

Article

Energetic Value of *Elymus elongatus* L. and *Zea mays* L. Grown on Soil Polluted with Ni²⁺, Co²⁺, Cd²⁺, and Sensitivity of Rhizospheric Bacteria to Heavy Metals

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Abstract: Plants, and microorganisms associated with them, offer an effective tool for removing pollutants, such as heavy metals, from the soil environment. The aim of this study was to determine changes caused by Ni²⁺, Co²⁺, and Cd²⁺ in the genetic diversity of soil-populating bacteria and the effect these heavy metals on the heating value of elongated coach grass (*Elymus elongatus* L.) and maize (*Zea mays* L.). Microorganisms support plants in removing heavy metals from soil. These plants can then be used for energetic purposes. The study aim was accomplished by determining counts of microorganisms and their resistance (RS) to Ni²⁺, Co²⁺, Cd²⁺, their colony development index (CD), ecophysiological diversity index (EP), and diversity established with the next generation sequencing (NGS) method. Further analyses aimed to establish test plants resistance to pollution with heavy metals and their heating value. Organotrophic bacteria turned out to be the most resistant to Co²⁺, whereas actinobacteria—to Cd²⁺ effects. At all taxonomic levels, the genetic diversity of bacteria was most adversely influenced by Cd²⁺ in the soil sown with *Zea mays* L. Bacteria belonging to *Arthrobacter*, *Rhodoplanes*, *Kaistobacter*, *Devosia*, *Phycococcus*, and *Thermomonas* genera showed high tolerance to soil pollution with Ni²⁺, Co²⁺, and Cd²⁺, hence they should be perceived as potential sources of microorganisms useful for bioaugmentation of soils polluted with these heavy metals. Ni²⁺, Co²⁺, and Cd²⁺ had no effect on the heating value of *Elymus elongatus* L. and *Zea mays* L. The heating value of 1 kg of air-dry biomass of the tested plants was relatively high and ranged from 14.6 to 15.1 MJ. *Elymus elongatus* L. proved more useful in phytoremediation than *Zea mays* L.



Citation: Boros-Lajszner, E.; Wyszowska, J.; Borowik, A.; Kucharski, J. Energetic Value of *Elymus elongatus* L. and *Zea mays* L. Grown on Soil Polluted with Ni²⁺, Co²⁺, Cd²⁺, and Sensitivity of Rhizospheric Bacteria to Heavy Metals. *Energies* **2021**, *14*, 4903. <https://doi.org/10.3390/en14164903>

Academic Editor: Hwai Chyuan Ong

Received: 5 July 2021

Accepted: 9 August 2021

Published: 11 August 2021

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Keywords: soil pollution; heavy metals; bacteria; energy crops

1. Introduction

Microbiological diversity of the soil environment is essential because species abundance ensures ecological stability of ecosystems and adaptation to harsh environmental conditions [1,2]. The development of specialized microorganisms promotes plant growth and development in soils contaminated with, e.g., heavy metals [3,4]. The cooperation between rhizospheric microorganisms and plants is highly beneficial as it mitigates toxic effects of heavy metals in soil and controls their penetration into and accumulation in cells of microorganisms and plants [5–7]. The appropriate choice of plant species for remediation of soils, particularly these contaminated with heavy metals, determines faster restoration of land functionality [8]. Such plants feature a natural ability to tolerate high pollutant loads in the soil, rapid growth, high biomass yield, ability to form compact plant cover, dense bundle root system, low nutrient requirement, and adaptation to local climatic conditions [9–12]. The above reasons were drivers of elongated coach grass (*Elymus elongatus* L.) and maize (*Zea mays* L.) choice of this research.

Elongated coach grass (*Elymus elongatus*) can be found at altitudes ranging from 1000 to 4000 m above sea level on mountain slopes and river banks in south-eastern Europe and

Asia [13–16]. Unlike couch grass (*Elymus repens*), it does not spread uncontrolled because it has no runners. Due to low soil and climatic requirements, it can grow on light, weaker, dry, and saline soils, while it does not tolerate wetlands and peat soils [17–19]. It is resistant to frost (up to $-20\text{ }^{\circ}\text{C}$ without snow cover), drought, and high summer temperatures. It starts growing early in the spring and can persist in one place for 7 to 10 and even 12 years. Elongated couch grass is not an invasive species, it does not spread, and poses no threat to neighboring soils. A deep root system allows its plants to perfectly counteract draught [20]. Its aerial parts are a viable substrate for biogas production or a raw material in the cellulose-paper industry. It can also be used to sow sedimentation plots in wastewater treatment plants and protective belts, and to remediate soils contaminated with, heavy metals. After 4–7 days, its biomass cut into swaths in September has not more than 12–20% of water, hence is suitable for baling, pressing, briquetting, and pelleting. It can be intended for energy purposes, as its heating value reaches $18\text{--}24\text{ MJ kg}^{-1}$, approximating values typical of certain tree species (willow—*Salix* L.; pine—*Pinus sylvestris* L.; alder—*Alnus* Mill.) and brown coal [21,22].

The second of the tested plants—maize (*Zea mays* L.)—is an annual cereal crop native to Mexico. Likewise, *Elymus elongatus* L. Its yield depends on soil fertility, water availability, and soil acidification (pH) [23]. It shows high tolerance to the site it grows in, and can be grown in the crop succession system. Its high yielding potential, reaching 12–15 Mg dry matter of whole plants per 1 ha, has spurred a growing interest in its use not only for generating biogas (fresh matter, silage) or bioethanol (grain) but also for direct combustion [24,25]. A high heating value of maize biomass (17.2 in the case of grain, 16.2 in the case of glumelles, and 15.5 MJ kg^{-1} in the case of straw) and ecological concerns speak for its application to produce heat energy [26–28].

Plants absorb heavy metals from soil with their root system and transfer them to their aerial parts, thereby enabling pollutants removal from the soil [12,29–31]. The above findings show that plants are able to absorb and neutralize chemically-active contaminants. This holds true especially for the pollution-tolerant annual crops, yielding high annual biomass that can further be used for energetic purposes [32–35]. Other advantages of heavy metal neutralization include expected economic profits and restoring the natural condition of soil. This process is feasible not only with plants tolerant to pollutants but also with those showing high capability of their transport to aerial parts—e.g., *Zea mays* L., *Brasica napus*, *Elymus elongatus* L., or *Helianthus* L. [22,36–38]. Plants and their accompanying microorganisms represent an effective tool for the treatment of contaminated environment [39]. Bacterial biodiversity is a complex phenomenon resulting from the competition between microorganisms. Certain species can inhibit the development of other species but may also cooperate with other higher organisms—plants [1]. Considering the latter case, microorganisms can not only stimulate plant growth and development and prevent diseases, but also increase plant resistance to stress induced by abiotic factors, including—e.g., heavy metals [3]. Also, various microbial consortia can adapt to specified environmental conditions, thereby representing a vast reservoir of genetic information concerning hosts colonizing environments exposed to various biotic and abiotic stress factors. This makes microorganisms able to easier adapt to stress conditions and trigger changes in biodiversity [40].

Considering the energetic potential of *Elymus elongatus* L. and *Zea mays* L., a study was undertaken to determine the usability of the heating value of plants growing on soil polluted with Ni^{2+} , Co^{2+} , and Cd^{2+} , and to establish changes caused by these heavy metals in microbial diversity in the polluted soil.

2. Materials and Methods

2.1. Soil Material Characteristics

The study was conducted with sandy loam collected from a depth of 0 to 20 cm from an arable field located in Tomaszkowo village near Olsztyn (north-eastern Poland; $53.7196\text{ }^{\circ}\text{N}$, $20.3969\text{ }^{\circ}\text{E}$). According to the FAO system [41], the soil was classified as Eutric

Cambisol. Its fraction composition was as follows: 69.41% of sand, 27.71% of silt, and 2.88% of loam. It contained (per 1 kg d.m.): 0.11 g of total nitrogen (N_{total}), 6.90 g of organic carbon (C_{org}), 7.50 mmol⁽⁺⁾ of exchangeable hydrogen ions (HAC), and 31 mmol⁽⁺⁾ of exchangeable base cations. Its exchangeable capacity (CEC) was at 38.50 mmol⁽⁺⁾ kg⁻¹, saturation with base cations (BS)—at 80.52%, and its pH in 1 mol KCl dm⁻³ was 7.0.

2.2. Experimental Design

A pot experiment was established in a greenhouse belonging to the University of Warmia and Mazury in Olsztyn (Poland) in four replications. The experiment was performed in a completely randomized design. The tested factors were: (1) type of contamination: control soil not contaminated with heavy metals, soil contaminated with: Ni²⁺, Co²⁺, Cd²⁺; (2) cultivated plant species: *Elymus elongatus* L. and *Zea mays* L. Firstly, the collected soil was passed through a sieve with a mesh size of 1 cm. Then, 3.5 kg of air-dry soil matter was weighed and contaminated with single doses of nickel, cobalt, and cadmium chlorides reaching Ni²⁺—400 mg kg⁻¹, Co²⁺—80 mg kg⁻¹, and Cd²⁺—8 mg kg⁻¹. The level of contamination was adopted on the basis of the obligatory qualifications of the Minister of the Environment in Poland [42]. Regulation of the Minister of the Environment [42] contains heavy metals in soils recognized as non-polluted agricultural soils, on which plants intended for fodder and food can be cultivated without any health consequences. In the research, the content of heavy metals was assumed as contamination four times higher than the limit content considered to be non-contaminated agricultural soil. The choice of the level of pollution was a consequence of our previous research and the state of point pollution in Poland [43–46] and in other European Union Member States [47,48]. Such a research model may contribute to the recognition of the response of all organisms to soil contamination with heavy metals. It can also contribute to the selection of an effective soil remediation method. Non-polluted soil served as the control. In addition, macrolelements (nitrogen, phosphorus, potassium, and magnesium) were applied into the soil in doses adjusted to the nutritional requirements of the test plants. Soil samples were thoroughly homogenized, placed in plastic pots (7.5 dm³), and hydrated to the capillary water capacity of 50%. Experimental conditions were continuously monitored. The average ambient temperature was 16.5 °C, air humidity reached 77.5% and daytime length ranged from 14 h 4 min to 16 h 30 min. After two days since the soil has been placed in pots, 24 seeds of *Elymus elongatus* L. were sown in half of the pots (16 pots) and 12 seeds of *Zea mays* L. in the other 16 pots. The emerged seedlings were thinned and 10 plants of *Elymus elongatus* L. as well as 5 plants of *Zea mays* L. were left in the pots. The first mentioned plants were harvested at the 31 stage of the Biologische Bundesanstalt Bundessortenamt und Chemical Scale—the first knot stage (knot that is usually perceptible 2 cm above the ground level); whereas, the second ones—at the BBCH 39 stage—formation of successive nodes and internodes, followed by leaf development, including the flag leaf. The resistance of *Elymus elongatus* L. and *Zea mays* L. plants (RS index) to soil contamination with Ni²⁺, Co²⁺, and Cd²⁺ and their energetic capacity were determined after harvest. In turn, soil samples were analyzed for the population numbers of colonizing microorganisms, their resistance to Ni²⁺, Co²⁺, and Cd²⁺, and composition of their communities.

2.3. Resistance of *Elymus elongatus* L. and *Zea mays* L. to Heavy Metals and Analysis of Their Energetic Yield

Plant resistance (RS) to contamination with Ni²⁺, Co²⁺, and Cd²⁺ was computed from the Orwin and Wardle formula [49]

$$RS = 1 - \frac{2|D_0|}{(|C_0| + |D_0|)} \quad (1)$$

where:

RS—plant resistance to Ni²⁺, Co²⁺, and Cd²⁺

C₀—biomass yield in control soil (non-polluted)

D_0 —difference between biomass yield produced on non-polluted soil and soil polluted with heavy metals.

The heating value of *Elymus elongatus* L. and *Zea mays* L. was estimated with the combustion method in a C-2000 calorimeter (IKA WERKE, USA). The heat of combustion (Q) and the heating value was determined acc. to the Polish Standard PN-EN ISO 18125:2017-07 [50]. Calculations were made using the formula by Kopetz et al. [51]

$$Hv = \frac{Q(100 - MC)}{100} - Mc \cdot 0.0244 \quad (2)$$

where:

Hv—heating value of air-dry plant biomass (MJ kg⁻¹)

Q—heat of combustion of air-dry plant biomass

MC—biomass moisture content (%)

0.0244—correction coefficient for water vaporization enthalpy (MJ kg⁻¹ per 1% moisture content).

Calculations were also made for energy yield produced by plants grown on 1 kg of soil, using the formula

$$Y_{EP} = Hv \cdot Y \quad (3)$$

where:

Y_{EP} —plant energy yield (MJ kg⁻¹)

Hv—heating value of air-dry plant biomass (MJ kg⁻¹)

Y—biomass yield (kg of air-dry plant biomass kg⁻¹ soil).

2.4. Microbiological Analysis of Soil

The counts of organotrophic bacteria, actinobacteria, and fungi were determined with the serial dilution method. Soil samples (10 g) were placed in glass bottles containing a sterile isotonic saline solution (0.85% NaCl) and shaken for 30 min (130 rpm). Two serial dilutions (three replications) were placed onto sterile Petri dishes. Organotrophic bacteria were isolated using a culture medium with soil extract acc. to Bunt and Rovira [52], actinobacteria—using Parkinson et al. medium [53] with nystatin and actidione, and fungi—using Martin medium [54] with the addition of Bengal Rose and aureomycin. The microorganisms were grown at a temperature of 28 °C for 10 days. Their counts were determined by counting colonies that emerged each day over a 10-day period and expressed in colony forming units (cfu).

Microbial counts were used to compute:

- (1) The microbial colony development index (CD) acc. to Sarathchandra et al. [55]

$$CD = \left[\frac{N_1}{1} + \frac{N_2}{2} + \frac{N_3}{3} \dots \dots \frac{N_{10}}{10} \right] \cdot 100 \quad (4)$$

where: $N_1, N_2, N_3 \dots N_{10}$ —is the total number of microbial colonies identified in days 1, 2, 3, ... 10 divided by the total number of colonies identified throughout the experimental period;

- (2) The microbial ecophysiological diversity index (EP) acc. to De Leij et al. [56]

$$EP = -\sum(\pi_i \cdot \log \pi_i) \quad (5)$$

where: π_i —is the number of microbial colonies identified in a given day divided by the number of all grown colonies;

- (3) The resistance (RS) of soil microorganisms to pollution with Ni²⁺, Co²⁺, and Cd²⁺ acc. to the formula described by Orwin and Wardle [49]

$$RS = 1 - \frac{2|D_o|}{(|C_o| + |D_o|)} \quad (6)$$

where: RS—resistance of microorganisms to Ni^{2+} , Co^{2+} , and Cd^{2+} ; C_0 —microbial count in the control soil (non-polluted); and D_0 —difference between microbial counts in the non-polluted soil and the soil polluted with heavy metals;

- (4) The influence of heavy metals (IF_{Hm}) on counts of soil microorganisms acc. to the following formula

$$\text{IF}_{\text{Hm}} = \frac{A_{\text{Hm}}}{A_0} - 1 \quad (7)$$

where: IF_{Hm} —heavy metal pollution index, A_{Hm} —microbial counts in the soil polluted with heavy metals, and A_0 —microbial counts in the non-polluted soil.

2.5. Metagenomic Soil Analysis

Genomic DNA of bacteria was extracted from 1 g of soil using the Genomic Mini AX Bacteria+™ kit, following producer's instructions. The metagenomic analysis was performed by the Genomed S.A. company (Warsaw, Poland) with the next generation sequencing (NGS) method using an MiSeq Reporter (MSR) v2.6 sequencer (Illumina v2). The 16S gene rRNA sequence was amplified with specific sequences of 341F and 785R primers. The metagenomic analysis of the gene encoding 16S rRNA was carried out based on the hypervariable region V3–V4. The bioinformatic analysis of results obtained was performed with the QIIME package based on GreenGenes v13_8 database compared to standard sequences.

2.6. Statistical Analysis

Data were processed statically using the Statistica 13.1 package [57]. Results were compared with ANOVA and post hoc Tukey test (HSD—Tukey's honest significant difference test). Homogenous groups were identified at a significance level of $p = 0.05$. The analyzed data had normal distribution and similar variance. Metagenomic profiles were analyzing with STAMP 2.1.3. software [58]. The principal component analysis (PCA) was conducted for the most abundant bacterial phyla and genera with the multivariate technique. The relative abundance of bacterial classes and orders prevailing in the soils samples with a difference between proportions at $\geq 1\%$ was presented using RStudio v1.2.5033 software [59], R project, and gplots v3.6.2 software used to generate a heat map [60].

3. Results

3.1. Sensitivity of Test Plants to Toxic Effects of Ni^{2+} , Co^{2+} , and Cd^{2+} and Their Energetic Value

Figure 1 presents results of determinations of *Elymus elongatus* L. and *Zea mays* L. resistance (RS) to soil pollution with Ni^{2+} , Co^{2+} , and Cd^{2+} . The RS values can range from 0 to 1, with 0 denoting a complete lack of resistance and 1 indicating complete plant resistance. In the case of aerial parts, both *Elymus elongatus* L. and *Zea mays* L. were the most resistant to soil pollution with Cd^{2+} , as indicated by RS values of 0.840 and 0.570, respectively. In turn, both tested plants were the least resistant to the toxic effect of Ni^{2+} , with RS values reaching 0.431 for *Elymus elongatus* L. and 0.258 for *Zea mays* L. Intermediate plant RS values were determined after soil contamination with Co^{2+} . In the case of roots, similar plant responses to the effects of heavy metals were noted for *Zea mays* L., whereas roots of *Elymus elongatus* L. were the most sensitive to Co^{2+} (RS = 0.255), and the most resistant to Cd^{2+} (RS = 0.478).

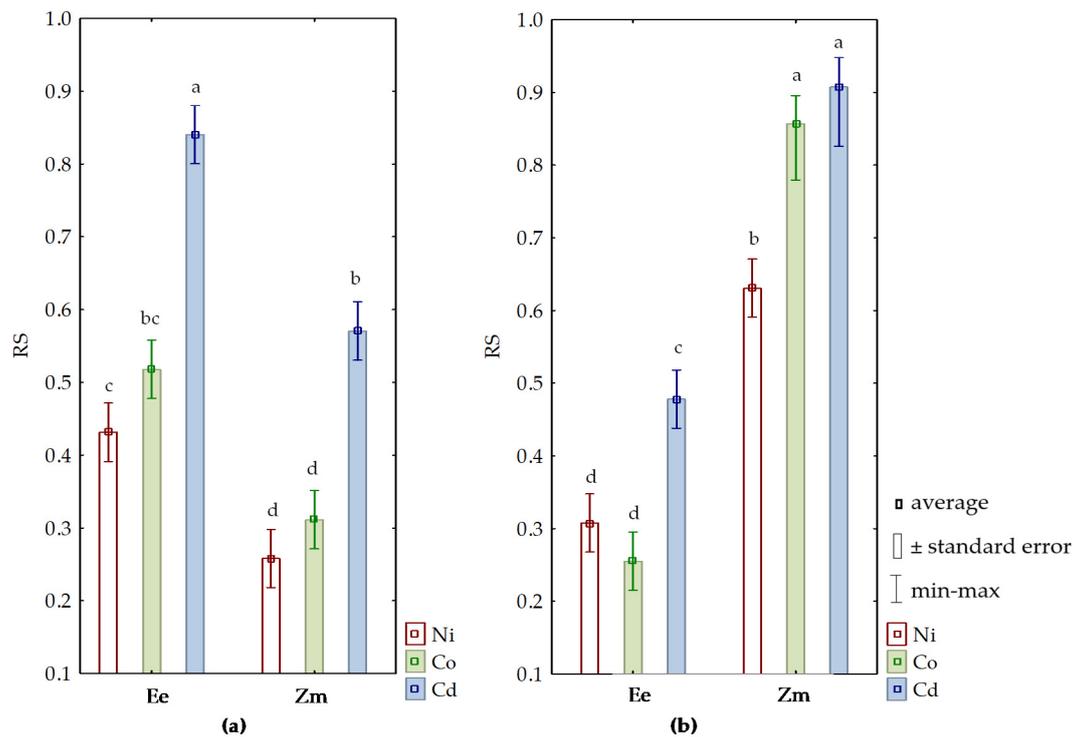


Figure 1. Resistance (RS) of *Elymus elongatus* L. and *Zea mays* L. to heavy metals. (a)—above-ground parts; (b)—roots; Ee—*Elymus elongatus* L.; Zm—*Zea mays* L.; Ni—soil contaminated with Ni²⁺; Co—soil contaminated with Co²⁺; Cd—soil contaminated with Cd²⁺. For each group of microorganisms, the same letters (a–d) are assigned to the same homogeneous groups. The same letters for above-ground parts and roots indicate no statistically significant differences.

The heat of combustion and the heating value of *Elymus elongatus* L. and *Zea mays* L. were at similar levels and ranged from 18.497 to 19.087 MJ kg⁻¹ air-dry matter, and from 14.604 to 15.052 MJ kg⁻¹ air-dry matter, respectively (Table 1). Although energy produced from 1 kg of soil sown with *Zea mays* L. was higher than that produced from soil sown with *Elymus elongatus* L., which was due to the biomass yield generated, the relative decrease in energy production caused by the toxic effects of heavy metals was significantly greater for *Zea mays* L. than for *Elymus elongatus* L.

Table 1. Heat of combustion and heating value of *Elymus elongatus* L. and *Zea mays* L. grown on the soil polluted with heavy metals.

| Heavy Metals | Energy Production (Q) | Heating Value (Hv) | Plant Energy Yield (Y _{EP}) |
|----------------------------|---|-----------------------------|---------------------------------------|
| | MJ kg ⁻¹ Air-Dry Matter Plants | | MJ kg ⁻¹ |
| <i>Elymus elongatus</i> L. | | | |
| C | 19.087 ^a ± 0.201 | 15.052 ^a ± 0.201 | 0.101 ^{cd} ± 0.020 |
| Ni ²⁺ | 18.836 ^{ab} ± 0.200 | 14.604 ^a ± 0.200 | 0.059 ^d ± 0.010 |
| Co ²⁺ | 18.889 ^{ab} ± 0.201 | 14.891 ^a ± 0.200 | 0.068 ^d ± 0.010 |
| Cd ²⁺ | 18.789 ^{ab} ± 0.201 | 14.810 ^a ± 0.200 | 0.091 ^{cd} ± 0.020 |
| <i>Zea mays</i> L. | | | |
| C | 18.351 ^b ± 0.201 | 14.791 ^a ± 0.200 | 0.265 ^a ± 0.030 |
| Ni ²⁺ | 18.546 ^{ab} ± 0.201 | 14.953 ^a ± 0.200 | 0.110 ^{cd} ± 0.020 |
| Co ²⁺ | 18.497 ^b ± 0.201 | 14.913 ^a ± 0.200 | 0.127 ^c ± 0.020 |
| Cd ²⁺ | 18.562 ^{ab} ± 0.201 | 14.967 ^a ± 0.200 | 0.194 ^b ± 0.030 |

Identical letters (a–d) in columns denote the same homogeneous groups. ±—standard error.

Energy production by *Elymus elongatus* L. grown on the soil polluted with Ni^{2+} was 41.5% lower and that produced by *Zea mays* L. was 58.5% lower than energy production by these plants grown on the non-polluted soil. As a result of soil pollution with Co^{2+} , energy production dropped by 32.7% and 52.1%, whereas after soil pollution with Cd^{2+} —by 9.9% and 26.8%, respectively.

3.2. Sensitivity of Soil Microorganisms to Toxic Effects of Ni^{2+} , Co^{2+} , and Cd^{2+}

The counts of microorganisms were affected by both test plant species and pollutant type (see Supplementary Materials: Table S1). The control soil (not contaminated with heavy metals) sown with *Elymus elongatus* L. was significantly more populated by organotrophic bacteria and significantly less populated by fungi than the control soil sown with *Zea mays* L. In turn, counts of actinobacteria were comparable in the soils sown with both test plant species. Ni^{2+} and Cd^{2+} ions significantly increased the count of organotrophic bacteria in the soils sown with both species of the test plants. All heavy metals analyzed increased the count of fungi in the soil sown with *Elymus elongatus* L., whereas actinobacteria proliferation was promoted only by Ni^{2+} . In the soil sown with *Zea mays* L., Ni^{2+} , Co^{2+} , and Cd^{2+} inhibited the proliferation of actinobacteria and fungi. In turn, in the soil sown with *Elymus elongatus* L., they caused the greatest increase in fungi count (Figure 2). Values of the heavy metal pollution index (IF_{Hm}) ranged from 0.446 (Co^{2+}) to 0.835 (Cd^{2+}). In the case of organotrophic bacteria, they reached 0.262 and 0.186 for Ni^{2+} and Cd^{2+} in the soil sown with *Elymus elongatus* L. as well as 0.188 and 0.392 in the soil sown with *Zea mays* L. The most adverse effects of Ni^{2+} and Co^{2+} on actinobacteria and fungi were observed in the soil sown with *Zea mays* L., as indicated by negative IF_{Hm} values.

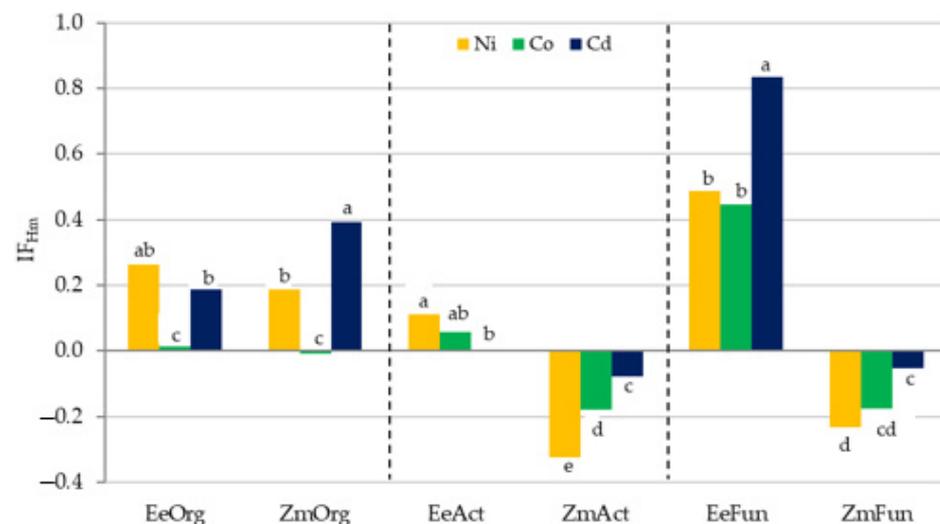


Figure 2. Effect of heavy metals (IF_{Hm}) on microbial counts in the soil sown with *Elymus elongatus* L. and *Zea mays* L. Ee—*Elymus elongatus* L.; Zm—*Zea mays* L.; Org—organotrophic bacteria; Act—actinobacteria; Fun—fungi. For each group of microorganisms, the same letters (a–e) are assigned to the same homogeneous groups. The same letters for each group of microorganisms indicate no statistically significant differences.

The resistance index (RS) represents a highly unbiased indicator of heavy metal effect on microorganisms (RS) (Figure 3). It provides information about the stability of microorganisms in the environment exposed to contamination. The RS values of 0.997 (soil exposed to Co^{2+} and sown with *Elymus elongatus* L.) and 0.983 (soil exposed to Co^{2+} and sown with *Zea mays* L.) indicate that organotrophic bacteria were highly resistant to the toxic effect of this heavy metal. Actinobacteria turned out to be highly resistant to all heavy metals tested; with their RS ranging from 0.802 (Ni^{2+}) to 0.997 (Cd^{2+}) in the soil sown with *Elymus elongatus* L. In the case of *Zea mays* L., high RS values were only noted for actinobacteria (0.854) and fungi (0.898) in the soil polluted with Cd^{2+} .

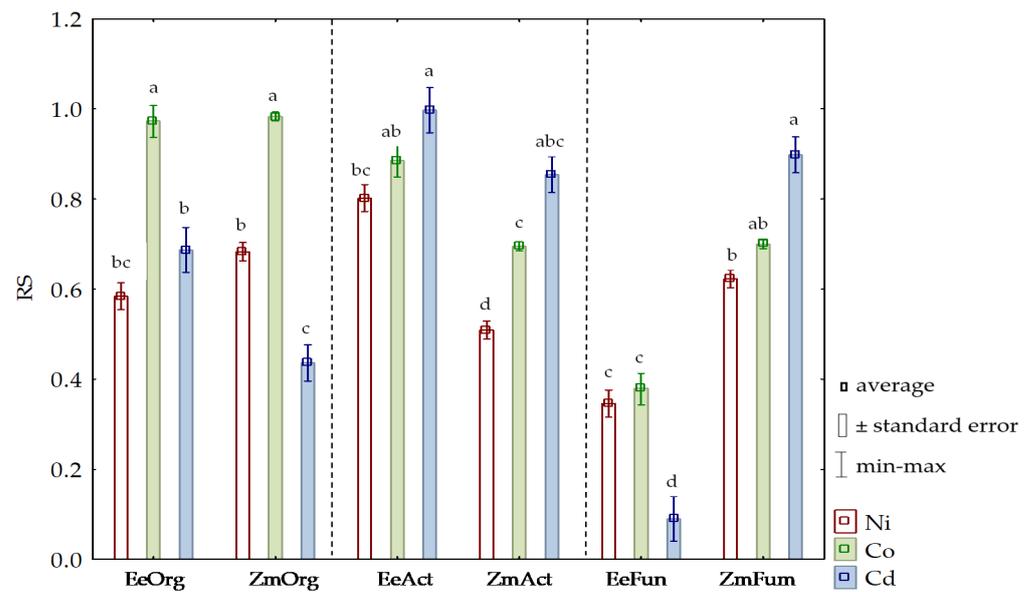


Figure 3. Resistance (RS) of microorganisms to heavy metals in the soil sown with *Elymus elongatus* L. and *Zea mays* L. Ee—*Elymus elongatus* L.; Zm—*Zea mays* L.; Org—organotrophic bacteria; Act—actinobacteria; Fun—fungi. For each group of microorganisms, the same letters (a–d) are assigned to the same homogeneous groups. The same letters for each group of microorganisms indicate no statistically significant differences.

Values of the ecophysiological diversity index (EP) of organotrophic bacteria and fungi populating the soil sown *Elymus elongatus* L. were higher compared to those of respective microorganisms colonizing the soil sown with *Zea mays* L. (Figure 4).

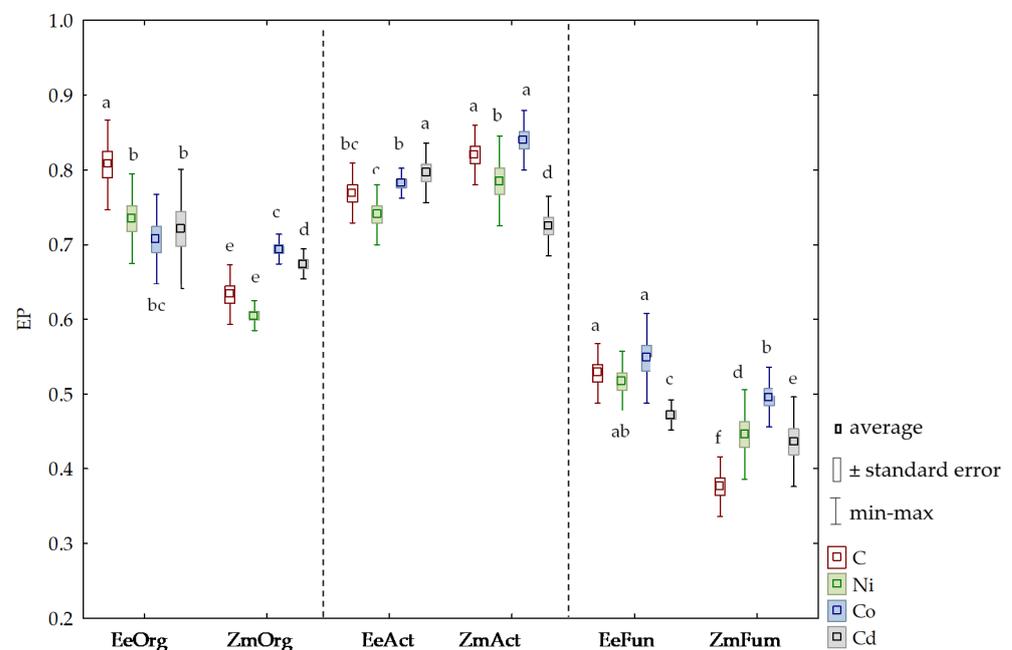


Figure 4. Microbial ecophysiological diversity index (EP) in the soil polluted with heavy metals. In general, high EP values were noted for actinobacteria and organotrophic bacteria, and lower ones for fungi. In the soil remediated with *Elymus elongatus* L., all heavy metals decreased EP of organotrophic bacteria, whereas Cd^{2+} additionally decreased EP of fungi. Cd^{2+} also adversely affected EP of actinobacteria in the soil sown with *Zea mays* L.

The heavy metals tested caused smaller changes in the microbial colony development index (CD) (Figure 5) than in EP values. In the polluted soil sown with *Elymus elongatus* L., the CD values ranged from 43.206 (Ni²⁺) to 51.798 (Co²⁺) for organotrophic bacteria, from 29.346 (Co²⁺) to 31.467 (Ni²⁺) for actinobacteria, and from 45.333 (Co²⁺) to 51.997 (Cd²⁺) for fungi. In the soil sown with *Zea mays* L., the CD values ranged from 54.754 (Co²⁺) to 57.565 (Ni²⁺) for organotrophs, from 24.251 (Co²⁺) to 29.520 (Cd²⁺) for actinobacteria, and from 50.259 (Co²⁺) to 54.625 (Cd²⁺) for fungi. In the pots sown with *Elymus elongatus* L., the CD of fungi and organotrophic bacteria increased upon heavy metal pollution, whereas that of actinobacteria decreased compared to the control non-polluted soil (Figure 5).

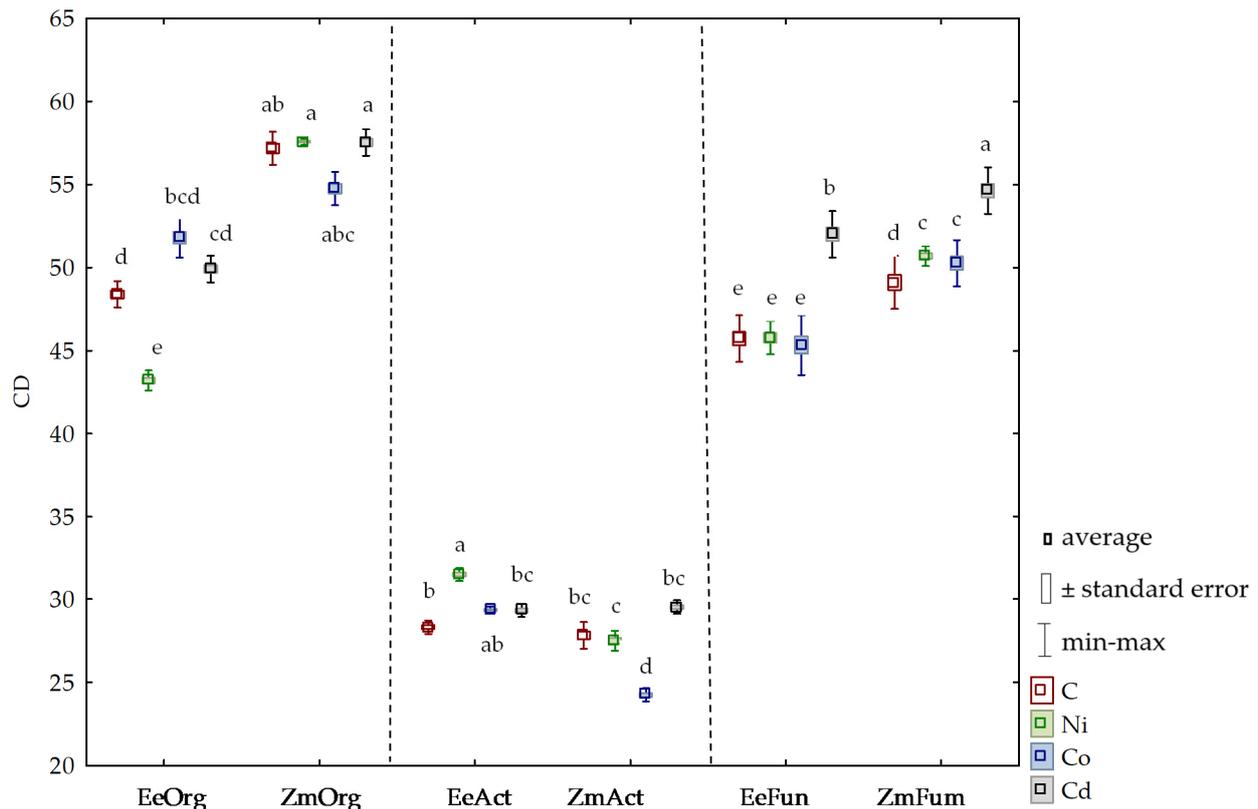


Figure 5. Microbial colony development index (CD) in the soil polluted with heavy metals and sown with *Elymus elongatus* L. and *Zea mays* L. Ee—*Elymus elongatus* L.; Zm—*Zea mays* L.; Org—organotrophic bacteria; Act—actinobacteria; Fun—fungi. For each group of microorganisms, the same letters (a–e) are assigned to the same homogeneous groups. The same letters for each group of microorganisms indicate no statistically significant differences.

The test plant species elicited a significant effect on bacteria diversity (Figure 6). In the soil not polluted with heavy metals, *Zea mays* L. had a more beneficial effect on the development of bacteria from the *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* phyla than *Elymus elongatus* L., which in turn stimulated development of *Acidobacteria*, *Planctomycetes*, *Chloroflexi*, *Firmicutes*, *Verrucomicrobia*, *Gemmatimonadetes*, and *Chlamydiae*. Soil pollution with Cd²⁺, Co²⁺, and Ni²⁺ significantly increased *Proteobacteria* count in the soil sown with *Elymus elongatus* L. compared to the soil sown with *Zea mays* L. The tested plants had an opposite effect on the abundance of *Actinobacteria* in the soils polluted with all heavy metals analyzed. Those two phyla prevailed in the tested soils, regardless of plant species grown and pollutant type.

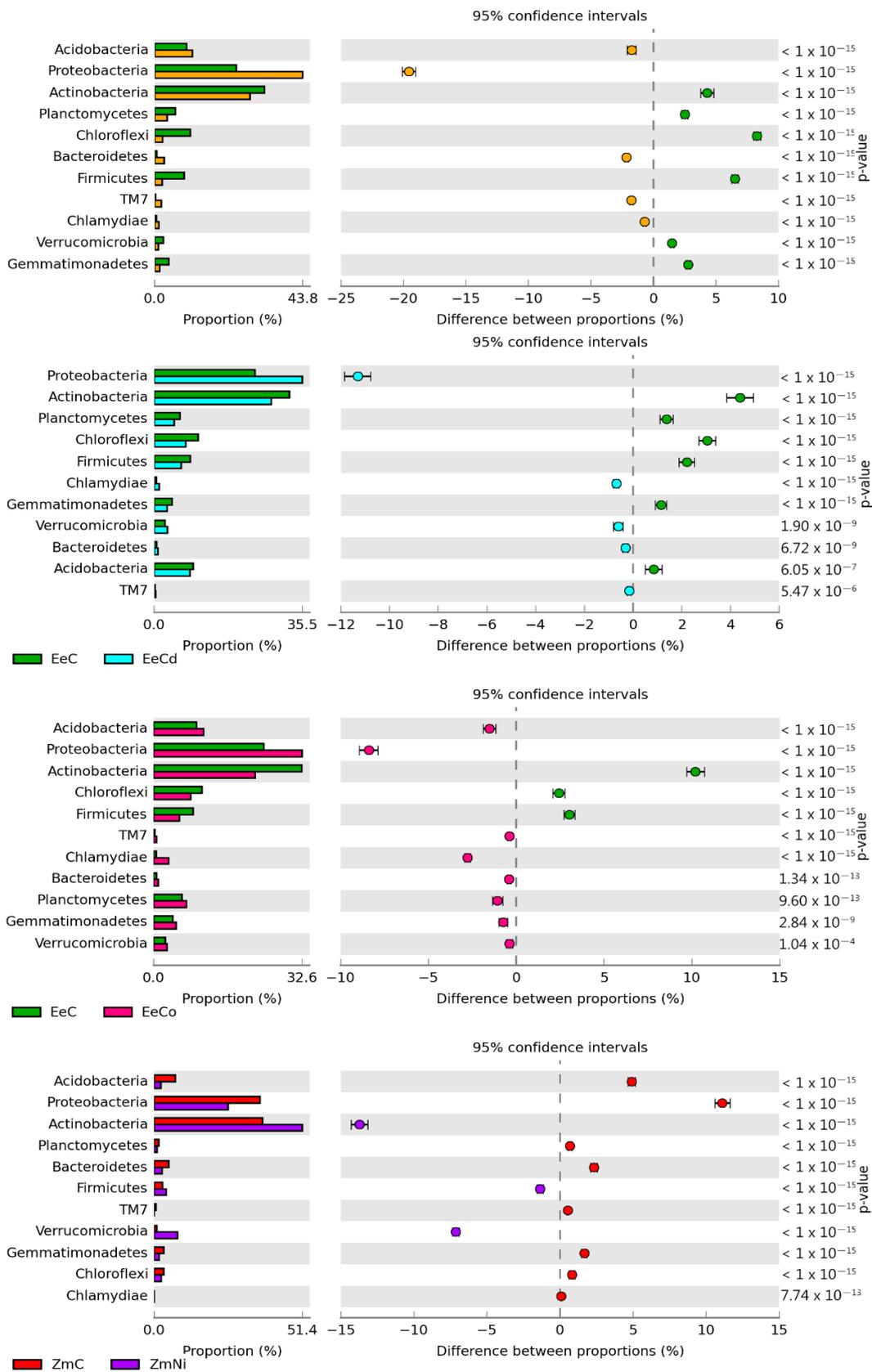


Figure 6. Cont.

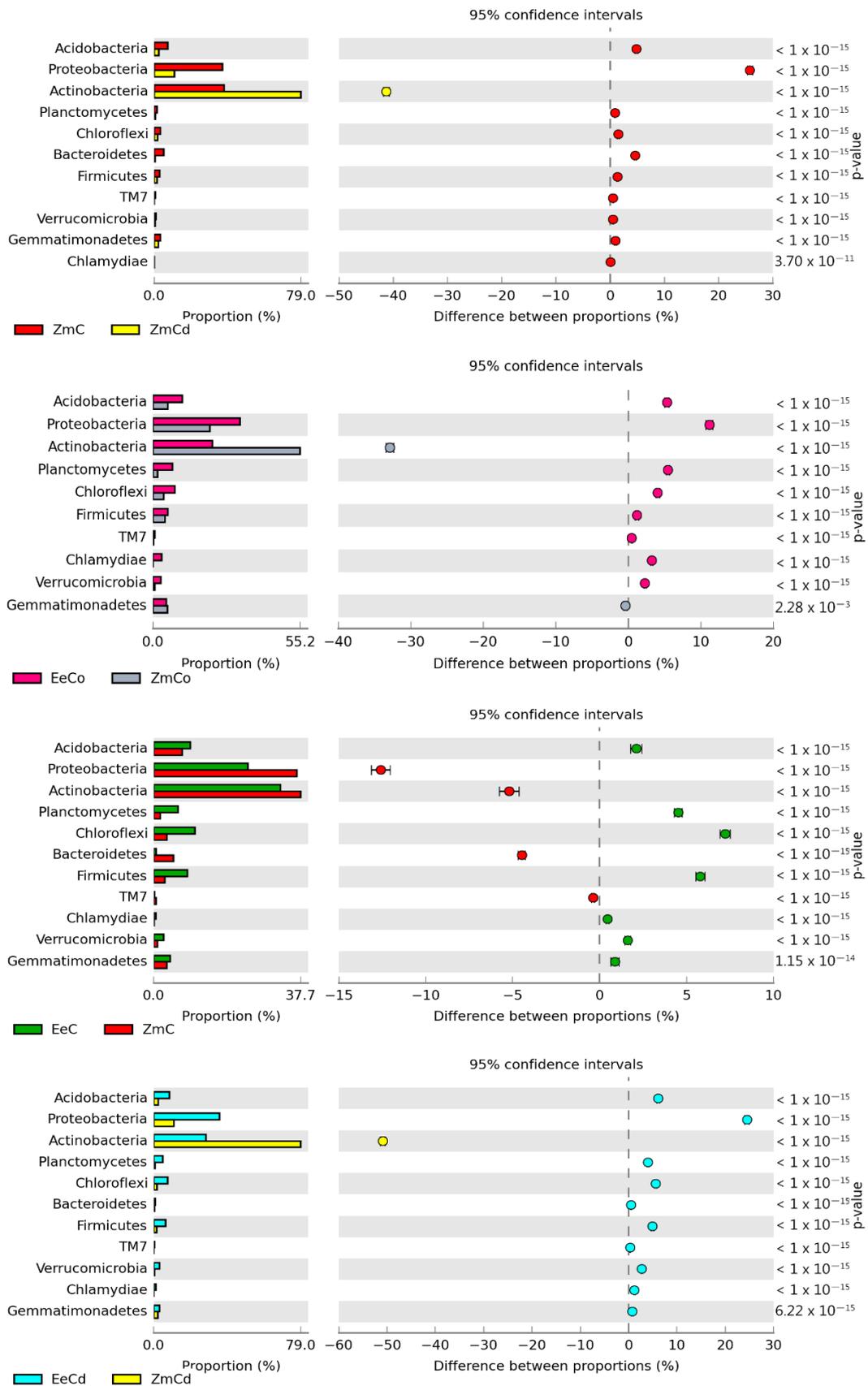


Figure 6. Cont.

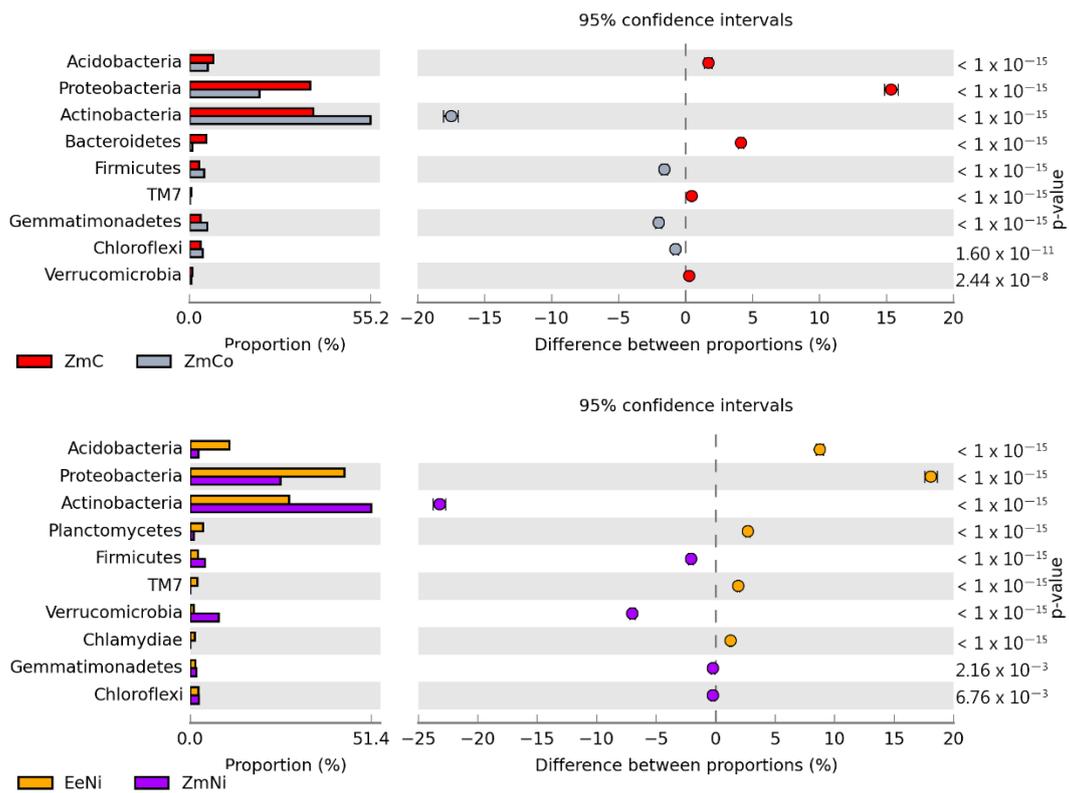


Figure 6. Relative abundance of bacterial phyla with OTU $\geq 1\%$ in the soil polluted with heavy metals. Ee—*Elymus elongatus* L.; Zm—*Zea mays* L.; Org—organotrophic bacteria; Act—actinobacteria; Fun—fungi. C—control soil; Ni—soil contaminated with Ni^{2+} ; Co—soil contaminated with Co^{2+} ; Cd—soil contaminated with Cd^{2+} .

The above observations were confirmed by results of the principal component analysis (PCA), in which the first two principal components explained 98.41% of the total variance of data (Figure 7). PCA proved very well in depicting beta diversity between soil treatments.

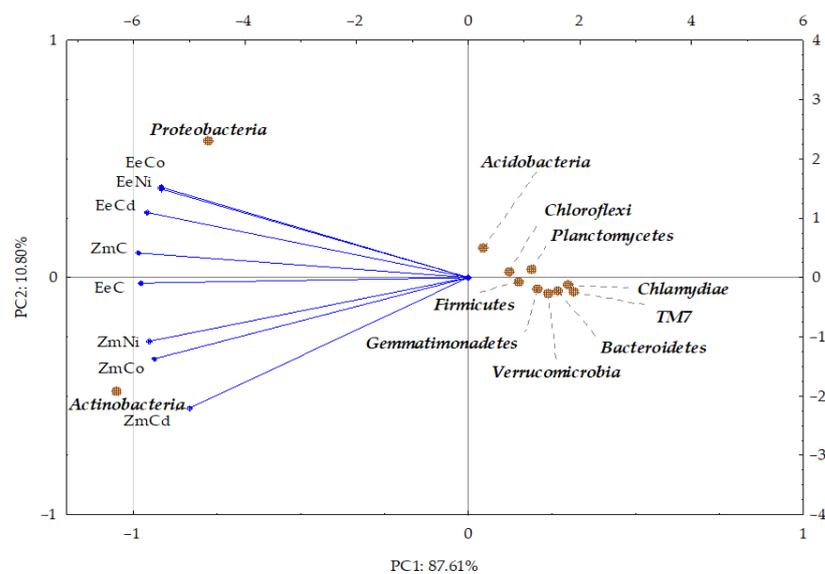


Figure 7. PCA of bacterial phyla colonizing the soil polluted with heavy metals. Ee—*Elymus elongatus* L.; Zm—*Zea mays* L.; Org—organotrophic bacteria; Act—actinobacteria; Fun—fungi. C—control soil; Ni—soil contaminated with Ni^{2+} ; Co—soil contaminated with Co^{2+} ; Cd—soil contaminated with Cd^{2+} .

It demonstrated prevalence of *Proteobacteria* in the soils polluted with Cd^{2+} , Co^{2+} , and Ni^{2+} and sown with *Elymus elongatus* L., and of *Actinobacteria* in the soil polluted with those heavy metals but sown with *Zea mays* L. The remaining phyla constituted a relatively homogenous group and were less abundant in the analyzed soils.

Significant differences in the soil microbiome were also observed at the class and order levels (Figure 8), as affected by the test plant species and soil pollution with Cd^{2+} , Co^{2+} , and Ni^{2+} . The class and order analysis of bacteria represented by at least 1% of total assigned sequences demonstrated the predominance of bacteria classified to the order *Actinomycetales* (c_Actinobacteria) and *Rhizobiales* (c_Alphaproteobacteria) in all soils tested. In turn, *Actinomycetales* bacterial predominated in the soil samples sown with *Zea mays* L. polluted with all heavy metals tested, whereas *Rhizobiales*—in the soil sown with *Elymus elongatus* L. and polluted with Cd^{2+} and in the unpolluted soil sown with *Zea mays* L. Attention should also be paid to the bacteria classified to orders *Bacillales* (c_Bacilli), *Acidimicrobiales* (c_Acidimicrobiia), iii1-15 (c_Acidobacteria-6) and *Gaiellales* (c_Thermoleophilia), *Xanthomonadales* (c_Gammaproteobacteria) and *Sphingomonadales* (c_Alphaproteobacteria), which formed two separate subclusters in the clusters of prevailing bacteria.

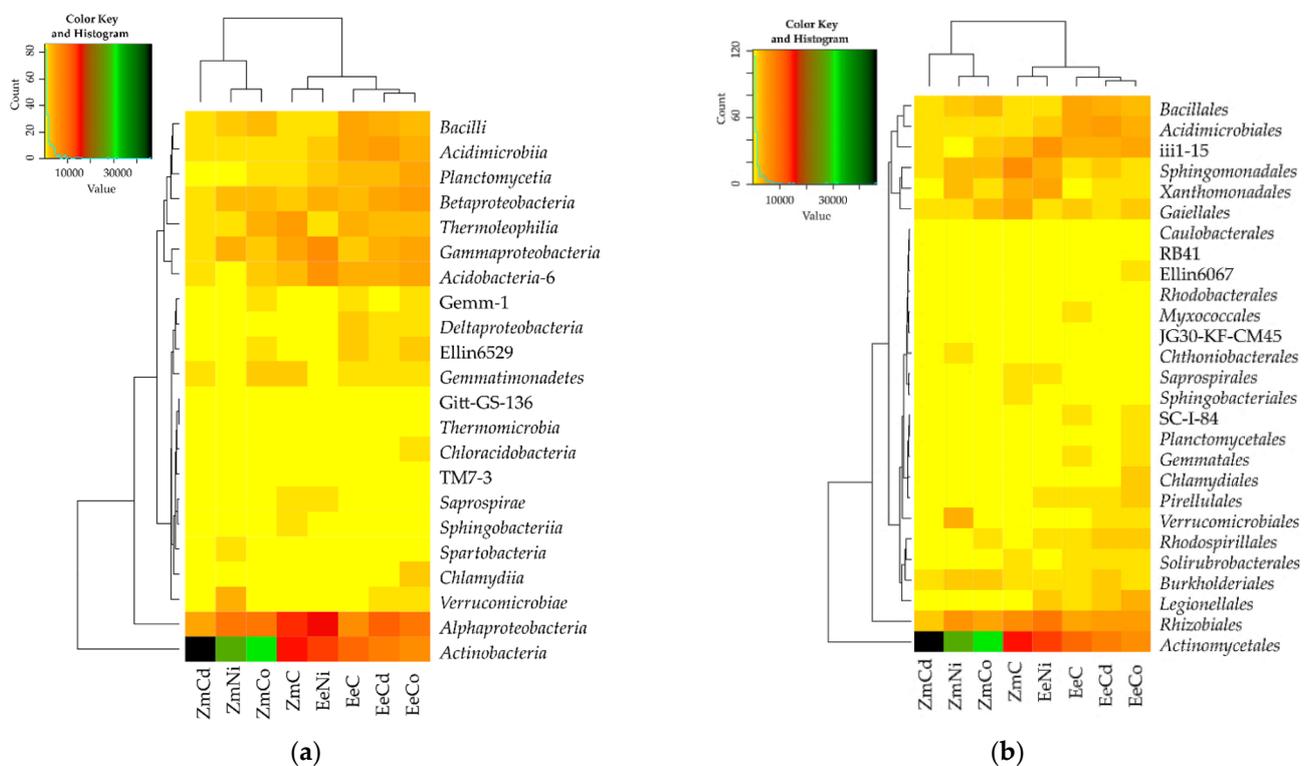


Figure 8. Relative abundance of classes (a) and orders (b) of bacteria with OTU $\geq 1\%$ in the soil polluted with heavy metals. Ee—*Elymus elongatus* L.; Zm—*Zea mays* L.; Org—organotrophic bacteria; Act—actinobacteria; Fun—fungi. C—control soil; Ni—soil contaminated with Ni^{2+} ; Co—soil contaminated with Co^{2+} ; Cd—soil contaminated with Cd^{2+} .

The Venne analysis enabled identifying 23 bacterial genera unique for individual soil samples (Figure 9). The unique bacterial genera identified in the non-polluted soil sown with *Zea mays* L. included *Arthrobacter*, *Sphingomonas*, *Lysobacter*, and *Sphingobium*, whereas those identified in the non-polluted soil sown with *Elymus elongatus* L. included *Luteolibacter*, *Methylibium*, *Iamia*, and *Pirellula*. In the soil polluted with Ni^{2+} and sown with *Zea mays* L., the unique genera included *Luteolibacter*, *Methylibium*, *Candidatus Xiphinematobacter*, and *Pseudoxanthomonas*, whereas in that sown with *Elymus elongatus* L., they included *Planctomyces*, *Aquicella*, *Iamia*, *Pirellula*, *Thermomonas*, *Rhodobacter*, and *Lysobacter*.

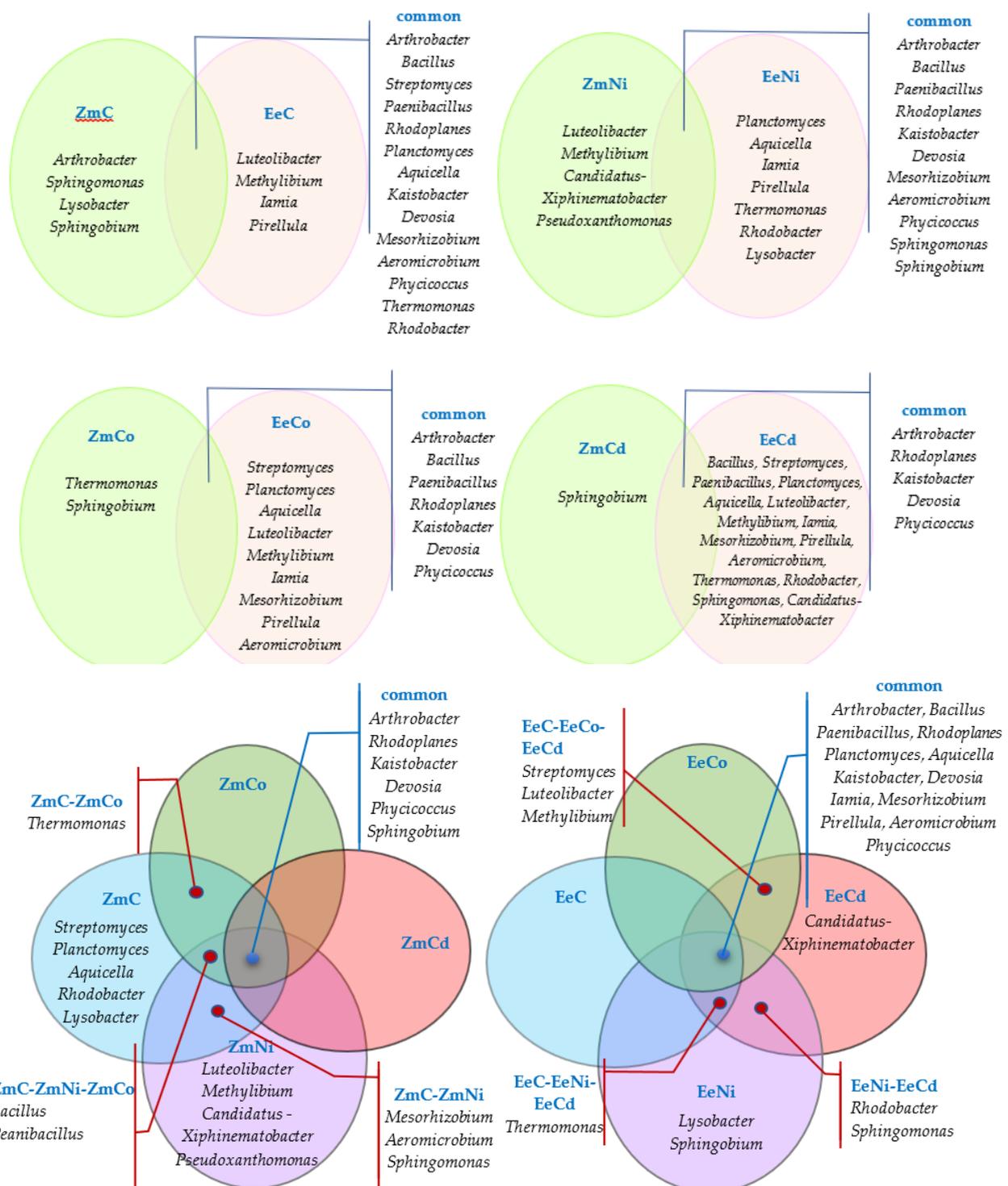


Figure 9. Unique and common bacterial genera in the soil polluted with heavy metals (Venn diagram). Ee—*Elymus elongatus* L.; Zm—*Zea mays* L.; Org—organotrophic bacteria; Act—actinobacteria; Fun—fungi. C—control soil; Ni—soil contaminated with Ni²⁺; Co—soil contaminated with Co²⁺. Cd—soil contaminated with Cd²⁺.

The soil polluted with Co²⁺ and sown with *Zea mays* L. was mainly populated by *Thermomonas* and *Sphingobium*, and that sown with *Elymus elongatus* L.—by *Streptomyces*, *Planctomyces*, *Aquicella*, *Luteolibacter*, *Methylibium*, *Iamia*, *Mesorhizobium*, *Pirellula*, and *Aeromicrobium*. In turn, *Sphingobium* was the unique genus found in the soil exposed to Cd²⁺ and sown with *Zea mays* L., whereas *Bacillus*, *Streptomyces*, *Paenibacillus*, *Planctomyces*, *Aquicella*, *Methylibium*, *Iamia*, *Mesorhizobium*, *Pirellula*, *Aeromicrobium*, *Thermomonas*, *Rhodobacter*, *Sph-*

ingomonas, *Candidatus Xiphinematobacter* were the unique genera found in the soil sown with *Elymus elongatus* L. When comparing soils unpolluted and polluted with heavy metals and sown with *Elymus elongatus* L., two bacterial genera were distinguished in the soil polluted with Ni²⁺ (*Lysobacter* and *Sphingomonas*) and one in the soil polluted with Cd²⁺ (*Candidatus Xiphinematobacter*). After soil sowing with *Zea mays* L., the *Streptomyces*, *Planctomyces*, *Aquicella*, *Rhodobacter*, and *Lysobacter* genera were found unique in the non-polluted soil, whereas *Luteibacter*, *Candidatus Xiphinematobacter*, *Mthylibium*, and *Pseudoxantomonas* in the soil polluted with Ni²⁺. Regardless of soil pollution with heavy metals and species of the cultivated plant, the common bacterial genera of all treatments included *Arthrobacter*, *Rhodoplanes*, *Kaistobacter*, *Devosia*, and *Phycococcus*. In turn, bacteria from the *Bacillus*, *Peaenibacillus*, *Planctomyces*, *Aquicella*, *Iamia*, *Mesorhizobium*, *Pirellula*, and *Aeromicrobium* genera represented the common microbiome of the soil sown with *Elymus elongatus* L. and polluted with Cd²⁺, Co²⁺, and Ni²⁺.

Recapitulating the role of heavy metals in amending the soil microbiome, it was found the bacterial diversity in the polluted soils was significantly affected by the test plant species (Table 2). At the phylum, class, and order levels, greater bacterial diversity was demonstrated in the soil sown with *Elymus elongatus* L. than with *Zea mays* L. Biodiversity of all taxa was diminished by heavy metals in the soil sown with *Zea mays* L., with the greatest adverse effect observed for Cd²⁺. In the soil sown with *Elymus elongatus* L., Ni²⁺ had a negative effect on bacterial diversity at the phylum, class, and order levels, whereas Co²⁺ at the family and genus levels. In turn, Cd²⁺ caused no major changes as it significantly increased bacteria diversity only at the order level.

Table 2. Bacterial diversity in the soil polluted with heavy metals, expressed with the value of Shannon–Wiener index (H').

| Taxon | Object | | | | | | | |
|--------|--------------------|-------------------|-------------------|--------------------|--------------------|--------------------|-------------------|-------------------|
| | EeC | EeNi | EeCo | EeCd | ZmC | ZmNi | ZnCo | ZnCd |
| Phylum | 2.02 ^b | 1.78 ^c | 2.16 ^a | 2.00 ^b | 1.67 ^{cd} | 1.54 ^d | 1.54 ^d | 0.90 ^e |
| Class | 3.08 ^b | 2.60 ^c | 3.18 ^a | 3.04 ^b | 2.51 ^c | 2.19 ^d | 2.24 ^d | 1.28 ^e |
| Order | 3.19 ^c | 3.00 ^d | 3.43 ^a | 3.35 ^b | 2.86 ^e | 2.39 ^f | 2.45 ^f | 1.38 ^g |
| Family | 3.29 ^a | 3.25 ^a | 3.11 ^b | 3.31 ^a | 3.26 ^a | 2.82 ^{cb} | 2.72 ^c | 1.84 ^d |
| Genus | 2.01 ^{bc} | 2.13 ^b | 1.86 ^c | 2.06 ^{bc} | 2.19 ^b | 2.37 ^a | 1.93 ^c | 1.55 ^d |

Ee—*Elymus elongatus* L.; Zm—*Zea mays* L.; Org—organotrophic bacteria; Act—actinobacteria; Fun—fungi. C—control soil; Ni—soil contaminated with Ni²⁺; Co—soil contaminated with Co²⁺; Cd—soil contaminated with Cd²⁺. The same letters (a–g) are assigned to the same homogeneous groups within individual taxa.

The genetic diversity of bacteria was only weakly correlated with parameters obtained with culture methods (Table 3). In the non-polluted soil, a significant correlation was demonstrated between H' index at the class level and bacterial count, bacterial CD, and EP of organotrophic bacteria. In the soil polluted with Ni²⁺, a significant negative correlation was found between ecophysiological diversity of organotrophic bacteria and H' at the phylum, class, and order levels. Soil pollution with Co²⁺ contributed to a positive correlation between actinobacteria count and H' index computed for class, order, and family. In the case of organotrophic bacteria, a significant but negative correlation was found between H' index at the order and family levels and the count of these microorganisms. The ecophysiological diversity of both organotrophic bacteria and actinobacteria was not positively correlated with H' index values calculated for all taxa. In the soil polluted with Cd²⁺, worthy of notice is the positive correlation between EP of actinobacteria and H' value and a negative correlation between counts of organotrophic bacteria and actinobacteria and H' value.

Table 3. Coefficient of correlation between bacterial count, colony development and ecophysiological diversity index, and the value of the Shannon–Wiener index (H') calculated for individual taxa, regardless of test plant species.

| Variables | Shannon–Wiener Index (H') | | | | |
|---|-------------------------------|----------|----------|----------|----------|
| | Phylum | Class | Order | Family | Genus |
| Control soil | | | | | |
| Org | −0.529 | −0.752 * | −0.444 | −0.073 | 0.375 |
| Act | −0.588 | −0.738 * | −0.525 | −0.229 | 0.167 |
| CD _{Org} | 0.563 | 0.791 * | 0.474 | 0.089 | −0.379 |
| CD _{Act} | −0.647 | −0.860 * | −0.562 | −0.180 | 0.306 |
| EP _{Org} | 0.592 | 0.814 * | 0.505 | 0.123 | −0.351 |
| EP _{Act} | 0.315 | 0.494 | 0.248 | −0.028 | −0.344 |
| Soil contaminated with Ni ²⁺ | | | | | |
| Org | −0.504 | −0.458 | −0.300 | 0.494 | 0.494 |
| Act | 0.237 | 0.201 | 0.077 | −0.482 | −0.482 |
| CD _{Org} | −0.112 | −0.149 | −0.266 | −0.670 | −0.670 |
| CD _{Act} | −0.225 | −0.233 | −0.255 | −0.248 | −0.248 |
| EP _{Org} | −0.767 * | −0.767 * | −0.751 * | −0.395 | −0.395 |
| EP _{Act} | −0.449 | −0.434 | −0.376 | 0.018 | 0.018 |
| Soil contaminated with Co ²⁺ | | | | | |
| Org | −0.406 | −0.655 | −0.737 * | −0.725 * | 0.325 |
| Act | 0.414 | 0.735 * | 0.845 * | 0.829 * | −0.466 |
| CD _{Org} | −0.787 * | −0.644 | −0.540 | −0.559 | −0.640 |
| CD _{Act} | 0.399 | 0.365 | 0.328 | 0.335 | 0.247 |
| EP _{Org} | −0.257 | 0.115 | 0.284 | 0.257 | −0.867 * |
| EP _{Act} | −0.096 | 0.133 | 0.234 | 0.218 | −0.507 |
| Soil contaminated with Cd ²⁺ | | | | | |
| Org | −0.912 * | −0.952 * | −0.959 * | −0.946 * | −0.806 * |
| Act | −0.828 * | −0.880 * | −0.889 * | −0.871 * | −0.706 |
| CD _{Org} | −0.190 | −0.256 | −0.270 | −0.244 | −0.073 |
| CD _{Act} | −0.757 * | −0.836 * | −0.851 * | −0.822 * | −0.593 |
| EP _{Org} | 0.524 | 0.546 | 0.549 | 0.542 | 0.465 |
| EP _{Act} | 0.899 * | 0.950 * | 0.959 * | 0.942 * | 0.773 * |

Org—organotrophic bacteria; Act—actinobacteria; CD—microbial colony development index; EP—ecophysiological diversity index; * r—coefficient of correlation significant at: $p = 0.05$, $n = 8$.

4. Discussion

4.1. Sensitivity of Test Plants to the Effects of Ni²⁺, Co²⁺, and Cd²⁺ and Their Energetic Value

Plants growing in the environment polluted with heavy metals are exposed to metabolic stress, specific symptoms, and consequences of which may vary depending on pollutant [61]. However, regardless of the heavy metal present, the main symptoms include growth inhibition and biomass reduction [62]. The basic vital processes, like photosynthesis, respiration, water metabolism, nitrogen metabolism, and ionic and hormonal homeostasis, are disturbed in cells as well [63,64]. The values of resistance index (RS) determined in the present study for the aerial parts of *Elymus elongatus* L. and *Zea mays* L. point to their greatest sensitivity to Ni²⁺ and the lowest one to Cd²⁺. Similar responses to soil contamination with heavy metals were observed for the roots of *Zea mays* L., whereas roots of *Elymus elongatus* L. were the most sensitive to Co²⁺. Toxicity of heavy metals can be due to overproduction of reactive oxygen species (ROS), blocking functional groups and structure of nucleic acids, and to displacement and replacement of ions of essential elements being enzyme co-factors [62–64]. In response to the effects of heavy metals, plants exhibit enhanced antioxidative activity. Enzymes of the antioxidative pathways are encoded by genes which are overexpressed [63,65]. The first line of plant defense against heavy metals

allows them to survive and grow further; however, it often fails under long-term exposure to contamination [65].

Elymus elongatus L. was more resistant to soil pollution with heavy metals than *Zea mays* L. The present study results corroborate earlier findings [66,67], indicating that *Zea mays* L. has a relatively high yielding potential but is sensitive to heavy metals. According to Kopecky et al. [68], tolerance of crops to increased levels of heavy metals is rather uncommon, probably due to limited variability during long-term selection aimed at attaining desirable functional features. Interestingly, despite the above observations, heavy metals did not affect the heat of combustion and the heating value of *Elymus elongatus* L. and *Zea mays* L., whereas differences observed in energy production could be attributable to the biomass yields of these plants produced in particular soil treatments.

4.2. Sensitivity of Soil Microorganisms to the Effects of Ni^{2+} , Co^{2+} , and Cd^{2+}

Like in the case of eukaryotic microorganisms, toxic effects of heavy metals on cells of microorganisms depend on metal type and concentration, and species of cultivated plant [69–71]. In the present study, heavy metals elicited various effects on microorganisms tested. Organotrophic bacteria were the most resistant to Co^{2+} , whereas actinobacteria—to Cd^{2+} . Our previous research [4] conducted with soil sown with *Brassica napus* demonstrated actinobacteria to be more resistant to the effects of Cd^{2+} than Co^{2+} and Ni^{2+} . The diversified effects of heavy metals on microorganisms were also pinpointed by Zaborowska et al. [69] and Giller et al. [72]. In turn, in experiments carried out by Zaborowska et al. [73] and Boros-Lajszner et al. [74], the adverse effect of Co^{2+} on the microbiome aggravated along with its increasing content in the soil.

In the present study, the values of the ecophysiological diversity index (EP) determined for actinobacteria and organotrophic bacteria were higher than those determined for fungi. This may be indicative of the faster development of microorganisms that adapted to the adverse environmental conditions [6]. Metal ions can also inhibit activities of enzymes by complexing sulfhydryl groups of active centers [75]. The toxic effects of heavy metals may also disturb protein folding and ionic equilibrium [76,77].

The rhizosphere of metallophytes represents an environment rich in metal-resistant microorganisms, which significantly affect phytoremediation effectiveness [78]. In the present study, *Proteobacteria* and *Actinobacteria* were the predominating phyla, regardless of the test plant species and heavy metal analyzed, with *Proteobacteria* prevailing in the soil samples exposed to Cd^{2+} , Co^{2+} , and Ni^{2+} and sown with *Elymus elongatus* L., whereas *Actinobacteria* in the soil samples polluted with these heavy metals but sown with *Zea mays* L. Also Gołębiewski et al. [79], Franke-Whittle et al. [80], Sun et al. [81], and Greening et al. [82] demonstrated that soils polluted with heavy metals were mostly populated by bacteria from *Actinobacteria* and *Proteobacteria* phyla.

In the present study, bacteria from *Arthrobacter*, *Rhodoplanes*, *Kaistobacter*, *Devosia*, and *Phycoccus* genera were little sensitive to soil pollution with Cd^{2+} , Co^{2+} , and Ni^{2+} . As reported by De et al. [83] and Sun et al. [81], some rhizospheric bacteria, e.g., these from the genus *Arthrobacter*, are the so-called plant growth-promoting rhizobacteria (PGPR), i.e., bacteria of the rhizosphere that stimulate plant growth by increasing nutrient uptake from the substratum; producing vitamins, phytohormones, and siderophores; and enlarging the root absorptive surface [84]. Metallophyte growth promotion by rhizospheric bacteria very often leads to increased production of plant biomass containing bound metals, which in turn results in an increased phytoremediation effectiveness [85]. Bacteria from the genus *Phycoccus* classified to *Actinobacteria*, o *Actinomycetales*, and f *Intrasporangiaceae*, whose highest OTUs were isolated from the soil samples polluted with Cd^{2+} , may be useful in biotechnology as they produce antibiotics, immunosuppressants, anti-carcinogens, and enzymes [86]. The present study results confirm findings reported earlier by Park et al. [87]; Lin et al. [88]; Borowik et al. [89], and Deng et al. [90]. According to Park et al. [87] and Deng et al. [90], *Actinobacteria* are able to degrade choline in soils polluted with petroleum and heavy metals. In the present study, the ability of *Rhodoplanes* genus bacteria

to adapt to stress conditions induced by heavy metals was probably because they produce hopanoids [91], which after being incorporated into the cytoplasmic membrane impart cell rigidity, stability, and resistance, similarly to ferns, mosses, protists, fungi, and lichens. Thereby, the higher resistance of this genus bacteria to the toxic effects of heavy metals in the environment allows them to grow and develop even in severely contaminated soils [91,92]. Also *Kaistobacter* genus bacteria proved tolerant to heavy metals. Their OTU in the soil polluted with Ni^{2+} and sown with *Elymus elongatus* L. was at 569, and in the soil sown with *Zea mays* L.—at 1122. In the soil polluted with Co^{2+} , their OUT reached 863 and 1463, and in that polluted with Cd^{2+} it reached 1151 and 733, respectively. This data indicates that the *Kaistobacter* genus bacteria cope better in the soils polluted with Ni^{2+} and Co^{2+} than in those exposed to Cd^{2+} . The results obtained in our study confirm those reported by Wu et al. [93], who also observed that these bacteria were able to survive in heavy-metal-polluted soils. The *Kaistobacter* bacteria were also capable of surviving in the soil contaminated with fungicides [94] and in soils polluted with petroleum-based products [95]. Additionally, *Devosia* genus bacteria showed a high bioremediating potential in habitats of polluted soils, as they survived in the soils exposed to heavy metals, with OTUs ranging from 938 to 3305. Their survivability under these stress conditions can be attributable to the genes they produce that are responsible for detoxification, chemotaxis, and response to stress [96]. Talwar et al. [96] emphasized the flexibility of *Devosia* spp. genome in adaptation, bioremediation, and ability to utilize a broad range of substrates.

In the present study, the test plant species had a significant effect on bacterial diversity. Greater diversity was observed at all taxonomic levels in the soil sown with *Elymus elongatus* L. than with *Zea mays* L., which can be due to the production of bioactive metabolites by the first one, affecting co-operation between plant and microorganisms and between microorganisms and requiring intensive communication [97]. *Quorum sensing*, enabling communication between bacterial cells, is one of such mechanisms. Gene expression is regulated depending on density of cells present in a given environment, which enables not only intra-species but also inter-species communication of bacteria, and even interactions with higher organisms [98]. Probably, this phenomenon did occur in the present study, which indicates that the cultivated plants, *Elymus elongatus* L. in particular, mitigated disorders in bacterial diversity induced by heavy metals. Soil microorganisms communicate through chemical signals—signaling molecules called autoinducers—which play the key role in the quorum sensing mechanism [99]. These molecules migrate from the cytoplasm outside of the cell and accumulate in a given environment along with bacteria count increase. Once the threshold value is exceeded, gene expression changes, resulting in the metabolic effect observed in all cells of a bacterial population [100,101]. This mechanism is involved in biodiversity regulation in the soil environment, including particularly the functional and metabolic biodiversity. It also plays an important role in the course of various cellular processes, including synthesis of enzymes, polysaccharides, antibiotics, or toxins [98].

5. Conclusions

Elymus elongatus L. proved more utile than *Zea mays* L. for the phytoremediation of soil polluted with Cd^{2+} , Co^{2+} , and Ni^{2+} because it is more resistant to the effects of heavy metals, as proved by the indices of these plants' resistance to the tested pollutants.

Biomass of *Elymus elongatus* L. and *Zea mays* L. grown in the soil polluted with Cd^{2+} , Co^{2+} , and Ni^{2+} can be used for combustion as the heat of combustion and heating value of these plants were comparable to those of the biomass produced in the non-polluted soil.

Organotrophic bacteria were more resistant to the effects of Co^{2+} , whereas actinobacteria to these of Cd^{2+} in the soil.

Elymus elongatus L. proved better than *Zea mays* L. in mitigating disorders in bacterial diversity caused by heavy metals, as indicated by Shannon index values.

Among all heavy metals tests, the most adverse effect on bacterial diversity at all taxonomic levels was observed for Cd^{2+} in the rhizosphere of *Zea mays* L.

Based on the study results, it can be concluded that the bacteria from *Arthrobacter*, *Rhodoplanes*, *Kaistobacter*, *Devosia*, and *Phycococcus* genera are little sensitive to soil pollution with Cd^{2+} , Co^{2+} , and Ni^{2+} , and should be perceived as sources of species useful for the remediation of soils polluted with heavy metals.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/en14164903/s1>, Table S1: Microbial count (cfu) in 1 kg d.m. of the soil sown with *Elymus elongatus* L. and *Zea mays* L.

Author Contributions: E.B.-L., J.W. and J.K. framed the methodology, conceived the ideas, and designed the paper. E.B.-L. conducted the experiments, collected and analyzed the data. A.B. conducted the bioinformatic analysis and visualization of data. All authors contributed significantly to the discussion of the results and the preparation of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The results presented in this paper were obtained as part of a comprehensive study financed by the University of Warmia and Mazury in Olsztyn, Faculty of Agriculture and Forestry, Department of Soil Science and Microbiology (grant no. 30.610.006-110). Project financially supported by Minister of Science and Higher Education in the range of the program entitled “Regional Initiative of Excellence” for the years 2019-2022, project no. 010/RID/2018/19, amount of funding 12,000,000 PLN.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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