Culturable soil microbial biodiversity in post-mining areas of the Konin lignite mine and its potential for ecosystem restoration

Magdalena Lewandowska, Agnieszka Richert, Natalia Hejda*

Department of Genetics, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Torun, Poland

*Corresponding author: nhejda@doktorant.umk.pl

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Abstract: Post-mining areas represent strongly transformed environments that provide unique conditions for studying microbial diversity and ecological succession. This study aimed to characterize the bacterial and fungal communities isolated from soil samples collected in the post-mining landscapes of the Konin Lignite Mine (central Poland). Cultivable microorganisms were isolated and identified using molecular methods based on 16S rRNA (bacteria) and ITS (fungi) gene sequencing. Taxonomic classification was conducted using BLAST and SILVA (bacteria) and UNITE (fungi) databases. Identified taxa included bacteria from genera *Pseudomonas*, *Bacillus*, *Micrococcus*, and *Stutzerimonas*, and fungi from genera *Cladosporium*, *Trichoderma*, and *Penicillium*. Many of these microorganisms exhibit traits relevant to bioremediation and supporting plant growth in degraded soils. Our findings highlight the ecological potential of soil microbiota in post-industrial ecosystems and their importance in restoration efforts.

Keywords: soil microbiota, post-mining areas, biodiversity, bioremediation, molecular identification, *Pseudomonas*, *Trichoderma*

1. Introduction

The environmental legacy of lignite mining includes extensive degradation of soil and landscape structure, often accompanied by long-term ecological consequences. Open-pit mining in particular leads to the removal of topsoil, compaction of subsurface layers, disruption of hydrology, and contamination with heavy metals and hydrocarbons (Fashola et al., 2016; Adamczyk et al., 2014). These disturbances create challenging conditions for biotic communities, particularly for soil microbial populations that are essential to ecosystem functioning.

Soil microorganisms perform vital ecosystem services including, organic matter decomposition, nutrient mineralization, nitrogen fixation, and regulation of plant health (Feng et al., 2019). Their community structure is highly sensitive to environmental stress, making them reliable indicators of soil health and recovery. In degraded environments such as post-

mining landscapes, microbial communities often undergo succession, with stress-tolerant and functionally specialized taxa dominating the early stages of recolonization (Neina, 2019).

Recent studies have shown that mining-altered soils, despite their poor physicochemical properties, may harbor microbial communities with unique metabolic capabilities. These include resistance to toxic compounds, ability to degrade xenobiotics, and facilitation of plant growth through hormone production and nutrient solubilization (Singh et al., 2020; Tomczyk-Zak et al., 2017). As a result, soil microbiota in post-mining areas are increasingly recognized not only as indicators of ecological restoration but also as potential agents in biotechnological applications such as bioremediation and phytoremediation.

The Konin Lignite Mine, once a major energy supplier in central Poland, is now a site of ongoing environmental recovery efforts. Following decades of excavation, the post-mining areas exhibit high heterogeneity in soil quality and microbial recolonization. Understanding the composition and functional potential of microbial communities in these areas is essential for designing effective reclamation strategies.

This study aims to assess the cultivable bacterial and fungal diversity in soils from the post-mining zones of the Konin Lignite Mine using molecular methods. By focusing on soil-derived data, we intend to highlight the role of edaphic microorganisms in ecosystem recovery and to identify microbial taxa with potential relevance for environmental biotechnology and land restoration practices.

2. Purpose of work

The aim of this study was to investigate the biodiversity of bacteria and fungi present in soil and water samples collected from the post-mining areas of the Konin Lignite Mine and to perform molecular identification of isolated and cultured strains. The research included the cultivation of microorganisms, their isolation, and detailed characterization. In order to identify selected bacterial strains, genetic material was extracted, amplified, and the 16S rRNA and ITS gene was sequenced. The results obtained may contribute to a better understanding of the microbiota of areas degraded by mining activities and provide a basis for assessing the potential use of isolated microorganisms in reclamation processes.

3. Materials and Methods

3.1 Study Area and Sampling

The study was conducted in the vicinity of the former Konin Lignite Mine, located in central Poland (Wielkopolska region). The mine was established in the early 1940s and remained in operation until the early 21st century.. Post-mining zones were selected based on visible

degradation, lack of vegetation, and accessibility. Soil samples were collected from six distinct sites representing different stages of post-mining succession. At each site, approximately 500 g of soil was collected from the top 0–10 cm layer using sterile tools, pooled into composite samples, and stored at 4°C until laboratory processing.

Analysis of the physicochemical properties of surface horizons of soils located near the planned lignite mine "Tomisławice" showed that the pH of most soils was neutral, ranging from 7.0 to 7.3. An exception was the mursh soil, which exhibited acidic conditions (pH 5.7). Exchangeable acidity (Hw) in the studied samples was relatively uniform, ranging from 10.6 to 16.9 mmol $H^+ \cdot kg^{-1}$, showed a significantly higher value of 38.1 mmol $H^+ \cdot kg^{-1}$ (Jakubus, Gajewski & Kaczmarek, 2013).

The surface horizons (0–25 cm) were analyzed for selected heavy metals, including Fe, Mn, Zn, Cu, and Ni. Iron had the highest content, ranging from 2.10 to 32.63 $g \cdot kg^{-1}$, while nickel had the lowest, ranging from 0.90 to 11.27 $mg \cdot kg^{-1}$. The remaining metals occurred in the following ranges: manganese 33.2–602.0 $mg \cdot kg^{-1}$, zinc 6.29 - 43.49 $mg \cdot kg^{-1}$, and copper 6.00 - 10.01 $mg \cdot kg^{-1}$. These results indicate chemical variability of soils in the studied area, with particular emphasis on the profile with high exchangeable acidity (Jakubus, Gajewski & Kaczmarek, 2013).

Table 1. Heavy metal content in the surface horizon of soil (0–25 cm) (Jakubas, Gajewski & Kaczmarek, 2013).

Metal	Concentration range	Remarks
Iron (Fe)	2.10 – 32.63 $g \cdot kg^{-1}$	highest content
Manganese (Mn)	33.2 – 602.0 $mg \cdot kg^{-1}$	–
Zinc (Zn)	6.29 – 43.49 $mg \cdot kg^{-1}$	–
Copper (Cu)	6.00 – 10.01 $mg \cdot kg^{-1}$	–
Nickel (Ni)	0.90 – 11.27 $mg \cdot kg^{-1}$	lowest content

3.2 Cultivation and Isolation of Microorganisms

For microbiological analysis, 10 g of each composite soil sample was suspended in 90 mL of sterile physiological saline and vortexed. Serial dilutions (10^{-1} to 10^{-10}) were prepared, and 100 µL aliquots from each dilution were plated onto R2A agar (bacteria) and PDA agar (fungi). Incubation was carried out at 37°C for bacterial plates and 23°C for fungal plates for

3 to 7 days. Colonies with distinct morphology were sub-cultured onto fresh plates to obtain pure isolates.

3.3 DNA Extraction and PCR Amplification

Genomic DNA was extracted using the GeneMatrix Bacterial & Yeast Genomic DNA Purification Kit (EURx, Poland), following the manufacturer's protocol. The 16S rRNA gene of bacteria was amplified using primers BAC16SF and BAC16SR (unpublished data). For fungi, the ITS region was targeted using ITS1 and ITS4 primers (White et al., 1990).

PCR reactions were performed in a total volume of 50 [µL], containing 5 [µL] of PCR buffer, 1 [µL] of dNTP, 1 [µL] of each primer, 0.25 [µL] of DNA polymerase, 1 [µL] of template DNA, and nuclease-free water to the final volume.

PCR amplification of fungal DNA was carried out under the following thermal cycling conditions: an initial denaturation at 95°C for 4 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 51°C for 30 s, and elongation at 72°C for 30 s. A final elongation step was performed at 72°C for 5 min, followed by holding at 4°C.

PCR amplification of bacterial DNA was performed using the following temperature program: an initial denaturation at 95°C for 2 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 53°C for 40 s, and elongation at 72°C for 1 min 40 s. The final elongation step was conducted at 72°C for 7 min, with a final hold at 4°C. PCR products were visualized on a 1.5% agarose gel stained with ethidium bromide.

3.4 DNA Sequencing and Bioinformatic Analysis

Amplicons were purified using the GeneMATRIX Tissue DNA Purification Kit (EURIX Molecular Biology Product, Gdańsk, Poland)and sequenced via Sanger sequencing by Genomed (Warsaw, Poland), with using BigDye ™ Terminator Cycle Sequencing kit. Chromatograms were manually inspected and edited using SeqTrace software 0.9.0 to generate consensus sequences. These sequences were aligned and compared to reference databases using BLASTn (NCBI) and taxonomically verified with SILVA (bacteria) and UNITE (fungi) databases. Only matches with ≥97% similarity and ≥90% coverage were accepted for taxonomic assignment.

4. Results

4.1 Soil Bacterial Communities

A total of 16 bacterial strains were successfully isolated from soil samples. The identified strains belonged to the following genera: *Pseudomonas*, *Stutzerimonas*, *Bacillus*, *Micrococcus*, *Achromobacter*, *Paraperlucidibaca*, and *Sphingomonas*. Among them,

Pseudomonas paeninsulae and *P. taeanensis* were most frequently detected, indicating their dominance in the sampled environments.

Table 2. Bacterial identification

NCBI: BLAST N	Silva
Pseudomonas Select seq AJ419674.1 similarity: 99,66% *Pseudomonas paeninsulae* Select seq NR_197735.1 similarity: 99,59%	*Pseudomonas* similarity: 100%
Paraperlucidibaca wandonensis Select seq NR_109730.1 similarity: 98,41%	*Moraxellaceae* similarity: 98,31%
Micrococcus luteus Select seq: MF523990.1 similarity: 98,55%	*Micrococcus* similarity: 98,27%
Moraxella osloensis CP014234.1 similarity: 97.92 %	*Enhydrobacter aerosaccus* similarity: 97,93%
Pseudomonas taeanensis Select seq: KY783359.1 similarity: 99,75%	*Pseudomonas* similarity: 99,92%
Bacillus mycoides JX500197.1 similarity: 99,75%	*Bacillus* similarity: 95,27%
Peribacillus psychrosaccharolyticus MF101012.1 similarity: 93,20%	-
Uncultured bacterium Select seq KP805877.1 similarity: 88,30%	-
Achromobacter piechaudii Select seq MK737340.1 similarity: 95,12%	*Achromobacter* similarity: 94,97%
Stutzerimonas stutzeri Select seq OQ892251.1 similarity: 99,73%	*Pseudomonas* similarity: 97,93%
Uncultured *Arthrobacter* sp. Select seq LT798852.1 similarity: 94,01%	-
Acinetobacter johnsonii Select seq MK629796.1 similarity: 85,28%	*Acinetobacter* similarity: 84,93%
Sphingomonas melonis Select seq MF681924.1 similarity: 96,71%	*Sphingomonas* similarity: 96,5%

4.2 Soil Fungal Communities

Twelve fungal isolates were characterized. Dominant genera included *Cladosporium*, *Trichoderma*, *Penicillium*, and *Clonostachys*. Species such as *Cladosporium cladosporioides* and *Trichoderma harzianum* were identified with high sequence similarity.

The fungal strains displayed morphological diversity and rapid growth on PDA medium.

Table 3. Fungal identification

NCBI: BLAST N	UNITE community BLAST
Cladosporium cladosporioides sequence ID: MZ303793.1 similarity: 100%	*Cladosporium cladosporioides* similarity: 100%
Absidia glauca sequence ID: AY944879.1 similarity: 99,39%	*Absidia glauca* similarity: 99,39%
Clonostachys rosea sequence ID: OM965347.1 similarity: 99,82%	*Clonostachys rosea* similarity: 99,82%
Trichoderma harzianum Sequence ID: KX632497.1 similarity: 98,92%	*Trichoderma harzianum* similarity: 99,27%
Penicillium lanosum Sequence ID: PV156930.1 similarity: 93,55% *Penicillium swiecickii* Sequence ID: KY228682.1 similarity: 93,55%	*Penicillium lanosum* similarity: 93,60% *Penicillium chrysogenum* similarity: 93,60%
Penicillium bialowiezense Sequence ID: MZ078713.1 similarity: 99,82% *Penicillium brevicompactum* Sequence ID: OW982543.1 similarity: 99,82%	*Penicillium bialowiezense* similarity: 99,82% *Penicillium brevicompactum* similarity: 99,82%
Penicillium brevicompactum Sequencje ID: KT876695.1 similarity: 100% *Penicillium bialowiezense* Sequencje ID: OU989430.1 similarity: 100%	*Penicillium bialowiezense* similarity: 100% *Penicillium brevicompactum* similarity: 100%
Cladosporium cladosporioides Sequencje ID: OQ851636.1 similarity: 100% *Ladosporium subuliforme* Sequencje ID: LN850753.1 similarity: 100%	*Cladosporium cladosporioides* similarity: 100% *Cladosporium xylophilum* similarity: 100%
Cladosporium cladosporioides Sequencje ID: MZ159836.1 similarity: 99,55% *Cladosporium crousii* Sequencje ID: ON712301.1	*Cladosporium cladosporioides* similarity: 99,55% *Cladosporium colocasiae*

similarity: 99,55%	similarity: 99,55% *Cladosporium angulosum* similarity: 99,55%
Trichoderma rossicum Sequencje ID: HQ115675.1 similarity: 97,27%	*Trichoderma rossicum* similarity: 97,27% *Trichoderma barbatum* similarity: 97,27%
Cladosporium cladosporioides Sequencje ID: MZ159836.1 similarity: 100%	-
Hypocreales sp. Sequencje ID: JF817338.1 similarity: 99,66% *Ustilaginoidea virens* Sequencje ID: OQ645526.1 similarity: 99,50%	*Simplicillium* similarity: 99,66% *Ustilaginoidea virens* similarity: 99,50%
Trichoderma stromaticum Sequencje ID: MK552406.1 similarity: 99,63% *Trichoderma barbatum* Sequencje ID: MW269168.1 similarity: 99,63%	*Trichoderma barbatum* similarity: 99,81% *Trichoderma stromaticum* similarity: 99,63%

Table 4. Cultivable soil microbial taxa isolated from the Konin post-mining area (da Silva, R., et al. (2022); Dubey, M. K., & Jensen, D. F. (2021); Yan, Q., Li, Y., & Xie, J. (2021); Stamm, W. E., & et al. (2006); Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., & Lorito, M. (2004)).

Domain	Genus	Species	Notable Functions/Traits
Bacteria	*Pseudomonas*	*P. paeninsulae*, *P. taeanensis*	Bioremediation, siderophore production
Bacteria	*Stutzerimonas*	*S. stutzeri*	Denitrification, hydrocarbon degradation
Bacteria	*Bacillus*	*B. mycoides*	Soil health, plant growth promotion
Bacteria	*Peribacillus*	*P. psychrosaccharolyticus*	Nutrient cycling, stress resistance
Bacteria	*Micrococcus*	*M. luteus*	Oligotrophic survival
Bacteria	*Achromobacter*	*A. piechaudii*	Nitrogen metabolism
Bacteria	*Paraperlucidibaca*	*P. wandonensis*	Alkane degradation, cold adaptation
Bacteria	*Sphingomonas*	*S. melonis*	Biotransformation, pollutant tolerance
Fungi	*Cladosporium*	*C. cladosporioides*	Metal tolerance, organic matter degradation
Fungi	*Trichoderma*	*T. harzianum*, *T. stromaticum*, *T. rossicum*	Biocontrol, enzyme production, rhizosphere competence
Fungi	*Penicillium*	*P. lanosum*, *P. bialowiezense*, *P. brevicompactum*	Antibiotic production, soil colonization
Fungi	*Clonostachys*	*C. rosea*	Biocontrol, root zone colonization

4. Discussion

The results of this study underscore the remarkable adaptability and ecological importance of microbial communities inhabiting post-mining soils. Despite severe anthropogenic disturbance, the soils of the Konin Lignite Mine harbor a taxonomically and functionally diverse set of microorganisms, many of which are known to participate in key ecological processes such as organic matter decomposition, nutrient cycling, and contaminant degradation.

The dominance of genera such as *Pseudomonas* and *Bacillus* among the bacterial isolates is consistent with findings from other disturbed environments. These taxa are frequently reported in reclaimed soils due to their broad metabolic capacities and resilience to stressors such as heavy metals, low nutrient content, and pH variability (Feng et al., 2019; Ezeokoli et al., 2020). *Pseudomonas* spp. are well known for their bioremediation potential, particularly in degrading hydrocarbons and enhancing plant growth through the production of siderophores and phytohormones.

One notable isolate, *Stutzerimonas stutzeri*, is known for its denitrifying ability and its capacity to degrade complex hydrocarbons. *Bacillus mycoides* and *Peribacillus psychrosaccharolyticus* were also identified, showing high resilience under oligotrophic and metal-contaminated conditions.

The presence of *Micrococcus luteus*, a bacterium typically found in nutrient-poor soils, suggests adaptation to the harsh post-mining environment. Less frequent isolates such as *Achromobacter piechaudii* and *Paraperlucidibaca wandonensis* may play roles in nitrogen and sulfur cycling under adverse environmental conditions.

The identification of *Stutzerimonas stutzeri* is particularly significant given its capacity for anaerobic respiration and involvement in nitrogen cycling, including denitrification processes (Wanga et al., 2023). Its presence suggests the possible restoration of functional soil microbial networks even in heavily altered substrates.

Fungal communities were similarly diverse, with *Trichoderma* spp. representing a key group of interest. These fungi are not only competitive colonizers of rhizosphere environments but also effective antagonists of plant pathogens and producers of extracellular enzymes that facilitate organic matter turnover (Tripathi et al., 2013). Their frequent occurrence in the studied samples highlights their ecological fitness and potential application in restoration practices.

Other isolates, including *Penicillium bialowiezense* and *Trichoderma stromaticum*, are associated with soil organic matter decomposition and plant health. Their prevalence suggests potential involvement in early successional stages of microbial recolonization of post-mining soils. In particular, the genus *Trichoderma* is notable for its role in promoting plant root development and suppressing soilborne pathogens, making it ecologically significant for restoration strategies.

The genus *Cladosporium*, typically associated with phyllosphere habitats, was also found in abundance. Its ability to tolerate extreme environmental conditions, including metal toxicity, may explain its prevalence in the harsh conditions of the Konin post-mining soils (Dusengemungu et al., 2022).

Our findings align with studies conducted in other post-industrial landscapes, suggesting that microbial succession and recolonization occur in predictable patterns, driven by selective pressures such as substrate availability, chemical toxicity, and microclimatic variation (Neina, 2019; Singh et al., 2020). The recovery of microbial functions, even in the absence of full vegetation cover, supports the notion that microbial communities can act as early bioindicators of soil restoration.

Further research should focus on evaluating the functional potential of these isolates in situ, including tests for plant-microbe symbiosis, pollutant degradation, and contributions to soil structure improvement. Metagenomic approaches could also complement culture-based methods to uncover the full extent of microbial biodiversity and ecosystem service potential.

5. Conclusions

The post-mining soils of the Konin Lignite Mine host a taxonomically diverse culturable microbiota with considerable potential for supporting ecological restoration. The presence of microbial taxa with known functional attributes—such as hydrocarbon degradation, nutrient cycling, and plant-growth promotion—suggests that even heavily degraded soils can serve as reservoirs of useful biodiversity.

Culturable representatives of genera such as *Pseudomonas*, *Bacillus*, *Trichoderma*, and *Cladosporium* demonstrate the microbial community's adaptability to stress and its ecological value. These taxa can play pivotal roles in improving soil fertility, facilitating plant succession, and detoxifying pollutants, which are essential aspects of sustainable post-mining land management.

Further investigation into the ecological roles and functional interactions of these microorganisms, including field-based trials and metagenomic profiling, is recommended. Such efforts will help establish tailored microbial-assisted strategies for restoring ecosystem functionality in mining-impacted landscapes.

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