microbiology research

MDPI

Article

The Effect of Heavy Metals on Microbial Communities in Industrial Soil in the Area of Piekary Śląskie and Bukowno (Poland)

Anna Katarzyna Jarosławiecka * and Zofia Piotrowska-Seget

Institute of Biology, Biochemistry and Environmental Protection, University of Silesia, Jagiellonska Street 28, 40-032 Katowice, Poland

* Correspondence: anna.jaroslawiecka@us.edu.pl; Tel.: +0048-32-2009-470; Fax: 0048-32-2009-361

Abstract: The aim of this study was to determine the activity and structure of microbial communities in soils contaminated with heavy metals (HMs). To achieve this goal, soil samples were taken from two contaminated sites (i.e., Piekary Śląskie and Bukowno) in Poland. A wide range of methods were applied, including: total and metal-tolerant culturable bacteria enumeration; microbial community structure analysis using the phospholipid fatty acid method (PLFA); denaturing gradient gel electrophoresis (PCR-DGGE); and metabolic activity using BIOLOG and EcoPlate^{TM}. Our studies showed that HMs negatively affected microbial community structure and activity in polluted soils. Apart from the contamination with HMs, other soil parameters like soil pH and water also impacted microbial community structure and growth. Metal-tolerant bacterial strains were isolated, identified and tested for presence of genes encoding HM tolerance using the polymerase chain reaction (PCR) methodology. Contamination with HMs in the tested areas was found to lead to development of metallotolerant bacteria with multiple tolerances toward Zn, Ni, Cd and Cu. Different genes (e.g., *czcA*, *cadA* and *nccA*) encoding HM efflux pumps were detected within isolated bacteria. Culturable bacteria isolated belonged to *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* genera. Among non-culturable bacteria in soil samples, a significant fraction of the total bacteria and phyla, such as *Gemmatimonadetes* and *Acidobacteria*, were found to be present in all studied soils. In addition, bacteria of the *Chloroflexi* genus was present in soil samples from Piekary Śląskie, while bacteria of the *Firmicutes* genus were found in soil samples from Bukowno.

Keywords: heavy metals; soil pollution; microorganisms; cadmium; zinc; copper; lead; nickel

Citation: Jarosławiecka, A.K.; Piotrowska-Seget, Z. The Effect of Heavy Metals on Microbial Communities in Industrial Soil in the Area of Piekary Śląskie and Bukowno (Poland). *Microbiol. Res.* **2022**, *13*, 626–642. https://doi.org/10.3390/microbiolres13030045

Academic Editor: Giacomo Zara

Received: 22 July 2022
Accepted: 2 September 2022
Published: 7 September 2022

1. Introduction

The environmental effects of heavy metals (HMs) have been studied for several years [1,2]. Nevertheless, despite various attempts to lower HM emissions, related environmental contamination remains a serious concern [3] because of their non-biodegradable nature and ability to accumulate in the food chain generating negative effects on human health [4]. As an example, cadmium accumulation in soil can be caused by natural processes, but is mainly the result of anthropogenic activities, including wastewater irrigation, fertilizers, agricultural usage of sewage sludge, smelting and mining [5]. Even microelements (e.g., copper, zinc and nickel) can become toxic to cells if their concentration exceeds cellular requirements [6].

Due to their size, ubiquity and key role in metabolic cycles, microorganisms are considered to be the best indicators of the effects of toxic compounds on soil [7]. The negative impacts of HMs on microbial communities in soil have been reported in several studies [8–11]. Heavy metals are well-known to lower/inhibit activity of soil enzymes as well as disturb carbon, nitrogen and organic matter transformation and reduce microorganism biodiversity and biomass [6], leading to dominance of specific HM-tolerating microorganisms in soils [12–15].

Bioremediation using microorganisms and their natural mechanisms responsible for tolerance/resistance to toxic metallic ions like intra and extracellular sequestration, active efflux from cells and reduction of HM ions is considered to be a very promising tool for HM removal/recovery in comparison with the conventional chemical and physical techniques, since it is an environmentally friendly (for example, retention of soil structure and no secondary pollution) and low-cost technique [6]. In particular, microbial biosorbents are very effective in removing contaminants, although other options such as bioleaching or phytoremediation are also available [6,16].

Many researchers are concerned about the environmental impact of untreated polluted areas, and various studies have been conducted to improve this situation and understanding on how microbial communities in areas contaminated with HMs are shaped and function [17–19] in order to further develop the optimal and most effective strategy for bioremediation [18,20–22].

The area selected for this study is described as a territory of ecological disaster and is located in the industrial region of Southern Poland. Both Mining and Metallurgical Plants 'Waryński' in Piekary Śląskie and 'Bolesław' in Bukowno were operating for over a century, leading to extensive contamination of soil with various HMs [23,24].

The aim of this study was to (1) determine the effects of HMs and other physicochemical properties of soils on microbial communities in industrial soils with a history of long-term contamination with various HMs; (2) evaluate the actual state of microbial diversity, metabolic activity and structure by culturable and non-culturable methods; (3) isolation and identification of strains potentially useful in bioremediation in this area, including tests on the presence of genes typically involved in HM efflux among those isolated.

The results obtained with this research can contribute to the development of ecofriendly bioremediation technology and monitoring of the environmental effect of soil pollution in this area.

2. Material and Methods

2.1. Soil Sample Collection

Soil samples were collected in the proximity of the former Mining and Metallurgical Plants 'Waryński' in Piekary Śląskie (Upper Silesia) and 'Bolesław' in Bukowno (Lesser Poland), Poland. Selected soil sampling sites were designed as P1 (50°21′54.4″ N, 18°58′02.5″ E), P2 (50°21′56.2″ N, 18°58′10.0″ E) and P3 (50°22′03.7″ N, 18°57′44.9″ E) in Piekary Śląskie and B1 (50°16′27″ N, 19°29′7″ E), B2 (50°16′4″ N, 19°29′13″ E) and B3 (50°15′51″ N, 19°29′22″ E) in Bukowno (Figure 1).

Five soil samples were taken from the top soil layer (0–0.2 m) from a 1 m × 1 m sampling area and pooled together to make a composite soil sample. The composite samples were transported to the laboratory, where the soil was air dried, sieved through a 2-mm mesh to remove the stones and plant debris and then stored at –20 °C until further use. All analyses were carried out in triplicate for every single soil sample.

2.2. Physicochemical Analysis

Different physicochemical soil parameters were analysed including granulometry, soil moisture, pH, soil organic matter (SOM), organic carbon, total nitrogen, total phosphorus, HM (Zn, Cd, Cu, and Ni) concentration and bioavailable forms, as well as K, P, Na, Mg and Ca.

Soil granulometry was determined using laser diffraction (Mastersizer 2000, Malvern Pananalytical, Malvern, UK) for particles ranging 0–1 mm in size and sieve analysis for particles above 1 mm. The soil samples were classified into a particular granulometric group according to PN-88/B-02480. The soil moisture content was determined in agreement with the ISO method 16586:2003 and soil pH was measured according to PN-ISO 10390:1997. The SOM and organic carbon were estimated using the Turin test [25].

(**a**)

(**b**)

Figure 1. Sample site geographical location in (**a**) Piekary Śląskie, and (**b**) Bukowno, Poland (source: https://www.google.pl/maps, modified, 21 July 2022).

Total nitrogen was quantified using colorimetry by the Slandi 300 (Slandi, Michałowice, Poland) photometer. The soil (0.2 g) was mixed with 2 mL of 30% H_2O_2 and left for 24 h. The sample was then boiled until complete evaporation of the solution. A volume of 20 mL of H_2O was added to the residual precipitate and brought to the boil. After cooling and filtering through a qualitative filter in the solution, the concentration of ammonium, nitrite and nitrate were measured in the solution and the results were added to obtain the total nitrogen value in the dry mass of the soil sample.

To quantify phosphate (PO_4^{3-}) and Na, K, Mg and Ca content, 0.5 g of soil sample was added to 3 mL of 60% HNO_3. The suspension was mineralised in a microwave oven (Mars 5, CEM, Matthews, NC, USA). The resulting solution was diluted to 50 mL. The concentration of phosphate (PO_4^{3-}) was measured using the colorimetric method on Slandi 300 (Slandi, Michałowice, Poland). The results were converted into phosphorus content in dry matter.

The concentration of Na, K, Mg and Ca were estimated using flame atomic absorption spectrometry (FAAS) (SavantAA Σ, GBC, Braeside, Melbourne, Australia). The results were given in mg/kg dry soil mass.

The total content of selected HMs (Cd, Zn, Ni and Cu) in soil was determined using FAAS (iCE 3500 FAAS, Thermo Fisher Scientific, Waltham, MA, USA). A quantity of 300 mg of soil was dried at 105 °C and then mineralised in a mixture of 8 mL 65% HNO_3 and 2 mL H_2O_2 at 190 °C for 50 min using an Ethos Up microwave oven (Milestone, Sorisole, Italy). After mineralisation, the solutions were filtered on 0.45 µm filter paper and diluted in water and analysed by FAAS. Results were given in mg of metal per kg of dry soil and compared with Polish regulations for the maximum allowed concentration of HMs in soils (Regulation of the Minister of Environment, 9 September 2002 on Soil Quality Standards 2002; further RME 2002) for soil class B: Cd 4 mg/kg dry soil mass, Zn 300 mg/kg dry soil mass, Cu 100 mg/kg dry soil mass and Ni 70 mg/kg dry soil mass.

To quantify water-extractable HMs in soil or their bioavailable fraction (which is the amount of metal extracted from soil using 0.01 M $CaCl_2$), 5 g of soil sample was dried at 105 °C and resuspended in 50 mL of DI water or 50 mL 0.01 M $CaCl_2$ solution, respectively, and then shaken for 2 h at 120 rpm. The suspension was filtered through 0.45 µm filter and metal content was measured using FAAS (iCE 3500 FAAS, Thermo Fisher Scientific, Waltham, MA, USA). Results were given in mg of metal per kg of dry soil.

2.3. Culturable Bacteria Enumeration

The total number of heterotrophic (HB) and oligotrophic bacteria (OB), as well as metal-tolerant bacteria were determined by the plate method using tryptic soy agar (TSA) (BTL, Warsaw, Poland) and broth agar (BTL, Warsaw, Poland) for HB and 10% TSA (BTL, Warsaw, Poland) for OB. All plates were supplemented with 200 µg/mL Nystatin to avoid fungal contamination. The metal-tolerant fraction of culturable bacteria was determined on 1/10 TSA (BTL, Warsaw, Poland), TSA (BTL, Warsaw, Poland) and broth agar (BTL, Warsaw, Poland) plates supplemented with 1 mM $CdCl_2$ (Sigma-Aldrich, Inc. St. Louis, MO, USA), 1 mM $ZnCl_2$ (Sigma-Aldrich, Inc., St. Louis, MO, USA), 1 mM $NiCl_2$ (Sigma-Aldrich, Inc., St. Louis, MO USA) or 1 mM $CuCl_2$ (Sigma-Aldrich, Inc., St. Louis, MO, USA), respectively. The inoculated plates were incubated for 7 days at 22 °C. The number of bacteria was expressed as log of colony forming unit (CFU) per g of dry soil.

2.4. Total Bacteria Enumeration

The total bacterial count in soil was quantified using the 16S rRNA gene in real-time PCR. The template used for amplification was genomic DNA isolated from 2 × 0.5 g of soil using GeneMATRIX Soil DNA Purification Kit (EURx Ltd., Gdańsk, Poland) according to the producer's protocol and combined as one sample.

A fragment of the 16S rRNA gene was amplified using primers pE (5′-AAA CTC AAA GGA ATT GAC GG-3′) and pF (5′-ACG AGC TGA CGA CAG CCA TG-3′) (Edwards et al., 1989). The reaction mixture (25 µL) consisted of 10 µL of LightCycler® 480 SYBR Green I

Master (Roche Diagnostic GmbH, Mannheim, Germany), 1 µL of pE primer (100 µM dil. 1:10 *v*/*v*) (Sigma-Aldrich, Inc., St. Louis, MO, USA), 1 µL of pF primer (100 µM dil. 1:10 *v*/*v*) (Sigma-Aldrich, Inc., St. Louis, USA), 2 µL of DNA and 6 µL of H_2O. The amplification conditions were: 95 °C for 10 min, 30 cycles of 10 s at 95 °C followed by annealing for 20 s at 57 °C and extension for 30 s at 72 °C. Fluorescence data were acquired at the end of each extension step at 81 °C to avoid detection of primer dimers. For the melting curve analysis of products, temperature was raised from 65 to 95 °C and the melting temperatures were determined using LightCycler® (Roche Diagnostic GmbH, Mannheim, Germany) following a modified procedure described elsewhere [26].

Amplification efficiency was estimated by serial dilution of the standard, which was a fragment of 16S rRNA cloned into the pTZ57R/T vector. The standard was prepared using a InsTAclone PCR Cloning Kit (Thermo Fisher Scientific, Waltham, MA, USA), following the manufacturer's instructions. The 16S rRNA gene copies quantification was calculated as described in [27].

2.5. Total Microbial Activity Measurement

General microbial activity was determined according to a procedure described elsewhere [28] with minor modification: centrifugation of suspension at 2000 rpm for 3 min. The concentration of free fluorescein in the filtered solution was measured at 490 nm using a spectrophotometer (Helios Epsilon, Thermo Fisher Scientific, Waltham, MA, USA). A calibration curve was used to calculate the amount (in µg) of fluorescein per g of dried soil, which corresponds to enzymatic activity in the soil sample.

2.6. Metallotolerant Bacteria Isolation and Identification

Metallotolerant bacteria were isolated from soil samples using 1/10 TSA, TSA and BA media supplemented with 1 mM $CdCl_2$ (Sigma-Aldrich, Inc., St. Louis, MO, USA)—to ensure metal-resistant bacteria isolation—and 200 µg/mL Nystatin (Sigma-Aldrich, Inc., St. Louis, MO, USA). After incubation for 5 days at 22 °C, morphologically different bacteria were taken for further studies. Strains were identified based on the 16S rRNA sequence. Genomic DNA was isolated using Genomic Mini (A&A Biotechnology, Gdańsk, Poland). The 16S rRNA fragment was amplified by PCR using 0.5 µL of starter 8F AGAGTTTGATC-CTGGCTCAG (100 µM, dil. 1:10 *v*/*v*) (Sigma-Aldrich, Inc., St. Louis, MO, USA), 0.5 µL of starter 1492R GGTTACCTTGTTACGACTT (100 µM, dil. 1:10 *v*/*v*) (Sigma-Aldrich, Inc., St. Louis, MO, USA), 0.5 µL of polymerase DreamTaq™ 500 U (Thermo Fisher Scientific, Waltham, MA, USA), 2.5 µL of 10× buffer DreamTaq (Thermo Fisher Scientific, Waltham, MA, USA), 0.5 µL of dNTP Mix (10 mM) (Thermo Fisher Scientific, Waltham, MA, USA) and 3 µL of DNA. The PCR amplification was done according to [27].

The PCR products were purified using Gel Out (A&A Biotechnology, Gdańsk, Poland) following the manufacturer's protocol. The isolated DNA was sequenced at Genomed S.A. (Warsaw, Poland) and compared with the GenBank using the BLAST [29] database to identify bacteria.

2.7. Minimal Inhibitory Concentration (MIC) of HMs

The MICs were determined by plating method using BA plates (BTL, Warsaw, Poland) supplemented with $ZnNO_3$ (Sigma-Aldrich, Inc., St. Louis, MO, USA), $CdCl_2$ (Sigma-Aldrich, Inc., St. Louis, MO, USA), $CuCl_2$ (Sigma-Aldrich, Inc., St. Louis, MO, USA) or $NiCl_2$ (Sigma-Aldrich, Inc., St. Louis, MO, USA) at different concentrations (e.g., 1–10 mM, 15 mM and 20 mM) with 200 µg/mL of Nystatin (Sigma-Aldrich, Inc., St. Louis, MO, USA). After inoculation, plates were incubated for 7 days at 22 °C. The MIC for each bacterial isolate was determined as the lowest concentration of HM at which no colonies were observed. Plates without added metals were used as positive control while *Cupriavidus metallidurans* AE104 was used as a negative control for MIC.

2.8. Microbial Community Structure

The structure of microbial communities in soil was estimated using phospholipid fatty acids (PLFAs) as described by other studies [30] with minor modifications. Lipids of microbial origin were extracted, fractioned, methylated and then analysed using gas chromatography (Hewlett-Packard 6890, Agilent, Santa Clara, CA, USA). Methylated fatty acids were identified and quantified using the MIDI-MIS software (Sherlock TSBA6 library, MIDI Inc., Newark, DE, USA) and nonadecanoic acid (19:0) as internal standard. Specific PLFAs for gram-negative bacteria (16:1ω7c, cy17:0, 18:1ω7, cy19:0) [31], gram-positive bacteria (i15:0, a15:0, i16:0, i17:0, a17:0) [31], actinomycetes (10Me17:0, 10Me18:0) [32] and fungi (18:2ω6,9) [33] were used to calculate the total microbial PLFAs and PLFAs for each group of microorganisms, expressed as nmol PLFA per g of dry weight of soil.

2.9. Microbial Community's Physiological Profile

Microbial community's catabolic abilities were determined using BIOLOG EcoPlates™ (Biolog Inc., Hayward, CA, USA). The soil solution was prepared with 10 g of dry soil and 90 mL of 0.85% NaCl. The samples were shaken for 2 h (140 rpm) and left for 30 min to settle. The 96-well BIOLOG microplates were inoculated with 120 µL (10^{-2}) dilution aliquots of soil solution supernatant and incubated at 22 °C for 5 d. The readings were performed twice per day using a Bio-Tek ELx808 (BioTek-Agilent, Santa Clara, CA USA) microplate reader at 590 nm. The average well colour development (AWCD) was calculated at 144 h and equalled the optical density value of each well, corrected by subtracting the blank well using Equation (1) [34]:

$$\text{AWCD} = \sum \text{OD}_\text{i}/31 \quad (1)$$

where AWCD is the average well colour development (units), ODi is optical density value from each well, corrected by subtracting the blank well (inoculated, but without a carbon source), and 31 is number of variables (C substrates).

The sum of the AWCD scores for individual substrates was used as a measurement of total microbial catabolic activity. All analyses were performed in triplicate.

2.10. Genetic Biodiversity of Microbial Community Structure

Total DNA was extracted from 2 × 0.5 g soil samples using a GeneMATRIX Soil DNA Purification Kit (EurX, Gdańsk, Poland) according to the manufacturer's recommendations and combined in to one sample. The 16S rRNA gene fragment V3–V5 was amplified using primers MF-341-GC CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCCG CCT ACG GGA GGC AGC AG with GC clamp [35,36] and MR-907 CCGTCAATTCMTTTGAGTTT [37]. The reaction mixture (25 µL) contained: 0.25 µL of primer MF-341-GC (100 µM, 1:10) (Sigma-Aldrich, Inc., St. Louis, MO, USA), 0.25 µL of primer MR-907 (100 µM, 1:10) (Sigma-Aldrich, Inc., St. Louis, MO, USA), 0.5 µL of polymerase DreamTaq™ 500U (Thermo Fisher Scientific, Waltham, MA, USA), 2.5 µL of buffer 10× DreamTaq™ (Thermo Fisher Scientific, Waltham, MA, USA), 0.5 µL of 10mM Mix dNTP (Thermo Fisher Scientific, Waltham, MA, USA), 4 µL of DNA and 17 µL of H_2O. The amplification conditions were: 94 °C for 5 min, and 36 cycles of 94 °C for 20 s, 58 °C for 20 s, 72 °C for 30 s with final 72 °C 5 min.

The electrophoresis of the PCR products was performed in 6% (*w/v*) polyacrylamide gel (37.5:1 acrylamide:bis-acrylamide) with a linear denaturing gradient ranging from 40 to 70% using DCode Mutation Detection System (Bio-Rad, Hercules, CA, USA). The band pattern was analysed using Image Lab 6.0.1 (Bio-Rad, Hercules, CA, USA) (see Figures S1 and S2 of the Supplementary Materials section). The selected bands were cut out, reamplified, and sequenced. Reamplification was performed using MF-341 CCTACGGGAGGCAGCAG and MR-907 CCGTCAATTCMTTTGAGTTT AG primers. The reaction mixture was prepared as described before and amplification conditions were: 94 °C for 5 min followed by 34 cycles of 94 °C for 20 s, 56 °C for 20 s, 72 °C for 30 s and finally 72 °C for 5 min. The biodiversity

of the soil bacterial community was expressed as the Shannon-Wiener index (H'), richness (Rs), and evenness (E_H), calculated according to methodology reported elsewhere [38].

2.11. CadA, CzcA, and NccA Genes Detection

Soil isolated strains were tested for presence of *cadA*, *czcA*, and *nccA* genes using PCR. The DNA isolated from the bacteria using Genomic Mini (A&A Biotechnology, Gdańsk, Poland) according to the manufacturer's instructions was the template for this reaction. The 605 bp fragment of *cadA* gene was amplified using degenerated primers cad1 5′ AAR ACI GGI ACI YTI ACI AAR GGI G 3′ and cad2 5′ GIG CRT CRT TIA CIC CRT CIC CIA 3′ [39]; the 581 bp *nccA* gene was amplified using 2555nccAF 5′AGCCGSGASAACGGCAAGCG 3′ and 3117nccAR 5′ CCGATCACCACCGTYGCCAG 3′; and the *czcA* gene with primers 1226czcAF 5′GACTTCGGCATCATCRTCGAYGG3′, 2026czcAR 5′CGTTGAASCGCARCTGGATCGG 3′ [40]. The reaction mixture (25 µL) contained 1.5 µL of MgCl2 (Thermo Fisher Scientific, Waltham, MA, USA), 2.5 µL of 10× buffer KCl (Thermo Fisher Scientific, Waltham, MA, USA), 0.5 µL of dNTP Mix (10 mM) (Thermo Fisher Scientific, Waltham, MA, USA), 0.25 µL of primer cad1 or 2555nccAF or 1226czcAF, respectively (100 µM, 1:10 *v*/*v*) (Sigma-Aldrich, USA), 0.25 µL of primer cad2, primer 3117nccAR or 2026czcAR 5′, respectively (100 µM, 1:10 *v*/*v*) ((Sigma-Aldrich, Inc., St. Louis, MO, USA), 0.5 µL of polymerase Taq (Thermo Fisher Scientific, Waltham, MA, USA), 3 µL of DNA and 16.5 µL of H_2O.

The amplification conditions were: 94 °C for 2 min, followed by 25 cycles at 94 °C for 1 min denaturation, 60 °C for 1 min annealing, 72 °C for 2 min and extension at 72 °C for 5 min. The pKPY11 plasmid with the cloned *cadA* gene was used as positive control [39].

The amplification conditions for *nccA* gene were: 94 °C for 2 min for initial denaturation, 25 cycles of 94 °C for 1 min, 59 °C for 1 min and 72 °C for 1 min followed by extension at 72 °C for 5 min. The positive control for the amplification was genomic DNA from *C. metallidurans* CH34 isolated with Genomic Mini (A&A Biotechnology, Gdańsk, Poland) according to the manufacturer's instructions.

The *czcA* gene fragment was amplified using 94 °C for 2 min initial denaturation, 25 cycles at 94 °C for 1 min, 60 °C for 1 min, 72 °C for 2 min and a last extension of 72 °C for 5 min. The positive control for the amplification was genomic DNA from *C. metallidurans* CH34. The amplification specificity was checked through sequencing of PCR products. Briefly, PCR products were purified using Gel Out (A&A Biotechnology, Gdańsk, Poland) according to the manufacturer's instruction. The eluted DNA were sequenced in Genomed S.A. (Warsaw, Poland) and BLAST with the GeneBank database.

2.12. Statistical Analysis

Statistically significant differences between samples were calculated using one-way ANOVA and post-hoc Tukey test ($p < 0.05$) with Excel and Real Statistics 2020. Kendall Tau correlation was used to investigate the effect of environmental factors on microbial communities.

3. Results

3.1. Physicochemical Analysis of Soil Samples

The results of physicochemical analysis of soil samples are summarised in Table 1. As shown, samples significantly differed in water content, with pH values ranging from slightly acidic to slightly alkaline. The characteristic feature of the tested soils was the high content of macro elements (i.e., Ca, Mg, Na, K and total N, see Table 1). The total content of HMs in different soil samples significantly exceeded acceptable standards for Cd and Zn (Table 1). The only elements whose values remained within acceptable limits were Ni and Cu (with the exception of Cu at point B1).

Table 1. Physicochemical characterization of soil samples.

	Piekary Śląskie			Bukowno		
	P1	**P2**	**P3**	**B1**	**B2**	**B3**
Water content (%)	26.21 ± 0.24 ^{b}	29.48 ± 0.12 ^{a}	8.54 ± 0.25 ^{c}	5.01 ± 0.28 ^{d}	5.34 ± 0.36 ^{d}	1.56 ± 0.21 ^{e}
pH (H_20)	7.71 ± 0.03 ^{b}	8.14 ± 0.02 ^{a}	7.62 ± 0.01 ^{c}	6.79 ± 0.01 ^{e}	6.96 ± 0.01 ^{d}	5.49 ± 0.01 ^{f}
pH (KCl)	8 ± 0.03 ^{b}	8.29 ± 0.01 ^{a}	7.15 ± 0.01 ^{c}	6.77 ± 0.01 ^{e}	6.81 ± 0.01 ^{d}	4.69 ± 0.01 ^{f}
pH ($CaCl_2$)	7.84 ± 0.01 ^{b}	8.15 ± 0.01 ^{a}	7.04 ± 0.01 ^{c}	6.78 ± 0.01 ^{e}	6.82 ± 0.01 ^{d}	5.07 ± 0.01 ^{f}
Total N (mg/kg)	829.9 ± 35.21 ^{a}	780.8 ± 15.41 ^{a}	997.8 ± 19.28 ^{a}	747.97 ± 950.41 ^{a}	215.63 ± 6.15 ^{a}	151.2 ± 7.81 ^{a}
Organic matter (%)	7.76 ± 0.02 ^{b}	7.89 ± 0.06 ^{b}	10.95 ± 0.55 ^{a}	2.62 ± 0.04 ^{c}	2.23 ± 0.07 ^{c}	0.54 ± 0.04 ^{d}
Organic carbon (%)	4.5 ± 0.01 ^{b}	4.58 ± 0.03 ^{b}	6.35 ± 0.32 ^{a}	1.52 ± 0.02 ^{c}	1.3 ± 0.04 ^{c}	0.32 ± 0.02 ^{d}
P (%)	0.83 ± 0.03 ^{a}	1.14 ± 0.04 ^{b}	0.38 ± 0.01 ^{c}	0.2 ± 0.02 ^{d}	0.29 ± 0.01 ^{e}	0.24 ± 0.01^{de}
Na (mg/kg)	547.77 ± 23.24 ^{a}	397.67 ± 63.28 ^{b}	131.1 ± 5.11 ^{c}	112.3 ± 10.87 ^{cd}	38.6 ± 6.35 ^{de}	15.17 ± 0.42 ^{e}
Mg (g/kg)	37.486 ± 0.877 ^{a}	14.965 ± 0.567 ^{b}	5.865 ± 0.636 ^{c}	19.257 ± 0.024 ^{d}	3.420 ± 0.441 ^{e}	0.277 ± 0.024 ^{f}
K (g/kg)	0.708 ± 0.152 ^{c}	1.093 ± 0.055 ^{ab}	1.219 ± 0.059 ^{a}	0.962 ± 0.111 ^{b}	0.333 ± 0.076 ^{d}	0.114 ± 0.056 ^{d}
Ca (g/kg)	109.124 ± 2.746 ^{a}	36.105 ± 3.384 ^{b}	9.396 ± 0.425 ^{d}	24.361 ± 1.402 ^{c}	3.230 ± 0.285 ^{e}	0.274 ± 0.049 ^{e}
Zn total (g/kg)	1.208 ± 0.106 ^{b}	1.282 ± 0.205 ^{b}	1.035 ± 0.083 ^{b}	1.8143 ± 0.00 ^{a}	1.777 ± 0.00 ^{b}	14.362 ± 0.00 ^{b}
Zn (H_20) (mg/kg)	0.49 ± 0.3 ^{f}	1.14 ± 0.69 ^{def}	3.47 ± 1.54 ^{c}	19.38 ± 0.00 ^{a}	1.98 ± 0.00 ^{cd}	6.25 ± 0.00 ^{b}
Zn ($CaCl_2$) (mg/kg)	0.34 ± 0.12 ^{e}	0.35 ± 0.11 ^{e}	7.92 ± 0.88 ^{d}	56.21 ± 0.00 ^{a}	14.34 ± 0.00 ^{b}	21.43 ± 0.00 ^{c}
Cd total (g/kg)	0.293 ± 0.016 ^{c}	1.215 ± 0.119 ^{b}	0.0459 ± 0.006 ^{d}	1.991 ± 0.00 ^{a}	0.100 ± 0.00 ^{d}	0.017 ± 0.00 ^{d}
Cd (H_20) (mg/kg)	0.06 ± 0.03 ^{c}	0.14 ± 0.07 ^{b}	0.03 ± 0.00 ^{c}	0.51 ± 0.00 ^{a}	0.02 ± 0.00 ^{c}	0.01 ± 0.00 ^{c}
Cd ($CaCl_2$) (mg/kg)	0.05 ± 0.01 ^{e}	0.11 ± 0.03 ^{d}	0.24 ± 0.01 ^{c}	3.32 ± 0.00 ^{a}	0.49 ± 0.00 ^{c}	0.21 ± 0.00 ^{b}
Ni total (mg/kg)	3.94 ± 0.08 ^{d}	3 ± 0.22 ^{d}	0.62 ± 0.08 ^{e}	54.16 ± 0.00 ^{a}	44.63 ± 0.00 ^{b}	15.25 ± 0.00 ^{c}
Ni (H_20) (mg/kg)	0.001 ± 0.001 ^{e}	0.002 ± 0.001 ^{de}	0.003 ± 0.001 ^{d}	0.02 ± 0.00 ^{c}	0.04 ± 0.00 ^{a}	0.03 ± 0.00 ^{b}
Ni ($CaCl_2$) (mg/kg)	0.001 ± 0.002 ^{a}	0.00	0.00	0.01 ± 0.00 ^{c}	0.02 ± 0.00 ^{b}	0.02 ± 0.00 ^{b}
Cu total (mg/kg)	580.68 ± 59.12 ^{b}	175.65 ± 13.7 ^{c}	14.59 ± 0.69 ^{d}	1116.87 ± 0.0 ^{a}	75.81 ± 0.00 ^{d}	0.00 ± 0.00
Cu (H_20) (mg/kg)	0.04 ± 0.02 ^{a}	0.05 ± 0.01 ^{a}	0.03 ± 0.02 ^{a}	0.00 ± 0.00 ^{b}	0.02 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}
Cu ($CaCl_2$) (mg/kg)	0.02 ± 0.01 ^{b}	0.05 ± 0.00 ^{a}	0.01 ± 0.01^{b}	0.01 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}	0.01 ± 0.00 ^{b}

The data represent the mean and standard deviation of three replicates. P1, P2 and P3 indicate individual sampling sites from Piekary Śląskie; and B1, B2 and B3 from Bukowno. Data with different lower-case letters indicate statistically significant differences between results of particular parameter (one-way ANOVA, Tukey test, $p < 0.05$) and the same letter refers to no significant difference.

3.2. Enumeration of Metal Tolerant Microorganisms in Soil Samples

From samples collected at Piekary Śląskie, 64–77% out of the total oligotrophic bacteria (OB) was identified as metal tolerant but no oligotrophic Cu-tolerant bacteria were detected. Neither oligotrophic Ni- and Cd-tolerating bacteria were found in P1, nor Cd-tolerating bacteria in soil sample P2. In soil samples from Bukowno, no Cu-tolerant OB were found. As in the case of Piekary Śląskie, the number of oligotrophic bacteria that tolerated Zn and Ni accounted for 64–93% out of the total bacterial counts from Bukowno.

Metal tolerant bacteria in soil from Piekary Śląskie accounted for 71–96% of all bacteria grown on TSA medium (Heterotrophic bacteria, HB) and 67–89% of bacteria grown on agar broth medium. Cd-tolerant bacteria were found in soil samples from Bukowno (B1 and B2) but Zn-tolerant bacteria were absent in the B2 sample. The number of metallo-tolerant bacteria in Bukowno samples grown on TSA medium ranged from 73% to 99% (Heterotrophic bacteria, HB). In the case of bacteria grown on agar broth, metal-resistant bacteria accounted for 67–89% of all bacteria (Table 2). The use of real-time PCR (RT-PCR) allowed the assessment of the total bacterial number including culturable and non-cultured fractions. Using this method, bacterial numbers were estimated to be over twice as large compared to bacteria able to grow on culture media (Table 2).

3.3. Bacteria Isolated from Soil Samples

A significant number of metallotolerant bacteria were isolated from soil samples obtained from Piekary Ślaskie and Bukowno sampling sites (18 and 12, respectively), according to their 16S rRNA sequence (Table S1 in Supplementary Materials). The different isolates were classified into three phyla: *Proteobacteria*, *Actinobacteria* and *Bacteroidetes. Proteobacteria* phylum found in the soil samples was represented by *Methylobacterium* from the

Alpha-Proteobacteria class, *Variovorax* from the *Betaproteobacteria* class, *Pseudomonas* from the *Gammaproteobacteria* and *Ralstonia* classes, and *Cupriavidus* from the *Betaproteobacteria* class. The *Actinobacteria* phylum was represented by three species from the class *Actinobacteria* (i.e., *Arthrobacter, Rhodococcus, Agromyces*). The *Bacteroidetes* phylum was represented by *Chryseobacterium* from the *Flavobacterium* class.

Table 2. Metallotolerant bacteria enumeration in soil samples.

Bacteria Count, (log_{10} CFU/g Dry Soil)		Piekary Śląskie			Bukowno		
		P1	P2	P3	B1	B2	B3
OB	Total	4.44 ± 0.09^{ce}	4.79 ± 0.14^{cd}	4.91 ± 0.03^{c}	3.86 ± 0.04^{f}	5.73 ± 0.43^{a}	5.53 ± 0.16^{ab}
	Cu	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Cd	0.00 ± 0.00	0.00 ± 0.00	3.80 ± 0.14^{a}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Ni	0.00 ± 0.00	3.51 ± 0.17^{a}	3.44 ± 0.35^{ab}	2.48 ± 0.15^{c}	0.00 ± 0.00	0.00 ± 0.00
	Zn	3.10 ± 0.00^{a}	3.11 ± 0.00^{a}	3.67 ± 0.31^{a}	3.59 ± 0.03^{a}	4.58 ± 0.49^{a}	2.83 ± 2.46^{a}
HB (TSA)	Total	4.35 ± 0.08^{cde}	4.50 ± 0.04^{ac}	4.64 ± 0.67^{ab}	3.71 ± 0.09^{f}	4.38 ± 0.10^{acd}	4.73 ± 0.67^{a}
	Cu	3.98 ± 0.34^{ce}	4.26 ± 0.06^{c}	4.38 ± 0.65^{ab}	3.28 ± 0.24^{f}	4.56 ± 0.21^{a}	4.19 ± 0.63^{cd}
	Cd	3.10 ± 0.00^{a}	3.59 ± 0.31^{a}	3.53 ± 0.52^{a}	0.00 ± 0.00	0.00 ± 0.00	3.47 ± 0.54^{a}
	Ni	3.99 ± 0.14^{a}	4.33 ± 0.25^{a}	4.42 ± 0.65^{a}	3.87 ± 0.89^{a}	5.12 ± 0.70^{a}	4.66 ± 0.65^{a}
	Zn	3.86 ± 0.28^{a}	3.47 ± 0.39^{a}	4.28 ± 0.63^{a}	3.87 ± 2.06^{a}	4.64 ± 0.83^{a}	4.63 ± 0.65^{a}
HB (Broth agar)	Total	4.65 ± 0.10^{ade}	4.77 ± 0.07^{ac}	4.80 ± 0.24^{ab}	4.65 ± 0.10^{ef}	4.77 ± 0.07^{ad}	4.80 ± 0.24^{a}
	Cu	4.14 ± 0.07^{a}	3.99 ± 0.09^{a}	4.03 ± 0.61^{a}	4.14 ± 0.07^{a}	3.99 ± 0.09^{a}	4.03 ± 0.61^{a}
	Cd	3.33 ± 0.40^{a}	3.21 ± 0.17^{a}	4.07 ± 0.22^{a}	3.33 ± 0.40^{a}	3.21 ± 0.17^{a}	4.07 ± 0.22^{a}
	Ni	3.99 ± 0.38^{ade}	4.23 ± 0.26^{ac}	4.16 ± 0.30^{ad}	3.99 ± 0.38^{ef}	4.23 ± 0.26^{a}	4.16 ± 0.30^{ab}
	Zn	3.77 ± 0.06^{a}	3.65 ± 0.21^{a}	4.27 ± 0.05^{a}	3.77 ± 0.06^{a}	3.65 ± 0.21^{a}	4.27 ± 0.05^{a}
RT-PCR	Total	9.04 ± 0.08^{bc}	9.11 ± 0.02^{ab}	9.16 ± 0.03^{a}	9.02 ± 0.03^{bd}	9.06 ± 0.03^{abd}	8.99 ± 0.03^{cd}

The data represent the mean and standard deviation of three replicates. P1, P2 and P3 indicate individual sampling sites from Piekary Śląskie; and B1, B2 and B3 from Bukowno. T: total number of heterotrophic bacteria; Cu, Cd, Ni, Zn: the number of Cu-, Cd-, Ni- and Zn-tolerant bacteria. Data with different lower-case letters indicate statistically significant differences between results of particular parameter (one-way ANOVA, Tukey test, $p < 0.05$) and the same letter refers to no significant difference.

Most isolated bacteria were tolerant towards all selected HMs. Special attention should be given to *Rhodococcus erythropolis* strain P3 4 TSA, which showed tolerance up to 15 mM Zn and 6 mM Cd, *Arthrobacter nitroguajacolicus* P2 1 TSA tolerated 10 mM Zn and 6 mM Cd, and *Arthrobacter oryzae* P1 2 TSA, which enabled growth on media containing 10 mM Zn and 4 mM Cd—all of which were isolated from the Piekary Śląskie sampling site. High MIC values were also noted for bacteria isolated from Bukowno. For example, *Agromyces* sp. p2ba, *Arthrobacter nitroguajacolicus* ba O3 and *Chryseobacterium* sp. 1/10 la1 were found to be tolerant of a wide variety of conditions (Table S1 in Supplementary Materials).

3.4. Microorganisms Structure and Metabolic Activity

PLFA analysis showed gram negative bacteria as the dominant group in the different samples, constituting 46–50% of microorganisms in Piekary Śląskie and 47–60% in Bukowno. *Actinomycetes* were the least numerous phylum of microorganisms in all tested soils (Table 3). Despite HM contamination, all soil samples showed microbial enzymatic activity (FDA) and microorganisms were found able to break down carbohydrates, carboxylic acids, amino acids, polymers, amines and amides. Their potential to degrade individual carbon substrates, however, varied significantly among tested microbial communities (BIOLOG) (Table 3). Carbohydrates and amino acids were identified as the most important carbon source for microorganisms in Piekary Śląskie and Bukowno. Amines and amides were the least metabolised and constituted 3–7% of the used carbon sources (Table 3).

Table 3. Metabolic activity of microorganisms in soil samples.

Method		Sampling Site					
		P1	**P2**	**P3**	**B1**	**B2**	**B3**
FDA (μg fluorescein/g dry soil)		0.23 ± 0.08^{b}	0.27 ± 0.06^{b}	0.58 ± 0.1^{a}	0.28 ± 0.1^{b}	0.15 ± 0.04^{b}	0.56 ± 0.07^{a}
PLFA	Total, nmol PLFA/g dry soil	2.83 ± 0.76^{bc}	8.27 ± 0.95^{ac}	12.64 ± 1.70^{a}	5.88 ± 5.89^{ac}	10.38 ± 1.26^{ac}	77.62 ± 2.78^{ac}
	Gram + (%)	38.74 ± 3.75^{a}	41.54 ± 0.46^{a}	38.84 ± 0.25^{a}	32.57 ± 19.47^{a}	38.19 ± 2.36^{a}	37.93 ± 3.01^{a}
	Gram − (%)	46.04 ± 4.33^{a}	50.49 ± 0.57^{a}	47.14 ± 0.65^{a}	59.84 ± 18.32^{a}	47.75 ± 1.73^{a}	47.33 ± 2.43^{a}
	Actinomycetes (%)	15.21 ± 1.91^{a}	4.65 ± 0.07^{a}	9.23 ± 0.53^{a}	6.71 ± 7.39^{a}	8.23 ± 0.81^{a}	8.43 ± 1.59^{a}
	Fungi (%)	0.00 ± 0.00	3.31 ± 0.67^{a}	4.80 ± 0.12^{a}	0.88 ± 0.76^{a}	5.83 ± 0.18^{a}	6.31 ± 1.37^{a}
BIOLOG	AWCD	0.82 ± 0.05^{ecd}	0.78 ± 0.04^{cd}	1.15 ± 0.17^{f}	0.09 ± 0.02^{a}	0.82 ± 0.08^{d}	1.38 ± 0.07^{b}
	Carbohydrates (%)	32.87 ± 7.14^{b}	19.18 ± 1.53^{a}	24.60 ± 0.10^{b}	61.69 ± 3.17^{b}	18.41 ± 2.74^{b}	25.05 ± 1.99^{b}
	Carboxylic Acids (%)	4.41 ± 3.81^{cdf}	17.09 ± 0.46^{c}	15.42 ± 1.13^{cd}	7.67 ± 5.37^{ce}	8.13 ± 3.66^{b}	18.97 ± 2.07^{a}
	Amino acids (%)	31.42 ± 3.52^{cf}	35.48 ± 0.34^{ce}	30.11 ± 1.85^{c}	12.16 ± 1.82^{cd}	41.28 ± 4.47^{b}	26.94 ± 1.13^{a}
	Polymers (%)	17.98 ± 0.66^{c}	16.81 ± 3.78^{cd}	16.45 ± 0.98^{ce}	12.24 ± 6.99^{cf}	18.90 ± 1.92^{b}	13.52 ± 0.58^{a}
	Amines and amides (%)	5.58 ± 3.97^{bcd}	4.36 ± 0.76^{bde}	5.99 ± 0.40^{cb}	2.58 ± 3.00^{bdf}	5.83 ± 0.66^{b}	7.93 ± 3.40^{a}
	Miscellaneous (%)	7.74 ± 5.31^{c}	7.07 ± 3.19^{ce}	7.44 ± 2.69^{cd}	3.66 ± 2.56^{cf}	7.45 ± 1.55^{b}	7.60 ± 2.27^{a}
DGGE	H′	1.02 ± 0.08^{fde}	0.70 ± 0.02^{b}	0.95 ± 0.04^{d}	1.01 ± 0.05^{ed}	0.49 ± 0.03^{cb}	0.04 ± 0.00^{a}
	Rs	26 ± 0.00^{a}	21 ± 0.00^{a}	21 ± 0.00^{a}	18 ± 0.00^{a}	24 ± 0.00^{a}	24 ± 0.00^{a}
	E_H	0.31 ± 0.02^{ed}	0.23 ± 0.01^{cb}	0.31 ± 0.01^{dc}	0.35 ± 0.02^{fe}	0.23 ± 0.01^{b}	0.01 ± 0.00^{a}

The data represent the mean and standard deviation of three replicates. P1, P2 and P3 indicate individual sampling sites from PiekaryŚląskie; and B1, B2 and B3 from Bukowno. Data with different lower-case letters indicate statistically significant differences between results of particular parameter (one-way ANOVA, Tukey test, $p < 0.05$) and the same letter refers to no significant difference. AWCD (Average Well Colour Development).

Genetic structure analysis suggested that *Proteobacteria*, *Gemmatimonadetes* and *Acidobacteria* were present in all studied soils. Representative types *Chloroflexi* and *Actinobacteria* were detected in soil from Piekary Śląskie, while in Bukowno sampling sites, *Bacteroidetes* and *Firmicutes* were found (Table S2 of Supplementary Materials).

3.5. Genes Encoding Mechanisms of HM Tolerance

Detection of *CzcA*, *NccA* and *CadA* genes, known as part of HM tolerance mechanisms [41], showed that the *czcA* (822 kb) gene encoding for Co-, Zn- and Cd-tolerance was present in P1 3 1/10 *Variovorax paradoxus*, P3 3 TSA *Variovorax paradoxus*, P2 4 ba *Arthrobacter sp.*, P3 1 ba *Variovorax sp.* and P3 2 ba *Arthrobacter sp.* isolated from Piekary Śląskie samples, as well as in ba 03 *Arthrobacter nitroguajacolicus*, 1/10 p2ba *Variovorax boronicumulans*, TSA 07 *Pseudomonas ficuserectae* and 1/10 O1 *Methylobacterium zatmanii* isolated from soil samples from Bukowno. The presence of the *nccA* (581 kb) gene encoding tolerance for Ni, Co and Cd was found in P1 2 TSA *Arthrobacter oryzae* (Piekary Śląskie), and ba 03 *Arthrobacter nitroguajacolicus*, TSA O6 *Arthrobacter oxydans*, TSA 05 *Pseudomonas sp.*, and1/10 O1 *Variovorax boronicumulans* (Bukowno). The *cadA* (1058 kb) gene, determining tolerance to Cd, Zn and Co, was only identified in TSA la3 *Pseudomonas* sp. (Bukowno). The bands were sequenced and compared with the sequences deposited in the GenBank database (Figure S3 of the Supplementary Materials).

3.6. Kendall Tau Correlation

Interaction between physicochemical and biological soil parameters was tested using the Kendall Tau correlation, Figures S4 and S5 (Supplementary Materials). As shown, negative correlation was observed between soil water content, pH and bacteria cultured on TSA medium, as well as Cd-tolerant and Zn-tolerant cultured bacteria on broth agar, and Zn-tolerant media on 1/10 TSA medium in samples from Piekary Śląskie (Figure S4, Supplementary Materials). The Zn and Cu content negatively affected the number of microorganisms as shown by PLFA and qPCR analysis, as well as culturable fraction of bacteria (TSA medium and 1/10 TSA). These elements also had a negative effect on FDA, BIOLOG index (AUC).

A positive correlation was found between the bioavailable heavy metal fraction (i.e., Zn, Cd) and PLFA (i.e., gram-positive, gram-negative, fungi, actinomycetes) as well as FDA, AUC and Rs determined by the BIOLOG method. Bioavailable Ni and Cd also affected qPCR values in the same way (Figure S4, Supplementary Materials).

In soils collected from Bukowno, soil moisture and pH negatively affected the number of microorganisms (TSA total), soil microorganism activity (FDA), and their ability to break down substrates (BIOLOG). Bioavailable Ni was found positively correlated with the number of cultured microorganisms, PLFA (gram-positive bacteria, actinomycetes, fungi) and microorganism biodiversity determined by DGGE, FDA, as well as parameters from the BIOLOG method (i.e., AUC, H and Rs) (Figure S5, Supplementary Materials).

4. Discussion

4.1. Physicochemical Characterization of Soils

Total metal content in soil is a useful indicator of the overall degree of contamination. It does not, however, provide information about metal mobility, bioavailability and toxicity [42]. Some studies have shown that both total and bioavailable HMs fraction shape microbial communities in contaminated sites [43]. The bioavailable HM fraction found in soil samples from Piekary Śląskie and Bukowno was relatively small compared to the HM total content, probably linked to soil pH, and other key factors determining HM availability in soil. Low pH values decrease HM absorption onto clay minerals, hydrated oxides and organic surfaces [44]. Nevertheless, other physicochemical soil parameters may also contribute to a negative HM effect on soil microbial communities. Results of this research agree with other studies [17,36,45] suggesting that pH and water content have a significant impact on structure and growth of microbial communities (PLFA and culturable bacterial fraction) and are negatively correlated to several biological processes, including enzymatic (FDA) and metabolic activity (BIOLOG), as well as HM availability. The SOM was found not only to be a carbon source but also to influence HM mobility as suggested in the past [42]. The SOM content in both studied soils was low, suggesting that other forms of metal precipitation or immobilisation may occur in the study area. The high amount of nitrogen in soil suggested low enzymatic activity involved in denitrification. Negative effect of Cd on nitrogen transformation has been reported in the past [46] and other studies have suggested that cadmium salts at a concentration of 200 mg/kg soil inhibited denitrification by almost 80% [47] in line with our results.

The studied soils were also characterised by a high content of macro- and microelement which, in addition to their biological role, can generate precipitation of nutrients. For example, Ca may generate phosphorus precipitation, necessary for microorganisms to grow [48]. Our results suggest that, regardless of HM contamination, other factors such as essential nutrient precipitation might influence growth and composition of microbial communities in the study area.

4.2. The Influence of HMs on Bacterial Counts

Over 60% of the total heterotrophic bacteria count showed tolerance towards selected HMs, independently of the collection site. Presence of metal-tolerant bacteria in soils exposed to prolonged HM exposure has been repeatedly reported in the past [15,49]. For example, metal-tolerant bacteria have been found accounting for almost 80% of total soil bacterial number in both laboratory and field experiments [50]. Long-term HM soil contamination undoubtedly leads to selection of microorganisms that possess appropriate mechanisms to enable them to survive under these stressful conditions [51]. The number of soil bacteria calculated by real-time PCR was twice as high as those obtained by culture methods, suggesting that non-cultivated bacteria constitute a large group of soil microbiome. Similar differences between total bacteria number measured either by plate count or real-time PCR methods were also noted [52].

4.3. Microbial Community Structure and Activity in HM-Contaminated Soils

As determined by PLFA, gram-negative bacteria dominated in all tested soils. Nevertheless, gram-positive bacteria also constituted a relatively large group. *Actinomycetes* accounted for the smallest microorganism group. Fungi are considered by some authors to have higher HM resistance and to be even capable of increasing their number under certain conditions [53]. In this work, however, only small amounts of fungal biomarkers were observed compared to bacteria biomarkers.

This type of controversial observation is frequently discussed. For example, the negative impact of Pb on fungi and fungal colony-forming units (CFUs) of PLFA biomarkers has been reported in the past [54]. Similarly, in another study [55], soil fungal population showed higher sensitivity to HM contamination than bacteria. In contrast, a higher fungal tolerance to metals was observed compared to bacteria by another study [56]. These apparently conflicting findings can be explained by the effect of other factors, such as soil pH [57], nutrient content [58], or presence of other contaminants [59] which can mask the harmful effects of HMs.

The test used for microorganism genetic biodiversity (PCR-DGGE) is particularly useful in environments with small biodiversity, and allows accumulation of data on non-culturable fractions known to be dominant in soil [60]. In soil samples from Piekary Śląskie, *Proteobacteria*, *Chloroflexi*, *Acidobacteria* and *Gemmatimonadetes* outweighed the others. For samples from Bukowno, *Bacteroidetes*, *Firmicutes*, *Acidobacteria*, *Proteobacteria* and *Gemmatimonadetes* were the dominant microorganisms. Similar bacteria groups were found using DGGE analysis in other HM contaminated areas. For example, *Bacteroidetes*, *Acidobacteria*, *Gemmatimonadetes*, *Actinobacteria* and *Betaproteobacteria*, with predominance of the former three, were observed in HM-polluted soils from Katowice, Poland [61]. Presence of *Acidobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Chloroflexi* in long-term HM contaminated soils was also reported by other studies [13].

Bacteria belonging to the *Gemmatimonadetes* are considered to make up 2% of all bacteria in soil. This phylum is often found in HM contaminated areas [62,63] and it is mostly detected by non-culturing methods [64]. Prevalence of bacteria from this phylum in soils is probably related to their ability to adapt to low water content and neutral or slightly acidic soil pH [64].

Moreover, *Acidobacteria* is a large, diverse phylum found in different soils with most of the bacteria being non-culturable [65,66], the abundance of which is also correlated with soil pH [67,68]. *Chloroflexi* phylum members have been reported to colonise a variety of environments, including contaminated soils, while being mainly non-culturable in agreement with our results [62,63,69,70]. *Firmicutes* have been found as the dominant phylum in Cr-contaminated environments [71] while *Proteobacteria* is a well-known ubiquitous phylum among HM contaminated soils [72,73], in line with our results using the DGGE method. Microorganism metabolic activity analysis using BIOLOG suggested that carbohydrates and amino acids were the main carbon source while amines and amides were metabolised to the least extent, which agrees with similar observations of carbohydrates and amino acids usage as a primary carbon source in HM contaminated areas [74].

4.4. Isolated Bacteria Characteristics

Proteobacteria, *Bacteroides* and *Actinobacteria* were detected using culturable methods. *Actinobacteria* represent one of the most dominant groups of microorganisms and is the most effective in decomposing organic matter, including hemicellulose and lignin [75,76]. Other studies have suggested that, among others, *Actinobacteria* tolerate much higher HM concentrations than any other soil bacteria for the same conditions [62,63,69,76]. Most bacteria isolated from both studied areas were found tolerant to at least three of the selected HMs tested (i.e., Cd, Cu, Ni, Zn), in addition to reaching high MIC values. We have also been able to show the genes encoding the mechanisms of tolerance to HMs in isolated bacteria. Thus, *czcA* and *nccA* were detected in *Variovorax* sp., *Arthrobacter* sp., *Pseudomonas* sp. and *Methylobacterium*, while *cadA* was found in *Pseudomonas* sp. These same genes

have been reported in other studies using culturable techniques by other authors [40,77,78]. Their absence in other bacteria inhabiting tested soils suggests presence of alternative mechanisms enabling them to survive under these conditions, including transporting HM out of the cell, metal precipitation outside or inside the cell, or HM binding through exopolysaccharides [79].

Some of the isolated bacteria were well-known for their high metabolic potential and HM tolerance, such as *Cupriavidus* sp. [80], *Arthrobacter* sp. [81], *Variovorax* sp. [82] and *Pseudomonas* sp. [83]. Because of their characteristics and origin, these microorganisms can be used in HM contaminated area restoration or laboratory-scale tests to identify the mechanisms enabling them to tolerate high HM concentrations. Furthermore, some of these bacteria (e.g., *Agromyces* or *Variovorax* sp.) remain poorly understood, therefore their isolation from soil and genome sequencing is expected to allow for better understanding of their role in contaminated environments.

5. Conclusions

It has been demonstrated in this study that the structure and function of microbial communities in both tested contaminated soils were shaped not only by the specific HM and its concentration, but also by the other physicochemical features of the soil.

It seems that pH and water were the key factors affecting not only the biological part of the soil, but also its structural properties such as bioavailability of HMs. Other factors including low amounts of SOM and high concentration of microelements together with HMs contributed in determining the microbial communities in this area.

Undoubtedly long-term contamination of the soils with HMs has led to the development and domination of metal tolerant fractions of bacteria in these areas. In addition, the non-culturable fraction of bacteria was found to be a significant component of microbial communities in the HM-polluted soils, although they are omitted by the traditional methods.

Nevertheless, culture techniques still having high value, as isolated bacteria may be used in bioremediation of contaminated sites and this method, being environmentally friendly, is currently favoured by many researchers.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microbiolres13030045/s1, Table S1. Bacterial strains isolated from Piekary Śląskie and Bukowno soil samples, including HMs-MIC values; Table S2. Sequence-based identification and taxonomic affiliation of DGGE gels bands excised from Piekary Śląskie and Bukowno. Figure S1. DGGE profile for Piekary Śląskie soil sample; Figure S2. DGGE profile for Bukowno soil sample; Figure S3. PCR amplification for (a) *czcA*, (b) *nccA*, and (c) *cadA*; Figure S4. Kendal Tau correlation for physicochemical and biological parameters in the tested soil samples from Piekary Śląskie; Figure S5. Kendal Tau correlation for physicochemical and biological parameters in the tested soil samples from Bukowno.

Author Contributions: Conceptualization, A.K.J. and Z.P.-S.; methodology, A.K.J. and Z.P.-S.; software, A.K.J.; validation, A.K.J.; formal analysis, A.K.J.; investigation, A.K.J.; resources, A.K.J.; data curation, A.K.J.; writing—original draft preparation, A.K.J.; writing—review and editing, A.K.J.; visualization, A.K.J.; supervision, Z.P.-S.; project administration, A.K.J. and Z.P.-S.; funding acquisition, A.K.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by National Science Center, Poland, Grant number 2013/11/N/NZ9/04630 A.J. was supported by scholarship provided by the "DoktoRIS—Scholarship programme for innovative Silesia", which was co-financed by the European Union under the European Social Fund covered by Human Capital Programme.

Data Availability Statement: Data supporting reported results are available on request.

Conflicts of Interest: The authors declare that they have no conflict of interest.

References

1. Mitra, S.; Chakraborty, A.J.; Tareq, A.M.; Emran, T.B.; Nainu, F.; Khusro, A.; Idris, A.M.; Khandaker, M.U.; Osman, H.; Alhumaydhi, F.A.; et al. Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. *J. King Saud Univ. Sci.* **2022**, *34*, 101865. [CrossRef]
2. Li, C.; Zhou, K.; Qin, W.; Tian, C.; Qi, M.; Yan, X.; Han, W. A Review on Heavy Metals Contamination in Soil: Effects, Sources, and Remediation Techniques. *Soil Sediment. Contam.* **2019**, *28*, 380–394. [CrossRef]
3. Ali, H.; Khan, E.; Ilahi, I. Environmental chemistry and ecotoxicology of hazardous heavy metals: Environmental persistence, toxicity, and bioaccumulation. *J. Chem.* **2019**, *2019*, 6730305. [CrossRef]
4. Rai, P.K.; Lee, S.S.; Zhang, M.; Tsang, Y.F.; Kim, K.H. Heavy metals in food crops: Health risks, fate, mechanisms, and management. *Environ. Int.* **2019**, *125*, 365–385. [CrossRef] [PubMed]
5. Raza, A.; Habib, M.; Kakavand, S.N.; Zahid, Z.; Zahra, N.; Sharif, R.; Hasanuzzaman, M. Phytoremediation of cadmium: Physiological, biochemical, and molecular mechanisms. *Biology* **2020**, *9*, 177. [CrossRef] [PubMed]
6. Ayangbenro, A.S.; Babalola, O.O. A new strategy for heavy metal polluted environments: A review of microbial biosorbents. *Int. J. Environ. Res. Public Health* **2017**, *14*, 94. [CrossRef] [PubMed]
7. Bouchez, T.; Blieux, A.L.; Dequiedt, S.; Domaizon, I.; Dufresne, A.; Ferreira, S.; Godon, J.J.; Hellal, J.; Joulian, C.; Quaiser, A.; et al. Molecular microbiology methods for environmental diagnosis. *Environ. Chem. Lett.* **2016**, *14*, 423–441. [CrossRef]
8. Giller, K.E.; Witter, E.; Mcgrath, S.P. Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: A review. *Soil Biol. Biochem.* **1998**, *30*, 1389–1414. [CrossRef]
9. Khan, S.; Hesham, A.E.L.; Qiao, M.; Rehman, S.; He, J.Z. Effects of Cd and Pb on soil microbial community structure and activities. *Environ. Sci. Pollut. Res.* **2010**, *17*, 288–296. [CrossRef]
10. Wang, Y.P.; Shi, J.Y.; Wang, H.; Lin, Q.; Chen, X.C.; Chen, Y.X. The influence of soil heavy metals pollution on soil microbial biomass, enzyme activity, and community composition near a copper smelter. *Ecotoxicol. Environ. Saf.* **2007**, *67*, 75–81. [CrossRef]
11. Wang, X.; Gao, P.; Li, D.; Liu, J.; Yang, N.; Gu, W.; He, X.; Tang, W. Risk assessment for and microbial community changes in Farmland soil contaminated with heavy metals and metalloids. *Ecotoxicol. Environ. Saf.* **2019**, *185*, 109685. [CrossRef] [PubMed]
12. Bååth, E. Effects of heavy metals in soil on microbial processes and populations (a review). *Water Air Soil Pollut.* **1989**, *47*, 335–379. [CrossRef]
13. Chen, J.; He, F.; Zhang, X.; Sun, X.; Zheng, J.; Zheng, J. Heavy metal pollution decreases microbial abundance, diversity and activity within particle-size fractions of a paddy soil. *FEMS Microbiol. Ecol.* **2014**, *87*, 164–181. [CrossRef]
14. Müller, A.K.; Westergaard, K.; Christensen, S.; Sørensen, S.J. The effect of long-term mercury pollution on the soil microbial community. *FEMS Microbiol. Ecol.* **2001**, *36*, 11–19. [CrossRef]
15. Pennanen, T.; Frostegård, Å.; Fritze, H.; Bååth, E. Phospholipid fatty acid composition and heavy metal tolerance of soil microbial communities along two heavy metal-polluted gradients in coniferous forests. *Appl. Environ. Microbiol.* **1996**, *62*, 420–428. [CrossRef]
16. Jin, Y.; Luan, Y.; Ning, Y.; Wang, L. Effects and mechanisms of microbial remediation of heavy metals in soil: A critical review. *Appl. Sci.* **2018**, *8*, 1336. [CrossRef]
17. Guo, H.; Nasir, M.; Lv, J.; Dai, Y.; Gao, J. Understanding the variation of microbial community in heavy metals contaminated soil using high throughput sequencing. *Ecotoxicol. Environ. Saf.* **2017**, *144*, 300–306. [CrossRef]
18. Zeng, X.Y.; Li, S.W.; Leng, Y.; Kang, X.H. Structural and functional responses of bacterial and fungal communities to multiple heavy metal exposure in arid loess. *Sci. Total Environ.* **2020**, *723*, 138081. [CrossRef]
19. Tipayno, S.C.; Truu, J.; Samaddar, S.; Truu, M.; Preem, J.K.; Oopkaup, K.; Espenberg, M.; Chatterjee, P.; Kang, Y.; Kim, K.; et al. The bacterial community structure and functional profile in the heavy metal contaminated paddy soils, surrounding a nonferrous smelter in South Korea. *Ecol. Evol.* **2018**, *8*, 6157–6168. [CrossRef] [PubMed]
20. Masum, S.A.; Islam, M.S. A case study on predicting the environmental impacts of untreated effluent generated from Tannery industrial estate in Dhaka, Bangladesh. *Geogr. Environ. Sustain.* **2020**, *13*, 22–31. [CrossRef]
21. Masum, S.A.; Thomas, H.R. Effects of microbial spatial distribution on organic biodegradation and immobilization of trace metals in co-contaminated soils. *Comput. Geotech.* **2021**, *133*, 104063. [CrossRef]
22. Wood, J.L.; Liu, W.; Tang, C.; Franks, A.E. Microorganisms in heavy metal bioremediation: Strategies for applying microbial-community engineering to remediate soils. *AIMS Bioeng.* **2016**, *3*, 211–229. [CrossRef]
23. Szrek, D.; Bajda, T.; Manecki, M. A comparative study of the most effective amendment for Pb, Zn and Cd immobilization in contaminated soils. *J. Environ. Sci. Health—Part A Toxic/Hazard. Subst. Environ. Eng.* **2011**, *46*, 1491–1502. [CrossRef] [PubMed]
24. Kucharski, R.; Sas-Nowosielska, A.; Małkowski, E.; Japenga, J.; Kuperberg, J.M.; Pogrzeba, M.; Krzyzak, J. The use of indigenous plant species and calcium phosphate for the stabilization of highly metal-polluted sites in southern Poland. *Plant Soil* **2005**, *273*, 291–305. [CrossRef]
25. Oleksynowa, K.; Tokaj, J.; Jakubiec, J. *Guide to Exercies in Soil Science and Geology*; Wydawnictwo AR: Kraków, Poland, 1991.
26. Edwards, U.; Rogall, T.; Blockerl, H.; Emde, M.; Bottger, E.C. Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. *Nucleic Acids Res.* **1989**, *17*, 7843–7853. [CrossRef] [PubMed]
27. Pacwa-Płociniczak, M.; Płaza, G.A.; Poliwoda, A.; Piotrowska-Seget, Z. Characterization of hydrocarbon-degrading and biosurfactant-producing Pseudomonas sp. P-1 strain as a potential tool for bioremediation of petroleum-contaminated soil. *Environ. Sci. Pollut. Res.* **2014**, *21*, 9385–9395. [CrossRef]

28. Sułowicz, S.; Cycoń, M.; Piotrowska-Seget, Z. Non-target impact of fungicide tetraconazole on microbial communities in soils with different agricultural management. *Ecotoxicology* **2016**, *25*, 1047–1060. [CrossRef] [PubMed]
29. Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. Basic local alignment search tool. *J. Mol. Biol.* **1990**, *215*, 403–410. [CrossRef]
30. Frostegard, A.; Tunlid, A.; Baath, E. Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. *Appl. Environ. Microbiol.* **1993**, *59*, 3605–3617. [CrossRef]
31. Willers, C.; Jansen van Rensburg, P.J.; Claassens, S. Phospholipid fatty acid profiling of microbial communities-a review of interpretations and recent applications. *J. Appl. Microbiol.* **2015**, *119*, 1207–1218. [CrossRef]
32. Kroppenstedt, R.M. Fatty Acid and Menaquinone Analysis of Actinomycetes and Related Organisms. In *Chemical Methods in Bacterial Systematics*; Goodfellow, M., Minnikin, D.E., Eds.; Academic Press: London, UK, 1985; pp. 173–199.
33. Frostegård, Å.; Tunlid, A.; Bååth, E. Use and misuse of PLFA measurements in soils. *Soil Biol. Biochem.* **2011**, *43*, 1621–1625. [CrossRef]
34. Magdalena, F.; Stefania, J.T. Agricultural utilisation of dairy sewage sludge: Its effect on enzymatic activity and microorganisms of the soil environment. *Afr. J. Microbiol. Res.* **2011**, *5*, 1755–1762. [CrossRef]
35. Muyzer, G.; De Waal, E.C.; Uitterlinden, A.G. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* **1993**, *59*, 695–700. [CrossRef] [PubMed]
36. Muyzer, G.; Smalla, K. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie Van Leeuwenhoek* **1998**, *73*, 127–141. [CrossRef] [PubMed]
37. Ishii, K.; Fukui, M.; Takii, S. Microbial succession during a composting process as evaluated by denaturing gradient gel electrophoresis analysis. *J. Appl. Microbiol.* **2000**, *89*, 768–777. [CrossRef]
38. Cycoń, M.; Markowicz, A.; Borymski, S.; Wójcik, M.; Piotrowska-Seget, Z. Imidacloprid induces changes in the structure, genetic diversity and catabolic activity of soil microbial communities. *J. Environ. Manag.* **2013**, *131*, 55–65. [CrossRef]
39. Oger, C.; Mahillon, J.; Petit, F. Distribution and diversity of a cadmium resistance (cadA) determinant and occurrence of IS257 insertion sequences in Staphylococcal bacteria isolated from a contaminated estuary (Seine, France). *FEMS Microbiol. Ecol.* **2003**, *43*, 173–183. [CrossRef]
40. Karelová, E.; Harichová, J.; Stojnev, T.; Pangallo, D.; Ferianc, P. The isolation of heavy-metal resistant culturable bacteria and resistance determinants from a heavy-metal-contaminated site. *Biologia (Bratisl)* **2011**, *66*, 18–26. [CrossRef]
41. Nies, D.H. Efflux-mediated heavy metal resistance in prokaryotes. *FEMS Microbiol. Rev.* **2003**, *27*, 313–339. [CrossRef]
42. Kim, R.Y.; Yoon, J.K.; Kim, T.S.; Yang, J.E.; Owens, G.; Kim, K.R. Bioavailability of heavy metals in soils: Definitions and practical implementation—a critical review. *Environ. Geochem. Health* **2015**, *37*, 1041–1061. [CrossRef] [PubMed]
43. Zhen, Z.; Wang, S.; Luo, S.; Ren, L.; Liang, Y.; Yang, R.; Li, Y.; Zhang, Y.; Deng, S.; Zou, L.; et al. Significant Impacts of Both Total Amount and Availability of Heavy Metals on the Functions and Assembly of Soil Microbial Communities in Different Land Use Patterns. *Front. Microbiol.* **2019**, *10*, 2293. [CrossRef]
44. Guo, X.; Zhao, G.; Zhang, G.; He, Q.; Wei, Z.; Zheng, W.; Qian, T.; Wu, Q. Effect of mixed chelators of EDTA, GLDA, and citric acid on bioavailability of residual heavy metals in soils and soil properties. *Chemosphere* **2018**, *209*, 776–782. [CrossRef] [PubMed]
45. Nicol, G.W.; Leininger, S.; Schleper, C.; Prosser, J.I. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. *Environ. Microbiol.* **2008**, *10*, 2966–2978. [CrossRef] [PubMed]
46. Afzal, M.; Yu, M.; Tang, C.; Zhang, L.; Muhammad, N.; Zhao, H.; Feng, J.; Yu, L.; Xu, J. The negative impact of cadmium on nitrogen transformation processes in a paddy soil is greater under non-flooding than flooding conditions. *Environ. Int.* **2019**, *129*, 451–460. [CrossRef]
47. Smolders, E.; Brans, K.; Coppens, F.; Merckx, R. Potential nitrification rate as a tool for screening toxicity in metal-contaminated soils. *Environ. Toxicol. Chem.* **2001**, *20*, 2469–2474. [CrossRef] [PubMed]
48. Richardson, A.E.; Simpson, R.J. Soil microorganisms mediating phosphorus availability. *Plant Physiol.* **2011**, *156*, 989–996. [CrossRef] [PubMed]
49. Azarbad, H.; van Straalen, N.M.; Laskowski, R.; Nikiel, K.; Röling, W.F.M.; Niklińska, M. Susceptibility to additional stressors in metal-tolerant soil microbial communities from two pollution gradients. *Appl. Soil Ecol.* **2016**, *98*, 233–242. [CrossRef]
50. Song, J.; Shen, Q.; Wang, L.; Qiu, G.; Shi, J.; Xu, J.; Brookes, P.C.; Liu, X. Effects of Cd, Cu, Zn and their combined action on microbial biomass and bacterial community structure. *Environ. Pollut.* **2018**, *243*, 510–518. [CrossRef]
51. Díaz-Raviña, M.; Bååth, E. Development of metal tolerance in soil bacterial communities exposed to experimentally increased metal levels. *Appl. Environ. Microbiol.* **1996**, *62*, 2970–2977. [CrossRef]
52. Borymski, S.; Cycon, M.; Beckmann, M.; Mur, L.A.J.; Piotrowska-Seget, Z. Plant species and heavy metals affect biodiversity of microbial communities associated with metal-tolerant plants in metalliferous soils. *Front. Microbiol.* **2018**, *9*, 1425. [CrossRef] [PubMed]
53. Rajapaksha, R.M.C.P.; Tobor-Kapłon, M.A.; Bååth, E. Metal toxicity affects fungal and bacterial activities in soil differently. *Appl. Environ. Microbiol.* **2004**, *70*, 2966–2973. [CrossRef] [PubMed]
54. Bååth, E.; Díaz-Raviña, M.; Bakken, L.R. Microbial biomass, community structure and metal tolerance of a naturally Pb-enriched forest soil. *Microb. Ecol.* **2005**, *50*, 496–505. [CrossRef] [PubMed]

55. Xu, Y.; Seshadri, B.; Bolan, N.; Sarkar, B.; Ok, Y.S.; Zhang, W.; Rumpel, C.; Sparks, D.; Farrell, M.; Hall, T.; et al. Microbial functional diversity and carbon use feedback in soils as affected by heavy metals. *Environ. Int.* **2019**, *125*, 478–488. [CrossRef] [PubMed]
56. Wang, F.; Yao, J.; Si, Y.; Chen, H.; Russel, M.; Chen, K.; Qian, Y.; Zaray, G.; Bramanti, E. Short-time effect of heavy metals upon microbial community activity. *J. Hazard. Mater.* **2010**, *173*, 510–516. [CrossRef]
57. Azarbad, H.; Niklińska, M.; Van Gestel, C.A.M.; Van Straalen, N.M.; Röling, W.F.M.; Laskowski, R. Microbial community structure and functioning along metal pollution gradients. *Environ. Toxicol. Chem.* **2013**, *32*, 1992–2002. [CrossRef]
58. Chodak, M.; Gołebiewski, M.; Morawska-Płoskonka, J.; Kuduk, K.; Niklińska, M. Diversity of microorganisms from forest soils differently polluted with heavy metals. *Appl. Soil Ecol.* **2013**, *64*, 7–14. [CrossRef]
59. Klimek, B.; Sitarz, A.; Choczyński, M.; Niklińska, M. The Effects of Heavy Metals and Total Petroleum Hydrocarbons on Soil Bacterial Activity and Functional Diversity in the Upper Silesia Industrial Region (Poland). *Water Air Soil Pollut.* **2016**, *227*, 265. [CrossRef]
60. Bodor, A.; Bounedjoum, N.; Vincze, G.E.; Erdeiné Kis, Á.; Laczi, K.; Bende, G.; Szilágyi, Á.; Kovács, T.; Perei, K.; Rákhely, G. Challenges of unculturable bacteria: Environmental perspectives. *Rev. Environ. Sci. Biotechnol.* **2020**, *19*, 1–22. [CrossRef]
61. Pacwa-Płociniczak, M.; Płociniczak, T.; Yu, D.; Kurola, J.M.; Sinkkonen, A.; Piotrowska-Seget, Z.; Romantschuk, M. Effect of Silene vulgaris and Heavy Metal Pollution on Soil Microbial Diversity in Long-Term Contaminated Soil. *Water Air Soil Pollut.* **2018**, *229*, 13. [CrossRef]
62. Navarro-Noya, Y.E.; Jan-Roblero, J.; González-Chávez, D.C.; Hernández-Gama, R.; Hernández-Rodríguez, C. Bacterial communities associated with the rhizosphere of pioneer plants (Bahia xylopoda and Viguiera linearis) growing on heavy metals-contaminated soils. *Antonie Van Leeuwenhoek* **2010**, *97*, 335–349. [CrossRef]
63. Pereira, L.B.; Vicentini, R.; Ottoboni, L.M.M. Changes in the bacterial community of soil from a neutral mine drainage channel. *PLoS ONE* **2014**, *9*, e96605. [CrossRef] [PubMed]
64. DeBruyn, J.M.; Nixon, L.T.; Fawaz, M.N.; Johnson, A.M.; Radosevich, M. Global biogeography and quantitative seasonal dynamics of Gemmatimonadetes in soil. *Appl. Environ. Microbiol.* **2011**, *77*, 6295–6300. [CrossRef] [PubMed]
65. Kalam, S.; Basu, A.; Ahmad, I.; Sayyed, R.Z.; El-Enshasy, H.A.; Dailin, D.J.; Suriani, N.L. Recent Understanding of Soil Acidobacteria and Their Ecological Significance: A Critical Review. *Front. Microbiol.* **2020**, *11*, 580024. [CrossRef] [PubMed]
66. Kielak, A.M.; Barreto, C.C.; Kowalchuk, G.A.; van Veen, J.A.; Kuramae, E.E. The ecology of Acidobacteria: Moving beyond genes and genomes. *Front. Microbiol.* **2016**, *7*, 744. [CrossRef] [PubMed]
67. Hartman, W.H.; Richardson, C.J.; Vilgalys, R.; Bruland, G.L. Environmental and anthropogenic controls over bacterial communities in wetland soils. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 17842–17847. [CrossRef] [PubMed]
68. Jones, R.T.; Robeson, M.S.; Lauber, C.L.; Hamady, M.; Knight, R.; Fierer, N. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. *ISME J.* **2009**, *3*, 442–453. [CrossRef]
69. Gołebiewski, M.; Deja-Sikora, E.; Cichosz, M.; Tretyn, A.; Wróbel, B. 16S rDNA pyrosequencing analysis of bacterial community in heavy metals polluted soils. *Microb. Ecol.* **2014**, *67*, 635–647. [CrossRef] [PubMed]
70. Tebo, B.M.; Davis, R.E.; Anitori, R.P.; Connell, L.B.; Schiffman, P.; Staudigel, H. Microbial communities in dark oligotrophic volcanic ice cave ecosystems of Mt. Erebus, Antarctica. *Front. Microbiol.* **2015**, *6*, 179. [CrossRef] [PubMed]
71. Miao, Y.; Liao, R.; Zhang, X.X.; Wang, Y.; Wang, Z.; Shi, P.; Liu, B.; Li, A. Metagenomic insights into Cr(VI) effect on microbial communities and functional genes of an expanded granular sludge bed reactor treating high-nitrate wastewater. *Water Res.* **2015**, *76*, 43–52. [CrossRef]
72. Liu, Y.; Gao, T.; Wang, X.; Fu, J.; Zuo, M.; Yang, Y.; Yin, Z.; Wang, Z.; Tai, X.; Chang, G. Effects of heavy metals on bacterial community surrounding Bijiashan mining area located in northwest China. *Open Life Sci.* **2022**, *17*, 40–54. [CrossRef] [PubMed]
73. Tseng, S.C.; Liang, C.M.; Chia, T.; Ton, S.S. Changes in the composition of the soil bacterial community in heavy metal-contaminated farmland. *Int. J. Environ. Res. Public Health* **2021**, *18*, 8661. [CrossRef] [PubMed]
74. Xie, Y.; Fan, J.; Zhu, W.; Amombo, E.; Lou, Y.; Chen, L.; Fu, J. Effect of heavy metals pollution on soil microbial diversity and bermudagrass genetic variation. *Front. Plant Sci.* **2016**, *7*, 755. [CrossRef] [PubMed]
75. Mawang, C.I.; Azman, A.S.; Fuad, A.S.M.; Ahamad, M. Actinobacteria: An eco-friendly and promising technology for the bioaugmentation of contaminants. *Biotechnol. Rep.* **2021**, *32*, e00679. [CrossRef] [PubMed]
76. Alvarez, A.; Saez, J.M.; Davila Costa, J.S.; Colin, V.L.; Fuentes, M.S.; Cuozzo, S.A.; Benimeli, C.S.; Polti, M.A.; Amoroso, M.J. Actinobacteria: Current research and perspectives for bioremediation of pesticides and heavy metals. *Chemosphere* **2017**, *166*, 41–62. [CrossRef] [PubMed]
77. Remenár, M.; Kamlárova, A.; Harichova, J.; Zámocky, M.; Ferianc, P. The heavy-metal resistance determinant of newly isolated bacterium from a nickel-contaminated soil in Southwest Slovakia. *Pol. J. Microbiol.* **2018**, *67*, 191–201. [CrossRef] [PubMed]
78. Harichová, J.; Karelová, E.; Pangallo, D.; Ferianc, P. Structure analysis of bacterial community and their heavy-metal resistance determinants in the heavy-metal-contaminated soil sample. *Biology* **2012**, *67*, 1038–1048. [CrossRef]
79. Bruins, M.R.; Kapil, S.; Oehme, F.W. Microbial resistance to metals in the environment. *Ecotoxicol. Environ. Saf.* **2000**, *45*, 198–207. [CrossRef]
80. Nies, D.H. The biological chemistry of the transition metal transportome of: Cupriavidus metallidurans. *Metallomics* **2016**, *8*, 481–507. [CrossRef]

81. Guo, X.; Xie, C.; Wang, L.; Li, Q.; Wang, Y. Biodegradation of persistent environmental pollutants by *Arthrobacter* sp. *Environ. Sci. Pollut. Res.* **2019**, *26*, 8429–8443. [CrossRef]
82. Han, J.I.; Choi, H.K.; Lee, S.W.; Orwin, P.M.; Kim, J.; LaRoe, S.L.; Kim, T.G.; O'Neil, J.; Leadbetter, J.R.; Lee, S.Y.; et al. Complete genome sequence of the metabolically versatile plant growth-promoting endophyte Variovorax paradoxus S110. *J. Bacteriol.* **2011**, *193*, 1183–1190. [CrossRef]
83. Hu, N.; Zhao, B. Key genes involved in heavy-metal resistance in Pseudomonas putida CD2. *FEMS Microbiol. Lett.* **2007**, *267*, 17–22. [CrossRef] [PubMed]