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Original article

THE USE OF LAWN GRASSES FOR PURIFICATION OF SOIL FROM TOXIC Cr(VI)

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Background. Environmental pollution with toxic compounds poses a danger to nature and humans. Various technologies for soil purification from toxic metals are being developed.
Objective. The work was aimed to study the possibility of lawn grass using for soil purification from Cr(VI).
Methods. Plant seeds (*Festuca rubra* L. 45%, *Festuca arundinacea* Schreb. 25%, *Lolium perenne* L. 20%, *Poa pratensis* L. 10%) were sown in a container at 24 °C and grown for two months to obtain a lawn. K_2CrO_4 was added to the soil (400 g) (variants: 1.0 g, No. 1 and 2.5 g, No. 2) The following parameters were determined in two, five and ten days after Cr(VI) addition to the soil: the content of Cr(VI) in the roots, aerial part, and the soil; the coefficient of Cr(VI) content reduction; total content of aerobic heterotrophic microorganisms (CFU/g).
Results. In 2 days after Cr(VI) adding the roots accumulated Cr(VI) in more significant amounts than the leaves (16.6 and 15 times in No. 1 and No. 2). In 5 days, an increase in Cr(VI) concentration in the plants was detected. Cr(VI) concentration in the soil decreased in 5 days by 45.8 and 13.57 times in variants No. 1 and No. 2, respectively. Inhibition of soil microorganisms growth was detected. CFU number in the control sample was 7.2×10^8, and in experimental variants No. 1 and No. 2 – 5×10^6 and 1×10^6, respectively.
Conclusions. The addition of K_2CrO_4 inhibited the growth of soil microorganisms. Lawn grasses composition was successfully used for Cr(VI) extraction from the soil. A notable (13.8 and 9.3 times) reduction in Cr(VI) content in the soil was observed in two days. This reduction progressed greatly in five days as well (91.8 and 85.0 times). Cr(VI) was accumulated in the root system and the leaves of the plants. Thus, the selected grasses can be used in bioremediation technologies to purify contaminated soil.

Keywords: heavy metal; chromium(VI); soil purification; *Festuca rubra* L.; *Festuca arundinacea* Schreb.; *Lolium perenne* L.; *Poa pratensis* L.; plant-used phytoremediation technology.

Introduction

Cr(VI) is the most toxic form of chromium, which negatively affects humans [1] and plants in case of soil and water pollution [2]. Cr(VI) – chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) ions – is a stable form, while trivalent Cr(III) is less mobile, less stable, and exhibits less toxicity. Cr(VI) can be reduced to Cr(III) by different chemicals with reducing properties. These features of transforming toxic metal into a less toxic form can be used in water and soil purification technologies [3].

Heavy metal uptake by plants is affected by many factors, such as environment, temperature, pH, aeration, plant type, size of the plant and its root system, leaf structure, and content of water in soil [4–7]. Cr(VI) has been reported to be transported and accumulated in plants via carrier ions such as sulphate or iron [8, 9]. Usually, plants are sensitive to the presence of chromate and dichromate in the soil [9–13]; therefore, the concentration of the metal is a critical parameter of their survival [14–17]. However, some plants are known as hyperaccumulators of toxic metals [18].

Since chromate is a compound produced during human economic activity as a waste of industrial production, special technologies are currently being developed to clean up contaminated water and soil. There are technologies using physical and chemical methods for removing the pollutant, which is based on reduction-oxidation, precipitation, accumulation, and sorption [19]. For these purposes, the use of both microorganisms and plants was proposed. In particular, different options for developing purification technologies using natural and selected strains of microorganisms are offered now [20, 21]. Plants can also be used in bioremediation technologies because they synthesise naturally numerous compounds with reducing activity [22]. Thus, bioremediation is considered an auspicious way of combating environmental pollution [23].

Pollution of soil by toxic metals became a significant problem because of its negative effect on plant growth and danger to human health [24]. Due to this fact, there is an increased interest in developing technologies for purifying metal-contaminated soil and/or reducing the toxicity of pollutants present there. Traditional methods for soil remediation include physical and chemical techniques. At the same time, these methods are expensive. Using the chemicals causes secondary soil pollution and negatively affects soil properties and plant growth [25, 26]. The application of biological methods is safer for the environment and allows us to avoid these problems. These methods are considered an effective technique for toxic metal remediation and include using of microorganisms (bioremediation) and plants (phytoremediation) to remove pollutants from soils [27]. The use of microorganisms is possible for these purposes because of the initiation of some processes including metals precipitation, chemical adsorption, ion exchange, the formation of complexes with organic ligands, redox reactions, mobilisation, and bio-oxidation [28]. At the same time, using microorganisms requires the selection of bacterial strains that are resistant to certain metals and, at the same time, safe for the environment, as well as the possibility of appropriate microbial preparations production.

The great prospects of bioremediation are based on the fact that using plants or microorganisms for such purposes excludes the use of any toxic compounds. In addition, this method is often more cost-effective, as it does not require complex and specific equipment. In this regard, the possibility of using plants of various species for soil purification from toxicants is being investigated [29–34]. Earlier, we studied the peculiarities of the duckweed *Lemna minor* L. cultivation *in vitro* in the presence of Cr(VI) [35]. It was determined that during duckweed plants' growth, Cr(VI) was reduced to Cr(III) in the culture medium. Cr(VI) was transported into the plant cells and reduced to Cr(III) directly in the cells. The Cr(VI) concentration time decreases to zero depending on its initial concentration.

In this work, we determined the possibility of using lawn grass to purify the soil contaminated with Cr(VI), including the Cr(VI) uptake by the roots and changes of its content in different parts of the plants in dynamics. This choice was motivated by the unpretentiousness of the grasses, the rapid and significant development of their root system, and the simplicity of these plants' cultivation.

Materials and Methods

Plant seeds (TM "Golden Garden", composition: *Festuca rubra* L. 45%, *Festuca arundinacea* Schreb. 25%, *Lolium perenne* L. 20%, *Poa pratensis* L. 10%) were sown at laboratory conditions in containers at 24 °C. Universal substrate Polissky (TM Rich Land, Ukraine, pH 5.5–6.6, N = = 100–200 mg/kg, P = 140–260 mg/kg, K = 120–200 mg/kg) was used for plant cultivation. The plants were grown for two months after sowing the seeds to obtain a lawn, the grass being cut to a height of 10 cm once a week. Then, K_2CrO_4 in the amount of 1.0 and 2.5 g of Cr(VI) was added to a container with soil (400 g), No. 1 and No. 2 variants of the experiment, respectively. The plants grown at the same conditions without Cr(VI) added were used as control samples. The following parameters were determined in two, five and ten days after the Cr(VI) adding to the soil: the content of Cr(VI) in the roots, the aerial part of the plants, and the soil; coefficient of Cr(VI) content decrease – the content in the soil to the amount of Cr(VI) at the beginning of the experiment and after two, five, and ten days; total content of aerobic heterotrophic microorganisms in the soil (Colony Forming Units, CFU/g).

To determine Cr(VI) content, the soil sample (10 g) was added to distilled water (30 ml), kept in a shaker for 1 h, and centrifuged at 9000 rpm (Eppendorf Centrifuge 5415C) for 10 min. After this procedure, the supernatant was collected. Next, the sediment was washed with water (10 ml), the sample was centrifuged at 9000 rpm for 10 min, and the supernatant was collected and added to the first one. Next, root and shoot samples (0.1 and 0.5 g, respectively) were ground with distilled water and centrifuged at 9000 rpm (Eppendorf Centrifuge 5415C) for 10 min, and then the supernatant was collected. The concentration of Cr(VI) was determined as follows: 2 ml of the sample (solution), 0.5 ml of nitric acid (1:3), and 0.5 ml of a 0.5% solution of diphenylcarbozide in 96% ethanol were mixed [25]. The optical density was determined at a wavelength of 546 nm using Fluorat-02 Panorama spectrophotometer. The calculation was carried out according to the calibration curve, using K_2CrO_4 solution in the reaction mixture (C = 0.0055x, R^2 = 0.9241).

For microbiological analysis, the soil samples from the control's root zone and two experimental variants were used. In each variant, 1 g of the soil was suspended in 10 ml of sterile water. In addi-

tion, 1 ml of the soil suspension was ten-fold diluted according to the standard method [26] and used for the study. The total content of aerobic heterotrophic microorganisms in the soil was evaluated via the calculation of colony forming units (CFU) with a standard technique on LB medium (28 °C) [36].

All analyses were performed in triplicate. Statistical analysis was provided in standard soft of Microsoft Excel (version 2003) and presented as a mean of confidence intervals ($P < 0.05$).

Results

Two days after adding the toxic solution to the soil, some noticeable changes in plant growth were observed. Determination of the content of Cr(VI) in the roots and aerial parts of plants revealed differences in the toxic compound's accumulation level in the two treatment variants. The content of Cr(VI) in the roots was 0.05 ± 0.01 and 0.24 ± 0.02 mg/g of DW, and the content in the aerial part of the plants was 0.003 ± 0.001 and 0.016 ± 0.009 mg/g of DW in variants No. 1 and No. 2, respectively (Fig. 1). At the initial stage of cultivation after the addition of the toxicant (two days), the roots accumulated Cr(VI) in a significantly greater amount of Cr(VI) per 1g of the plant material than the leaves (16.6 and 15.0 times in the first and second variants, respectively).

The content of the metal in the soil was 0.20 ± 0.03 and 0.68 ± 0.08 mg/g, being equal to 0.08 and 0.27 g in terms of the entire weight of the soil in the containers. This is much less than the soil's total initial Cr(VI) content (1.0 and 2.5 g, respectively). Thus, there was a significant reduction in the content of Cr(VI) in the soil simultaneously with the accumulation of the metal in the plants. Moreover, the toxic compound was accumulated both in the roots and leaves of the plants (Fig. 1). It should be noted that the relative content of Cr(VI) in the roots in both versions of the experiment exceeded the content of the compound in the leaves by 15.8 and 15.1 times, respectively. It is evident that, first of all, toxic metal accumulation occurs precisely in the root system, which is in direct contact with the soil contaminated with the toxic compound. The coefficient of reduction of Cr(VI) content in two days was 13.8 in the first variant and 9.3 in the second one. Thus, this parameter was bigger in the variant of the experiment with a lower initial Cr(VI) content. It is important to emphasise that the plants were sufficiently resistant to the effect of the toxic metal. Despite the accumulation of Cr(VI) in plants (both in roots and leaves), their growth in two days did not differ from the control sample, even when Cr(VI) was added to the container at a content of 2.5 g (see Fig. 1).

In 5 days, an increase in Cr(VI) concentration in the plant cells was detected (Fig. 2). In the variant with a lower initial concentration of Cr(VI) in the soil, the content of Cr(VI) in the roots and leaves of plants was 0.08 ± 0.01 and $0.012 \pm \pm 0.002$ mg/g, and in the second variant – 0.69 ± 0.08 and 0.21 ± 0.012 mg/g. At the same time, the concentration of Cr(VI) in the soil decreased (see Fig. 2). Therefore, the accumulation of Cr(VI) in the plants continued, and the content of the toxicant increased in two variants: in the roots by 1.6 and 2.9 times and the leaves by 4 and 14 times compared to the previous measurements (in two days of growth).

The concentration of Cr(VI) in the soil decreased in 5 days by 45.8 and 13.57 times, respectively, in variants No. 1 and No. 2, and the coefficient of Cr(VI) content reduction in five days was 51 in the first variant and 56 in the second one.

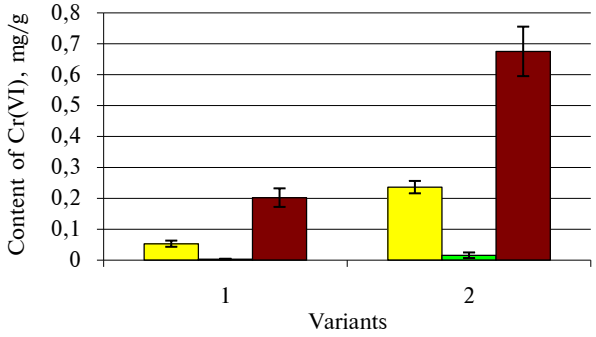

Figure 1: Cr(VI) content in roots, leaves, and soil samples in 2 days after Cr(VI) solution adding to the soil: – roots, – shoots, – soil

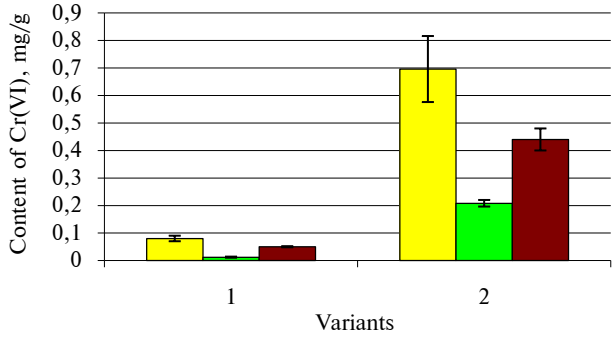

Figure 2: Cr(VI) content in roots, leaves, and soil samples in 5 days after Cr(VI) solution adding to the soil: – roots, – shoots, – soil

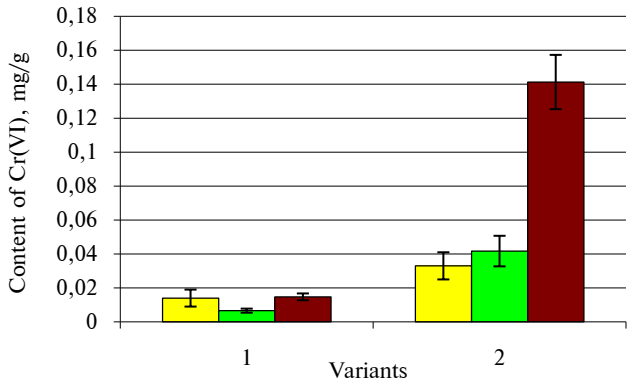

Figure 3: Cr(VI) content in roots, leaves, and soil samples in 10 days after Cr(VI) solution adding to the soil: – roots, – shoots, – soil

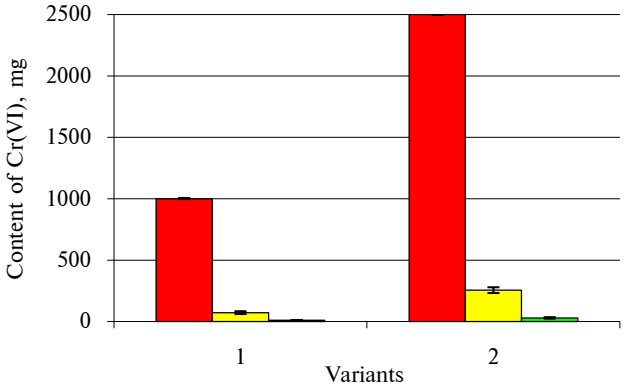

Figure 4: Decrease of Cr(VI) content in the soil (totally in the containers) in 2 and 5 days after Cr(VI) solution adding to the soil: – 0 days, – 2 days, –5 days

In 10 days, a further presence of the chromium(VI) content in the roots of plants of variant No. 1 was observed – up to 0.013 ± 0.03 mg/g (Fig. 3). However, it was determined that the concentration of Cr(VI) in the leaves of these plants and also in the plants of variant No. 2 was lower than the one observed in 5 days. At the same time, the plants themselves did not die. Such result can be explained by the fact that "detoxification" processes can occur in plant cells, i.e., the reduction of toxic Cr(VI) to a less toxic Cr(III). The similar effect was studied earlier using duckweed plants in the experiment [35].

As seen in Fig. 4, the total content of detected Cr(VI) in the containers decreased significantly, and such a decrease was observed in both variants of the experiment.

Bacterial analysis of the soil revealed notable differences in the number of microorganisms in control (without Cr(VI) adding) and experimental (with Cr(VI)) variants. In particular, on LB medium, the CFU in control was 7.2×10^8, and in the experimental variants No. 1 and No. 2 – 5×10^6 and 1×10^6, respectively. This indicates a considerable suppression of the growth of soil microorganisms in Cr(VI) soil pollution. At the same time, such data also demonstrate the possibility of soil microorganisms' survival when chromate is added to the soil in appropriate concentrations.

Discussion

Plants can be used for soil remediation due to the excretion of some metabolites into the soil, toxic metals transportation, accumulation, and reduction in plant cells [37]. The choice of plants for research was determined by the characteristics of their growth, particularly the rate of formation of a developed root system. In addition, the dense grass lawn can be used to cover the surface of the soil, which is contaminated with toxic compounds. The principle mechanism of detoxification of the toxic compound can be explained by the fact that several processes occur simultaneously. We observed these processes in the conducted experiments. First, chromate anion can be transported across the membranes through the sulfate transport system [38], thus reaching the cells [39]. The toxicant can be accumulated in the roots and leaves of the plants [39], which was also observed in our previous experiment [35]. We observed a similar process in the experiments described above. We can assume that this is the mechanism by which Cr(VI) was transported in the lawn grass plants in our study: from the soil to the roots and then to the leaves. This is confirmed by the fact that even in two days lawn grass roots accumulated Cr(VI) – up to 0.23 ± ± 0.02 mg/g. This is also evidenced by the increase in the amount of accumulated Cr(VI) in roots and leaves over time. At the same time, the amount of Cr(VI) in the leaves increased more intensively closer to the middle of the experiment (5 days) than at its beginning. This indicates that the amount of metal accumulating in the roots reaches a critical value over time. The detection of this limit as well as the study of the process of reduction of Cr(VI) to Cr(III) in the roots can be a topic for further research. Such study will allow a better understanding of the nature of the mechanism of Cr(VI) accumulation and reduction by lawn grass plants and the development of optimal conditions for applying this method for soil purification from heavy metal ions. Since plant cells contain various components (chemical compounds – plant

metabolites) with reducing activity, Cr(VI) can be reduced to less toxic Cr(III) directly in the cells [40, 41]. Thus, there is the process of accumulation of the toxic compound in plants with a simultaneous decrease of its content in the soil. The fact of a reduction in the amount of toxic chromium in the leaves on the 10th day of the experiment in comparison with the 5th day may indicate precisely the presence of such a transformation of Cr(VI). In addition, plants excrete their own metabolites (e.g., organic acids) that can reduce Cr(VI), thus reducing its concentration in the soil [42, 43].

Plants can absorb ions from the soil even at low concentrations through their root system [44]. This process was also detected in our study: accumulation of Cr(VI) was studied in plant roots even in two days (Fig. 1). It must be noted that the level of the accumulation increased during the plant cultivation (Figs. 2, 3). After the absorption by the root system, metal ions were to be transported to the shoots [45, 46]. This process was also detected in our study. In particular, in three days (from the 2nd to the 5th), the content of Cr(VI) in the shoots of the grass increased by 4 and 14 times in two versions of the experiment with different concentrations of Cr(VI) in the soil.

Previously, the peculiarities of the growth of plants of various species in the soil in the presence of Cr(VI) were investigated [47]. In particular, the ability of *Chrysopogon zizanioides* to remove Cr(VI) was determined [45]. The authors studied 51% Cr(VI) removal in seven weeks using low initial metal concentration (10 ppm). In our study, the most effective process was proposed because Cr(VI) content in the soil decreased by 86 times in 5 days in the case of using lawn grass.

An important result obtained during the experiment is that the accumulation of Cr(VI) in plants did not cause the yellowing of the experimental plants. Such results confirm that the same detoxification process is taking place: the transformation of the toxic Cr(VI) into low toxic Cr(III). In our experiments, the plants selected for research did not die even at higher concentrations of Cr(VI) in the soil, which indicates their high potential for use in phytoremediation technologies. It is also important to note that with an increase in the amount of toxic Cr(VI) in the soil, the amount of CFU decreased, but microorganisms were detected even in the case of a higher concentration of the toxic metal in the soil. This phenomenon allows us to summarise that, probably, soil microflora can be regenerated after soil purification from Cr(VI).

Despite the complexity of the process described above, using lawn grass is a mechanism for soil renewal by detoxifying and reducing the concentration of the toxic compound. Thus, the conducted studies confirmed the possibility of using lawn grass, which is relatively easy to grow, to reduce soil contamination with a toxic Cr(VI).

Conclusions

So, adding toxic K_2CrO_4 to the soil in the amount of 1.0 or 2.5 g of Cr(VI) inhibited the growth of the soil microorganisms. At the same time, the plants survived under such conditions. Therefore, the possibility of effective use of *Festuca rubra* L., *Festuca arundinacea* Schreb., *Lolium perenne* L., *Poa pratensis* L. grasses (lawn grass) for extracting toxic Cr(VI) compounds from the soil was shown. A notable (13.8 and 9.8 times in two variants) decrease in the Cr(VI) content in the soil was observed already in two days. This decrease progressed significantly in five days as well (91.8 and 85.0 times). Besides this, it was determined that Cr(VI) was accumulated in the root system and the plants' leaves. In the version of the experiment with a lower concentration of chromium in the soil (1 g per container), its accumulation in plants occurred more quickly and efficiently. As a result, the toxic compound accumulated in the plants with a simultaneous decrease in its content in the soil. Thus, the selected combination of grasses can survive at sufficiently high concentrations of Cr(VI) in the soil and accumulate the toxicant, thereby purifying the substrate. This plant mixture can be used in environmental phytoremediation technologies to purify areas contaminated with Cr(VI). At the same time, further development of the technology of the practical application of the specified plants for purifying contaminated soil from toxic metal is necessary.

Interests disclosure

The authors have no conflicts of interest to declare.

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ВИКОРИСТАННЯ ГАЗОННИХ ТРАВ ДЛЯ ОЧИЩЕННЯ ҐРУНТУ ВІД ТОКСИЧНОГО Cr(VI)

Проблематика. Забруднення навколишнього середовища токсичними сполуками становить небезпеку для природи і людини. Тому нині розробляються різноманітні технології очищення ґрунту від токсичних металів.
Мета. Вивчення можливості використання газонної трави для очищення ґрунту від Cr(VI).
Методика реалізації. Насіння рослин (*Festuca rubra* L. 45 %, *Festuca arundinacea* Schreb. 25 %, *Lolium perenne* L. 20 %, *Poa pratensis* L. 10 %) висівали в контейнер за 24 °C і вирощували протягом двох місяців до отримання газону. До ґрунту (400 г) вносили K_2CrO_4 (варіанти: 1,0 г, № 1, та 2,5 г, № 2). Через 2, 5 та 10 діб після внесення Cr(VI) визначали такі показники: вміст Cr(VI) у коренях, надземній частині та ґрунті; коефіцієнт зменшення вмісту Cr(VI); загальний вміст аеробних гетеротрофних мікроорганізмів (КУО/г).
Результати. Через 2 доби після додавання розчину, що містив Cr(VI), корені накопичували його у більшій кількості, ніж листки (у 16,6 і 15 разів у № 1 і № 2 відповідно). Через 5 діб виявлено підвищення концентрації Cr(VI) у рослинах. Концентрація Cr(VI) у ґрунті зменшувалася за 5 діб у 45,8 та 13,57 разу у варіантах № 1 та № 2 відповідно. Виявлено значне пригнічення росту ґрунтових мікроорганізмів. Число КУО в контролі становило $77{,}2\times10^8$, а в дослідних варіантах № 1 і № 2 – 5×10^6 та 1×10^6 відповідно.
Висновки. Додавання K_2CrO_4 пригнічувало ріст ґрунтових мікроорганізмів. Газонна трава успішно вилучала Cr(VI) із ґрунту. Помітне (у 13,8 та 9,3 разу) зниження вмісту Cr(VI) у ґрунті спостерігалося за 2 доби. Це зниження також значно прогресувало за 5 діб (у 91,8 і 85,0 разу). Cr(VI) накопичувався у кореневій системі та листках рослин. Таким чином, використана композиція (газонна трава) може бути застосована в технологіях біоремедіації для очищення забрудненого ґрунту.

Ключові слова: токсичний метал; хром(VI); очищення ґрунту; *Festuca rubra* L.; *Festuca arundinacea* Schreb.; *Lolium perenne* L.; *Poa pratensis* L.; технологія біоремедіації.