Bioremediation of Copper- and Chromium-Contaminated Soils Using *Agrostis capillaris* L., *Festuca pratensis* Huds., and *Poa pratensis* L. Mixture of Lawn Grasses

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Abstract: Environmental pollution by toxic metals is a common ecological problem. Chromium and copper compounds released into the environment as a result of human-made stress pose a serious threat to living organisms. Phytoremediation is a promising method of toxic metals removal from contaminated sites. The concentration of metals in grass biomass—in the roots and aerial parts—was determined by X-ray fluorescence analysis. The estimation of numbers of microorganisms was conducted by a tenfold dilution and spread-plating method. It was shown that lawn grass accumulated from 69.1 ± 13.2 to 497.7 ± 74.1 mg/kg Cu and Cr during the growth in the contaminated soil with 50, 100, and 200 mg/kg of metals. In general, there was a pattern of accumulation of copper in the aerial part of the grass and chromium in the roots. Thus, the total copper concentration in the aerial part ranged from 105.2 ± 23.8 to 497.7 ± 74.1 mg/kg of plant biomass. The total chromium concentration in the roots ranged from 156.4 ± 47.9 to 426.8 ± 62.5 mg/kg. The viability of the soil microbiome was not inhibited at such metal concentrations. The obtained data allow lawn grass to be considered as promising for the phytoremediation of contaminated areas.

Keywords: environmental pollution; heavy metals contamination; copper; chromium; phytoremediation; lawn grass; soil microbiome

1. Introduction

Pollution of the environment by toxic metals is constantly growing [1]. Developed countries in Europe [2], the United States, and China are particularly affected. Pollution of agricultural lands, natural ecosystems, and groundwater is the most hazardous for humans. Copper and chromium are among the most dangerous and common heavy pollutants. Natural and anthropogenic processes are the main sources of contamination by these metals. Natural sources include erosion and weathering of rocks [2]. Heavy metals are released into the environment from deposits, mining sites, or as a result of industrial activities and the development of transport infrastructure [3,4]. Copper industrial mines are a powerful source of copper pollution in the world [5]. The tailing ponds of copper mines contain a high level of copper [6,7]. A significant danger to the environment is acid mine drainage, which is an outflow of acidic metal-containing solution from the mines of metal mining. Acid mine drainage contains a high concentration of copper and...
iron\cite{5}. The negative effects of drainage leakage are exacerbated by microorganisms that mobilize metal-containing compounds and cause the destruction of rocks by exometabolites (organic acids). Together, this leads to the contamination of soils and groundwater with metals\cite{8}. The industrial extraction of chromium is very developed in some countries: South Africa > Kazakhstan > Turkey > India > Iran. It is extracted in the form of chromite and ferrochrome. South Africa holds 72–80\% of the world’s viable chromite ore reserves\cite{9}. The areas adjacent to the mining sites are saturated with chromium compounds in high concentrations and pose significant danger to the environment and living organisms. Thus, the concentration of chromium significantly exceeds the maximum allowable norms in the soils adjacent to the Baghjar Chromite Mine of Sabzevar Ophiolite Belt, Northeastern Iran\cite{10}. The landfills of electrical and electronic equipment, and the wastewater of electroplating plants and the chemical industry contain toxic chromium compounds in high concentrations\cite{11}.

Evidence shows that the problem of contamination of biogeocenoses with toxic metals is more serious than a modern society seems to accept. The danger of metals is that when they are released into the environment, they inhibit the growth of other living organisms\cite{12}, including plants\cite{13}, animals\cite{14}, reptiles\cite{15}, and microorganisms\cite{16}, as well as adversely affect human health\cite{17}. Metals can enter the human body from contaminated soils along with plants and drinking water\cite{18}. Plants are able to accumulate toxic metals along with the necessary macronutrients\cite{19}. Metals enter drinking water by leaching from contaminated ecosystems and leaking into groundwater\cite{20}. Chromium and copper compounds also have a negative effect on microorganisms\cite{21}. Microbiomes of various biogeocenoses (rivers, fields, forests, agrocenoses, etc.) are some of the most important components responsible for the sustainable development and functioning of integral natural ecosystems. Soil fertility directly depends on their balanced microbial composition\cite{22,23}. Actinomycetes, nitrogen-fixing nodule bacteria, phosphate-mobilizing\cite{24}, and facultative and strict anaerobes are plant-associated microorganisms\cite{25} that supply nutrients and essential elements for optimal plant growth. The presence of toxic metals, even at low concentrations, leads to disruption of biogeochemical cycles with the participation of microorganisms, plants, and nutrient substrates, which leads to partial or complete devastation of biogeocenoses and the emergence of infertile soils\cite{23}.

Despite all the dangers, very little attention has been paid to the development of methods of protection and purification of contaminated sites from chromium and copper. Physico-chemical methods of treatment of contaminated wastewater and contaminated soils are common: adsorption\cite{26}, membrane filtration\cite{27}, cementation\cite{28}, photocatalysis, and electrodialysis\cite{29}. There are a number of physico-chemical methods available for purifying contaminated soils, such as thermal vitrification, soil washing, solidification, and geological disposal\cite{30,31}. These methods are extremely expensive and are only suitable for treating small contaminated areas. In recent decades, there has been considerable interest in biological methods of wastewater and soil treatment, including bioremediation and phytoremediation\cite{31}. In contrast to physico-chemical methods, they are cost-effective and environmentally friendly. Active research into bioremediation methods began in the 1990s\cite{32,33}. In situ soil bioremediation can reduce the level of pollution of bottom sediments of rivers, ground and surface waters, as well as adjacent agricultural lands. This will restore the sustainable functioning of natural ecosystems in mining areas. Bioremediation can be promising as an independent technology for purification of contaminated ecosystems, and in combination with physical and chemical methods\cite{32}. However, each of the above approaches still requires further detailed research to improve the efficiency of purification of the contaminated areas. The bioremediation approach based on the use of plants and microorganisms as the main biotechnological agents is being applied to detoxify toxic metals\cite{34}. A number of studies has shown that plants and microorganisms have the genetic potential to remove toxic metals from the environment\cite{35–37}. The main participation of microorganisms in the transformation of copper and chromium compounds in soils is their mobilization by metabolites–chelators. The availability of metals to plant roots is
enhanced not only by the exometabolites of microorganisms that are biocatalysts of this process but also by their own exometabolites, such as phytochelatins (PC), organic acids, amino acids, and enzymes [37, 38]. Undoubtedly, the removal of chromium and copper compounds is carried out simultaneously with the participation of the soil microbiome and the plant by integrated mechanisms of interaction between them.

Various plants have been used for the bioremediation of contaminated soil from metal compounds. For example, *Alyssum murale* [39] and *Rhazya stricta* L. [40] plants can efficiently accumulate toxic metals from the soil. Lawn grasses are especially promising for the phytoremediation of contaminated areas due to their resistance to extreme factors, a fast growth rate [41], and an ability to accumulate metals [42, 43]. For example, *Festuca rubra* grass has a high phytoremediation potential for removal of Cu, Mo, Se, etc., from fly ash [44]. The synergistic combination of metal-resistant bacteria and plants is used to promote the effect of metal phytoremediation. For example, cadmium-resistant microorganisms increased the performance of Napier grass on cadmium phytoremediation [45]. Thus, for the development of methods for the bioremediation of the environment, there is a need to carry out a detailed study of the possibility of the accumulation of metals by plants as well as their impact on the growth and development of plants and associated microorganisms.

In this regard, the aim of our work was to theoretically substantiate and experimentally confirm the possibility of bioaccumulation of copper and chromium compounds from contaminated soils with a mixture of lawn grasses and to determine the effect of metals on plant growth and the soil microbiome. To summarize, we consider the use of lawn grass to be an efficient measure to solve the problem of environmental contamination by toxic metals.

2. Materials and Methods

2.1. Theory/Calculation

The main pathways of metal ions removal from the soil have been theoretically substantiated by a thermodynamic prognosis concept. According to thermodynamic prognosis, plants are able to accumulate toxic metals due to the stereochemical analogy with macroelements (K\(^+\), Na\(^+\), Mg\(^{2+}\), Ca\(^{2+}\), SO\(_4^{2-}\), NO\(_3^{-}\), etc.) [46] through the same transport systems of their absorption. For example, Cu\(^{2+}\) is a stereochemical analog of Mg\(^{2+}\) because they have an equal ionic radius of 0.075 nm. That is why Cu\(^{2+}\) should be accumulated by plants due to its stereochemical analogy with macroelement Mg\(^{2+}\).

2.2. Soil Physico-Chemical Properties

Chernozem soil from agricultural land in Kyiv region was used for the grass growing. Subsequently, the main soil physico-chemical properties of soil were determined: pH value, soil dissolved organic content (DOC), total Cu and Cr concentrations, and macroelement content (K, Mg, Ca, N, P, S).

The pH of soil was measured potentiometrically [47] using an ionometer universal EZODO MP-103 with remote electrodes. The soil dissolved organic content was determined by the permanganate method [48]. The total concentrations of Cu, Cr and macroelements in the soil were determined by X-ray fluorescence analysis [49]. The initial soil parameters were: pH = 6.8 ± 0.2; DOC = 85 ± 5 mg/kg; 15 ± 3.2 mg/kg Cu and 22 mg/kg Cr. The concentrations of macroelements in soil were as follows: 25 ± 3.2 g/kg N; 23 ± 4.1 g/kg K; 24.8 ± 5.4 g/kg Ca; 6 ± 0.4 g/kg Mg; 2 ± 0.1 g/kg P; 1.2 ± 0.1 g/kg S.

2.3. Preparations of Cu\(^{2+}\) and CrO\(_4^{2-}\) Solutions

Aqueous solutions of metals were previously prepared for the insertion of metals into the soil. Salts of the metals (CuSO\(_4\) · 5H\(_2\)O and K\(_2\)CrO\(_4\), Sigma Aldrich, pure for analysis) were used as sources of Cu\(^{2+}\) and CrO\(_4^{2-}\) ions in the forms of soluble compounds. Salts of the metals were added to the soil only once after 25 days, since the process of grass growing had started so that the final concentrations of 50, 100, and 200 mg/kg would be reached. Concentrations were indicated for cationic forms of the metals, not for total salt content.
The amount of metals to be injected into the soil was determined by calculating the molar masses of metal-containing salts and atomic masses of metals. Thus, the conversion factor \((k)\) for \(\text{Cu(II)}\) was 3.93, and for \(\text{Cr(VI)}\) it was 3.73. The calculated amount of the metal salts was dissolved in 500 mL of distilled water and applied to the soil with lawn grass with a syringe evenly throughout the soil.

### 2.4. Plant Sowing and Cultivation in the Presence of \(\text{Cu(II)}\) and \(\text{Cr(VI)}\)

The plants were grown in plastic boxes with soil in the greenhouse in autumn. Lawn grass, which is undemanding to the physico-chemical conditions of the environment, was used as plant-accumulators of metals. The mixture of herbs used as lawn grass included the following species of plants: \(\text{Agrostis capillaris} \) L., \(\text{Festuca pratensis} \) Huds., and \(\text{Poa pratensis} \) L. Before the experiment, soil moisture was determined. To do this, 100 g of moist soil was dried at a constant temperature \((105^\circ \text{C})\) for 3 h. After a complete drying and cooling process had been carried out, the conversion factor of moist soil mass \((k)\) to absolute dry mass \((\text{ADM})\) was determined. Plastic containers with a total volume of 14 L were used to plant lawn grass. Planting was performed at the rate of 1 kg of seeds per 30 m\(^2\). The depth of the soil layer before sowing the seeds was 9 cm. The soil was watered before sowing. The seeds were covered with an even layer of soil (2 cm). The soil surface was moistened with water by a sprayer. The plants were grown for a month until the lawn was formed. Only after that (25 days of grass growing) were aqueous solutions of metal salts added. Aqueous solutions of \(\text{Cu(II)}\) and \(\text{Cr(VI)}\) were added directly into the soil in sections using a syringe. The soil with plants without metals and the soil without metals and without plants were used as controls. After the insertion of metals, the plants were grown for 30 days. Grass samples were taken during the 30 days growth for the purpose of analyzing the dynamics of the accumulation of chromium and copper compounds in plant biomass. Samples were taken at 1, 7, 14, and 28 days after the insertion of metals. Plants were removed in full and then dried and weighed to determine their total mass. The soil was thoroughly mixed in a container and sampled for analysis. The temperature of grass growing ranged from 16 \(^\circ\text{C}\) to 25 \(^\circ\text{C}\). Watering of plants in the greenhouse was carried out as the soil dried up (2–3 times a week). The lighting of the plants took place in accordance with the autumn light day of the temperate climate zone.

The determination of the effect of toxic metals on the growth of lawn grass was performed by measuring the length and weight of control and experimental plants at the end of the experiment (after the removal of the plants from the soil). After removing the lawn grass, it was thoroughly washed with cold water to be completely removed of the soil and dried with filter paper. The soil and moist plants were dried to a completely dry mass in a dry oven at 105 \(^\circ\text{C}\). Before the analysis was carried out, the dry lawn grass and the soil were ground in a porcelain mortar. The process of growing the plants is shown in Figure 1.

### 2.5. Determination of the Effect of \(\text{Cu(II)}\) and \(\text{Cr(VI)}\) on the Amount of Microorganisms in the Soil Microbiome

Quantitative accounting of microorganisms was conducted by cultivation on agar nutrient medium NA (HiMedia Laboratories Pvt. Ltd., India). The estimation of numbers of microorganisms was conducted by a tenfold dilution [50] and the spread-plating method [51]. To obtain isolated colonies, a series of tenfold dilutions of both control and experiment soil samples were prepared in sterile saline (0.85% NaCl). Plates with sterile agar medium (HiMedia) and the investigated metals were pre-prepared and dried. The appropriate dilution of soil (0.2 mL) was added by a sterile sampler on the surface of the nutrient medium and dispensed with a sterile Drygalsky glass spatula. Microorganisms were cultivated at 30 \(^\circ\text{C}\).
The concentration of metals in grass biomass, which included the roots and leaves (aerial part) was determined by X-ray fluorescence analysis [49]. The analysis was performed using an ElvaX CEP-01 X-ray fluorescence spectrometer. The peculiarity of the method was that building an accurate calculation procedure requires use of an array of standard samples with a certain concentration of metals that are similar in chemical composition to the investigated samples. Therefore, the samples with a certain concentration of test metals were previously prepared to determine the concentration of Cu and Cr in soil and plants. Soil samples were kept for 1 day in a 10% citric acid solution for extraction of metal compounds into the solution and washing of the soil. Samples of plants and soil were dried to a constant weight at 105 °C for 2 h. To create a calibration graph, aqueous solutions of metals were added to dry the soil and the plants. To obtain standard calibration soil samples in 10 g of each sample, metals were added to a final concentration of 10, 50, 100, and 200 mg/kg of soil. To obtain standard calibration samples of plants in 1 g of each sample, metals were added to a final concentration of 10, 50, 100, 200, 300, and 400 mg/kg of plants. After re-drying to a constant weight, the samples were analyzed by an X-ray fluorescence spectrometer. To do this, 0.5 g of each sample was placed in an analysis cuvette to obtain spectra at a voltage of 45 watts. The correct calibration of the spectrometer was

$$ M = \frac{(a \cdot 10^n)}{V} $$

where $M$—the number of colony-forming units in 1 mL (CFU/mL); $a$—the average number of colonies grown on the plate; $10^n$—dilution factor; $V$—volume of suspension in mL. The number of microorganisms was converted from CFU/g of moist soil to CFU/g of absolutely dry soil mass. The determination of the effect of Cr(VI) and Cu(II) on the microbiomes (total amount of microorganisms detected at such conditions) of control and contaminated soils was conducted by comparing the number of colonies of microorganisms on control plates and on plates with metals.

2.6. X-ray Fluorescence Analysis of the Samples

The number of microorganisms was determined after 10 days by direct counting of colonies in the plates. The number of cells in 1 mL of suspension was calculated by the equation:

$$ \text{number of cells} = \left( \frac{n}{V} \right) \times 10^a $$

where $n$—dilution factor; $V$—volume of suspension in mL. The number of microorganisms was converted from CFU/g of moist soil to CFU/g of absolutely dry soil mass. The determination of the effect of Cr(VI) and Cu(II) on the microbiomes (total amount of microorganisms detected at such conditions) of control and contaminated soils was conducted by comparing the number of colonies of microorganisms on control plates and on plates with metals.
obligatorily checked. To determine the concentration of metals in the samples, the spectra of the studied and standard model spectra were compared.

The ElvaX program used a complete quadratic regression model to describe the dependence of the concentrations of the studied elements on the line intensities of all elements present. At the same time, the certified values of concentrations in the calibration standards were indicated only for the investigated elements. The statistical significance of the regression coefficients was determined by the method of a multiple regression in the automatic mode.

2.7. Statistical Analysis

All data were analyzed by ANOVA (analysis of variance) using the statistical platform of Microsoft Excel. One-way ANOVA, determination of average values (AV) and standard deviations (SD) with a 95% confidence level were performed. Significance of differences between average values in the studied grasses were established using post hoc tests (Bonferroni correction). Each value was presented as the mean ± standard deviation (SD). Different diagrams were constructed using Microsoft Excel software.

3. Results

3.1. Accumulation of Copper and Chromium Compounds by Lawn Grass

The analysis showed a high efficiency of copper and chromium accumulation by lawn grass. The concentration of Cu and Cr in the control roots and aerial part (Figure 2) of the grass was very low (2.1 ± 1.2 mg/kg and 3.2 ± 1.5 mg/kg Cu as well as 4.1 ± 1.3 mg/kg and 2.2 ± 1.2 mg/kg Cr, respectively). It should be noted that the plants began to accumulate metals on the first day after their insertion into the soil with full-grown plants. As a result, the intensive accumulation of Cu(II) in both roots and aerial part was observed after the insertion of 50 mg/kg Cu(II) with a significant \( p = 0.00003 \) difference compared with control plants. This rapid accumulation can be explained by the high need of lawn grass for moisture. Together with the transport of water and macronutrients dissolved in it, metals accumulated in the roots and aerial part of the plants. In the presence of different concentrations of chromium and copper in the soil, the effectiveness of their accumulation by plants differed. Thus, the concentration in the roots was 69.1 ± 13.2, in the aerial part of the plant—105.2 ± 23.8 mg/kg ADW of plant biomass in the presence of 50 mg/kg Cu(II) in soil (Figure 2a).

The concentration of copper in grass increased 2.9 times \( p = 0.0002 \) from 69.14 ± 13.2 to 197.2 ± 55 mg/kg in the roots and 2.6 times \( p = 0.000003 \) from 105.2 ± 23.8 to 271.4 mg/kg ± 40.3 mg/kg in the leaves from 1 to 30 days of growth in copper-contaminated soil (50 mg/kg) (Figure 2a). The concentration of chromium in the grass was slightly different from the concentration of copper. Thus, the grass accumulated more quantity of chromium than copper in the roots during growth in the presence of 50 mg/kg Cu(II). The concentration of chromium increased two times, from 156.4 ± 47.9 to 320.12 ± 67.0 mg/kg, in the roots \( p = 0.002 \). This was 1.6 times more than the copper content. The concentration of chromium did not change significantly in the aerial part from 1 to 30 days of growth (Figure 2b). The maximum amount of chromium compounds was accumulated by the roots of the grass on the 30th day of growth in the presence of 50 mg/kg Cr(VI), which was 320.1 ± 67.0 mg/kg (Figure 2b). This was significantly higher \( p = 0.00004 \) than the chromium content in control plants. The concentration of chromium in the aerial part was at a constant level and ranged from 169.5 ± 51.4 to 175.9 ± 38.5 mg/kg for 30 days of growing after the insertion of metal. Thus, chromium compounds accumulated more in the roots than in the aerial part of the grass (Figure 2). On the contrary, copper compounds accumulated more in the aerial part of the grass (Figure 2a).

The intensive accumulation of copper compounds also occurred at an initial concentration of 100 mg/kg Cu(II) in the soil. Thus, on the first day of growth in copper-contaminated soil, 172.3 ± 32.3 mg/kg Cu (Figure 2c) was accumulated in the roots, which was 2.5 times higher than the amount accumulated at the initial concentration of 50 mg/kg.
of soil. However, the concentration of total copper in the roots increased \((p = 0.047)\) to \(220.5 \pm 45.0 \text{ mg/kg}\) after 30 days of growing. It was only 1.1 times higher than its concentration when grass was growing in the presence of 50 mg/kg Cu(II) in the soil. At the same time, the level of copper accumulation remained unchanged throughout the observed period (Figure 2c). Changes in the copper concentration under such conditions were recorded more in the aerial part of the grass. Thus, on the first day after the insertion of 100 mg/kg Cu(II), its concentration in the aerial part was \(213.3 \pm 43.0 \text{ mg/kg}\), on the 7th day it was \(300.7 \pm 32.9 \text{ mg/kg}\). On days 7, 14, and 30, no statistically significant changes in the concentration of copper in the aerial part, which ranged from \(300.7 \pm 39.2\) to \(313.2 \pm 55.0 \text{ mg/kg}\), were recorded (Figure 2c).

**Figure 2.** Accumulation of metals by lawn grass during growth at their different concentrations in the soil: (a) 50 mg/kg Cu(II); (b) 50 mg/kg Cr(VI); (c) 100 mg/kg Cu(II); (d) 100 mg/kg Cr(VI); (e) 200 mg/kg Cu(II); (f) 200 mg/kg Cr(VI), \((\bar{x} \pm \text{SD}, n = 5)\) with different letters significantly differ from each other based on Bonferroni corrections at \(p < 0.05\).
The patterns of accumulation of chromium compounds by grass during growth in the 100 mg/kg Cr(VI) of contaminated soil differed from the accumulation of copper compounds. Thus, a large amount of chromium was accumulated by plants within 1 day after its insertion. The distribution of chromium in the roots and the aerial part was similar, which was confirmed by statistical analysis. Thus, the roots accumulated 278.5 ± 52.1 mg/kg, and the aerial part accumulated 282 ± 28.5 mg/kg of chromium after 1 day of its insertion (Figure 2d). On the 30th day, the concentration in the roots increased (p = 0.049) to 372.8 ± 74.8 mg/kg, which was 1.3 times more than on the 1st day and in the aerial part it significantly (p = 0.004) increased to 406.7 ± 63.2 mg/kg (Figure 2d).

As expected, at the highest concentration of copper and chromium (200 mg/kg) in the soil, the plants accumulated them most intensively. Thus, the concentration of Cu on the first day after the insertion was 299.2 ± 57.5 in the roots and 301.2 ± 27.3 mg/kg in the aerial part. They remained at this level for 30 days of grass growth in the presence of copper (Figure 2e). As with the two previous variants, copper compounds accumulated more in the aerial part of the grass. On the first day, the distribution of copper in the roots and in the aerial part was similar. However, the concentration in the aerial part increased almost twice and amounted to 490.0 ± 47.3 mg/kg and 497.7 ± 74.1 mg/kg of plant biomass on the 14th and 30th day, respectively (Figure 2e).

The plants accumulated chromium both in the roots and in the leaves at its concentration of 200 mg/kg in the soil. The amount of accumulated chromium on the first day in the roots was 282.7 ± 75.4 mg/kg (Figure 2f). It increased 1.5 times (p = 0.01) and amounted to 426.84 mg/kg at 30 days of growth. A similar amount of chromium was accumulated in the aerial part of the grass (Figure 2f).

In general, there was a pattern of accumulation of copper in the aerial part of the grass and chromium in the roots. The intensive accumulation of metals was observed during the first day of grass growing. The increase in the concentration of copper and chromium in parts of plants correlated with their concentration in the soil. The duration of grass growth in the presence of metals also affected the level of metal accumulation. In most cases, the highest concentration was accumulated on the 30th day of cultivation.

### 3.2. The Influence of Metals on the Growth of Lawn Grass

The effect of copper and chromium on the growth and development of lawn grass was studied by changes in the length (Figure 3) and weight (Figure 4) of plants. At such metal concentrations, no significant inhibition of plant growth was observed (Figure 3). The effect of copper and chromium on the weight was manifested significantly (p = 0.0002 and p = 0.0001, respectively) only at their concentrations in the soil of 200 mg/kg. Yellowing of the aerial part of the grass, single necrosis, and wilting of plants were observed. As expected, the control grass growing in metal-free soil showed the most intensive growth. Thus, during the period of 30 days of growth, the average length of control grass significantly increased, from 27 ± 2.0 cm to 43.0 ± 2.0 cm (Figure 3).

In all other variants of the experiment, the same growth rate of grass was observed. A significant decrease in length compared to the control was observed on the 14th day after the insertion of copper and chromium. For example, at a concentration of 200 mg/kg Cr(VI) in the soil, the length of the grass was 33 ± 1.3 cm, and the control grass was 38 ± 3 cm (Figure 3). Similar patterns of inhibition of grass growth were observed in the presence of 200 mg/kg Cu(II). There was an even more significant delay in their growth compared to the control group during the 30 days of growing the plants after the insertion of metals. The length of the plants ranged from 34 ± 3 cm (100 mg/kg Cr (VI)) to 38 ± 1.2 cm (Figure 3). That is, there was a decrease in growth in length by 11.6–21% compared with the control group. The presence of metals in the soil inhibited plant growth compared to the control, but the intensity of inhibition did not differ statistically under the influence of Cu(II) or Cr(VI) in the concentration range of 50–200 mg/kg. Another parameter that was tested for metal exposure was plant weight. The conversion factor of grass to absolutely dry weight was 3.55.
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The length of the plants ranged from 34 ± 3 cm (100 mg/kg Cr (VI)) to 38 ± 1.2 cm (Figure 3). That is, there was a decrease in growth in length by 11.6–21% compared with the control group during the 30 days of growing the plants after the insertion of metals. For example, at a concentration of 200 mg/kg Cu(II) or Cr(VI). There was an even more significant delay in their growth compared to the control group during the 30 days of growing the plants after the insertion of metals. A significant decrease in length compared to the control was observed on the 14th day after the insertion of copper and chromium. For example, at a concentration of 200 mg/kg Cu(II), the length of the grass was 33 ± 1.3 cm, and the control grass was 38 ± 3 cm (Figure 3). Similar patterns of inhibition of grass growth were observed in the presence of Cu and Cr (Figure 4).

3.3. The Influence of Metals on the Amount of Microorganisms in the Soil Microbiome

The presence of metals in the soil inhibited the increase of the plant weight compared to the control group, but the intensity of suppression did not differ statistically under the influence of 50–200 mg/kg of Cu(II) or Cr(VI). The average dry matter yield of control grass removed from the containers was 107.8 ± 6.9 g. The weight of grass that continued to grow in the presence of toxic metals ranged from 62.8 ± 9.3 g (200 mg/kg Cr) to 85.5 ± 14.2 g (50 mg/kg Cr). It is obvious that there was an inhibition of plant weight compared to the control group by 20.7–41.8% in the presence of Cu and Cr (Figure 4).

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3.3. The Influence of Metals on the Amount of Microorganisms in the Soil Microbiome

The presence of chromium and copper compounds in the soil at concentrations of 50, 100, and 200 mg/kg was shown not to inhibit the growth of the soil microbiome. It was found that both the control soil and the experimental soil with metals contained numerous viable microorganisms. In the control soil without metals, the number of microorganisms was 1.4 × 10^7 CFU/g (Figure 5).

The concentration of cells ranged from 1.3 × 10^7 to 1.8 × 10^7 CFU/g in the presence of 50–200 mg/kg of Cu(II) or Cr(VI). No statistically significant difference in the values of the number of microorganisms between control and experimental soil samples was recorded (Figure 5).
The obtained results showed that their growth was not significantly inhibited, even at plants, and therefore it is less toxic. In nutrient media, copper is usually contained in the precipitated when interacting with soil components and not completely bioavailable to roni correction.

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The conversion factor of grass to absolutely dry weight was 3.55.

trol group. The presence of metals in the soil inhibited plant growth compared to the con-

4. Discussion

Lawn grass used for sowing urban landscapes usually contains a mixture of different species of grasses because it grows thicker and is resistant to abiotic factors and mechanical damage [52]. The study of stress-resistant plants is of considerable interest to modern scientists.

A study of the isolation of copper-resistant bentgrass Agrostis stolonifera, which is able to grow well at 100 mg/kg Cu(II) [53], has already been published. The same authors selected in vitro Agrostis stolonifera L. and Festuca rubra rubra, which are also lawn grasses resistant to Cu(II) compounds, at the concentration of 150 mg/L in nutrient medium [54]. In our study, a mixture of lawn grasses, namely Agrostis capillaris L., Festuca pratensis Huds., and Poa pratensis L., which were not adapted to copper compounds, was investigated. The obtained results showed that their growth was not significantly inhibited, even at 200 mg/kg of Cu(II) and Cr(VI) in the soil. Thus, we can assume that copper is partially precipitated when interacting with soil components and not completely bioavailable to plants, and therefore it is less toxic. In nutrient media, copper is usually contained in the form of the soluble cation Cu²⁺. Thus, the experimental results outdoors are significantly different from the results in vitro. Plants that are resistant to metal compounds are industrially promising because they are typically able to remove them from contaminated media. In recent decades, the ability of plants to remove toxic metals has been actively studied. It has been shown that the roots and tissues of plants contain organic acids that act as chelators for metals such as chromium and promote its absorption by plant tissues [55]. It has long been known that the artificial insertion of organic acids into the hydroponic system where tomatoes are grown has led to statistically significant results in increasing the accumulation of chromium by plants [56]. We have previously shown that Nicotiana tabacum L. plants are able to accumulate Cu, Cr, Ni, Co, and Cd in very high concentrations when grown in soil contaminated with 500 mg/kg of each of the metals. Thus, the amount of accumulated cobalt and nickel in the plant mass reached more than 10 g/kg, and copper and chromium about 8 g/kg [57]. A number of different plants species are able to accumulate compounds of copper and chromium. For example, water lilies (Nymphaea spontanea) are capable for removing chromate ions from aqueous solutions and electroplating waste. At the same time, they are able to accumulate large amounts of chromium in biomass—2100 mg/kg [58]. Sunflower is also able to accumulate chromium compounds, with citric acid playing a key role as a chelating agent. Thus, the concentration of chromium in sunflower roots ranged from 20.53 ± 2.04 mg/kg to 97.83 ± 0.76 mg/kg [59]. In this study, lawn grass accumulated chromium in the roots at 150–400 mg/kg without the insertion of chelating agents. The reason for this may be the high concentration of microorganisms in both control and chromium- and copper-contaminated soils. Microorganisms are associated with
plants and are able to synthesize a number of organic compounds, antibiotics, vitamins, amino acids, enzymes [60], and chelating compounds, including acetic acid [61], citric acid [62], etc. Organic acids, amino acids, and other compounds that can be synthesized by microorganisms increase the bioavailability of nutrients for plants. Chelators are also able to dissolve inaccessible insoluble compounds of toxic metals, which contribute to their accumulation in plants [59].

The quantitative characteristic of the soil microbiome can reflect the level of soil metal pollution [63]. Research shows that the presence of toxic metals in soil can inhibit the growth of microorganisms [64]. This effect can be assessed by both the number [65,66] and the diversity [67] of microorganisms in the soil. In our case, there was no effect of metals on the number of microorganisms in the soil. This indicates the ability of the microbiome to adapt to copper and chromium and to maintain the stability of the microbial population in contaminated soil.

Plants are also being actively studied for their ability to accumulate copper, as it is a very common contaminant. Thus, 16 wild species of plants growing in a region of metal mining in Armenia were studied. The concentration of Cu ranged from 55 mg/kg (Hypericum perforatum) to 775 mg/kg (Thymus kotschyanus) in the roots of dominant plant species growing in Cu-contaminated areas [68]. The studied plants have significant phytoremediation potential for the treatment of polluted environments. The medicinal plant Eclipta alba L. is also able to accumulate copper compounds. The roots of this plant accumulated 471 ± 15.2 mg/kg Cu, and the leaves 382 ± 14.42 mg/kg Cu during cultivation in contaminated soil containing 200 mg/kg Cu(II) for a period of 30 days [69], the results of which are very similar to our study. Lawn grass accumulated 497.6 ± 51.7 mg/kg Cu in the upper part and 308.5 ± 74.1 mg/kg Cu in the roots. Therefore, the plants described above are promising for the development of soil phytoremediation technology. It is worth noting that lawn grass is a more attractive object for biotechnology because it is easier to care for, resistant to low and high temperatures, and can grow in different types of soils.

5. Conclusions

The presented quantitative patterns of distribution of toxic copper and chromium in biomass of lawn grass (mixture of species Agrostis capillaris L., Festuca pratensis Huds., and Poa pratensis L.) confirmed the possibility of its application for bioremediation of contaminated soils. Lawn grass has been shown to accumulate the toxic copper and chromium compounds very quickly and efficiently without any significant inhibition with respect to its growth. The number of microorganisms in contaminated soils did not decrease under the influence of metals, which indicates their high adaptive potential. Since microorganisms could be involved in the transformation of metals in the soil, further research of the mechanisms of microbial–plant interactions and microbial biodiversity under the influence of metals is needed to determine the most effective way of carrying out the bioremediation of contaminated soil. The obtained results allow consideration of lawn grass as promising for phytoremediation of contaminated areas.

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