soil systems

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Prospects for the Use of *Echinochloa frumentacea* for Phytoremediation of Soils with Multielement Anomalies

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Abstract: In a model experiment, some adaptive characteristics, the bioaccumulation of toxic elements from technogenically-contaminated soils with polyelement anomalies, and rhizosphere microflora of Japanese millet, *Echinochloa frumentacea*, were studied using biochemical, microbiological, physicochemical (AAS, ICP-MS, INAA), and metagenomic (16S rRNA) methods of analysis. Good adaptive characteristics (the content of photosynthetic pigments, low molecular weight antioxidants) of *E. frumentacea* grown on the soils of metallurgical enterprises were revealed. The toxic effect of soils with strong polyelement anomalies (multiple excesses of MPC for Cr, Ni, Zn, As, petroleum products) on biometric parameters and adaptive characteristics of Japanese millet were shown. The rhizosphere populations of *E. frumentacea* grown in the background soil were characterized by the lowest taxonomic diversity compared to the rhizobiomes of plants grown in contaminated urban soils. The minimal number of all groups of microorganisms studied was noted in the soils, which contain the highest concentrations of both inorganic (heavy metals) and organic (oil products) pollutants. The taxonomic structure of the rhizospheric microbiomes of *E. frumentacea* was characterized. It has been established that *E. frumentacea* accumulated Mn, Co, As, and Cd from soils with polyelement pollution within the average values. V was accumulated mainly in the root system (transfer factor from roots to shoots 0.01–0.05) and its absorption mechanism is rhizofiltration. The removal of Zn by shoots of *E. frumentacea* increased on soils where the content of the element exceeded the MPC and was 100–454 mg/kg of dry weight (168–508 g/ha). Analysis of the obtained data makes it possible to recommend *E. frumentacea* for phytoremediation of soil from Cu and Zn at a low level of soil polyelement contamination using grass mixtures.

Keywords: *Echinochloa frumentacea*; contaminated soils; toxic elements; heavy metals; adaptation; AOS; ascorbic acid; GSH; phytoremediation; bioaccumulation; rhizosphere microflora

Citation: Gorelova, S.V.; Muratova, A.Y.; Zinicovscaia, I.; Okina, O.I.; Kolbas, A. Prospects for the Use of *Echinochloa frumentacea* for Phytoremediation of Soils with Multielement Anomalies. *Soil Syst.* **2022**, *6*, 27. https://doi.org/10.3390/soilsystems6010027

Academic Editors: Matteo Spagnuolo, Paola Adamo and Giovanni Garau

Received: 31 January 2022
Accepted: 14 March 2022
Published: 16 March 2022

1. Introduction

Global climate changes, pollution levels, and high-impact farming have induced a strong decline in soil quality, making the sustainable use of land more challenging than in the past [1]. For soils contaminated with trace element (TE) there are various phytomanagement options for reducing the environmental risks. Phytoextraction in conjunction with the application of conditioners can be considered as the way to minimize the dispersion

and biological action of TE on soil and to increase vegetation cover on polluted soils [2–4]. Recently, the cultivation of non-food crops for the production of plant-based feedstock, the bioremediation of metal-contaminated soils, and risk management have been developing at the field scale [5–9].

Chemically-induced hyperaccumulation is impaired by various environmental risks, e.g., metal leaching from the root zone and toxic effects on the microbiome [7,10]. Regarding secondary TE accumulators, the desirable characteristics for these plant species are (1) relatively fast growth and high biomass; (2) extended root system for exploring large soil volumes; (3) good tolerance to high concentrations of TE in plant tissues; (4) high translocation factor; (5) adaptability to specific environments/sites; (6) easy agricultural management [11], (7) good interaction with associated bacteria [12,13].

Global climate change makes it possible to introduce relatively thermophilic new biomass crops for phytoremediation, especially species of the *Poaceae* family [14]. These plants, due to C_4 assimilation pathways [15], are of particular interest for phytoremediation due to the production of significant biomass and, as a consequence, the accumulation of significant amounts of carbon and heavy metals.

The selective accumulation of Pb, Cu, Zn, and Cd in the roots and the possibility to remove the root remains makes technical sorghum, sugar sorghum, and *Sudan grass* extremely suitable for phytoremediation purposes [16]. Some sorghum cultivars show a high antioxidant status during the bioaccumulation of Pb and Cu on anthropogenically disturbed substrates with polyelement contamination [17].

The capacity of *Miscanthus* species to accumulate inorganic contaminants into the root system and to reduce the dissipation of persistent organic contaminants makes them good candidates for soil phytostabilization and phytodegradation. The noninvasive hybrid *Miscanthus giganteus*, with high lignocellulosic content, has a high potential in the biorefinery and bioenergy industries [18]. The applicability of *Arundo donax* for cadmium removal from contaminated soil and water was shown by Sabeen et al. [19].

Echinochloa frumentacea is a cost-effective crop through seeds and biomass production. It can be applied for sustainable crop rotation for the enhancement of soil development processes, nutrient cycles, and microbial community.

Echinochloa frumentacea was previously described as an effective extractor (As) ([20], responsive to fertilizers, hymexazole, and rhizobacteria inoculation [21]. There is the experience of using this species in the phytoremediation of polyelement-contaminated substrates such as the stabilization of municipal wastewater sludge [22] and phytoremediation of soil contaminated with cadmium, copper, and polychlorinated biphenyls [23]. *Echinochloa* species can also be used efficiently for chromium and cadmium extraction [24].

Other advantages are the sufficient diversity of varieties, their high seed germination (up to 10 years), that they develop well on soils poor in mineral nutrition, give from two to eight mows, and provide grain and green mass with average yields of 1.5–3 t/ha and 30–50 t/ha, respectively. The latter fact is very important for effective phytoextraction since TE removal by plants arises from two factors: (1) TE concentration in dry plant tissue [25] and (2) the amount of harvested biomass [9].

The acceptable concentration of TEs in plant raw material significantly expands the area of its valorization. Potential plant-based products are (1) non-food products such as biofuels [26], fibers, wood, essential oils, etc., and (2) animal feed, depending on the contamination level. Plant parts generally accumulate metals to different degrees [27], and some parts are usually less contaminated and may often be used for consumption or at least for non-food purposes even if the plant has been grown on contaminated or marginal soil [28]. Seeds generally accumulate the lowest TEs concentrations in a plant [29].

Other harvestable parts can be used in various processes, i.e., composting to fertilize TE-deficient soil, incineration, ashing, vacuum and oxidative pyrolysis, liquid extraction, the synthesis of hydrogen fuel, biofuels such as bioethanol, biogas, and activated carbons, hydrothermal oxidation, gasification, etc. In addition, metals can be recovered and reused for economic purposes by phytomining [30].

The objective of this study is to determine the phytoremediation potential and prospects for the use of *Echinochloa frumentacea* on urban soils with polyelement anomalies (especially for large urbanized ecosystems with high levels of industrial and vehicular pollution). During the study, the tasks were:

1. to assess the adaptive potential of *Echinochloa frumentacea* on HM-contaminated soils;
2. to assess bioaccumulation and the transfer factor of heavy metals (HMs) from polyelement-contaminated soils to *Echinochloa frumentacea*;
3. to study the rhizosphere microflora of *Echinochloa frumentacea* on HM-contaminated soils;
4. on the basis of the data obtained, to assess the possibility of using *Echinochloa frumentacea* for the phytoremediation of HM-contaminated chernozems.

2. Materials and Methods

2.1. Soil Sampling

Model laboratory experiments with urban ecosystem soils characterized by polyelement anomalies [17] were performed. To carry out the experiments, the soils were collected in the following sites: the sanitary protection zones (SPZ) of large metallurgical enterprises: Kosogorsky Metallurgical Plant (KMP), Tulachermet PJSC (TCh), the embankment opposite the arms and machine-building plants (Embankment), as well as a major highway and a sanitary protection zone of the central avenue of the city (Lenin Ave.). The gray forest soils of the state museum–estate of Leo N. Tolstoy "Yasnaya Polyana" served as background (Background). Sampling and soil preparation for determination of toxic elements was carried out in accordance with RNS R 53123-2008 [31]. The sampling depth was 0–25 cm.

2.2. Soil Characterization

To characterize the soil used for analysis of the pH, content of petroleum products, total carbon, mobile phosphorus, available forms of nitrogen–nitrates from water soluble ammonia were determined.

Preparation of soil extract and determination of its acidity were performed according to Russian National Standard (RNS) 26483-85 [32] using a Metler Toledo Delta 320 pH meter. Determination of the total organic carbon in soil was performed according to [33]. The content of mobile (available) phosphorus P_2O_5 in mg/100 g was determined by the photocolorimetric method according to Kirsanov [34]. The determination of nitrates and water-soluble ammonium in the soil was carried out by standard photocolorimetric methods according to RNS 26489-85 [35] and RNS 26488-85 [36]. Petroleum products were determined by the gravimetric method [37]. Elemental analysis of soil was determined using X-ray fluoresce in the chemical analytical laboratory of the Geological Institute of the Russian Academy of Sciences.

2.3. Experiment Design

The object of the study was the Japanese millet, *Echinochloa frumentacea* Link.

The soils of sampling points for the model experiment were placed in plastic containers with drainage that did not have a drain for water. Sowing of seeds and observation of plants was carried out in laboratory conditions at a temperature of 21–23 °C using natural light. Watering was carried out with distilled water as the topsoil dried up. The seed germination was determined on the 7th day.

2.4. Pigments Determination

A spectrophotometric method was used to determine the quantitative content of pigments in plants. The determination of pigments was carried out in an ethanol extract using 1 cm thick cuvettes in triplicate biological and analytical repetition. Optical density was determined at wavelengths of 665 nm, 649 nm, and 470 nm. Calculation of the quantitative content of chlorophylls and carotenoids was carried out according to the formulas [38], followed by conversion to g of fresh weight [39].

2.5. Plants Analysis

Several analytical techniques were applied to determine the elemental composition of plants materials. Content of As, Br, K, La, Na, Mo, Sm, U, W, Ba, Ce, Co, Cr, Cs, Hf, Ni, Rb, Sb, Sc, Sr, Ta, Tb, Th, Yb, Zn, Al, Ca, Cl, I, Mn, and V was determined using instrumental neutron activation analysis (INAA) at the fast-pulsed reactor IBR-2 in FLNP JINR. For Al, Ca, Cl, I, Mn, and V determination, samples of about 0.3 g were irradiated for 3 min and measured for 15 min. To determine As, Br, K, La, Na, Mo, Sm, U, W, Ba, Ce, Co, Cr, Cs, Hf, Ni, Rb, Sb, Sc, Sr, Ta, Tb, Th, Yb, and Zn, samples were irradiated for 3 days and measured after 4 and 20 days of irradiation. Gamma spectra of induced activity were measured using three spectrometers based on HPGe detectors with an efficiency of 40–55% and resolution of 1.8–2.0 keV for total absorption peak 1332 keV of the isotope ^{60}Co and Canberra spectrometric electronics. The analysis of the spectra was performed using the Genie2000 software from Canberra, while the calculation of concentration was carried out using software "Concentration" developed in FLNP. The quality control of measurement was assured by the use of the following reference materials: IAEA-336 (Lichen), NIST SRM 1572 (*Citrus* Leaves), NIST SRM 1575 (*Pine* Needles).

The content of Cu, Pb, and Cd in samples was determined by using an iCE 3400 Atomic Absorption Spectrometer (AAS) with electrothermal (graphite furnace) atomization (Thermo Fisher Scientific, Waltham, MA, USA). Details of samples preparation for analysis and measurement can be found in [40].

Along with INAA, elemental composition of plant samples grown on contaminated was determined using an Element 2 mass spectrometer (Thermo Fisher Scientific of GmbH, Dreieich, Germany) after adding indium as an internal standard. Before the measurement, the instrument was adjusted in such a way that the sensitivity was at least 1,000,000 cps when analyzing a solution of indium with a concentration of 1 μg/L. The instrument was calibrated using multielement standard solutions ICP-MS-68A Solution B, ICP-MS-E, and ICP-MS-B (High-Purity Standards, Charleston, SC, USA). The good correlation of the concentration of elements obtained by two technique was attained.

To determine Mg and Fe content, a KVANT-2 spectrometer (KORTEK, Moscow, Russia) was used. The measurement was carried out in an air-acetylene flame using absorption lines of 248.3 nm for Fe and 285.2 for Mg. The instrument was calibrated using a multielement standard solution ICP-MS-68A Solution A (High-Purity Standards, USA).

For ICP-MS and AAS analysis, plant samples were digested using a microwave system (MARS5, CEM Corporation, Charlotte, NC, USA) in XP-1500 Teflon liners. The liners were preliminarily kept with 10.0 mL of an aqueous solution of nitric acid (1:1) at 160 °C, then cooled and rinsed with deionized water (18.2 MΩ.cm, Milli-Q, ADVANTAGE A10, Millipore Corporation, Molsheim, France). A 0.5 g sample was placed in a liner and nitric acid was added, then the mixture was kept at room temperature for 48 h. Then, hydrogen peroxide was added, and after the end of the vigorous reaction, decomposition was carried out in a microwave system at 180 °C. The resulting solution was analyzed after dilution with deionized water. Each average plant sample was analyzed twice, the content of the element in the sample was calculated as the average of two independent values.

Simultaneously with the plants, the analysis of "blank" samples and standard samples of plants certified for microelement composition was carried out. Elodea canadensis EK-1, birch leaf LB-1, and grass mixture Tr-1 (Institute of Geochemistry SB RAS, Irkutsk, Russia) were used as standard samples. For every 10 routine samples, one blank sample and one standard sample were analyzed.

2.6. Determination of Ascorbic Acid (AA) and Glutathione (GSH)

Determination of ascorbic acid and glutathione was carried out by titrimetric method using fresh shoots of *Echinochloa frumentacea*. To determine the content of ascorbic acid, an aliquot of the centrifuged supernatant of plant shoots was titrated in a solution of metaphosphoric acid (5 mL) with 0.001 N solution of 2,6-dichlorophenolindophenol (2,6-DCPIP) to a slightly pink color.

To determine the amount of glutathione, 2–3 drops of a 15% KI solution and 5 drops of a 1% starch solution were added to an aliquot of the supernatant (5 mL) and titrated with a 0.001 N KIO_3 solution until a faint blue color was obtained.

The content of AA and GSH was calculated using the following formulas and expressed in mg/g of fresh weight

$$c\ (AA) = [(a \times K) \times 0.88 \times M] \div (m \times n)$$

$$c\ (GSH) = [(b - a) \times K \times 0.307 \times M] \div (m \times n)$$

where c(AA), c(GSH) is content of ascorbic acid and glutathione in plant material (mg/g fresh weight), a is the volume of titrated 2,6-dichlorophenolindophenol (mL), b is the volume of titrated KIO_3 (mL), K is the ratio of volumes of titrated KIO_3 and 2,6-dichlorophenolindophenol (in the present study K = 1.5 ± 0.02), n is the sample weight (mg), M is the total volume of extract (mL), m is the volume of aliquot (mL), 0.88 is the volume of AA (mL), equivalent to 1 mL of 0.001 N solution of 2,6-dichlorophenolindophenol, and 0.307 is the volume of GSH (mL), (equivalent to 1 mL of 0.001 N solution of KIO_3) [41].

2.7. Microbiological Analysis of Rhizosphere Soil

To analyze the rhizosphere soil, the plants were taken out from the pot, the bulk soil was shaken off from the plant roots, and the roots with the remaining adhering soil (thickness, no more than 2–3 mm) were used for analysis. The sampling was made in three replicates per pot. The sample of root with attached rhizosphere soil (1.5–2.0 g) was placed into 0.25-mL Erlenmeyer flask with 100 mL of sterile tap water and was shaken for 30 min. Then, the roots were taken out and the suspension was kept to let the soil particles settle out, after which a range of dilutions was prepared for isolation of rhizosphere microorganisms.

The total numbers of culturable heterotrophic bacteria, actinomycetes, and micromycetes after plant growth were estimated. For isolation and enumeration of the total number of culturable heterotrophs, we used the GRM-agar medium (State Research Center for Applied Biotechnology and Microbiology, Obolensk, Russia) of the following composition: fish meal pancreatic hydrolysate 12 g/L, enzymatic peptone 12 g/L, NaCl 6 g/L, and agar-agar 10–12 g/L. The number of actinomycetes was determined using a starch–ammonium agar medium of the following composition: $(NH_4)_2SO_4$ 1.0 g/L, $MgSO_4 \cdot 7H_2O$ 1.0 g/L, NaCl 1.0 g/L, $CaCO_3$ 3.0 g/L, and agar-agar 20 g/L. The number of microscopic fungi was determined using Martin's medium of the following composition: glucose 10 g/L, KH_2PO_4 5 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, peptone 5 g/L, agar-agar 20 g/L, and tap water 1 L (pH 5.5). The number of microorganisms resistant to Zn^{2+} and Pb^{2+} ions was determined on LB agar medium [42]. After sterilization, the water-soluble salts of heavy metals $ZnSO_4 \cdot 7H_2O$, $Pb(CH_3COO)_2$, or $CuSO_4$ were added to the culture medium to a final metal concentration of 0.5 mmol/L. The inoculated plates were incubated at 28–30 °C for 5–10 days, after which microbial colonies and colony-forming units (CFU) were counted and the morphological diversity of the microorganisms was measured.

The study of the taxonomic structure of rhizosphere microbial communities was carried out using metagenomic analysis of rhizosphere soil samples for the 16S rRNA gene. The purified DNA preparation was used as a template in the PCR reaction with universal primers (27f/533r) to the variable V4 region of the 16S rRNA gene using GS Junior Technology (Roche 454 Life Sciences, Branford, New Haven, CT, USA). Sequencing was performed on a MiSeq platform (Illumina, San Diego, CA, USA). The obtained data were analyzed using the QIIME v. 1.9.1.

2.8. Statistical Analysis

All experiments were performed in triplicate and the results are presented as mean values ± standard deviations. Basic statistics were completed by using Microsoft Office Excel Microsoft, Redmond, Washington, DC, USA), and all the others were performed with STATISTICA StatSoft (New York, NY, USA). The Student's *t*-test was performed to

reveal the differences between the values obtained for background and experimental soils ($p < 0.05$).

3. Results

The main characteristics of the analyzed soils are given in Table 1.

Table 1. Characteristics of urban soils used in the study.

Soil Sample	Background (Yasnaya Polyana)	KMP	Tulachermet	Highway (Lenin Ave.)	Embankment
Soil type	clay loam	clay loam	sandy loam	clay loam	sandy loam
pH	6.20	7.26	7.35	7.29	7.31
Content of essential elements, mg/kg					
$N\text{-}NO_3^-$	20.5 ± 1.2	28.2 ± 1.6	38.6 ± 3.5	41.1 ± 2.4	5.1 ± 1.8
$N\text{-}NH_4^+$	12.9 ±1.2	6.9 ± 0.7	9.5 ± 0.3	2.3 ± 0.2	5.2 ± 0.4
P_2O_5	51 ± 5	160 ± 4	98 ± 8	218 ± 7	305 ± 7
total carbon, % to air dry soil	4.87 ± 0.05	4.41 ± 0.11	4.84 ± 0.33	3.83 ± 0.06	4.21 ± 0.08
water-soluble carbon, % to the total	0.14 ± 0.05	0.13 ± 0.02	0.14 ± 0.06	0.12 ± 0.03	0.16 ± 0.06
humus, % to air dry soil	8.12 ± 0.16	7.14 ± 0.14	7.62 ± 0.15	6.16 ± 0.12	5.61 ± 0.11
humus carbon, %	4.72 ± 0.09	4.15 ± 0.08	4.43 ± 0.09	3.58 ± 0.07	3.26 ± 0.07
MgO, %	1.06 ± 0.09	0.94 ± 0.08	1.33 ± 0.11	0.62 ± 0.05	1.12 ± 0.09
CaO, %	1.17 ± 0.11	2.83 ± 0.25	2.24 ± 0.23	11.68 ± 1.32	5.48 ± 0.47
Na_2O, %	0.69 ± 0.07	0.55 ± 0.05	0.51 ± 0.05	0.63 ± 0.06	0.59 ± 0.05
K_2O, %	2.48 ± 0.24	2.42 ± 0.21	1.55 ± 0.18	1.21 ± 0.12	2.41 ± 0.22
Trace elements, mg/kg					
Mn	900 ± 87	**6600** ± 260	950 ± 87	700 ± 58	**1600** ± 156
Fe	39,250 ± 1960	**45,750** ± 2290	**116,650** ± 5830	**45,300** ± 2260	**97,700 ± 8754**
V	65 ± 3.2	55 ± 4	**145** ± 7.2	59 ± 2.9	91 ± 5
Cr	**116** ± 8	**137** ± 12	**117** ± 4.5	**70** ± 2.8	**1260** ± 80
Ni	41 ± 3	45 ± 4	**42** ± 3.3	35 ± 2.8	**285** ± 23
Cu	29 ± 2	52 ± 4	27 ± 0.8	30 ± 0.9	**1188** ± 89
Zn	23 ± 2	**192** ± 9	**71** ± 3.6	106 ± 5.3	**4579** ± 230
Pb	28 ± 2	**71** ± 6	24 ± 0.7	36 ± 1.1	**185** ± 14
As	5.1 ± 0.4	**9.9** ± 0.5	4.3 ± 0.2	5.4 ± 0.3	**12.5** ± 0.6
petroleum products, g/kg	1.5 ± 0.6	2.6 ± 0.4	**4.1 ± 1.0**	2.5 ± 0.7	**9.5 ± 3.2**

* Values exceeding MPC are marked in bold ([43,44]).

The KMP soil was characterized by a high content of Fe (45,750 mg/kg), exceeding the MPC for Mn (by 4.4 times) and Zn (by 41%); Tulachermet soil was characterized by a very high content of Fe (116,650 mg/kg), exceeding the MPC (APC) for V-Mn (by 40–50% for V and 10% for Mn), Ni (by 110–175%), Cu (by 127%), Zn (by 29–192%), As (by 115–220%), and oil products (by 4 times); the soil of Lenin Avenue was characterized by a high content of Fe (45,300 mg/kg), exceeding the permissible concentrations of Mn (by 6%) and Cu (by 186%). The soil of the embankment opposite the arms factory was characterized by the greatest pollution, it exceeded the permissible content of the complex of heavy metals, Mn (by 6%), Cr, Ni (3.6 times), Cu (36 times), Zn (83 times), As (by 25%), and petroleum products (by 9.5 times). In the background soils, the excess of MPC and APC for normalized elements was not noted.

In the performed study, the adaptive characteristics were studied and data on the sowing qualities of seeds and biometric parameters of *Echinochloa frumentacea* on soils contaminated with heavy metals were obtained. The results obtained are presented in Table 2. The soils of sanitary protection zones of metallurgical companies did not affect significantly the decrease in the germination of *Echinochloa frumentacea*. However, the soils of the SPZ of the highways and the soils of the embankment most contaminated with toxic elements caused a decrease in seed germination by 21–23% (Table 2).

Table 2. Effect of soil pollution with heavy meals on germinating ability and biometric parameters of *Echinochloa frumentacea*.

Characteristics	Background (Yasnaya Polyana)	TCh	KMP	Highway Lenin Ave.	Embankment
Germinating ability, %	87 ± 9	80 ± 7	80 ± 8	67 * ± 6	69 ± 7
Height of shoots, cm	15.1 ± 1.3	9.8 * ± 1.2	10.1 ± 1.3	8.6 * ± 0.9	4.6 * ± 0.6
root length, cm	7.6 ± 0.8	4.5 * ± 0.3	4.3 * ± 0.4	3.5 * ± 0.3	4.3 * ± 0.3

*—$p < 0.05$ for the difference between experimental and background samples.

The biometric parameters of *Echinochloa frumentacea* grown on the soils of the experimental zones were measured one month after germination. The results of biometric measurements showed that soil contamination with trace elements had a toxic effect on plants, which was expressed in a decrease in their growth parameters. The maximum toxic effect on the experimental plants was exerted by the soils of the embankment, where there were multiple excesses of MPC (MAC) for a complex of toxic components: Cr, Ni (4 times), Cu (36 times), Zn (83 times), and oil products (more than 9 times). The shoot length of plants on the most polluted soils decreased 3.3 times compared to the background. The soils of the SPZ of metallurgical industries caused a decrease in the growth parameters of the shoot up to 35% compared to the background. The soils of the sanitary protection zone of the highway had an increased toxic effect, reducing the shoot length of the experimental plants by 43% compared to the background (Table 2).

The development of the root system, which performs the function of supplying photosynthetic organs with minerals and water, also suffered from the presence of toxic elements in soils. The length of roots on the soils of the sanitary protection zone of metallurgical industries and the arms factory (embankment) was lower than on the background soils by 19–20%. The most polluted soils of the highway had the maximum inhibitory effect on the development of the root system, reducing the length of the roots by 54% compared to the background (Table 2, Figure A1). Such pronounced impact of the soils of the SPZ of the highway on the growth and development of the root system can also be due to its salinization caused by the use of reagents during the winter.

The formation of plant biomass, which is important to take into account when carrying out phytoremediation measures, depends on the leaf surface area formed by plants, the work of photosynthetic pigments, and enzymatic activity. The quantitative characteristics of the photosynthetic apparatus are the most important indicators of plant adaptation to oxidative stress in the condition of soil contamination. The obtained results showed that the soils of the sanitary protection zone of metallurgical plants had a stimulating effect on the pigment apparatus of *Echinochloa frumentacea*.

The content of chlorophyll a in plants grown on the soils of Tulachermet was higher than in plants grown on the background soils by 36% and on the soils of KMP by 19% (Table 3). The content of chlorophyll a in plants grown on the soils of Lenin Avenue was lower than on the background soils by 7%. The greatest depressing effect on the content of pigments was exerted by the soils of the most polluted experimental zone, the embankment near the arms plant. The content of chlorophyll a in the shoots of Japanese millet was lower by 22%. At the same time, the content of chlorophyll b decreased by 34% compared with the control. On the soils of the embankment, the development of chlorosis and apical necrosis of the leaves of *Echinochloa frumentacea* was observed.

Table 3. Effect of the polyelemental pollution of soils on the quantitative characteristics of the photosynthetic pigments of *Echinochloa frumentacea*.

Pigments' Content, mg/g and Their Ratio	Background	TCh	KMP	Lenin Ave.	Embankment
chlorophyll a	1.46 ± 0.12	1.99 * ± 0.14	1.74 ± 0.15	1.37 ± 0.11	1.14 * ± 0.07
chlorophyll b	0.32 ± 0.03	0.49 * ± 0.05	0.35 ± 0.03	0.24 ± 0.03	0.21 * ± 0.02
chlorophyll sum	1.78 ± 0.15	2.48 * ± 0.19	2.09 ± 0.18	1.61 ± 0.14	1.35 * ± 0.08
carotenoids	0.49 ± 0.05	0.66 * ± 0.07	0.57 ± 0.05	0.43 ± 0.04	0.40 ± 0.03
chlorophyll a/b	4.6	4.1	5.1	5.6	5.4
chlorophyll/ carotenoids	3.6	3.8	3.7	3.7	3.4

*—$p < 0.05$ for the difference between experimental and background samples.

The content of chlorophyll b in the seedlings of *Echinochloa frumentacea* grown on the soils of metallurgical industries was higher than on the soils of the background zones by 9 and 53% for the soils of the SPZ KMP and Tulachermet, respectively. However, in plants growing on the soils of the highway, the content of chlorophyll b decreased by 10% compared to the control.

The same trends were observed for the content of carotenoids in the shoots of *Echinochloa frumentacea*: on the soils of the SPZ KMP and Tulachermet, it increased by 16 and 35%, respectively, compared to the background soils (Table 3). On the contrary, on soils with the highest pollution, it decreased significantly (by 19%). Carotenoids are one of the components of the antioxidant system of the plant, as well as a component of the pigment systems. Therefore, a decrease in their content can lead not only to a decrease in plant biomass but also to the development of severe oxidative stress [45].

The study showed that the pigment apparatus of *Echinochloa frumentacea* is well adapted to soils with a low degree of polyelement pollution (SPZ of metallurgical industries); however, on soils with strong polyelement anomalies (Embankment), the photosynthetic apparatus of the plant suffers due to a drastic decrease in the chlorophylls level (up to 2 times).

An important adaptive characteristic of plants grown on soils contaminated with HMs is their ability to produce antioxidant compounds that prevent the development of oxidative stress, which is accompanied by the oxidation of membrane lipids and disruption of transport and homeostasis processes [46]. The plant antioxidant system (AOS) includes low molecular weight antioxidants such as ascorbic acid (AA) and glutathione (GSH) [47,48]. Due to the ability to reversibly oxidize and reduce, ascorbic acid is involved in the most important plastic and energy processes of the plant cell: photosynthesis and respiration [47,49–51]; it is a recognized antioxidant [52–54], participates in growth and development processes [48,55], and forms plants' resistance to many adverse factors: UV radiation, pathogens [56], ozone [55,57], low temperatures, drought [48], soil salinity [48,58], heavy metals [59,60], and petroleum products [61].

The content of ascorbic acid and glutathione as components of AOS that prevent the development of oxidative stress in seedlings of *Echinochloa frumentacea* grown on soils with polyelement pollution was studied. The results are presented in Table 4.

Table 4. Effect of the polyelemental pollution of soils on the content of ascorbic acid and glutathione in *Echinochloa frumentacea* shoots.

Experimental Soils	Content of Ascorbic Acid, mg/g	Content of Glutathione, mg/g
Background	6.86 ± 0.52	1.45 ± 0.12
TCh	7.12 ± 0.64	1.52 ± 0.14
KMP	8.18 * ± 0.79	1.67 * ± 0.16
Highway	8.05 ± 0.74	1.58 ± 0.15
Embankment	6.45 ± 0.58	1.35 ± 0.12

*—$p < 0.05$ for the difference between experimental and background samples.

The content of low-molecular antioxidants ascorbic acid and glutathione in the shoots of *Echinochloa frumentacea* was 2–2.5 times higher than in *Poa pratensis* grown on the same soils and varied within 6.9–8.2 mg/g (AA) and 1.35–1.67 mg/g (GSH) (Table 4). On the soils of the SPZ Tulachermet, KMP, and the highway, it was higher than in the control by 4, 19, and 17%, respectively. On the soils of the embankment, a slight decrease in the content of AA was observed compared to the control (by 6%). The content of glutathione in the shoots of *Echinochloa frumentacea* varied within 1.35–1.67 mg/g and was also higher on the soils of three experimental zones by 5, 15, and 9% for Tulachermet, KMP, and Lenin Avenue, respectively. The content of glutathione slightly decreased in comparison with the control on the soils of the embankment.

Study of the formation of the biomass of *Echinochloa frumentacea* showed that after 1.5 months of growth on the soils of SPZ of metallurgical plants, the plant formed more biomass than on the background soils. The biomass of Japanese millet grown on experimental soils varied in the range of 428–1024 g/m^2 (Table 5). The increase in shoot biomass relative to plants grown on background soils was 39% for plants on KMP soils and 48% for plants on Tulachermet soils. The biomass of plants grown on the soils of the SPZ of Lenin Ave. and the embankment was 12.6% and 20% less than on the background soils, respectively. The moisture content in the shoots on the background soils and Tulachermet was 82–84%, on the soils of KMP and highway in the experimental plants, the accumulation of dry matter increased and the water content decreased to 78–65%.

Table 5. Formation of *Echinochloa frumentacea* biomass on the soils with polymetal anomalies.

Soil Samples	M of the Shoot, g	Shoots Biomass, kg/ha	Dry Content, %	Moisture Content, %	Biomass of Dry Weight (Shoots), kg/ha
Background	1.34 ± 0.12	5360 ± 487	15.3	84.8	820
TCh	1.98 * ± 0.17	7920 * ± 713	17.6	82.4	1393
KMP	1.87 * ± 0.19	7480 * ± 778	22.4	77.6	1675
Highway (Lenin Ave.)	1.17 ± 0.11	4680 ± 423	35.3	64.7	1652
Embankment	1.07 * ± 0.09	4280 * ± 419	27.8	72.2	1190

*—$p < 0.05$ for the difference between experimental and background samples.

To assess the phytoremediation capacity of plants, it is important to determine the bioaccumulation of toxic elements in plant organs and to calculate the efficiency of removal of the element from contaminated soils.

Since there are no MPCs established for plants, and the data on the composition of elements in different works differ greatly depending on the applied methods of measurement and sample preparation techniques [62–65] (some studies were performed on unwashed plant material), when determining the bioaccumulative characteristics of plants, a comparison was made with the average data obtained for different plants species and published in Markert [66]—Reference Plants (RP).

The accumulative capacity of the shoots and roots of adult plants (4.5 months) grown on contaminated soils was assessed using atomic absorption spectroscopy.

The content of V in the roots of *Echinochloa frumentacea* grown on contaminated soils varied within 8.9–47.8 mg/kg of dry weight, which is 43–78 times higher than in the aboveground parts of the plant (Table 6). On the soil with a high content of V (Tulachermet), the accumulation of elements by the root system of Japanese millet increased significantly, while in the shoots the content of the element was at a low level of 0.61 mg/kg of dry weight.

Table 6. The average content of heavy metals in the shoots and root system of *Echinochloa frumentacea* grown on soils with polyelement anomalies (full vegetation period), mg/kg dry weight (AAS).

Collection Site	Organ	Pb mg/kg	Cd mg/kg	Cu mg/kg	V mg/kg
Background	shoot	0.20 ± 0.006	0.46 ± 0.014	11.02 ± 0.3	0.40 ± 0.02
	root	3.30 ± 0.09	0.27 ± 0.008	27.84 ± 0.8	8.85 ± 0.4
TCh	shoot	0.14 ± 0.004	0.25 ± 0.008	11.47 ± 0.3	0.61 ± 0.02
	root	1.57 ± 0.05	0.18 ± 0.05	16.44 ± 0.5	47.79 * ± 2.4
KMP	shoot	7.42 * ± 0.22	0.25 ± 0.015	16.34 ± 0.5	0.87 ± 0.04
	root	7.38 * ± 0.22	0.51 ± 0.08	41.05 * ± 1.2	19.26 * ± 1.0
Highway	shoot	0.18 ± 0.05	0.28 ± 0.08	27.56 * ± 0.8	0.43 ± 0.02
	root	6.93 * ± 0.02	1.01 ± 0.03	96.60 * ± 2.9	18.47 * ± 0.9
Reference Plant, Markert, 1992		1	0.05	10	0.5

*—$p < 0.05$ for the difference between experimental and background samples.

The root system of *Echinochloa frumentacea* is an active barrier that prevents the transport of V into the photosynthetic organs of the plant. The coefficient of transfer of the element from roots to shoots was 0.01–0.05. A possible way to clean V-contaminated soil using Japanese millet is rhizofiltration.

The content of Pb in the roots of *Echinochloa frumentacea* was 1.6–7.4 mg/kg, and in the shoots, 0.14–7.42 mg/kg dry weight (Table 6). An analysis of the obtained data showed that the root system of *Echinochloa frumentacea* ceases to perform barrier functions with respect to Pb when the threshold concentrations of the element in soils are exceeded. The content of Pb in Japanese millet grown on soils, in which Pb content exceeded the MPC value (71 mg/kg soil) was the highest and the same for shoots and roots, 7.4 mg/kg dry weight (Table 6). At the obtained values of the removal of the element from soils, which exceeded RP by more than 7 times, it should be expected that the content of the element in the soil during phytoremediation measures will come to acceptable values in 4–5 years.

For most plants, the critical level of copper content is 10–20 mg/kg dry weight [67]. Cu accumulation by the shoots and roots of *Echinochloa frumentacea* exceeded the threshold of normal regulation for plants on KMP-contaminated soils, where the content of the copper was quite high (52 mg/kg) and amounted to 16.3 mg/kg in shoots and 41 mg/kg in roots. In general, the root system of Japanese millet had a high affinity for copper. Therefore, one of the ways to clean soils from Cu in the case of polyelement pollution using *Echinochloa frumentacea* is rhizofiltration. However, in the shoots of KMP, highway, and embankment soils, the content of Cu varied from 16.3 to 96.6 mg/kg of dry weight (Tables 6 and 7). These values are higher than those given in Murillo et al. [68] for sorghum of 4.6–11.7 mg/kg; therefore, it can be assumed that Japanese millet can also be used for the phytoextraction of Cu from soils with polyelement contamination.

Table 7. Bioaccumulation of toxic elements by *Echinochloa frumentacea* grown on soils with polyelement anomalies (vegetation period 1.5 months) (average data for ICP-MS and INAA), mg/kg.

Element	TCh	KMP	Highway	Embankment	Background	RP (Markert, 1992)
Sc	0.070 ± 0.002	0.059 ± 0.002	0.076 ± 0.0021	0.047 ± 0.001	0.057 ± 0.002	0.02
Rb	6.4 ± 0.6	4.8 ± 0.4	3.0 ± 0.3	5.7 ± 0.5	38.3 ± 0.2	50
Mo	2.2 * ± 0.01	12.7 * ± 0.6	3.5 * ± 0.01	4.2 * ± 0.02	0.8 ± 0.02	0.5
Cd	0.31 ± 0.009	0.28 ± 0.008	0.47 ± 0.01	0.37 ± 0.01	0.52 ± 0.02	0.05
Sb	0.023 ± 0.001	0.019 ± 0.001	0.044 * ± 0.002	0.074 * ± 0.003	0.010 ± 0.001	0.1
Ba	6.7 ± 0.34	9.6 ± 0.48	12.3 ± 0.62	4.8 ± 0.24	10.8 ± 0.54	40
Pb	0.50 ± 0.02	0.25 ± 0.01	0.44 ± 0.01	0.88 * ± 0.03	0.22 ± 0.02	1
Th	0.074 ± 0.004	0.050 ± 0.003	0.075 ± 0.004	0.045 ± 0.003	0.063 ± 0.003	0.005
U	0.023 ± 0.001	0.014 ± 0.001	0.037 * ± 0.001	0.036 * ± 0.001	0.015 ± 0.001	0.01
V	0.61 ± 0.03	0.64 ± 0.03	0.74 ± 0.04	0.66 ± 0.03	0.50 ± 0.02	0.5
Cr	1.01 ± 0.06	0.93 ± 0.06	1.62 ± 0.1	4.57* ± 0.27	0.93 ± 0.06	1.5
Co	0.19 ± 0.01	0.19 ± 0.01	0.27 ± 0.02	0.10 ± 0.01	0.16 ± 0.01	0.2
Ni	1.4 ± 0.1	2.4 ± 0.2	3.6 ± 0.3	4.7 ± 0.4	3.4 ± 0.3	1.5
Cu	20.2 * ± 0.6	12.3 ± 0.4	31.3 * ± 0.9	32.1 * ± 0.9	21.2 ± 0.6	10
As	0.29 * ± 0.01	0.21 ± 0.01	0.15 ± 0.01	0.20 ± 0.01	0.12 ± 0.01	0.1
Se	0.15 ± 0.01	0.13 ± 0.01	0.06 ± 0.005	0.19 ± 0.01	0.13 ± 0.01	0.02
Al	295 ± 8.8	322 ± 9.7	390 * ± 12	251 ± 7.5	275 ± 8.2	80
Fe	291 ± 15	379 * ± 129	324 ± 16	327 ± 16	282 ± 14	150
Mn	173 ± 8.6	101 ± 5.5	199 ± 10	572.8 * ± 30	195 ± 9.7	200
Zn	100 * ± 4	41 ± 1.6	109 * ± 4.3	454 * ± 18	53	50
Sr	36 ± 2.9	91 * ± 7.2	48 ± 3.8	45 ± 3.6	33 ± 2.6	

*—$p < 0.05$ for the difference between experimental and background samples.

However, on soils with a high content of V and Cr, the absorption of the element by the plant decreased, which should be taken into account when carrying out remediation measures. According to the literature data, when the content of copper in plants exceeds the critical level, the signs of toxicity may develop: the concentration of chlorophyll decreases, and the growth of shoots and roots is suppressed [69]. The complex of HM pollutants in the experimental soils suppressed the growth parameters of Japanese millet; however, on the soils of the sanitary protection zone of metallurgical industries, a compensatory mechanism was observed and the content of photosynthetic pigments increased (Table 3).

Accumulation of Mo by shoots of *Echinochloa frumentacea* on the soils of Tulachermet up to 12.7 mg/kg dry weight, which is 3–6 times more than on the soils of other experimental zones and 12 times more than on the background soils (Table 7). High Mo-uptake can be explained by the slightly alkaline pH of analyzed soils [70].

The accumulation of Cd did not exceed 0.28–0.52 µg of dry weight in most of the experimental variants. These values are 5.5–10.4 times higher than the average data for RP. The Cd enrichment factor for *Echinochloa frumentacea* is 5.6–19 and is at its maximum on the soils of the sanitary protection zone of the highway. Low Cd uptake can be associated with the presence of zinc in soil, which inhibits Cd uptake and translocation under cadmium/zinc combined stress [71].

The accumulation of Cr by the shoots of *Echinochloa frumentacea* on soils with the highest polyelement pollution, where the content of the element was many times higher than APC (1260 mg/kg), was higher than for other soils (Cr content up to 117 mg/kg) and amounted to 4.7 mg/kg of dry weight (Table 7). These values were 3 times higher than RP; however, with the soils' contamination with Cr when its content exceeds TEC 4 times, this level of bioaccumulation of the element is not enough for effective biological treatment in terms less than 10 years. On the soils of other sampling sites, Cr accumulated in the shoots of *Echinochloa frumentacea* was up to three times higher than the upper limit of normal values (0.1–0.5 mg/kg, [62]): 0.93–1.62 mg/kg.

The critical level of Co in most plants starts from 0.4 mg/kg. Cereals are most sensitive to their excess [72] The Co content in Japanese millet ranged from 0.10 to 0.27 mg/kg dry weight and lies within the average values characteristic for vegetation, 0.02–1 mg/kg [62].

The content of Ni in *Echinochloa frumentacea* plants varied in the range of 1.4–4.7 mg/kg dry weight and its maximum accumulation was attained on the most polluted soils. These values are 2–3 times higher than the average values for vegetation grown on the soils of Tulachermet, the highway, and embankment.

The content of As in the shoots of *Echinochloa frumentacea* was 0.15–0.29 mg/kg of dry weight. Its content on the soils of the SPZ of metallurgical industries and the embankment was 2–3 times higher than RP values. However, based on the data on the bioaccumulation and removal of As, Japanese millet is not recommended for the phytoremediation of soils contaminated with arsenic.

The critical concentrations of manganese in plants vary from 220 to 5300 mg/kg dry weight [67]. Japanese millet is not an Mn bioaccumulator. The content of the element in the shoots of *Echinochloa frumentacea* falls within the limits of average values and amounts to 57–199 mg/kg of dry weight and lies below the limits of critical values (Table 7).

Visible symptoms of zinc toxicity appear with its concentrations in plants above 300 mg/kg but are sometimes possible at lower concentrations (100 mg/kg) [67]. The content of Zn in the shoots of *Echinochloa frumentacea* was 53 mg/kg on the background soils and varied in the range of 41–457 mg/kg dry weight on the soils of the experimental zones. The content of the element exceeded the critical concentrations for plants on the most polluted soils of the embankment, which could cause the observed toxic effects. The factor *Echinochloa frumentacea* enrichment with Zn was two on the soils of the KMP and Lenin Avenue and eight on the soils of the SPZ of the arms plant (embankment). These are a good indicator for the phytoextraction of the element from soils, taking into account the accumulation of biomass by plants.

The content of Fe in the shoots of *Echinochloa frumentacea* varied within 282–389 mg/kg of dry weight and was below the critical concentration limits for plants (500 mg/kg [62,67]) but higher than the average data for RP 2 or more times. Apparently, for Fe, which is a soil pollutant in the region, there is no barrier at the level of the root system of the plant.

Culture-Based Assessment of Microbial Communities in the Rhizosphere of E. frumentacea

At the end of the experiment, the total number of heterotrophic bacteria, and the number of actinomycetes and micromycetes, were determined in the rhizosphere of plants. The results of the analysis of the main groups of cultivated heterotrophic soil microorganisms in the rhizosphere of *E. frumentacea* are shown in Figure A2.

The abundance of rhizospheric microorganisms depended on the kind of soil in which the plants were grown. The number of bacteria in the plant rhizosphere varied from 0.8 to 3.3×10^7 CFU/g of soil sampled from the Embankment and Background, respectively. It was found that the soil of the Embankment near the Tula Arms Plant was the most polluted (Table 1). The high content of almost all metals analyzed clearly caused the toxicity of this soil, which led to the inhibition of the number of all groups of microorganisms studied, bacteria, actinomycetes, and micromycetes. In addition, this soil was characterized by a minimum content of humus carbon (Table 1), which is important for the development of soil microflora.

The number of cultivated heterotrophic bacteria in the rhizosphere soil from the Embankment (Figure 1a) was two times lower than in the background soil (Yasnaya Polyana). The almost two-fold excess of the number of bacteria in the Tulachermet soil may be due to the presence of an additional carbon source in this soil (oil products' content was 4.1 g/kg, and humus carbon was higher than in the Embankment soil). However, an even higher concentration of oil products (9.5 g/kg) did not support the number of bacteria in the soil of the Embankment, which was associated with the toxic effect of heavy metals on oxidative enzymes involved in the hydrocarbon degradation. The soil from Lenin Ave. also stimulated the rhizospheric bacteria; however, there was no obvious explanation of this effect from the data of analyses of this soil (Table 1). We can only assume that in terms of background pollution, the Lenin Ave. soil, just like the KMP soil, was the closest to the background one.

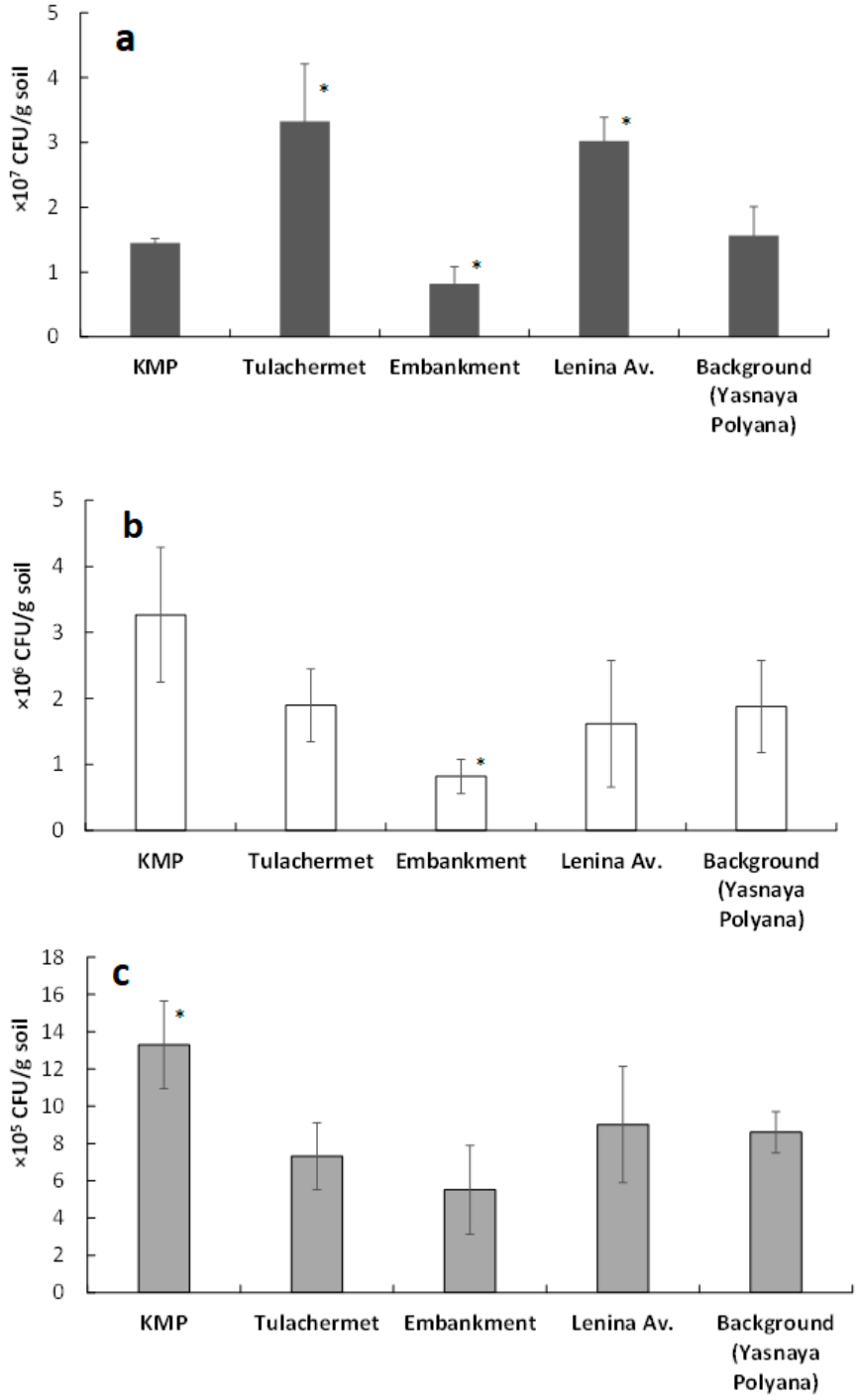

Figure 1. The number of cultivable heterotrophic bacteria, actinomycetes, and micromycetes in the rhizosphere of *E. frumentacea*. (**a**)—bacteria; (**b**)—actinomycetes; (**c**)—micromycetes. Error bars mean standard errors/*—$p < 0.05$ for the difference between experimental and background samples.

The number of actinomycetes in the rhizosphere of *E. frumentacea* varied from 0.8 to 3.3 × 10^6 CFU/g of the soil in the Embankment and KMP samples, respectively. The influence of urban soils on the actinomycete population in the rhizosphere was most noticeable in the soils of the Embankment and KMP (Figure 1b). In the first case, in relation to bacteria, the inhibitory effect of the contaminated urban soil was visible: the number of actinomycetes in the rhizosphere decreased by 2.3 times compared to the control soil. However, in the KMP soil, stimulation of actinomycetes (1.7 times) was observed. Probably, this could be associated with an increase in the actinomycete taxa resistance to heavy metals

and the ability to participate in remediation of the environment [73]. This explanation was also confirmed by our data on the taxonomic structure of the rhizosphere community of *E. frumentacea* grown in the KMP soil. There were no significant differences between the abundance of actinomycetes in the Tulachermet and Lenin Ave. soils and the control soil of Yasnaya Polyana.

The number of micromycetes in the rhizosphere of *E. frumentacea* reached 5.5–13.3 $\times 10^5$ CFU/g of the soil of the Embankment and KMP samples, respectively (Figure 1c). The changes in the abundance of micromycetes depending on the soil type were similar to those described for actinomycetes. The minimal number of micromycetes was observed for the Embankment soil (a 1.6-fold decrease from the control), and the maximal number was observed for the KMP soil (a 1.5-fold increase in comparison with the control), which could be associated with specific stimulation by the plant of micromycetes taxa resistant to heavy metals.

The sequencing of 16s rRNA gene from rhizosphere samples resulted in 133.776 reads. After data denoising and chimera screening, a total of 92.527 sequences were used for further identification. The average number of nucleotide sequences in the library per sample was 15.728.

To characterize biodiversity and carry out a comparative analysis of the communities, the parameters of α-diversity were calculated, the taxonomic composition of the community in all samples was determined and compared, and taxa that reliably decreased or increased their number in the rhizospheres of the plants studied were identified.

The calculation of the sampling effort indicated that, on average, 73.7% of the true diversity of the rhizosphere communities of *E. frumentacea* were covered (Table 8). The α-diversity was assessed using species richness indices (observed OTUs) and the Shannon and the Simpson indices in the rhizosphere of *E. frumentacea* grown in the control soil, and the average number of OTUs was minimal (2013 on average) in comparison with other soils. The species richness index of plants grown in Tulachermet was maximal (3816 observed OTUs) (Table 8).

Table 8. The α-diversity indices for rhizospheric microbial communities of *E. frumentacea* grown on different soils.

Soil Samples	Observed OTUs	PD Whole Tree	Chao1	Shannon	Simpson	Sampling Effort
KMP	3437	94.08	4820.03	10.39	0.99829	71.3
Tulachermet	3816	102.17	5321.48	10.64	0.99842	71.7
Embankment	3059	98.08	4056.96	10.07	0.99738	75.4
Lenin Av.	3403	87.14	4620.18	10.38	0.99811	73.7
Background	2013	66.99	2635.74	8.94	0.99331	76.4

The Shannon and Simpson indices indicated a high taxonomic diversity of bacterial communities in the rhizosphere of plants grown in contaminated urban soils, while the background soil (Yasnaya Polyana) was characterized by the lowest taxonomic diversity (Table 8).

Figure A2 shows the bacterial types that dominate in the rhizosphere of *E. frumentacea*. The Proteobacteria, Actinobacteria, Planctomycetes, Acidobacteria, as well as Bacteroides and Chloroflexi phyla occupied the dominant positions in the taxonomic structure of the rhizosphere community of *E. frumentacea*. However, the ratio of these types was different for plants grown on different soils. Thus, the share of Proteobacteria in the total taxonomic structure of the microbial community in the background soil (Yasnaya Polyana) reached 52%, but it significantly decreased when plants were grown on urban soils. The minimal amount of Proteobacteria was noted in the soils of KMP (27%) and Tulachermet (28%), followed by the soils of Lenin Ave. (30%) and the Embankment (36%). At the same time, the share of another dominant phylum, Actinobacteria, increased from 18% (in control) to 22, 26, 29, and 31% in the soils of the Embankment, Tulachermet, KMP, and Lenin Ave.,

respectively. In the rhizospheric communities of plants grown in the polluted urban soils, the share of other phyla also clearly increased. Thus, the share of Acidobacteria in the control soil was only 4% but increased to 5, 8, 9, and 9.2 in the soils of Lenin Ave., the Embankment, Tulachermet, and KMP, respectively. The Planctomycetes phylum in the rhizospheric community of *E. frumentacea* grown in the control soil was only 4%, while in contaminated urban soils it increased to 6, 9, and 10% (in the soils of the Embankment, Tulachermet, Lenin Ave., and KMP, respectively). In contaminated soils, an increase in Chloroflexi share from 3% in the control to 6–7% in urban soils was noted. In addition to Proteobacteria, under the influence of pollution, the share of another phylum, Firmicutes, decreased (albeit less significantly), which was more than 4% in clean soil and decreased to 2–3% in polluted soil. Changes in the share of other phyla of the microbial community in the rhizosphere of *E. frumentacea* under the influence of pollution were unsignificant. Thus, a comparative analysis of the taxonomic structure of the rhizosphere microbial community at the phylum level revealed a distinct influence of urban soils characterized by technogenic polyelement anomalies.

The taxonomic analysis of the dominant phylum Actinobacteria made it possible to identify 39 families, among which 16 families occupied a fundamental position, presented in Table A1 and constituting from 60% to 77% of all found families of Actinobacteria in the rhizosphere of *E. frumentacea*. The Gaiellaceae family had the maximal share in the population of rhizospheric actinomycetes in almost all soil samples; only in the soil of the Embankment was the abundance of Gaiellaceae minimal. At the same time, this soil was characterized by an increased share of the Micrococcaceae family (Table A1). Nocardioidaceae, Micromonosporaceae (0.9–3.4%), as well as representatives of the Solirubrobacterales and 0319-7L14 orders were other notable actinomycetes in the rhizosphere of *E. frumentacea*.

As part of another dominant phylum, Proteobacteria, 66 families of bacteria were identified. Thirteen families accounting for 72–86% of all OTUs assigned to Proteobacteria made the most significant contribution to the rhizosphere microbiome structure of *E. frumentacea*, (Table A1). Alphaproteobacteria is the most numerous class of the Proteobacteria phylum, accounting for 41 to 53% of all Proteobacteria. Among Alphaproteobacteria, the dominant position was occupied by the Sphingomonadaceae (2.6–12.7%) and Hyphomicrobiaceae (2.0–5.6%) families. The Betaproteobacteria class accounted for 15 to 32% of all OTUs assigned to the Proteobacteria phylum, and the Comamonadaceae (0.8–5.1%) and Oxalobacteraceae (1.3–3.5%) families were its dominant representatives. The Gammaproteobacteria class accounted for 16 to 30% of all OTUs assigned to the Proteobacteria phylum, and the Xanthomonadaceae family, whose share reached 1.6 to 13.5%, was its dominant representative. It can be assumed that, along with Sphingomonadaceae, the Xanthomonadaceae family can also form the basis of the rhizobiome of *E. frumentacea*. The share of these families decreased when plants were grown in all urban soils, except for Lenin Ave., where it distinctly increased, as did the share of the Hyphomicrobiaceae and Oxalobacteraceae families. Among other bacterial taxa the dominant position in the soil of the Embankment was occupied by representatives of Comamonadaceae, and in the soil of KMP, by Sphingomonadaceae, Hyphomicrobiaceae, and Xanthomonadaceae. The share of Oxalobacteraceae increased in all urban soils compared to the control soil. In the rhizosphere of plants grown in Tulachermet soil, it reached a maximum (3.5%).

In the course of this study, 22 morphologically different strains of bacteria were isolated from the rhizosphere of *E. frumentacea* plants. All isolates were characterized by their resistance to heavy metals in the environment and plant growth-promoting potential. The strains combining these properties were transferred to the collection of rhizospheric microorganisms of the Institute of Biopharmaceutics, Russian Academy of Sciences (http://collection.ibppm.ru, accessed on 14 March 2022).

4. Discussion

Summarizing the data on the quantitative content of the studied plant antioxidant system components of *Echinochloa frumentacea* showed that the plant is characterized by

a good adaptive potential on soils with polyelement anomalies caused by technogenic pollution of metallurgical industries: the content of carotenoids increases from 16 to 34% on the soils of the SPZ of metallurgical enterprises. The amount of AA and GSH in plants grown on experimental soils are 4–19% higher compared to the soils of the background zone (Table 9).

Table 9. Components of the *Echinochloa frumentacea* antioxidant system on soils with polyelement anomalies.

Sample of Soils	Carotenoids	AA	GSH
TCh	34%↑	4%↑	5%↑
KMP	16%↑	19%↑	15%↑
Lenin Av.	No significant differences	17%↑	9%↑
Embankment	19%↓	6%↓	7%↓

↑ higher or ↓ lower compared to background soil.

However, intense pollution of the soils of the Embankment caused inhibition of the synthesis of carotenoids and consumption of the synthesized low molecular weight antioxidants (ascorbic acid and glutathione) necessary for the urgent adaptive response of the plant to prevent the effects of oxidative stress [74]. In bean and rice HM-stressed plants, other authors also observed a significant increase in the content of these antioxidants compared to the control [75,76].

Echinochloa frumentacea on soils with multielement contamination accumulated similar amounts of Mn compared to the literature data for *Echinochloa colona* (L.) Link (213 mg/kg) [15]. The accumulation of Zn by shoots of Japanese millet was 2 times lower than for *Echinochloa colona*; however, on soils with the highest Zn content, its accumulation increased by 1.7 times.

The calculation of the removal of elements from soils by *Echinochloa frumentacea* during the growing season of 1.5 months is presented in Table 10.

Table 10. Removal of elements from soils *Echinochloa frumentacea* in the case of polyelement pollution, g/ha.

Element	KMP	TCh	Lenin Av.	Embankment	Background
Mo	3.69 *	17.7 *	5.78 *	4.70 *	0.66
Cd	0.52	0.39	0.78 *	0.41	0.43
Sb	0.04	0.03	0.07 *	0.08 *	0.01
Ba	11.2	13.4 *	20.3 *	5.37	8.86
Pb	0.84 *	0.35	0.73 *	0.98 *	0.18
Th	0.12 *	0.07	0.12 *	0.05	0.05
U	0.04	0.02	0.06 *	0.04	0.01
V	1.02 *	0.89	1.22 *	0.74	0.41
Cr	1.69	1.30	2.68 *	5.11 *	0.76
Co	0.32 *	0.26	0.45 *	0.11	0.13
Ni	2.35	3.35	5.95 *	5.26	2.79
Cu	33.8	17.1	51.7*	35.9 *	17.4
As	0.49 *	0.29 *	0.25	0.22	0.10
Se	0.25 *	0.18	0.10	0.21 *	0.11
Al	494 *	449	644 *	281	226
Fe	488 *	528 *	535 *	366	231
Mn	290 *	141	329 *	64 *	160
Zn	168 *	57.1	180 *	508 *	43.5
Sr	60.3 *	127 *	79.3 *	50.4	27.1

*—$p < 0.05$ for the difference between experimental and background samples.

The calculation of the removal of elements on the dry biomass of plants showed that on experimental soils with polyelement anomalies, the analyzed plant is not suitable for

Pb and As phytoremediation. The removal of these elements from the soil by shoots was 0.35–084 g/ha and 0.22–0.49 g/ha, respectively. Bioaccumulation of Pb was at its maximum in the root system and constituted 1.57–7.38 mg/kg dry weight. The content of Pb in the shoots was lower and varied within 0.14–0.88 mg/kg dry weight. At the same time, in plants grown on soils with a multiple excess of the MPC for Pb by the end of the growing season, up to 7.42 mg/kg of dry weight of Pb was accumulated in the aboveground mass. These figures are lower compared to those available in the literature for other cereals, e.g., sorghum 107–378 g/ha [77].

The content of V in the root system of *E. frumentacea* on contaminated soils reached 9–48 mg/kg dry weight, depending on the soil. At the same time, the root system performs a barrier function and the transfer of V into the shoots was very low, 0.01–0.05. The content of V in the shoots was 0.43–0.87 mg/kg dry weight. The removal of V from contaminated soils by shoots was 0.89–1.12 g/ha.

The amount of Ni and Cu removed from the polluted soils increased with an increase in pollution level and was 5.26–5.95 g/ha for Ni and 34–52 g/ha for Cu. The content of Cu in the shoots was 11.5–27.6 mg/kg of dry weight, and in the root system, 16.5–96.6 mg/kg of dry weight. The plant can be used for long-term phytoextraction of the indicated elements when the MPC is exceeded up to 2 times.

The removal of manganese from soils, in which its content exceeds the MPC more than 4 times, amounted to 290 g/ha. These values are not sufficient for remediation of the soil when MPC is multifold exceeded because the remediation period can be more than 20 years.

The removal of Zn from contaminated soils was 168–508 g/ha and it was maximal on the most polluted soils. The content of zinc in the shoots of *E. frumentacea* grown on soils, where the MPC was exceeded by less than twice, was 109 mg/kg; while at multiple excess of MPC (4.4 times) this increased to 454 mg/kg of dry weight. Zinc is a mobile element and is well accumulated by the plant. The removal of the element for *Sorghum bicolor* and *Zea mais* due to the formation of the maximum biomass, according to the literature data, was 1223–1998 g/ha [77]. An increase in the accumulation of zinc and the removal of the element from contaminated soils makes it possible to recommend this culture for phytoremediation in grass mixtures with other crops, for example, with a representative of cruciferous plants, *Brassica napus*.

Studies on the rhizosphere microflora of *Echinochloa* plants are rare [78–80], and we did not find any data on the impact of technogenic soil pollution on the structure of microbial communities in the root zone of *E. frumentacea*. At the same time, the study of the root microflora of remediating plants and the isolation of rhizospheric microbial strains that are resistant to the presence of pollutants (metals) in the environment and have a plant growth stimulating potential will make it possible to find a suitable microbial inoculant as a tool to increase the effectiveness of the remediation capabilities of *E. frumentacea* used for the restoration of technogenically disturbed soil ecosystems. Previously, a positive effect on the growth of *E. frumentacea* when inoculated with PGPR (plant growth-promoting rhizobacteria) strains was shown both in pure soil conditions [81,82] and in the presence of heavy metals (Cd, Ni, Pb, Cu) and As [83].

It is known that heavy metals have a great effect on bacterial communities of soil, contributing to an increase or decrease in bacterial abundance, species diversity, and alterations in dominant and subordinate species [84]. However, a negative effect of heavy metals on the composition and abundance of soil actinomycetes and fungal communities is also noted [85–87].

In this study, we demonstrated that the abundance of the main groups of microorganisms (bacteria, actinomycetes, and micromycetes) in the rhizosphere of *E. frumentacea* depended on the type of soil in which the plants were grown and, probably, was determined by its characteristics. The minimal number of all groups of microorganisms studied (on average, two times lower than in the uncontaminated control soil) was noted in the Embankment soil, which contains the highest concentrations of both inorganic (heavy

metals) and organic (oil products) pollutants. A two-fold increase in the number of bacteria was observed in the rhizosphere of plants grown in the soils of Tulachermet and Lenin Ave., a 1.7-fold increase in the number of actinomycetes, and 1.5-fold increase in the number of micromycetes were observed in the soil of KMP, which could be due to specific plant stimulation of microbial taxa resistant to heavy metals.

The rhizosphere populations of *E. frumentacea* grown in the background soil of Yasnaya Polyana were characterized by the lowest taxonomic diversity compared to the rhizomes of plants grown in contaminated urban soils. The species richness index was maximal for rhizosphere communities of plants grown on Tulachermet soils. The taxonomic structure of the rhizospheric microbiomes of *E. frumentacea* was represented by the dominant bacterial phyla Proteobacteria and Actinobacteria, as well as the phyla Planctomycetes, Chloroflexi, Acidobacteria, and Bacteroides. Compared to the control soil, the cultivation of *E. frumentacea* plants in urban soils led to a change in the ratio of the main phyla. The share of Proteobacteria (~52% in control, 27–36% in urban soils) and Firmicutes (>4% in control, <2–4% in urban soils) decreased, but the shares of Actinobacteria (~18% in control, 22–31% in urban soils), Planctomycetes (~4% in control, 6–10% in urban soils), Chloroflexi (~3% in control, 6–7% in urban soils), Acidobacteria (~4% in control, 5–9% in urban soils), and Bacteroides (~5% in control, 4–8% in urban soils) increased (Table A1).

The data of metagenomic analysis characterizing the rhizospheric microbiome of *E. frumentacea* plants grown on different soils make it possible to assume that the dominant families are Gaiellaceae and Nocardioidaceae (Actinobacteria), Sphingomonadaceae, Hyphomicrobiaceae, Comamonadaceae, Oxalobacteraceae, and Xanthomonadaceae (Proteobacteria), and the families Pirellulaceae (Planctomycetes), Cytophagaceae, and Chitinophagaceae (Bacteroides) can participate in the formation of the rhizobiome of *E. frumentacea* (Table A1). Sun et al. (2018) suggested a potential ecological role for Gaiellaceae in metal-contaminated soils, finding their increased numbers in sequencing libraries and a close significant correlation with various metals' or metalloids' content. In our study, in four out of five soil samples, the Gaiellaceae family was dominant, reaching a maximum in the Tulachermet soil. It can be assumed that representatives of the Gaiellaceae family can form the so-called "core" rhizobiome of *E. frumentacea* plants, being present in its rhizosphere under various conditions. Representatives of the Planctomycetes and Bacteroides phyla also seem to contribute to the formation of the "core" rhizobiome of *E. frumentacea*. Thus, Pirellulaceae (Planctomycetes), Cytophagaceae, and Chitinophagaceae (Bacteroides) families make up a significant share in the rhizospheric microbial populations of plants grown in various soils.

Thus, the qualitative and quantitative changes in the rhizospheric microbial populations of *E. frumentacea* under the influence of technogenic soil pollution have been found.

5. Conclusions

The study of the adaptive characteristics of *Echinochloa frumentacea* revealed that soils with a high degree of contamination with a complex of HMs and petroleum products have an inhibitory effect on the germination and growth processes of *Echinochloa frumentacea*: on the most polluted soils of the Embankment, a reduction in seed germination by 21–23% and a decrease in growth parameters up to 3 times were observed.

A low level of contamination with the complex of HM in the sanitary protection zones of metallurgical industries did not have a toxic effect on seed germination and stimulated the quantitative characteristics of photosynthetic pigments and AOS of *Echinochloa frumentacea*: the content of chlorophylls and low molecular antioxidants such as ascorbic acid and glutathione (GSH) increased by 9–53%, 17–19%, and 5–15% respectively. The data obtained indicate good adaptive characteristics of *Echinochloa frumentacea* to technogenic pollution of soils by metallurgical enterprises.

The accumulation of *Echinochloa frumentacea* biomass on contaminated soils varied within 4280–7920 kg/ha. The increase in shoot biomass in relation to plants on background soils was shown for plants grown on metallurgical enterprises soils. The polyelement

pollution of soils of the SPZ and of the highway contributed to the accumulation of the dry mass of plants compared to background soils.

Echinochloa frumentacea is not suitable for Mn, Co, As, and Cd accumulation from soils with polyelement contamination. The bioaccumulation of Pb was maximum in the root system and constituted 1.57–7.38 mg/kg dry weight. V from polluted soils mainly accumulated in the roots. The main method of V accumulation from soils is rhizofiltration. The accumulation of Cu by the shoots and root system of plants makes it possible to consider *Echinochloa frumentacea* as a Cu phytoremediant. The removal of Zn from contaminated soils during a short vegetation period (1.5 months) was 168–508 g/ha. The obtained values make it possible to recommend *Echinochloa frumentacea* for Zn removal from soils with polyelement anomalies in combination with other species accumulating the element, in intercrop mixtures.

The minimal number of all groups of microorganisms studied (on average, two times lower than in the uncontaminated background soil) was noted in the Embankment soil, which contains the highest concentrations of both inorganic (heavy metals) and organic (petroleum products) pollutants. A two-fold increase in the number of bacteria was observed in the rhizosphere of plants grown in the soils of Tulachermet and Lenin Ave., and a 1.7-fold increase in the number of actinomycetes and a 1.5-fold increase in the number of micromycetes were observed in the soil of KMP, which could be due to specific plant stimulation of microbial taxa resistant to heavy metals.

The rhizosphere populations of *Echinochloa frumentacea* grown in the background soil of Yasnaya Polyana were characterized by the lowest taxonomic diversity compared to the rhizobiomes of plants grown in contaminated urban soils. The species richness index was maximal for rhizosphere communities of plants grown on Tulachermet soils.

The data of metagenomic analysis characterizing the rhizospheric microbiome of *E. frumentacea* plants grown on different soils make it possible to assume that the dominant families are Gaiellaceae and Nocardioidaceae (Actinobacteria), Sphingomonadaceae, Hyphomicrobiaceae, Comamonadaceae, Oxalobacteraceae, and Xanthomonadaceae (Proteobacteria), and the families Pirellulaceae (Planctomycetes), Cytophagaceae, and Chitinophagaceae (Bacteroides) can participate in the formation of the rhizobiome of *E. frumentacea*.

Further detailed study of the properties of the selected microorganisms will make it possible to select a promising inoculant to improve plant growth on contaminated soil to increase the efficiency of its phytoremediation.

Author Contributions: Conceptualization S.V.G., A.K. and A.Y.M.; methodology S.V.G., A.Y.M., I.Z. and O.I.O.; software A.Y.M., I.Z. and O.I.O.; validation A.Y.M., S.V.G., I.Z. and O.I.O.; formal analysis S.V.G., A.Y.M., I.Z. and O.I.O.; investigation S.V.G., A.Y.M., I.Z. and O.I.O.; resources RFBR; writing—S.V.G., A.K., A.Y.M. and I.Z.; visualization, S.V.G., A.Y.M. and I.Z.; supervision S.V.G.; project administration S.V.G.; funding acquisition S.V.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Russian Foundation for Basic Research project No 19-29-05257 "Technogenic soil pollution with toxic elements and possible methods for its elimination".

Acknowledgments: The study was carried out with financial support by the Russian Foundation for Basic Research project No 19-29-05257 "Technogenic soil pollution with toxic elements and possible methods for its elimination".

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

Appendix A

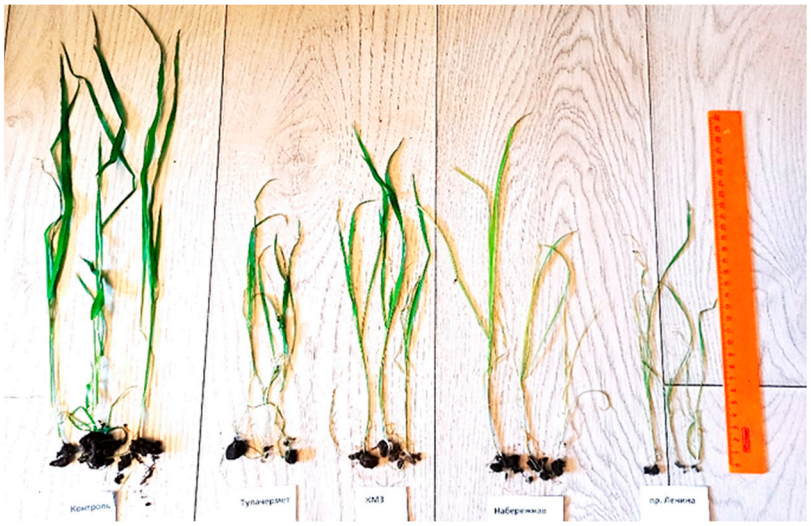

Figure A1. *Echinochloa frumentacea* shoots (1.5 monthly) grown on the soils of the experimental zones: from left to right: background, Tulachermet, KMP, Embankment, highway (Lenin Ave.).

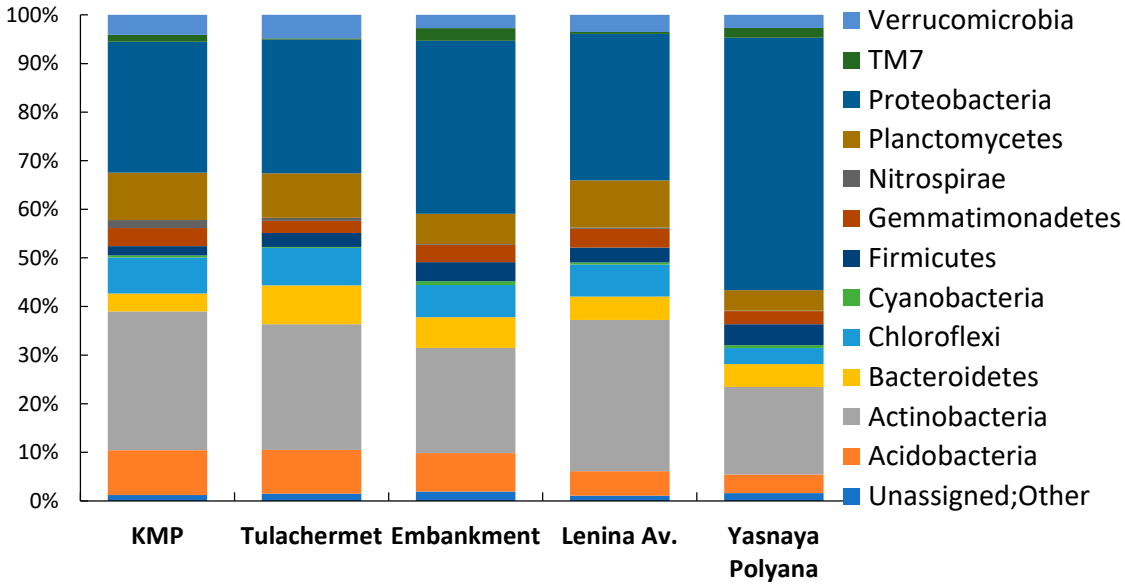

Figure A2. The relative abundances at the phylum level of the microbial communities in the rhizosphere of *E. frumentacea* grown on different soils.

Table A1. Taxonomic structure of rhizosphere microbial communities of *E. frumentacea* (%).

Taxonomic Associations of OTUs	Soil Samples				
	1	2	3	4	5
Unassigned; Other; Other; Other; Other	27.30	28.40	33.10	20.50	23.20
k__Bacteria;p__Acidobacteria;c__Acidobacteria-6;o__iii1-15;f__	4.10	4.10	2.50	0.30	2.40
k__Bacteria;p__Acidobacteria;c__Acidobacteriia;o__Acidobacteriales;f__Acidobacteriaceae	0.00	0.00	0.00	1.50	0.00
k__Bacteria;p__Acidobacteria;c__Acidobacteriia;o__Acidobacteriales;f__Koribacteraceae	0.00	0.00	0.20	1.00	0.00
k__Bacteria;p__Acidobacteria;c__Sva0725;o__Sva0725;f__	0.50	0.10	1.00	0.00	0.20
k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__	2.20	1.40	1.70	0.30	1.20
k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__C111	1.70	1.90	0.40	0.00	1.00
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__	0.20	0.50	0.30	2.40	0.20
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Cellulomonadaceae	0.10	0.30	1.60	0.00	0.10
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Intrasporangiaceae	0.80	1.50	0.60	0.70	1.00
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae	0.90	1.50	3.60	1.10	1.60

Table A1. *Cont.*

Taxonomic Associations of OTUs	Soil Samples				
	1	2	3	4	5
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae	1.00	0.80	0.40	1.30	1.50
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Mycobacteriaceae	0.90	0.10	1.30	0.20	1.40
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardiaceae	0.50	0.00	0.10	0.20	1.60
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae	3.10	2.00	1.60	1.80	3.70
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Pseudonocardiaceae	0.80	0.70	0.10	0.40	1.30
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Streptomycetaceae	1.20	0.10	0.30	0.80	1.80
k__Bacteria;p__Actinobacteria;c__MB-A2-108;o__0319-7L14;f__	3.20	2.30	0.10	0.30	1.00
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__Gaiellaceae	3.80	6.10	1.00	4.00	4.00
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__	3.10	1.80	1.40	1.50	3.80
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Solirubrobacteraceae	0.80	0.40	0.00	0.10	1.90
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae	1.40	3.00	2.00	0.40	2.20
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae	1.00	3.00	1.50	3.50	1.10
k__Bacteria;p__Chloroflexi;c__Ellin6529;o__;f__	2.30	2.80	2.30	0.30	2.10
k__Bacteria;p__Chloroflexi;c__Gitt-GS-136;o__;f__	1.40	1.20	0.80	0.10	0.90
k__Bacteria;p__Chloroflexi;c__Thermomicrobia;o__JG30-KF-CM45;f__	1.00	1.60	0.90	0.10	1.60
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae	0.80	1.20	1.40	2.80	0.90
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae	0.20	0.50	1.10	0.00	0.00
k__Bacteria;p__Gemmatimonadetes;c__Gemm-1;o__;f__	1.60	0.60	1.50	0.10	1.10
k__Bacteria;p__Gemmatimonadetes;c__Gemmatimonadetes;o__;f__	0.40	0.50	1.00	1.50	0.90
k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__0319-6A21	1.40	0.30	0.00	0.00	0.10
k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__Nitrospiraceae	0.30	0.20	0.10	0.10	0.20
k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__WD2101;f__	3.50	3.90	1.40	2.90	2.90
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Gemmatales;f__Gemmataceae	1.10	1.30	0.80	0.20	1.00
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Pirellulales;f__Pirellulaceae	3.20	2.40	3.00	0.50	3.80
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Planctomycetales;f__Planctomycetaceae	0.50	0.60	0.70	0.10	1.20
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae	0.80	0.70	1.70	2.00	0.90
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae	0.40	1.10	1.40	1.70	1.00
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae	3.50	2.00	2.70	5.60	2.20
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae	0.40	0.20	0.40	0.50	1.20
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae	0.10	0.10	1.30	0.40	0.20
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae	1.20	1.10	0.80	1.90	0.90
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales; f__Sphingomonadaceae	4.10	2.60	3.60	12.70	5.40
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae	0.80	1.60	5.10	0.90	1.10
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae	2.70	3.50	2.50	3.30	1.30
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__	0.80	2.90	1.80	0.30	1.00
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales; f__Pseudomonadaceae	0.10	0.10	2.40	1.00	0.40
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales; f__Sinobacteraceae	2.10	1.30	0.70	0.50	1.30
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales; f__Xanthomonadaceae	3.20	2.30	1.60	13.50	6.60
k__Bacteria;p__TM7;c__TM7-1;o__;f__	0.50	0.20	2.10	1.30	0.20
k__Bacteria;p__Verrucomicrobia;c__[Spartobacteria];o__[Chthoniobacterales]; f__[Chthoniobacteraceae]	1.40	1.50	0.70	2.20	1.70

Notes: Soil samples: 1—KMP; 2—Tulachermet; 3—Embankment; 4—Lenin Ave.; 5—Background (Control).

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