

Article

The Role of Cellulose in Microbial Diversity Changes in the Soil Contaminated with Cadmium

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Abstract: Cadmium is an essential element for plant growth and development. Its accumulation in soil is more hazardous to human and animal health than to plants and microorganisms. A pot greenhouse experiment was conducted to determine the usability of *Sinapis alba* L. and *Avena sativa* L. for the phytoremediation of soil contaminated with cadmium and to verify cellulose viability in the remediation of soil under cadmium pressure in doses from 4 to 16 mg Cd²⁺ kg⁻¹ soil d.m. (dry matter) The effect of cadmium on soil microbiome was investigated with the culture method and the variable region sequencing method. *Sinapis alba* L. and *Avena sativa* L. were found viable in the phytoremediation of soil contaminated with Cd²⁺. *Avena sativa* L. was more potent to accumulate Cd²⁺ in roots than *Sinapis alba* L. Although the fertilization of Cd²⁺-contaminated soil with cellulose stimulated the proliferation of microorganisms, it failed to mitigate the adverse effects of Cd²⁺ on bacterial diversity. Bacteria from the *Sphingomonas*, *Sphingobium*, *Achromobacter*, and *Pseudomonas* genera represented the core microbiome of the soils sown with two plant species, contaminated with Cd²⁺ and fertilized with cellulose. Stimulation of the growth and development of these bacteria may boost the efficacy of phytoremediation of cadmium-contaminated soils with *Sinapis alba* L. and *Avena sativa* L.

Keywords: soil contamination; microorganisms; phytoremediation; cadmium



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1. Introduction

Cadmium is a heavy metal posing severe threat to human and animal health. Because of its high toxicity, the risks stemming from its adverse impacts are considered globally [1–4]. Its levels recorded in uncontaminated soils range from 0.01 to 1 mg kg⁻¹, with a global average of 0.36 mg kg⁻¹ [5], and are observed to increase successively due to geological weathering of rocks [6] and anthropogenic activity, such as mining, metallurgy, fuel combustion, and soil amendment with phosphorus-based fertilizers [3,7,8]. The increasing environmental contamination with this element contributes to its accumulation in all links of the food chain [6,9,10].

Cadmium is toxic to both prokaryotic and eukaryotic organisms. Its toxicity is due to the binding of its free ions with atoms of sulfur, oxygen, and hydrogen present in tissues, which modifies the morphology of plant and animal cells. It may also disturb the circulation of such elements as: Fe, Cu, Mg, Ca, Zn, and Se [11]. The toxic effects of cadmium on microorganisms entail chiefly blocking the active centers of enzymes, reactions with -SH groups, competition with basic metabolites, and cell membrane damage [12]. In turn, its toxic effects on plants are triggered by disorders in the uptake of micro- and macroelements indispensable for proper body function. In plants, cadmium is bound by complexing compounds (metallothioneins and phytochelatins) and accumulated in vacuoles, cell membrane, cell compartments, Golgi apparatus, and endoplasmic reticulum [13].

Cadmium is very quickly transported throughout the trophic chain: soil—plant—man [14,15]. Its ions aggravate oxidative stress in plant cells because it is very easily

absorbed by roots and transported to the aerial parts of plants, inhibiting their growth and potentially leading to their death [11–13,16]. The strongly adverse effects of cadmium on higher organisms are corroborated by the fact that it is included in the group 1 carcinogenic substances by the International Agency for Research on Cancer (IARC) [17].

Cadmium occurs in the environment mainly in the second oxidation state [18,19]. It is a white metal with bluish tint. Cadmium compounds feature various solubility in water, ranging from very good solubility (e.g., acetate, chloride, sulfate) to almost insolubility (e.g., oxide, sulfide). Similar to other heavy metals, considering its binding force, cadmium extends to seven geochemical fractions varying in mobility and, thereby, in bioavailability [18,20]. These fractions, beginning from the most available one to organisms, may be ordered as follows: ionic—occurring in soil water; ionic—adsorbed by the sorptive complex; carbonate; the one bound with manganese oxides; the one bound with iron oxides; organic; and the nondigestible one occurring in primary metals [21,22]. The amount of cadmium available to plants is determined by mobilization and immobilization processes, which are in turn dependent on the physicochemical properties of soil (pH, cation exchange capacity, fraction size distribution, and organic matter content and type), activities of soil microorganisms, and species of grown plants [19,20,23].

Root secretions may provide a source of nutrients to microorganisms which are more abundant and active in this rhizospheric zone of the soil than in the rootless one [24]. Soil microorganisms mineralize nutrients dissolved in the soil solution [25] and secrete their own metabolites (e.g., organic acids, siderophores, and biosurfactants), forming complexes with heavy metals which differ in stability and mobility [23,26,27]. They affect soil properties, such as sorption and desorption processes, which in turn determine the heavy metal accumulation in plant tissues. They may form a protective barrier preventing penetration of harmful substances to plant roots [28], and bolster physiological functions of plants by providing growth hormones and facilitating the uptake of iron, phosphorus, and potassium compounds [29].

Contaminated environments are most frequently recultivated via phytoextraction and phytostabilization. Hence, the choice of appropriate recultivation plant species for specified pollutants is of utmost importance [30,31]. Preparatory measures, such as liming, organic and mineral fertilization, and application of various sorbents, are strongly recommended before the exact recultivation [19,32–34].

In order to aid the phytostabilization of cadmium-contaminated soil, cellulose has been used in this study, as it is the most abundant organic compound in nature [35]. Cellulose is the major constituent of plant cell walls, a nonbranched polysaccharide containing 3000 to 4000 of D-glucose residues linked with β -1,4 glycosidic bonds [36]. It undergoes two-stage microbiological hydrolysis in the soil, firstly to cellobiose and then to glucose [37]. The rate of cellulose degradation is largely dependent on the activity of soil microorganisms for which it serves as a nutrient [38]. Its application allows capturing the microbiological variability of soil under strictly controlled conditions. After modification, cellulose can also serve as a good sorbent [39].

Considering the above, an experiment was established which combined remediation techniques applicable to soils contaminated with cadmium. The following research hypotheses were formulated: (1) crystalline cellulose stimulates the proliferation of autochthonous soil microorganisms, and (2) changes in the abundance and diversity of bacteria may increase *Sinapis alba* L. and *Avena sativa* L. tolerance to cadmium effects. Novelty of the study lies in the cultivation of two crops directly one after another, which is especially important in the case of soils contaminated with cadmium as its effects on the microbiome and the crop are long-standing. In addition, *Sinapis alba* L.—as the main crop—“cleans” the soil environment of cadmium by accumulating it in its roots and aerial parts [40], whereas *Avena sativa* L.—as the successive crop—serves as a phytosanitary plant that secretes an alkaloid, scopoletin, which prevents the development of fungi-inducing, take-all diseases of cereals [41].

Therefore, this study focuses on alternative techniques of remediation, including phytoremediation and phytostabilization of cadmium in soil, both deemed highly prospective owing to their simplicity, high efficacy, in situ applicability, and cost-effectiveness.

2. Materials and Methods

2.1. Soil

A pot experiment was conducted with soil having pH_{KCl} 7.0, classified as sandy loam (<0.002 mm fraction—2.88%, 0.002–0.050 mm fraction—27.71%, and 0.050–2.000 mm fraction—69.41%). The soil material was collected from an area located in northeastern Poland, Tomaszkowo village, in the Mazurian Lake District (53.7161° N, 20.4167° E). In-depth characteristics of the sandy loam used in the experiment were provided in a work by Boros-Lajszner et al. [32].

2.2. Experimental Scheme

The study was carried out with the following independent variables: (1) extent of soil contamination with cadmium and (2) addition of cellulose as a substance mitigating adverse effects of cadmium. The soil was contaminated with cadmium in the form of an aqueous solution of cadmium chloride (CdCl_2), in doses of 0, 4, 8, and 16 $\text{mg Cd}^{2+} \text{ kg}^{-1}$ soil d.m. Microcrystalline cellulose with a particle size of 90 μm (ACROS ORGANICS, Geel, Belgium, extra pure) was used in a dose of 15 g kg^{-1} soil d.m. As background, soil from all pots was fertilized with urea, monopotassium phosphate, potassium chloride, and magnesium sulfate in the following doses: N—160 mg, P—21 mg, K—73 mg, Mg—15 mg kg^{-1} soil d.m. Following study design, all components (cadmium chloride, cellulose, and fertilizers) were thoroughly mixed with soil (in the amount of 3.5 kg) and then the soil was transferred to pots. Analyses were carried out in 4 replications. White mustard (*Sinapis alba* L.) of Rota cultivar served as an experimental plant (8 plants per pot). Its aerial parts and roots were harvested after 40 days of growth. Next, a successive plant was sown, namely oat (*Avena sativa* L.) of Bingo cultivar (12 plants per pot). Oat crop was harvested after another 40 days. During white mustard and oat growth, the soil humidity was maintained at 50% of the maximum water-holding capacity. After the harvest of both crops, their aerial parts and roots were analyzed for Cd^{2+} content. *Sinapis alba* L. belongs to the family *Brassicaceae*. It is an annual plant characterized by a very high growth rate. It has been ranked third among the oil-producing crops in terms of acreage area in Poland. An additional advantage of *Sinapis alba* L. in its use as a phytoremediating plant is its high tolerance to soil contamination with cadmium [42,43]. *Avena sativa* L. belongs to the family *Poaceae*. It was selected as a successive crop because *Avena sativa* L. is a phytosanitary plant. This cereal species exerts two types of effects on soil: it significantly reduces weed infestation and prevents the development of take-all diseases in successive crops.

2.3. Soil and Plant Analysis

Soil graining was determined by the aerometric method [44,45], and soil pH was analyzed using the potentiometric method in an aqueous KCl solution with 1 mol dm^{-3} soil concentration [46]. Cadmium content was determined in the aerial parts and roots of *Sinapis alba* L. and *Avena sativa* L. by atomic absorption spectrometry following microwave mineralization, according to the Polish standard PN-EN 14084:2004(N) [47].

2.4. Indicators Valorizing the Usability of Plants for the Remediation of Cadmium-Contaminated Soil

The harvested crops of white mustard (*Sinapis alba* L.) and oats (*Avena sativa* L.) allowed for determining their resistance to cadmium based on the formula described by Orwin and Wardle [48]:

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

where:

RS—plant resistance to heavy metal;
 C_0 —biomass yield in control soil (nonpolluted);
 D_0 —difference between biomass yield produced on nonpolluted soil and soil polluted with heavy metal.

In addition, cadmium uptake by plants (D) was computed using the following formula:

$$D = (Y_A \times C_A) + (Y_R \times C_R) \quad (2)$$

and its redistribution in plants according to the following formula:

$$R_A = \frac{C_A}{C_A + C_R} \times 100\% \quad (3)$$

$$R_R = \frac{C_R}{C_A + C_R} \times 100\% \quad (4)$$

where:

Y_A —aboveground parts yield (g kg^{-1});
 Y_R —root yield (g kg^{-1});
 C_A —heavy metal content in aboveground parts ($\mu\text{g g}^{-1}$);
 C_R —heavy metal content in roots ($\mu\text{g g}^{-1}$);
 R_A —redistribution of heavy metals in aboveground parts (%);
 R_R —redistribution of heavy metals in roots (%).

2.5. Microbiological Analyses

Microbiological analyses were carried out following the procedures described by Wyszowska et al. [49]. The growth rate of individual groups of microorganisms and the counted number of grown colonies were used to compute the colony development index (CD) and ecophysiological diversity index (EP) of microorganisms in the cadmium-contaminated soil. The formulas have been described in the manuscript by De Leij et al. [42], Sarathchandra et al. [43], and Wyszowska et al. [49].

In turn, the population counts of soil microorganism were used to calculate the index of their resistance (RS) to Cd^{2+} contamination according to the formula described by Orwin and Wardle [48] and the index of the impact of cadmium (IF_{Cd}) and cellulose (IF_{Ce}) on their abundance. Detailed description of particular indices was provided in the work by Boros-Lajszner et al. [50]. In addition, the control soil and soil contaminated with a cadmium dose of $16 \text{ mg Cd}^{2+} \text{ kg}^{-1}$ were analyzed for bacterial diversity with the variable region sequencing method. DNA was extracted using the Bead-Beat Micro Gravity kit (A & A Biotechnology, Gdansk, Poland). PCR was performed using Q5 Hotstat High-Fidelity DNA Polymerase (NEBNext, Ipswich, MA, USA). The metagenomic analysis, automatic data analysis, and preparation of the reference sequence database are presented in the paper by Borowik et al. [51].

2.6. Statistical Analyses

Data were processed statistically with the analysis of variance ANOVA at a significance level of $p \leq 0.05$ using STATISTICA 13.1 software (StatSoft, Tulsa, OK, USA) [52]. The analyzed data had normal distribution and similar variance. Homogenous groups were computed using the post hoc Tukey test (HSD—Tukey's honestly significant difference test).

Metagenomic data $\geq 1\%$ were visualized with 4 types of computer software: bacteria phyla were visualized using STAMP 2.1.3. (St Lucia, Queensland, Australia) with two-way G-test for statistical hypotheses (w/Yates) + Fisher test [53]; bacterial classes, orders, and families—using an internet tool, Circos 0.68 [54]; bacterial genera—using Rstudio v1.2.5033 (JJ Allaire, Boston, MA, USA) with R project [55,56], gplots v3.6.2 library [57], and an internet tool for data set analysis InteractiveVenn (Irvine, CA, USA) [58]. In addition, bacteria biodiversity was determined based on the Shannon–Wiener index (H') and Simpson index (D) [59,60].

3. Results

3.1. Effect of Cadmium and Cellulose on Plants

Sinapis alba L. (Figure 1) and *Avena sativa* L. (Figure 2) proved to be relatively resistant to soil contamination with cadmium doses from 4 to 16 mg Cd²⁺ kg^{−1} soil d.m. (Table 1). Figure 1 presents *Sinapis alba* L. at the BBCH 65 stage, i.e., at the full blossom stage when 50% of flowers are opened on the main inflorescence and older petals fall down. In turn, Figure 2 depicts *Avena sativa* L. at the BBCH 51 stage, i.e., at the panicle ejection stage.



Figure 1. The effect of cadmium on the growth of *Sinapis alba* L. (a,e)—0 mg Cd²⁺; (b,f)—4 mg Cd²⁺; (c,g)—8 mg Cd²⁺; (d,h)—16 mg Cd²⁺.

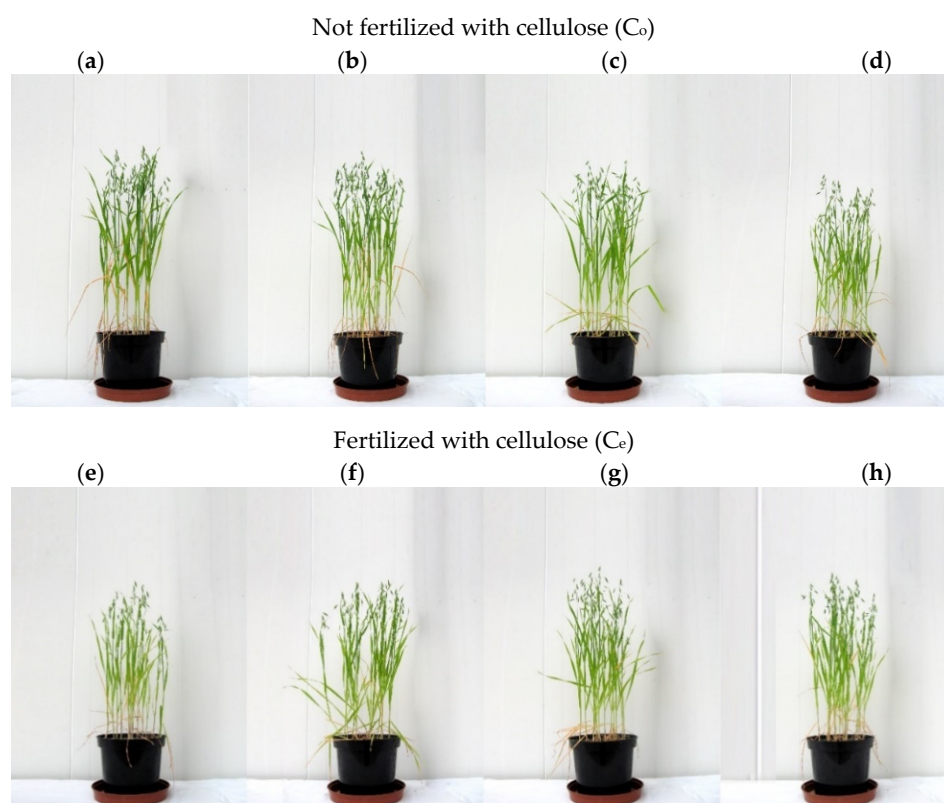


Figure 2. The effect of cadmium on the growth of *Avena sativa* L. (a,e)—0 mg Cd²⁺; (b,f)—4 mg Cd²⁺; (c,g)—8 mg Cd²⁺; (d,h)—16 mg Cd²⁺.

Table 1. The index of resistance (RS) of *Sinapis alba* L. and *Avena sativa* L. to soil contamination with cadmium.

Dose Cd ²⁺ (mg kg ⁻¹ soil)	<i>Sinapis alba</i> L.		<i>Avena sativa</i> L.	
	Aerial Parts	Roots	Aerial Parts	Roots
not fertilized with cellulose (C ₀)				
4	0.735 ^b	0.644 ^a	0.870 ^a	0.920 ^a
8	0.697 ^c	0.523 ^{bc}	0.771 ^c	0.655 ^c
16	0.642 ^c	0.469 ^d	0.718 ^c	0.655 ^c
$\bar{X}$	0.691 ^B	0.545 ^C	0.786 ^A	0.743 ^B
fertilized with cellulose (C _e)				
4	0.959 ^a	0.564 ^b	0.829 ^b	0.792 ^b
8	0.955 ^a	0.506 ^c	0.815 ^b	0.755 ^b
16	0.792 ^b	0.312 ^e	0.529 ^d	0.578 ^d
$\bar{X}$	0.902 ^A	0.461 ^D	0.724 ^B	0.708 ^C

The same letters (a–e) in the columns are assigned to the same homogeneous groups; separate homogeneous groups were calculated for means regardless of the plant part (A–D).

The plants' response to the heavy metal tested varied depending on cellulose application to the soil. In the series without polysaccharide application, *Avena sativa* L. (mean RS of aerial parts = 0.786) was more resistant to cadmium presence in the soil environment than *Sinapis alba* L. (mean RS of aerial parts = 0.691). In the series with cellulose, the plants' response was opposite, i.e., the mean RS of the aerial parts reached 0.902 for *Sinapis alba* L. and 0.724 for *Avena sativa* L. The response of roots for both tested plants to the tested doses of the heavy metal was stronger. The lower values of the resistance index (RS) of

Sinapis alba L. and *Avena sativa* L. roots compared to the aerial parts are indicative of more destructive effect of the heavy metal on their roots.

Sinapis alba L. featured a significantly higher cadmium uptake from the soil contaminated with its dose of $16 \text{ mg Cd}^{2+} \text{ kg}^{-1}$ than *Avena sativa* L. (Table 2). Fertilization with cellulose caused a 54% decrease in cadmium uptake by *Sinapis alba* L. and a 28% decrease in its uptake by *Avena sativa* L. Values of redistribution indicators (R_A and R_R) prove that *Sinapis alba* L. accumulated 2.4-fold to 6.7-fold more cadmium in its roots than aerial parts, whereas *Avena sativa* L. accumulated even more cadmium in respective morphological parts, showing from 8.9-fold to 30.2-fold higher accumulation in roots than aerial parts.

Table 2. Cadmium uptake (D) ($\mu\text{g kg}^{-1} \text{ d.m.}$) by *Sinapis alba* L. and *Avena sativa* L. and its redistribution (%) in the aerial parts (R_A) and roots (R_R) of plants.

Dose Cd^{2+} (mg kg^{-1} soil)	<i>Sinapis</i> <i>alba</i> L.	<i>Avena</i> <i>sativa</i> L.	<i>Sinapis</i> <i>alba</i> L.		<i>Avena</i> <i>sativa</i> L.	
	D		R_A	R_R	R_A	R_R
not fertilized with cellulose (C_0)						
0	17.141 ^e	0.519 ^g	13.035 ^d	86.965 ^c	10.069 ^e	89.931 ^c
16	196.392 ^a	43.137 ^c	29.080 ^a	70.920 ^f	6.741 ^f	93.259 ^{ab}
fertilized with cellulose (C_e)						
0	4.822 ^f	5.396 ^f	24.670 ^b	75.330 ^e	3.203 ^g	96.797 ^a
16	90.824 ^b	31.069 ^d	20.703 ^c	79.297 ^d	9.120 ^e	90.880 ^{bc}

Identical letters (a–g) in columns denote the same homogeneous groups.

3.2. Effect of Cadmium and Cellulose on Soil Microorganisms

Cadmium significantly affected the population numbers of the analyzed microorganisms (Table 3). The highest counts of microorganisms were determined in the soil samples not contaminated with cadmium. Generally, higher counts of microorganisms were found in the control and contaminated soil sown with the successive crop, i.e., *Avena sativa* L., than in the soil with the main crop, i.e., *Sinapis alba* L. Soil contamination with the tested metal contributed to a significant decrease in the abundance of all soil microorganisms under study, as evidenced by the negative coefficient of correlation between the extent of soil contamination with cadmium and population numbers of microorganisms, ranging from -0.741 (actinobacteria in the soil sown with *Avena sativa* L.) to -0.999 (organotrophic bacteria in the soil sown with *Avena sativa* L.).

The adverse effect of cadmium on the communities of oligotrophic bacteria, actinobacteria, and fungi was also indicated by negative values of IF_{Cd} indices (Figure 3), which were observed to decrease along with an increasing Cd doses applied to the soil. In the soil sown with *Sinapis alba* L., the values of IF_{Cd} indices ranged from -0.262 ($4 \text{ mg Cd}^{2+} \text{ kg}^{-1}$ soil d.m.) to -0.575 ($16 \text{ mg Cd}^{2+} \text{ kg}^{-1}$ soil d.m.) for organotrophic bacteria, from -0.084 to -0.458 for actinobacteria, and from -0.048 to -0.624 for fungi, respectively. In the soil sown with *Avena sativa* L., the respective values ranged from -0.115 to -0.513 for organotrophic bacteria, from -0.614 to -0.678 for actinobacteria, and from -0.194 to -0.565 for fungi. The substantially higher IF_{Cd} values noted for organotrophs and fungi in the soil sown with *Avena sativa* L. prove that the impact of cadmium on their communities was weaker in the soil sown with the successive crop. In turn, the impact of cadmium on actinobacteria proliferation not only did not diminish with time of the experiment but increased, as indicated by the lower IF_{Cd} values determined in the soil sown with the successive plant. Population numbers of microorganisms were significantly determined not only by the extent of soil contamination and crop species but also by soil fertilization with cellulose (Figure 4). The polysaccharide tested significantly stimulated the proliferation of organotrophic bacteria, actinobacteria, and fungi in the noncontaminated soil and the soil contaminated with cadmium. The only exceptions were actinobacteria in the noncontaminated soil sown with *Avena sativa* L. and fungi in the soil contaminated with a cadmium

dose of 4 mg Cd²⁺ kg^{−1} soil d.m. and sown with *Avena sativa* L. Fertilization with cellulose caused greater, positive changes in the population numbers of all groups of microorganisms in the soil sown with the main rather than successive crop. In the soil sown with *Sinapis alba* L., the mean value of the IF_{Cd} index reached 0.647 for organotrophic bacteria, 2.409 for actinobacteria, and 1.528 for fungi, whereas the respective values determined in the soil sown with *Avena sativa* L. were 0.383, 0.182, and 0.472.

Table 3. Microbial counts in the soil sown with *Sinapis alba* L. and *Avena sativa* L. contaminated with cadmium.

Dose Cd ²⁺ (mg kg ^{−1} soil)	Organotrophic bacteria cfu × 10 ⁸		Actinobacteria cfu × 10 ⁸		Fungi cfu × 10 ⁶	
	<i>Sinapis alba</i> L.	<i>Avena sativa</i> L.	<i>Sinapis alba</i> L.	<i>Avena sativa</i> L.	<i>Sinapis alba</i> L.	<i>Avena sativa</i> L.
not fertilized with cellulose (C ₀)						
0	12.382 ^c	14.441 ^c	2.430 ^d	10.260 ^a	5.373 ^b	9.120 ^b
4	9.757 ^d	12.774 ^d	2.226 ^d	3.960 ^e	5.115 ^b	7.353 ^c
8	6.822 ^f	10.766 ^e	1.776 ^{de}	3.745 ^{ef}	2.710 ^c	4.634 ^{ef}
16	5.263 ^g	7.029 ^f	1.316 ^e	3.302 ^f	2.021 ^c	3.963 ^f
$\bar{X}$	8.556 ^D	11.253 ^C	1.937 ^C	5.317 ^B	3.805 ^D	6.268 ^C
r	−0.954 [*]	−0.999 [*]	−0.990 [*]	−0.741	−0.926 [*]	−0.927 [*]
fertilized with cellulose (C _e)						
0	22.759 ^a	20.973 ^a	12.571 ^a	6.193 ^b	14.347 ^a	19.847 ^a
4	16.848 ^b	15.750 ^b	9.043 ^b	5.470 ^c	14.087 ^a	6.319 ^d
8	10.062 ^d	12.262 ^d	4.339 ^c	4.933 ^d	5.484 ^b	6.155 ^d
16	8.153 ^e	11.997 ^{de}	2.577 ^d	4.703 ^d	5.388 ^b	6.047 ^d
$\bar{X}$	14.456 ^B	15.246 ^A	7.132 ^A	5.325 ^B	9.827 ^A	9.592 ^B
r	−0.924 [*]	−0.861 [*]	−0.942 [*]	−0.921 [*]	−0.854 [*]	−0.694

r—correlation coefficient significant for * $p = 0.05$. The same letters (a–g) in the columns are assigned to the same homogeneous groups; separate homogeneous groups were calculated for means regardless of the plant (A–D).

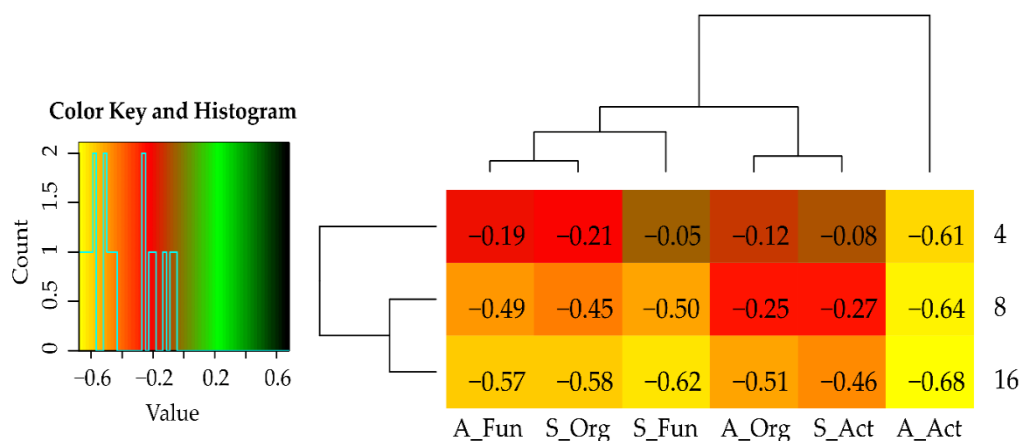


Figure 3. Index of cadmium effect (IF_{Cd}) on microorganisms in the soil sown with *Sinapis alba* L. and *Avena sativa* L. S—*Sinapis alba* L.; A—*Avena sativa* L.; Org—organotrophic bacteria; Act—actinobacteria; Fun—fungi.

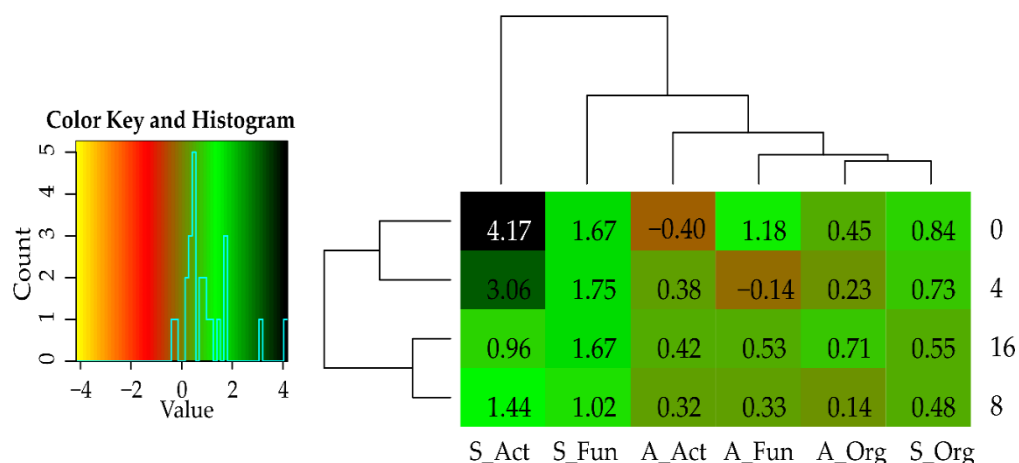


Figure 4. Index of cellulose effect (IF_{Ce}) on microorganisms in the soil sown with *Sinapis alba* L. and *Avena sativa* L. Refer to the key in Figure 1.

The effects of the independent variables on the communities of organotrophic bacteria, actinobacteria, and fungi were very well mirrored by the index of their resistance (RS) to soil contamination with cadmium (Table 4). The analysis of RS index values allowed concluding that the organotrophic bacteria and fungi were more resistant to cadmium effects in the soil not fertilized with cellulose, regardless of crop species, whereas actinobacteria behaved the same way only in the soil sown with *Sinapis alba* L.

Table 4. The index of resistance (RS) of microorganisms to soil contamination with cadmium and sown with *Sinapis alba* L. and *Avena sativa* L.

Dose Cd ²⁺ (mg kg ⁻¹ soil)	Organotrophic Bacteria		Actinobacteria		Fungi	
	<i>Sinapis alba</i> L.	<i>Avena sativa</i> L.	<i>Sinapis alba</i> L.	<i>Avena sativa</i> L.	<i>Sinapis alba</i> L.	<i>Avena sativa</i> L.
not fertilized with cellulose (C ₀)						
4	0.650 ^a	0.793 ^a	0.845 ^a	0.239 ^d	0.908 ^a	0.675 ^a
8	0.380 ^b	0.594 ^b	0.576 ^b	0.223 ^d	0.337 ^b	0.341 ^b
16	0.270 ^{cd}	0.322 ^d	0.371 ^c	0.192 ^e	0.232 ^c	0.278 ^c
$\bar{X}$	0.433 ^B	0.570 ^A	0.597 ^B	0.218 ^D	0.492 ^A	0.431 ^B
fertilized with cellulose (C _e)						
4	0.588 ^a	0.601 ^b	0.562 ^b	0.791 ^a	0.964 ^a	0.189 ^d
8	0.284 ^c	0.413 ^c	0.209 ^d	0.662 ^b	0.236 ^c	0.184 ^d
16	0.218 ^d	0.401 ^c	0.114 ^e	0.612 ^{bc}	0.231 ^c	0.180 ^d
$\bar{X}$	0.382 ^C	0.472 ^B	0.295 ^C	0.688 ^A	0.477 ^A	0.184 ^C

The same letters (a–e) in the columns are assigned to the same homogeneous groups; separate homogeneous groups were calculated for means regardless of the plant (A–D).

Soil contamination with cadmium triggered changes in soil microbiome diversity, as evidenced by the values of the colony development index (CD) and the physiological diversity index (EP) (Tables 5 and 6). In the soil not fertilized with cellulose, the CD value computed for organotrophic bacteria was higher than the respective values determined for actinobacteria and fungi (Table 5). Regardless of Cd-contaminating dose, the CD values determined in the soil sown with *Sinapis alba* L. reached 49.634 for organotrophic bacteria, 21.520 for actinobacteria, and 30.613 for fungi, whereas the respective values noted in the soil sown with *Avena sativa* L. were 38.855, 21.232, and 35.611, respectively. Soil fertilization with cellulose exerted an inexplicit effect on CD values of the analyzed bacterial communities, as it decreased the CD value of organotrophic bacteria, increased that in fungi, and did not modify that in actinobacteria.

Table 5. Colony development index (CD) of microorganisms in the soil contaminated with cadmium and sown with *Sinapis alba* L. and *Avena sativa* L.

Dose Cd ²⁺ (mg kg ⁻¹ soil)	Organotrophic Bacteria		Actinobacteria		Fungi	
	<i>Sinapis alba</i> L.	<i>Avena sativa</i> L.	<i>Sinapis alba</i> L.	<i>Avena sativa</i> L.	<i>Sinapis alba</i> L.	<i>Avena sativa</i> L.
not fertilized with cellulose (C ₀)						
0	44.394 ^{bc}	36.931 ^b	21.644 ^a	21.627 ^{ab}	30.128 ^d	35.389 ^d
4	48.026 ^b	37.690 ^b	21.926 ^a	19.508 ^c	30.059 ^{de}	37.399 ^{cd}
8	38.606 ^c	38.453 ^{ab}	21.908 ^a	22.942 ^a	33.388 ^{cd}	34.278 ^d
16	67.508 ^a	42.346 ^a	20.603 ^b	20.852 ^b	28.876 ^e	35.379 ^d
$\bar{X}$	49.634 ^A	38.855 ^C	21.520 ^A	21.232 ^A	30.613 ^D	35.611 ^C
fertilized with cellulose (C _e)						
0	33.972 ^c	36.927 ^{bc}	21.069 ^{ab}	21.537 ^{ab}	50.389 ^a	50.680 ^a
4	44.827 ^{bc}	32.589 ^c	21.574 ^a	20.329 ^b	47.700 ^b	46.712 ^b
8	49.403 ^b	33.680 ^c	20.416 ^b	20.205 ^b	45.342 ^b	39.479 ^c
16	46.905 ^b	38.941 ^{ab}	19.047 ^c	20.510 ^b	29.621 ^e	44.952 ^b
$\bar{X}$	43.777 ^B	35.534 ^D	20.527 ^B	20.645 ^B	43.263 ^B	45.456 ^A

The same letters (a–e) in the columns are assigned to the same homogeneous groups; separate homogeneous groups were calculated for means regardless of the plant (A–D).

Table 6. Ecophysiological diversity index (EP) of microorganisms in the soil contaminated with cadmium and sown with *Sinapis alba* L. and *Avena sativa* L.

Dose Cd ²⁺ (mg kg ⁻¹ soil)	Organotrophic Bacteria		Actinobacteria		Fungi	
	<i>Sinapis alba</i> L.	<i>Avena sativa</i> L.	<i>Sinapis alba</i> L.	<i>Avena sativa</i> L.	<i>Sinapis alba</i> L.	<i>Avena sativa</i> L.
not fertilized with cellulose (C ₀)						
0	0.858 ^b	0.856 ^b	0.873 ^a	0.871 ^{ab}	0.689 ^b	0.697 ^b
4	0.833 ^c	0.824 ^c	0.887 ^a	0.872 ^{ab}	0.716 ^b	0.640 ^c
8	0.842 ^{bc}	0.846 ^{bc}	0.811 ^d	0.853 ^b	0.739 ^{ab}	0.781 ^{ab}
16	0.645 ^e	0.840 ^{bc}	0.851 ^b	0.893 ^a	0.772 ^a	0.824 ^a
$\bar{X}$	0.795 ^B	0.842 ^A	0.856 ^A	0.872 ^A	0.729 ^A	0.736 ^A
fertilized with cellulose (C _e)						
0	0.895 ^a	0.762 ^d	0.843 ^b	0.842 ^b	0.502 ^d	0.521 ^d
4	0.851 ^b	0.914 ^a	0.885 ^a	0.896 ^a	0.569 ^c	0.567 ^{cd}
8	0.780 ^d	0.915 ^a	0.885 ^a	0.850 ^b	0.695 ^b	0.712 ^b
16	0.846 ^{bc}	0.841 ^b	0.826 ^c	0.827 ^c	0.764 ^a	0.624 ^c
$\bar{X}$	0.843 ^A	0.858 ^A	0.860 ^A	0.854 ^A	0.633 ^B	0.606 ^B

The same letters (a–e) in the columns are assigned to the same homogeneous groups; separate homogeneous groups were calculated for means regardless of the plant (A,B).

Regardless of crop species grown in pots, the cadmium contamination of soil not fertilized with cellulose diminished the ecophysiological diversity of organotrophic bacteria and actinobacteria, while increasing that in fungi (Table 6). The effect of cadmium on EP values of the analyzed microorganisms was more diverse in the polysaccharide-enriched soil. Generally, cadmium decreased the EP value of organotrophic bacteria in the soil sown with *Sinapis alba* L., and increased it in the soil sown with *Avena sativa* L. It also increased EP values computed for actinobacteria and fungi in soils sown with both crops tested. The analysis of data presented in Table 6 indicates that cellulose increased the ecophysiological diversity only in the case of organotrophic bacteria.

All independent variables (soil contamination with cadmium, soil fertilization with cellulose, and crop species) caused changes in the structure of the genetic diversity of bacteria at all taxonomic levels (Figures 5–14). Of all identified phyla, the highest OTU was

determined for *Proteobacteria* and *Actinobacteria* (Figures 5–8). *Proteobacteria* turned out to be the prevailing phylum in the soil sown with *Sinapis alba* L., both in the noncontaminated and contaminated one as well as without and with cellulose addition (Figure 5).

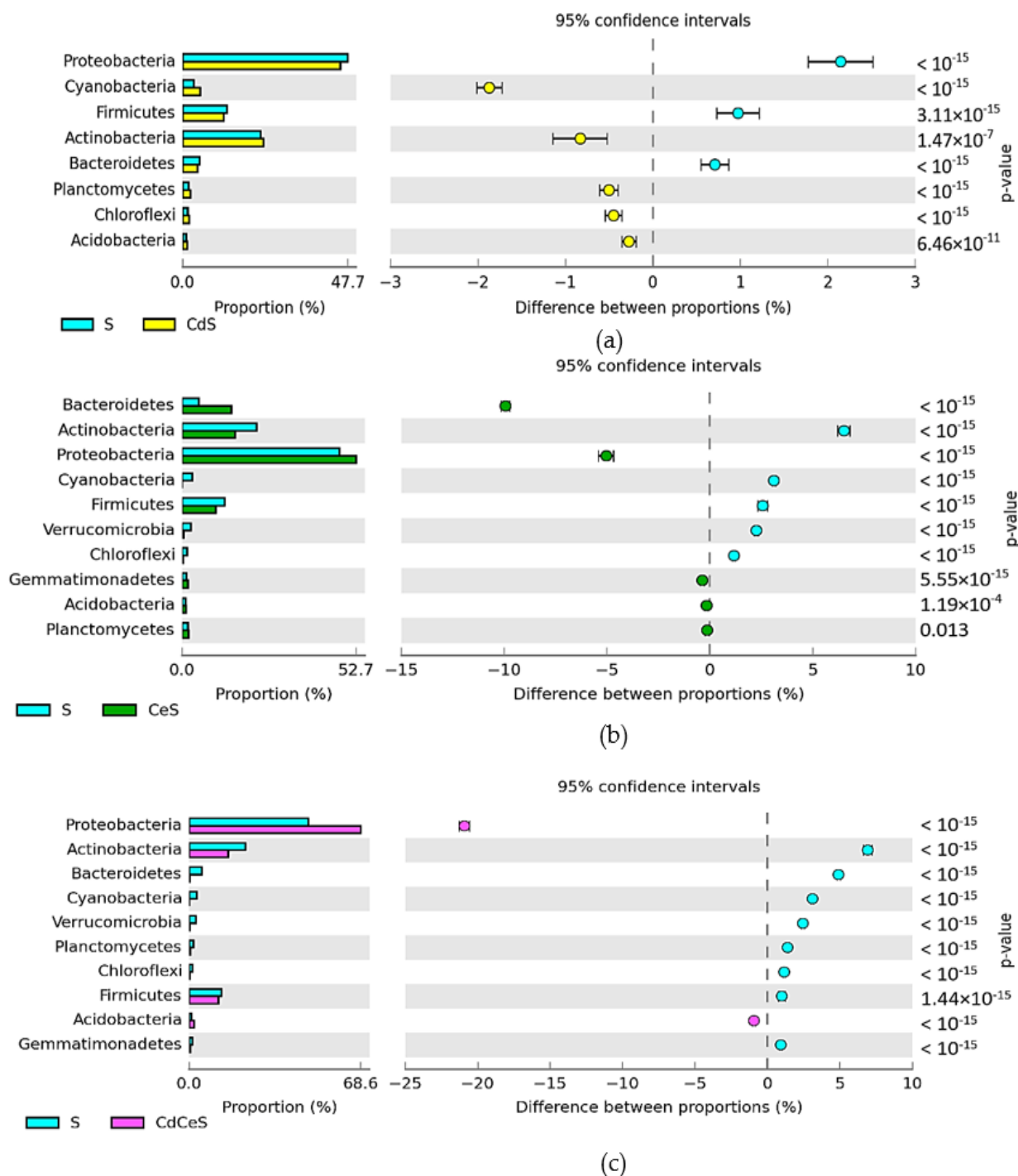


Figure 5. A comparative analysis of the effects of soil contamination with cadmium (a), soil fertilization with cellulose (b), and soil contamination with cadmium + soil fertilization with cellulose (c) on changes in bacterial phyla in the soil sown with *Sinapis alba* L. (S). Differences between ratios of bacterial phyla, OTU $\geq 1\%$. Cd—ion Cd^{2+} ; Ce—cellulose.

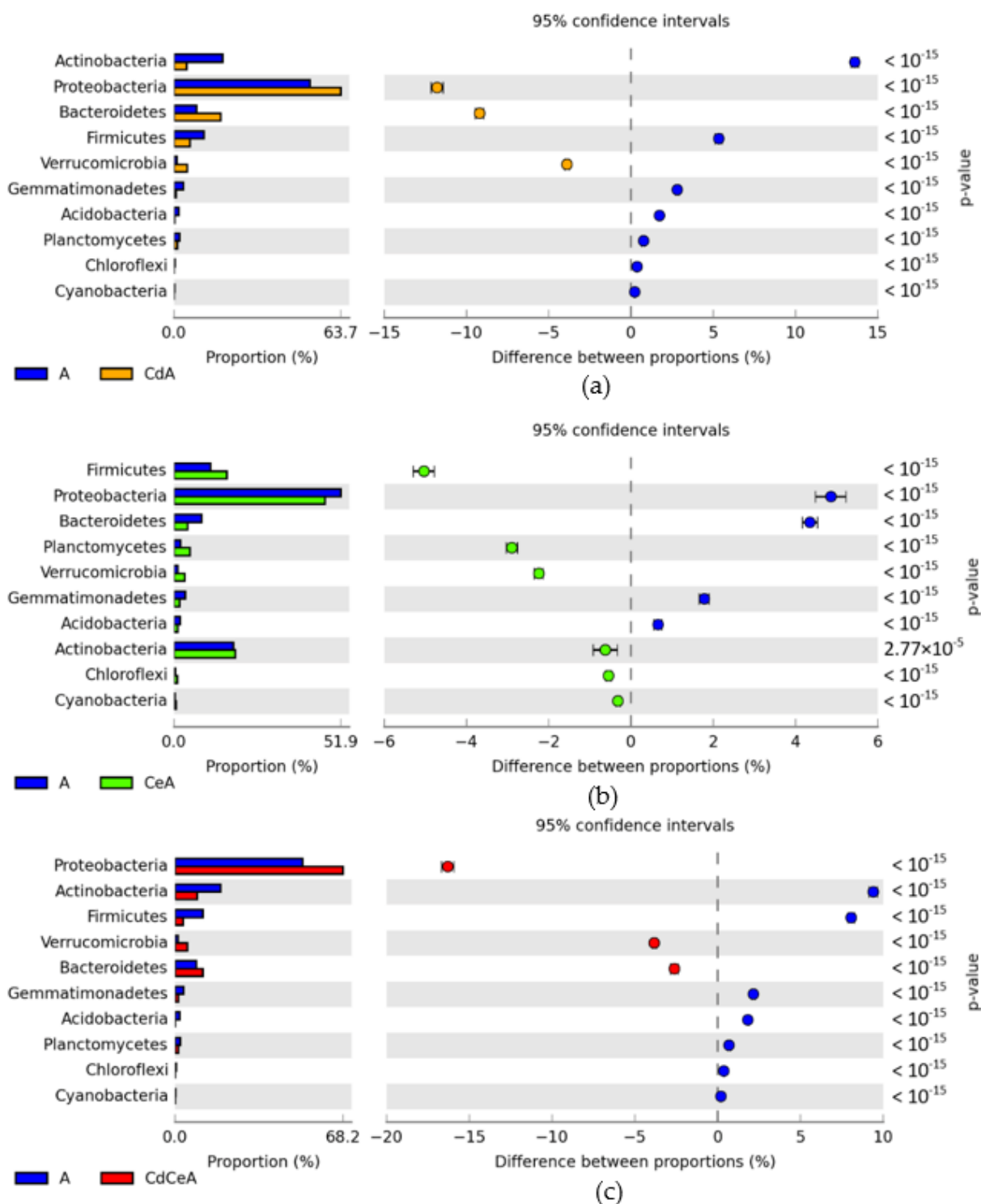


Figure 6. A comparative analysis of the effects of soil contamination with cadmium (a), soil fertilization with cellulose (b), and soil contamination with cadmium + soil fertilization with cellulose (c) on changes in bacterial phyla in the soil sown with *Avena sativa* L. (A). Differences between ratios of bacterial phyla, OTU $\geq 1\%$. Cd—ion Cd²⁺; Ce—cellulose.

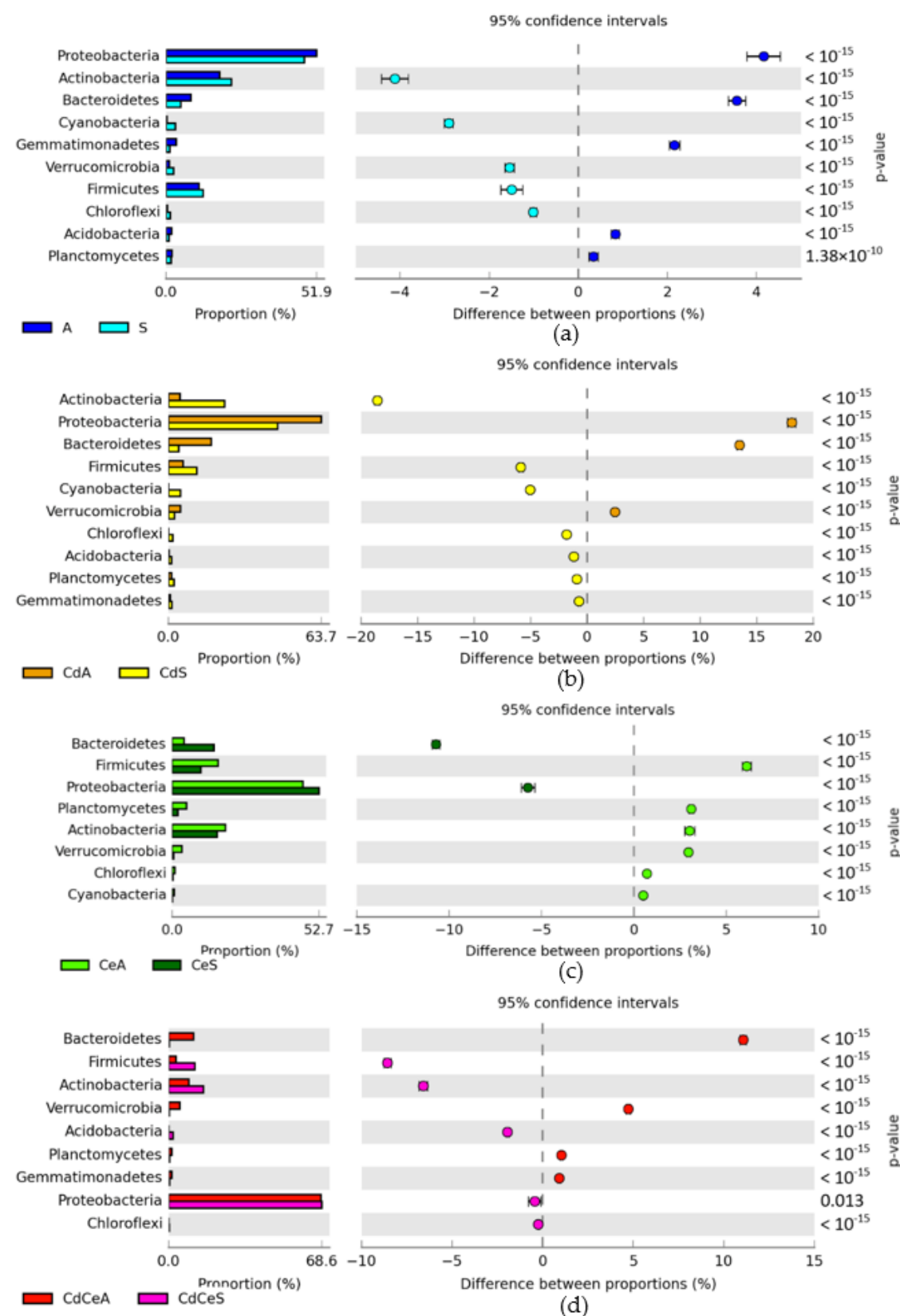


Figure 7. A comparative analysis of the effects of crop species (a), soil contamination with cadmium (b), soil fertilization with cellulose (c), and soil contamination with cadmium + soil fertilization with cellulose (d) on changes in bacterial phyla in the soil. Cd—ion Cd^{2+} ; Ce—cellulose; S—*Sinapis alba* L.; A—*Avena sativa* L.

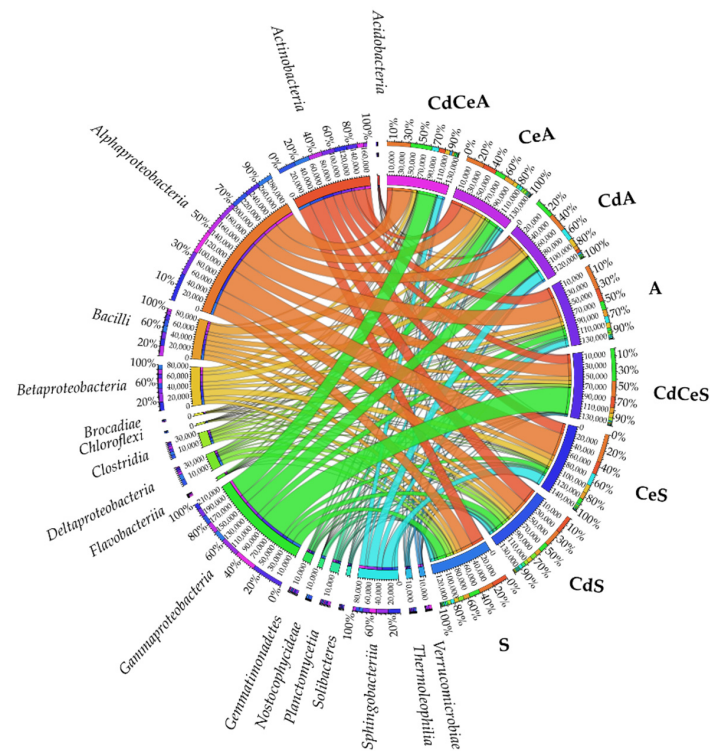


Figure 8. Relative abundance of dominating bacterial classes in the samples of soil sown with *Sinapis alba* L. (S) and *Avena sativa* L. (A). Cd—ion Cd^{2+} ; Ce—cellulose.

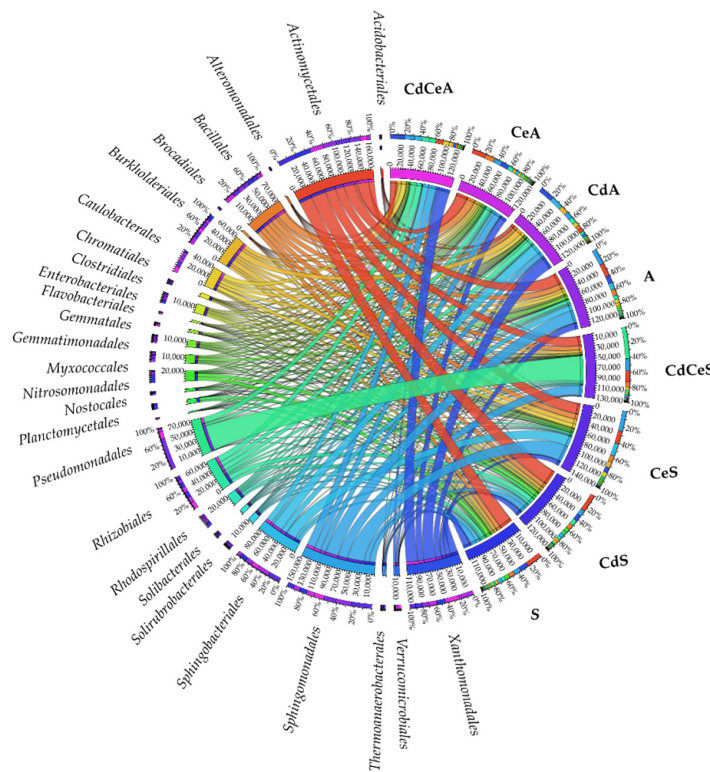
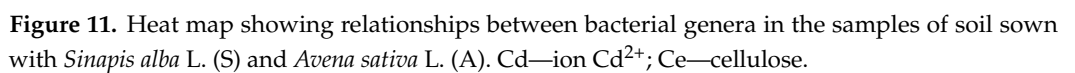
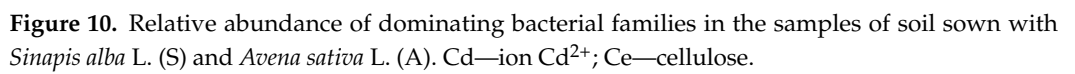


Figure 9. Relative abundance of dominating bacterial orders in the samples of soil sown with *Sinapis alba* L. (S) and *Avena sativa* L. (A). Cd—ion Cd^{2+} ; Ce—cellulose.



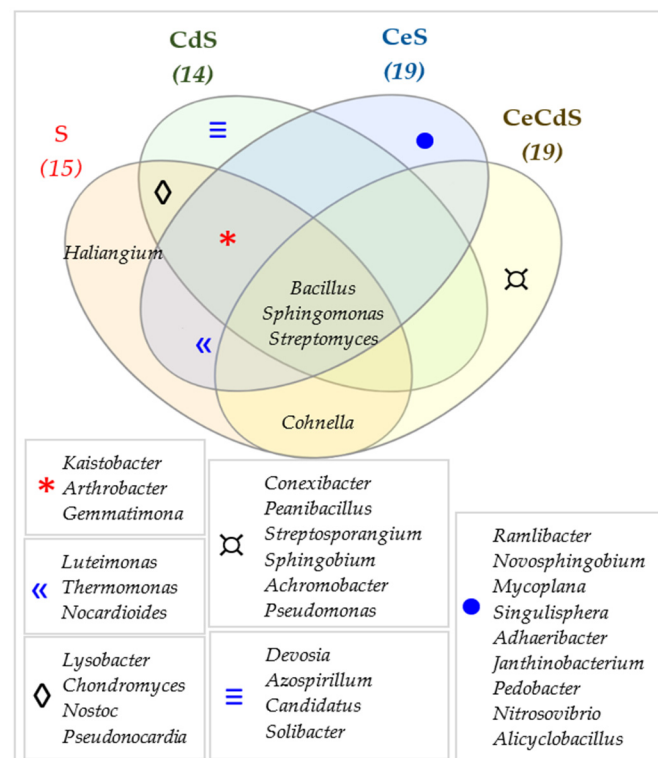


Figure 12. Venn diagram presenting unique and common bacterial genera in the samples of soil sown with *Sinapis alba* L. (S). Cd—ion Cd^{2+} ; Ce—cellulose.

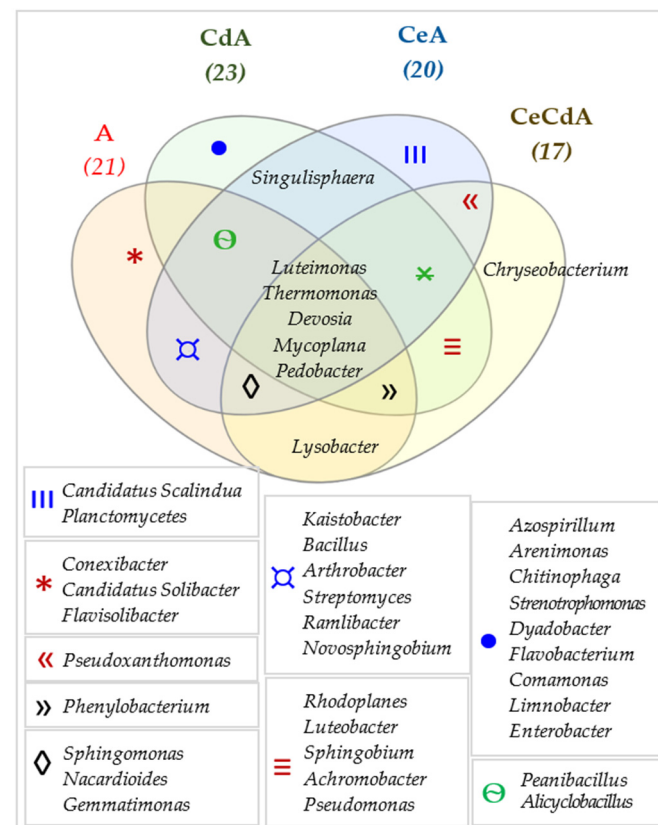


Figure 13. Venn diagram presenting unique and common bacterial genera in the samples of soil sown with *Avena sativa* L. (A). Cd—ion Cd^{2+} ; Ce—cellulose.

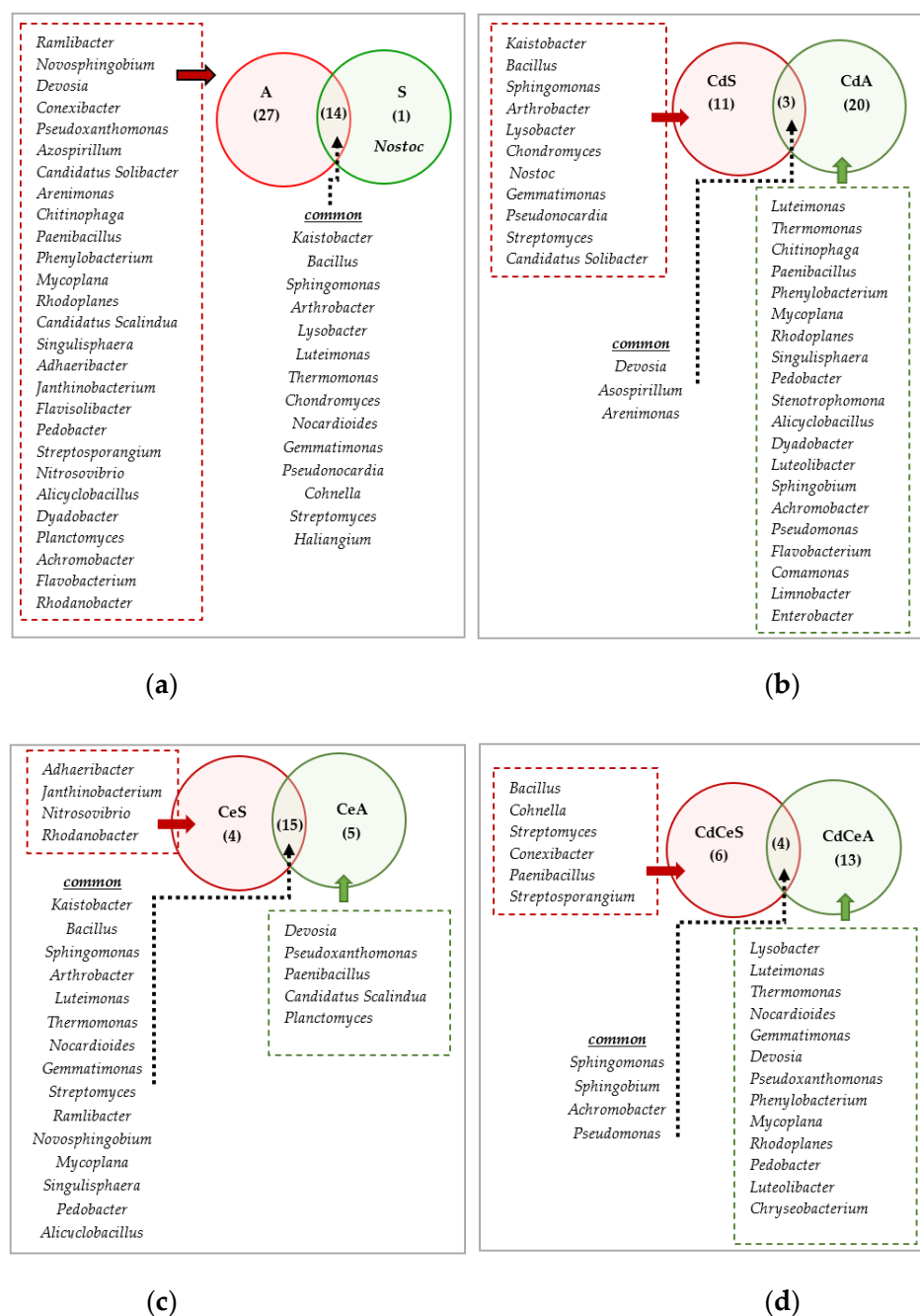


Figure 14. A comparative analysis of the effects of crop species (a), soil contamination with cadmium (b), soil fertilization with cellulose (c), and soil contamination with cadmium + soil fertilization with cellulose (d) on changes in the unique and common bacterial genera in the soil. Cd—ion Cd^{2+} ; Ce—cellulose; S—*Sinapis alba* L.; A—*Avena sativa* L.

In the control soil not fertilized with cellulose and sown with *Sinapis alba* L. (S), the *Proteobacteria* accounted for 47.71%, whereas in the cadmium-contaminated soil (CdS) accounted for 45.56%. Cellulose application promoted *Proteobacteria* proliferation, boosting their contribution in the soil microbiome to 52.74% in CeS soil and to 68.61% in CdCeS soil. Contributions of the second ranked phylum, i.e., *Actinobacteria*, in the soil microbiome were as follows: S—22.63%, CdS—23.46%, CdCeS—16.11%, and CdCeS—15.70%. In the nonfertilized and noncontaminated soil sown with *Avena sativa* L. (A), *Proteobacteria* constituted 51.87%; in the cadmium-contaminated soil (CdA), they accounted for 63.65%; in the soil fertilized with cellulose (CeA), 47.01%; whereas, in the fertilized and contaminated soil

(CdCeA) they accounted for 68.17% of all bacteria (Figure 4). *Actinobacteria* accounted for, respectively: 18.51% (A), 4.90% (CdA), 19.14% (CeA), and 9.11% (CdCeA). Soil contamination with cadmium triggered changes in the bacterial structure. It increased the OTU of *Proteobacteria* by 11.78% in the soil not fertilized with cellulose and by 16.30% in the fertilized soil.

Polysaccharide application to the soil sown with *Avena sativa* L. increased the difference in *Proteobacteria* proportion by 5%. Soil contamination with a cadmium dose of 16 mg Cd²⁺ kg^{−1} soil d.m. in the pots sown with *Avena sativa* L. (CdA) contributed to decreased abundance of *Actinobacteria* by 13.61% in the series without cellulose and by 9.4% (CdCeA) in the series with cellulose application (Figure 6). Other relatively abundant taxa identified in the noncontaminated and contaminated soil included *Firmicutes* and *Bacteroidetes*. A comparative analysis of the relative abundance of bacterial phyla in the samples of soil sown with *Sinapis alba* L. (S) and *Avena sativa* L. (A) allowed concluding that *Avena sativa* L. cultivation significantly reduced the OTU number of *Proteobacteria* (by 4.2%), while increasing that of *Actinobacteria* (by 4.2%) (Figure 7a). Exposure of the soil sown with *Avena sativa* L. to cadmium pressure decreased the relative abundance of *Proteobacteria* (by 18.1%) and *Bacteroidetes* (by 13.5%), and increased that in *Actinobacteria* (by 18.6%) (Figure 7b). Soil fertilization with cellulose (Figure 7c) promoted the proliferation of *Bacteroidetes* (OTU increase by 10.7%) and *Proteobacteria* (OTU increase by 5.7%), but suppressed that of *Actinobacteria* (by 3.0%). Cellulose application to the cadmium-contaminated soil diminished the relative abundance of *Bacteroidetes* (by 11.1%) and increased that in *Firmicutes* (by 8.6%) and *Actinobacteria* (by 6.6%) (Figure 7d).

Considering OTU $\geq 1\%$, the most representative at the class level turned out to be: *Alphaproteobacteria*, *Gammaproteobacteria*, and *Betaproteobacteria* from the phylum *Proteobacteria*; *Actinobacteria* from the phylum *Actinobacteria*; *Bacilli* from the phylum *Firmicutes*; and *Sphingobacteriia* from the phylum *Bacteroidetes* (Figure 8). The greatest differences among soil samples: A—43.785 OTU, CdA—32.744 OTU, CeA—32.760 OTU, CdCeA—42.843 OTU, S—27.133 OTU, CdS—30.325 OTU, CeS—50.320 OTU, and CdCeS—27.733 OTU, were observed in OTUs representing the class *Alphaproteobacteria*. Interestingly, in the soil sown with *Sinapis alba* L. and fertilized with cellulose (CeS) as well as in the cadmium-contaminated soil sown with *Avena sativa* L. (CdA), high OTU numbers were generated in the *Betaproteobacteria* and *Sphingobacteriia* classes.

Cadmium also affected the lower taxon, i.e., order (Figure 9). *Actinomycetales* from the phylum *Actinobacteria* turned out to be the prevailing order in the present study. Its abundance was high in all soil samples. Successive orders (in terms of abundance) representing the phylum *Proteobacteria* were predominated by *Sphingomonadales* and *Xanthomonadales*. High abundance was also demonstrated for the order *Bacillales* belonging to the phylum *Firmicutes* and the order *Sphingobacteriales* from the phylum *Bacteroidetes*. Worthy of attention is the order *Pseudomonadales* from the phylum *Proteobacteria*, which turned out to be abundant in the soil sown with *Sinapis alba* L. fertilized with cellulose and contaminated with cadmium (CdCeS).

The greatest changes identified in the structure of bacteria classified to families were observed for *Xanthomonadaceae*, *Sphingomonadaceae*, and *Sphingobacteriaceae* (Figure 10). The *Pseudomonadaceae* family bacteria turned out to be highly abundant in the soil samples contaminated with cadmium, fertilized with cellulose, and used to grow both crops studied (CdCeS and CdCeA).

The OTU number assigned to individual bacterial genera was found to depend on crop species grown, cellulose fertilization, and soil contamination with cadmium (Figure 11). The prevailing genus in the soil sown with *Sinapis alba* L. turned out to be: *Kaistobacter*, *Bacillus*, *Sphingomonas*, *Arthrobacter*, *Lysobacter*, and *Luteimonas*. Soil contamination with cadmium led to diminished abundance of the *Arthrobacter*, *Lysobacter* and *Luteimonas* genus bacteria and increased abundance of *Nostoc* and *Devosia*, whereas cellulose application to the noncontaminated soil had the most beneficial effect on the *Kaistobacter*, *Pedobacter*, *Mycoplana*, and *Novosphingobium* genus bacteria. This positive impact of cellulose on

the mentioned bacterial genera was substantially diminished by soil contamination with cadmium. Bacteria most abundant in the soil sown with *Avena sativa* L. were representatives of the following genera: *Kaistobacter*, *Bacillus*, *Gemmatimonas*, *Luteimonas*, *Sphingomonas*, and *Streptomyces*. Except for *Luteimonas*, all of them were adversely affected by cadmium. In contrast, soil contamination with cadmium promoted the development of bacteria from the following genera: *Pedobacter*, *Sphingobium*, *Achromobacter*, *Pseudomonas*, *Arenimonas*, *Rhodoplanes*, *Luteimonas*, and *Thermomonas*. Soil fertilization with cellulose modified the OTUs of individual bacterial genera, but did not mitigate the adverse effects of cadmium on *Kaistobacter*, *Bacillus*, *Gemmatimonas*, *Sphingomonas*, and *Streptomyces*.

The Venn analysis allowed identifying unique bacterial genera typical of particular soils under study (Figures 12–14). In the case of the soil sown with *Sinapis alba* L., the prevailing genus identified in the noncontaminated and nonfertilized samples (S) turned out to be *Haliangium*; the prevailing genera in the cadmium-contaminated soil (CdS) included: *Devosia*, *Azospirillum*, *Candidatus*, and *Solibacter*; those in the soil with cellulose addition (CeS) included: *Ramlibacter*, *Novosphingobium*, *Mycoplana*, *Singulisphaera*, *Adhaeribacter*, *Janthinobacterium*, *Pedobacter*, *Nitrosovibrio*, and *Alicyclobacillus*; whereas, those identified in the soil additionally contaminated with cadmium (CeSCd) included: *Conexibacter*, *Paenibacillus*, *Streptosporangium*, *Sphingobium*, *Achromobacter*, and *Pseudomonas*.

The common genera for all soil samples included: *Bacillus*, *Sphingomonas*, and *Streptomyces* (Figure 12). In the case of the soil sown with *Avena sativa* L., *Conexibacter*, *Candidatus*, *Solibacter*, and *Flavisolibacter* were the unique genera in the noncontaminated soil samples (A); *Candidatus*, *Scalindua*, and *Planctomyces*—in the noncontaminated soil fertilized with cellulose (CeA); *Azospirillum*, *Arenimonas*, *Chitinophaga*, *Stenotrophomonas*, *Dyadobacter*, *Flavobacterium*, *Comamonas*, *Limnobacter*, and *Enterobacter*—in the cadmium-contaminated soil (CdA); and *Chryseobacterium*—in the soil fertilized with cellulose and contaminated with cadmium (CeCdA).

In turn, *Luteimonas*, *Thermomonas*, *Devosia*, *Mycoplana*, and *Pedobacter* turned out to be common genera for all soil samples. The first four belong to the phylum *Proteobacteria*, whereas the latter to the phylum *Bacteroidetes* (Figure 12). Figure 14 depicts unique bacterial genera and those common for crop species, for soils fertilized with cellulose, for those contaminated with cadmium, and for those contaminated with cadmium and fertilized with cellulose. In total, 14 genera were identified as common for both crops (A and S), i.e.: *Kaistobacter*, *Bacillus*, *Sphingomonas*, *Arthrobacter*, *Lysobacter*, *Luteimonas*, *Thermomonas*, *Chondromyces*, *Nocardioides*, *Gemmatimonas*, *Pseudonocardia*, *Cohnella*, *Streptomyces*, and *Haliangium*. Three common genera were identified in the soils contaminated with cadmium (CdA and CdS): *Devosia*, *Azospirillum*, and *Arenimonas*. The common microbiome of the soils sown with *Sinapis alba* L. and *Avena sativa* L. fertilized with cellulose (CeA and CeS) included 15 genera: *Kaistobacter*, *Bacillus*, *Sphingomonas*, *Arthrobacter*, *Luteimonas*, *Thermomonas*, *Nocardioides*, *Gemmatimonas*, *Streptomyces*, *Ramlibacter*, *Novosphingobium*, *Mycoplana*, *Singulisphaera*, *Pedobacter*, and *Alicyclobacillus*, whereas that found in the soils contaminated with cadmium and fertilized with cellulose (CdCeA and CdCeS) included four genera: *Sphingomonas*, *Sphingobium*, *Achromobacter*, and *Pseudomonas*.

In summary of the above findings, it may be concluded that the changes observed in the genetic diversity of bacteria upon the influence of soil contamination with cadmium and its fertilization with cellulose, and upon cultivation of *Sinapis alba* L. and *Avena sativa* L. were reflected in the values of the Shannon–Wiener (Table 7) and Simpson (Table 8) indices.

Table 7. Effect of cadmium (Cd) and cellulose (Ce) on the values of Shannon–Wiener diversity index of bacteria in the soil.

Taxa	S	CdS	CeS	CdCeS	A	CdA	CeA	CdCeA
Phylum	1.777 ^e	1.845 ^e	1.493 ^e	1.095 ^c	1.635 ^e	1.248 ^e	1.785 ^e	1.220 ^d
Class	2.605 ^d	2.614 ^d	2.118 ^d	1.922 ^b	2.333 ^d	2.125 ^d	2.562 ^d	2.035 ^c
Order	3.138 ^c	3.146 ^c	2.573 ^c	2.280 ^b	2.865 ^{cd}	2.755 ^{cd}	3.198 ^c	2.728 ^{bc}
Family	4.019 ^b	3.999 ^b	3.346 ^b	2.799 ^{ab}	3.631 ^b	3.254 ^b	4.001 ^b	3.187 ^b
Genus	4.608 ^a	4.559 ^a	3.988 ^a	3.137 ^a	4.324 ^a	4.036 ^a	4.614 ^a	3.902 ^a

S—*Sinapis alba* L., A—*Avena sativa* L. Identical letters (a–e) in columns denote the same homogeneous groups.

Table 8. Effect of cadmium (Cd) and cellulose (Ce) on the values of Simpson diversity index of bacteria in the soil.

Taxa	S	CdS	CeS	CdCeS	A	CdA	CeA	CdCeA
Phylum	0.738 ^c	0.762 ^d	0.675 ^d	0.515 ^d	0.698 ^d	0.565 ^d	0.742 ^c	0.528 ^d
Class	0.891 ^b	0.892 ^c	0.824 ^c	0.773 ^c	0.850 ^c	0.831 ^c	0.883 ^b	0.800 ^c
Order	0.925 ^{ab}	0.923 ^{bc}	0.881 ^b	0.805 ^b	0.907 ^b	0.904 ^b	0.930 ^{ab}	0.908 ^b
Family	0.963 ^a	0.958 ^a	0.923 ^{ab}	0.824 ^a	0.943 ^{ab}	0.929 ^{ab}	0.963 ^a	0.925 ^{ab}
Genus	0.959 ^a	0.945 ^a	0.950 ^a	0.832 ^a	0.963 ^a	0.965 ^a	0.967 ^a	0.958 ^a

S—*Sinapis alba* L., A—*Avena sativa* L. Identical letters (a–d) in columns denote the same homogeneous groups.

The Shannon–Wiener and Simpson indices are one of the most popular indices used to determine biodiversity. They provide information about population numbers and homogeneity of organisms constituting a given community. The higher their values are, the greater is the diversity. Cadmium exposure decreased the value of the Shannon–Wiener index in the soil sown with *Avena sativa* L., but did not modify it in the soil sown with *Sinapis alba* L. (Table 7). In turn, cellulose application to the noncontaminated soil used to grow *Sinapis alba* L. diminished the genetic diversity of bacteria at all taxonomic levels, while an opposite effect was observed in the soil sown with *Avena sativa* L. Cellulose application to the cadmium-contaminated soil did not improve bacterial diversity, regardless of crop species grown in the soil.

The Simpson’s index values confirm the effects of cadmium and cellulose on bacterial diversity (Table 8) indicated by Shannon–Wiener index values.

4. Discussion

4.1. Plant Response to Soil Contamination with Cadmium and Fertilization with Cellulose

Although cadmium is commonly believed to be a toxic element inhibiting plant growth and development [61], some authors [30,31,62] have concluded that its excess in the soil elicited a positive effect on the growth and development of grasses and legumes. In the study by Xu and Wang [63], *Poa pratensis* and *Festuca arundinacea* responded with a growth of aerial parts to cadmium doses of 50 and 100 mg Cd²⁺ kg^{−1}, whereas Zhang et al. [64] reported the growth of aerial parts of *Pennisetum americanum* and *Pennisetum purpureum* in response to cadmium doses ranging from 1 to 8 mg Cd²⁺ kg^{−1}. Both the main crop—*Sinapis alba* L. and the successive crop—*Avena sativa* L., tested in the present study, were relatively resistant to the applied cadmium doses. This resistance may be due to greater tolerance of certain species belonging to *Poaceae* and *Brassicaceae* families to the contamination with this heavy metal developed through, e.g., synthesis of protein substances (phytochelators), which neutralize cadmium by its fixation [65,66]. The present study results and the findings reported by Deram et al. [67]; Aibibu et al. [68]; Zhang et al. [69]; Xu, Wang [63]; Guo et al. [70]; Zhang et al. [71]; Quezada Hinojosa et al. [72]; and Stanisławska-Głubiak et al. [73] prove that greater amounts of cadmium are accumulated in the roots than in aerial parts of plants. This may be due to the activation of defense mechanisms counteracting stress posed by cadmium presence in the soil, which is manifested by the

inhibited transport of this heavy metal from roots to aerial parts [74]. Soil fertilization with cellulose mitigated the adverse effects of cadmium only in the case of *Sinapis alba* L., whereas the response of *Avena sativa* L. to cadmium was similar regardless of polysaccharide application. This is most likely due to variable physical stability resulting from various abnormalities, such as, e.g., collapses, microfibril kinks or voids (micropores), and the low capacity of unmodified cellulose to adsorb heavy metals [75–77]. Summarizing, it may be concluded that cellulose mitigated the adverse effect of cadmium on the growth of aerial parts only in the case of the main crop—*Sinapis alba* L., whereas the response of the successive crop—*Avena sativa* L., was similar, regardless of soil amendment with the polysaccharide.

4.2. Response of Microorganisms to Soil Contamination with Cadmium and Fertilization with Cellulose

Microorganisms not only play a key role in the course of biochemical processes in soil but may also affect the health status of plants by synthesizing phytohormones, increasing availability of nutrients, facilitating nutrient uptake by plants, and reducing toxicity of heavy metals in plants [28,29]. However, these functions may be impaired by various pollutants, such as heavy metals (including cadmium), pervading the natural environment and may be manifested by reduced abundance and diversity of microorganisms, which significantly affects the appropriate soil quality [6,9,10]. According to Zaborowska et al. [78] and Haddad et al. [35], cadmium may exert various effects on the growth and development of microorganisms; however, these effects are mainly determined by the contamination level. The present study results demonstrate that cadmium contributed to diminished abundance of organotrophic bacteria, actinobacteria, and fungi, as indicated by negative coefficients of correlation between the extent of soil contamination with cadmium and population numbers of the studied microorganisms, and by negative values of the cadmium effect index (IF_{Cd}). Cadmium not only affects the abundance of soil microbiota but may also trigger changes in their biodiversity [79]. Regardless of cadmium dose, in the case of the soil samples not amended without cellulose, higher values of the colony development index (CD) were determined for organotrophic bacteria and actinobacteria in pots sown with *Sinapis alba* L., and those of fungi in those sown with *Avena sativa* L. Soil fertilization with cellulose exerted various effects on the microorganisms as it decreased CD of bacteria, increased that in fungi, and had no significant effect on CD of actinobacteria. Values of the ecophysiological diversity index (EP) also changed upon cadmium influence. This heavy metal contributed to lower EP values computed for organotrophic bacteria and actinobacteria, and to higher EP values noted for fungi in the soil not fertilized with cellulose, whereas its effects varied in the fertilized soil. Boros-Lajszner et al. [50,80] reported similar results in their studies into the effect of nickel, cobalt, and cadmium on soil microbiome diversity. Similar conclusions were formulated by Wyszowska et al. [49] in their experiment with heavy metals. Soil microorganisms usually respond rapidly to changes in the environment; hence, they are deemed to be viable indicators of soil quality and fertility [81,82]. In the present study, cellulose application to the soil increased the ecophysiological diversity of organotrophic bacteria. This increase was due to the fact that the organotrophs were predominated by r-strategists [42]. Organotrophic bacteria respond rapidly to the supply of nutrients. Hence, they play an important role in cellulose degradation to simple sugars [35,37].

Rhizospheres of *Sinapis alba* L. and *Avena sativa* L. were characterized by a specific composition of bacterial communities, which suggests that each species may develop a unique microbiome in the soil contaminated with cadmium [83]. Despite the above finding, the prevailing bacterial phyla identified in the soil used to grow both crop species were *Proteobacteria* and *Actinobacteria*, followed by *Firmicutes* and *Bacteroidetes*. This is consistent with findings reported by Hou et al. [84], Fu et al. [85], and Wang et al. [86]. According to Min et al. [87], bacteria belonging to these phyla play a key role in the restoration of microbial communities in the cadmium-contaminated soil. In the present study, the relative

abundance of *Proteobacteria* and *Actinobacteria* was higher in the soil fertilized with cellulose. As posited by Li et al. [88], this may suggest that the higher organic matter content facilitates bacteria survival in extreme conditions [89]. Microorganisms which respond rapidly to changes in nutrients in the rhizosphere are fast-growing microorganisms (r-strategists) [90]. *Actinobacteria*—being k-strategists, are the so-called slow-growing microorganisms [91,92] and together with *Firmicutes*, *Bacteroidetes* and *Acidobacterie* may be actively engaged in organic matter transformation [93,94]. Therefore, a hypothesis may be advanced that the changes in bacteria abundance may increase tolerance to and accumulation of cadmium in the soil sown with *Sinapis alba* L. and *Avena sativa* L.

In the present study, the most tolerant to soil contamination with Cd^{2+} turned out to be *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria* from the phylum *Proteobacteria* together with *Actinobacteria* and *Bacilli* from the phylum *Firmicutes*. Additionally, Duan et al. [95] confirmed their high tolerance to contamination with Cd^{2+} . Previous investigations by Liang et al. [96] and Borowik et al. [97] proved high resistance of the bacteria from *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria* to contaminants.

The present study results showed that soil contamination with cadmium led to a reduction in OTU numbers of bacteria representing the *Luteimonas*, *Thermomonas*, and *Devosia* genera from the phylum *Proteobacteria*, together with *Nostoc* bacteria belonging to the phylum *Cyanobacteria*. The bacteria from the genus *Luteimonas* and *Thermomonas*, and *Nocardioide*s belonging to the phylum *Actinobacteria*, were also highly resistant to soil contamination with Cd^{2+} . Bacteria from the genus *Luteimonas* were identified in soils amended with compost produced from food waste [98], in wastewater [99], in dump soils [100], and in fresh litter material at poultry farms [101], which suggests their high capability for degradation of insoluble large-molecular-weight organic matter. Likewise, *Thermomonas* genus bacteria are involved in degradation of sugar cane straw and goat manure [102]. These bacteria also colonize soils contaminated with heavy metals [50,80] and petroleum-based products [103–106], exhibiting a high bioremediating potential. In our previous studies [50,80], the *Thermomonas* genus bacteria also showed high tolerance to soil contaminated with Ni^{2+} , Co^{2+} , and Cd^{2+} , and therefore should be perceived as potential microorganisms useful in bioaugmentation of soils contaminated with these heavy metals. Bacteria from the genus *Nocardioide*s degrade aromatic amines, using them as a source of carbon, nitrogen, and energy. They boost their growth with vitamins, which are an indispensable micronutrient aiding the biodegradation process [107]. Microorganisms using C from the organic material take the greatest part in modeling ecosystems because they facilitate organic matter degradation and increase humus content by degrading cellulose, thereby reinforcing interactions between microorganisms [108]. In the present study, the fertilization of cadmium-contaminated soil with cellulose had definitely the most positive effect on the bacteria from *Kaistobacter* *Mycoplana*, and *Novosphingobium* genera belonging to the class *Alphaproteobacteria*, phylum *Proteobacteria*, together with *Pedobacter* belonging to the class *Sphingobacteriia*, phylum *Bacteroidetes*.

An advantage of the *Kaistobacter* genus bacteria is their potential for alleviating plant diseases, e.g., in tobacco [109]; *Mycoplana* genus bacteria were demonstrated to positively affect cultivation of orchard grass in petroleum-polluted soil [110], whereas *Pedobacter* bacteria enhanced tomato resistance to bacterial wilting [111]. This fundamental importance of reducing the risk of plant disease development in sustainable agriculture is suggested by a possible approach to modeling disease suppression rates [109,112]. The analysis of microorganisms at the genus level allowed identifying bacteria from the *Sphingomonas*, *Sphingobium*, *Achromobacter*, and *Pseudomonas* genera belonging to the phylum *Proteobacteria*, which were common for the soils sown with *Sinapis alba* L. and *Avena sativa* L. fertilized with cellulose and contaminated with cadmium. According to Altimira et al. [112] and Nilgiriwala et al. [113], *Sphingomonas* encode genes of multiple oxidases and enable Cd recovery from soil by means of enzymatic bioprecipitation. Likewise, the species belonging to the genus *Pseudomonas* are usually involved in the nitrogen cycle and contaminant degradation, and may enhance plant growth and development [114,115]. In turn, Gomez-

Balderas et al. [116] demonstrated that soil contamination with Cd^{2+} might significantly diminish the relative abundance of *Pseudomonas*.

Qiong et al. [117] posited that bacteria response to cadmium contamination depended on soil type. The fluvoaquic soil severely contaminated with cadmium was mainly populated by *Novosphingobium*, *Sphingobium*, *Pseudomonas*, *Rheinheimera*, and *Corynebacterium* genera. Therefore, it is very important to continue research that would inform about the response of microorganisms in different types of soil and different regions, to identify the possibilities of improving the condition of contaminated soils.

5. Conclusions

Soil contamination with cadmium doses ranging from 4 to 16 $\text{mg Cd}^{2+} \text{ kg}^{-1}$ soil did not impair the growth and development of *Sinapis alba* L. and *Avena sativa* L., which proves that both crop species may be harnessed for the phytoremediation of soils contaminated with this metal. Given the greater capability of *Avena sativa* L. than *Sinapis alba* L. for Cd^{2+} accumulation, it was found more viable for soil remediation from the ecological standpoint as it reduces the risk of cadmium penetration into the food chain. Although Cd^{2+} -contaminated soil fertilization with cellulose stimulated the proliferation of microorganisms, it did not mitigate the adverse effect of Cd^{2+} on bacterial diversity. Soil phytoremediation by *Avena sativa* L. may be aided by biostimulation with cellulose coupled with bioaugmentation using *Chryseobacterium* bacteria, whereas phytoremediation by *Sinapis alba* L., by bioaugmentation with the *Sphingomonas*, *Sphingobium*, *Achromobacter*, and *Pseudomonas* genera bacteria, which represented the unique microbiome in the soil contaminated with Cd^{2+} and fertilized with cellulose.

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