicals. Numerous research works have been performed using microorganisms such as *Acidithiobacillus ferrooxidans* [6] and *Acidithiobacillus thiooxidans* [1], which can be used either individually or together [7, 8]. Several optimizations can be applied to this process. One of them is the adaptation period, which allows the bacteria to adjust to the processed material, this adaptation period may be an important step, as it was carried in previous studies for the recovery of different elements such as Cu and Ni [9, 10]. It is done by cultivating bacteria with a small amount of material and then the adapted bacteria are reinoculated and used for bioleaching.

The aim of this study was to experimentally optimize bioleaching process by using an adaptation period for the bacteria. Pure of *Acidithiobacillus ferrooxidans* culture was used to recover indium from waste LCD screens. Two sets of experimental data were obtained, data from bioleaching with and without adapted bacteria. These data sets were analysed and compared in order to assess which approach is more effective for the leaching of indium.

2 Material and methods

2.1 Material preparation

The target material consisted of ITO layers obtained from liquid crystal displays (LCDs) of mobile phones. The displays used in the experiment were manually dismantled to separate the indium-containing layers from the other components. In LCDs, the first ITO layer, referred to as the Back Glass (BG), is located beneath the borosilicate glass that forms the outer surface touched by the user. This is followed by layers containing transistors, colour pigments, and other components. The second ITO layer is deposited on a plastic film. Both ITO-containing layers have the appearance of thin, almost iridescent glass, as shown in Figure 1(a) and (b).

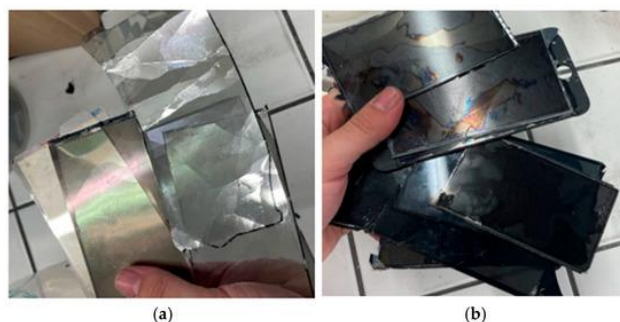


Fig. 1. Layers of ITO inside LCD

The mechanical processing of the separated layers was carried out using an LHM 20/16 hammer crusher (CONDUX-WERK, Mankato, MI, USA), in which the layers were crushed into smaller fractions and thus prepared for the subsequent processing step. The crushed material was then ground using a ball mill (LAC, MK Labor, Rajhrad, Czech Republic). After milling, the material was separated by sieving into several size fractions: <1 mm, 1–1.5 mm, 1.5–2 mm, and >2 mm. Based on previous experiments, which demonstrated that finer particle-size fractions were more suitable for the leaching process, only the <1 mm fraction was selected for the present experiment [6].

2.2 Adaptation and bioleaching

Pure strain of bacteria called *Acidithiobacillus ferrooxidans* was used, isolated from Zlaté Hory (Czech Republic) from acidic mine waters. Strain was obtained from the Czech Collection of Microorganisms in Prague. The pH for *A. ferrooxidans* is optimal from 1.5–2.5 and temperature from 30 to 35 °C. Energy is obtained through oxidation of compounds such as (Fe^{2+}) or sulphur (S^0). For cultivation and leaching sterile 9K medium was used, then 3 x 100 ml of it was inoculated with 5 ml of the bacteria in sterile Erlenmeyer flasks and left to cultivate in laboratory thermostat for 4 days at 30 °C. After primary cultivation 1 g of the prepared milled ITO layer was added to each flask with bacteria, and the adaptation process had started at the same temperature as primary cultivation. Whole adaptation lasted for 3 weeks.

Three experimental sets were prepared: three flasks inoculated with adapted *A. ferrooxidans*, three flasks inoculated with non-adapted bacteria, and two abiotic controls. Each biotic flask contained 285 ml of 9K medium and 15 ml of bacterial culture and was incubated for 4 days at 30 °C. The abiotic set contained 300 ml of 9K medium. Then 3 g of milled LCD material was added to each flask, and bioleaching was carried out at 30 °C and 150 rpm for 28 days. During the process samples of solutions were collected for indium analysis. At the end of the experiment, all samples were filtered, and the solid residues were dried and subjected to elemental analysis.

3 Results and discussion

3.1 Analysis of indium in collected samples of leaching solutions by ICP-OES

The concentration of In increased during the initial stage of bioleaching in all samples with adapted *A. ferrooxidans* (samples In + a, three replicates) as shown in figure 2. Between days 1 and 7, the In concentration increased from 2.00–2.35 mg/L to 3.64–4.44 mg/L. After day 7, the concentrations gradually stabilized. The highest In concentration of 4.66 mg/L was observed in sample In + a 1 on day 21. At the end of the 28-day bioleaching period, In concentrations ranged from 3.93 to 4.62 mg/L.

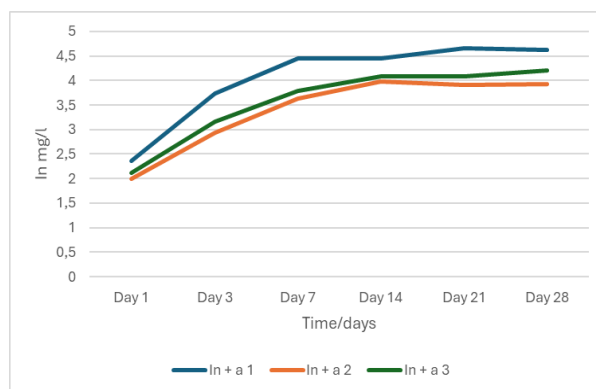


Fig. 2. Graph of indium concentrations leached by adapted bacteria

For leaching with non-adapted *A. ferrooxidans* (samples In - a, three replicates) as shown in Figure 3 the concentration increased rapidly during the initial stage of bioleaching in all samples with non-adapted *A. ferrooxidans*. It increased from 2.2–2.3 mg/L on day 1 to approximately 3.8–3.9 mg/L on day 7. After day 7, the increase became considerably less and the In concentrations gradually stabilized. Samples In - a 2 and In - a 3 showed a slight further increase, reaching 4.0–4.1 mg/L by day 28, whereas a slight decrease was observed in sample In - a 1 at the end of the experiment.

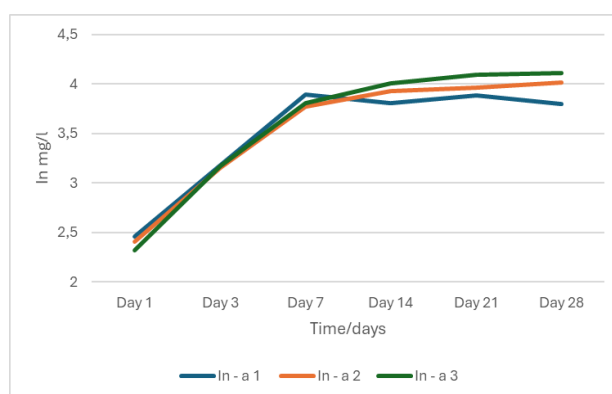


Fig. 3. Graph of indium concentrations leached by non-adapted bacteria

The adapted culture showed higher mean In concentrations than the non-adapted culture from day 3 onwards. The difference increased from 3.2% on day 3 to 6.9% at the end of the 28-day bioleaching period with adapted *A. ferrooxidans*. On day 28, the mean In concentration reached 4.252 mg/L for the adapted culture compared with 3.977 mg/L for the non-adapted culture. These results suggest a positive effect of adaptation on bioleaching of indium.

4 Conclusions

Sets of experiments were carried out to investigate if adaptation process for *A. ferrooxidans* can enhance leaching of indium from LCD screens of mobile phones. The aim was not to maximize In recovery, but to assess the potential effect of the adaptation period by comparing the In concentrations obtained by leaching with adapted and non-adapted *A. ferrooxidans*.

Both adapted and non-adapted *A. ferrooxidans* were capable of mobilizing In from the LCD material, with increase in In concentration occurring during the first 7 days of bioleaching. The adapted culture reached slightly higher In concentrations, with a maximum of 4.66 mg/L, suggesting a potential positive effect of the adaptation period. However, the differences between the experimental sets were relatively small and further statistical evaluation is required to confirm the significance of the observed effect.

Acknowledgements

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FLAX CULTIVAR VARIABILITY UNDER METAL STRESS: RELEVANCE TO PHYTOREMEDIATION OF CONTAMINATED ENVIRONMENTS

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Abstract

Cadmium (Cd) and zinc (Zn) are important stress factors that can affect plant growth and physiological processes. The response of two flax varieties (*Linum usitatissimum* L.)—Jitka and Agram—to Cd and Zn exposure was evaluated. Plants were cultivated in 1/4-strength Hoagland's nutrient solution supplemented with 20 mg·L⁻¹ Cd or 60 mg·L⁻¹ Zn. Stem length, tolerance index (TI), total superoxide dismutase (SOD) activity, and SOD activity-band profiles obtained by polyacrylamide gel electrophoresis and in-gel activity staining were evaluated. The results revealed variety- and metal-dependent responses. Under Cd treatment, Jitka exhibited a higher TI (88.51%) than Agram (79.41%), whereas under Zn treatment, Agram showed a higher TI (71.96%) than Jitka (61.23%). The inhibitory effect of Zn on stem growth was generally more pronounced than that of Cd. Total SOD activity also varied depending on the applied metal and flax variety: Cd treatment generally decreased SOD activity, whereas Zn treatment had a predominantly stimulatory effect. PAGE combined with in-gel SOD activity staining provided complementary information on changes in the profiles of individual SOD activity bands. These findings highlight the importance of genotypic variability in assessing flax tolerance to metal stress.

Keywords: cadmium, zinc, flax

1 Introduction

Heavy metals are important abiotic stress factors that can negatively affect plant growth, development, and metabolism. Cadmium (Cd) has no known physiological function in plants and, at elevated concentrations, can cause growth inhibition, metabolic disturbances, and oxidative stress [1–3]. Zinc (Zn), although an essential micronutrient, can also become toxic at excessive concentrations and disrupt plant physiological processes [4]. Plants respond to metal stress through antioxidant defence mechanisms involving enzymes such as superoxide dismutase (SOD), guaiacol peroxidase (GPX), and catalase (CAT) [2,5,6]. SOD represents a primary enzymatic defence against oxidative stress, while CAT and peroxidases contribute to the subsequent removal of hydrogen peroxide [5,6]. Changes in antioxidant enzyme activity can therefore provide important indicators of plant responses to metal stress [2,5]. Total SOD activity is commonly determined spectrophotometrically; however, this approach reflects the combined activity of several isoforms and does not distinguish their individual contributions [1–3]. Native polyacrylamide gel electrophoresis followed by SOD zymography enables isoform-specific activity patterns to be assessed and may therefore provide a more sensitive approach for monitoring changes induced by metal stress [1,2].

Flax (*Linum usitatissimum* L.) can tolerate and accumulate elevated concentrations of certain metals, although its response varies considerably among genotypes [7,8]. Ilavská et al. (2024) demonstrated substantial variability in Cd tolerance and accumulation among 31 flax varieties, based on growth and photosynthetic parameters [7]. These findings highlight the importance of combining growth characteristics with biochemical parameters, including antioxidant enzyme activity, to obtain a more comprehensive assessment of genotype-specific responses to metal stress [2,5].

The aim of this study was to evaluate the effects of cadmium and zinc on the growth and selected biochemical characteristics of two flax varieties—Jitka and Agram. Particular attention was paid to changes in the morpho-physiological parameters, and the activities of the antioxidant enzymes, including superoxide dismutase (SOD), with emphasis on its isoform-level evaluation using native PAGE and zymography.

2 Material and methods

2.1 Experimental setup

Two varieties of flax (*Linum usitatissimum* L.)—Jitka and Agram—were used in the experiment. Plants were cultivated for 21 days in 1/4-strength Hoagland's nutrient solution supplemented with 20 mg·L⁻¹ Cd or 60 mg·L⁻¹ Zn. Control plants were grown without the addition of metals. Cultivation was carried out under a 16/8 h photoperiod at a temperature of 28/18 °C. The experiment was conducted in three biological replicates. At the end of the cultivation period, stem length was measured from the base of the stem to its apex. The tolerance index (TI) was calculated separately for each variety and metal treatment according to the following equation: TI (%) = (mean stem length of treated plants / mean stem length of control plants) × 100.

2.2 Assessment of SOD activities

After 21 days of cultivation, crude protein extracts were prepared from plant material for the analysis of antioxidant defence enzymes. Total superoxide dismutase (SOD) activity was determined spectrophotometrically and expressed as specific activity (U mg⁻¹ protein). The protein concentration of each extract was determined before the SOD activity assay.

For the separation and visualization of individual SOD isoforms, 15 µg of protein from each sample was loaded onto a 12% (w/v) polyacrylamide gel (PAGE). Following electrophoretic separation, SOD activity was detected by in-gel activity staining (zymography). The results were obtained from three independent biological replicates and are presented as the mean ± standard error of the mean (SE).).

3 Results and discussion

The measured stem length and tolerance index values indicated a differential response of the Jitka and Agram cultivars to Cd and Zn exposure. Under Cd treatment, Jitka showed a higher tolerance index (88.51%) than Agram (79.41%), indicating that Jitka maintained its stem growth more effectively under the applied Cd treatment. Similar genotype-dependent variability in Cd tolerance has been reported by Pavlovičová et al. (2020), who recorded tolerance indices ranging from 63 to 89% among five flax cultivars, including Jitka. [9] Under Zn treatment, the tolerance index was 61.23% in Jitka and 71.96% in Agram. Compared with the corresponding Cd treatments, Zn caused a greater numerical reduction in stem growth in both cultivars, particularly in Jitka. In contrast to their responses to Cd, Agram exhibited a higher tolerance index than Jitka under Zn exposure. These results demonstrate that the relative tolerance of the two cultivars depended on the metal applied, supporting the genotype- and metal-dependent nature of the flax response to metal stress [10].

The preliminary results showed that Cd and Zn treatments altered SOD activity in a metal- and genotype-dependent manner (Fig. 1A). Cd treatment generally resulted in a decrease in SOD activity in both varieties, whereas Zn treatment had a predominantly stimulatory effect, but statistical proof is required.

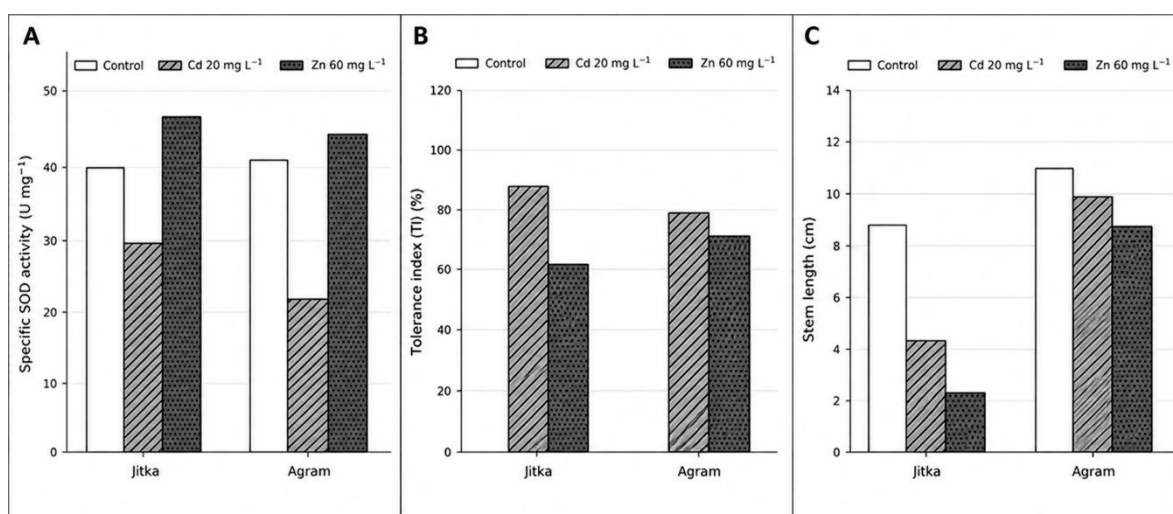


Fig. 1. Effect of cadmium and zinc on physiological parameters of flax genotypes Jitka and Agram. (A) Specific superoxide dismutase (SOD) activity (U mg⁻¹) in leaves. (B) Tolerance index (TI, %). (C) Stem length (cm). Plants were grown under control conditions or in the presence of 20 mg L⁻¹ Cd or 60 mg L⁻¹ Zn.

Spectrophotometric determination provides information about total SOD activity but does not distinguish the contributions of individual SOD forms present in the tissue. Therefore, SOD activity was also evaluated following protein separation by native PAGE. In-gel activity staining revealed a characteristic profile of distinct SOD activity bands and allowed treatment-induced changes in this profile to be monitored. Native PAGE separates proteins in their non-denatured state according to a combination of their charge, size, and conformation; consequently, the position of a band cannot be interpreted solely as its molecular mass. Moreover, assignment of individual bands to Fe-SOD, Mn-SOD, or Cu/Zn-SOD requires appropriate selective-inhibition assays.

A representative zymogram showing the profile of SOD isoforms in the tissues of the tested plants is presented in Fig. 2.

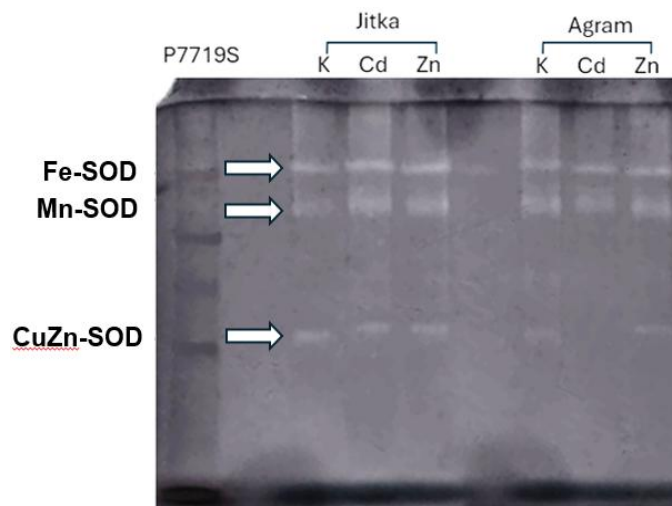


Fig. 2. Representative zymogram showing superoxide dismutase (SOD) activity in the leaves of selected flax varieties Jitka and Agram (*Linum usitatissimum* L.) cultivated under control conditions (K) or exposed to 20 mg L⁻¹ Cd or 60 mg L⁻¹ Zn. The Fe-SOD, Mn-SOD, and Cu/Zn-SOD isoforms are identified on the gel by arrows.

Given the sensitivity of this method to changes induced by metal exposure, the SOD activity-band profile could potentially be used as one of the biochemical indicators of plant exposure to elevated metal concentrations. However, its potential use as a bioindicator requires further validation across a wider range of plant species, environmental conditions, metal concentrations, and exposure periods. In further research, we plan to extend this approach to the evaluation of other antioxidant enzymes, such as catalase and peroxidases, and to assess its applicability under different experimental conditions, including the use of different plant species, metal concentrations, and exposure periods.

4 Conclusions

The results revealed variety-specific responses of flax (*Linum usitatissimum* L.) to cadmium and zinc exposure. Changes in growth and biochemical parameters confirmed the importance of genotype in plant responses to metal stress. These findings may contribute to a better understanding of the mechanisms underlying metal tolerance in flax.

The results confirm that Cd and Zn present in the cultivation medium affect the total activity of superoxide dismutase in the tissues of the tested plants, indicating that the antioxidant defence system responds to metal-induced stress. The direction and magnitude of these changes depended on both the applied metal and the flax variety. However, spectrophotometric determination of total SOD activity does not allow changes at the level of individual enzyme forms to be assessed. Therefore, the applied methodology, which combines polyacrylamide gel electrophoresis with subsequent in-gel detection of SOD activity, represents a suitable complementary approach for monitoring changes in the antioxidant response of plants. In comparison with the measurement of total SOD activity alone, this approach provides additional information on the activity profiles of individual SOD bands.

Acknowledgements

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THE REMEDIATION SCULPTURE

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Abstract

The Remediation Sculpture is an architectural and artistic intervention in an orchard located on the premises of the Waldorf Elementary School in Bratislava, in an area affected by long-term contamination from the chemical industry. Using traditional polycultural techniques and remediation biotechnologies, we launched a process of transforming the polluted soil, leading not only to an increase in biodiversity, testing of phyto- and mycoremediation techniques and evaluation of data for the scientific community, but, above all, to a change in the attitude of the community. The main idea is the transformation of environmental values into cultural values and vice versa. The design follows the principles of polycultural relations and of the edible forest garden. It combines plants capable of restarting chemical processes in the soil, improving soil structure, supporting the underground nutrient web, increasing organic matter and supporting water retention, with nitrogen-fixing plants, bee attractors and wildlife plants, supplemented with mycorrhizal fungi and oyster mushroom (*Pleurotus ostreatus*) to prolong the life of the old orchard trees. The intervention was evaluated as a phytostabilisation project. Its main benefits include increased biodiversity, revitalisation of the site, probable accumulation of pollutants in plant roots, support of microbial life in the soil and immobilisation of metals in the soil.

Keywords: remediation sculpture, mycorrhizal family, mixed environmental strategy, polyculture

1 Introduction

Groundwater and soil contamination is a longstanding problem rooted in more than 150 years of chemical industry activity and irresponsible political decisions. It also affects the orchard of an elementary school situated within the broader area of the Istrochem company, which children use daily.

The Remediation Sculpture is an architectural and artistic intervention into this environmentally and socially stagnant environment. It is an artistic argument that changes the environment through a critical pedagogy of toxicity, based on direct action in the field and not only on traditional forms of education. Using traditional polycultural techniques and remediation environmental biotechnologies [1–4], we launched a transformation process that led not only to an increase in local biodiversity, testing of combined phyto- and mycoremediation techniques, and testing and evaluation of data for the scientific community, but, above all, to a change in the attitude of the community.

The aim of this paper is to present the concept of the Remediation Sculpture, the plant and fungal species used and the first results of the laboratory analyses.

2 Material and methods

2.1 Study site

The intervention was carried out on the premises of the Waldorf Elementary School in Bratislava, where two old orchards are located. The first one (500 m²) contains fruit trees – apple, pear, mulberry and cherry; the second one (50 m²) contains horse chestnut (*Aesculus hippocastanum*). Apart from the trees, the land was continuously grassed.

According to the Hydrosan analysis from 2006 [14], the topsoil (0.0–0.3 m) is loamy sand with pH 6.4–6.7 (slightly acidic) and mineralisation of 1 755–2 052 mg/l. According to a geological survey from 1986, the profile consists of fill (gravel, concrete, loam) at 0.0–0.4 m, black-brown to black humic loam of almost firm consistency at 0.4–1.9 m, and well-compacted grey-brown sandy river gravel at 1.9–2.3 m.

The site is affected by long-term contamination; its duration and depth have not been determined. The contamination is very slight (borderline) and includes organic substances and metals: the pesticide metabolite chloridazon-desphenyl is present, and 4,4'-DDE and arsenic occur at limit concentrations.

2.2 Conceptual strategy

The orchard, as a landscape space with visible anthropogenic intervention and its own rhythm, has always played an important role in the cultivation of the landscape. It no longer belongs to the wild

landscape but to the management of cultural soil – “techno soil”. We proposed interventions in the form of “mycorrhizal families” – remediation circles understood as additional underplanting in this space (Fig. 1). Conceptually, however, this underplanting is precisely the moment that brings the existing trees into the cultural space as “exhibition artefacts” – sculptures. The main idea of the intervention is the transformation of environmental values into cultural values and of cultural values into environmental ones. The orchard as a gallery, the gallery as an orchard – the trees acquire a cultural identity, and this identity becomes the subject of environmental questions.



Fig. 1. Remediation circle – “mycorrhizal family” around an old orchard tree

2.3 Techniques used for the Remediation Sculpture

Principle of polycultural relations. The principle is based on the traditional cultivation practices of the original inhabitants of the oldest civilisations, i.e. on traditional ecological knowledge (TEK). High species diversity is used to create a functional ecosystem that supports the underground economy and fosters beneficial mutualistic relationships.

Edible forest garden principles. The design follows the structure of a natural forest [5, 6]. It uses different species with important chemical and physiological mechanisms that act in synergy with each other.

Plants supporting soil dynamics (dynamic accumulators). These plants were used to restart chemical processes in the soil, improve soil structure, bind soil communities together, support the underground nutrient web, increase organic matter and support water retention [7, 8]. Species used included *Mentha piperita*, *Solidago virgaurea*, *Calendula officinalis*, *Origanum vulgare*, *Rumex hydrolapathum*, *Achillea millefolium*, *Melissa officinalis*, *Symphytum officinale*, *Myrrhis odorata*, *Pulmonaria officinalis*, *Rubus fruticosus*, *Sambucus nigra* and *Cornus alba*.

Nitrogen-fixing plants. Biological nitrogen fixation, particularly the symbiotic type, plays a crucial ecological role in maintaining adequate nitrogen levels [9]. These plants create root nodules, are partners of mycorrhizal fungi, accumulate minerals, improve the soil and help to prevent diseases. Species used included *Lupinus* sp., *Glycyrrhiza glabra*, *Ceanothus* sp., *Galega officinalis* and *Elaeagnus multiflora*.

Bee attractors. Beneficial, mostly perennial species that attract bees and butterflies. Species used included *Angelica gigas*, *Valeriana officinalis*, *Buddleja davidii*, *Echinacea purpurea*, *Amelanchier canadensis*, *Myrrhis odorata*, *Verbena bonariensis*, *Ceanothus* sp., *Alcea rosea*, *Alchemilla vulgaris* and *Digitalis purpurea*.

Wildlife plants. These species provide shelter for animals, increase biodiversity, help to preserve native species, support symbiotic relations and increase organic matter; they also attract insects and act as natural insecticides. Species used included *Iris sibirica*, *Hemerocallis* sp., *Saponaria officinalis*, *Dipsacus fullonum*, *Nicotiana tabacum*, *Stipa tenuissima*, *Dryopteris wallichiana*, *Carex* sp., *Luzula nivea*, *Hakonechloa macra* and *Hosta* sp.

Mycorrhiza as a social concept of planting. Mycorrhiza is a mutualistic relationship between plants and fungi [10, 11]. Mycorrhizal fungi improve the access of plants to mineral nutrients, secrete digestive enzymes, help plants to tolerate stress, break down toxic substances and improve plant resistance.

Oyster mushroom. Together with the plants, the mycorrhizal fungi and the tree, the oyster mushroom completes the mycorrhizal family. The remediation effects of white rot fungi (Basidiomycota) have been known for a long time [12, 13]; they use their own enzymes to break down toxic substances in soil and wastewater. Mycelium of *Pleurotus ostreatus* was used to decrease the content of organic pollutants in the topsoil.

2.4 Laboratory analyses

Samples of *Cynara cardunculus* and *Malus domestica* were analysed in a laboratory for Pb, As and pesticides.

3 Results and discussion

The results of the laboratory analyses are given in Table 1. In *Cynara cardunculus*, the content of Pb was 0.178 mg/kg and that of As 0.12 mg/kg. In *Malus domestica*, the contents of Pb and As were lower than 0.01 mg/kg and 0.1 mg/kg, respectively. As these are single measurements, further monitoring is needed to confirm the observed trends.

Table 1. Content of Pb, As and pesticides in plant samples

Plant species	Pb	As	Pesticides
	(mg/kg)	(mg/kg)	
<i>Cynara cardunculus</i>	0.178	0.12	–
<i>Malus domestica</i>	< 0.01	< 0.1	–

4 Conclusions

The remediation method was evaluated as a phytostabilisation project. The planting, combined with mycoremediation techniques, first of all increased biodiversity around the trees and contributed to the revitalisation of the site. It initiated new mutualistic relationships that will benefit the existing trees and pollinators and supported the growth of certain plant species; lupin (*Lupinus* sp.) supports microbial life in the soil. The evaluation also indicated probable accumulation of pollutants in plant roots and immobilisation of metals in the soil. Further tests and evaluations of the plants, soil and groundwater are necessary, as well as the addition of important seasonal plants to the Remediation Sculpture.

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EVALUATION OF FUNGAL POTENTIAL IN CADMIUM BIOLEACHING FROM SOLID MINING WASTES AND ITS REMOVAL USING CHEMICALLY MODIFIED MANGANESE OXIDES

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Abstract

Bioleaching represents a promising approach for mobilizing metal(loid)s from mining residues and waste materials that may pose environmental risks. In this study, flotation waste from the Lintich tailing pond (Banská Štiavnica, Slovakia) was subjected to fungal bioleaching using adapted and non-adapted *Aspergillus fischeri* strains, achieving Cd extraction efficiencies of 3.38 % and 3.63 %, respectively. Although the resulting Cd concentrations in the leachates remained low, the Toxicity Characteristic Leaching Procedure (TCLP) test confirmed that measurable Cd mobilization persist under simulated landfill conditions. To assess removal options of Cd from the leachates, a synthetically prepared manganese-based sorbent was tested, achieving preliminary Cd removal of 22–30 % across tested concentrations. These results demonstrate the potential of fungal bioextraction and provide a basis for ongoing optimization of sorption-based strategies aimed at improving the removal of Cd from mining waste leachates.

Keywords: fungal bioleaching, cadmium, mine tailings, sorption

1 Introduction

Mining wastes represent a long-term environmental concern due to the presence of potentially toxic elements that may be released into surrounding ecosystems [1]. Among these elements, cadmium (Cd) is of particular concern because of its high toxicity, mobility and tendency to accumulate in living organisms [2]. Although a substantial proportion of metals in mine tailings may be incorporated into relatively stable mineral phases, changes in environmental conditions can promote their remobilization and subsequent transfer into soil and water systems [3]. The environmental mobility of Cd is of particular interest because it frequently exhibits a strong association with the readily soluble fraction in standard sequential solid-phase extraction procedures [4, 5], indicating a potential for mobilization under changing geochemical conditions.

Biotechnological approaches have attracted increasing attention as environmentally friendly alternatives for the remediation and valorization of metal-containing wastes. Fungal bioleaching utilizes the metabolic activity of microorganisms to mobilize metals from solid substrates, primarily through the production of organic acids and other extracellular metabolites. These processes may involve mineral dissolution through acidolysis and complexolysis, while fungal biomass can additionally contribute to metal immobilization through biosorption [6]. Species of the genus *Aspergillus* are frequently investigated because of their ability to tolerate elevated metal concentrations, produce metal-mobilizing metabolites and interact with mineral substrates [6–8].

Therefore, the aim of this study was to evaluate the potential of adapted and non-adapted *Aspergillus fischeri* strains to mobilize cadmium from mine tailing waste originating from Banská Štiavnica. In addition, the applicability of a synthesized manganese-based sorbent for the removal of dissolved Cd from leachates was preliminary assessed as a potential post-treatment step.

2 Material and methods

2.1 Mine tailing material

The flotation waste material used in this study was collected from the Lintich tailing pond located in the Banská Štiavnica mining district, central Slovakia [9]. Prior to the experiments, the material was sieved to obtain particle fractions below 0.8 mm and 2 mm and was characterized for total metal content using flame atomic absorption spectrometry (F-AAS; Perkin-Elmer Model 1100, USA).

2.2 Fungal strain and bioleaching experiments

An *Aspergillus fischeri* strain obtained from the culture collection of the Institute of Experimental Biology, Faculty of Natural Sciences, Masaryk University, was used for bioleaching experiments. The fungus was routinely maintained on Sabouraud Dextrose Agar (SDA) plates at 25 °C.

To obtain an adapted fungal strain, the strain was subjected to gradual exposure to the tailing material over several sub-cultivation cycles. The amount of mine waste added into 25 ml of SDA medium was progressively increased from 1 g to 20 g over four consecutive one-week adaptation stages.

Bioleaching experiments were carried out in 250 ml reagent bottles containing 150 ml of Sabouraud Dextrose Broth (HiMedia, India) supplemented with 7.5 g of mining waste. Both adapted and non-adapted *A. fischeri* strains were investigated. Biotic controls (inoculated medium without tailings) and abiotic controls (tailings without fungal inoculation) were prepared under identical conditions. All treatments were performed in triplicate. Following sterilization at 121 °C for 15 min, the media were inoculated and incubated statically in the dark at approx. 25 °C for 14 days. After incubation, fungal biomass was separated from the liquid phase, and the pH of the residual media was measured. These leachates were filtered through 0.45 µm membrane filters and analysed for cadmium concentration by F-AAS.

The recovered fungal biomass was rinsed with distilled water and dried at 60 °C to constant weight. For metal determination, dried biomass samples were digested in closed PTFE vessels using concentrated 5 ml HNO₃ and 2 ml H₂O₂ at 150 °C for 3 h prior to F-AAS analysis.

2.3 Toxicity Characteristic Leaching Procedure (TCLP)

To assess the potential mobility of Cd under simulated landfill conditions, the mine tailing material was subjected to the Toxicity Characteristic Leaching Procedure (TCLP) according to EPA Method 1311 (U.S. Environmental Protection Agency, 1992). Based on preliminary pH measurements, Extraction Fluid No. 1 (0.1 M acetic acid, pH 4.93±0.05) was selected as the extraction solution. For each test, 1.5 g of dried sample was mixed with 30 ml of extraction fluid and agitated for 18 h at 23±2 °C and 30 rpm. The resulting suspensions were filtered through 0.45 µm membrane filters and the concentration of Cd in the leachates was determined by F-AAS.

2.4 Synthesis of MnO₂/Mn(II)-oxalate and sorption experiments

A manganese-based sorbent was synthesized by thermal precipitation from manganese sulphate (MnSO₄·H₂O) and oxalic acid (H₂C₂O₄·2H₂O) solutions. Prior to synthesis, the pH of the oxalic acid solution was adjusted to 2.7 using 10 % NaOH. The reaction was carried out under reflux at 80 °C for 3 h in the presence of MnO₂ as a nucleation agent at a MnO₂:Mn-oxalate molar ratio of 1:1. After cooling and maturing overnight, the precipitate was recovered by filtration (0.45 µm), washed with distilled water and dried at 60 °C to constant weight.

Sorption experiments were performed using stock solution prepared from Cd(NO₃)₂·4H₂O. Batch sorption experiments were carried out in 50 ml tubes by adding 0.1 g of a prepared manganese-based sorbent to the cadmium solutions with initial concentrations of 2, 10, 20, 35 and 50 mg/l at pH 4 (adjusted with HNO₃) using a rotatory shaker. After 24 h the suspensions were filtered (0.45 µm) and the residual Cd concentration in the filtrates was determined by F-AAS.

3 Results and discussion

The total content of Cd in the analysed mine tailing was 27 mg/kg, which is consistent with the ranges reported in previous studies from the same site, which was 14–42 mg/kg [10, 11]. Cadmium often binds to carbonates, which increases its availability under acidic conditions [12], so given its higher mobility, the U.S. EPA (EPA-SW846/1311) sets a limit of 1 mg/l for Cd. The Cd concentration in our TCLP extracts reached 0.48 mg/l, which is below the regulatory limit, and the material thus meets the criteria for safe landfilling.

The presence of mine tailings promoted fungal growth in both *A. fischeri* strains. Biomass production increased by approx. 1.4-fold from ~0.6 g to ~0.9 g for non-adapted and from ~0.7 g to ~1 g for adapted strain. Despite enhanced growth, only limited acidification of the medium was observed, with both strains reaching approximately the same final pH in the presence of the material (~5.88), which is higher, compared to the tailing-free samples (~5.32).

Figure 1 shows that the total extraction efficiency of Cd, including metal concentrations in the culture medium and fungal biomass after the cultivation, was relatively low, reaching extraction efficiencies of 3.63

% and 3.38 % for the non-adapted and adapted strains, respectively. The comparable, statistically insignificant results indicate that adaptation did not significantly improve Cd bioleaching under the tested conditions.

To assess whether residual Cd could be removed from the leachates (TCLP and fungal bioleaching), batch sorption experiments were conducted using the synthesized sorbent across a range of Cd concentrations (Fig. 2). Overall, the Cd removal efficiency was relatively low, ranging from 22.2 % to 30.9 %. Nevertheless, the observed removal indicates an affinity of manganese oxide surface towards cadmium, which is consistent with previous findings reported by Dolinská et al. [13], who observed improved Cd^{2+} adsorption after modification of bentonite with manganese oxides. In the present study, Cd removal varied with the initial metal concentration, with the highest value observed at the lowest tested concentration (2 mg/l). This trend is consistent with the commonly reported decrease in removal efficiency at increasing metal concentrations, which may result from the progressive saturation of available sorption sites [14, 15]. However, the relatively higher removal observed at 50 mg/l deviates from this general trend and may indicate the contribution of additional processes, such as precipitation, although this cannot be confirmed based on the present dataset alone.

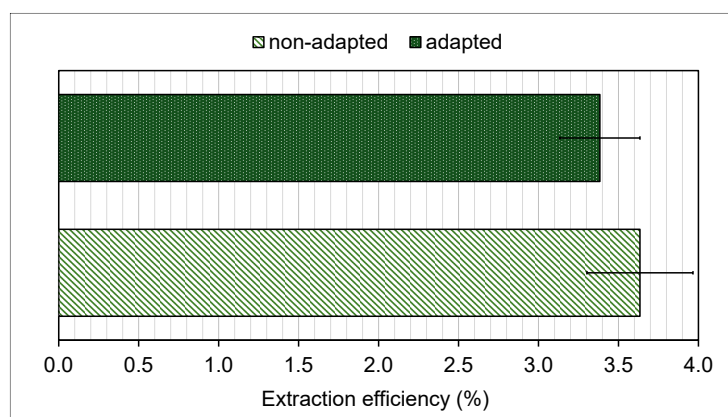


Fig. 1. Comparison of total extraction efficiency of cadmium between non-adapted and adapted *A. fischeri* strains

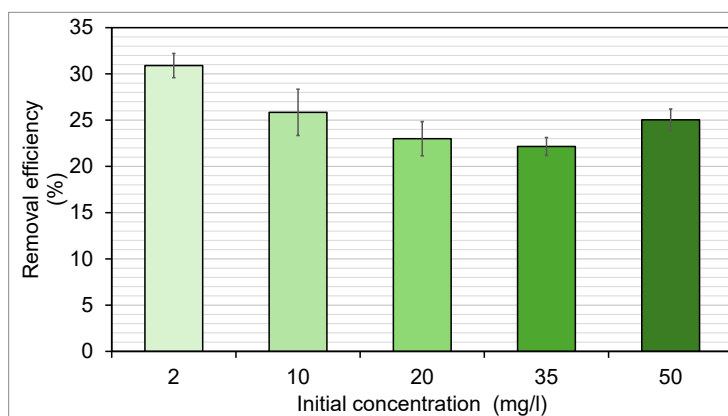


Fig. 2. Removal efficiency (%) of the synthesized manganese-based sorbent at different initial concentrations of cadmium

4 Conclusions

Series of experiments were carried out to evaluate the potential of fungal bioleaching for cadmium mobilization from mine tailing originating from the Lintich tailing pond and to assess the applicability of a manganese-based sorbent for subsequent Cd removal from leachates. Both adapted and non-adapted *A. fischeri* strains were able to mobilize cadmium, however it was observed that adaptation had no significant effect on the resulting extraction efficiencies. Although the bioleaching efficiency was relatively low, the TCLP testing confirmed the persistence of Cd mobility under simulated landfill conditions. Despite the favourable pH conditions for cadmium adsorption, the $\text{MnO}_2/\text{Mn(II)}$ -oxalate sorbent exhibited only moderate performance (~25 %), suggesting that further optimization of sorbent synthesis or operating conditions is

required to enhance the efficiency of this integrated remediation approach and to increase its potential for practical application in mining waste management.

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EFFECTS OF TEMPERATURE AND PH ON THE FUNCTIONAL DIVERSITY OF MICROSCOPIC FUNGI IN A THERMALLY ACTIVE SPOIL HEAP

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Abstract

Microscopic fungi represent an important component of soil microbial communities and may contribute to ecological processes in post-mining environments. In this study, 15 soil substrate samples were collected at the thermally active Hedvika coal-mining spoil heap in Ostrava, Czech Republic. The samples originated from both a thermally stabilised reclaimed zone and a thermally active zone, covering a pH range of 5.1–8.5. Functional diversity and metabolic activity of microscopic fungi were assessed using the Biolog EcoPlate™ system. Principal component analysis (PCA) of community-level physiological profiles, evaluated in relation to soil pH and temperature, suggested that both variables contributed independently to the differentiation of fungal metabolic patterns, which is consistent with key ecological concepts of the soil microbiome. The results indicate that temperature and pH may act as distinct ecological drivers shaping the functional characteristics of fungal communities in thermally active post-mining substrates. These findings may support the interpretation of microbial processes in thermally affected spoil heaps and can be applied in reclamation planning and environmental monitoring of post-mining sites.

Keywords: thermally active spoil heap, microscopic fungi, functional diversity, Biolog EcoPlate™, soil temperature

1 Introduction

1.1 Thermally active spoil heaps in the Ostrava region

Spoil heaps formed by the deposition of mining waste and other unused material represent a significant environmental burden in post-mining landscapes. Their impact is mainly related to changes in substrate physicochemical properties, the possible release of potentially hazardous elements and the long-term instability of deposited material. In the Ostrava-Karviná Coal District, thermally active spoil heaps include Heřmanice, Hedvika and Ema. The Hedvika spoil heap covers 40.6 ha, has a reported volume of 4.9 million m³, contains areas affected by endogenous combustion and has been largely reclaimed [1].

Thermal activity in spoil heaps is driven mainly by exothermic reactions in the deposited material, particularly oxidation of residual coal and sulphide minerals. These processes create strong temperature gradients and alter substrate chemistry, including pH. Such conditions form a specific ecological environment in which organisms tolerating low pH, elevated temperature or combined stress may be favoured. Thermally active spoil heaps therefore provide a suitable model for studying relationships between substrate properties and functional characteristics of soil microorganisms, including microscopic fungi [2].

1.2 Role of microscopic fungi in thermally active substrates

Microscopic fungi are important soil microorganisms involved in the release, mobility and distribution of elements in natural and technogenic substrates. By colonising rock surfaces, soil particles and other substrates, they contribute to the mechanical and chemical decomposition of organic and inorganic materials. They also produce organic acids, which lower pH near hyphae, promote dissolution of poorly soluble minerals, including phosphates, and support nutrient release and mineral biotransformation [3].

In thermally active post-mining substrates, fungal activity is influenced mainly by temperature, pH, nutrient availability and potentially hazardous elements. These factors may act as selective pressures affecting fungal community composition and metabolic activity. Although many microscopic fungi prefer mesophilic conditions, some species or strains are thermotolerant, including representatives of *Aspergillus*, *Penicillium* and *Trichoderma* [4]. Fungal survival under variable temperature and pH conditions has also been reported from other thermally affected soil and rock substrates [5, 6].

The importance of microscopic fungi in post-mining substrates is also linked to their response to metals and other potentially toxic elements. Metal resistance is species- and strain-specific and may involve extracellular and intracellular mechanisms, such as metal binding to organic acids and extracellular polymeric substances or deposition in cellular compartments [7–10]. These properties support the role of microscopic fungi in functional processes in thermally affected post-mining substrates.

2 Material and methods

Soil substrate samples were collected from the thermally active Hedvika spoil heap (49.8299239° N, 18.3615956° E) in Ostrava, Czech Republic. Substrate temperature was measured and pH was determined for all 15 samples. The samples were suspended in phosphate-buffered saline (PBS). The obtained suspensions were used for the cultivation of the main groups of cultivable microorganisms: bacteria on Tryptone Soya Agar, microscopic fungi on 2% Malt Extract Agar, and actinomycetes on Küster's agar. All culture media were supplied by HiMedia Laboratories, India.

The prepared soil suspensions were further used to inoculate Biolog EcoPlate™ microtitre plates to assess the utilisation of individual carbon sources. The plates were incubated at 30 °C, and absorbance was measured at regular intervals. For comparison among samples, the 72h time point was selected, as the colour response was sufficiently developed to differentiate the samples but did not show excessive saturation. Metabolic activity was expressed as AWCD (Average Well Colour Development), representing the mean colour response of EcoPlate™ wells. Functional diversity was assessed using the CMD Index, which expresses the number of carbon substrates utilised after 72 h of incubation.

Principal component analysis (PCA) was used as an exploratory ordination method to evaluate the relationship between community-level metabolic profiles, temperature, pH and the fungal component of the analysed samples. Isolated microscopic fungi were preliminarily identified based on macro- and micromorphological characteristics. Spatial visualisation of the sampling sites at the Hedvika spoil heap was created in Python (Python Software Foundation, <https://www.python.org>) using the Matplotlib library. Sampling points and a colour scale corresponding to substrate temperature in individual samples were manually added to the graph.

3 Results and discussion

The samples collected from the thermally active Hedvika spoil heap covered a pronounced temperature gradient from 15.1 °C (sample 01) to 52.7 °C (sample 15), together with soil pH variability ranging from 5.1 to 8.5 (Fig. 1, Table 1). The spatial distribution of samples showed that the highest temperatures were recorded in the northern, thermally active part of the spoil heap (samples 11–15), whereas samples from the southern, stabilised slopes with forest reclamation showed temperatures close to ambient conditions (samples 01–03). Metabolic activity and functional diversity did not show a simple linear relationship with either temperature or pH. The lowest CMD Index (2) and AWCD (0.04) values were recorded in sample 15, which had the highest temperature (52.7 °C), while the highest CMD Index (31) and AWCD (1.78) values were found in sample 07 at 36.4 °C. This pattern suggests that community metabolic activity reached its maximum within the intermediate temperature range, whereas it markedly decreased at the highest recorded temperature.

The abundance of cultivable microscopic fungi was not directly proportional to AWCD and CMD Index values, indicating that fungal abundance and the community-level functional profile reflect different aspects of the microbial community. The use of a non-selective soil suspension preserved the community context in which microscopic fungi occurred together with bacteria and actinomycetes. AWCD and CMD Index values therefore represented the overall metabolic response of the cultivable microbial community and were interpreted in relation to fungal abundance, temperature and pH.

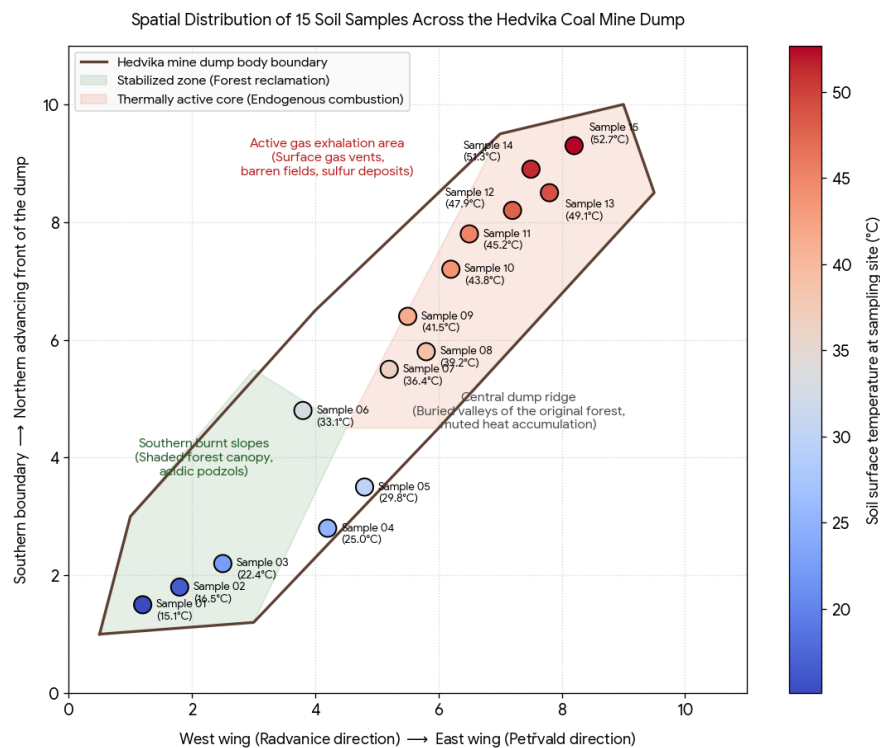


Fig. 1. Spatial distribution of 15 samples from the thermally active Hedvika spoil heap along a temperature gradient from 15.1 °C (sample 01) to 52.7 °C (sample 15)

Table 1. Soil sample characteristics, including temperature, pH and microbial abundance, together with metabolic activity (AWCD) and functional diversity (CMD Index) determined using Biolog EcoPlate™ after 72 h of incubation

Sample ID	Soil Temp. (°C)	Soil pH	Bacteria (CFU/g)	Fungi (CFU/g)	Actinomycetes (CFU/g)	AWCD	CMD Index (Richness)
01	15.1	5.1	3.8	21.2	4.2	0.18	6
02	16.5	7.2	14.1	8.4	8.5	0.52	15
03	22.4	5.5	8.1	18.9	11.2	0.45	11
04	25.0	8.0	25.2	3.9	17.4	1.05	22
05	29.8	6.8	22.8	12.1	21.5	1.48	28
06	33.1	5.2	14.9	22.4	22.1	0.72	16
07	36.4	7.0	31.8	7.1	31.5	1.78	31
08	39.2	8.4	41.0	2.1	35.8	0.65	14
09	41.5	6.0	23.2	16.5	34.3	0.95	21
10	43.8	7.5	39.4	4.5	40.0	0.82	18
11	45.2	5.6	24.2	19.5	38.3	0.41	10
12	47.9	6.9	34.5	10.6	45.1	0.55	13
13	49.1	8.2	48.0	1.8	49.9	0.21	5
14	51.3	6.5	32.8	11.0	47.4	0.28	7
15	52.7	8.5	53.1	0.8	54.7	0.04	2

Principal component analysis (PCA) was used to evaluate the relationship between the observed metabolic profiles, temperature, pH and the fungal component of the samples (Fig. 2). The different orientation of the temperature and pH vectors in the PCA space supports the interpretation of these variables as two distinct environmental factors contributing to the differentiation of metabolic profiles and the fungal component of the analysed samples. Several interpretative regions, labelled D0–D4, could be distinguished in the PCA space, corresponding to different combinations of temperature, pH, microscopic fungal abundance and the presence of selected fungal taxa.

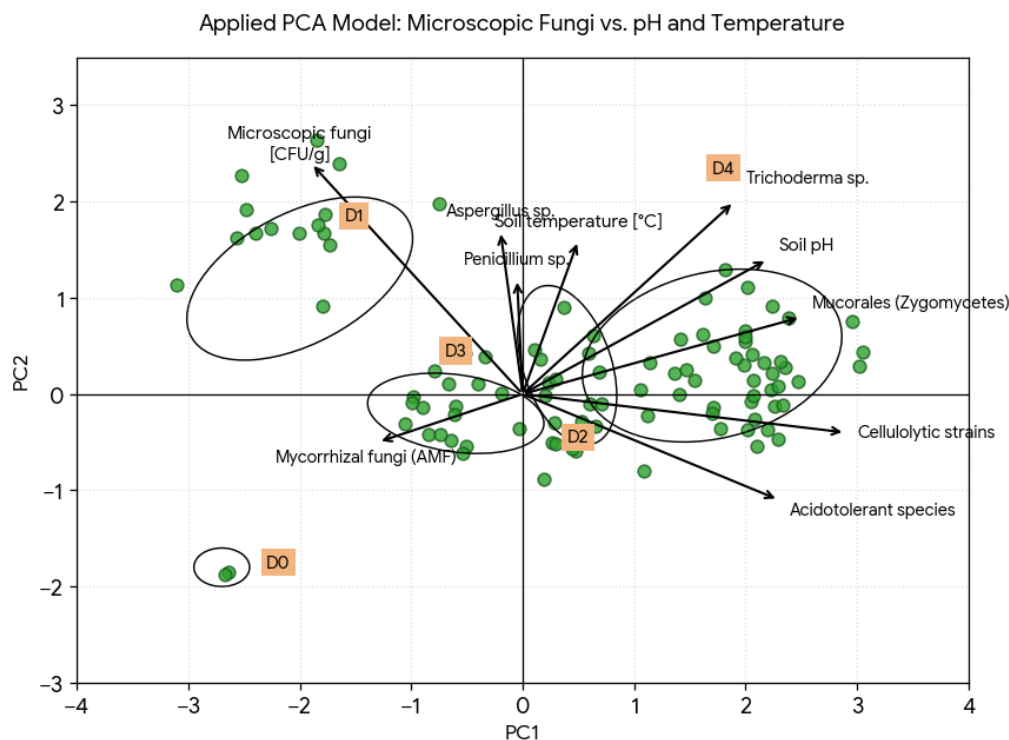


Fig. 2. Applied principal component analysis (PCA) demonstrating the distribution of soil fungal communities and individual taxa in relation to soil pH and sample temperature

In the PCA space, region D1 was associated with higher cultivable abundance of microscopic fungi and with lower temperature and lower pH conditions. This part of the ordination space may correspond to an environment in which acidotolerant or more cold-adapted components of the fungal community are better represented. This interpretation is consistent with the general understanding that soil pH is an important factor shaping soil microbial communities [11]. The orientation of vectors in the PCA space suggests that the temperature gradient contributed substantially to the differentiation of part of the samples and the observed fungal characteristics, while the influence of pH was expressed in a different direction. The position of the genera *Aspergillus* and *Penicillium* near the temperature vector may be interpreted in relation to their broader ecological tolerance; for *Aspergillus*, this variability has also been described in a review by Krijgsheld et al. [12]. Region D4 was associated with higher temperature and neutral to alkaline pH. In this part of the PCA space, vectors related mainly to *Trichoderma*, representatives of Mucorales and cellulolytic strains were located, suggesting their association with warmer parts of the spoil heap. Region D3 occupied a transitional position between the main ordination regions. The morphological appearance of selected microscopic fungal isolates obtained from the analysed samples is shown in Fig. 3.



Fig. 3. Selected representatives of isolated microscopic fungi from the thermally active Hedvika spoil heap: colonies of (A) *Paracorniculantispora psalliotae*, (B) *Talaromyces luteus*, (C) *Penicillium erubescens* and (D) *Aspergillus niger* cultivated on 2% Malt Extract Agar (MEA)

4 Conclusions

This study confirmed a pronounced temperature gradient ranging from 15.1 to 52.7 °C and pH variability from 5.1 to 8.5 at the thermally active Hedvika spoil heap. AWCD and CMD Index values differed markedly among samples and did not show a simple linear relationship with either temperature or pH. The highest metabolic activity and functional richness were recorded at 36.4 °C, whereas both parameters decreased substantially at the highest observed temperature. PCA suggested that temperature and pH contributed differently to the differentiation of community-level physiological profiles. Several interpretative regions were distinguished in the ordination space, reflecting different combinations of temperature, pH and cultivable microscopic fungal abundance. Since Biolog EcoPlate™ was applied to non-selective soil suspensions, the results were interpreted as the community-level metabolic response of the samples in relation to the fungal component of the microbial community. Overall, the findings indicate that combining cultivation-based fungal data with community-level physiological profiling can support the interpretation of microbial processes in thermally affected post-mining substrates.

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POSSIBILITIES OF MINE WASTES BIOLEACHING FROM OLD MINING BURDENS

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Abstract

Low-grade sulphidic mining wastes, in the form of heaps and tailings, are a negative impact of the active but mainly finished mining activity on ore deposits. They are subject to different natural chemical and biological transformation processes that result in the leaching of various toxic elements, primarily metals and metalloids into the soils and waters. However, these mining wastes also have a positive side. Although they contain low concentrations of elements, by applying appropriate methods they can be used as alternative raw materials sources of metals and metalloids, including critical metals. Several technologies are known for processing mining dumps and heaps. These include also the bioleaching by acidophilic iron- and sulphur-oxidizing bacteria and archaea. The focus of the paper was to evaluate preliminary bioleaching tests on solid mine wastes collected from the mine heap of an abandoned mining area, Zlatá Baňa (Slovak Republic). In the experiment, non-autochthonous and autochthonous bacterial cultures of *Acidithiobacillus sp.* were used. Bioleaching experiments were carried out under laboratory aerobic conditions at 30 °C. The acid sulphate media 9KA (without iron) and 9K (complete medium) were used. Mine wastes were characterized by X-ray fluorescence analysis, FT-IR spectrometry, and XRD diffractometry. Concentrations of elements in leachates were analysed using atomic absorption spectrometry and sulphate was analysed using ion chromatography. The obtained results show that higher leaching of Fe, Al, Zn, and Ca was achieved with the application of the complete nutrient medium, and no difference in metal leaching was observed between the use of autochthonous and non-autochthonous bacterial cultures.

Keywords: Sulphidic mining wastes, bioleaching, metals, *Acidithiobacillus sp.*

1 Introduction

Heaps, dumps, and tailings of low-grade sulphide mining waste are an integral part of ongoing, mostly completed mining activities on ore deposits. They consist of heterogeneous materials deposited here over many years of intensive mining activity and mineral processing [1]. They are subject to different mechanical processes as well as natural chemical and biological transformation processes that result in the leaching of various toxic elements (mainly metals and metalloids) into the environment. Solid mine wastes present a long-term source of water and soil contamination. The main risk is the bioavailability of metals and their entry into the food chain [2]. Environmental contamination is indicated by the presence of secondary minerals, which are found in a metastable form in the surface areas of the ore bodies and waste heaps after mining [3].

Typical examples of old mining loads in Slovakia are mainly abandoned deposits in Smolník, Pezinok, Ľubietová, waste deposit in Šobov, and others [4]. The abandoned Zlatá Baňa deposit is among the mining sites that receive little attention from the point of view of environmental contamination. This deposit has been used for the extraction of Au, Sb, Hg, and also opal, whose deposits have been unique till the 19th century. The continuation of mining activity was the mining of antimony. At the beginning of the 20th century, after the end of the mining activities, the area was devastated by numerous remains of mining activities [5].

At present several technologies are known for processing mining dumps and heaps. These include also the bioleaching using acidophilic iron- and sulphur-oxidizing bacteria (FeSOB) and archaea. Bioleaching of the sulphide ores, ore concentrates and mining wastes represents a natural process of interaction between

FeSOB and minerals to leach metals from insoluble form (metal sulphides) to soluble form (metal sulphates). The bioleaching process is an environmentally friendly, technically feasible, and cost-effective process to extract valuable metals mainly from low-grade sulphide mining wastes on an industrial scale using the heap, dump, in-situ, and stirred tank bioreactor leaching processes [6–7].

Chemolithotrophic, acidophilic FeSOB *Acidithiobacillus sp.* are often used to extract metals from sulphide ores. These bacteria oxidize ferrous iron (Fe^{2+}) and sulphur compounds to produce ferric iron (Fe^{3+}) and sulphuric acid (H_2SO_4) which are effective oxidizing agents for metal solubilization [8].

Besides sulphidic forms, metals are found as oxides, hydroxides, carbonates, silicates, and organically bound forms. Almost all acidophiles can manage to solubilize metals from these metallic forms at suitable conditions [9].

The focus of the paper was to realize and evaluate the preliminary bioleaching tests of solid mine wastes taken from the mine heap of an abandoned mining area - Zlatá Baňa.

2 Material and methods

2.1 Microorganisms

Chemolithotrophic, iron- and sulphur-oxidizing bacteria *Acidithiobacillus sp.* (Asp) were used in the bioleaching experiments. Bacterial cultures were isolated from two different localities of Slovakia: 1 - from the acid mine drainage effluent from the Pech shaft, the Smolník deposit (Asp-S) [10]; 2 - from the seepage water flowing out of the mine waste heap at the Zlatá Baňa deposit (Asp-ZB) [11]. Due to the location of the studied mining waste sampling, Asp-S represents a non-autochthonous bacterial culture and an Asp-ZB autochthonous bacterial culture. A selective nutrient medium 9K containing ferrous iron was applied for the isolation, cultivation and preparation of the active bacterial culture [12].

2.2 Samples of solid mine wastes

The four mine waste samples were collected from the mine waste heap from the Zlatá Baňa abandoned mining area. Sampling was performed from a 30 cm depth of the mine waste heap. All collected material was dried at laboratory temperature, quartered, grounded, and sieved under 63 μm . The elemental composition of the mine waste samples was analysed by XRF spectrometry using SPECTRO iQ II (Ametek, Germany). FTIR spectroscopy was performed to identify functional groups and molecular vibrations by Bruker Alpha Platinum-ATR spectrometer (BRUKER OPTICS, Ettingen, Germany). XRD analysis was used to determine the crystalline phases present in the sample using the diffractometer Bruker D2 Phaser (Bruker AXS, GmbH, Germany). The XRD patterns were processed using the software Diffac. EVA v. 2.1. The ICDD PDF database (ICDD PDF – 2 Release 2009) was utilized for the phase identification.

2.3 Bioleaching experiments

Based on the results of XRF, FTIR, and XRD analyses, one representative sample of the mine waste (ZB) was chosen for the bioleaching tests. Experiments were carried out under aerobic conditions at pH 2.0 in an acidic environment, at 150 rpm in 250 ml shake flasks at 30 °C. The liquid medium 9K composition was the following (amounts per litre): Conditions - 1 (medium without ferrous iron) – 0.4 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g $(\text{NH}_4)_2\text{SO}_4$, 0.04 g K_2HPO_4 ; Conditions – 2 (medium with ferrous iron) - 0.4 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g $(\text{NH}_4)_2\text{SO}_4$, 0.04 g K_2HPO_4 and 33.4 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. The medium was acidified to pH 2.0 by the H_2SO_4 . Pulp density was 4 %. The solid mining waste was not sterilized before bioleaching. The total volume of the liquid phase was 100 ml. The inoculum of bacterial culture Asp-S and Asp-ZB was 20 %. Inoculated samples were designated as ZB-Asp-S and ZB-Asp-ZB. In non-inoculated samples i.e. abiotic control, both the inoculum and nutrient medium volumes were replaced with an equal volume of deionized water (ZB-DW). Experiments were realized in duplicate for 60 days, and the mean values were reported. The concentration of metals in leachates was determined by atomic absorption spectrometry (AAS) using a Varian 240FS/240 Z spectrometer (Varian, Australia) and sulphates by ion chromatography using Dionex ICS 5000 (Sunnyvale, CA, USA), equipped with an IonPac AS11-HC anion column and suppressed conductivity detector. pH values were determined by pH meter PHM210 (MeterLab, France).

3 Results and discussion

The mineralogical and elemental composition as well as functional groups and molecular vibrations of four mine waste solid samples collected from the abandoned mining area Zlatá Baňa were similar and did not show significant differences. Therefore, in the bioleaching tests, only one representative sample was used

(ZB). The characterization of this representative sample using X-ray fluorescence (XRF), Fourier-transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD) analyses is presented in Table 1 and Fig. 1–2.

Table 1. The elemental composition of mine waste sample from Zlatá Baňa (ZB) tested in the bioleaching experiments (majority elements, XRF analysis)

Sample	Si	Al	Fe	Ca	S	As	Zn
ZB	(mg/kg)						
	270200	109000	42430	22870	17630	338	284

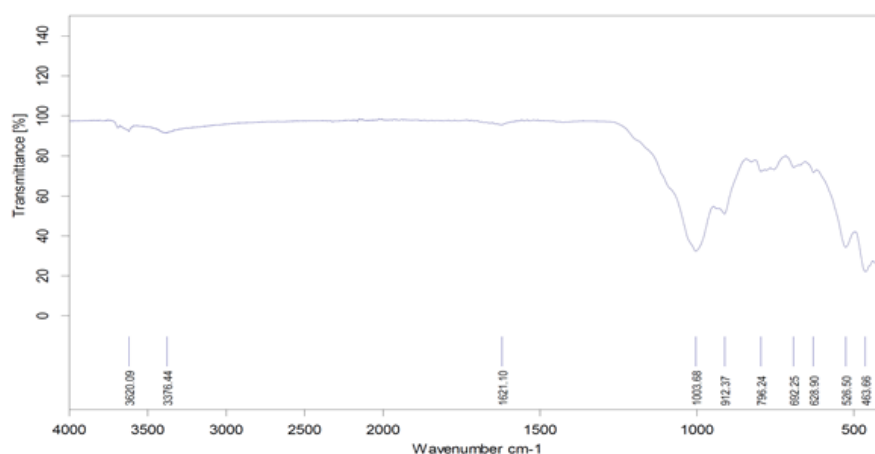


Fig. 1. FTIR spectrum of the mining waste sample ZB

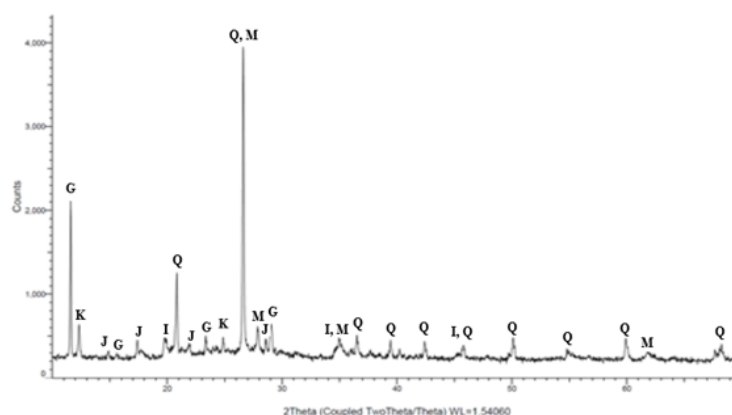


Fig. 2. XRD pattern of the mining waste sample ZB (G - gypsum, K – kaolinite, M – muscovite, I – illite, Q – quartz, J – jarosite)

XRF analysis revealed that the major elements in the waste sample were silicon (Si), aluminium (Al), iron (Fe), calcium (Ca), potassium (K), and sulphur (S). Minor elements included magnesium (Mg), zinc (Zn), and arsenic (As).

The FTIR spectrum of the sample exhibited prominent absorption bands at: 3620 cm⁻¹ and 3376 cm⁻¹ - attributed to O-H stretching, suggesting the presence of hydroxyl-bearing minerals; 1621 cm⁻¹ - assigned to H-O-H bending, indicating adsorbed water or clay minerals; 1003 cm⁻¹ - Si-O stretching, indicative of silicate minerals; 912 to 628 cm⁻¹ - typical of Al-OH bending and Fe-O vibrations, suggesting the presence of phyllosilicates and iron oxides; 526, 463, and 413 cm⁻¹ - assigned to metal-oxygen vibrations in aluminosilicate or sulphate minerals. These results are consistent with characteristic spectral features reported for kaolinite, gypsum, muscovite, and jarosite [13].

The identified mineral phases by XRD analysis include quartz, kaolinite, muscovite, illite, gypsum, and jarosite. Jarosite is a common secondary iron sulphate mineral that forms under acidic and oxidizing conditions, often in mining environments. Its identification has implications for acid mine drainage (AMD) potential [14].

Results of bioleaching of sample ZB obtained under Conditions -1 (incomplete medium 9K - without ferrous iron) are not shown, the extraction of metals was negligible (concentration level in ng/l) [11]. Results of bioleaching of sample ZB obtained under Conditions -2 (complete medium 9K - with ferrous iron) are presented in Figs. 3–10, respectively.

Figs. 3 and 4 show the changes in pH and sulphate concentration of liquid phases during the bioleaching at Conditions II. There was a decrease in pH from the start to the end of experiments from 2.0 to 1.84 and 1.85 in ZB-Asp-S and ZB-Asp-ZB, respectively. The pH in ZB-DW decreased from its initial value of 4.6 to its end value of 2.0. A gradual increase in sulphate concentration from 15.67 g/l to 20.97 g/l and 20.50 g/l in ZB-Asp-S and ZB-Asp-ZB, respectively (Fig. 4). Sulphate concentration in the ZB-DW increased gradually from 0.67 g/l to 3.90 g/l by the 60th day. A decrease in the pH values and a gradual increase in sulphate concentration in liquid phases indicate bacterial activity and oxidation of Fe^{2+} during bioleaching in inoculated samples [15]. The slow increase in sulphate in the first 3 days in ZB-Asp-S and ZB-Asp-ZB indicates the oxidation of sulphide minerals by the acidic medium [16] and the bacterial oxidation of Fe^{2+} present in the nutrient medium to Fe^{3+} [17]. Later, a gradual increase in sulphate concentration points to thiosulphate and polysulphide pathways of sulphide mineral oxidation [18]. The decrease in pH of ZB-DW throughout the experiment was due to its content of acid-generating minerals [19].

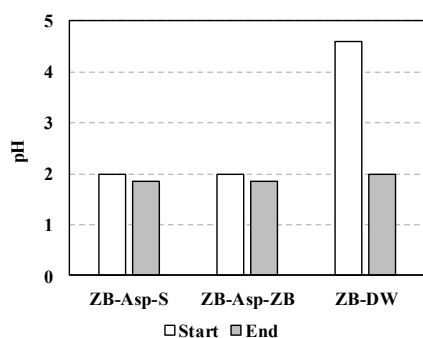


Fig. 3. The changes of pH at experiments

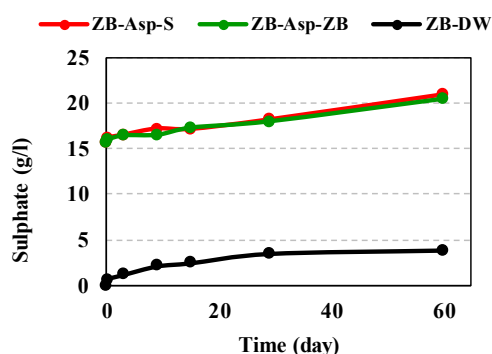


Fig. 4. Sulphate concentration in liquid phases during the experiments

There was a marked decrease in total dissolved Fe in the first 4 days from its initial concentration of 7.7 g/l to 5.2 g/l and 5.0 g/l for ZB-Asp-S and ZB-Asp-ZB, respectively (Fig. 5). Then it gradually increased to 6.6 g/l for ZB-Asp-S and 6.2 g/l for ZB-Asp-ZB by the 15th day. Then another decrease followed, to 5.5 g/l for ZB-Asp-S and 5.2 g/l for ZB-Asp-ZB by the 26th day. Next followed the increase in Fe concentration finally to 8.0 g/l (ZB-Asp-S) and 9.1 g/l (ZB-Asp-ZB) on the 60th day in the bioleaching assays. There was minimum Fe dissolution in the deionized water (0.2–0.8 g/l) and its final value was 0.1 g/l. The oxidation of sulphide minerals during bioleaching by the Fe^{3+} generated from the oxidation of Fe^{2+} by the iron- and sulphur-oxidizing bacteria resulted in metal(loid)s dissolution.

Zn solubilization was already detectable on the 1st day of incubation with the Asp using both the autochthonous and the non-autochthonous bacterial cultures, which gradually increased, after 15 days decreased, later increased over time, reaching its maximum 8.3 mg/l and 8.6 mg/L for ZB-Asp-S and ZB-Asp-ZB, respectively for after 60 days of the experiment (Fig. 6). The solubilization of Zn was observed in the abiotic controls, too. Its leaching of Zn was caused by acid-generating minerals occurring in the mining waste [19].

Solubilization of As by the acidophilic bacteria Asp was detected only in XRF analysis of the solid phase after the bioleaching tests (Fig. 7). The occurrence of As in the leachates was not detected. At the beginning of bioleaching, there was a decrease in Fe (Fig. 5), the jarosite was formed as a new mineral at bioleaching which co-precipitated As [20]. We cannot explain the increased concentration of As in the solid phase of the abiotic control.

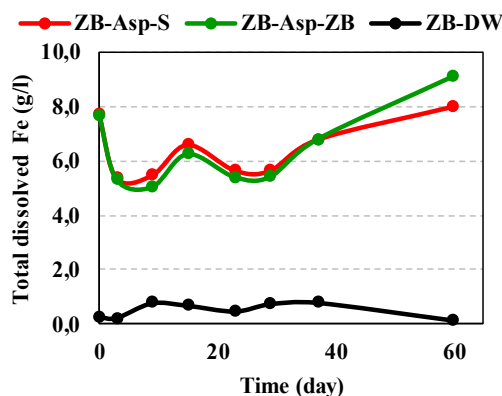


Fig. 5. Total dissolved Fe in liquid phases during the tests

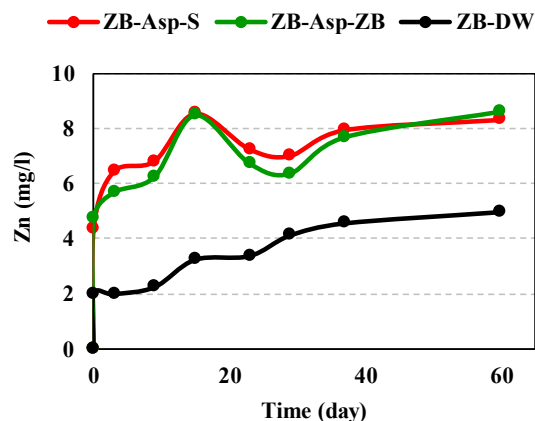


Fig. 6. Concentration of Zn in liquid phases during the tests

Silicate minerals were also partially leached from studied mine waste, evident in the dissolution of Al in both inoculated and non-inoculated liquid phases. Al solubilization was already observed on the starting day of incubation with the Asp using both the autochthonous and non-autochthonous bacterial cultures, later increased over time, reaching its maximum 83.8 mg/l and 80.1 mg/l for ZB-Asp-S and ZB-Asp-ZB, respectively after 60 days of the experiment (Fig. 8). The solubilization of Al was observed in the abiotic controls approximately on the 5th day, then followed by the increase in final concentration to 57.5 mg/l.

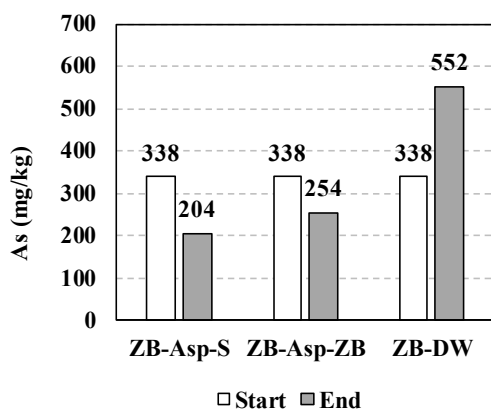


Fig. 7. Concentration of As in solid phase before and after tests (XRF analysis)

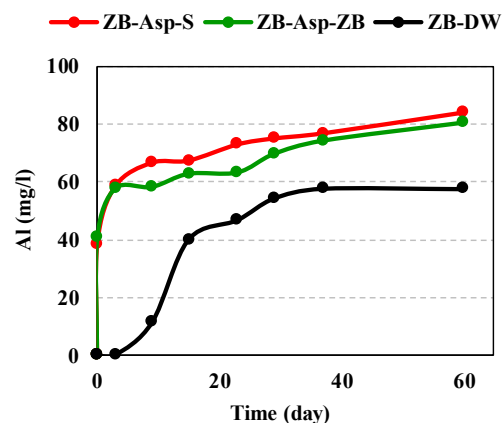


Fig. 8. Concentration of Al in the liquid phases during the experiments

XRF analysis of the solid phase before and after experiments indicated significant Ca leaching in inoculated samples as well as in abiotic control (Fig. 9). The identified mineral phases by XRD analysis of the studied sample mine waste ZB include gypsum (Fig. 2). At the biotic conditions, the acidic medium dissolved gypsum, and Ca was extracted to a liquid phase. In abiotic conditions, the leaching of Ca was caused by acid-generating minerals present in the solid phase ZB, a decrease in pH from 4.6 to a final value of 2.0 [19].

The colouration of the liquid phases after the experiments is shown in Fig. 10a. Both inoculated samples (ZB-Asp-S and ZB-Asp-ZB) showed distinct orange colours. The abiotic control had a faint yellow colouration (Fig. 10a), similar to the mine waste ZB sample (Fig. 10b).

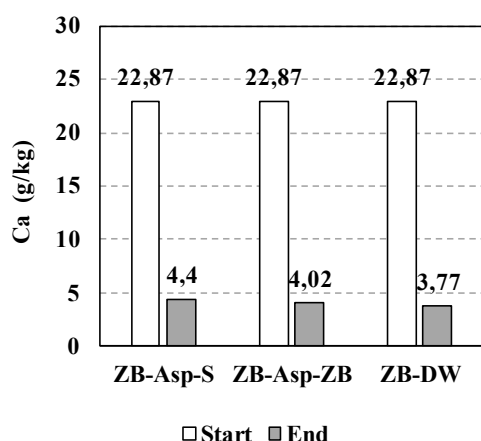


Fig. 9. Concentration of Ca in the solid phase before and after tests (XRF analysis)

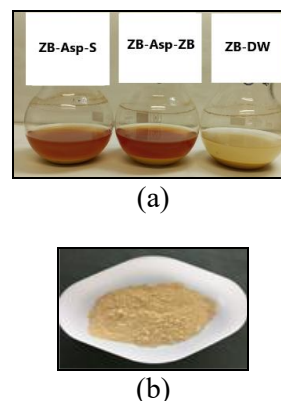


Fig. 10. (a) Colouration of the liquid phases after experiments
(b) Mine waste ZB before experiments

4 Conclusions

This study investigated the possibilities of mine waste bioleaching of solid mine wastes taken from the mine heap of an abandoned mining area - Zlatá Baňa in the Slovak Republic. The achieved results of the preliminary bioleaching tests documented that: the applied iron- and sulphur-oxidizing bacteria *Acidithiobacillus sp.* (Asp) showed active metabolism during bioleaching, as evidenced by changes in pH and an increase in sulphate concentration in the leachates; the studied sample of mining waste (ZB) contain acid-generating minerals, as acidification of the abiotic control occurred; the oxidation of minerals during bioleaching by the iron- and sulphur-oxidizing bacteria resulted in Fe, Zn, As and Al dissolution; significant Ca leaching was detected in inoculated samples as well as in abiotic control; both inoculated samples showed distinct orange colours formed under the influence of soluble Fe compounds; higher leaching of Fe, Al, Zn and Ca was achieved at the application of the complete nutrient medium; of the metals leaching difference in the application of autochthonous and non-autochthonous bacterial culture was not observed.

In the abandoned mining area Zlatá Baňa are a huge number of extensive heaps, open mine tunnels, and several shafts. Despite the persisting environmental problems, no scientific research attention has been paid to this area. It is necessary to focus on obtaining a comprehensive set of knowledge regarding the environmental status of this former mining area, which has not yet been evaluated for this purpose. Self-improvement of the situation in this abandoned mining site is not possible, but it is possible to focus on methods of remediation.

Acknowledgements

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EVALUATION OF ACIDITHIOBACILLUS FERRIDURANS GROWTH AND BIOLEACHING PERFORMANCE ON ARSENIC-BEARING MINE TAILINGS

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Abstract

Arsenic-bearing mine tailings represent both an environmental concern and a potential secondary source of valuable metals. Bioleaching experiments were performed with *Acidithiobacillus ferridurans* at pulp densities of 1; 5 and 10% (w/v), while bacterial response to tailings addition was monitored online using a Hamilton Incyte permittivity sensor. At 1% pulp density, Cu recovery reached 59.2% after two days and remained 54.6% on day 6. At pulp density 5%, Cu recovery increased from 16.6% on day 2 to 61.3% on day 6, indicating delayed mobilisation, whereas recovery remained below 4% at pulp density 10%. Mn was readily mobilised in both inoculated and sterile systems, while measurable As mobilisation occurred only in the inoculated systems and reached 12.8% at pulp density 1% on day 6. Online monitoring showed that tailings addition caused a pronounced pH increase and a decline in permittivity, which means the decline in viable cell density. After readjustment to pH 2.5 in a repeated experiment, the permittivity increased and pH subsequently decreased without further acid addition. The results indicate that pulp density, pH and exposure time strongly influence microbial activity and elements mobilisation from the investigated tailings.

Keywords: *Acidithiobacillus ferridurans*; bioleaching; mine tailings; arsenic; permittivity monitoring

1 Introduction

Mine tailings may contain residual valuable metals together with potentially hazardous elements, and their long-term storage can therefore represent both an environmental concern and a secondary-resource opportunity. Bioleaching uses microbial activity to promote the solubilisation of metals under comparatively mild conditions. Iron- and sulphur-oxidising *Acidithiobacillus* species are among the best-known microorganisms used in such processes. *Acidithiobacillus ferridurans* is an obligately chemolithotrophic, acidophilic and mesophilic species capable of using ferrous iron, reduced sulphur compounds and hydrogen as electron donors [1].

Arsenic-bearing mine tailings are a particularly challenging substrate because process conditions strongly influence both microbial activity and arsenic mobilisation. Park et al. [2] showed with *A. ferrooxidans* that arsenic bioleaching from mine tailings was strongly affected by initial pH and pulp density; higher pulp density was associated with lower bacterial growth and lower As extraction. Similar work with *A. thiooxidans* demonstrated decreasing As leaching with increasing solid concentration and indicated that jarosite formation and As co-precipitation may reduce the amount of dissolved arsenic [3]. Studies on polymetallic mine tailings have also shown that Cu, Mn and As can respond differently to the applied *Acidithiobacillus* culture [4]. These observations emphasise that pulp density is a crucial parameter, and cannot be evaluated independently from the chemical conditions developing in the leaching suspension.

Besides analysing dissolved metals, direct observation of the microbial response can provide additional information on the limitations of a bioleaching system. In our previous work, online dielectric spectroscopy using a Hamilton Incyte sensor was applied to follow the growth of *Acidithiobacillus* cultures in real time [5]. The method was subsequently used for inhibition analysis in the presence of battery black mass, where the permittivity signal was used as an indicator of the condition of the bacterial population following solid addition [6]. This approach is especially useful in *Acidithiobacillus* cultures, where precipitates formation can complicate conventional microscopic monitoring.

The present study investigates the bioleaching of arsenic-bearing polymetallic mine tailings by *A. ferridurans* at pulp densities of 1; 5 and 10% (w/v). The mobilisation of Cu, Mn and As was compared with the sterile controls, while a separate online monitoring experiment was used to examine the response of the bacterial culture to tailings addition. Particular attention was given to the pH increase induced by the tailings and to whether readjustment of pH could restore the viable-biomass permittivity signal.

2 Material and methods

2.1 Sample preparation and characterisation

The investigated material was representatively sampled from a historical polymetallic mine tailings deposit located in the Eastern Alps, Austria. The received sample was divided into representative subsamples using a Jones riffle splitter and subsequently wet-sieved into different particle-size fractions for mineralogical characterisation and liberation studies. Based on the determined liberation size, a representative subsample was wet-ground in a laboratory rod mill to a particle size below 63 μm . Grinding conditions were selected to minimise overgrinding and the formation of ultrafine particles (<20 μm), which could unnecessarily hinder solid–liquid separation during the subsequent bioleaching experiments. Chemical analysis of the prepared material is summarised in Table 1.

Table 1. Chemical composition of the investigated mine tailings

Element	Cu	Zn	Pb	As	Au	Ag	Mn
Concentration (mg/kg)	1950	24.9	1.8	1930	1.04	0.74	2160

2.2 Bacterial culture and bioleaching experiments

Acidithiobacillus ferridurans (Karitas strain, originally from Slovakia and maintained at the University of Miskolc) was cultivated in standard Silverman-Lundgren 9K medium. The initial pH was adjusted to 2.3 with H_2SO_4 and 10% (v/v) inoculum was used. Growth was monitored in real time using a Hamilton Incyte sensor, and bioleaching was initiated at the end of the exponential growth phase, when the pH was approximately 2.0.

Bioleaching experiments were performed at pulp densities of 1; 5 and 10% (w/v), with parallel reactors. Sterile fresh 9K medium at the same initial pH and pulp densities served as abiotic control. No further pH adjustment was applied. Experiments were carried out in 250 mL Erlenmeyer flasks for 13 days at 30 °C and 150 rpm in a WiseCube orbital shaker. Suspension samples (20 mL) were collected after 2, 6, 9 and 13 days. To minimise contamination, pH and oxidation-reduction potential (ORP) measurements followed the sampling in the inoculated systems, whereas the sterile controls were measured only at the end of the experiment. The removed volume was replaced with an equal volume of sterile 9K medium. Liquid samples were acidified with HNO_3 and refrigerated until analysis. The reported recovery values for the inoculated (AF) systems represent mean values of the parallel experiments. At the end of the experiment, suspensions were filtered; the solid residues were washed with deionised water, dried and weighed.

2.3 Online monitoring of bacterial growth

Bacterial response to mine tailings addition was monitored online using a Hamilton cell monitoring system (Hamilton Bonaduz AG, Switzerland) equipped with an Incyte sensor. The sensor applies dielectric spectroscopy to monitor viable biomass based on the permittivity of intact cells in suspension. Inhibition experiments were performed with *A. ferridurans* at 5% (w/v) pulp density. Tailings were added at the end of the exponential growth phase and permittivity was continuously recorded. In the first experiment, no pH adjustment was applied after tailings addition. The experiment was then repeated with the pH adjusted to 2.5 using H_2SO_4 after tailings addition, allowing comparison of the permittivity and pH responses.

2.4 Chemical analysis and recovery calculation

Dissolved Cu, Mn and As concentrations were determined by ICP-OES according to MSZ 1484-3:2006, Chapter 5. Cumulative metal recovery was calculated from the initial elemental content of the solid sample and the measured liquid-phase concentrations, accounting for metal removed during previous sampling. Dissolved As concentrations below 0.002 mg/L were treated as below the analytical limit.

3 Results and discussion

3.1 Online monitoring of bacterial response to mine tailings

Online permittivity monitoring revealed a pronounced response of *A. ferridurans* to the addition of the mine tailings. In the experiment without pH adjustment, the permittivity signal decreased markedly after addition of 5% (w/v) tailings and subsequently stabilised at a substantially lower level (Fig. 1a). Tailings addition was accompanied by an increase in pH from approximately 2.0 to 3.5. A distinctly different response was observed when the experiment was repeated with pH adjustment. After addition of the same

amount of tailings, the pH was adjusted to 2.5 with H_2SO_4 , after which the permittivity increased, indicating recovery and continued growth of the viable bacterial population (Fig. 1b). The pH subsequently decreased without further acid addition, reaching 2.04 by the end of the 11-day experiment. These observations suggest that the decrease in bacterial activity was strongly associated with the tailings-induced pH increase rather than being caused solely by direct toxicity of the arsenic-bearing substances. A contribution of dissolved toxic elements, however, cannot be excluded.

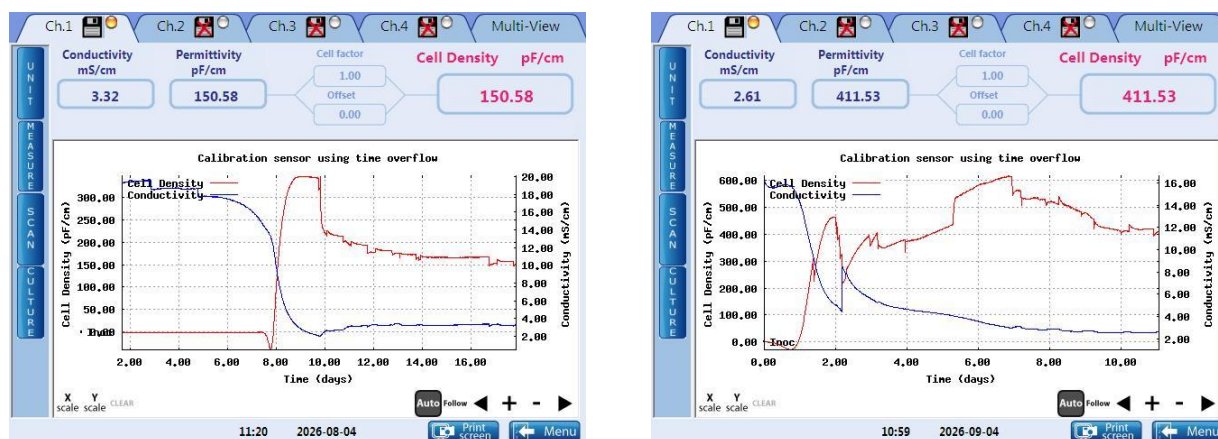


Fig. 1. Online monitoring of *A. ferridurans* response to the addition of 5% (w/v) mine tailings: (a) without pH adjustment and (b) after adjustment of pH to 2.5 with H_2SO_4 .

3.2 Effect of pulp density on metal recovery

The recovery results revealed distinct effects of pulp density and leaching time on the mobilisation of Cu, Mn and As (Table 2). After two days, at 1% (w/v) pulp density, Cu recovery reached 59.2% in the inoculated system compared with 11.3% in the sterile control. Initial Cu recovery decreased to 16.6% at 5% and 4.0% at 10% (w/v) pulp densities. Manganese was more readily mobilised: an apparent recovery of 103.9% was calculated at 1% (w/v) pulp density, decreasing to 56.0 and 32.6% at 5 and 10%, respectively. Substantial Mn dissolution also occurred in the sterile controls, indicating a strong abiotic contribution to Mn mobilisation. Arsenic behaved differently: dissolved As remained below the analytical limit (<0.002 mg/L) in the sterile controls, whereas 5.75% As recovery was obtained in the inoculated 1% pulp density system. Recovery decreased to 0.34% at 5% and 0.03% at 10% (w/v) pulp densities.

By day 6, Cu recovery in the 5% pulp density inoculated system increased markedly to 61.3%, whereas the 1 and 10% systems showed little change (54.6 and 3.7%, respectively). In the sterile controls, Cu recovery reached 22.1% at 1% pulp density, but remained low at 5 and 10% (1.58 and 0.33%, respectively) pulp densities. Thus, the effect of pulp density on Cu mobilisation was not simply a monotonic inhibition: at 5% (w/v) pulp density, Cu dissolution in the inoculated system was delayed compared with the 1% system, but increased substantially between days 2 and 6. Mn recovery increased to 105.5, 128.6 and 42.2% at 1, 5 and 10% (w/v) pulp density, respectively. It should be noted, that values exceeding 100% are apparent recoveries and likely reflect uncertainty in the initial solid composition and the analytical mass balance. Sterile Mn recovery also increased with time, reaching 95.2, 40.5 and 23.1%, confirming a substantial abiotic contribution. As mobilisation remained strongly dependent on pulp density: recovery increased to 12.79% at 1% pulp density, but reached only 0.92% at 5% and 0.04% at 10% pulp densities, while dissolved As remained below the analytical limit in all sterile controls. These trends are consistent with the previous studies showing strong effects of pulp density and pH on bioleaching of arsenic-bearing mine tailings [2,3]. Endpoint measurements on day 13 showed higher ORP values in the inoculated systems than in the corresponding sterile controls, particularly at 5 and 10% pulp density (615 vs. 326 mV and 550 vs. 293 mV, respectively). Final pH increased with pulp density and exceeded 3 at 10% pulp density in both systems. Since a considerable variability occurred between parallel endpoint measurements, these pH and ORP values were used only as supporting observations and not for quantitative interpretation of the day 6 recovery data.

Table 2. Recovery of Cu, Mn and As after 2 and 6 days of leaching

Day	System	Cu (%)	Mn (%)	As (%)
2	AF 1%	59.23	103.94	5.75
2	S 1%	11.33	84.72	<DL
2	AF 5%	16.62	55.97	0.34
2	S 5%	0.64	29.81	<DL
2	AF 10%	3.97	32.62	0.03
2	S 10%	0.82	20.19	<DL
6	AF 1%	54.58	105.54	12.79
6	S 1%	22.13	95.21	<DL
6	AF 5%	61.33	128.55	0.92
6	S 5%	1.58	40.50	<DL
6	AF 10%	3.68	42.15	0.04
6	S 10%	0.33	23.14	<DL

DL: analytical limit for dissolved As, 0.002 mg/L; S: sterile control. AF values represent means of parallel bioleaching experiments. Apparent Mn recoveries above 100% are reported as calculated and should be interpreted with consideration of uncertainty in the initial solid composition and analytical mass balance.

4 Conclusions

The results demonstrate a strong interaction between pulp density, pH conditions and bioleaching performance of *A. ferridurans* on arsenic-bearing polymetallic mine tailings. At 1% (w/v) pulp density, Cu mobilisation was rapid and As recovery increased to 12.8% by day 6. At 5% pulp density, Cu mobilisation was delayed, but reached 61.3% by day 6, whereas 10% pulp density strongly limited Cu and As recovery. Mn showed substantial dissolution in both inoculated and sterile systems, indicating an important abiotic contribution. Online permittivity monitoring showed that the tailings-induced pH increase was associated with a marked decline in viable biomass signal, whereas readjustment to pH 2.5 was followed by renewed increase in permittivity and spontaneous acidification to pH 2.04. These findings prove that pH is a very important factor controlling microbial activity in this tailings system. Nevertheless, direct effects of dissolved toxic elements cannot be excluded.

Acknowledgements

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CHARACTERIZATION OF DISSOLVED ORGANIC MATTER IN NIŽNÁ SLANÁ MINE WATER BY HPLC-FLD, HPLC-HRMS AND GC-MS

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Abstract

Mine water from the flooded Nižná Slaná siderite deposit (Slovakia) was characterized prior to biological Fe(II) oxidation. The slightly acidic water (pH 5.62; about 30 °C) was highly mineralized, containing 29328 mg L⁻¹ sulphate and 1064.7 mg L⁻¹ Fe ions. HPLC-FLD and HPLC-HRMS analyses after SPE extraction, and GC-MS analyses after dichloromethane extraction provided complementary information about the organic fraction. Comparison with 2, 5 and 7 µg L⁻¹ PAH standards supported ten retention-time-consistent signals. HPLC-HRMS yielded 20 empirical formulas (maximum mass error 5.52 ppm), and GC-MS gave six tentative library assignments. This untreated fingerprint enables direct comparison with biologically treated samples.

Keywords: mining-influenced water, dissolved organic matter, HPLC-FLD, HPLC-HRMS, GC-MS, PAHs

1 Introduction

Mining influenced water commonly contain sulphate, Fe and other dissolved metals generated through interactions among groundwater, minerals, oxidants and microorganisms [1, 2]. Their dissolved organic fraction is less frequently examined, although it may affect complexation, adsorption, precipitation and constituent mobility [3]. The flooded Manó-Gabriela siderite mine at Nižná Slaná discharges highly mineralized water to the Slaná River [4, 5]; its regional geology is described in [6], and Fig. 1 shows the discharge from the Marta adit. This work establishes an untreated water fingerprint by HPLC-FLD, HPLC-HRMS and GC-MS for later comparison with biological Fe(II)-oxidation experiments [7].



Fig. 1. Mine water discharge from the flooded Manó–Gabriela siderite mine at the Marta adit, Nižná Slaná, Slovakia.

2 Material and methods

2.1 Sampling and physicochemical characteristics

Mine water was collected from the Gabriela shaft within the mine; the water subsequently discharges through the Marta adit (Fig. 1). Major anions and cations were determined by ion chromatography (Dionex ICS 5000). The mine water contained (mg L⁻¹) SO₄²⁻ 29,328, Cl⁻ 82.6, Mg²⁺ 5,703, Ca²⁺ 475, K⁺ 105, Na⁺ 54.3, and NH₄⁺ 38.5. Transition metals and metalloids were determined by atomic absorption spectrometry (VARIAN AA240FS) yielding (mg L⁻¹) Fe 1064.7, Mn 69.1, As 20.23, Ni 2.6, Zn 0.37, and Co 0.3016. Fe³⁺ was determined by UV spectrophotometry at 300 nm and Fe²⁺ by a modified o-phenanthroline method insensitive to Fe³⁺. The composition agrees with earlier reports [4, 5].

2.2 SPE and HPLC-FLD

Ultrapure water (UPW; 18.2 MΩ·cm) served as the procedural blank. Three 500 mL of mining-influenced water replicates and a 500 mL UPW blank were extracted on CHROMABOND Easy SPE cartridges (Macherey-Nagel). Cartridges were conditioned with 3 mL each of methanol and water, loaded

under vacuum, washed with 3 mL methanol/water (5:95, v/v), and eluted with 2 mL each of methanol and acetone. Eluates were dried under nitrogen at 40 °C, reconstituted in 1 mL acetonitrile (500-fold concentration), and filtered through 0.22 µm PTFE filters. HPLC-FLD analysis used a Dionex UltiMate 3000 RS with DAD 3000-RS and FLD 3400-RS detectors and a Nucleosil 100-5 C18 PAH column (250 × 3 mm, 5 µm, 100 Å). Water/acetonitrile gradient elution was performed at 0.50 mL min⁻¹ and 25 °C with a 10 µL injection. A 16-EPA-PAH mixture was used to prepare 2, 5 and 7 µg L⁻¹ standards.

2.3 HPLC-HRMS

Samples were filtered through 0.22 µm PTFE membranes and analysed without extraction using a Dionex UltiMate 3000 HPLC-DAD coupled to a Bruker compact QTOF with positive-mode Apollo II electrospray ionization. Separation used an Acclaim 120-3 C18 column (100 × 2.1 mm) at 40 °C, a 200 µL min⁻¹ flow rate, and a 20 µL injection. The water (A) and methanol (B) mobile phases each contained 0.1% formic acid; the gradient was 5% B (0–5 min), 5–100% B (5–20 min), 100–5% B (20–25 min), and 5% B (25–30 min). A 4 s offset aligned the UV and MS chromatograms. Accurate protonated masses were assigned empirical formulas, not unambiguous structures [8, 9].

2.4 GC-MS

A 200 mL aliquot was extracted with 10 mL dichloromethane (2 × 5 mL), dried over anhydrous Na₂SO₄, filtered through 0.22 µm PTFE and analysed using an Agilent 7890A GC-5975C inert MSD GC-MS system. A 2 µL splitless injection at 270 °C was made onto an HP-5 column (30 m × 0.32 mm × 0.25 µm) with He at 1 mL min⁻¹. The oven was heated from 40 to 265 °C over 15 min within an 18 min programme; MS acquisition used a 4 min solvent delay and m/z 30–350 scan range. Table 1 reports tentative spectral-library assignments.

3 Results and discussion

3.1 HPLC-FLD characterization

Figure 2 compares the HPLC-FLD chromatograms of the 5 µg L⁻¹ PAH standard and the 500-fold-concentrated mine-water extract.

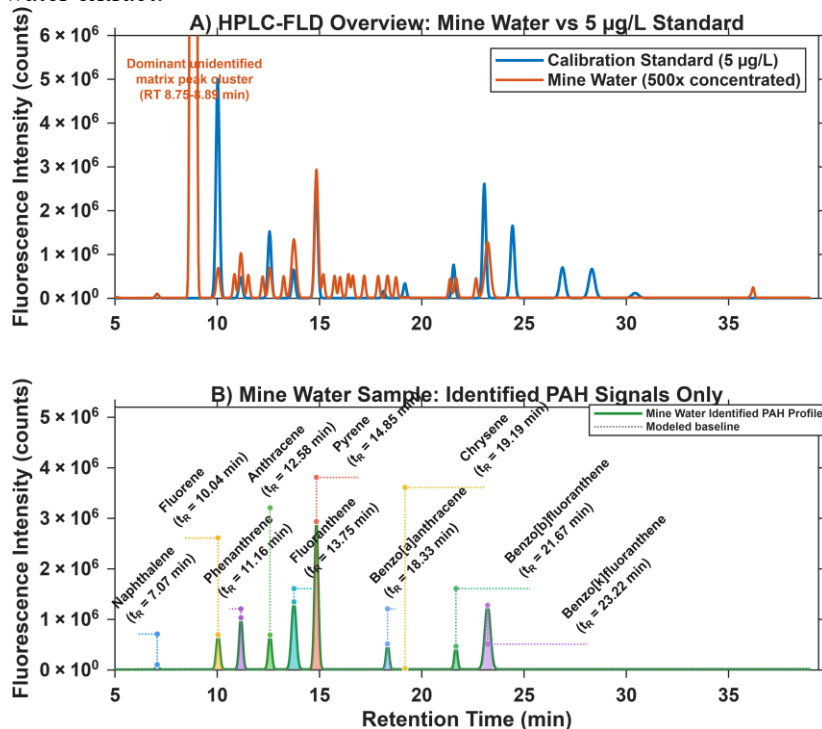


Fig. 2. HPLC-FLD chromatograms of the 5 µg L⁻¹ PAH standard and the mine-water extract concentrated 500-fold. (A) Overlay of the standard and mine-water extract. (B) Retention-time-supported PAH signals on an expanded vertical scale.

The mine-water extract showed an unidentified cluster at 8.75–8.89 min and ten signals consistent in retention time with naphthalene (7.07 min), fluorene (10.04 min), phenanthrene (11.16 min), anthracene

(12.58 min), fluoranthene (13.75 min), pyrene (14.85 min), benzo[a]anthracene (18.33 min), chrysene (19.19 min), benzo[b]fluoranthene (21.67 min), and benzo[k]fluoranthene (23.22 min). No qualifying matches were retained for benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene, or indeno[1,2,3-c,d]pyrene; acenaphthylene had no accepted assignment. These are qualitative retention-time matches, not confirmed compound identifications.

3.2 HPLC-HRMS and GC-MS characterization

HPLC-HRMS yielded 20 empirical formulas with absolute mass deviations of 0.33–5.52 ppm (Table 1). The oxygen-containing, nitrogen-containing and carbon-rich formulas indicate diversity among detected LC-ESI(+) features. Considering the fact that ionization is compound-class selective, these features do not represent bulk dissolved organic matter, and accurate mass alone cannot establish molecular structures [8, 9].

GC-MS gave six tentative library matches (Table 1), including three branched alkanes, 9H-fluoren-9-ol, “Tetraphen-7-ol,” and 1,6-dioxacyclododecane-7,12-dione. The C9–C12 alkanes could reflect legacy mining inputs or natural sources [10, 11]. If confirmed, 9H-fluoren-9-ol could be a fluorene transformation product [12]; the cyclic dione has been reported in a polybutylene adipate terephthalate (PBAT)-related context [13]. The source of each feature remains unresolved, and the “Tetraphen-7-ol” library name requires confirmation. The three methods provide complementary, but not mutually confirming evidence.

Table 1. HPLC-HRMS empirical formulas and tentative GC-MS library assignments in the untreated mine-water sample.

HPLC-HRMS: unique mine-water empirical formulas							
RT (min)	Formula	Found [M+H] ⁺	Error (ppm)	RT (min)	Formula	Found [M+H] ⁺	Error (ppm)
2.1	C ₄ H ₆ N ₂ O ₆	179.0302	+2.234	18.5	C ₁₅ H ₃₅ N ₈ O ₉	472.2602	+0.635
8.6	C ₁₂ H ₂₂ N ₂ O ₃	243.1697	-2.467	18.6	C ₈ H ₂₂ N ₃ O ₇	273.1534	+1.464
13.0	C ₁₀ H ₂₀ N ₅ O ₂	243.1688	-0.411	18.7	C ₇ H ₈ N ₇ O ₂	223.0806	-2.690
14.1	C ₂₂ H ₂₃ N ₂	316.1929	-1.581	18.7	C ₁₈ H ₁₀ N ₂ O ₂	287.0801	-4.877
14.4	C ₁₅ H ₂₅ NO ₅	300.1804	-0.333	18.9	C ₁₀ H ₁₈ O ₂	171.1381	+1.169
14.9	C ₁₆ H ₂₇ N ₆ O ₇	416.2018	+1.201	18.9	C ₁₆ H ₁₀ N ₂	231.0911	-2.164
15.1	C ₁₄ H ₂₂ N ₆ O ₂	307.1881	+1.302	19.1	C ₃₀ H ₃₅ N ₃ O ₃	486.2762	+2.262
15.4	C ₁₆ H ₂₇ NO ₅	314.1959	-0.637	19.4	C ₁₀ H ₁₉ N ₅ O ₂	242.1601	-4.129
16.4	C ₁₉ H ₂₇ N ₄ O ₂	344.2225	+5.520	19.9	C ₃ H ₄ N ₇ O ₅	219.0338	-3.652
18.5	C ₇ H ₁₄ N ₇ O ₂	229.1275	-2.619	20.02	C ₉ H ₂₅ N ₈	246.2278	+1.624
GC-MS: tentative spectral-library assignments							
RT (min)		Compound				Formula	
7.494		Nonane, 4-ethyl-5-methyl				C ₁₂ H ₂₆	
7.494		3-Ethyl-3-methylheptane				C ₁₀ H ₂₂	
12.221		Hexane, 2,3,5-trimethyl				C ₉ H ₂₀	
11.891		9H-Fluoren-9-ol				C ₁₃ H ₁₀ O	
12.626		Tetraphen-7-ol				C ₁₈ H ₁₂ O	
12.937		1,6-Dioxacyclododecane-7,12-dione				C ₁₀ H ₁₆ O ₄	

Note: HRMS formulas and GC-MS assignments are tentative; lower-score GC-MS peaks were excluded.

3.3 Relevance to the biological treatment comparison

This untreated-water reference will be compared with identically processed treated samples; no treatment effect is inferred here, and Fe redox and mineralogical changes will be evaluated independently [7].

4 Conclusions

The untreated Nižná Slaná mining influenced water sample showed a chemically heterogeneous extractable organic fraction. HPLC-FLD supported ten retention-time-consistent PAH signals, HPLC-HRMS yielded 20 empirical formulas (maximum absolute mass deviation 5.52 ppm), and GC-MS provided six tentative assignments. The GC-MS matches are compatible with broad source possibilities including legacy mining hydrocarbons, natural background, transformation products or polymer-associated inputs, without

assigning compound-specific provenance. The dataset provides a pre-treatment analytical fingerprint for direct comparison with identically processed treated samples.

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BIODEGRADATION OF PET MICROPLASTICS IN A TEST MEDIUM

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Abstract

Polyethylene terephthalate-based microplastics represent a persistent component of environmental pollution, and microbial biodegradation is considered one of the promising approaches to their elimination. The aim of this study was to evaluate the effect of *Ideonella sakaiensis* on PET microplastics and PES fibres in a model liquid medium. The five-week experiment was conducted in triplicate and evaluated gravimetrically and by ATR-FTIR analysis; intact PET and PES samples were also examined as a supplementary part of the study. A statistically significant increase in relative weight loss over time was demonstrated for PET microplastics, whereas the effect observed for PES fibres was weaker. FTIR analysis indicated surface modifications of PET while its fundamental chemical characteristics remained preserved. More pronounced changes were observed in selected parts of the intact PET sample, probably due to differences in the structure and crystallinity of the material. The study provides baseline data for further optimization of cultivation conditions and targeted material pretreatment.

Keywords: PET, PES, microplastics, biodegradation, ATR-FTIR spectroscopy, *Ideonella sakaiensis*

1 Introduction

Environmental pollution by microplastics represents a long-term problem associated with the high persistence of plastic materials and their gradual fragmentation. Significant sources include polyethylene terephthalate (PET), widely used in the packaging industry, and polyester (PES) fibres, which are also predominantly based on PET from a chemical perspective [1–3]. The complete removal of these particles from the environment is difficult; therefore, in addition to mechanical and chemical methods, biological approaches are also being investigated [4].

Figure 1 shows the bacterium *Ideonella sakaiensis*, one of the most important model microorganisms capable of hydrolysing PET and using its degradation products as a source of carbon and energy. The degradation process occurs mainly through the action of the enzymes PETase and MHETase [5,6]. However, the efficiency of the process is strongly influenced by substrate properties, particularly crystallinity, available reaction surface area, and surface treatment of the material [7]. The aim of this study was to experimentally compare the biodegradation behaviour of beverage-grade PET and PES fibres in a model liquid medium and to evaluate differences between the microplastic and intact forms of the materials.

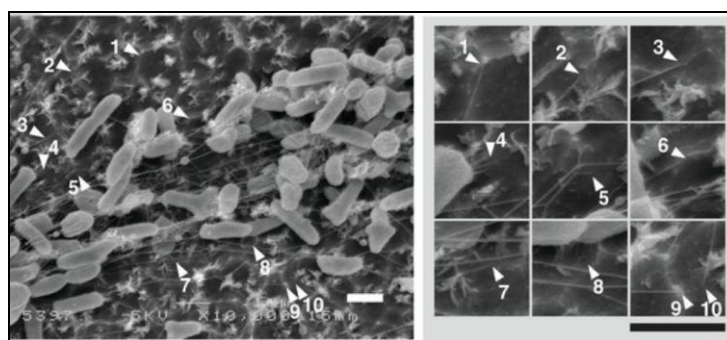


Fig. 1. *Ideonella sakaiensis* on the PET surface; extracellular appendages and contact points [5]

2 Material and methods

2.1 Tested materials

The first test material was PET obtained from conventional beverage bottles. The bottles were washed, dried, mechanically ground in an IKA MF 10 basic mill at 4 500 rpm using a 1 mm sieve, and the resulting material was homogenized. The second material consisted of TESIL® T84M PES fibres manufactured from recycled PET; the fibres used had a linear density of 3.3 dtex, a length of 38–85 mm, and a hygienic surface

finish. For the PES-MP variant, the fibres were cut into approximately 1 cm segments. Intact PET samples (PET-C; bottle neck, bottom, and a strip of the bottle wall) and intact PES fibres (PES-C) were also tested as supplementary variants.

2.2 Microorganism and cultivation conditions

The natural strain *Ideonella sakaiensis* DSM 112585 (201-F6) was used in the experiment. The working culture was propagated in liquid R2A medium for 72 h at 35 °C and 150 rpm. The biodegradation experiment itself was carried out in a YSV model liquid medium with a limited content of readily available organic carbon; the pH was maintained at approximately 7.0–7.2.

2.3 Experimental design and incubation conditions

The experiment was conducted as a five-week time-course study. A total of 34 reactors were prepared in 250 mL Erlenmeyer flasks: 15 PET-MP BIO reactors, 15 PES-MP BIO reactors, two blank reactors without inoculum, and two supplementary reactors containing PET-C and PES-C. Each MP and PES-C reactor contained 100 mL of YSV and 1.00 g of polymer. The PET-C reactor contained 100 mL of YSV and 7.55 g of polymer. For PET-MP and PES-MP, three parallel replicates were evaluated each week by destructive sampling. All reactors were incubated under aerobic conditions at 35 °C and 150 rpm. In week 3, culture purity was additionally checked microscopically; no bacterial cells were observed in the blank reactors.

2.4 Sample processing and analytical methods

At the end of cultivation, the samples were filtered through a 0.2 µm filter, washed with deionized water and 70% ethanol, and the biomass was removed by oxidative digestion with H₂O₂ at 60 °C for 24 h. This was followed by drying at 60 °C and gravimetric determination of weight loss. Chemical changes on the surfaces of PET-MP and PES-MP were evaluated by ATR-FTIR spectroscopy at weeks 0, 3, and 5. Data from the MP variants were statistically evaluated using two-way ANOVA, separate trend analysis, Spearman's correlation, and, additionally, the Wilcoxon test.

3 Results and discussion

From week 1 onward, turbidity of the medium and a characteristic microbial odour were observed in the inoculated reactors, whereas the blank reactors remained without visible changes. As shown in figure 2, gravimetric analysis showed no weight loss in either the PET-MP or PES-MP blank reactors. For PET-MP, the values were zero in weeks 1 and 2; in week 3, the mean relative weight loss reached approximately 0.88%, increasing to 1.40% in week 4 and 1.99% in week 5. For PES-MP, the weight loss remained zero during the first four weeks and became apparent only in week 5, when the mean value reached 1.30%.

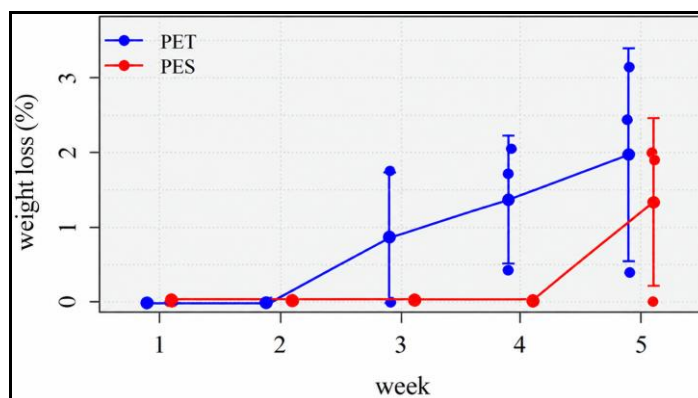


Fig. 2. Development of percentage weight loss of PET-MP and PES-MP during five weeks of cultivation.

Two-way ANOVA demonstrated significant effects of material ($p = 0.029$) and cultivation week ($p = 0.003$) on relative weight loss, while the material $\times$ week interaction was not significant ($p = 0.370$). Trend analysis confirmed an increase for PET-MP (slope 0.539% per week; $R^2 = 0.536$; $p = 0.002$) and PES-MP (slope 0.260% per week; $R^2 = 0.307$; $p = 0.032$). Spearman's correlation was stronger for PET-MP ($\rho = 0.817$; $p = 0.0002$) than for PES-MP ($\rho = 0.553$; $p = 0.032$). Wilcoxon comparison of weeks 1 and 5 showed

higher median weight loss in week 5 for both PET-MP and PES-MP, but the differences were not significant ($p = 0.064$ and $p = 0.197$, respectively). Overall, the time-dependent biodegradation effect was more pronounced for PET-MP.

For the supplementary intact PET-C sample, a relative weight loss of 10.327% was recorded in week 5, whereas no weight loss was detected for PES-C. In PET-C, surface changes were most apparent on the bottle neck and bottom, where a higher proportion of the amorphous phase can be expected [8]. However, as the intact samples were tested without replication, these results are only indicative and cannot be evaluated statistically in the same way as the MP variants.

As shown in figure 3, ATR-FTIR analysis confirmed that the fundamental chemical characteristics of PET were preserved throughout the experiment. The characteristic PET bands remained clearly detectable, particularly the ester carbonyl band at approximately 1715 cm^{-1} and the C–O bands around 1240 cm^{-1} and $1090\text{--}1110\text{ cm}^{-1}$. For PET-MP, the spectral library match decreased in weeks 3 and 5, accompanied by an increased baseline, higher spectral noise and greater heterogeneity. These changes indicate surface modification during biodegradation while the main PET bands remained preserved, suggesting predominantly surface-level alterations rather than extensive transformation of the polymer matrix.

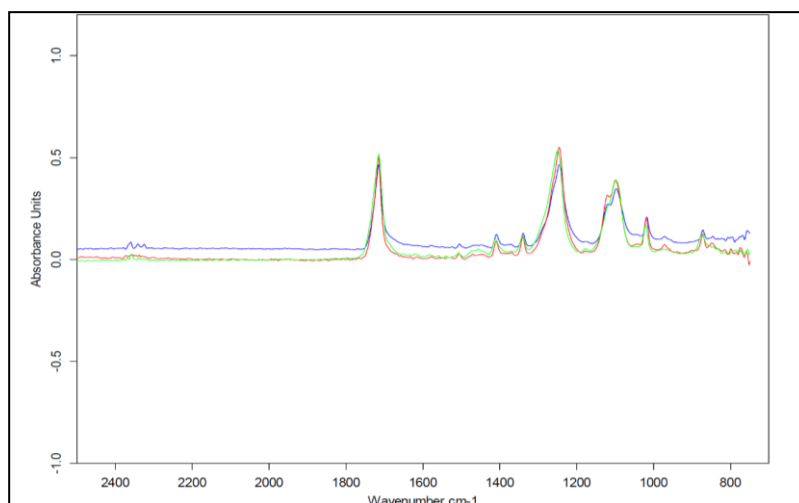


Fig. 3. Comparison of FTIR spectra of PET samples obtained at weeks 0, 3, and 5 of the biodegradation experiment; the green curve corresponds to week 0, the red curve to week 3, and the blue curve to week 5.

As shown in figure 4, no clear progressive trend was observed for PES-MP; differences between time points were more consistent with the natural heterogeneity of the fibres and variability of the measured areas. FTIR therefore supports a greater extent of surface changes in PET-MP, but not complete transformation of the polymer matrix.

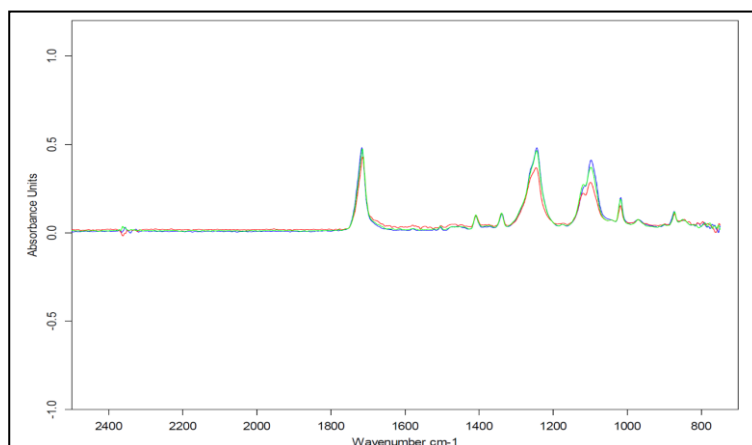


Fig. 4. Comparison of FTIR spectra of PES samples obtained at weeks 0, 3, and 5 of the biodegradation experiment; the green curve corresponds to week 0, the red curve to week 3, and the blue curve to week 5.

The difference between PET and PES can be explained primarily by material structure. Due to spinning and drawing, PES fibres are generally more oriented and crystalline, while their surface finish may further restrict access of microorganisms and enzymes to the polymer. In contrast, homogenized PET from bottles contained regions with different degrees of crystallinity. Gravimetric results may also be affected by minor particle losses during filtration, washing, and handling. Since the cultivation conditions were not optimized for maximum degradation, further experiments should consider longer cultivation, culture adaptation, static cultivation, and improved aeration control.

4 Conclusions

The results indicate that *I. sakaiensis* has measurable biodegradation potential towards beverage-grade PET, with degradation-associated changes increasing over the five-week period. The effect on PES fibres was substantially weaker and appeared only at the end of the experiment, suggesting that the physical form and structural properties of the substrate strongly influence biodegradation efficiency. Gravimetric and statistical analyses demonstrated significant effects of material type and cultivation duration, while FTIR revealed surface changes in PET-MP without altering the fundamental chemical characteristics of the polymer. Results for intact PET suggested greater susceptibility of more amorphous bottle regions, indicating that local differences in polymer structure may affect the extent of microbial attack. PET biodegradability is therefore influenced not only by chemical composition, but also by crystallinity, substrate form, surface accessibility, and surface treatment. The limited response observed for PES also indicates that fibre morphology, processing history, and possible additives may reduce the accessibility of the polymer to microbial degradation. These findings support the need for a more detailed comparison of industrial PET-based materials under controlled conditions. Further research should focus on optimizing cultivation conditions, adapting the microorganism, evaluating suitable pretreatment methods, and comparing materials with defined crystallinity and surface treatments in order to improve degradation efficiency and clarify the main limiting factors of the process.

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INDIUM REMOVAL FROM SOLUTION BY *CHLORELLA VULGARIS* BIOMASS

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Abstract

Indium is a critical metal used mainly as indium tin oxide (ITO) in flat panel displays (FPDs), touch screens and photovoltaic cells, and its recovery from secondary sources is of growing interest. This contribution presents preliminary results on the biosorption of In(III) from synthetic solution using non-living biomass of the green microalga *Chlorella vulgaris*. Batch experiments were performed at an initial In concentration of 8.8 mg/l, pH 2.9, a biomass dose of 1 g/l and a contact time of 48 h, at four temperatures (10, 25, 40 and 50 °C). Removal efficiency exceeded 90 % under all tested conditions, with the highest value (97 %) obtained at 25 °C. The results indicate that *C. vulgaris* biomass is a promising, low-cost biosorbent for In recovery from dilute solutions.

Keywords: biosorption, indium, green alga

1 Introduction

Indium is regarded as a critical raw material because of its growing industrial demand, driven mainly by the use of indium tin oxide (ITO) as a transparent conductive layer in flat panel displays, touch screens and photovoltaic cells, together with the limited availability of primary ore deposits. Waste electrical and electronic equipment, and spent flat panel displays in particular, therefore represent an important secondary source of indium that can be recovered through appropriate leaching and metal-recovery processes [1].

Among the technologies proposed for the recovery of indium and other metals from leachates obtained after the leaching of such waste, biological methods based on biosorption and bioaccumulation offer an environmentally friendly and potentially low-cost alternative to conventional physico-chemical approaches. The two processes differ mainly in their underlying mechanism (Fig. 1). Biosorption is a fast, metabolism-independent process in which metal ions bind passively onto functional groups present on the surface of non-living biomass; it is generally reversible and can reach a relatively high binding capacity. Bioaccumulation, in contrast, typically proceeds in two steps: an initial rapid surface-binding step similar to biosorption, followed by a slower, energy-requiring transport of metal ions across the cell membrane into living cells. From a technological point of view, however, a simpler distinction is often used, in which biosorption refers to processes based on non-living biomass and bioaccumulation to processes based on living cells. Using dried, non-living biomass is technologically advantageous, since biosorption is a fast process that does not require the cultivation and maintenance of a viable culture, and the biomass can be stored and dosed similarly to a conventional sorbent material [2, 3].

From a broader perspective, using microalgal biomass as a biosorbent is attractive not only because of its metal-binding properties, but also because microalgae can be cultivated on a relatively small footprint, using simple growth media, which makes biomass production for this purpose scalable and compatible with the concept of a circular economy.

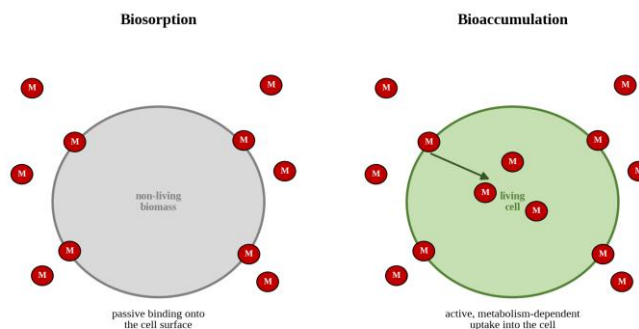


Fig. 1. Schematic comparison of biosorption (non-living biomass) and bioaccumulation (living biomass) as biological routes for the recovery of In and other metals from leachates (M; symbol for metal)

Only some studies have reported uptake of indium by microbial biomass. Bioaccumulation of In(III) by the living bacterium *Shewanella algae* has been reported from model chloride solutions as well as from real leachate obtained by leaching of spent LCD monitors, with the bacterium showing notable selectivity towards In over other metals present in the leachate [4]. Indium uptake from synthetic solution has also been reported for a psychrophilic yeast biomass of *Cryptococcus laurentii* [5]. Biosorption studies using non-living biomass are even less common; the available data, mostly obtained with brown algae and modified plant biomass, indicate that the process is strongly pH-dependent, with an optimum typically around pH 3, and that maximum sorption capacities in the range of tens of mg of metal per gram of biomass can be achieved [6, 7]. Reports on indium uptake by green microalgal biomass, such as *Chlorella vulgaris*, are, to the best of our knowledge, still missing from the literature.

The aim of this contribution is to present preliminary results on the biosorption of In(III) from synthetic solution by non-living biomass of the green microalga *Chlorella vulgaris*, focusing on the effect of temperature on the removal efficiency.

2 Material and methods

2.1 Biomass

Non-living (dried) biomass of the green microalga *Chlorella vulgaris* bought from Institute of Microbiology of the Czech Academy of Sciences was used as the biosorbent throughout this study.

2.2 Characteristics of the biosorption experiment

Batch kinetic biosorption experiments were carried out in Erlenmeyer flasks using synthetic In(III) solutions. The initial In concentration was 8.7 mg/l for the experiments performed at 10, 25 and 40 °C, and 8.8 mg/l for the experiment performed at 50 °C. The pH of the solutions was adjusted to 2.9 prior to biomass addition, as biosorption of In is known to be strongly pH-dependent. The biomass dose was 1 g/l, and the flasks were agitated for a contact time of 48 h at the respective temperature (10, 25, 40 and 50 °C). After the contact period, the biomass was separated from the solution by filtration and the residual In concentration in the filtrate was determined by ICP-MS. The removal efficiency (%) was calculated from the difference between the initial and final In concentration in solution, related to the initial concentration.

3 Results and discussion

The effect of temperature on the biosorption of In(III) by *Chlorella vulgaris* biomass is shown in Fig. 2. Final removal efficiency was high (92–97 %) achieved after 48 hours over the entire tested temperature range of 10–50 °C, indicating that, under the conditions used, final In biosorption efficiency onto *C. vulgaris* biomass is not strongly temperature-dependent. The highest removal efficiency (97 %) was obtained at 25 °C, while slightly lower values were recorded at 10 °C (92 %) and 40 °C (94 %); at 50 °C the removal efficiency increased again to 96 %.

The relatively small fluctuations in removal efficiency across the tested temperature range suggest that, similarly to other biosorption systems, physico-chemical sorption mechanisms such as ion exchange,

complexation and electrostatic interaction with functional groups on the algal cell wall (e.g. carboxyl, hydroxyl and phosphate groups) dominate over purely diffusion-controlled uptake, which would be expected to be more sensitive to temperature [3]. The slightly lower removal efficiency observed at 10 °C may reflect a moderate reduction in ion mobility and in the rate of surface binding at lower temperature, while the slight decrease at 40 °C compared with 25 and 50 °C is within the range that can be attributed to normal experimental variability at this stage of the study.

These results are promising, considering the very low initial In concentration used (below 9 mg/l), which is comparable to the concentrations that can be expected in real leachates after dilution or pre-treatment. Compared with bioaccumulation by living bacterial or yeast biomass, the biosorption process applied in this work has the additional advantage of being metabolism-independent and technologically simpler, as it does not require the cultivation and maintenance of viable cell cultures, and the loaded biomass can subsequently be processed further (e.g. by incineration) to concentrate and recover the bound indium.

These preliminary results confirm the potential of *C. vulgaris* biomass as an effective, low-cost biosorbent for In removal from dilute solutions across a broad temperature range. A more detailed evaluation of biosorption kinetics, isotherms and the effect of further process parameters (pH, biomass dose, initial concentration) is necessary to understand the process of indium biosorption by algal biomass.

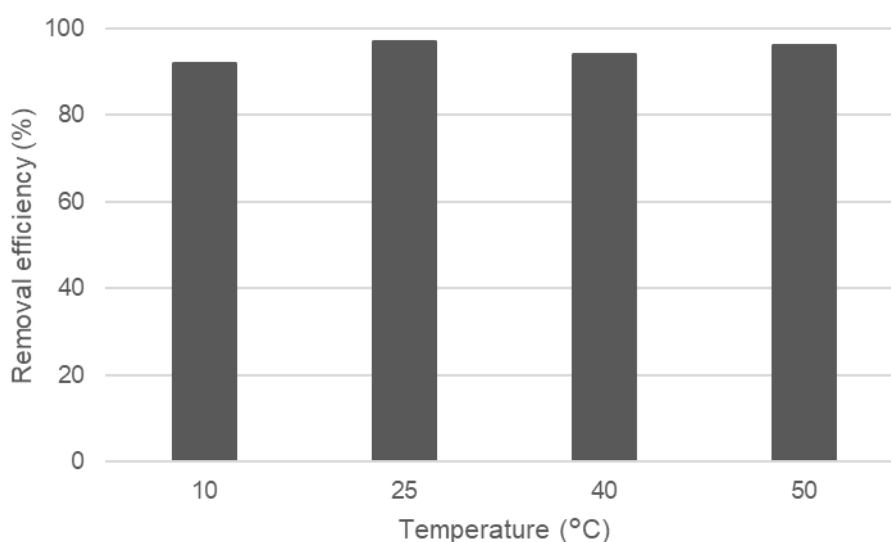


Fig. 2. Effect of temperature on the removal efficiency of In(III) by *Chlorella vulgaris* biomass (initial In concentration ~8.8 mg/l, pH 2.9, biomass dose 1 g/l, contact time 48 h)

4 Conclusions

Preliminary batch experiments showed that non-living biomass of the green microalga *Chlorella vulgaris* is able to remove more than 90 % of In(III) from dilute synthetic solution (initial concentration ~8.8 mg/l, pH 2.9, biomass dose 1 g/l) within 48 h, across a temperature range of 10–50 °C. The highest removal efficiency (97 %) was achieved at 25 °C, while the removal efficiency remained above 92 % at all other tested temperatures, indicating a robust and only weakly temperature-dependent biosorption process.

These findings support further investigation of *C. vulgaris* biomass as a promising, low-cost biosorbent for indium recovery from secondary sources, such as leachates obtained from the processing of spent flat panel displays. Future work will focus on the effect of additional process parameters, including pH, biomass dose and initial In concentration, on the biosorption efficiency and capacity, as well as on biosorption kinetics and equilibrium modelling.

Acknowledgements

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GROWTH AND FERROUS-IRON OXIDATION KINETICS OF *ACIDITHIOBACILLUS FERROOXIDANS* UNDER A 5 mT STATIC MAGNETIC FIELD

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Abstract

Acidithiobacillus ferrooxidans regenerates Fe³⁺, the main oxidant in indirect bioleaching. We examined whether a 5 mT static magnetic field accelerates growth and Fe²⁺ oxidation relative to the local geomagnetic field (~50 μT). Three independent paired experiments were analysed at 24 h; one continued through 48 h of exposure and 24 h of geomagnetic recovery. At 24 h, Fe²⁺ depletion averaged 37.5 ± 6.2% at 5 mT and 21.1 ± 9.2% in controls (p = 0.103). OD₄₃₀ and Fe³⁺ were 32.5% and 30.7% higher, respectively, but neither comparison remained significant after Holm correction (both adjusted p = 0.081). The consistent directional trend suggests faster early culture kinetics, but requires confirmation with greater biological replication and sham-coil and abiotic controls.

Keywords: *Acidithiobacillus ferrooxidans*; bioleaching; ferric iron; ferrous-iron oxidation; static magnetic field

1 Introduction

Acidithiobacillus ferrooxidans is an acidophilic chemolithoautotroph that obtains energy by oxidising Fe²⁺ to Fe³⁺. The resulting ferric iron is a principal oxidant in indirect bioleaching, linking bacterial growth kinetics to the rate at which metal-bearing substrates can be attacked [1]. The iron-dependent redox metabolism of *A. ferrooxidans* may also contribute to its susceptibility to changes in the external magnetic field. Paramagnetic iron ions and potentially magnetically interacting iron-rich nanoscale assemblies could provide a substrate capable of weakly biasing electron-transfer or membrane-associated processes, although this mechanism remains hypothetical and has primarily been considered in other microorganisms [2]. Variables that alter the timing of Fe²⁺ oxidation may therefore influence reactor start-up and process duration even when the final extent of oxidation is unchanged.

Magnetic-field responses of microorganisms are heterogeneous and depend on field intensity, exposure system and biological context [3]. In *A. ferrooxidans*, hypomagnetic exposure suppressed growth relative to the geomagnetic field [2], whereas several low-millitesla studies reported faster culture development, Fe³⁺ regeneration or metal recovery [4–6]. However, growth, iron speciation and metal solubilisation are not interchangeable outcomes: a lower residual Fe²⁺ concentration can arise from more biomass, a higher oxidation rate per cell, or altered abiotic chemistry and mass transfer.

From a biotechnological perspective, controlled magnetic-field exposure could serve as a non-chemical process variable for modulating microbial iron oxidation in bioleaching reactors. If reproducible across independent cultures and larger working volumes, earlier Fe³⁺ regeneration could shorten reactor start-up, improve process predictability and potentially accelerate metal recovery [2,4–6].

This study examined whether exposure to a 5 mT static magnetic field shifts the 48 h growth and Fe²⁺-to-Fe³⁺ conversion kinetics of *A. ferrooxidans* relative to the local geomagnetic field, and whether the profiles converge during a subsequent 24 h recovery period under the geomagnetic field.

2 Material and methods

2.1 Microorganism and cultivation

Acidithiobacillus ferrooxidans was obtained from the Institute of Geotechnics of the Slovak Academy of Sciences, Košice, Slovakia. Cells were cultivated at 30 °C with continuous air bubbling in sterile 9K medium containing 0.4 g L⁻¹ (NH₄)₂SO₄, 0.1 g L⁻¹ KCl, 0.5 g L⁻¹ K₂HPO₄, 0.5 g L⁻¹ MgSO₄·7H₂O, 0.01 g L⁻¹ Ca(NO₃)₂ and 44.2 g L⁻¹ FeSO₄·7H₂O. In each independent experiment, a common starter culture was divided into matched 100 mL aliquots assigned to the geomagnetic control or 5 mT. Three experiments were conducted on separate dates and sampled at 2 and 24 h. In one experiment, exposure continued to 48 h and

was followed by 24 h under the geomagnetic field. Three or four analytical measurements were obtained per culture and time.

2.2 Magnetic-field exposure

The 5 mT static field was generated by direct current in a custom copper coil surrounding the culture vessel. The coil comprised 800 turns of 1 mm enamelled copper wire wound in four layers on a cylindrical plastic former (50 mm outer diameter; 215 mm length). Magnetic flux density at the sample position was verified with a CYGM99C Hall-effect Gaussmeter/Teslameter (ChenYang Technologies, Germany; DC accuracy $\pm 1.0\%$). Controls were maintained under the local geomagnetic field ($\sim 50 \mu\text{T}$).

2.3 Growth and iron measurements

Growth was monitored as dilution-corrected OD₄₃₀ using an Agilent 8453 spectrophotometer; cell density was estimated as $3.2623 \times 10^6 \times \text{OD}_{430}$ cells mL⁻¹. Fe³⁺ was measured at 300 nm after 0.2 μm filtration. For Fe²⁺, samples were diluted 10- or 50-fold in acidified water; 100 μL was mixed with 1.0 mL NaF reagent, 0.4 mL 1,10-phenanthroline/acetate reagent and 1.0 mL ultrapure water, incubated for 30 min in darkness, and measured at 510 nm against a reagent blank.

2.4 Data analysis

The independent cultivation run was the experimental unit. Analytical measurements were averaged within each culture before analysis and were not treated as biological replicates.

The prespecified primary outcome was Fe²⁺ depletion from 2 to 24 h, calculated as $100 \times ([\text{Fe}^{2+}]_{2\text{h}} - [\text{Fe}^{2+}]_{24\text{h}})/[\text{Fe}^{2+}]_{2\text{h}}$. Greater depletion under 5 mT was evaluated by a one-sided paired t-test across the three run-level pairs; an exact paired sign-flip test assessed sensitivity to the parametric assumption.

OD₄₃₀ and Fe³⁺ were secondary outcomes evaluated by one-sided paired t-tests with Holm correction. Results are means $\pm$ SD of the three independent run means. Significance was assessed at $\alpha = 0.05$, but inference was exploratory because $n = 3$. Later time points were evaluated descriptively because only one experiment included the complete exposure and recovery period.

3 Results and discussion

3.1 Ferrous-iron depletion and ferric-iron accumulation

At 24 h, all three independent experiments showed responses in the predicted direction: greater Fe²⁺ depletion and higher OD₄₃₀ and Fe³⁺ concentrations under 5 mT than in the corresponding geomagnetic controls (Table in Figure 1). Fe²⁺ depletion was on average 16.4 percentage points greater under 5 mT, but the primary directional test did not reach statistical significance ($p = 0.103$). OD₄₃₀ and Fe³⁺ were respectively 32.5% and 30.7% higher under 5 mT; however, neither secondary comparison remained significant after correction for multiple testing (both Holm-adjusted $p = 0.081$).

In the single experiment covering the complete time course, the separation in Fe²⁺ depletion and cell-density estimates became more pronounced after 48 h of continuous 5 mT exposure (Fig. 1). Following the subsequent 24 h recovery period under the geomagnetic field, Fe²⁺ was nearly exhausted in both cultures and their cell-density estimates converged. Because these later observations were obtained from one matched culture pair, they were interpreted descriptively. Although the differences were not statistically significant, the cultures exposed to 5 mT consistently showed faster early iron oxidation and growth. During the recovery phase, the exposed and control cultures became more similar after 24 h under the geomagnetic field.

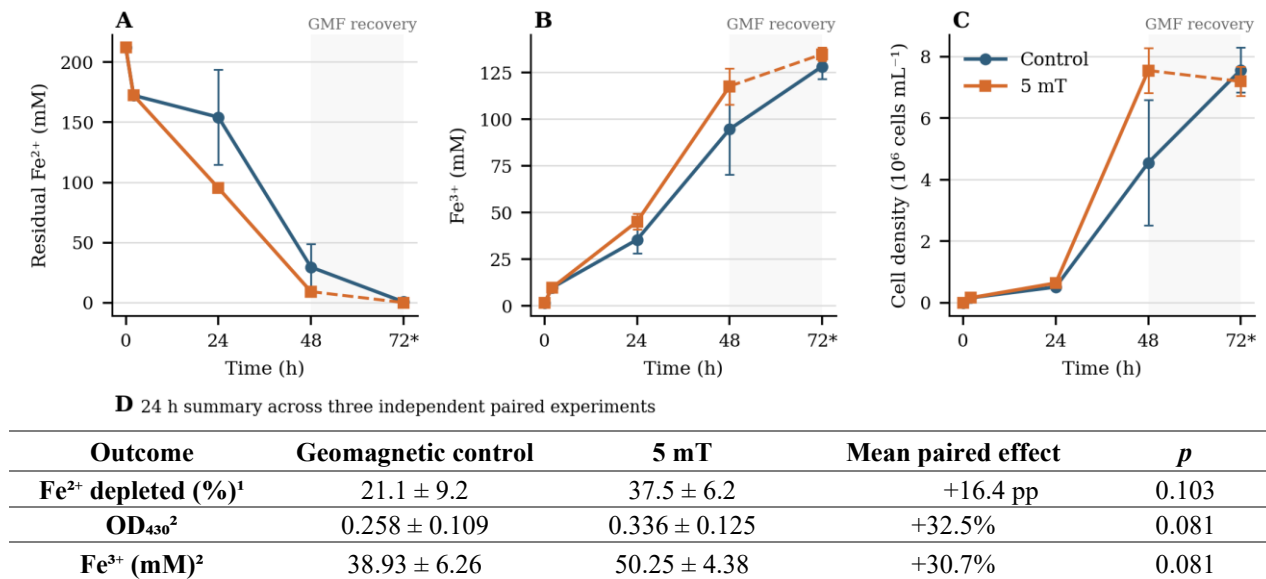


Fig. 1. Growth and iron responses of *A. ferrooxidans* to 5 mT. Panels A–C show one matched culture pair during 48 h exposure and 24 h geomagnetic recovery (dashed segment); error bars denote analytical SD. Panel D summarises the 24 h responses across three independent paired experiments (mean $\pm$ SD).¹ Prespecified primary outcome; p from a one-sided paired t -test. The exact paired sign-flip sensitivity test gave $p = 0.125$. ² Secondary outcomes; reported p values are Holm-adjusted across the two secondary comparisons. The corresponding unadjusted values were 0.056 for OD_{430} and 0.041 for Fe^{3+} .

Exposure to the 5 mT static magnetic field was associated with greater Fe^{2+} depletion and higher OD_{430} and Fe^{3+} concentrations after 24 h in all three independent experiments. However, neither the primary Fe^{2+} -depletion test nor the multiplicity-adjusted secondary comparisons reached statistical significance. The differences between the exposed and control cultures decreased during recovery under the geomagnetic field. A similar pattern was observed after hypomagnetic exposure ($<1 \mu\text{T}$) in our previous study [2]. Despite the opposite changes in magnetic-field intensity, the growth and metabolic parameters approached those of the geomagnetic-field controls during recovery in both experiments. These observations suggest that altered magnetic-field intensity may act as a temporary environmental factor.

The dependence of *A. ferrooxidans* on Fe^{2+} oxidation for energy generation provides a physiological context for the observed trend. Changes in external magnetic conditions could potentially affect iron-dependent electron transfer, ion transport or membrane-associated processes, but mechanisms involving paramagnetic ions, radical pairs or iron-rich cellular structures remain hypothetical. Furthermore, lower residual Fe^{2+} can result from greater biomass production, increased oxidation activity per cell, or changes in abiotic iron oxidation and mass transfer. The corresponding trends in OD_{430} and Fe^{3+} support an acceleration of the overall culture response, but they do not distinguish among these possibilities. The limited biological replication and absence of sham-coil and abiotic controls mean that the present experiment cannot conclusively attribute the observed differences to magnetic-field exposure. Nevertheless, their consistent direction across the independent experiments reveals a clear trend that warrants further investigation under more rigorously controlled conditions.

Although metal solubilisation was not examined, the observed trend in Fe^{2+} oxidation is relevant to bioleaching because microbial regeneration of Fe^{3+} is a key step in indirect mineral dissolution. A 3.14 mT static magnetic field increased Cu extraction from chalcopyrite-processing residues from 15.76% to 18.88% during 25 days of cultivation [7]. Fields of 2, 5, 8 and 11 mT also increased As and Cd recovery by approximately 2–8% compared with non-exposed controls [8]. More recently, fields of 3 and 5 mT improved metal recovery from spent lithium-ion batteries inoculated with *A. ferrooxidans* [5,6].

These studies measured the combined outcome of microbial activity, substrate dissolution, mineral magnetic properties, cell–surface interactions and abiotic chemistry. In contrast, the present experiment contained no solid metal-bearing substrate and therefore did not directly evaluate bioleaching. By examining growth together with Fe^{2+} oxidation and Fe^{3+} formation, it instead isolated microbial regeneration of ferric iron, which represents a key step in indirect bioleaching. If the observed trend is confirmed in adequately replicated experiments, temporary magnetic-field exposure could potentially accelerate reactor start-up or Fe^{3+} regeneration.

4 Conclusions

Across three independent experiments, 5 mT exposure consistently produced greater 24 h Fe²⁺ depletion and higher OD₄₃₀ and Fe³⁺, but none of the corrected comparisons was significant. The results therefore identify a directional trend, not a confirmed field effect. Recovery data suggest a temporary kinetic response, but were available for one culture pair. Greater replication, sham-coil and abiotic controls, and experiments with metal-bearing substrates are required to determine whether magnetic exposure can accelerate Fe³⁺ regeneration or bioleaching.

Acknowledgements

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ACTIVATED CARBON-BASED FLUIDIZED-BED REACTOR FOR IRON OXIDATION BY ACIDOPHILIC AUTOTROPHS: IMPLICATIONS FOR BIOLEACHING AND AMD TREATMENT

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Abstract

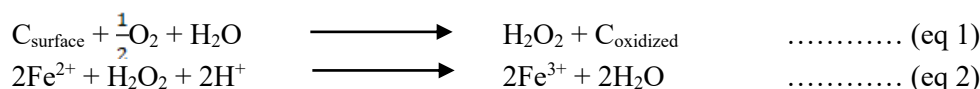
To enhance ferrous iron (Fe^{2+}) oxidation rates and prevent biomass washout, iron-oxidizing acidophilic autotrophs were cultivated in a fluidized-bed reactor (FBR) using activated carbon (AC) as a support matrix. Activated carbon was selected due to its high specific surface area, porosity, chemical inertness, mechanical strength, and resistance to attrition under fluidization conditions. Its uniform particle size distribution and relatively high density promoted stable hydrodynamics, minimizing channelling, particle segregation, and carrier loss. Autotrophic growth and biomass accumulation on the carrier were monitored through online off-gas analysis of O_2 consumption and CO_2 uptake. The high biomass retention achieved within the reactor supported elevated iron oxidation rates under adequate oxygen supply. This configuration is particularly attractive for bioleaching processes, where rapid iron cycling and high redox potentials are essential, as well as for environmental applications such as acid mine drainage (AMD) remediation. By separating biological Fe^{2+} oxidation from chemical sulphide leaching, each process can be operated under optimal conditions, reducing microbial stress and enhancing mineral dissolution and metal recovery.

Keywords: Heterogeneous catalysis, Activated carbon, Respirometry, Electron shuttling, Fluidized bed reactor, Abiotic oxidation kinetics.

1 Introduction

High-rate continuous bio-oxidation of ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) is essential for producing active leaching agents in biohydrometallurgy and managing iron-rich industrial and mine waters. However, continuous high-rate operation without biomass retention often leads to cell washout, limiting volumetric productivity. Fluidized Bed Reactors (FBRs) using solid carrier materials resolve this limitation by retaining high biomass concentrations while providing excellent fluid dynamics and rapid mass transfer [1].

Granular activated carbon (AC) is a superior carrier matrix due to its extensive specific surface area, porous architecture, chemical stability in acidic media, and resistance to particle attrition during continuous fluidization. Its high skeletal density establishes uniform bed fluidization, preventing bed channelling, particle segregation, and media loss. In addition to serving as a physical support for microbial biofilms, activated carbon possesses intrinsic surface conductivity, acting as a heterogeneous redox mediator that accelerates electron transfer from dissolved Fe^{2+} to molecular oxygen (O_2) producing reactive oxygen species such as hydroxyl radicals ($\bullet\text{OH}$) [2,3]:



It was reported by [4] that activated carbon is able to directly oxidize Fe^{2+} to Fe^{3+} through its surface oxygenated groups and/or indirectly by producing H_2O_2 from O_2 according to equation 1 and 2.

Despite widespread deployment of AC-FBRs, conventional reactor modelling often assigns 100% of Fe^{2+} turnover to microbial pathways (e.g., *Leptospirillum ferriphilum*), ignoring the abiotic catalysis occurring on the carrier surface. Furthermore, online respirometric monitoring of carbon dioxide evolution (CO_2) in autotrophic biological systems can be skewed by the chemical oxidation of the carbon support matrix itself under low pH and strongly oxidizing conditions [5].

This paper quantifies the abiotic kinetic rate constants (abiotic) and surface degradation dynamics of steam-activated carbon in an uninoculated FBR. By comparing these abiotic metrics against baseline autotrophic growth kinetics (*L. ferriphilum*), we established a decoupled mass-balance framework that separates physical surface reactions from biological uptake which reveals the impact of AC-FBR in bioleaching and acid mine drainage (AMD) treatment.

2 Materials and Methods

2.1 Support Material and Reactor Hydrodynamics

Norit® ROW 0.8 mm steam-activated carbon pellets (CAS No. 7440-44-0) were pre-treated (conditioned) by acid washing with 1M H₂SO₄, rinsing with deionized water, and drying at 40°C. Dried carrier material (250 mL) was loaded into a bottom-up fluidization column hydraulically connected to an aeration reservoir.

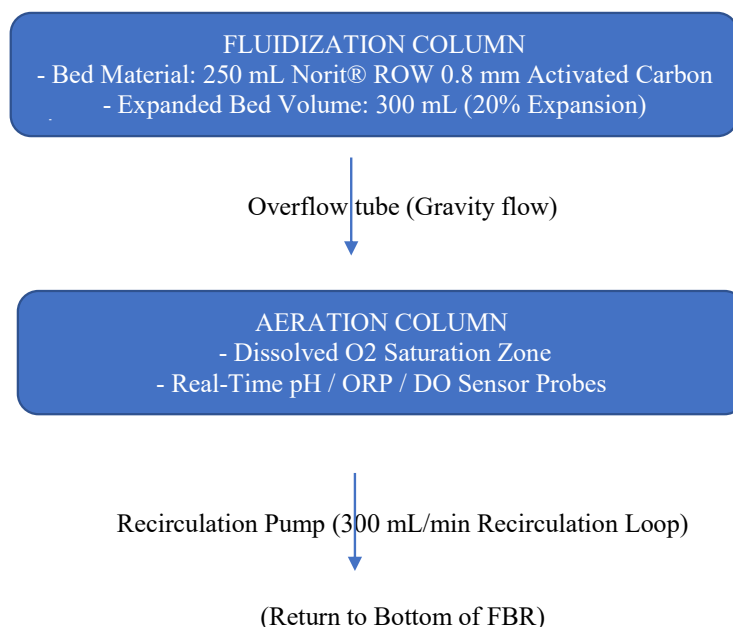


Fig. 1. Flow diagram of the fluidized bed reactor set-up

Fluidization was maintained by recirculating liquid at 300 mL min⁻¹, yielding a steady 20% bed expansion (expanded bed volume: 300 mL; total system liquid inventory: 620 mL). Hydrodynamic stability was verified by uniform bed expansion without channelling or particle carryover.

2.2 Bacterial Cultivation & Online Respirometry

Iron-oxidizing acidophilic autotrophs (*Leptospirillum ferriphilum*, DSM 14647) were cultivated in an 80 mM H₂SO₄ mineral medium (pH 1.2, initial Fe²⁺ approx 160 mM at 40°C). Low pH effectively reduced the amount of ferric iron precipitation. Respirometric kinetics were monitored using online off-gas analyses to measure oxygen uptake rates O₂ and carbon dioxide uptake/fixation rates CO₂. Dissolved oxygen (DO), pH, and oxidation-reduction potential (ORP); using Pt/Ag/AgCl combined electrode, were recorded continuously using Consort D230 data acquisition units. Aqueous iron concentrations Fe²⁺ and total iron (Fe_{total}) were analysed via o-phenanthroline and UV spectrophotometry (300 nm, 510 nm for Fe³⁺).

3 Results and Discussion

3.1 Respirometric Kinetics of Autotrophic Growth

In the batch biological controlled system, real-time monitoring of off-gas dynamics provided continuous measurements of autotrophic growth kinetics. *L. ferriphilum* exhibited clear exponential respiration phases.

During exponential growth on Fe²⁺ at 40°C, the biological culture achieved a maximum specific growth rate (μ) of 0.11 h⁻¹. Peak CO₂ fixation (CO₂) reached 0.12 mmol L⁻¹ h⁻¹ at 23 hours of incubation, directly mirroring biomass accumulation within the system. This is similar to the growth rate in [6].

Oxygen consumption (O₂) peaked at 3.5 mmol L⁻¹ h⁻¹, corresponding to a total cumulative oxygen consumption of 33 mmol L⁻¹ upon complete Fe²⁺ oxidation. The sharp drop in both O₂ and CO₂ signals at 23 hours marked the exact point of substrate (Fe²⁺) exhaustion.

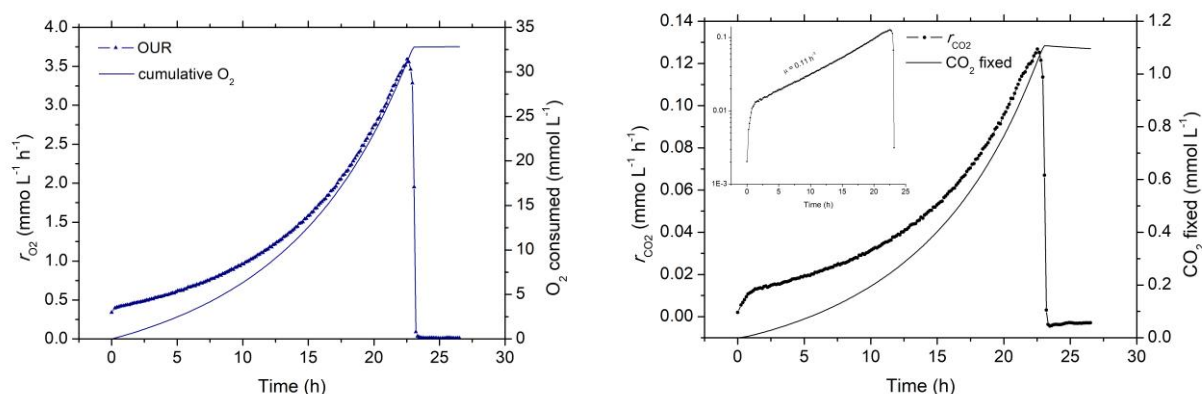


Fig. 2. Biological Respirometric Baseline of *L. ferriphilum*. Oxygen consumption rates r_{O_2} ($\text{mmol L}^{-1} \text{h}^{-1}$) (A). Carbon fixation kinetics r_{CO_2} ($\text{mmol L}^{-1} \text{h}^{-1}$) (B). Both O_2 & CO_2 consumption are expressed as positive values.

3.2 Abiotic Heterogeneous Catalysis in AC-FBR

In the uninoculated FBR containing 250 mL AC, abiotic oxidation of Fe^{2+} proceeded at rates significantly higher than homogeneous aqueous chemical oxidation. The kinetics observed appears to be in two stages, an initial rapid decrease in the early hours and a slow deceleration approaching nearly constant level in the later hours (as seen in fig 3, blue), this is similar to the findings in [3], AC acted as a surface electron shuttle, facilitating electron transfer from dissolved Fe^{2+} to O_2 and generating secondary reactive radicals ($\cdot OH$). This behaviour has been described by [4], they document that activated carbon (AC) facilitates heterogeneous Fe^{2+} oxidation through surface oxygen functional groups and electron-transfer pathways (via quinone/hydroquinone redox cycling). The calculated Fe^{2+} consumption curve derived from off-gas O_2 declined just as biological oxidation.

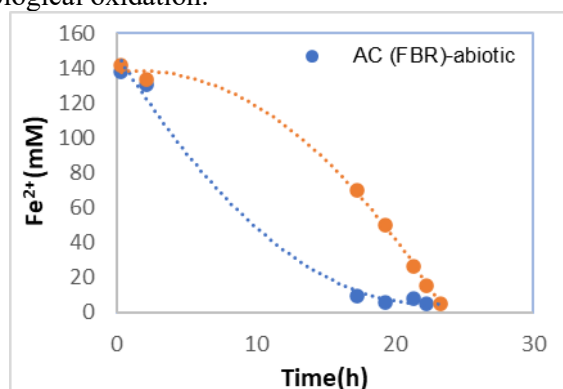


Fig. 3. Biological batch system Fe concentration (mM) (Red). FBR carrier system Fe concentration (mM) (Blue)

As detailed in Table 1 the abiotic FBR system converted over 95% of the initial 160 mM Fe^{2+} within 23.5 hours, maintaining redox potentials above +950 mV (Ag/AgCl), without biological activity.

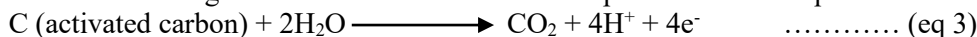
Table 1. Kinetic performance and respirometric parameters of biological and AC-FBR operational modes

Parameter / Operational Metric	Biological Batch System	Activated Carbon FBR System
Primary Catalyst	<i>L. ferriphilum</i> cells	Norit® ROW 0.8 mm AC Pellets
Dominant Mechanism	Autotrophic enzymatic electron transfer	Surface-mediated electron shuttling ($\cdot OH$)
Operating Temperature	40°C	40°C
Time to Complete Oxidation	23.0 hours	23.5 hours
Peak Oxygen Utilization Rate (O_2)	3.5 $\text{mmol L}^{-1} \text{h}^{-1}$	2.8 $\text{mmol L}^{-1} \text{h}^{-1}$
Peak Gas Exchange Behaviour (CO_2)	Net fixation (0.12 $\text{mmol L}^{-1} \text{h}^{-1}$)	Net evolution (0.04 $\text{mmol L}^{-1} \text{h}^{-1}$)

3.3 Carbon Matrix Degradation Kinetics and Respirometric Interference

A notable finding from off-gas respirometry was the continuous evolution of positive CO₂ signals in the uninoculated AC-FBR (data not shown). In control experiments running acidified mineral salt solutions (80 mM H₂SO₄) over AC without iron, steady CO₂ production occurred (0.02–0.04 mmol L⁻¹ h⁻¹).

This confirms that the steam-activated carbon undergoes direct oxidative degradation under warm, strongly acidic conditions (see equation 3), a phenomenon consistent with the abiotic electrochemical oxidation and baseline gasification of activated carbon reported in acidic aqueous environments [7].



In a hybrid bio-assisted FBR where *L. ferriphilum* fixes atmospheric CO₂, this abiotic CO₂ release offsets measured biological consumption. Uncorrected off-gas carbon balance calculations can therefore underestimate true biological cell synthesis rates (μ) by up to 25–30% if background matrix oxidation is ignored.

4 Conclusions

Activated carbon pellets (Norit® ROW 0.8 mm) provided stable hydrodynamics without particle segregation, attrition, or media loss during continuous fluidization. Uninoculated AC functioned as a highly effective heterogeneous catalyst, converting >95% of 160 mM Fe²⁺ within 23.5 hours. However, background oxidative degradation of the AC support matrix generates continuous baseline CO₂ off-gassing (0.02–0.04 mmol L⁻¹ h⁻¹) under acidic, warm conditions. Accounting for this matrix degradation is essential to prevent a 25–30% underestimation of biological cell growth and biomass accumulation when using respirometric carbon balances in bio-assisted AC-FBRs for bioleaching and AMD treatment.

Acknowledgements

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IRON OXIDE NANOPOWDER TRANSFORMATIONS TRIGGERED BY ASPERGILLI

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Abstract

Fungal strains of *Aspergillus* genus naturally possess a high capacity to alter the physicochemical composition of nearby minerals. This is typically initiated and facilitated by the acidolysis, a process involving the secretion of acidic or redox active compounds that promote mineral dissolution, extracting metallic ions into the extracellular environment. These naturally occurring processes affect the mineralogy of iron oxides, e.g., magnetite, in the vicinity of growing microorganisms to various extent. Our study offers insights into the mineralogical and structural alterations induced by fungal interactions with magnetite nanopowder.

Keywords: bioleaching, biosorbent, iron oxides, filamentous fungi

1 Introduction

Metabolically active filamentous fungi naturally possess a high capacity to alter the physicochemical composition of nearby minerals [1]. This is typically initiated and facilitated by the acidolysis, a process involving the secretion of acidic or redox active compounds that promote mineral dissolution, extracting metallic ions into the extracellular environment. The biochemically induced extraction of elements from solid (mineral) phases is commonly known as bioleaching [2], which, besides chemical interactions, also involves physical interplay between microbial cells and solid mineral surfaces [3].

These naturally occurring processes affect the mineralogy of iron oxides, e.g., magnetite, in the vicinity of growing microorganisms to various extent [4]. And thus, there is an understandable notion that the structural and mineralogical properties of the iron oxides, natural scavengers of potentially toxic metals and metalloids [5–8], can be altered. To evaluate these alterations, the implementation of a highly sensitive methods to determine the iron structural properties in solid samples are needed.

2 Material and methods

Our evaluation of iron oxides' transformation involved dynamic cultivation of *Aspergillus* fungal strain in the presence of 0.5 g of commercially available magnetite nanopowder ($\text{FeO} \cdot \text{Fe}_2\text{O}_3$, Sigma-Aldrich), suspended in the sterile Czapek-Dox culture medium for 8 days to reach early stage of stationary growth phase.

After the desired cultivation period, the grown biomasses with immobilized magnetite were filtered using a membrane filter with pore sizes of 0.1 μm (Merck Millipore, USA) and lyophilized before conducting XRD and Mössbauer analysis of these biocomposite. The filtrate was collected and after stabilization using concentrated HNO_3 , the total bioextracted iron was measured using FAAS.

3 Results and discussion

The presence of magnetite within fungal biomasses has been confirmed using powder XRD analysis. The Figure 1 highlights diffraction peaks corresponding in the angles and intensities to the standard crystallographic data of magnetite (JCPDS Card No. 19–0629).

The incubation with *Aspergillus* strain has increased background noise and the broad diffraction peak centred around the 2Theta of 20° suggests a probable transformation of magnetite into a low-ordered biogenic crystallites with crystal-core diameter up to 7 nm.

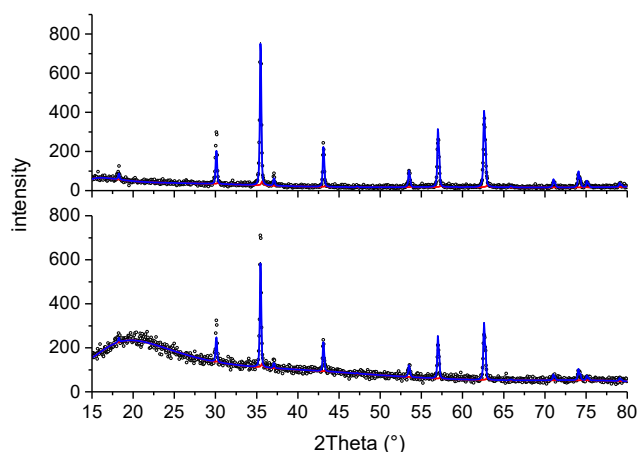


Fig. 1. X-ray diffraction patterns of magnetite nanopowder (up) before and (down) after treatment with *Aspergillus* strain. Open circles represent the experimental diffraction data, while the solid lines correspond to the Rietveld-refined profiles for magnetite and maghemite.

The analysis of the hyperfine parameters calculated from recorded spectrum of Mössbauer analysis (Figure 2) confirmed the presence of magnetite as a major component (90%), while maghemite can be considered a minor component (9%) in the nanopowder. The remaining 1% of iron in sample is associated with nanosized superparamagnetic particles.

While the fungus did not significantly impact the stability of the magnetite nanopowder resulting in negligible release of iron ($2.7 \text{ mg} \cdot \text{L}^{-1}$), it displayed the ability to distort the crystal lattice of magnetite nanoparticles as indicated by Mössbauer analysis (Figure 2) at room temperature highlighting the increased occurrence of the particles with size $\sim 10 \text{ nm}$. However, no significant changes were observed in the valence state of iron in the sample after incubation with fungus.

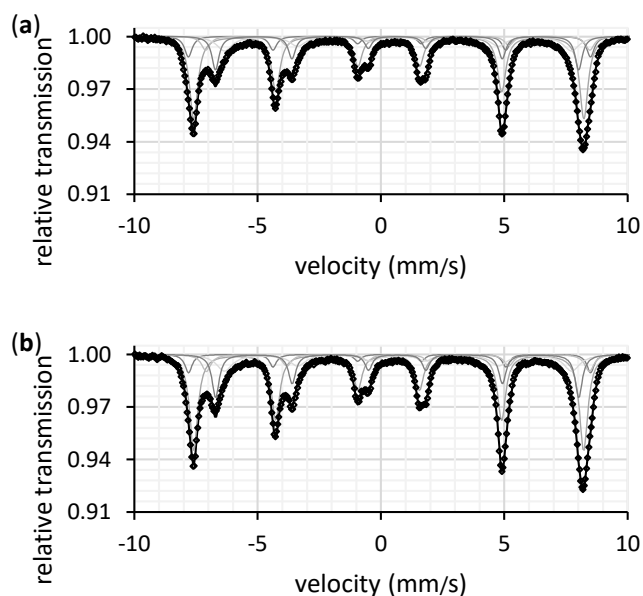


Fig. 2. Mössbauer spectra of magnetite nanopowder (a) before and (b) after treatment with *Aspergillus* strain.

4 Conclusions

In conclusion, our study offers insights into the mineralogical and structural alterations induced by fungal interactions with magnetite nanopowder. *Aspergillus* strain showcased the ability to trigger crystal lattice distortions in magnetite, suggesting its potential to induce structural changes even with limited bioleaching activity.

Acknowledgements

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PHOTOVOLTAIC PANELS IN THE ENVIRONMENTAL CONTEXT – MATERIAL DEGRADATION AND RECYCLING CHALLENGES

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Abstract

The environmental impact of photovoltaic panels was presented in two main aspects: material degradation and challenges associated with PV module recycling. The analysis focused on degradation processes occurring during panel operation under real environmental conditions, with particular emphasis on microcracks. Potential risks resulting from the possible migration of metals and critical elements into soil and water environments were also presented. In addition, the issue of photovoltaic panel recycling was presented, with particular attention paid to the promising possibilities of applying bioleaching methods for metal recovery from PV waste, as well as the potential biodegradation of EVA layers using microorganisms.

Keywords: photovoltaic panels, degradation, recycling, bioleaching, TCE, metals migration

1 Introduction

Photovoltaic technology is currently the fastest-growing technology for generating electricity from renewable sources. It is estimated that the volume of photovoltaic waste could reach between 1.7 and 8 million tonnes by 2030 [1]. Consequently, the end-of-life (EoL) management of photovoltaic (PV) panels will become a key challenge for the circular economy in the coming decades. Despite the significant environmental benefits associated with the use of photovoltaic systems, increasing attention is being paid to the impact of PV modules throughout their entire life cycle. This focus centres primarily on material ageing processes occurring during long-term operation and on the challenges associated with handling panels after their service life has ended. Material degradation not only leads to a gradual decline in the energy efficiency of the modules but also affects their mechanical integrity, durability, and the feasibility of effectively recovering valuable raw materials. Furthermore, structural damage can increase the risk of metals and other constituents leaching into the environment, particularly in cases of improper disposal or uncontrolled waste management [2,3]. Modern crystalline silicon (c-Si) photovoltaic modules consist primarily of tempered glass (approximately 65–75% of the module mass), an aluminium frame (10–20%), polymer encapsulation materials—most notably ethylene-vinyl acetate (EVA) copolymer - a backsheet, silicon cells, and conductive metals such as aluminium, copper, and silver (c-Si accounts for 98% of global production). Depending on the technology employed (e.g., CIGS - CuInGaSe or CdTe), they may also contain technology-critical elements (TCE) such as In, Ga, Ge, and Te, which are primarily used in thin-film photovoltaic modules [2]. The article highlights the environmental aspects of the use and management of spent photovoltaic modules, focusing particularly on material degradation processes and their potential environmental consequences. It also presents recycling methods, including emerging biological approaches that could offer a more selective and environmentally beneficial alternative to traditional chemical methods in the future.

2 Material degradation and defects in photovoltaic modules

Material degradation of PV modules during the operational phase results from the long-term impact of environmental, mechanical, thermal, and chemical factors, as well as their interaction with the specific materials constituting the module's structure. The processes involved can lead to a gradual deterioration of the module's material integrity and functionality; furthermore, individual degradation mechanisms can trigger and exacerbate one another, creating coupled degradation pathways [4]. The literature [5–7] clearly demonstrates that a decline in electrical parameters is a common phenomenon of module ageing. However, prolonged exposure can also increase the likelihood of material degradation and structural defects, which can compromise the protective integrity of the PV modules and potentially increase the availability of the metal-

containing inner layers. Lillo-Sánchez et al. [6] examined 56 silicon modules after 22 years of operation in Seville. They found an average decline in peak power of 30.9%, or about 1.4% per year, and numerous serious defects, including severe browning, a "milky pattern," and oxidation of the metallization grid. Importantly, the authors noted that these serious defects increased during the final years of operation and contributed to an accelerated degradation rate. Long-term operation may progressively compromise the structural integrity of PV modules through mechanisms such as microcracking, delamination, encapsulant degradation, and corrosion, potentially increasing the exposure of internal material layers to environmental agents. Table 1 summarizes selected PV degradation defects, categorized by their causes, impact on module efficiency, and consequences for subsequent recycling.

Table 1. Main degradation mechanisms of PV and their potential operational, environmental and recycling implications [2,3]

Degradation mechanism	Main causes	Effect during operation	Environmental implications	Recycling implications
Microcracks in silicon cells	Mechanical and thermal stress	Hot spots, power loss, cell isolation	Potential metal exposure and leaching	Lower Si recovery, higher losses
EVA encapsulant degradation	UV, heat and moisture	Yellowing, transparency loss, embrittlement	Release of degradation products; increased material exposure	Difficult glass/cell separation
Delamination	Ageing, heat and moisture	Layer separation, loss of integrity	Increased water access to internal layers	Reduced glass and Si recovery
Corrosion of metallic interconnections	Moisture, oxygen and temperature changes	Higher resistance, electrical failure	Potential metal release and mobilization	Lower metal purity
Backsheet cracking and degradation	UV, heat and mechanical stress	Cracking, embrittlement, loss of insulation	Increased exposure to rainwater	Difficult polymer separation
Potential-induced degradation (PID)	High voltage and humidity	Leakage currents, power loss	Potential increase in PV waste	Earlier EoL of modules

3 Potential environmental consequences of PV degradation

One of the most frequently discussed environmental aspects associated with the operation of photovoltaic modules is the potential release of metals and critical elements into soil and water environments. Under normal operating conditions, this risk is considered very low because the module's active layers are hermetically sealed between glass sheets and polymer layers, effectively limiting material contact with the surrounding environment. However, the situation may change in the event of mechanical damage to the panels, long-term aging, improper storage, or uncontrolled destruction (shredding) after the end of their service life. Potential sources of contamination include, first and foremost, metals used in electrical connections (Pb, Sn, Cu, Ag) and, in the case of thin-film modules, technologically critical elements such as Cd, Te, In, Ga, or Se [8]. It must be emphasized, however, that their mobility depends on numerous factors, including the module type, the degree of material degradation, environmental pH, the chemical composition of infiltrating waters, and the duration of exposure.

Field and laboratory studies conducted to date indicate that intact photovoltaic modules do not cause a significant increase in metal concentrations in the soil beneath photovoltaic installations. Robinson et al. [9], analysing soil samples taken from beneath silicon modules and reference sites, found no statistically significant enrichment of the soil with lead or cadmium, although they did observe local variations for certain trace elements. Similar conclusions were presented in a review by Kwak et al. [8], indicating that the potential risk of metal release during the normal operation of the panels remains low. Field observations by Robinson et al. [9] revealed elevated concentrations of selenium, strontium, lithium, nickel, and barium in soils beneath c-Si photovoltaic systems compared to adjacent control soils, suggesting local enrichment associated with the photovoltaic infrastructure. However, mean concentrations remained below levels considered ecologically hazardous. At the same time, laboratory studies have shown that under conditions simulating module damage—particularly in acidic environments or when the material is fragmented—increased leaching of certain elements may occur [8,9]. This phenomenon is of particular significance for decommissioned panels that have not undergone proper recycling. In such cases, the degradation of protective layers can facilitate contact between active materials and rainwater, thereby increasing the mobility of selected metals and critical elements.

4 PV recycling: conventional methods and emerging biological approaches

Current recycling technologies encompass mechanical, thermal, and chemical processes, often employed in hybrid systems. A major challenge remains the effective removal of the EVA encapsulation layer, which undergoes cross-linking after years of service, creating a strong bond between the glass and the photovoltaic cells. This phenomenon significantly hinders the separation of individual module components and increases the energy intensity of thermal processes or the demand for aggressive chemical reagents, thereby raising both recycling costs and environmental impact [3,10]. Recovering high-purity materials also presents a significant challenge. While mechanical processes allow for the efficient recovery of aluminium and glass, the recovery of Ag and TCE requires advanced hydrometallurgical methods or hybrid technologies [10]. Further complications arise from the progressive degradation of modules during their service life - including cell micro-cracking, corrosion of electrical connections, and polymer material ageing - which can reduce the quality of recovered raw materials and increase material losses.

Biotechnological methods for the recovery of metals from PV waste remain relatively underexplored compared with conventional hydrometallurgical and pyrometallurgical routes. Recent studies [11–13] have demonstrated the feasibility of bioleaching for selected metals from crystalline-silicon PV waste, with the majority of experimental work focusing on silver recovery from end-of-life c-Si solar cells. Lin et al. [11] proposed an acid-free process combining pyrolytic decapsulation, bioleaching and subsequent silicon recovery; bioleaching of the resulting c-Si cells achieved 44.7% Ag dissolution in a single cycle. Pang et al. [12] investigated cyanogenic bioleaching using *Pseudomonas fluorescens* for Ag and Al recovery from c-Si PV cells, demonstrating that metal dissolution was governed by both reaction and diffusion processes. In the further works, they demonstrated that coupling cyanogenic bioleaching with a low-intensity direct electric current markedly enhanced Ag dissolution, highlighting the potential of process intensification for overcoming the inherently slow kinetics of conventional bioleaching. Overall, the available literature remains limited and is predominantly focused on Ag recovery from c-Si cells, whereas the selective biological recovery of other technology-critical elements from complex PV waste streams remains insufficiently investigated. This research gap is particularly relevant for elements such as In, Ga, Ge and Te, as well as for integrated processes capable of treating heterogeneous PV materials rather than isolated cell fractions. The recent review by Johnston et al. [13] confirms that microbial and enzyme-based recovery from solar-panel waste is promising, but still requires substantial development in terms of selectivity, kinetics, feedstock preparation and process scalability.

One of the significant problems limiting the biological processing of PV is EVA degradation. Although direct studies on PV-grade EVA are still scarce, several studies have demonstrated the susceptibility of EVA to microbial degradation. Abrusci et al. [14] investigated photodegraded EVA films using a bacterial consortium composed of *Bacillus cereus*, *Bacillus megaterium* and *Bacillus subtilis*, as well as *Brevibacillus borstelensis*. After 90 days of incubation, biodegradation assessed by CO₂ evolution reached approximately 15% for pure photodegraded EVA and 23–26% for EVA containing Ca- or Fe-stearate pro-oxidant additives, indicating that prior oxidative ageing substantially enhanced microbial degradation. Maidarjav et al. [15] demonstrated direct biodegradation of EVA by *Klebsiella aerogenes* EM011. The bacterium was able to grow in carbon-free medium using EVA as the sole carbon/energy source, resulting in decomposition of approximately $0.65 \pm 0.04\%$ of the EVA film mass after 30 days. Other microbial systems have also shown activity toward EVA. Abdulina et al. [16] reported 2.2–8.8% EVA destruction after 180 days using hydrocarbon-oxidizing bacteria and 0.21–0.6% after 90 days using sulphate-reducing bacteria. Their results, including measured lipolytic activity and spectroscopic evidence of changes in the ester groups of EVA, suggest that extracellular microbial enzymatic activity may contribute to polymer-chain degradation.

5 Conclusions

Despite the rapid development of photovoltaic technologies, the long-term environmental consequences of PV degradation remain insufficiently understood, particularly regarding the mobility and potential release of TCE (In, Ga, Ge, Te). Long-term module degradation involves interconnected physical, chemical, and mechanical processes; their intensification can increase the access of environmental agents to the module's internal layers and potentially facilitate the mobilization of the metals contained within them. The growing number of modules reaching the end of their service life highlights the need for further research into the release, mobility, and speciation of these elements in soil and aquatic environments, as well as into effective recovery methods. At the same time, the bioleaching of PV waste remains in the early stages of

development, with existing studies focusing primarily on silver recovery from silicon cells. Biological methods for the selective recovery of In, Ga, Ge, and Te from PV waste remain far less understood, indicating a significant research gap and a need for further investigation.

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PHOTOVOLTAIC PANELS AS A POTENTIAL SOURCE OF TECHNOLOGY-CRITICAL ELEMENTS IN THE ENVIRONMENT

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Abstract

Photovoltaic panels are currently an important green source of energy production. However, exposure to atmospheric conditions and mechanical damage may cause the loss of their compact structure, resulting in the formation of so-called snail trail defects. These defects may lead to the leaching of elements contained in the panels and consequently contribute to environmental contamination. The aim of the study was to investigate whether operating photovoltaic panels affect the surrounding soils. Soil samples were collected in the immediate vicinity of photovoltaic panels installed on a private property in Jasienica (near Bielsko-Biała), Poland. A total of 24 soil cores, each 30 cm long, were collected. Basic physicochemical properties of the soils were analysed. Inductively coupled plasma mass spectrometry (ICP-MS) was used to determine the elements including Technology Critical Elements (TCE) in soils. The study provides one of the first field-based assessments combining soil magnetometry and TCE determination around a photovoltaic installation in Poland and contributes to the growing body of evidence supporting the environmental sustainability of photovoltaic energy systems.

Keywords: Technology Critical Elements, PV, Photovoltaic Panels, soil contamination, magnetic susceptibility

1 Introduction

The rapid expansion of photovoltaic (PV) technology has become one of the key pillars of the global transition toward renewable energy systems and climate neutrality. PV installations play a crucial role in achieving international climate goals and promoting sustainable development. The sustainable development of photovoltaics, however, requires not only technological innovation and increasing energy efficiency but also a comprehensive assessment of their environmental impacts throughout the entire life cycle of PV systems. Such assessments should include the extraction of raw materials, manufacturing processes, operational phases, and end-of-life management of photovoltaic modules. The full environmental benefits of photovoltaics can only be realized when both energy production and potential environmental risks associated with the materials used in PV technologies are carefully considered [1–3]. Modern photovoltaic modules contain a variety of elements, including technology-critical elements (TCEs), which are essential to produce advanced energy technologies. Depending on the type of photovoltaic cell, these elements may include silver, indium, gallium, tellurium, selenium, and other metals that contribute to the efficiency and durability of PV systems. Although the concentrations of these elements in individual modules are relatively low, the rapidly growing number of installations worldwide raises questions regarding their long-term environmental behaviour and potential release into surrounding ecosystems. Photovoltaic cells are designed to minimize the release of potentially harmful substances through encapsulation within protective layers of glass and polymeric materials. These barriers protect the cells from ultraviolet radiation, moisture, oxygen, and mechanical damage, thereby ensuring long-term operational stability [4]. Nevertheless, prolonged exposure to environmental factors such as UV radiation, temperature fluctuations, precipitation, and mechanical stress may gradually degrade the protective layers. Damage occurring during installation, operation, extreme weather events, or improper maintenance may further compromise module integrity and potentially facilitate the migration of selected elements into soils and other environmental compartments. In recent years, increasing attention has been paid to the environmental consequences of photovoltaic infrastructure, particularly regarding the possible accumulation of metals and technology-critical elements in soils surrounding PV installations. Although photovoltaics is generally considered environmentally friendly technologies, the long-term effects of module weathering under field conditions remain insufficiently

understood. This issue is especially important in the context of the anticipated growth of photovoltaic capacity and the increasing number of ageing installations that will remain in operation for several decades.

Our preliminary studies conducted at a photovoltaic farm in Ruda Śląska indicated elevated concentrations of selected elements in soils collected from areas surrounding the PV installation. However, the investigated site was in Upper Silesia, a region characterized by a long history of mining and metallurgical activities, which may significantly influence soil geochemistry and obscure the identification of potential contamination sources [5]. Consequently, distinguishing between historical industrial impacts and possible contributions from photovoltaic infrastructure remains challenging in such areas.

Therefore, the present study aims to evaluate the potential influence of photovoltaic panels on the surrounding soil environment in a location that is not affected by significant anthropogenic pressure. By investigating soils in the vicinity of monocrystalline photovoltaic panels situated in a relatively uncontaminated area, this research seeks to provide a clearer assessment of whether photovoltaic installations may act as a source of technology-critical elements and other trace elements in the environment. The findings may contribute to a better understanding of the long-term environmental sustainability of photovoltaic technologies and support the development of monitoring strategies for rapidly expanding solar energy infrastructure.

2 Material and methods

2.1 Sampling and research area

The study focused on the areas surrounding monocrystalline photovoltaic panels (located on private property since 2023 in Jasienica, near Bielsko-Biala, Poland). Soil samples were collected on the basis of the results of the preliminary in situ soil magnetic screening performed in accordance with the ISO Standard (ISO: 21226:2019, 2019). The magnetic susceptibility meter MS2 Bartington was equipped with MS2D loop sensor (Bartington Instruments Ltd., Witney, UK). At each individual point, the volume magnetic susceptibility (κ) value was calculated as mean value of 11 readings within a 2m² square. The κ value was expressed in 10⁻⁵ SI units. Surface magnetic susceptibility measurements were carried out at 12 points in the areas around the PV panels. Localization of individual points was confirmed using Garmin GPS. Sampling was performed using soil sampler Humax SH 300 (Baumann Saatzuchtbedarf) enabling collection of undisturbed 30-cm long soil cores (two from each place). A total of 24 soil core samples were taken into diamagnetic plastic tubes at different distances from the contamination source. Figure 1 shows the location and method of soil sampling.

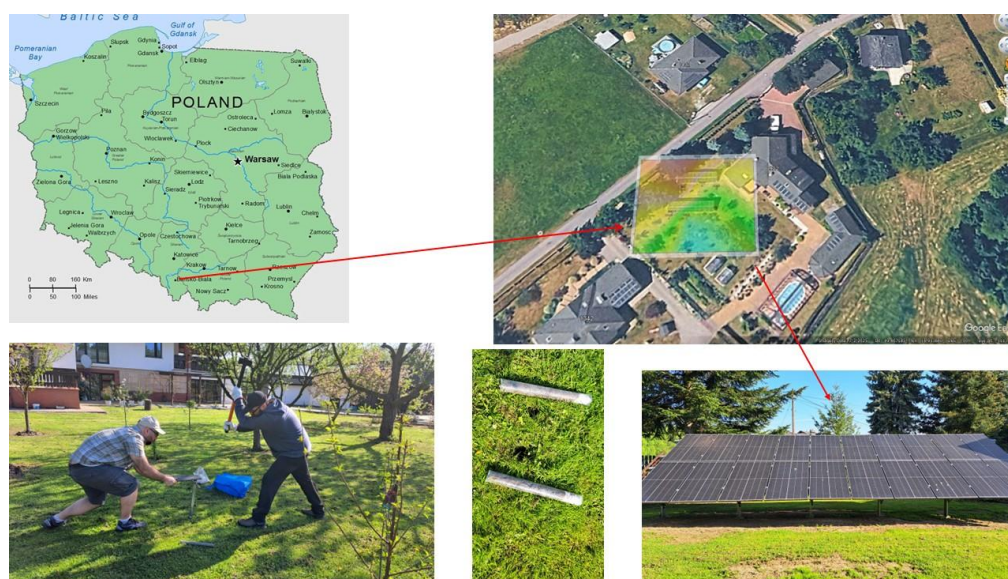


Fig. 1. The location and method of soil sampling

2.2 Physicochemical analysis of soils

The soil samples (from soil cores) were then subdivided, resulting in 50 laboratory samples. After drying, the samples were sieved through a 2 mm mesh to ensure uniform particle size. This standardized preparation process ensures that the samples are adequately prepared for consistent pH analysis and accurate

measurements of other soil properties. The conductivity and pH parameters were measured using the CX-401 multi-meter, along with the electrodes mentioned above according to PN-EN ISO 10390:2022-09 procedure [6]. In order to determine the total element content, soil samples were digested in a microwave oven (Microwave 5000, Anton Paar). 0.2 g of soil was prepared and digested with 5 ml of HNO₃ (spectral purity, Merck, Germany), 2 ml of H₂O₂ and 3 ml of HF (spectral purity, Merck, Germany). The digestion program consisted of heating to 210°C over 40 min followed by a 25 min hold period. After digestion, the solution was transferred to a polypropylene volumetric flask and diluted to a final volume of 50 ml. The concentrations of metals and metalloids were determined using an Elan 6100 DRC-e ICP-MS instrument (PerkinElmer, USA) equipped with a cross-flow nebulizer, Scott-type spray chamber, nickel sampler and skimmer cones, and a dynamic reaction cell (DRC).

3 Results and discussion

The concentrations of technology-critical elements (TCEs) and trace metals determined in soils collected in the immediate vicinity of the investigated photovoltaic installation remained generally low and showed limited spatial variability. No clear evidence of local enrichment attributable to photovoltaic panel operation was observed. Elements commonly associated with photovoltaic technologies, including Ag, In, Ga, Ge, Mo, Te and Sn, occurred at concentrations characteristic of natural or slightly elevated geochemical backgrounds. Their concentrations did not indicate significant release from the photovoltaic modules during the investigated period of operation. In particular, the median concentrations of Te (0.235 mg/kg) and In (0.054 mg/kg), regarded as key TCEs in thin-film photovoltaic modules, were low and did not indicate enrichment of the soil environment. Concentrations of all regulated potentially toxic elements remained below the permissible limits specified in the current Polish legislation. Potentially toxic elements regulated under Polish legislation, including Cd, As, Ni, Pb, Cu, Zn, Cr, Co, and Ba, were present at concentrations below the permissible limits.

Table 1. The analysis of soil samples (ND No Data in legislation)

Element/Isotope	Unit	Minimum N=50	Maximum N=50	Median N=50	Permissible concentration in Polish legislation ^[7] (mg/kg)	Background values ^[8] (mg/kg)
Tl205	mg/kg	0.306	0.986	0.735	ND	0.15
Ag107	mg/kg	0.375	0.749	0.579	ND	0.15
Cd114	mg/kg	0.269	0.851	0.571	2	0.15
As75	mg/kg	4.33	7.25	5.42	25	5
Sb121	mg/kg	0.636	2.51	0.919	ND	0.33
Sn118	mg/kg	1.78	3.68	2.46	20	0.8
Mo98	mg/kg	0.297	1.05	0.468	50	0.41
Te126	mg/kg	0.126	0.368	0.235	ND	0.03
Ge74	mg/kg	0.589	2.39	0.804	ND	-
In115	mg/kg	0.040	0.073	0.054	ND	0.02
V51	mg/kg	41.3	58.7	47.8	ND	42
Sr88	mg/kg	19.8	38.2	26.4	ND	95
Ba138	mg/kg	223	340	265	400	88
Co59	mg/kg	5.70	8.20	6.82	50	13
Ni60	mg/kg	13.1	20.4	16.4	150	31
Pb208	mg/kg	21.5	46.2	35.4	200	17
Mn55	mg/kg	256	510	340	ND	490
Cu65	mg/kg	10.2	39.9	21.4	200	22
Ga71	mg/kg	8.18	11.9	9.7	ND	3.3
Zn66	mg/kg	62.8	197	117	500	80
Cr52	mg/kg	33.8	53.2	39.9	200	40

The highest median concentrations were recorded for Mn (340 mg/kg) and Ba (265 mg/kg), which are likely related to the natural geochemical composition of the parent material rather than anthropogenic inputs.

This confirms the good environmental quality of the investigated soils and indicates the absence of significant anthropogenic contamination sources at the study site.

The application of soil magnetometry as a preliminary screening tool proved useful for selecting sampling locations around photovoltaic installations. The method enabled rapid characterization of spatial variability and may support future environmental monitoring strategies in PV facilities.

The investigated photovoltaic installation has been operating only since 2023; therefore, the obtained results should be regarded as representative of the early operational stage of photovoltaic systems. Long-term monitoring is necessary to assess whether weathering processes, microcrack formation, or panel aging may increase the release of selected elements over time.

The findings support the view that modern monocrystalline photovoltaic panels do not constitute a significant source of technology-critical elements to surrounding soils during the initial years of operation. Nevertheless, continued environmental assessment is recommended in view of the rapidly increasing number of photovoltaic installations and their expected service life exceeding 20–30 years.

4 Conclusions

The obtained results differ from observations reported in some preliminary studies conducted in industrialized regions, where elevated concentrations of selected elements were identified in soils surrounding photovoltaic installations [5]. In the present study, performed in a relatively uncontaminated area, no such enrichment was observed. This suggests that local geochemical background and historical anthropogenic pressure may strongly influence the interpretation of environmental data collected near photovoltaic systems. The results also indicate that potential releases of TCEs from modern monocrystalline modules, if occurring, are currently too limited to produce measurable accumulation in the surrounding soils. However, as photovoltaic panels undergo long-term weathering and mechanical degradation, future studies should investigate older installations and compare different PV technologies to better assess their long-term environmental impact.

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EFFECT OF IODINE BIOFORTIFICATION ON PHOTOSYNTHETIC PIGMENTS IN MINT PLANTS

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Abstract

Iodine is an essential micronutrient required for the synthesis of thyroid hormones, and iodine deficiency remains a significant public health concern in many European countries. Biofortification of crops during cultivation represents a promising strategy to increase dietary iodine intake through the application of mineral fertilizers to soil or directly onto plants. Foliar application is considered particularly effective because iodine added to soil is often rapidly immobilized by organic matter, reducing its availability for root uptake. Mints (*Mentha* spp.) are aromatic and medicinal plants of the Lamiaceae family, widely valued for culinary and medicinal uses, making them a suitable target for iodine biofortification studies. This study investigated the effect of foliar application of KI and KIO₃ on the chlorophyll content of mint. Stock solutions of potassium iodide (KI) and potassium iodate (KIO₃) at concentrations of 1, 5, 10, 50, and 100 ppm were applied to the mint plants. Chlorophyll content was determined by homogenizing 0.1 g of fresh leaf tissue (without major veins) in 7 mL of 80% acetone. Pigment concentrations were measured with an Epoch Microplate Spectrophotometer, using 80% acetone as the blank. Total chlorophyll content showed a declining trend with increasing KI concentration, while KIO₃ showed a similar decline followed by an increase at the highest concentration tested. None of these differences from the control were statistically significant. These preliminary results indicate that foliar iodine application within the tested concentration range had no clear and significant impact on chlorophyll content.

Keywords: iodine, mint, foliar spray, biofortification, photosynthetic pigments.

1 Introduction

Mints are aromatic herbs belonging to the family Lamiaceae, distributed mainly in temperate and tropical/subtropical regions and are believed to have originated in Europe and the Mediterranean. Mint requires a high level of nutrients to thrive; loam to sandy loam soils with a pH in the range of 6.5–8.0 are ideal for its cultivation. Mint tea is also used to relieve stress and aid sleep, and it acts as a strong diuretic [1].

The elimination of iodine deficiency is still a serious health issue in many European countries. Iodine is an essential element for humans and has important effects on hormones and brain development. In addition, insufficient or excessive iodine intake can cause abortion, cretinism, goitre, endemic mental retardation, impaired mental function, and autoimmune thyroid diseases [2].

One solution to solve iodine deficiency in humans is the biofortification of plants with iodine. By applying iodine-containing compounds to soils, crops can increase the absorption and accumulation of this trace element, and iodine in plants can be easily assimilated [3]. Biofortification with potassium iodide can increase iodine accumulation in plants, while its effects on biological quality depend on the applied dose and plant cultivar [4].

2 Material and methods

2.1 Cultivation of Mint

Stock solutions of potassium iodide (KI) and potassium iodate (KIO₃) were prepared at the tested concentrations of 0, 1, 5, 10, 50, and 100 ppm and applied to mint plants three times at 10-day intervals. Throughout the experiment, the mint plants were maintained at room temperature under indirect light.

2.2 Determination of photosynthetic pigments

0.1 g of fresh mint leaf tissue, after removal of the major veins, was extracted with 7 mL of 80% acetone. Absorbance of the samples was measured using an Epoch Microplate Spectrophotometer at wavelengths of 645 and 663 nm, with 80% acetone used as the blank. Chlorophyll a, chlorophyll b, and total chlorophyll content were calculated using the equations provided by Arnon [5].

3 Results and discussion

Changes in chlorophyll a, b, and total in mint leaves after foliar spraying with KI and KIO₃ are shown in Figure 1.

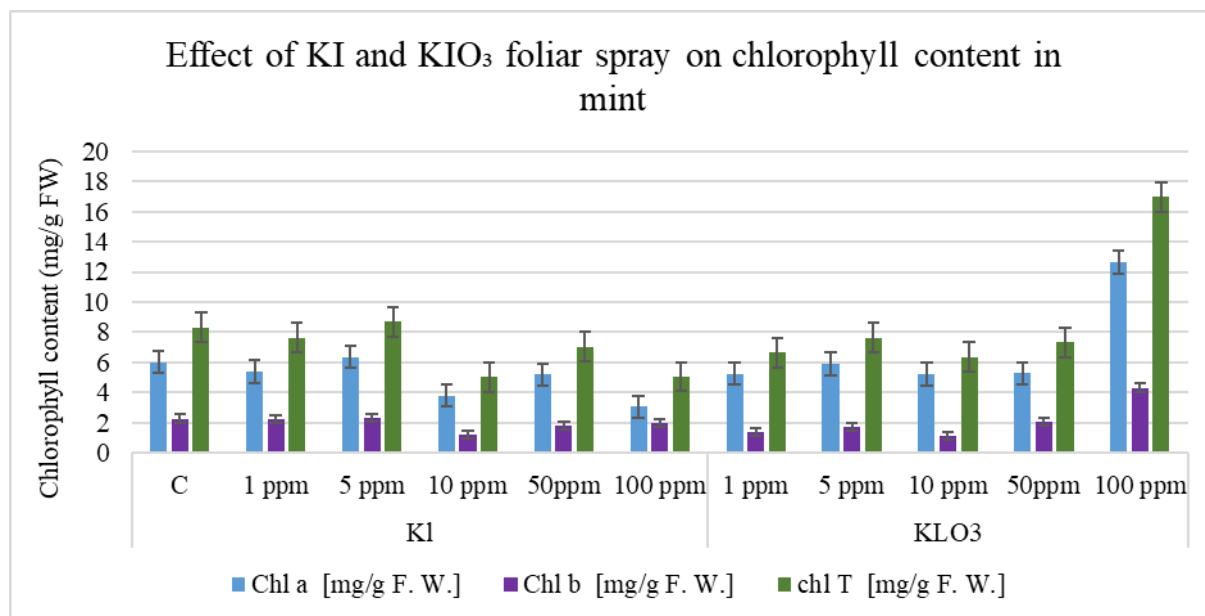


Fig. 1. Chlorophyll a, b and total content in mint following foliar spray with KI and KIO₃ at 0, 1, 5, 10, 50 and 100 ppm.

The results showed that in the potassium iodide (KI) treatment, total chlorophyll content showed a declining trend with increasing iodine concentration. A similar trend was observed in the KIO₃ treatment series, except that a marked increase in total chlorophyll content occurred at 100 ppm.

However, this increase was driven mainly by one of the two replicates and was accompanied by high variance, so it may reflect either a genuine biological response or simply natural variability between samples. The t-test showed that none of these differences from the control were statistically significant ($p > 0.05$), which may be related to the limited number of replicates ($n = 2$). Clarifying this would require increasing the number of biological replicates in future studies.

The results obtained may be consistent with previous studies indicating that iodide has a destructive effect on plant chlorophyll. In dwarf bean (*Phaseolus vulgaris*), researchers reached a similar conclusion and attributed this effect to the following mechanism: iodide does not inhibit chlorophyll synthesis; rather, the damage results from intracellular oxidation of iodide to molecular iodine, which leads to the destruction of existing chlorophyll and strong inhibition of photosynthetic reactions [6].

Furthermore, a study on maize showed that foliar application of potassium iodate under stress conditions resulted in a significant increase in chlorophyll content, which could possibly be attributed to the role of iodine in the photosynthetic process and in stimulating carbohydrate and protein synthesis. However, further investigation is needed to confirm this [7].

4 Conclusions

The preliminary results of this study indicate that mint, at this growth stage, tolerates foliar iodine application within the tested concentration range without a statistically significant effect on chlorophyll content. However, firm conclusions regarding the optimal iodine species or concentration for biofortification would require confirming whether this pattern holds across different growth stages. The absence of a significant adverse effect on chlorophyll can be considered a promising finding. Future studies should include more biological replicates.

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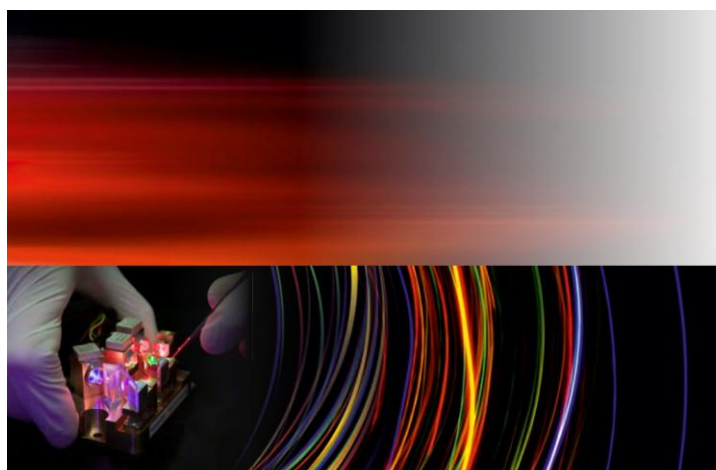
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