Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article OPEN ACCESS

Effect of Toxic Potassium Chromate on *in vitro* Growth of *Artemisia tilesii* Ledeb. Hairy Roots

Nadiia Matvieieva[1], Volodymyr Duplij*[1], Liliia Luchko[2], Taisa Bohdanovych[1], Mykola Kuchuk[1]

[1]Institute of Cell Biology and Genetic Engineering of the National Academy of Sciences of Ukraine, Department of Genetic Engineering, Laboratory of Adaptational Biotechnology, Kyiv, Ukraine
[2]Taras Shevchenko National University of Kyiv, National Scientific Center "Institute of Biology and Medicine", Kyiv, Ukraine

Nadiia Matvieieva: https://orcid.org/0000-0002-4877-5222
Volodymyr Duplij: https://orcid.org/0000-0002-7479-7257
Liliia Luchko: https://orcid.org/0009-0001-5048-1952
Taisa Bohdanovych: https://orcid.org/0000-0002-1834-523X
Mykola Kuchuk: https://orcid.org/0000-0001-7365-7474

Article Details:
Received: 2025-11-03
Accepted: 2025-11-28
Available online: 2025-11-30

DOI: https://doi.org/10.15414/ainhlq.2025.0030

Chromium contamination, prevalent due to its extensive industrial application, poses a significant environmental threat. Hexavalent chromium is notably more toxic than its trivalent form. Phytoremediation, leveraging plants' natural detoxification mechanisms like oxidative stress counteraction, synthesis of reducing compounds, and conversion of Cr(VI) to less toxic Cr(III), offers a promising remediation strategy. Consequently, exploring plant species for their phytoremediation potential is crucial for environmental sustainability. This study investigates the phytoremediation capacity of *Artemisia tilesii* Ledeb. hairy roots in media contaminated with Cr(VI). Hairy roots were cultured for 7 days in Murashige and Skoog medium supplemented with Cr(VI) at concentrations of 25, 50, and 100 mg·l^{-1}. Key parameters assessed included root biomass, flavonoid content, reducing activity, Cr(VI) content in both roots and medium, tolerance index, and growth-corrected bioconcentration factor. Results demonstrated that at 25 mg·L^{-1} Cr(VI), *A. tilesii* hairy roots exhibited a normal growth rate, with no adverse effects on flavonoid content or reducing activity. Remarkably, these roots efficiently accumulated chromium, removing nearly 90% of the Cr(VI) from the medium. However, higher Cr(VI) concentrations proved toxic, inhibiting root growth and biosynthetic activity. In conclusion, *Artemisia tilesii* hairy roots show significant potential for phytoremediation of Cr(VI)-contaminated environments, particularly when chromium concentrations do not exceed 25 mg·L^{-1}. This highlights its promise as a sustainable and effective tool for environmental clean-up.

Keywords: *Artemisia tilesii*, Cr(VI), hairy roots, reducing activity, flavonoids

***Corresponding Author:** Volodymyr Duplij, Institute of Cell Biology and Genetic Engineering of the National Academy of Sciences of Ukraine, Department of Genetic Engineering, Laboratory of Adaptational Biotechnology, Academika Zabolotnoho St 148., 03143, Kyiv, Ukraine
duplijv@icbge.org.ua

Introduction

Heavy metals, including arsenic (As), chromium (Cr), cadmium (Cd), copper (Cu), mercury (Hg), lead (Pb), and zinc (Zn), are chemical elements characterized by high atomic mass and inherent toxicity to living organisms. The accelerating pace of industrial development has exacerbated environmental pollution from these elements, particularly their accumulation in soils adjacent to metal ore processing facilities (Tchounwou et al., 2012; Anyanwu et al., 2018; Barathi et al., 2023). When heavy metals enter human and animal bodies, either through direct contact or via the food chain (Năstăsescu et al., 2020; Nedelescu et al., 2017), they disrupt cellular metabolism and impair vital organ functions, including those of the liver, kidneys, brain, and reproductive system (Afzal and Mahreen, 2024).

Chromium occurs in the environment primarily in two oxidation states: Cr(III), an essential micronutrient required in trace amounts, and Cr(VI) – highly toxic, mobile, and carcinogenic. The last form induces oxidative stress, damaging lipids, proteins, DNA, and membranes. In plants, this leads to chlorosis, growth inhibition, and root damage (Srivastava et al., 2021). Its toxicity arises from the ease with which Cr(VI) enters cells, where it undergoes intracellular reduction, generating reactive oxygen species (ROS) and causing DNA damage. Plants can mitigate Cr(VI) contamination through uptake, reduction to the less toxic Cr(III), and sequestration, making them valuable tools for bioremediation of polluted soils and waters (Han et al., 2023).

In plants, elevated concentrations of toxic metals manifest as stress, primarily through growth inhibition and leaf chlorosis (Clemens, 2006). At the molecular level, this stress induces alterations in protein expression (Cvjetko et al., 2014). For instance, Cr(VI) compounds, a significant environmental pollutant from industrial emissions of leather factories, oil refineries, and mining operations (Tumolo et al., 2020), specifically inhibit seed germination, stem and root elongation, and ultimately reduce overall plant biomass (Saud et al., 2022).

Current strategies for remediating heavy metal-contaminated water and soil in many countries largely rely on chemical and physical methods. However, these approaches are often hampered by significant disadvantages: high equipment and energy costs, the necessity of chemical reagents, and the generation of secondary pollutants (Elnabi et al., 2023; Sarker et al., 2023). While adsorbents and nanomaterials show promise for water decontamination due to their reusability, their economic viability remains debatable (Bayuo et al., 2023; Yang et al., 2019).

The inherent detoxification mechanisms of biological organisms propose an attractive alternative to conventional physical and chemical methods for cleaning heavy metal-contaminated environments (Chen et al., 2022). While plants are relatively easy to employ as accumulators of toxic compounds compared to microorganisms, their phytoremediation efficiency can be limited by toxicological stress, which restricts growth and biomass production (Ojuederie and Babalola, 2017). To soften these challenges, strategies like soil enrichment with silicates and biochar can reduce abiotic and biotic stress (Emamverdian et al., 2018; Haider et al., 2022), or the use of bacteria as "mediators" between the toxic environment and plant roots can be explored (Riseh et al., 2023).

The genetic transformation of plants presents a powerful approach to enhancing their resistance to stress factors. This involves incorporating genes of microorganisms that encode enzymes and metabolites with antioxidant and reducing properties. A particularly promising technique involves the co-cultivation of plants with *Rhizobium* (*Agrobacterium*) *rhizogenes*. This interaction induces plant transformation and the formation of hairy roots, characterized by negative geotropism, an enlarged surface area for environmental contact, and an elevated synthesis of secondary metabolites, including compounds with reducing properties. Genetic transformation occurs when the bacterial Ri-plasmid penetrates plant cells and integrates into the plant genome, resulting in the expression of bacterial *rol* and *aux* genes (Favero et al., 2021). The advantages of the transgenic hairy roots include rapid growth (without the need for exogenous growth regulators), increased production of secondary metabolites, and the expression of additional genes of different origin in plant cells due to Ri-plasmid modification (Morey and Peebles, 2022).

Hairy roots are valuable tools for toxic metal extraction in phytoremediation because they combine high growth rate, genetic stability, and strong biosorption capacity – traits essential for accumulating and tolerating heavy metals. They detoxify pollutants through mechanismssuch as accumulation, chelation, and degradation, making them useful in both research and applied remediation. Induced hairy roots can efficiently absorb and accumulate metals, and their biochemical properties can be studied *in vitro* to better understand detoxification pathways.

Their large surface area enhances contact with metal ions, improving uptake and accumulation efficiency. Additionally, hairy roots exude organic acids, amino acids, and phenolic compounds that mobilize metals, alter rhizosphere pH, and enhance metal bioavailability. This mimics natural root-soil interactions but under controlled laboratory or bioreactor conditions. Consequently, hairy roots can be used as research tools for screening plant species with the potential to tolerate, accumulate, and/or remove environmental pollutants (Ontañon et al., 2014; Perotti et al., 2020).

Artemisia tilesii is a perennial herb native to North America, particularly adapted to cold climates, tundra, and alpine meadows. Its resilience to extreme extreme environmental conditions is thought to rely on a specialized antioxidant defense system, making this species a promising candidate for phytoremediation research.

In studies of metal toxicity, biomass growth serves as a key parameter for evaluating plant tolerance at different contaminant concentration. Meanwhile, the flavonoid content and associated reducing activity provide indicators of the plant's ability to convert toxic chromium(VI) into the less toxic chromium(III). This reduction process not only mitigates damage to the plants itself but also decreases ecological risk, highlighting *A. tilesii* as a potential tool for remediating chromium-contaminated environments.

The objective of our study was to determine the effect of various Cr(VI) concentrations on the growth rate, flavonoid content, and reducing activity of *Artemisia tilesii* hairy roots.

Material and Methodology

Plant Material

Two lines of *A. tilesii* hairy roots (1–4 and 1–9) from the collection of the Institute of Cell Biology and Genetic Engineering of the National Academy of Sciences of Ukraine were used in this study. These roots were obtained via *Agrobacterium rhizogenes* – mediated genetic transformation and studied previously (Bohdanovych et al., 2022).

Transgenic Root Cultivation

Terminal root sections (5–15 mm in length) were cultured in glass vials containing Murashige and Skoog (MS) liquid nutrient medium (Murashige & Skoog, 1962). The medium was prepared with half-strength macrosalts (1/2 MS) and supplemented with 30 $g \cdot L^{-1}$ sucrose. Roots were cultivated at 25 °C with 145 $oscillations \cdot min^{-1}$ on a G-25 Incubator Shaker (New Brunswick Scientific) for 5 weeks.

Following this initial growth phase, potassium chromate (K_2CrO_4) solution, sterilized using a 0.2 µm pore diameter Minisart syringe filter (Sartorius), was added to the vials to achieve final Cr(VI) concentrations of 12.5, 25, 50, and 100 $mg \cdot L^{-1}$. Root incubation continued under the same conditions for an additional 7 days.

Determination of Hairy Root Weight

Upon completion of the incubation period, roots were taken out of from the vials and blotted dry with filter paper. Wet weight (WW) was determined using a Sartorius L 420 P electronic balance.

Extracts Preparation

Exactly 0.3 g of roots was homogenized in 3 ml of deionized water using a mortar and pestle. The homogenates were centrifuged at 14,000 rpm for 8 minutes in an Eppendorf Centrifuge 5415 C. The resulting supernatants were collected and utilized for the Cr(VI) content study. The same procedure of extraction was done using 70% ethanol for the extracts obtained for subsequent analyses, including total flavonoid content and reducing activity assay.

Determination of Specific Flavonoid Content

The specific flavonoid content in root extracts was determined using a modified method based on the work of Pękal and Pyrzynska (2014). To 1 ml of deionized water, 250 µl of extract and 75 µl of 5% $NaNO_2$ solution were added. After mixing, 75 µl of 10% $AlCl_3$ solution, 500 µl of 1M NaOH, and 600 µl of deionized water were added at 5-minute intervals. Optical density was measured at 510 nm using a Fluorat-02-panorama spectrofluorimeter (Lumex). Specific flavonoid content was calculated in rutin equivalent (mg $RE \cdot g^{-1}$ WW), utilizing a calibration curve generated on various dilutions of rutin solution ($y = 1.042x$; $R^2 = 0.826$).

Reducing Activity Assay

To determine the reducing activity, 312 µl of 1% $K_3Fe(CN)_6$ solution and 62–250 µl of extracts were added to 312 µl of 0.2 M phosphate buffer. The reaction mixtures were incubated in a MICROmed VB-20 water bath for 20 min at 50 °C. Subsequently, 312 µl of 10% trichloroacetic acid solution, 1.25 ml of deionized water, and 250 µl of 0.1% $FeCl_3$ solution were added to the mixture. Optical density was measured at a wavelength of 700 nm. Reducing activity was

calculated as the equivalent concentration ($EC_{0.5}$), defined as the mass of hairy roots required to affect an optical density of 0.5 in the reaction mixture. This value was determined by applying linear regression analysis.

Determination of the Specific Content of Cr(VI) in Roots and Medium

To determine the specific content of Cr(VI), a method with diphenylcarbazide (DPC) was used (Wurster et al., 2012). Thus, 0.2 ml of a 0.1N sulfuric acid solution and 0.5 ml of a 0.5% DPC solution were added to 2 ml of the medium samples or the obtained extracts, the preparation of which is described above. The optical density was measured at a wavelength of 546 nm. To construct a calibration graph of the dependence of the optical density on the concentration of Cr(VI) in the reaction mixture, K_2CrO_4 solution at different concentrations was used instead of the studied samples ($y = 0.0067x$; $R^2 = 0.9812$). The value of the Cr(VI) content in the samples was calculated using the calibration graph.

Determination of Total Cr(VI) Content in Roots and Medium

The total Cr(VI) content in the medium (mg) was determined based on its final concentration and the residual volume of the medium in the vial. The total Cr(VI) content in the roots was determined as the specific content multiplied by the mass of roots in the vial.

Determination of Tolerance Index (TI) and Modified Cr(VI) Bioconcentration Factor (mBCF)

The tolerance index (TI) was calculated as the ratio of the wet weight of roots grown in the presence of Cr(VI) to the wet weight of roots in the control group (without Cr(VI) addition). The bioconcentration factor (BCF) was calculated as the ratio of Cr(VI) concentration in hairy roots to the initial concentration in the medium. The modified (growth-corrected) bioconcentration factor (mBCF) was calculated as BCF multiplied by a tolerance index.

Statistical Analysis

Experiments were performed in triplicate for all assays. Data are presented as the mean ± standard error (SE) of the mean, unless explicitly noted. To assess statistical significance, one-way analysis of variance (ANOVA) was utilized, followed by Tukey's honest significant difference (HSD) post-hoc test for pairwise multiple comparisons. P values less than 0.05 were considered significant. All computations were carried out in R version 4.4.2 (R Foundation for Statistical Computing, Vienna, Austria). Calibration curves were constructed, and $EC_{0.5}$ values were calculated using regression analysis.

Results and Discussion

Firstly, we conducted the preliminary experiment for testing the sensitivity of the roots to Cr(VI) at a relatively low concentration (12.5 $mg \cdot L^{-1}$). In this experiment, two independent transgenic hairy root lines (1–4 and 1–9) were used. Root growth inhibition was not observed under these specific conditions, indicating an inherent resilience to abiotic stress induced by hexavalent chromium. Visual inspection after three-day incubation with potassium chromate revealed only some darkening of the roots, especially noticeable in the 1–9 root line (Figure 1).

Quantification of flavonoid content (Figure 2) indicated a significant increase in their concentration to 5.14 ±0.33 and 3.66 ±0.19 $mg \cdot g^{-1}$ WW following incubation of 1–4 and 1–9 hairy root lines with potassium chromate. These concentrations exceeded the control values (2.56 ±0.05 and 2.69 ±0.05 mg RE·g^{-1} WW) for 2.0 and 1.4-fold in 1–4 and 1–9 hairy root lines, respectively. This physiological response suggests the induction of secondary metabolite synthesis, specifically flavonoids, as a possible protective mechanism by the hairy roots in the presence of a toxic compound in the medium.

Effects of Cr(VI) on the Growth Rate of Hairy Roots

As the previous experiment showed no discernible impact on root growth, the concentration of the toxic metal was subsequently increased. Hairy roots from *A. tilesii* line 1–9 were exposed for seven days to a medium containing Cr(VI) at concentrations of 25, 50, and 100 $mg \cdot L^{-1}$. This exposure led to a visible darkening of the roots (Figure 3).

Cr(VI) had a varied impact on root growth depending on its concentration. At 25 $mg \cdot L^{-1}$ Cr(VI), the wet weight of hairy roots (3.14 ±0.11 g) was statistically similar to that of the control group (3.12 ±0.25 g), suggesting no significant effect at this lower concentration.

However, increasing the concentration to 100 $mg \cdot L^{-1}$ Cr(VI) resulted in a significant reduction in root wet weight (2.15 ±0.32 g). This indicates that higher concentrations of Cr(VI) inhibit root growth and are toxic to the studied samples (Figure 4). An intermediate

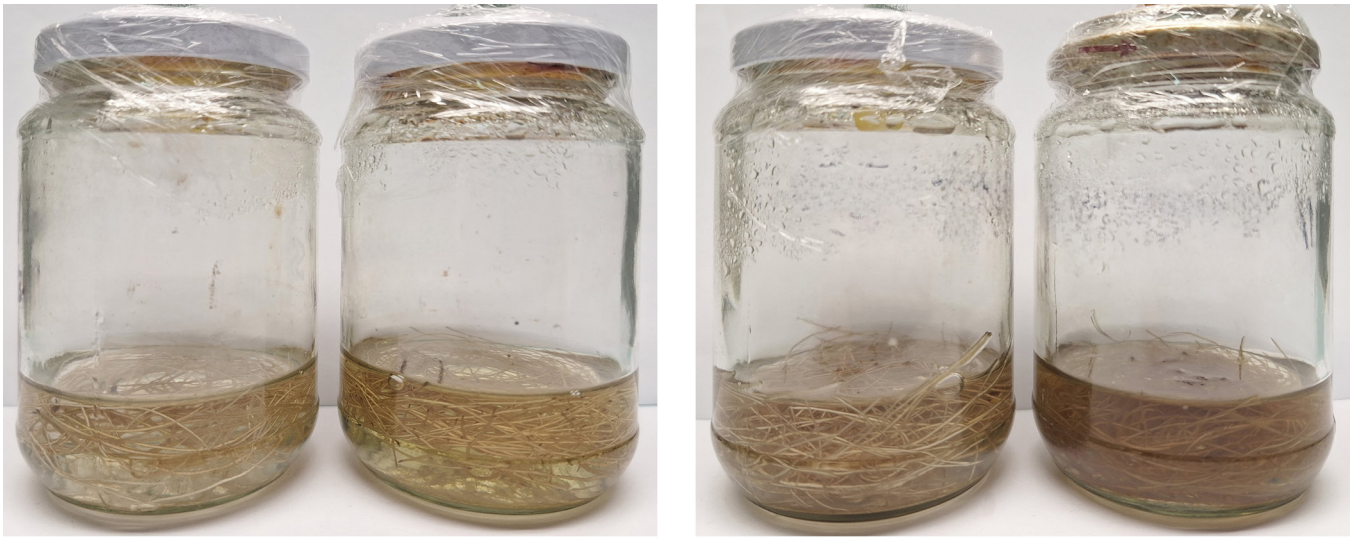

Figure 1 Growth of *Artemisia tilesii* Ledeb. hairy root lines 1–4 (A) and 1–9 (B) under control conditions (0) and on a medium supplemented with Cr(VI) at 12.5 mg·L^{-1} for 3 days

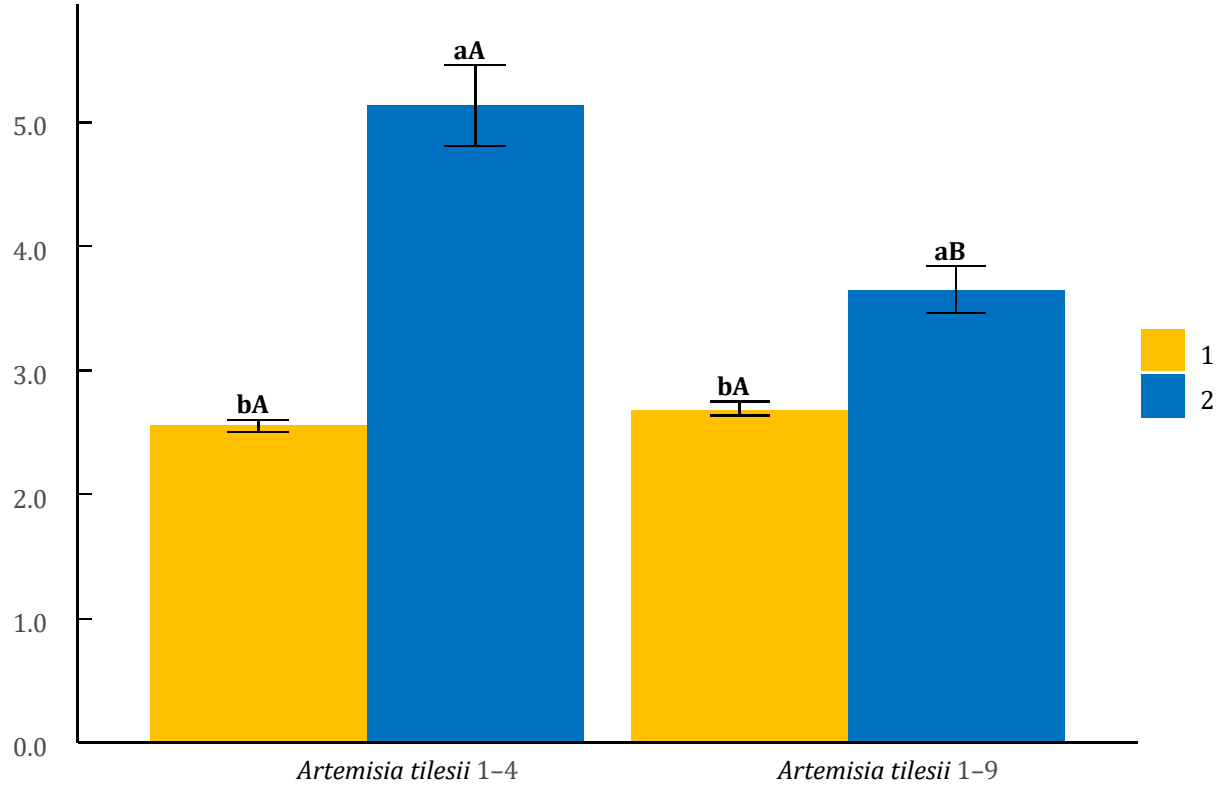

Figure 2 Specific flavonoid content in *Artemisia tilesii* Ledeb. hairy roots (lines 1–4 and 1–9) grown on media without (control, 1) and with 12. mg·L^{-1} of chromium (2)
the same letters indicate no significant differences: uppercase for comparisons between lines, and lowercase for comparisons between Cr(VI) concentrations

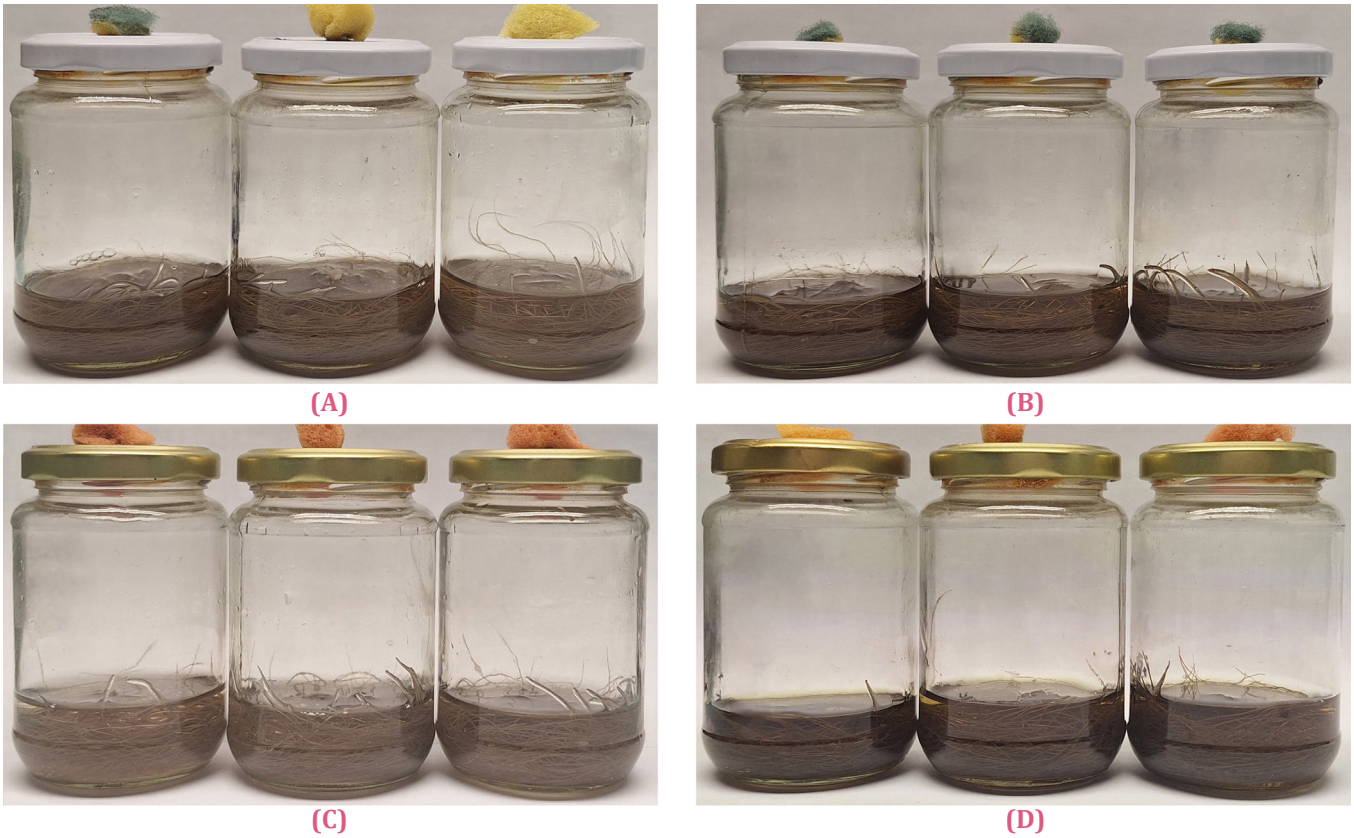

Figure 3 *Artemisia tilesii* Ledeb.hairy roots
control (A) and treated with Cr(VI) for 7 days at 25 (B), 50 (C), and 100 mg·L^{-1} (D)

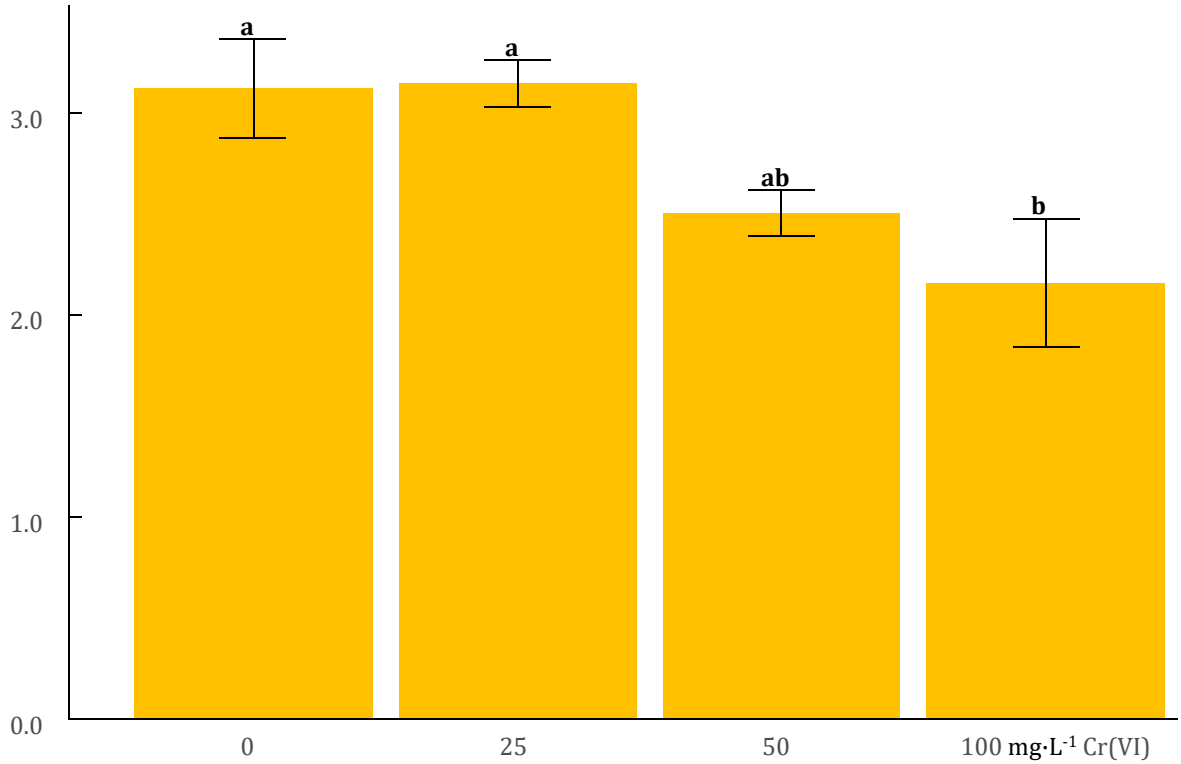

Figure 4 Wet weight of hairy roots of *Artemisia tilesii* Ledeb. incubated with potassium chromate for 7 days with the addition of Cr(VI) in the medium at concentrations of 25, 50, and 100 mg·L^{-1}; 0 – control variant without Cr(VI)

effect on root weight (2.50 ±0.11 g) was observed at 50 mg·L^{-1} Cr(VI). The regression analysis performed on all individual measurements showed the possibility of explaining the decrease in the growth of hair roots by a linear dependence on the added chromium (y = 3.20–0.01x, R^2 = 0.52).

The tolerance index (TI), which compares the wet weight of hairy roots in toxic conditions to that in control conditions, further supports these findings. When hairy roots were cultivated with 25 mg·L^{-1} Cr(VI), the TI was 1.02, reinforcing the observation that this concentration did not impede growth. However, doubling the concentration led to a noticeable decrease in the TI. At 50 mg·L^{-1} Cr(VI), the TI dropped to 0.76, and at 100 mg·L^{-1} Cr(VI), it further decreased to 0.68. These results consistently demonstrate the inhibitory effect of hexavalent chromium on the *in vitro* growth of *A. tilesii* hairy roots.

Cr(VI) Accumulation in *A. tilesii* Hairy Roots

The specific content of Cr(VI) in extracts from *A. tilesii* hairy roots showed distinct patterns based on initial chromium concentrations. At low and medium concentrations (25 and 50 mg·L^{-1}) in the growth medium, the average specific Cr(VI) content was nearly identical, measuring 0.0407 ±0.0050 and 0.0395 ±0.0001 mg·g^{-1} WW, respectively (Figure 5A). However, when roots were cultivated in 100 mg·L^{-1} Cr(VI), this indicator almost doubled to 0.0708 mg·g^{-1} WW.

To assess accumulation efficiency, the bioconcentration factor (BCF) was calculated as the ratio of chromium concentration in hairy roots to the initial concentration in the medium. The BCF values were 1.628, 0.79, and 0.71 for initial Cr(VI) concentrations of 25, 50, and 100 mg·L^{-1}, respectively. Since root mass growth decreased with increasing initial chromate concentration, the growth-corrected BCF (mBCF) showed an even more pronounced decline, registering 1.66, 0.60, and 0.48 for 25, 50, and 100 mg·L^{-1} initial Cr(VI) concentrations, respectively.

Conversely, the total Cr(VI) content in *A. tilesii* line hairy roots (Figure 5B) after a 7-day incubation with potassium chromate did not exhibit statistically significant differences across the tested concentrations. The total Cr(VI) content was 0.125 ±0.019, 0.098 ±0.006, and 0.152 ±0.022 mg Cr(VI) for initial concentrations of 25, 50, and 100 mg·L^{-1}, respectively. This suggests that, when considering only the accumulation process in hairy roots, the overall elimination of Cr(VI) from the medium remains consistent within the 25 to 100 mg·L^{-1} concentration range.

Residual Cr(VI) in Culture Medium

After a 7-day incubation of hairy roots with potassium chromate, total Cr(VI) content in the medium was 0.14 ±0.02, 1.98 ±0.11, and 5.17 ±0.04 mg (Figure 6) in the variants with the initial addition of 1.34, 2.68, and 5.36 mg of Cr(VI) (for the initial concentrations of 25, 50, and 100 mg·L^{-1}, respectively). These values indicate that 10.09% (95% CI: 6.05–16.35%), 74.18% (95% CI: 52.26–88.29%), and 96.62% (95% CI: 91.56–98.69%) of the initial Cr(VI) remained in the culture medium for initial concentrations of 25, 50, and 100 mg·L^{-1}, respectively. Thus, the hairy roots most effectively removed Cr (VI) when the metal concentration was the lowest. For effective Cr(VI) phytoremediation of polluted water using hairy roots, the water should be diluted to reduce Cr(VI) concentrations to 25 mg·L^{-1}.

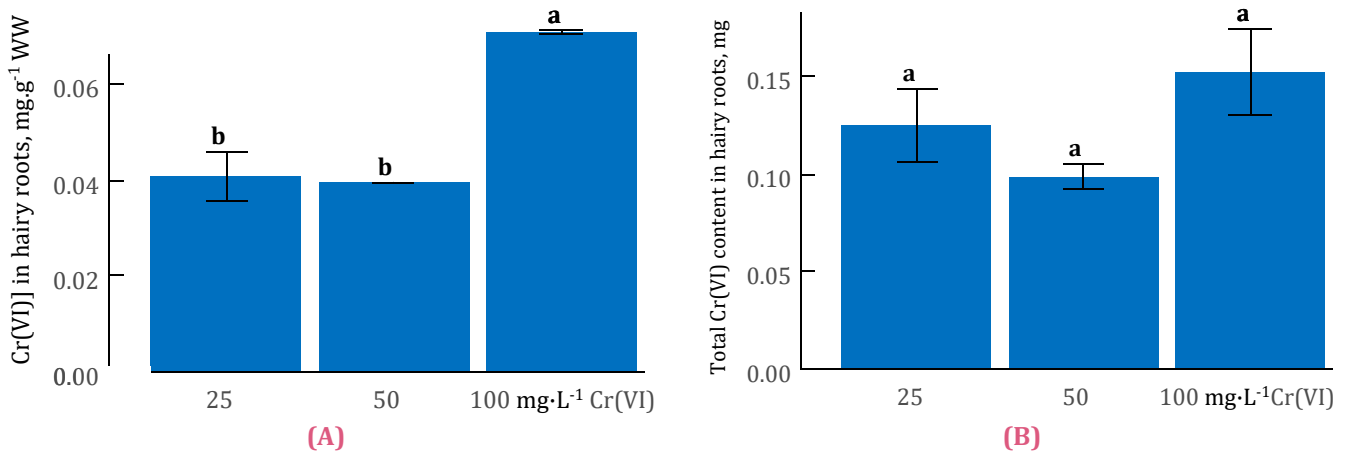

Figure 5 Specific (A) and total (B) Cr(VI) content in hairy roots of *Artemisia tilesii* Ledeb. after incubation for 7 days with potassium chromate

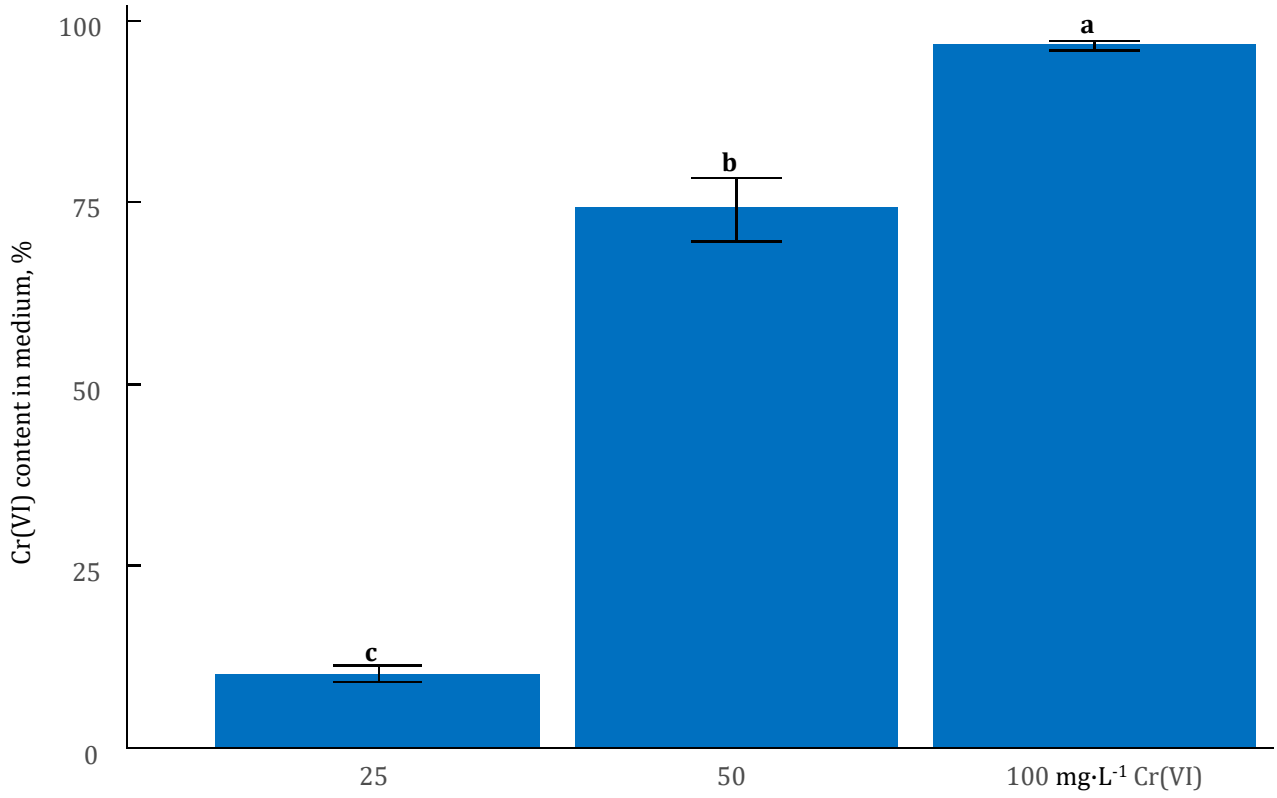

Figure 6 Total Cr(VI) content in the medium after 7-day incubation of hairy roots

The Flavonoid Content in Hairy Roots

Low doses of chromium stimulated the accumulation of flavonoids. After 7-day incubation with 25 mg·L^{-1} of Cr(VI), the specific flavonoid content was 3.21 ±0.13 mg RE·g^{-1} WW, which was significantly higher than the same parameter in the control (2.45 ±0.23 mg RE·g^{-1} WW) (Figure 7). Further increasing the Cr(VI) concentration in the medium to 50 and 100 mg·L^{-1} progressively suppressed the synthesis of these secondary metabolites. The specific flavonoid content in these samples was 1.76 ±0.13 and 1.05 ±0.14 mg RE·g^{-1} WW, respectively.

A linear regression analysis, encompassing all individual measurements of specific flavonoid content across Cr(VI) concentrations from 25 to 100 mg·L^{-1}, demonstrated a significant inverse relationship (y = 3.59–0.03x). The high coefficient of determination (R^2 = 0.80) supports the strong dependence of flavonoid accumulation on Cr(VI) concentration within this range.

Evaluation of Reducing Activity in Hairy Root Extracts

When assessing the bioactivity of the extracts of transgenic roots in the reaction with ferric chloride solution (Figure 8), it was found that the reducing power ($EC_{0.5}$ = 3.62 ±0.50 mg WW) at a low Cr(VI) concentration (25 mg·L^{-1}) was not statistically different from that of the control group (4.45 ±0.61 mg WW). However, a significant decline in the reducing power of extracts from these hairy roots was observed with increasing Cr(VI) initial concentrations, reaching 8.56 ±0.87 and 10.50 ±0.71 mg WW at 50 and 100 mg·L^{-1}, respectively. It should be mentioned that higher $EC_{0.5}$ values indicate that more plant material is needed for the reaction, reflecting lower sample activity.

A significant inverse linear correlation was observed between the $EC_{0.5}$ values and the specific flavonoid content of the *A. tilesii* hairy root extracts (Figure 9). This strong relationship is supported by a high coefficient of determination (R^2 = 0.94) for the established regression model (y = 14.05–3.43x). The inverse nature of this correlation suggests that higher flavonoid concentrations are directly associated with enhanced reducing activity. Consequently, flavonoids are identified as key contributors to the observed reducing activity within these extracts.

Thus, in this study, the effect of hexavalent chromium on the growth of *A. tilesii* hairy roots was concentration-dependent. At 25 mg·L^{-1} Cr(VI), root weight gain

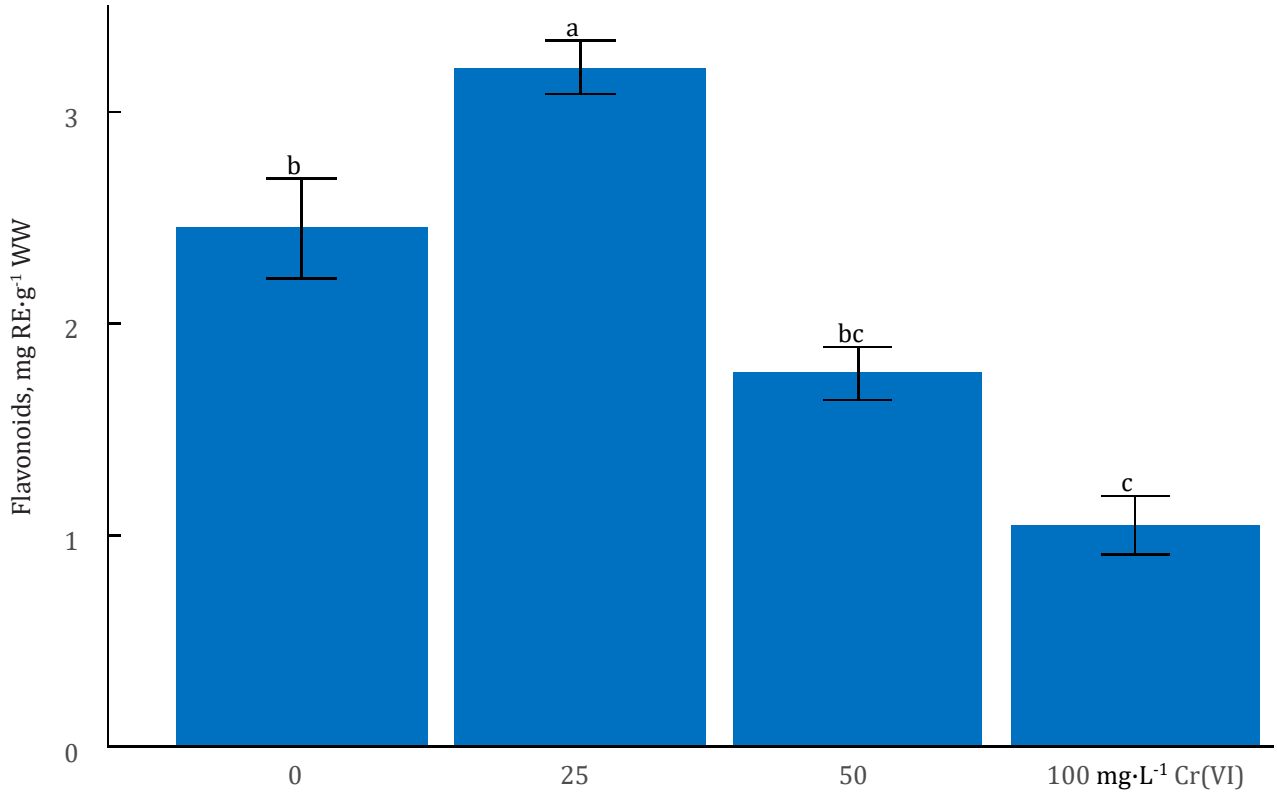

Figure 7 Specific flavonoid content in the hairy roots of *Artemisia tilesii* Ledeb. after a 7-day incubation with potassium chromate at concentrations of 25–100 mg·L^{-1} Cr(VI), compared with control samples (0)

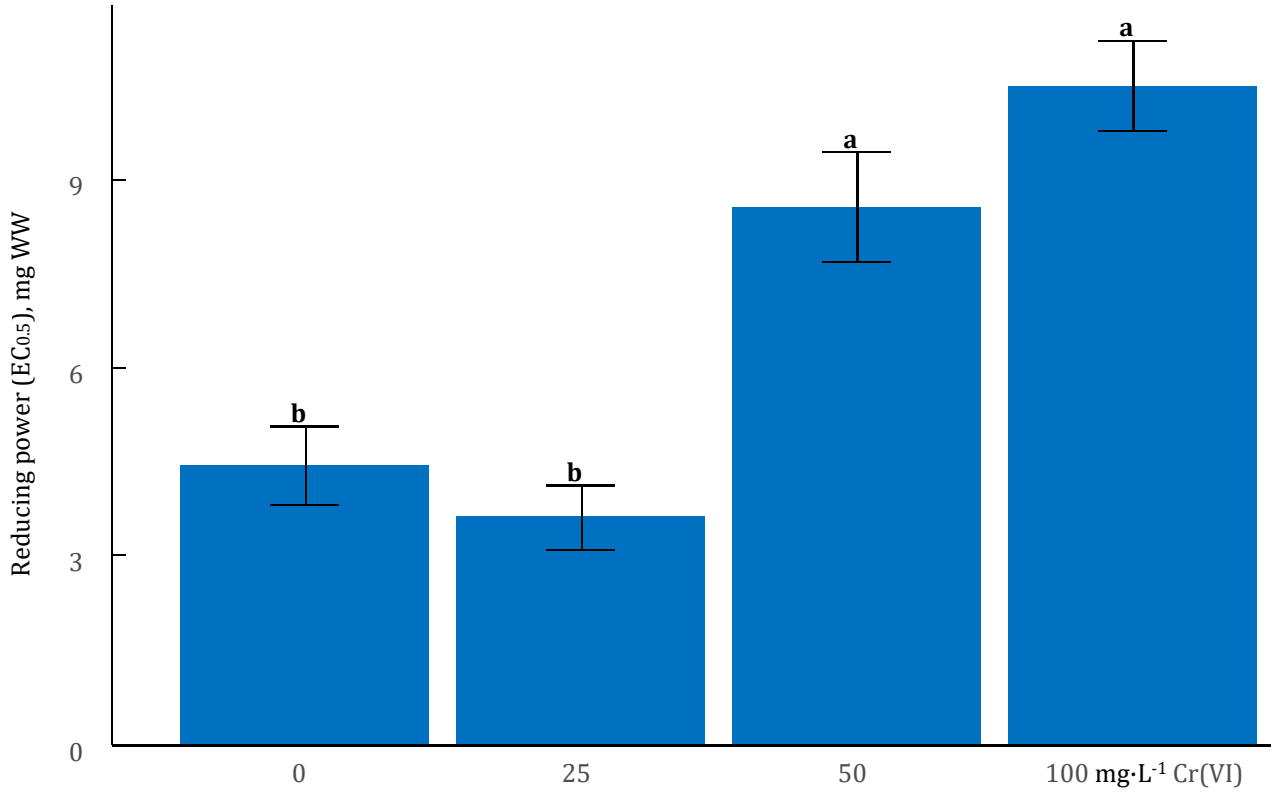

Figure 8 Reducing power of the extracts after a 7-day incubation of *Artemisia tilesii* Ledeb. hairy roots with potassium chromate at concentrations of 25–100 mg·L^{-1} Cr(VI), compared with control samples (0)

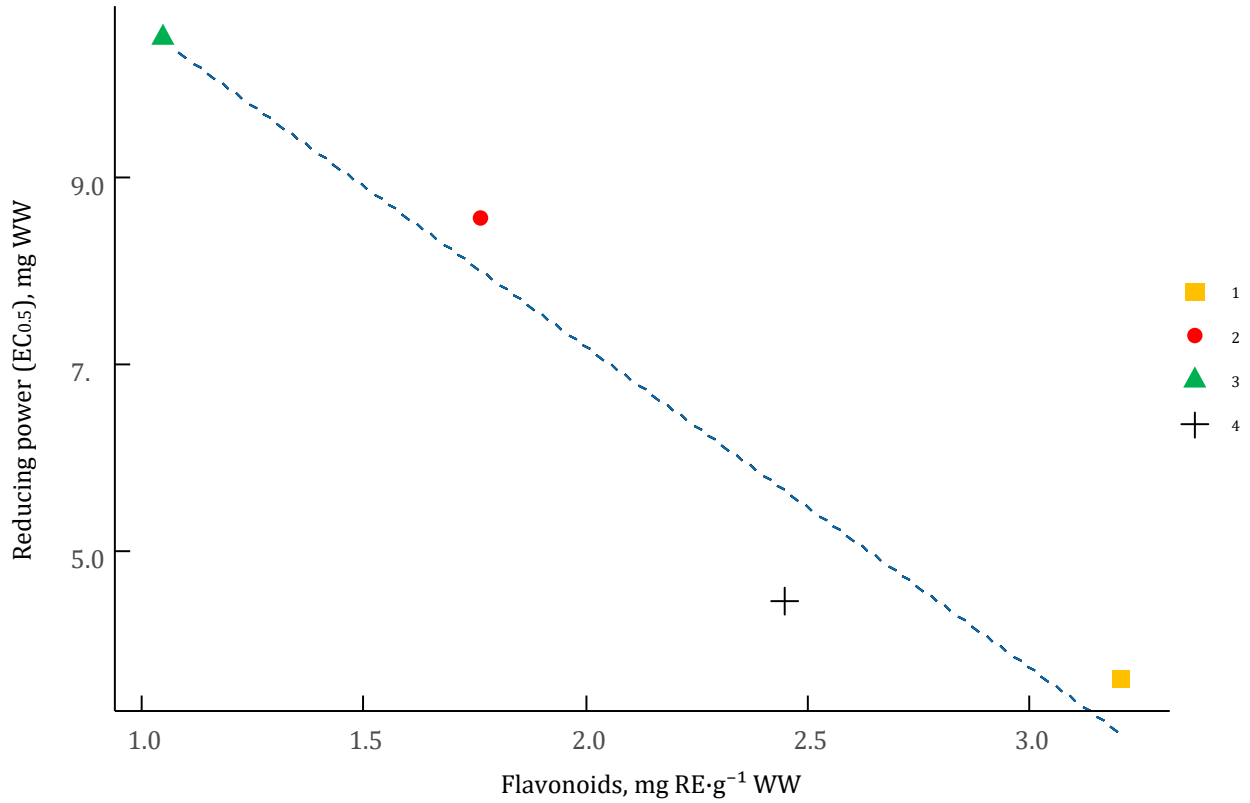

Figure 9 Regression analysis of the relationship between $EC_{0.5}$ and specific flavonoid content in *Artemisia tilesii* Ledeb. hairy root extracts after a 7-day incubation with potassium chromate at concentrations of 25 (1), 50 (2), or 100 (3) mg·L^{-1} Cr(VI), compared with control samples (4)

was statistically indistinguishable from the control, whereas higher concentrations significantly inhibited growth. This type of response is well-documented in the literature. For example, high concentrations of Cr(VI) have been shown to cause growth arrest and biomass reduction in *Brassica juncea* (Fengxiang et al., 2004). The research demonstrated that excessive Cr(VI) levels affected sweet potato (Gao et al., 2021) and wheat(Wang et al., 2017).

The values of the tolerance index confirmed the significant toxicity of Cr(VI) in high concentrations. In the case of roots grown in the medium with a lower Cr(VI) concentration,the TI was 1.02. This parameter decreased to 0.76 and 0.68 when the roots were grown with Cr(VI) in higher concentration (50 and 100 mg·L^{-1} Cr(VI), respectively). These results are consistent with studies conducted on other plants (Redondo-Gómez et al., 2011; Sinha et al., 2014; Srivastava et al., 2021; Boros-Lajszner et al., 2023). Perotti et al. (2020) also evaluated Cr(VI) remediation by hairy roots of *Brassica napus*. They found that the roots grew well at concentrations up to 10 mg·L^{-1} Cr(VI), while higher concentrations were toxic for the hairy roots (Perotti et al., 2020). In our work, it was found that *A. tilesii* hairy roots are significantly more resistant to the effects of Cr(VI), since the weight gain in the presence of even 25 mg·L^{-1} of Cr(VI) did not differ from that of the control, and the tolerance index under such conditions was high (TI = 1.02).

Plants of the *Artemisia* genus are known to produce compounds with antioxidant and reducing properties, including various phenoxy acids (gallic, coumaric, chlorogenic, syringic acids) and flavonoids such as quercetin and rutin (Ferreira et al., 2010; Stambulska et al., 2018; Bisht et al., 2021; Sharifi et al., 2022; Sadowska et al., 2023; Abdullajanov et al., 2025). While heavy metals are typically toxic, low concentrations can sometimes stimulate the synthesis of these protective secondary metabolites. For instance, the flavonoid content in *Lycopersicon esculentum* Mill. (tomato) increased in response to low levels of copper and zinc in the soil (Shomali et al., 2022).

Our results revealed a similar pattern. The addition of a low concentration of Cr(VI) to the medium significantly increased the flavonoid content in our plant cultures to 3.21 ±0.13 mg RE·g^{-1} WW, compared to 2.45 ±0.23 mg RE·g^{-1} WW in the control. However, this stimulatory effect was reversed at higher concentrations. Increasing

the Cr(VI) levels to 50 and 100 mg·L^{-1} inhibited both plant growth and flavonoid biosynthesis, reducing the flavonoid content to 1.76 ±0.13 and 1.05 ±0.14 mg RE·g^{-1} WW, respectively. This inhibition also diminished the associated reducing activity of the plant extracts. $EC_{0.5}$ value for reducing activity increased from 3.62 ±0.50 mg WW in the control to 4.45 ±0.61, 8.56 ±0.87, and 10.50 ±0.71 mg WW at Cr(VI) concentrations of 25, 50, and 100 mg·L^{-1}, respectively, indicating a weaker antioxidant capacity. These findings are consistent with previous research showing that Cr(VI) toxicity inhibits growth and alters secondary metabolism in plants (Dubey et al., 2018; Kumar et al., 2022).

Previous research by Ontañon et al. (2014) demonstrated the capacity of *Brassica napus* hairy roots in a hydroponic system to accumulate Cr(VI). Our experiments with *A. tilesii* hairy roots similarly showed Cr(VI) accumulation. The total Cr(VI) content in *A. tilesii* hairy roots was independent of the applied Cr(VI) dose for initial concentrations between 25 and 100 mg·L^{-1}, ranging from 0.098 to 0.152 mg.

The observed higher efficiency of Cr(VI) reduction by hairy roots at lower initial medium concentrations, coupled with the absence of growth inhibition, may be attributed to several factors. As suggested by Matsia et al. (2022), this could involve the formation of specific flavonoid complexes and the reduction of Cr(VI) to Cr(III) by plant metabolites. These metabolites possess significant reducing and antioxidant activity, enabling them to neutralize reactive oxygen species and, consequently, better mitigate cellular damage induced by oxidative stress.

Conclusions

Thus, our research explored how varying concentrations of Cr(VI) in the nutrient medium affect *A. tilesii* hairy roots. We found several key differences:

- No growth inhibition at low concentrations. *A. tilesii* hairy roots showed no growth inhibition when Cr(VI) concentrations did not exceed 25 mg·L^{-1}.
- High tolerance at low concentrations. The roots exhibited a high tolerance index in growth conditions where Cr(VI) concentrations did not exceed 25 mg·L^{-1}.
- Sensitivity to higher concentrations. Conversely, *A. tilesii* hairy roots demonstrated high sensitivity to Cr(VI) at higher concentrations.
- Increased flavonoid content at low Cr(VI). Roots exposed to relatively low Cr(VI) levels (up to 25 mg·L^{-1}) in the medium showed a higher specific content of flavonoids compared to the control group.
- Efficient bioremediation at low concentration. Light more than 10% of the initial total Cr(VI) content remained in the culture medium when its starting concentration was 25 mg·L^{-1}.

Our results show that *Artemisia tilesii* hairy roots can efficiently phytoremediate Cr(VI)–contaminated media when the initial concentration does not exceed 25 mg·L^{-1}. At or below this threshold, root viability remains uncompromised, enabling the removal of up to 90% of soluble Cr(VI) during the cultivation period. These results highlight the practical potential of *A. tilesii* hairy-root cultures as a biotechnological tool for treating moderately chromium-polluted water.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Statement

This article does not contain any studies that would require an ethical statement.

Funding

This work was funded by a National Academy of Sciences of Ukraine grant No 0125U000980 "Biotechnological plants for combating infectious bacterial diseases and phytodestruction of military-origin pollutants".

Acknowledgements

Not applicable.

References

Abd Elnabi, M. K., Elkaliny, N. E., Elyazied, M. M., Azab, S. H., Elkhalifa, S. A., Elmasry, S., Mouhamed, M. S., Shalamesh, E. M., Alhorieny, N. A., Abd Elaty, A. E., Elgendy, I. M., Etman, A. E., Saad, K. E., Tsigkou, K., Ali, S. S., Kornaros, M., & Mahmoud, Y. A. (2023). Toxicity of heavy metals and recent advances in their removal: A review. *Toxics*, 11(7), 580. https://doi.org/10.3390/toxics11070580

Abdullajanov, O.A., Ganiev, A.A., Turak, A., Begmatov, N.B., Bobakulov, Kh.M., Abdullaev, N.D., Zhao, J., He, F. (2025). Flavonoids and phenolic compounds from *Artemisia porrecta*. *Chemistry of Natural Compounds*, 61, 548–550 https://doi.org/10.1007/s10600-025-04698-z

Afzal, A., & Mahreen, N. (2024). Emerging insights into the impacts of heavy metals exposure on health, reproductive and productive performance of livestock. *Frontiers in Pharmacology*, 15, 1375137. https://doi.org/10.3389/fphar.2024.1375137

Anyanwu, B. O., Ezejiofor, A. N., Igweze, Z. N., & Orisakwe, O. E. (2018). Heavy metal mixture exposure and effects

in developing nations: An update. *Toxics*, 6(4), 65. https://doi.org/10.3390/toxics6040065

Barathi, S., Lee, J., Venkatesan, R., & Vetcher, A. A. (2023). Current status of biotechnological approaches to enhance the phytoremediation of heavy metals in India: A review. *Plants* (Basel, Switzerland), 12(22), 3816. https://doi.org/10.3390/plants12223816

Bayuo, J., Rwiza, M. J., Sillanpää, M., & Mtei, K. M. (2023). Removal of heavy metals from binary and multicomponent adsorption systems using various adsorbents – a systematic review. *RSC Advances*, 13(19), 13052–13093. https://doi.org/10.1039/d3ra01660a

Bisht, D., Kumar, D., Kumar, D., Dua, K., & Chellappan, D. K. (2021). Phytochemistry and pharmacological activity of the genus artemisia. *Archives of Pharmacal Research*, 44(5), 439–474.
https://doi.org/10.1007/s12272-021-01328-4

Bohdanovych, T. A., Morgun, B. V., Lakhneko, O. R., Shakhovsky, & A. M., Matvieieva, N. A. (2022). Peculiarities of the growth of *Artemisia tilesii* Ledeb. "hairy" roots with different foreign genes. *Biotechnologia Acta,* 15(3), 13–24.
http://jnas.nbuv.gov.ua/article/UJRN-0001377783

Boros-Lajszner, E., Wyszkowska, J., & Kucharski, J. (2023). Evaluation and assessment of trivalent and hexavalent chromium on *Avena sativa* and soil enzymes. *Molecules*, 28(12), 4693.
https://doi.org/10.3390/molecules28124693

Chen, R., Tu, H., & Chen, T. (2022). Potential application of living microorganisms in the detoxification of heavy metals. *Foods*, 11(13), 1905.
https://doi.org/10.3390/foods11131905

Clemens, S. (2006). Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. *Biochimie*, 88(11), 1707–1719.
https://doi.org/10.1016/j.biochi.2006.07.003

Cvjetko, P., Zovko, M., & Balen, B. (2014). Proteomics of heavy metal toxicity in plants. *Arhiv za Higijenu Rada i Toksikologiju*, 65(1), 1–18.
https://doi.org/10.2478/10004-1254-65-2014-2443

Dubey, S., Gupta, A., Khare, A., Jain, G., Bose, S., & Rani, V. (2018). Long- and short-term protective responses of rice seedling to combat Cr(VI) toxicity. *Environ Sci Pollut Res,* 25, 36163–36172.
https://doi.org/10.1007/s11356-018-3422-z

Emamverdian, A., Ding, Y., Xie, Y., & Sangari, S. (2018). Silicon Mechanisms to Ameliorate Heavy Metal Stress in Plants. *BioMed Research International*, 2018, 8492898. https://doi.org/10.1155/2018/8492898

Favero, B. T., Tan, Y., Lin, Y., Hansen, H. B., Shadmani, N., Xu, J., He, J., Müller, R., Almeida, A., & Lütken, H. (2021). Transgenic Kalanchoë blossfeldiana, containing individual rol genes and open reading frames under 35S promoter, exhibit compact habit, reduced plant growth, and altered ethylene tolerance in flowers. *Frontiers in Plant Science*, 12, 672023.
https://doi.org/10.3389/fpls.2021.672023

Ferreira, J.F.S., Luthria, D.L., Sasaki, T., & Heyerick, A. (2010). Flavonoids from *Artemisia* annua L. as antioxidants and their potential synergism with artemisinin against malaria and cancer. *Molecules*, 15(5), 3135–3170. https://doi.org/10.3390/molecules15053135

Gao, J., Zhang, D., Proshad, R., Uwiringiyimana, E., and Wang, Z. (2021). Assessment of the pollution levels of potential toxic elements in urban vegetable gardens in southwest China. *Scientific Reports,* 11, 22824.
https://doi.org/10.1038/s41598-021-02069-6

Haider, F. U., Wang, X., Farooq, M., Hussain, S., Cheema, S. A., Ain, N. U., Virk, A. L., Ejaz, M., Janyshova, U., & Liqun, C. (2022). Biochar application for the remediation of trace metals in contaminated soils: Implications for stress tolerance and crop production. *Ecotoxicology and Environmental Safety*, 230, 113165.
https://doi.org/10.1016/j.ecoenv.2022.113165

Han, F. X., Sridhar, B. B. M., Monts, D. L., & Su, Y. (2004). Phytoavailability and toxicity of trivalent and hexavalent chromium to *Brassica juncea*. *New Phytologist*, 162(2), 489–499.
https://doi.org/10.1111/j.1469-8137.2004.01027.x

Han, L., Gu, H., Lu, W., Li, H., Peng, W., Ma, N.L., Lam, S.S., Sonne, C. (2023). Progress in phytoremediation of chromium from the environment. *Chemosphere*, 344, 140307. https://doi.org/10.1016/j.chemosphere.2023.140307

Kumar, S., Wang, M., Fahad, S., Qayyum, A., Chen, Y., & Zhu, G. (2022). Chromium induces toxicity at different phenotypic, physiological, biochemical, and ultrastructural levels in sweet potato (*Ipomoea batatas* L.) plants. *International Journal of Molecular Sciences*, 23(21), 13496.
https://doi.org/10.3390/ijms232113496

Matsia, S., Tsave, O., Hatzidimitriou, A., & Salifoglou, A. (2022). Chromium flavonoid complexation in an antioxidant capacity role. *International Journal of Molecular Sciences*, 23(13), 7171. https://doi.org/10.3390/ijms23137171

Merecz-Sadowska, A., Sitarek, P., Kowalczyk, T., Palusiak, M., Hoelm, M., Zajdel, K., & Zajdel, R. (2023). *In vitro* evaluation and *in silico* calculations of the antioxidant and anti-Inflammatory properties of secondary metabolites from *Leonurus sibiricus* L. root extracts. *Molecules*, 28(18), 6550.
https://doi.org/10.3390/molecules28186550

Morey, K. J., & Peebles, C. A. M. (2022). Hairy roots: An untapped potential for production of plant products. *Frontiers in Plant Science*, 13, 937095.
https://doi.org/10.3389/fpls.2022.937095

Murashige, T., & Skoog, F. (1962). A Revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, 15(3), 473–497.
https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Năstăsescu, V., Mititelu, M., Goumenou, M., Docea, A. O., Renieri, E., Udeanu, D. I., Oprea, E., Arsene, A. L., Dinu-Pîrvu, C. E., & Ghica, M. (2020). Heavy metal and pesticide levels in dairy products: Evaluation of human health risk. *Food and Chemical Toxicology*, 146, 111844.
https://doi.org/10.1016/j.fct.2020.111844

Nedelescu, M., Baconi, D., Neagoe, A., Iordache, V., Stan, M., Constantinescu, P., Ciobanu, A., Vardavas, A. I., Vincenti, M., & Tsatsakis A. M. (2017). Environmental metal contamination and health impact assessment in two industrial regions of Romania. *Science of The Total Environment,* 580, 984–995. https://doi.org/10.1016/j.scitotenv.2016.12.053

Ojuederie, O. B., & Babalola, O. O. (2017). Microbial and plant-assisted bioremediation of heavy metal polluted environments: A review. *International Journal of Environmental Research and Public Health*, 14(12), 1504. https://doi.org/10.3390/ijerph14121504

Ontañon, O. M., González, P. S., Ambrosio, L. F., Paisio, C. E., & Agostini, E. (2014). Rhizoremediation of phenol and chromium by the synergistic combination of a native bacterial strain and *Brassica napus* hairy roots. *International Biodeterioration & Biodegradation*, 88, 192–198. https://doi.org/10.1016/j.ibiod.2013.10.017

Pękal, A., Pyrzynska, K. (2014). Evaluation of aluminium complexation reaction for flavonoid content assay. *Food Analytical Methods*, 7(9), 1776–1782. https://doi.org/10.1007/s12161-014-9814-x

Perotti, R., Paisio, C.E., Agostini, E., & González, P.S. (2020). CR(VI) phytoremediation by hairy roots of *Brassica napus*: assessing efficiency, mechanisms involved, and post-removal toxicity. *Environmental Science and Pollution Research*, 27, 9465–9474. https://doi.org/10.1007/s11356-019-07258-5

Redondo-Gómez, S., Mateos-Naranjo, E., Vecino-Bueno, I., & Feldman, S. R. (2011). Accumulation and tolerance characteristics of chromium in a cordgrass Cr-hyperaccumulator, *Spartina argentinensis*. *Journal of Hazardous Materials*, 185, 962–969. https://doi.org/10.1016/j.jhazmat.2010.09.101

Riseh, R. S., Vazvani, M. G., Hajabdollahi, N., & Thakur, V. K. (2023). Bioremediation of heavy metals by *Rhizobacteria*. *Applied Biochemistry and Biotechnology*, 195(8), 4689–4711. https://doi.org/10.1007/s12010-022-04177-z

Sarker, A., Masud, M. A. A., Deepo, D. M., Das, K., Nandi, R., Ansary, M. W. R., Islam, A. R. M. T., & Islam, T. (2023). Biological and green remediation of heavy metal contaminated water and soils: A state-of-the-art review. *Chemosphere*, 332, 138861. https://doi.org/10.1016/j.chemosphere.2023.138861

Saud, S., Wang, D., Fahad, S., Javed, T., Jaremko, M., Abdelsalam, N. R., & Ghareeb, R. Y. (2022). The impact of chromium ion stress on plant growth, developmental physiology, and molecular regulation. *Frontiers in Plant Science*, 13, 994785. https://doi.org/10.3389/fpls.2022.994785

Sharifi-Rad, J., Herrera-Bravo, J., Semwal, P., Painuli, S., Badoni, H., Ezzat, S. M., Farid, M. M., Merghany, R. M., Aborehab, N. M., Salem, M. A., Sen, S., Acharya, K., Lapava, N., Martorell, M., Tynybekov, B., Calina, D., & Cho, W. C. (2022). *Artemisia* spp.: An update on its chemical composition, pharmacological and toxicological profiles. *Oxidative Medicine and Cellular Longevity*, 2022, 5628601. https://doi.org/10.1155/2022/5628601

Shomali, A., Das, S., Arif, N., Sarraf, M., Zahra, N., Yadav, V., Aliniaeifard, S., Chauhan, D. K., & Hasanuzzaman, M. (2022). Diverse physiological roles of flavonoids in plant environmental stress responses and tolerance. *Plants*, 11(22), 3158. https://doi.org/10.3390/plants11223158

Sinha, V., Pakshirajan, K., & Chaturvedi, R.(2014). Chromium(VI) accumulation and tolerance by *Tradescantia pallida*: biochemical and antioxidant study. *Applied Biochemistry and Biotechnology*, 173(8), 2186–2196. https://doi.org/10.1007/s12010-014-1035-7

Srivastava, D., Tiwari, M., Dutta, P., Singh, P., Chawda, K., Kumari, M., Singh, P., Chawda, K., Kumari, M., Chakrabarty, D. (2021). Chromium stress in plants: toxicity, tolerance and phytoremediation. *Sustainability* 13, 4629. https://doi.org/10.3390/su13094629

Stambulska, U. Y., Bayliak, M. M., & Lushchak, V. I. (2018). Chromium(VI) toxicity in legume plants: modulation effects of rhizobial symbiosis. *BioMed Research International*, 2018, 8031213. https://doi.org/10.1155/2018/8031213

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy metal toxicity and the environment. *Experientia Supplementum*, 101, 133–164. https://doi.org/10.1007/978-3-7643-8340-4_6

Tumolo, M., Ancona, V., De Paola, D., Losacco, D., Campanale, C., Massarelli, C., & Uricchio, V. F. (2020). Chromium pollution in European water, sources, health risk, and remediation strategies: An overview. *International Journal of Environmental Research and Public Health*, 17(15), 5438. https://doi.org/10.3390/ijerph17155438

Wang, S., Wu, W., Liu, F., Liao, R., & Hu, Y. (2017). Accumulation of heavy metals in soil-crop systems: A review for wheat and corn. *Environmental Science and Pollution Research*, 24, 15209–15225. https://doi.org/10.1007/s11356-017-8909-5

Wurster, S., Kratz, E., Lachenmeier, D. W., & Mildau, G. (2012). Spectrophotometric quantification of toxicologically relevant concentrations of chromium(VI) in cosmetic pigments and eyeshadow using synthetic lachrymal fluid extraction. *International Journal of Spectroscopy*, 2012(1). https://doi.org/10.1155/2012/985131

Yang, J., Hou, B., Wang, J., Tian, B., Bi, J., Wang, N., Li, X., & Huang, X. (2019). Nanomaterials for the removal of heavy metals from wastewater. *Nanomaterials*, 9(3), 424. https://doi.org/10.3390/nano9030424