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Calorific Value of *Festuca rubra* Biomass in the Phytostabilization of Soil Contaminated with Nickel, Cobalt and Cadmium Which Disrupt the Microbiological and Biochemical Properties of Soil

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Abstract: The choice of optimal plant species for phytoremediation and organic fertilization plays an important role in stabilizing the functions of soils contaminated with heavy metals. The influence of nickel, cobalt and cadmium on the biomass yield and calorific value of *Festuca rubra*, heavy metal concentrations in soil and plants and the microbiological, biochemical and physicochemical proprieties of soil were analyzed in a pot experiment. The tolerance index (TI) describing *Festuca rubra*'s ability to tolerate heavy metals, as well as the translocation (TF), accumulation (AF) and bioaccumulation (BF) factors of heavy metals in *Festuca rubra* were calculated. The experiment was conducted in two series: In soil fertilized and not fertilized with compost. Nickel and cobalt significantly inhibited the growth and development of *Festuca rubra*. The experiment demonstrated that this plant species can be grown on soil contaminated with heavy metals. *Festuca rubra* contained on average 46.05% C, 34.59% O, 5.91% H, 3.49% N, 0.19% S and 9.76% ash. *Festuca rubra* has a stable calorific value which is not affected by heavy metals; therefore, biomass harvested from heavy metal-polluted soil can be used for energy generation. The calorific value of *Festuca rubra* ranged from 15.924 to 16.790 MJ kg^{-1} plant d.m., and the heat of combustion from 17.696 to 18.576 MJ kg^{-1}. It has a stable calorific value which is not affected by heavy metals, therefore biomass harvested from heavy metal-polluted soil can be used for energy generation. *Festuca rubra* is particularly useful for the phytostabilization of soil contaminated with cadmium and cobalt. Compost minimizes the adverse effects of heavy metal pollution on the microbiological, biochemical and physicochemical properties of soil.

Keywords: heat of combustion; calorific value of grass; heavy metals; soil biological properties

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1. Introduction

Plants have the ability to accumulate heavy metals and can be used to remove these pollutants from the soil environment. This process is known as phytoremediation [1]. Plants used for phytoremediation should be characterized by a high growth rate, high biomass yields, high accumulation, tolerance to heavy metals, resistance to pathogens and pests and adaptability to changing climatic conditions [1,2]. *Festuca rubra* L. (red fescue) is a plant of the family Poaceae that can be used to establish vegetative cover on post-mining land and spoil tips. The species has low trophic requirements and is resistant to adverse climatic conditions [3,4]. *Festuca rubra* is used in assisted phytoremediation [5]. According to Prasad and Freitas [3] and Gołda and Korzeniowska [4], *Festuca rubra* is a suitable species for the remediation of heavy metal-polluted soils in industrial sites such as mines or power plants. *Festuca rubra* readily accumulates Cu, Pb, Mn and Zn [6–8]; therefore, it is suitable for phytoextraction and phytostabilization of contaminated environments [9–11].

Phytoextraction is a phytoremediation process where heavy metals are removed from the soil and accumulated in shoots [1,12]. In turn, phytostabilization immobilizes pollutants in the root zone and prevents their further migration [13–15]. Assisted phytostabilization is a method that removes heavy metals from the soil, reduces their bioavailability and restores the biological quality of soil [16]. During this process, heavy metals are chemically stabilized by selected plant species, and soil quality is enhanced through the addition of substances such as compost, iron and manganese oxides or hydroxides, aluminosilicates, clay absorbents and zeolites [1,17,18].

Grasses are highly suitable for energy generation because they efficiently convert solar energy to biomass and are characterized by very high dry matter yield. The suitability of plant biomass for energy production is determined not only by its yield, but also by its calorific value, heat of combustion and chemical composition. These parameters considerably influence biomass processing and the quality of the end product [19]. Grass biomass has a high calorific value in the range of 14–18 MJ kg^{-1}, and equally importantly, its ash content is low after combustion [20]. Energy crops should also be characterized by high nutrient and water use efficiency, as well as a favorable energy balance between energy inputs and outputs. These crops should also be resistant to pests, disease and adverse environmental conditions [21].

Biomass is the oldest and the most popular source of renewable energy. It involves solid and liquid substances of plant and animal origin, and biodegradable wastes from agricultural production, forestry and the agri-food industry [22]. Plant- and animal-derived biomass can be processed into biofuels or directly incinerated [23]. In addition to biomass from dedicated energy crops, agricultural by-products (such as straw or other plant parts that are not processed into food or feed, surplus grass, poultry manure, slurry and slaughterhouse wastes) can be also used for energy generation [24].

The biomass of energy crops differs considerably in physicochemical properties, including state of matter, volume, moisture content and calorific value. Therefore, specialist logistic operations and processing treatments are required to optimize these parameters [25]. Different types of biomass also require specific conversion processes [26–30].

The results of this study could facilitate the selection of energy crops for cultivation on fallow land, in former industrial sites and on contaminated soil. These energy crops can be used for biofuel production. The cultivation of energy crops in polluted areas also has positive social implications.

Microorganisms also play an important role in increasing the phytoremediation potential of plants [1]. The bioavailability of heavy metals determines the extent to which these pollutants can be effectively removed from the soil. Factors such as the granulometric composition, pH, moisture content, organic matter content and redox potential of soil affect the bioavailability of heavy metals in the soil environment. For this reason, heavy metal concentrations in the bioavailable fraction can differ in soils with similar pollution levels. Numerous research studies have demonstrated that microorganisms play an important role in phytoremediation by secreting specific metabolites, siderophores and ligands [31–33]. A key role is played by endophytes and rhizosphere bacteria [16]. Mycorrhizal fungi can be also applied in assisted phytoremediation of long-term contaminated soils [34,35]. Microorganisms also secrete enzymes that facilitate the decomposition of plant residues, in particular cellulose and lignin degradation, and promote the cycling of phosphorus, nitrogen and sulfur compounds [36]. The biochemical properties of soil are also influenced by endoenzymes (intracellular enzymes) and exoenzymes (extracellular enzymes). The activity of dehydrogenases is a robust indicator of the respiratory activity of soil microorganisms [37]. Heavy metals can inhibit the activity of these enzymes by as much as 90%. Catalase, urease, β-glucosidase, arylsulfatase, alkaline phosphatase and acid phosphatase are also sensitive to heavy metals [38–41].

In Poland, the threshold values for heavy metals in soil are set by the Regulation of the Minister of the Environment of 1 September 2016 on the method of evaluating soil pollution [42]. The regulation divides land into various land-use categories depending on soil contamination levels. Four groups of land were identified. Cadmium content ranges from 2 (groups I and II) to 15 mg kg^{-1} (group IV); cobalt content ranges from 20 (group II) to 50 mg kg^{-1} (group IV); and nickel content ranges from 100 (group II) to 500 mg kg^{-1} (group IV).

The present study contributes new knowledge about changes that occur in soils contaminated with Ni^{2+}, Co^{2+} and Cd^{2+} and the phytoremediation potential of *Festuca rubra*. The results were used to determine the effectiveness of *Festuca rubra* as a hyperaccumulator [3,4] in soil fertilized with compost. The novelty of the presented research stems from the fact that soil processes were analyzed comprehensively by describing the impact of heavy metals on *Festuca rubra* plants, the role played by microorganisms and enzymes and the physicochemical properties in soil subjected to assisted phytoremediation.

The aim of this study was to determine: (1) The biomass yield and calorific value of *Festuca rubra*, (2) heavy metal content of soil and plants, (3) microbial counts in soil, (4) changes in the structure and diversity of cultured microorganisms, (5) activity of soil enzymes, (6) physicochemical properties of soil.

2. Materials and Methods

2.1. Soil Samples

Soil for the experiment was sampled in Tomaszkowo near Olsztyn, Region of Warmia and Mazury, Poland (53.716° N, 20.3969° E). Soil samples (Eutric Cambisols) were collected from arable land at a depth of 0–20 cm. The analyzed soil had the following granulometric composition: Sand—69.41%; silt—27.71%; clay—2.88%. Soil pH (1 mol KCl dm^{-3}) was 7.0. The following soil parameters were determined: Organic carbon content (C_{org})—14.30 g; total nitrogen content (N_{total})—0.98 g; hydrolytic acidity (HAC)—6.40 $mmol^{(+)}$; exchangeable base cations (EBS)—165.90 $mmol^{(+)}$; and cation exchange capacity (CEC)—172.30 $mmol^{(+)}$ in 1 kg of soil dry matter (d.m.). Base saturation (BS)v was 96.28%. The compost used in the experiment was produced by Ekokonsorcjum-Effekt (Kraków, Poland; license No. 21/02) from green waste (grass, shrubs) collected in parks, green squares, home gardens; fruits and vegetables collected in markets; and organic waste from food processing plants. Compost had the following chemical composition (g kg^{-1} d.m.): Organic carbon—232.0; total nitrogen—13.0; phosphorus—2.6; potassium—12.4; magnesium—3.0; calcium—14.3.

2.2. Experimental Design

A greenhouse experiment was conducted in a greenhouse, in 3.5 dm^3 pots, in four replications. The experimental variables were: (1) Soil contamination with heavy metals—Ni^{2+}, Co^{2+} and Cd^{2+}; (2) compost applied at a rate of 0 and 10 g kg^{-1}; (3) soil use—pots sown and not sown with *Festuca rubra*. Soil contamination levels were as follows: Control was uncontaminated soil, and soil contaminated with 400 mg Ni^{2+} kg^{-1}, 80 mg Co^{2+} kg^{-1}, and 8 mg Cd^{2+} kg^{-1} soil. Heavy metal contamination levels were selected based on the work of Barbieri [43], Kabata-Pendias and Mukherjee [44], Marchand et al. [45] and Nadgórska-Socha et al. [46–48]. Nickel was applied as $NiCl_2 \cdot 6H_2O$; cobalt—as $CoCl_2 \cdot 6H_2O$; and cadmium—as $CdCl_2 \cdot 2^1/_2H_2O$. All treatments were fertilized with the following chemical compounds (in mg kg^{-1} soil): $CO(NH_2)_2$—150.07; KH_2PO_4—131.81 mg; and $MgSO_4 \cdot 7H_2O$—101.41. Soil was thoroughly homogenized with heavy metals, fertilizers and compost, and it was placed in pots (3.5 kg soil d.m. per pot). Soil was brought to a moisture content of 60% (capillary water capacity). In the experimental series involving *Festuca rubra*, 10 plants were sown per pot. Soil moisture content was kept constant at 60% throughout the growing season. *Festuca rubra* was harvested three times during the growing season, and the dry matter yield of aerial plant parts was determined. The root yield of *Festuca rubra* was also determined after the third harvest. Calorific value and the content of nickel, cobalt and cadmium were determined in aerial plant parts, whereas roots were analyzed only for heavy metal concentrations. After harvest, soil samples were collected for microbiological, enzymatic, physicochemical and chemical analyses.

2.3. Microbiological Analyses of Soil Samples

The counts of organotrophic bacteria, actinobacteria and fungi were determined by serial dilution. All analyses were performed in four replicates. Organotrophic bacteria were plated on the growth medium proposed by Bunt and Rovira [49], actinobacteria were on the growth medium developed by Kusters and Williams with the addition of nystatin and actidione antibiotics [50] and fungi were on Martin's agar [51]. The cultures were incubated at a temperature of 28 °C. The emerged colonies were counted daily for 10 days.

2.4. Biochemical Analyses of Soil Samples

Soil samples were analyzed for enzyme activity: Dehydrogenase (Deh)—according to the method developed by Lenhard and modified by Öhlinger [52]; catalase (Cat), urease (Ure), β-glucosidase (Glu) and arylsulfatase (Aryl)—according to the method proposed by Alef and Nannipieri [53]; acid phosphatase (Pac) and alkaline phosphatase (Pal)—according to the method of Alef and Nannipieri [53] and Eivazi and Tabatabai [54] The following substrates were used in measurements of enzyme activity: Dehydrogenase—3% 2,3,5-triphenyl-tetrazolium chloride; catalase—3% hydrogen peroxide; urease—10% urea, acid and alkaline phosphatase—4-nitrophenyl phosphate disodium salt hexahydrate; β-glucosidase—4-nitrophenyl β-D-glucopyranoside; arylsulfatase—4-nitrophenyl phosphate disodium salt hexahydrate. The procedures for determining the activity of the analyzed soil enzymes have been described in detail by Zaborowska et al. [55].

2.5. Physicochemical and Chemical Analyses of Soil Samples

The granulometric composition of soil was measured with an aerometer [56,57]; soil pH was determined by potentiometric titration in aqueous KCl solution with a concentration of 1 mol·dm^{-3} [58]; organic carbon content was determined by Tiurin's method [59]; total nitrogen content—by the Kjeldahl method [60]; hydrolytic acidity (HAC) and exchangeable base cations (EBC)—according the method proposed by Kappen [61]; cation exchange capacity (CEC) and base saturation (BS)—by the method described by Klute [62]. Nickle, cobalt and cadmium content was determined in aqua regia extracts by flame and electrothermal atomic absorption spectrometry (AAS) with the Thermo Scientific iCE 35Z atomic absorption spectrometer according to Standard PN-ISO 11047:2001(A) [63].

2.6. Chemical Analyses of Plant Samples

Nickle, cobalt and cadmium concentrations in the aerial parts and roots of *Festuca rubra* were determined by AAS after mineralization in a microwave oven according to Standard PN-EN 14084:2004 [64].

The heat of combustion (Q) of aerial plant parts was measured with the IKA WERKE C2000 calorimeter system (IKA, Cincinnati, OH, USA). Heat of combustion (Q) was determined according to standard PN-EN ISO 18125:2017, and calorific value (Hv) according to standard PN-EN ISO 18125:2017 [65]. The following procedure was applied to measure heat of combustion: 0.5 g of *Festuca rubra* was placed in a quartz crucible. The crucible containing the sample was placed in a bomb calorimeter, and heat of combustion was measured.

The content of carbon, hydrogen and sulfur was measured simultaneously with the ELTRA CHS 500 analyser (Neuss, Germany) according to standards PN-G-04584 and PN-G-04517 [66,67]. Biomass samples were incinerated in an atmosphere of pure oxygen, and the produced gases were measured in a beam of infrared light.

The nitrogen content of biomass was determined based on standard PN-EN ISO 20483:2014-02 [68]. The analytical sample was mineralized in sulfuric (VI) acid in the presence of a catalyst. The reaction products were alkalized and distilled. The released ammonia was trapped in a hydrochloric acid solution. Nitrogen content was measured by the Kjeldahl method with the use of the K-424 digester unit and B-324 distillation unit (Buchi Labotechnik AG, Flawil, Switzerland).

The oxygen content of biomass was estimated based on the content of the remaining elements that were determined experimentally with the use of the following formula [69]:

$$O = 100 - (A + C + H + S + N) \quad (1)$$

where:

A—total ash content (% d.m.)
C—total carbon content (% d.m.)
H—total hydrogen content (% d.m.)
S—total sulfur content (% d.m.)
N—total nitrogen content (% d.m.)

Ash content was measured in the ELTRA THERMOSTEP Thermogravimetric Analyzer, (Neuss, Germany).

2.7. Statistical Analysis and Calculations

The results were processed statistically by analysis of variance (ANOVA) at $p \leq 0.05$ using Statistica 13.1 software [70]. Homogeneous groups were identified by Tukey's test.

The following indicators were calculated based on microbial counts:

The colony development (CD) index according to Sarathchandra et al. [71]:

$$CD = [\frac{N_1}{1} + \frac{N_2}{2} + \frac{N_3}{3} \ldots\ldots \frac{N10}{10}] \times 100 \quad (2)$$

where N_1, N_2, N_3 … N_{10} are the number of microbial colonies identified on each day of the experiment (days 1–10) divided by the total number of microbial colonies identified during the entire experiment.

The following is the ecophysiological diversity (EP) index of soil-dwelling microorganisms according to De Leij et al. [72]:

$$EP = -\Sigma(pi \times \log pi) \quad (3)$$

where pi is the number of microbial colonies identified on each day of the experiment divided by the total number of microbial colonies identified during the entire experiment.

The following indicators were calculated based on biomass yields and heavy metal concentrations in aerial plant parts, roots and soil: Heavy metal uptake (D) by *Festuca rubra* (Fr), tolerance index (TI), translocation factor (TF), bioaccumulation factor in aerial plant parts (BF_A), bioaccumulation factor in roots (BF_R) and the accumulation factor (AF). The above parameters were calculated with the use of the following formulas:

$$D = (\text{heavy metal content in aerial plant parts} \times \text{aerial biomass yield}) + (\text{heavy metal content of roots} \times \text{root yields}) \quad (4)$$

$$TI = \frac{\text{Yield from contaminated treatment}}{\text{Yield from uncontaminated treatment}} \quad (5)$$

$$TF = \frac{\text{Heavy metal content of the aerial parts of } \textit{Festuca rubra}}{\text{Heavy metal content in } \textit{Festuca rubra} \text{ roots}} \quad (6)$$

$$BF_A = \frac{\text{Heavy metal content of the aerial parts of } \textit{Festuca rubra}}{\text{Heavy metal content of soil}} \quad (7)$$

$$BF_R = \frac{\text{Heavy metal content of } \textit{Festuca rubra} \text{ roots}}{\text{Heavy metal content of soil}} \quad (8)$$

$$AF = \frac{\text{Heavy metal content of the aerial parts and roots of } \textit{Festuca rubra}}{\text{Heavy metal content of soil}} \quad (9)$$

The calorific value of *Festuca rubra* was calculated with a formula proposed by Kopetz et al. [73]:

$$Hv = \frac{Q\,(100 - Mc)}{100} - Mc \times 0.0244 \quad (10)$$

where:

Hv—calorific value of air-dry biomass (MJ kg^{-1})

Q—heat of combustion of air-dry biomass

M_C—biomass moisture content (%)

0.0244—correction coefficient for water vaporization enthalpy (MJ kg^{-1} per 1% moisture content)

The energy yield of *Festuca rubra* biomass per 1 kg of soil was calculated with the following equation:

$$Y_{EP} = Hv \times Y \quad (11)$$

where:

Y_{EP}—energy yield of *Festuca rubra* biomass (MJ)

Hv—calorific value of air-dry *Festuca rubra* biomass (MJ kg^{-1})

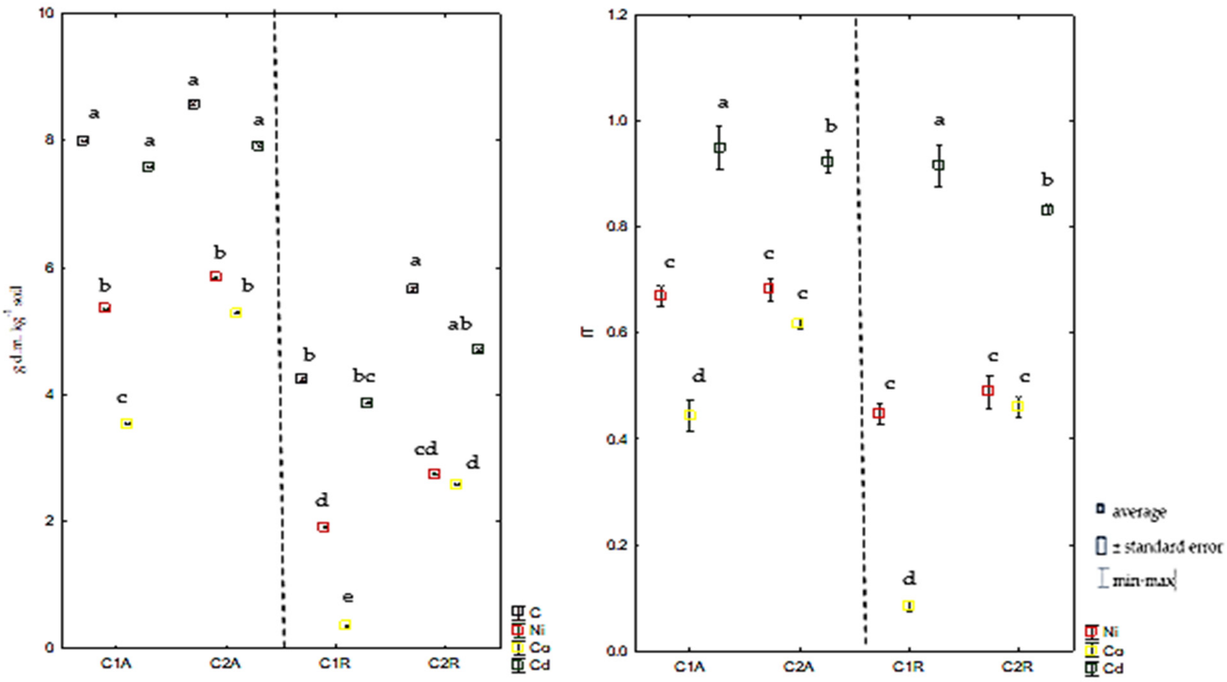
Y—aerial biomass yield of (kg) *Festuca rubra* per 1 kg of soil

3. Results

3.1. Response of Festuca rubra to Soil Contamination with Ni^{2+}, Co^{2+} and Cd^{2+}

Soil contamination with Ni^{2+} and Co^{2+} hindered the growth and development of *Festuca rubra* (Figure 1). Aerial biomass and root yields and the calculated heavy metal tolerance index (TI) revealed that the growth and development of *Festuca rubra* plants was most severely compromised by Co^{2+}, in particular in the treatment without compost, where the root yield was reduced by 91.64% and the aerial biomass yield—by 55.59% relative to the control treatment. In contaminated soil amended with compost, the aerial biomass yield was 54.08% lower and the root yield was 31.88% lower than in uncontaminated soil. Nickel decreased aerial biomass and root yields by 33.03% and 55.19%, respectively, in the treatment without compost, and by 31.88% and 51.14%, respectively, in the treatment where soil was amended with compost. Cadmium did not induce a significant reduction in aerial biomass or root yields. Based on the varied responses of *Festuca rubra* to the tested heavy metals, their toxicity was arranged in the following order: $Co^{2+} > Ni^{2+} > Cd^{2+}$. Compost increased *Festuca rubra's* tolerance to cobalt, but it did not significantly affect its tolerance to nickel (Figure 1).

Festuca rubra grown in soil contaminated with Ni^{2+}, Co^{2+} and Cd^{2+} was characterized by significantly higher heavy metal concentrations in roots than in aerial plant parts, and the application of compost increased the heavy metal content of both plant organs (Table 1). Despite the fact that the heavy metal content of plants increased under the influence of compost, their concentrations in soil were not reduced.



(a) **(b)**

Figure 1. Aerial biomass and root yields of *Festuca rubra* in soil contaminated with heavy metals (in g d.m. kg $^{-1}$ soil) (**a**) and the heavy metals tolerance index (TI) of *Festuca rubra* (**b**). Heavy metal doses in 1 kg d.m. soils: C—control; Ni—400 mg; Co—80 mg; Cd—8 mg; Compost: C1—soil not fertilized with compost; C2—soil fertilized with compost; A—aerial plant parts; R—roots; homogeneous groups within above-ground parts and roots are marked with identical letters (a–e).

Table 1. Content of nickel, cobalt and cadmium in the aerial parts and roots of *Festuca rubra* and in soil (in mg kg^{-1} plant/soil d.m.).

Heavy Metals	Aerial Parts	Roots	Soil
Soil not contaminated with metals, not fertilized with compost			
Nickel	3.34 ^{g}	8.59 ^{f}	7.55 ^{f}
Cobalt	5.00 ^{e}	5.00 ^{h}	9.75 ^{e}
Cadmium	0.17 ^{k}	0.82 ^{j}	0.60 ^{i}
Soil contaminated with metals, not fertilized with compost			
Nickel	17.34 ^{b}	73.53 ^{d}	320.03 ^{b}
Cobalt	7.45 ^{d}	99.04 ^{b}	51.49 ^{d}
Cadmium	1.68 ^{i}	2.07 ^{i}	1.53 ^{i}
Soil not contaminated with metals, fertilized with compost			
Nickel	2.00 ^{h}	13.30 ^{e}	5.39 ^{h}
Cobalt	5.00 ^{e}	6.81 ^{g}	9.50 ^{e}
Cadmium	0.97 ^{j}	0.84 ^{f}	0.60 ^{i}
Soil contaminated with metals, fertilized with compost			
Nickel	27.39 ^{a}	82.55 ^{c}	328.04 ^{a}
Cobalt	14.45 ^{c}	101.07 ^{a}	58.53 ^{c}
Cadmium	3.73 ^{f}	5.12 ^{h}	6.55 ^{g}

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

Heavy metal uptake by *Festuca rubra* differed across treatments (Table 2). In both contaminated and uncontaminated treatments, Ni^{2+} uptake by plants was highest and Cd^{2+} uptake was lowest. The remediation potential of *Festuca rubra* was determined based on the calculated values of TF, AF, BF_A and BF_R factors. The values of TF were lowest in soil contaminated with Co^{2+} (0.075–0.143) and highest in soil contaminated with cadmium (0.729–0.812). The values of accumulation (AF) and bioaccumulation (BF_A, BF_R) factors were lowest in *Festuca rubra* grown in soil contaminated with nickel. *Festuca rubra* grown in soil contaminated with Co^{2+} was characterized by the highest values of AF (1.974–3.068) and BF_R (1.727–1.923), whereas the grass grown in soil contaminated with Cd^{2+}—by the highest values of BF_A (0.569–1.098).

Table 2. Heavy metal uptake (D), translocation factor (TF), bioaccumulation factor in aerial plant parts (BF_A), bioaccumulation factor in roots (BF_R) and accumulation factor (AF).

Heavy Metals	D µg kg^{-1}	TF	BF_A	BF_R	AF
Soil not contaminated with metals, not fertilized with compost					
Ni^{2+}	63.090 ^{f}	0.389 ^{d}	0.442 ^{bc}	1.138 ^{e}	1.580 ^{e}
Co^{2+}	61.157 ^{g}	1.000 ^{b}	0.513 ^{b}	0.513 ^{h}	1.026 ^{h}
Cd^{2+}	4.833 ^{k}	0.207 ^{ef}	0.283 ^{de}	1.367 ^{d}	1.650 ^{e}
Soil contaminated with metals, not fertilized with compost					
Ni^{2+}	232.418 ^{c}	0.236 ^{e}	0.054 ^{f}	0.230 ^{i}	0.284 ^{i}
Co^{2+}	61.542 ^{g}	0.075 ^{f}	0.145 ^{ef}	1.923 ^{b}	2.068 ^{c}
Cd^{2+}	20.774 ^{i}	0.812 ^{c}	1.098 ^{a}	1.353 ^{d}	2.451 ^{b}
Soil not contaminated with metals, fertilized with compost					
Ni^{2+}	92.321 ^{d}	0.150 ^{fg}	0.371 ^{cd}	2.468 ^{a}	2.839 ^{a}
Co^{2+}	81.379 ^{e}	0.734 ^{c}	0.526 ^{b}	0.717 ^{g}	1.243 ^{g}
Cd^{2+}	13.074 ^{j}	1.153 ^{a}	1.128 ^{a}	0.978 ^{f}	2.106 ^{c}
Soil contaminated with metals, fertilized with compost					
Ni^{2+}	387.968 ^{a}	0.332 ^{d}	0.083 ^{f}	0.252 ^{i}	0.335 ^{i}
Co^{2+}	338.670 ^{b}	0.143 ^{fg}	0.247 ^{de}	1.727 ^{c}	1.974 ^{d}
Cd^{2+}	53.642 ^{h}	0.729 ^{c}	0.569 ^{b}	0.782 ^{g}	1.351 ^{f}

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

The heat of combustion of *Festuca rubra* ranged from 17.696 to 18.576 MJ kg^{-1} plant d.m., and the calorific value from 15.924 to 16.790 MJ kg^{-1} plant d.m. (Table 3). Neither of the above parameters was influenced by compost application or heavy metal contamination, and heat of combustion increased significantly only under exposure to cobalt in composted soil. Despite the above, cobalt had no significant effect on the calorific value of *Festuca rubra*. Biomass yield was reduced under exposure to nickel and cobalt, therefore the energy yield of *Festuca rubra* biomass was lowest in these treatments, and it was not correlated with its calorific value.

Table 3. Heat of combustion and calorific value of *Festuca rubra*.

Heavy Metals	Heat of Combustion (Q)	Calorific Value (Hv)	Energy Yield (Y_{EP}) MJ kg^{-1}
	MJ kg^{-1} Air-Dry Matter Plants		
Soil not fertilized with compost			
Control	18.210 ^{bc}	16.310 ^{ab}	0.130 ^{ab}
Ni^{2+}	18.211 ^{bc}	16.311 ^{ab}	0.087 ^{bc}
Co^{2+}	18.452 ^{ab}	16.645 ^{a}	0.059 ^{c}
Cd^{2+}	18.495 ^{ab}	16.454 ^{ab}	0.125 ^{ab}
Soil fertilized with compost			
Control	18.137 ^{bc}	16.306 ^{ab}	0.140 ^{a}
Ni^{2+}	17.696 ^{c}	15.924 ^{b}	0.093 ^{bc}
Co^{2+}	18.576 ^{a}	16.790 ^{a}	0.089 ^{bc}
Cd^{2+}	18.402 ^{ab}	16.557 ^{ab}	0.131 ^{ab}

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

The carbon-content of *Festuca rubra* biomass ranged from 43.57% in treatments contaminated with Ni^{2+} and fertilized with compost to 47.37% in uncontaminated and unfertilized treatments (Table 4). Sulfur is an important parameter in evaluations of the calorific value of biomass. In *Festuca rubra*, sulfur content was determined in the range of 0.15% (control soil without compost fertilization) to 0.24% (soil contaminated with Ni^{2+} and fertilized with compost).

Table 4. Elemental composition and ash content of *Festuca rubra*.

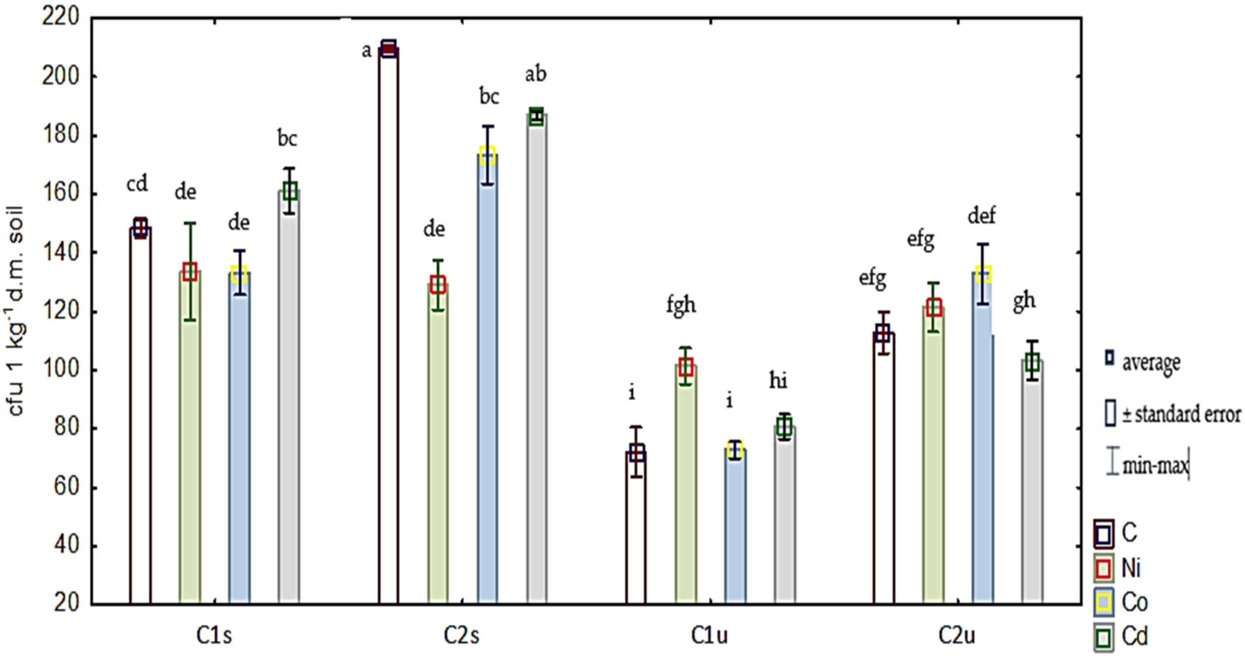
Heavy Metals	Carbon	Hydrogen	Sulfur	Nitrogen	Oxygen	Ash
	% d.m.					
Soil not fertilized with compost						
Control	47.37 ^{a}	6.07 ^{a}	0.15	3.08 ^{bc}	34.36 ^{bc}	8.98 ^{d}
Ni^{2+}	44.26 ^{c}	5.81 ^{a}	0.23 ^{a}	3.88 ^{a}	36.04 ^{a}	9.78 ^{bc}
Co^{2+}	46.47 ^{b}	5.95 ^{a}	0.18 ^{b}	3.84 ^{a}	34.13 ^{bc}	9.43 ^{c}
Cd^{2+}	46.64 ^{b}	5.99 ^{a}	0.20 ^{ab}	3.36 ^{b}	35.06 ^{b}	8.74 ^{d}
Soil fertilized with compost						
Control	47.02 ^{a}	6.00 ^{a}	0.17 ^{b}	3.14 ^{bc}	33.47 ^{c}	10.20 ^{b}
Ni^{2+}	43.57 ^{d}	5.56 ^{ab}	0.24 ^{a}	4.33 ^{a}	33.69 ^{c}	12.60 ^{a}
Co^{2+}	46.65 ^{b}	5.90 ^{a}	0.19 ^{ab}	3.42 ^{b}	34.64 ^{bc}	9.20 ^{c}
Cd^{2+}	46.43 ^{b}	6.02 ^{a}	0.17 ^{b}	2.87 ^{c}	35.36 ^{b}	9.16 ^{cd}

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

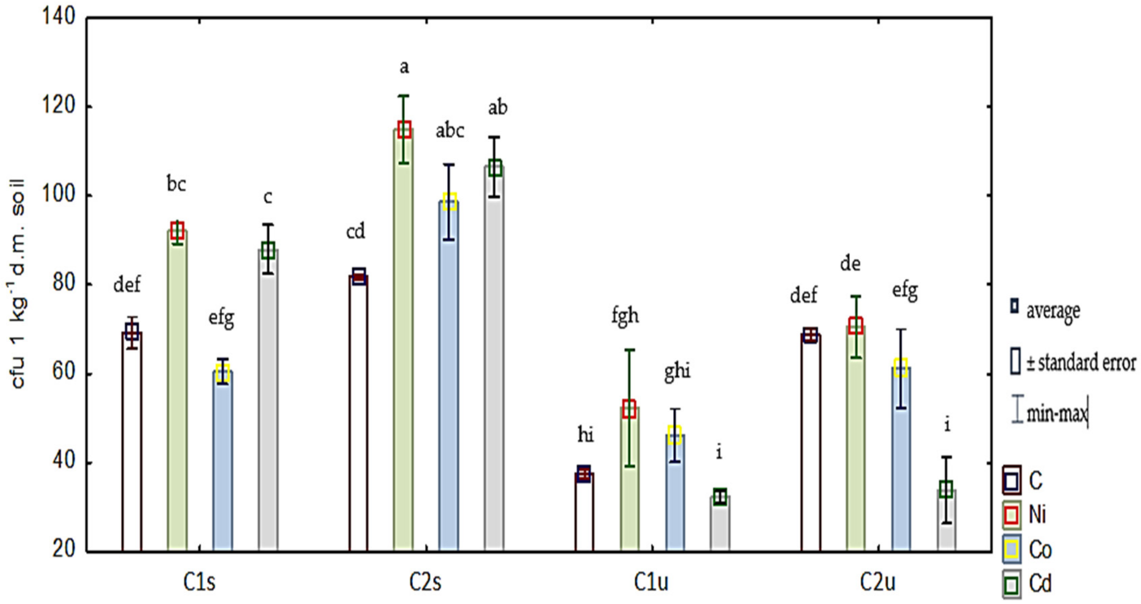
Nitrogen content was highest in biomass harvested from soil contaminated with Ni^{2+} and fertilized with compost, and it was lowest in biomass from soil polluted with Cd^{2+} and fertilized with compost. Soil contamination with Ni^{2+}, Co^{2+} and Cd^{2+} and compost fertilization had no effect on the hydrogen content of *Festuca rubra* biomass and had a minor impact on oxygen levels. Ash content was highest (8.98% on a d.m. basis) in *Festuca rubra* grown on uncontaminated and unfertilized soil. Ash content was significantly highest in biomass from treatments that were not contaminated with heavy metals and were supplied with compost.

3.2. Responses of Microorganisms to Soil Contamination with Ni^{2+}, Co^{2+} and Cd^{2+}

Microbial counts were affected by heavy metal contamination, compost application and the presence of *Festuca rubra* (Figure 2). In uncontaminated soil, compost stimulated the development of organotrophic bacteria, actinobacteria and fungi. Microbial proliferation was generally higher in soil sown with *Festuca rubra* than in unsown pots. Heavy metals exerted varied effects on microbial counts. In unamended soil sown with *Festuca rubra,* none of the tested heavy metals exerted a significant influence on organotrophic bacteria. Ni^{2+} increased the counts of actinobacteria and fungi, Co^{2+} increased the counts of fungi, whereas Cd^{2+} had no significant effect on any of the analyzed microbial groups. In pots not sown with *Festuca rubra*, heavy metals induced even smaller changes in microbial counts. Only the counts of organotrophic bacteria increased significantly under exposure to Ni^{2+}, and fungal counts decreased under exposure to Cd^{2+}.



(a)



(b)

Figure 2. *Cont*.

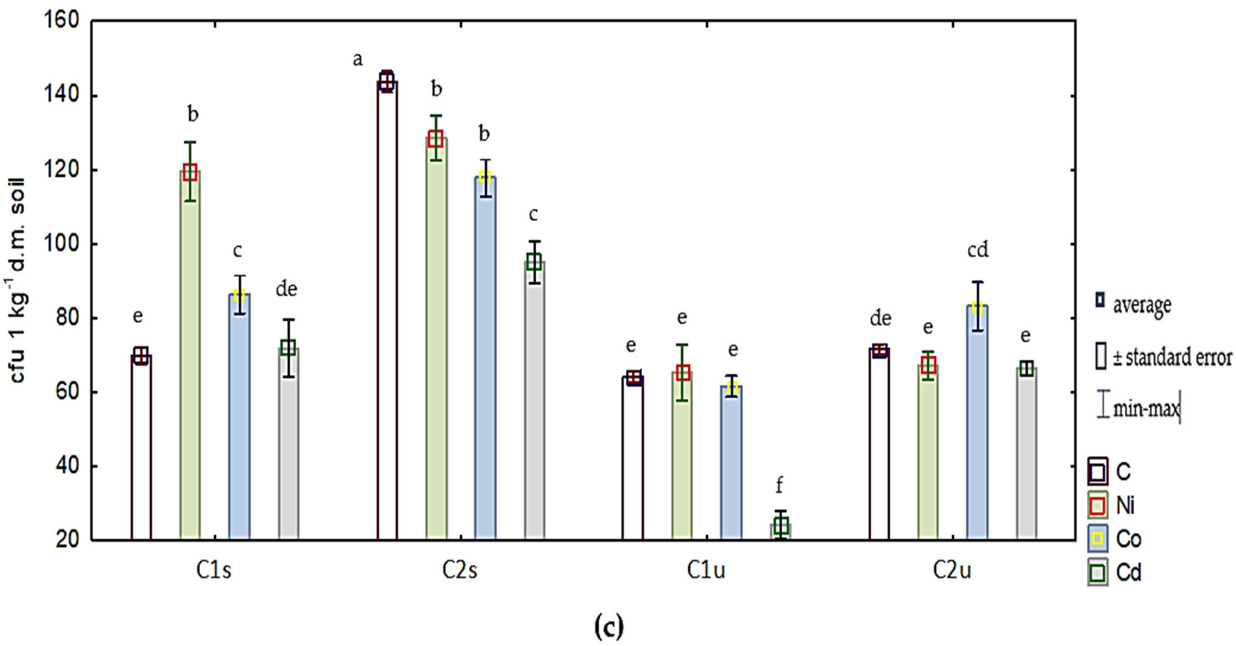


Figure 2. Microbial counts (CFU) in 1 kg soil d.m. (**a**) organotrophic bacteria 10^9; (**b**) actinobacteria 10^9; (**c**) fungi 10^7; heavy metal dose in 1 kg d.m. soils: C—control; Ni—400 mg; Co—80 mg; Cd—8 mg; C1s—soil not fertilized with compost, sown with *Festuca rubra*; C2s—soil fertilized with compost, sown with *Festuca rubra*; C1u—soil not fertilized with compost, not sown with *Festuca rubra*; C2u—soil fertilized with compost, not sown with *Festuca rubra*; homogeneous groups within each microbial group are marked with identical letters (a–i).

Heavy metals induced greater changes in the microbiome of soil amended with compost than in unamended soil. In this experimental series, in soil sown with *Festuca rubra*, nickel and cobalt significantly decreased the counts of organotrophic bacteria, and all heavy metals increased the counts of actinobacteria and reduced fungal counts. In soil not sown with *Festuca rubra*, the only significant changes were an increase in the counts of organotrophic bacteria under exposure to Co^{2+} and a decrease in the counts of actinobacteria in response to Cd^{2+}. Heavy metals had a relatively weak impact on the ecophysiological diversity of soil microorganisms. In soil amended with compost, only nickel in the treatment sown with *Festuca rubra* significantly decreased the EP index of fungi (Figure 3). In all experimental series, the remaining heavy metals did not decrease the EP index.

In unamended soil, exposure to Cd^{2+} decreased the EP index of organotrophic bacteria in both sown and unsown treatments, the EP index of actinobacteria in unsown soil and the EP index of fungi in soil sown with *Festuca rubra*. The EP index of actinobacteria in unsown soil was also adversely affected by Co^{2+}. In the remaining treatments, the EP index of soil microorganisms did not decrease significantly under exposure to heavy metals. The EP index was not significantly affected by the presence of *Festuca rubra* plants and, in most treatments, by compost application.

Heavy metals exerted a greater influence on the CD index (Figure 4) than the EP index, and an inverse response was noted in some cases. For example, all heavy metals increased the CD index of organotrophic bacteria isolated from unamended soil sown with *Festuca rubra*, but decreased the CD index of organotrophic bacteria isolated from soil amended with compost. All heavy metals decreased the CD index of fungi isolated from soil amended with compost and sown with *Festuca rubra*. The CD index of actinobacteria was least affected by heavy metals. In this group of microorganisms, the CD index was not significantly modified by heavy metals in unamended soil sown with *Festuca rubra*, whereas in soil amended with compost, this parameter increased only in response to nickel. The remaining heavy metals had no significant effect on the CD index of actinobacteria.

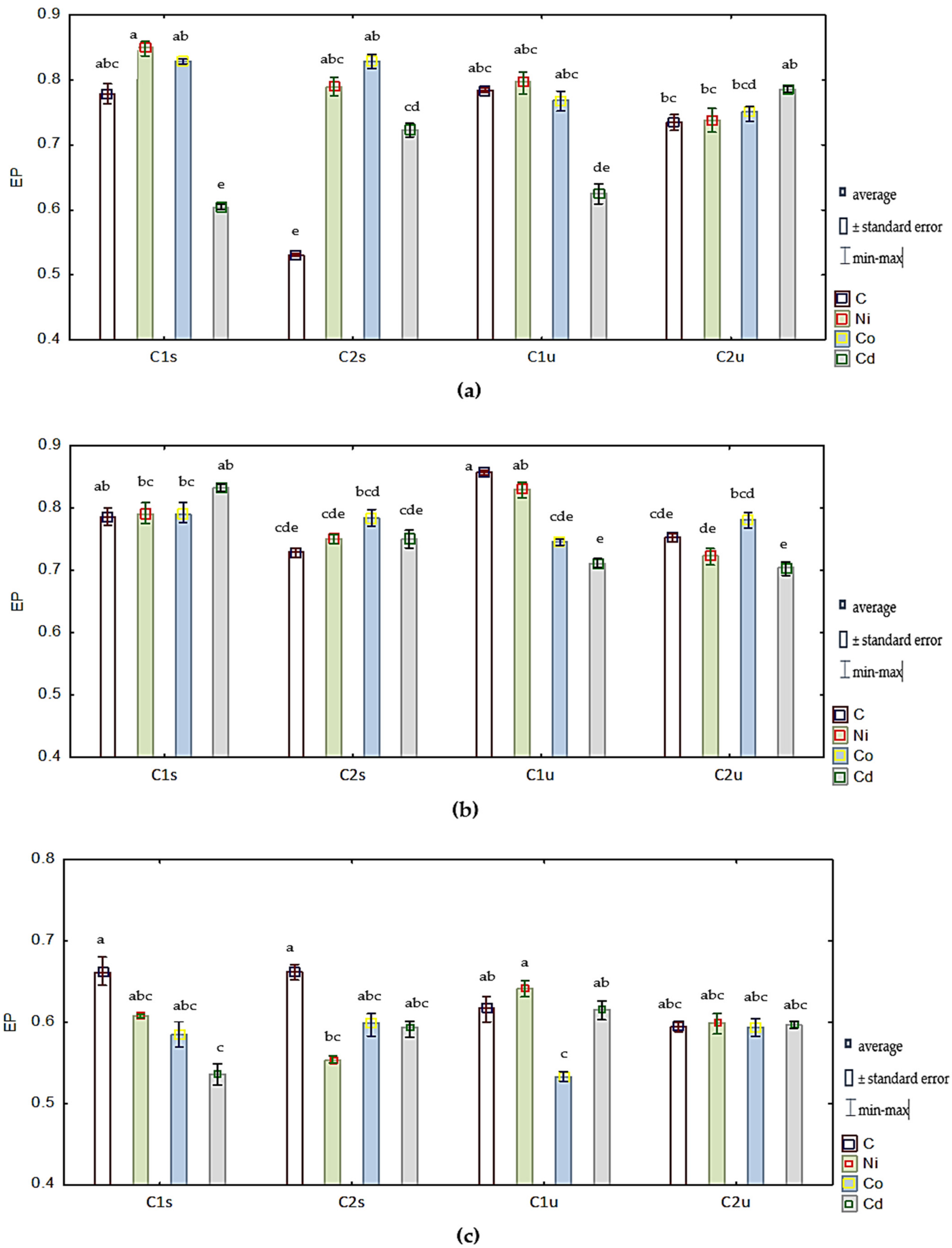
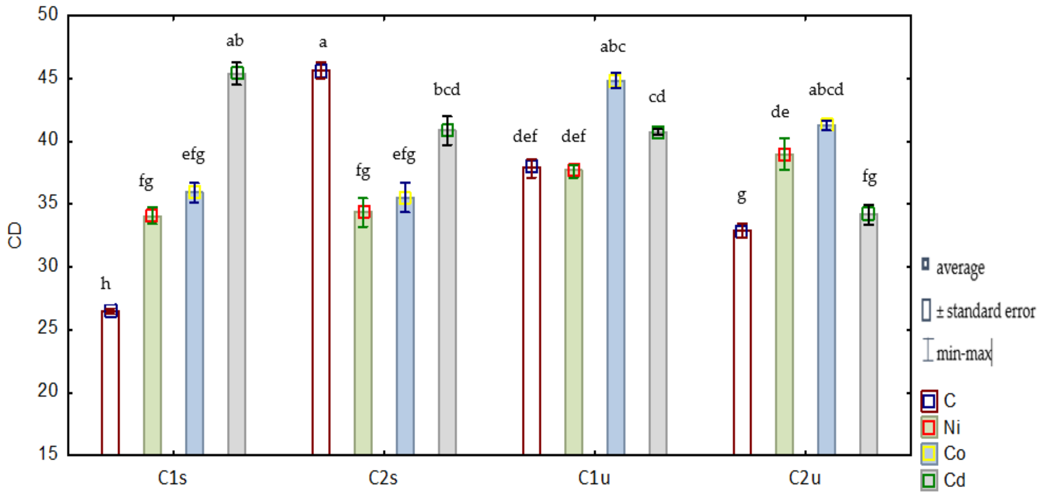
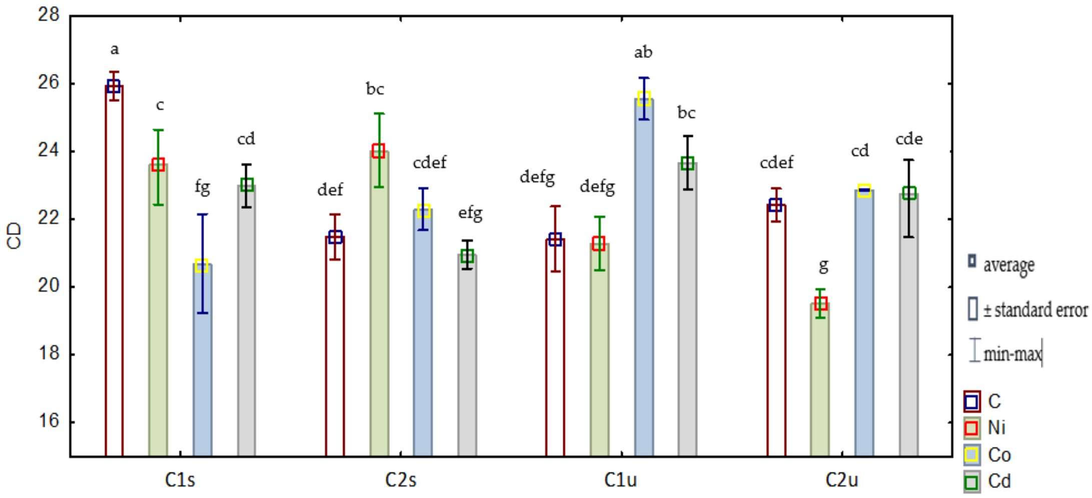


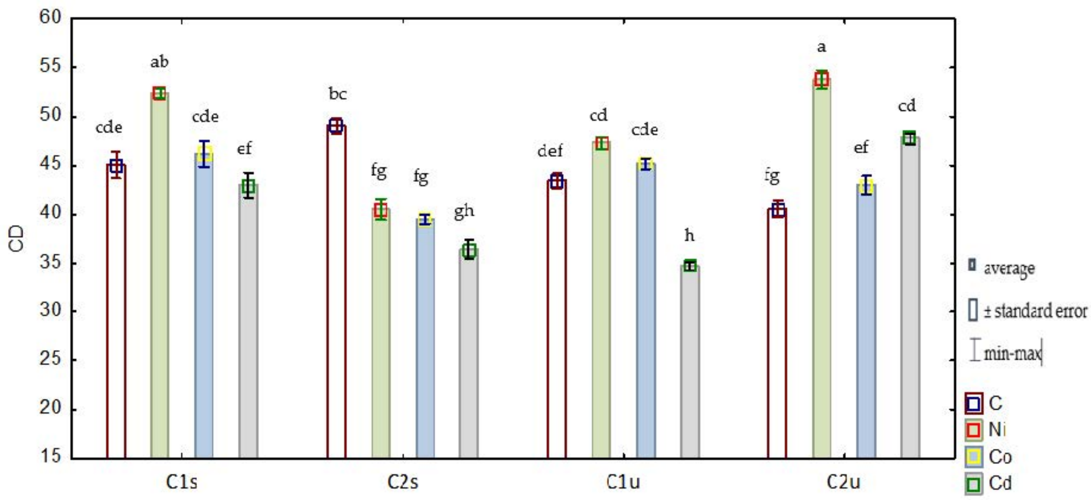
Figure 3. Ecophysiological diversity (EP) index of soil microorganisms. Refer to the key in Figure 2.



(a)



(b)



(c)

Figure 4. Colony development (CD) index of soil microorganisms. Refer to the key in Figure 2.

3.3. Enzyme Responses to Soil Contamination with Ni^{2+}, Co^{2+} and Cd^{2+}

In soil sown with *Festuca rubra*, the application of compost stimulated the activity of dehydrogenases, catalase, urease, acid phosphatase, alkaline phosphatase, β-glucosidase and arylsulfatase (Table 5). In unsown soil, compost significantly increased only the activity of urease, acid phosphatase and arylsulfatase, whereas the activity of the remaining enzymes was not affected. In unamended soil, the presence of *Festuca rubra* plants stimulated the activity of all enzymes, excluding catalase. In soil amended with compost, catalase activity was also stimulated by the grass. All heavy metals inhibited the activity of dehydrogenases, acid phosphatase and β-glucosidase in soil amended with compost and sown with *Festuca rubra*. In unamended soil sown with *Festuca rubra*, β-glucosidase activity decreased under exposure to Ni^{2+}, Cd^{2+} and Co^{2+}. Beta-glucosidase activity was also inhibited by heavy metals in unsown soil. Catalase was relatively most resistant to heavy metals, and its activity did not change significantly in unsown soil, regardless of compost application, and it decreased only under the influence of Co^{2+} in soil sown with *Festuca rubra* and amended with compost. The analysis of urease activity produced surprising results. In unamended soil, urease activity was not significantly inhibited by any of the tested heavy metals, and in some treatments it was enhanced by Ni^{2+}, Co^{2+} and Cd^{2+}.

In soil amended with compost, urease activity was most strongly suppressed by Ni^{2+}, followed by Co^{2+}, but it was not inhibited by Cd^{2+}. Regardless of compost application and soil use, Ni^{2+} and Cd^{2+} negatively affected the activity of acid phosphatase. In unamended soil sown with *Festuca rubra*, all of the tested heavy metals stimulated the activity of alkaline phosphatase, and in soil amended with compost the activity of this enzyme was stimulated only by Ni^{2+}. In unsown and unamended soil, alkaline phosphatase activity was stimulated only by Cd^{2+}, and in soil amended with compost by Co^{2+} and Cd^{2+}. These results indicate that Ni^{2+} exerted the most inhibitory effect, whereas Cd^{2+} had the least disruptive impact on the activity of the tested soil enzymes. Dehydrogenase was least resistant, and urease was most resistant to all of the analyzed heavy metals.

Table 5. Enzyme activity in soil contaminated with heavy metals.

Heavy Metals	Dehydrogenases µmol TFF kg^{-1} d.m. h^{-1}		Catalase mol O_2 kg^{-1} d.m. h^{-1}		Urease mmol N-NH_4 kg^{-1} d.m. h^{-1}		Acid Phosphatase		Alkaline Phosphatase		β-Glucosidase		Arylsulfatase	
							mmol PNP kg^{-1} d.m. h^{-1}							
	Sown Soil	Unsown Soil	Sown Soil	Unsown Soil	Sown Soil	Unsown Soil	Sown Soil	Unsown Soil	Sown Soil	Unsown Soil	Sown Soil	Unsown Soil	Sown Soil	Unsown Soil
Soil not fertilized with compost														
Control	4.147 ^{c}	1.976 ^{fg}	0.225 ^{bc}	0.203 ^{bc}	0.725 ^{e}	0.539 ^{g}	1.388 ^{de}	1.201 ^{fg}	0.435 ^{fg}	0.292 ^{gh}	1.183 ^{b}	1.119 ^{ef}	0.186 ^{b}	0.091 ^{ef}
Ni^{2+}	1.409 ^{gh}	0.606 ^{i}	0.220 ^{bc}	0.183 ^{bc}	0.824 ^{d}	0.501 ^{g}	1.027 ^{hi}	0.998 ^{i}	0.784 ^{bc}	0.206 ^{h}	1.126 ^{e}	1.095 ^{h}	0.160 ^{cd}	0.081 ^{fg}
Co^{2+}	2.911 ^{e}	1.453 ^{gh}	0.208 ^{bc}	0.198 ^{bc}	0.693 ^{ef}	0.685 ^{ef}	1.435 ^{de}	1.419 ^{de}	0.788 ^{bc}	0.301 ^{gh}	1.125 ^{e}	1.072 ^{i}	0.169 ^{bc}	0.072 ^{g}
Cd^{2+}	3.951 ^{cd}	1.756 ^{fg}	0.209 ^{bc}	0.171 ^{c}	0.690 ^{ef}	0.648 ^{f}	1.073 ^{ghi}	1.036 ^{hi}	0.606 ^{de}	0.580 ^{def}	1.107 ^{fgh}	1.099 ^{h}	0.177 ^{b}	0.072 ^{g}
Soil fertilized with compost														
Control	7.020 ^{a}	2.048 ^{f}	0.297 ^{a}	0.229 ^{bc}	1.031 ^{a}	0.687 ^{ef}	2.198 ^{a}	1.502 ^{d}	0.790 ^{bc}	0.406 ^{g}	1.320 ^{a}	1.128 ^{e}	0.219 ^{a}	0.145 ^{d}
Ni^{2+}	2.047 ^{f}	0.924 ^{hi}	0.246 ^{ab}	0.213 ^{bc}	0.841 ^{d}	0.500 ^{g}	1.138 ^{gh}	1.116 ^{ghi}	1.405 ^{a}	0.446 ^{efg}	1.129 ^{de}	1.104 ^{gh}	0.178 ^{b}	0.090 ^{ef}
Co^{2+}	3.520 ^{d}	1.683 ^{fg}	0.218 ^{bc}	0.225 ^{bc}	0.913 ^{c}	0.686 ^{ef}	1.740 ^{c}	1.718 ^{c}	0.796 ^{bc}	0.646 ^{cd}	1.141 ^{cd}	1.120 ^{ef}	0.209 ^{a}	0.099 ^{e}
Cd^{2+}	5.445 ^{b}	2.130 ^{f}	0.248 ^{ab}	0.218 ^{bc}	1.000 ^{ab}	0.949 ^{bc}	2.023 ^{b}	1.313 ^{ef}	0.891 ^{b}	0.709 ^{cd}	1.148 ^{c}	1.116 ^{efg}	0.207 ^{a}	0.095 ^{ef}

Homogeneous groups within each microbial and enzyme group are marked with identical letters (a–i).

3.4. *Physicochemical Properties of Soil Contaminated with Ni^{2+}, Co^{2+} and Cd^{2+}*

Compost increased the content of organic carbon, exchangeable base cations and cation exchange capacity of soil sown with *Festuca rubra* and unsown soil (Table 6).

Table 6. Physicochemical properties of soil contaminated with heavy metals.

Heavy Metals	pH_{KCl}	C_{org}	N_{total}	HAC	EBC	CEC	BS
		(g kg^{-1})		(mmol$^{(+)}$ kg^{-1} soil)			%
Unsown soil							
Soil not fertilized with compost							
Control	7.35^{bcd}	7.13^{bcd}	0.84^{ab}	4.13^{f}	114.00^{b}	118.13^{b}	96.51^{a}
Ni^{2+}	7.15^{ef}	6.40^{gh}	0.76^{def}	6.19^{bc}	89.00^{gh}	95.19^{gh}	93.50^{g}
Co^{2+}	7.05^{f}	6.35^{hi}	0.82^{abcd}	7.13^{a}	90.00^{g}	97.13^{g}	92.66^{h}
Cd^{2+}	7.30^{cd}	6.07^{i}	0.75^{efg}	5.81^{cd}	87.00^{h}	92.81^{h}	93.74^{g}
Soil fertilized with compost							
Control	7.40^{abc}	7.66^{a}	0.88^{a}	4.13^{f}	119.00^{a}	124.06^{a}	95.92^{bc}
Ni^{2+}	7.30^{cd}	6.98^{bcde}	0.87^{a}	5.44^{de}	109.00^{c}	114.44^{c}	95.25^{de}
Co^{2+}	7.25^{de}	7.16^{bc}	0.65^{abc}	5.06^{e}	106.00^{d}	110.13^{d}	96.25^{ab}
Cd^{2+}	7.30^{cd}	7.09^{bcde}	0.85^{ab}	5.06^{e}	113.00^{b}	118.06^{b}	95.71^{c}
Sown soil							
Soil not fertilized with compost							
Control	7.45^{ab}	6.95^{cde}	0.75^{efg}	4.50^{f}	90.00^{g}	94.50^{gh}	95.24^{de}
Ni^{2+}	7.25^{de}	6.65^{fg}	0.69^{g}	6.56^{b}	66.00^{k}	72.56^{k}	90.96^{i}
Co^{2+}	7.15^{ef}	6.81^{ef}	0.73^{fg}	7.50^{a}	67.00^{k}	74.50^{k}	89.93^{j}
Cd^{2+}	7.40^{abc}	6.29^{hi}	0.73^{fg}	6.19^{bc}	74.00^{j}	80.19^{j}	92.28^{h}
Soil fertilized with compost							
Control	7.50^{a}	7.89^{a}	0.88^{a}	4.50^{f}	101.00^{e}	106.44^{e}	94.89^{e}
Ni^{2+}	7.40^{abc}	7.10^{bcde}	0.80^{bcde}	5.81^{cd}	83.00^{i}	88.81^{i}	93.45^{g}
Co^{2+}	7.35^{bcd}	7.25^{b}	0.78^{cdef}	5.44^{de}	98.00^{f}	102.50^{f}	95.61^{cd}
Cd^{2+}	7.40^{abc}	6.85^{def}	0.78^{cdef}	5.44^{de}	91.00^{g}	96.44^{g}	94.36^{f}

C_{org}—total organic carbon, N_{total}—total nitrogen, HAC—hydrolytic acidity, EBC—total exchangeable cations, CEC—total exchange capacity of soil, BS—basic cation saturation ratio in soil. Identical letters (a–k) in columns denote the same homogeneous groups.

In both sown and unsown soil, all heavy metals increased hydrolytic acidity and decreased the content of exchangeable base cations and cation exchange capacity. Heavy metals also decreased base saturation in unamended soil. Co^{2+} induced the greatest decrease in soil pH. Ni^{2+} decreased soil pH in some treatments, whereas cadmium had no significant effect on this parameter (Table 6).

4. Discussion

4.1. *The Effectiveness of Festuca rubra in Remediating Soil Contaminated with Ni^{2+}, Co^{2+} and Cd^{2+}*

Grasses form a dense sward and therefore are highly effective in removing various types of contaminants from soil [10]. The applicability of various grass species for soil remediation was analyzed by Renella et al. [74], Lopareva-Pohu et al. [75], Padmavathiamma and Li [76] and Siebielec and Chaney [77]. They tested the effectiveness of *Lolium perenne*, *L. multiflorum*, *Festuca arundinacea*, *Poa pratensis*, *Festuca ovina* and *Holcus lanatus* in assisted phytostabilization. *Festuca rubra* has also attracted considerable interest as a promising species for removing pollutants from the soil environment [4,5,78–81]. The present study demonstrated that *Festuca rubra* had a varied tolerance to soil contamination with different heavy metals, and it was most resistant to Cd^{2+}, and least resistant to Co^{2+}. The average tolerance index (TI) of aerial plant parts was determined at 0.94 in soil contaminated with Cd^{2+}, 0.68 in soil contaminated with Ni^{2+} and 0.53 in soil contaminated with Co^{2+}. However, the translocation factor (TF) was much higher in soil polluted with Cd^{2+} than with the remaining heavy metals, which indicates that *Festuca rubra* has a limited potential for remediating cadmium-contaminated soils because aerial plant parts accumulated more Cd^{2+} than Co^{2+} or Ni^{2+}. The above observation was confirmed by the fact that the bioaccumulation factor was highest (BF_A = 0.57–1.10) in soil contaminated with Cd^{2+}. Nonetheless, the high values of BF_A indicate that the tested grass species can be effectively used to remove Co^{2+} and Cd^{2+} from soil and that aerial biomass is suitable for energy generation because plants grown in contaminated and uncontaminated treatments were characterized by similar calorific value. Our previous study [82] also revealed that the calorific value of *Elymus elongatus* was similar in soil contaminated with Cd^{2+}, Co^{2+} and Ni^{2+} and in uncontaminated soil. In this study, the content of C, H, S, N, O and ash was determined in *Festuca rubra* biomass. In the group of the analyzed elements, carbon plays the most important role because it is the main biomass component, and its energy is released during incineration. The calorific value of biomass is determined by its carbon content. The amount of ash remaining after biomass incineration should be as low as possible because ash is difficult to manage [19]. According to Vassilev et al. [83,84], grasses have the following average elemental composition: C—49.2% (min 46.1%, max 52.0%); O—43.7% (min 42.5%, max 44.5%); H—6.1% (min 5.1%, max 6.5%); N—0.9% (min 0.3%, max 2.6%); S—0.13% (min 0.04%, max 0.27%); and ash—4.8%. Similar results were obtained in the present study. Ash content was the only parameter that exceeded the values given in the literature [83,84]. Stolarski et al. [85] reported higher ash content in the biomass of leafy plants and grasses than in woody plants. It should be noted that *Festuca rubra* was characterized by higher values of BF_R than BF_A, which indicates that it can be used for the phytostabilization of soils contaminated with Cd^{2+} and Co^{2+}. Pusz et al. [81] also reported on the high phytoremediation potential of *Festuca rubra* in soils contaminated with heavy metals.

Heavy metals compromise the growth and development of plants, and effective methods for minimizing the environmental impact of these pollutants are being sought [80,81]. Various substances can be added to soil to speed up the remediation of soils contaminated with heavy metals [86–88]. The findings of other authors [87] and the results of this study indicate that compost minimizes the negative effect of heavy metals on plant yields. In the current study, compost was most effective in increasing *Festuca rubra* yields in soil contaminated with Co^{2+}. Compost supported the growth and development of *Festuca rubra* and, in consequence, heavy metals were more effectively removed from compost-amended than unamended soil. This observation was confirmed by the overall difference in total heavy metal uptake by the aerial parts and roots of *Festuca rubra* between treatments without and with compost application. Therefore, compost can be effectively used in the process of assisted phytoremediation.

The calorific value of *Festuca rubra* was compared with other types of biomass in Table 7. *Festuca rubra* analyzed in this study was characterized by lower calorific value than other plants. In biomass, this parameter is determined mainly by the type of biomass (chemical composition) [19]. Despite the fact that *Festuca rubra* is a perennial grass, plants native to Asia and North America are more suitable for energy generation because they are more efficient, bind more CO_2 and contain less ash as a by-product of combustion [20,21]. While the calorific value of *Festuca rubra* is lower than the plants shown in Table 7, its advantage is the fact that it is relatively resistant to soil contamination with heavy metals. Moreover, it has a beneficial effect on soil structure and water–air conditions [1–5]. For the above reasons, *Festuca rubra* is suitable for the reclamation of degraded areas. Its cultivation in such areas contributes to the biological regeneration of soils, and because the biomass cannot be used for feed purposes, its usefulness as an energy biomass is justified.

Table 7. Calorific value of various biomass types.

Plant	Calorific Value MJ kg^{-1}	Reference
Euphorbia nerrifolia	21.487	[20]
Nerium indicum	18.443	[20]
Mimusops elengi L.	19.217	[20]
Miscanthus sinensis	17.840; 17.960	[21,89]
Sida hermaphrodita	17.430	[21]
Silphium perfoliatum L.	16.820	[21]
Salix alba	19.200	[90]
Virginia fanpetals	17.170; 18.500	[91,92]
Festuca rubra	16.310	Own research

4.2. The Effects of Ni^{2+}, Co^{2+}, Cd^{2+} and Compost on the Microbiological and Biochemical Properties of Soil

Soil-dwelling microorganisms and enzymes play a highly significant role in evaluations of the quality of soils contaminated with heavy metals [93–96] because they are reliable indicators of heavy metal-induced changes in soil functions [97]. Microorganisms and enzymes are also closely associated with the physicochemical properties of soils which are compromised under exposure to high heavy metal concentrations. In the present study, microbial and enzyme responses to heavy metal contamination varied across treatments. The analyzed heavy metals negatively affected the development of organotrophic bacteria, and the activity of dehydrogenases, acid phosphatase and β-glucosidase in soil sown with *Festuca rubra*. In contaminated soil, the application of compost increased the counts of organotrophic bacteria, actinobacteria and fungi, stimulated the activity of dehydrogenases, catalase, urease, acid phosphatase and alkaline phosphatase, increased the content of C_{org}, increased EBC and CEC and decreased HAC. Compost also alleviated the toxic effects of heavy metals on the microbiological and biochemical properties of soil. Other researchers also reported on the beneficial influence of compost on the microbiological properties of soil [98–101]. Assisted phytoremediation has also attracted growing interest in recent years [102–104]. Monitoring of soil microbial activity is a useful tool in the process of remediating soils contaminated with heavy metals [93–96].

Enzymes are closely linked with the microbiological properties of soil and play an important role in assessments of the quality of the soil environment. Soil enzymes promote organic matter decomposition, redox reactions and nutrient cycling. Microbial activity is indicative of the dynamics of biochemical processes in soil [104,105]. Similar observations regarding soil enzymatic activity were made by other researchers [106–108]. In this study, compost stimulated enzyme activity, which suggests that the evaluated phytostabilization method had a positive impact on the biological parameters of soil. The above changes resulted from disruptions in the physicochemical parameters of soil [94,105,109].

An analysis of the content of Ni^{2+}, Co^{2+} and Cd^{2+} in soli after *Festuca rubra* harvest revealed that the concentrations of all heavy metals decreased, but land-use category changed only in the case of cadmium [42]. Lowering the category of soils contaminated with cadmium from group IV before phytoremediation into group II as a result of phytoremediation is a very beneficial phenomenon, because the land classified in group IV is industrial, traffic areas and fossil land, and the lands classified in group II are agricultural lands.

5. Conclusions

Festuca rubra has a stable calorific value that is not modified by high concentrations of Ni^{2+}, Co^{2+} or Cd^{2+} in soil. The calorific value of *Festuca rubra* ranged from 15.924 to 16.790 MJ kg^{-1} d.m. The heat of combustion of *Festuca rubra* ranged from 17.696 to 18.576 MJ kg^{-1} d.m. This observation indicates that *Festuca rubra* can be grown on soils contaminated with heavy metals, and that its aerial biomass can be used for energy generation. *Festuca rubra* biomass contained 43.57–47.37% carbon, 33.69–36.04% oxygen, 5.56–6.07% hydrogen, 2.87–4.33% nitrogen and 0.15–0.24% sulfur. The suitability of *Festuca rubra* biomass for energy generation was limited due to its relatively high ash content (8.74–12.60%). The calculated values of the accumulation factor (AF) clearly indicate that *Festuca rubra* has considerable phytoremediation potential in soils contaminated with cadmium and cobalt. This grass species delivers phytostabilizing effects, as demonstrated by higher values of the bioaccumulation factor in roots (BF_R) than in aerial plant parts (BF_A), as well as a translocation factor (TF) of less than one. *Festuca rubra* responded differently to soil contamination with heavy metals (400 mg Ni^{2+}, 80 mg Co^{2+} and 8 mg Cd^{2+} kg^{-1} d.m. soil). Cobalt exerted the strongest inhibitory effect on the growth and development of this grass species, the effect exerted by nickel was significantly less pronounced, whereas the impact of cadmium was not significant. Compost was effective only in increasing *Festuca rubra*'s tolerance to cobalt. Compost improves the microbiological, biochemical and physicochemical properties of soil, and it can significantly stabilize soil functions when soil homeostasis is disrupted by heavy metals.

Author Contributions: J.W., E.B.-L. and J.K. framed the methodology, conceived the ideas, and designed the paper. E.B.-L. conducted the experiments, collected the data. All authors analyzed the data and 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.

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Abbreviations

The following abbreviations are used in this manuscript:

Q—heat of combustion; Hv—calorific value of air-dry biomass; Y_{EP}—energy yield of Festuca rubra biomass; TI—tolerance index; D—heavy metal uptake; TF—translocation factor; BF_A—bioaccumulation factor in aerial plant parts; BF_R—bioaccumulation factor in roots; AF—accumulation factor; EP—ecophysiological diversity index; CD—colony development index; C_{org}—total organic carbon; N_{total}—total nitrogen; HAC—hydrolytic acidity; EBC—total exchangeable cations; CEC—total exchange capacity of soil; BS—basic cation saturation ratio in soil.

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