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MODELING OF CHROMIUM EFFECT ON ECOPHYSIOLOGICAL PARAMETERS OF SOIL–PLANT SYSTEM

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Ecophysiological parameters of soil–plant system under the effect of trivalent chromium and hexavalent chromium were studied using small artificial ecosystems – the microcosms. Emission of carbon dioxide (CO₂) and nitrogen oxides (NO_x) from soil and soil–plant system, morphometry and carbon concentration in shoots of *Phaseolus vulgaris* L. were determined. We established that effect of Cr(VI) decreased CO₂ emission and increased NO_x emission from soil microcosms and soil–plant microcosms. In turn, the effect of Cr(III) did not cause statistically significant changes on the emission of investigated gases in microcosms models. An inverse relationship between CO₂ and NO_x emissions was found by the correlation analysis. The emission intensity of investigated gases from soil without plants was higher than that from soil with plant cover. This fact stresses that the soil devoid of plant cover is an additional source of greenhouse gases emissions. Statistically significant changes effect in of Cr(VI) on morphometry and carbon concentration in the shoots of investigated plants was not found. In turn, Cr(III) decreased leaf growth in length and carbon concentration in shoots of *P. vulgaris*. Changes of investigated parameters showed that the problem of contamination of soil and groundwater with Cr(III) is important as pollution by Cr(VI).

Keywords: contamination with chromium compounds, ecophysiological parameters, microcosm models, soil–plant system, *Phaseolus vulgaris* L.

INTRODUCTION

Chromium (Cr) – is a chemical element that is almost always present in the soil, plant and animal tissues. Many countries, including Ukraine, are facing problems of soil and water contamination with Cr from different sources for example mining, electroplating in steel industry, refractory materials production, tanning in leather industry, oxidation in organic synthesis of drugs and oxidative dyeing in textile industry. Worldwide annual mining of the chromate (FeCr₂O₄) level of 10 million tons have been exceeded. Active scattering of Cr is also associated with burning fossil fuels, mainly coal. The

leather industry is the major cause of the high influx of Cr to the biosphere, accounting for 40 % of the total industrial use [1].

There are two predominant stable oxidation states of chromium: Cr^{6+} and Cr^{3+} with Cr^{6+} being considered more toxic than Cr^{3+} . In addition, Cr^{6+} can be reduced to Cr^{3+} in redox reactions. Cr is not considered as an essential element for plant nutrition. Both forms, Cr(III) and Cr(VI), might be taken up by plants. Uptake of Cr(III) is considered to be passive, while that of Cr(VI) is considered to be active [22]. The physiological role of Cr(III) as well as toxic effects of Cr(VI) on plants, animals and microorganisms have been repeatedly investigated. In case of humans, Cr(III) is regarded as a micronutrient; on the other hand, Cr(VI) has toxic effects on biological systems and has been classified by the International Agency for Research on Cancer (IARC) as a Group-1 human carcinogen [1].

Plants have a remarkable ability to absorb, translocate and accumulate heavy metals and organic compounds from the environment. In order to maintain their charge balance, roots release protons whenever they take up more cations than anions, and take up protons when the opposite occurs [2]. When Cr enters plant body, it causes oxidative stress, compete with essential elements for their binding sites in metalloenzymes ultimately reducing plant growth and yield [4].

Unfortunately, there is a small number of investigations, comparing the impact of different valences of Cr on ecological and physiological plant parameters. Singh et al (2001) investigated the effect of Cr(III) and Cr(VI) on spinach and reported that Cr(VI), applied at 60 mg/kg^{-1} and higher concentration, reduced the leaf size, caused burning of leaf tips or margin, and slowed leaf growth rate, resulting in reduced dry biomass [20]. Vernay et al. (2008) reported that plants of *Datura innoxia* Mill grown under Cr(VI) showed reduced growth, leading to reduction in root and shoot biomass. The addition of Cr(III) into the nutrient solution restricted the shoot and root growth but at a lower level than Cr(VI). The decrease of shoot growth was observed from 0.05 and 0.5 mM for Cr(VI) and Cr(III), respectively. Different Cr levels also remarkably affected growth physiology of plants. The photosynthetic rate was reduced by 21–62 %, while the transpiration rate was reduced by 5–59 % [24]. Sauerbeck et al (1991) reported that lower concentration of Cr in grains compared to roots was probably due to reduction of Cr(VI) to Cr(III), which reduced its mobility from roots to shoots [18].

It is recognized that the change in respiration intensity of living organisms under stress is a sensitive bioindication characteristic. The intensity of carbon dioxide emission (respiration) of soil and plants grown under contamination with chromium compounds scantily described in the literature. Even less information about nitrogen oxides (NO_x) emissions by plants and soil. It is now established that NO is a multifunctional signaling molecule, that is generated and is active in all organisms — from bacteria to plants and animals. The investigation of NO_x emissions from plants began in the 70s of last century, with the establishment of the phenomenon of NO emissions from plant tissues. Wildt et al. (1997) detected NO emission by plant tissues under physiological conditions of plant growth and increased emission of NO under high concentrations of nitrate, herbicides, salicylic acid and other biologically active substances in soils [8].

Chromium compounds are part of enzymes and can accumulate in microorganisms, which influence the emission of carbon dioxide and nitrogen oxides. Therefore, studies of emissions of ground gases require temporarily closed containers [5–13; 15; 19]. The use of microcosm models, in this regard, is advantageous. Microcosms are

small artificial ecosystems which are used for modeling and prognosis of changes of natural ecosystems in the future. The advantages of microcosm models include high reproducibility, clear boundaries and convenience for experimentation [16; 23].

The goal of this study is to model the effect of trivalent and hexavalent Cr compounds on the ecophysiological parameters of the soil-plant system using microcosm models.

MATERIALS AND METHODS

The objects of our investigation were ecophysiological parameters of the soil-plant system in conditions of small artificial ecosystems – microcosms. The type of microcosms “Terra-Aqua column” was used for this aim. These microcosms were made of 5-liter transparent plastic bottles. The Terra-Aqua column consisted of aquatic, terrestrial and air modules. To avoid soil profile lighting, surface-transparent walls of terrestrial modules were covered with black polyethylene bags. The soil was supplied with water by capillary forces using nylon rope with a diameter of 4 mm. Ropes had a length that is equal to the distance from the bottom of the aquatic module to the upper layer of soil. One end the rope was pressed to the walls along the soil profile. For the investigation of soil gaseous emission the peat-sand mix was used. To prevent falling out of soil particles to the water, the layer of gravel layer with 5 mm-height and particle size of 3 mm was stacked around the rope.

Plants of common bean (*Phaseolus vulgaris* L.) were chosen for the investigation of gaseous emission, morphometrical parameters and carbon concentration in dry weight of shoots (aboveground parts). One half of soil remained without plants and was used to study the emission of gasses from the soil due to activity of microorganisms. The second half of the soil was seeded with eight seeds of *P. vulgaris* and was used investigate the emission of gases from the soil-plant system. Seeds were placed into distilled water and were kept for 4 hours at 25 °C before sowing. Then, seeds were clamped horizontally into the soil to a depth that is equal to their thickness. Microcosms were then placed in the growth room with 16-hour of artificial lighting, temperature 23±2 °C, relative humidity 35±3 % and atmospheric pressure 743±5 mm Hg.

On the next day, one aquatic modules of microcosm was injected with a solution containing potassium dichromate $K_2Cr_2O_7$ for modeling the effect of Cr(VI). Another aquatic modules were injected with a solution containing chromium potassium alum $KCr(SO_4)_2 \cdot 12H_2O$ for modeling the influence of Cr(III). Both solutions were injected in equivalent dose of Cr in an amount of 0.5 mg/l. These compounds were chosen because they contain the same amount of potassium. Aquatic modules filled with distilled water were used as controls.

Emission of CO_2 and NO_x was measured in all microcosms after 14 days. In addition, morphometrical parameters of *P. vulgaris*, such stem length, the length and width of first true leaf were measured in microcosms with plants (Fig. 1). The emission intensity of nitrogen oxides was determined photometrically using the Griess-Ilosvay reagent. Carbon dioxide emission (respiration) was detected by Sharkov's method. Both methods were adapted by us for using in bottle microcosm models. Solutions of 1M NaOH in an amount of 5 ml for CO_2 absorption and 8% KI in the amount of 6 ml for NO_x absorption were poured into 5 ml vessels with height and diameter of 5 cm. Both solutions were placed on the soil surface of terrestrial modules. Then terrestrial models were covered with air modules and sealed with adhesive tape. All microcosms were covered with black polyethylene bags to stop photosynthesis (Fig. 2). After 10 hours,

vessels with 0.1 M NaOH solution were titrated with 0.2 M HCl containing phenolphthalein (for determining CO_2 concentration), and for vessels with KI we began preparations for the simultaneous determination concentration of NO_x using the Griess-Ilosvay reagent [17; 19].

After determining the emission of gases in microcosms and morphometric parameters of seedlings, the concentration of organic carbon in aboveground dry weight of *P. vulgaris* was analyzed by the Tyurin method [14].



Fig. 1. Seedlings of *Phaseolus vulgaris* L. in microcosms on 14th day after sowing

Рис. 1. Паростки *Phaseolus vulgaris* L. у мікрокосмах на 14-й день після посіву



Fig. 2. Preparation of microcosms for measuring emission of gases

Рис. 2. Підготовка мікрокосмів для вимірювання емісії газів

The statistical processing of data was performed using of programs MS Excell 2007 and Statst. For testing the significance we calculated *t*-criterion of Student. Correlation was performed using program Statistica Version 6.1. [17]. Results of the correlation analysis are presented by correlation coefficients in the cells of the pair correlations matrix (Table 3) at the intersection of the horizontal and vertical columns with the names of the investigated parameters. For calculating of the correlation coefficients were used general data of parameters all experimental and control options of soil–plant microcosms models (containing soil–plant system).

RESULTS AND DISCUSSION

We found that among morphometric parameters of *P. vulgaris* statistically significant changes undergone just the leaf length which was reduced under the effect of Cr(III). Under effect of Cr(VI) did not find any significant (statistically) changes in morphometric parameters of *P. vulgaris*. (Table 1). Significant decrease of organic carbon concentration in shoots of *P. vulgaris* under effect of Cr(III) was found. Effect of Cr(VI) did not cause any changes of carbon accumulation by shoots of investigated plants (Table 2). Thus for bean Cr(III) was more toxic than Cr(VI), that is considered more toxic by majority of authors [1–3; 21].

The emission intensity of carbon dioxide and nitrogen oxides in the soil microcosms and in the soil–plant microcosms under effect of Cr(III) did not undergone significant changes relative to controls. Instead, Cr(VI) decreased carbon dioxide emission and simultaneously increased the nitrogen oxides emission in soil microcosms and in soil–plant microcosms. The emission intensity of the investigated compounds that are well-known

greenhouse gases was significantly higher in soil microcosm models than in soil-plant analogues (Fig. 3, 4). This is due to the fact that the activity of soil microbocenosis is changed under the plant cover effect [9; 10].

Table 1. Morphometrical parameters of *Phaseolus vulgaris* L. on 14th day after sowing in the microcosms (n = 32)

Таблиця 1. Морфометричні параметри *Phaseolus vulgaris* L. через 14 днів після посіву насіння в мікрокосми (n = 32)

Experimental conditions	Length of stems, cm	Length of leaves, cm	Width of leaves, cm
Control	17.5±0.8	2.75±0.12	2.83±0.14
Chromium (III) 0.5 mg/l	15.2±0.6	2.33±0.09*	2.72±0.11
Chromium (VI) 0.5 mg/l	17.1±0.7	2.67±0.11	2.78±0.12

Comment: Asterisks (*) indicate statistically significant difference with regard to control conditions ($p \leq 0.05$).

Примітка: (*) – наявність статистично достовірної різниці щодо контролю при $P \leq 0,05$.

Table 2. Content of organic carbon in shoots of *P. vulgaris* L. on 14th day after sowing in the microcosms (n = 8)

Таблиця 2. Вміст Карбону в пагонах *P. vulgaris* L. через 14 днів після посіву в мікрокосми (n = 8)

Experimental conditions	Concentration of organic carbon in shoots (g/kg DW)
Control	202±9
Chromium (III) 0.5 mg/l	171±5*
Chromium (VI) 0.5 mg/l	193±7

Comment: Asterisks (*) indicate statistically significant difference with regard to control conditions ($p \leq 0.05$).

Примітка: (*) – наявність статистично достовірної різниці щодо контролю при $P \leq 0,05$.

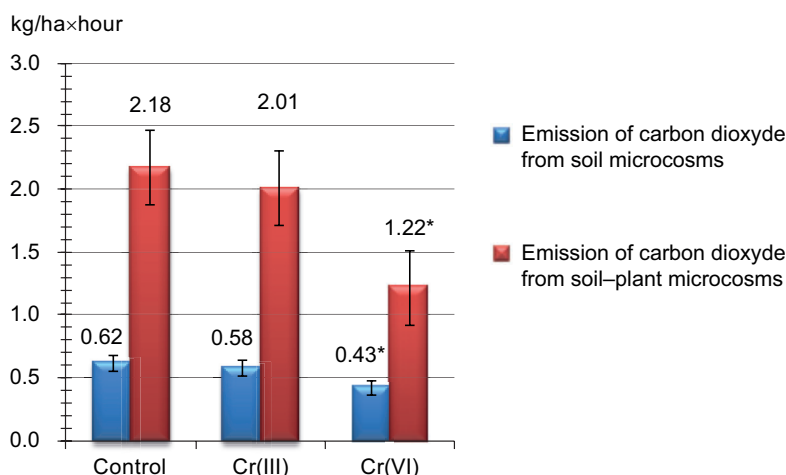


Fig. 3. Changeability of carbon dioxide emission in the darkened microcosms under the effect of Cr(III) and Cr(VI) compounds (n = 8)

Рис. 3. Мінливість емісії діоксиду карбону під впливом сполук Cr(III) і Cr(VI) у затемнених мікрокосмах (n = 8)

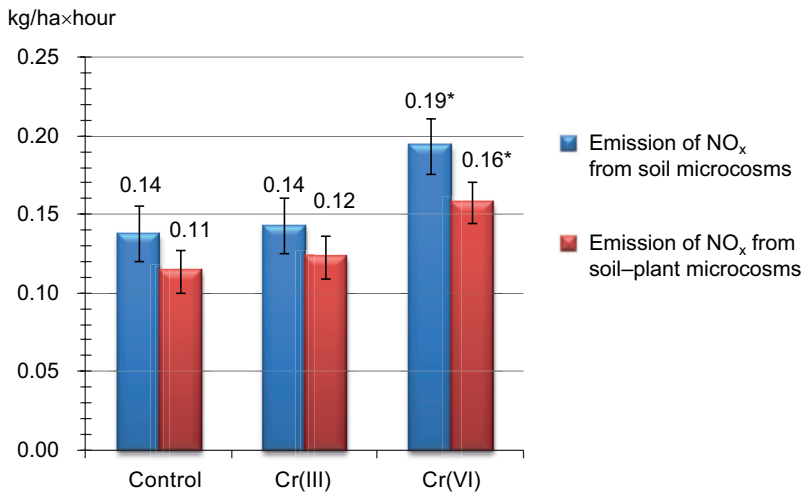


Fig. 4. Changes in nitrogen oxides emission in darkened microcosms under the effect of Cr(III) and Cr(VI) compounds (n = 8)

Рис. 4. Мінливість емісії оксидів нітрогену під впливом сполук Cr(III) та Cr(VI) у затемнених мікрокосмах (n = 8)

Table 3. The correlation coefficients between the ecophysiological parameters of soil-plant microcosms

Таблиця 3. Коефіцієнти кореляції між екофізіологічними параметрами ґрунтово-рослинних мікрокосмів

	Length of stems	Length of leaves	Width of leaves	Emission CO ₂ from soil-plant system	Emission NO _x from soil-plant system	Content of carbon in shoots
Length of stems		0.72*	-0.14	0.27	-0.07	0.74*
Length of leaves	0.72*		0.74*	-0.65*	0.67*	0.81*
Width of leaves	-0.14	0.74*		-0.83*	0.79*	0.23
Emission CO ₂ from soil-plant system	0.27	-0.65*	-0.83*		-0.96*	0.26
Emission NO _x from soil-plant system	-0.07	0.67*	0.79*	-0.96*		-0.14
Content of carbon in shoots	0.74*	0.81*	0.23	0.26	-0.14	

Comment: Asterisks (*) indicate statistically significant correlation ($p \leq 0.05$).

Примітка: (*) – наявність достовірної кореляції при $P \leq 0,05$.

The inverse strong correlation ($r = -0.96$) between carbon dioxide emission and nitrogen oxides emission (Table 3) that was found due to the correlation analysis of the ecophysiological parameters of soil-plant microcosms confirmed our assumption about the inverse relationship between CO_2 and NO_x emissions. In other words when the carbon dioxide emission (respiration) intensity is decreasing we can observe the increasing of nitrogen oxides emission and vice versa. On the basis of above mentioned correlation link we made hypothesis about the compensatory role of NO in the case of decreasing the respiration intensity (CO_2 emission) by the action of stress factors.

CONCLUSIONS

The effect of Cr(III) and Cr(VI) compounds differently change ecophysiological parameters of soil-plant system was established. The effect of Cr(VI) decreased CO_2 emission intensity and simultaneously increased NO_x emission intensity in the soil microcosms and in soil-plant microcosms. Instead, the effect of Cr(III) did not change emission intensity in all experimental conditions. An inverse relationship between CO_2 and NO_x emissions was found. Emission intensity of investigated gases from the soil without plants is greater than from the soil with plant cover. This fact stresses that the soil devoid of plant cover is an additional source of greenhouse gases emission. The effect of Cr(III) reduced leaf length and organic carbon concentration in shoots of *Phaseolus vulgaris* L. Changes in both mentioned parameters showed that the problem of contamination of soil and groundwater with Cr(III) is actual as pollution by toxic Cr(VI) which did not change leaf growth in length and carbon accumulation in shoots of *P. vulgaris*. Thus physical modeling using microcosm "Terra-Aqua column" is an effective method suitable to compare effect of different substances on the soil-plant system.

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МОДЕЛЮВАННЯ ВПЛИВУ ХРОМУ НА ЕКОФІЗІОЛОГІЧНІ ПАРАМЕТРИ СИСТЕМИ ҐРУНТ–РОСЛИНА

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Досліджено екофізіологічні параметри системи ґрунт–рослина під впливом хрому (Cr) різних валентностей в умовах малих штучних екосистем — мікрокосмів. Модельовані ґрунтові води одних мікрокосмів містили Cr(III), а інших — Cr(VI). Вимірювались емісія діоксиду вуглецю (CO₂) й оксидів азоту (NO_x) з ґрунту і системи ґрунт–рослина, морфометрія і вміст карбону в пагонах квасолі звичайної (*Phaseolus vulgaris* L.). Встановлено, що вплив Cr(VI) зменшив інтенсивність емісії CO₂ (дихання) і підвищив інтенсивність емісії NO_x з ґрунтових і ґрунтово-рослинних мікрокосмів, тоді як інтенсивність емісії досліджених газів не зазнала змін за дії Cr(III). За допомогою кореляційного аналізу виявлено зворотний зв'язок між емісіями CO₂ та NO_x. Інтенсивність емісії газів із ґрунту без рослин виявилася більшою, ніж із ґрунту з рослинним покривом. Цей факт ще раз вказує на те, що ґрунт, позбавлений рослинного покриву, є додатковим джерелом емісії парникових газів. Морфометрія і нагромадження вуглецю пагонами *P. vulgaris* не зазнали статистично достовірних змін під впливом Cr(VI), тоді як за дії Cr(III) зменшився ріст листя в довжину і нагромадження карбону пагонами *P. vulgaris*. Зміни обох досліджених параметрів свідчать про актуальність забруднення ґрунту і ґрунтових вод, як Cr(III), так і Cr(VI). Зміни досліджених параметрів свідчать про актуальність проблеми забруднення ґрунту і ґрунтових вод як сполуками Cr(VI), так і сполуками Cr(III).

Ключові слова: забруднення сполуками хрому, екофізіологічні параметри, мікрокосмні моделі, система ґрунт–рослина, *Phaseolus vulgaris* L.

МОДЕЛИРОВАНИЕ ВЛИЯНИЯ ХРОМА НА ЭКОФИЗИОЛОГИЧЕСКИЕ ПАРАМЕТРЫ СИСТЕМЫ ПОЧВА–РАСТЕНИЕ

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Исследованы экофизиологические параметры системы почва–растение при действии хрома (Cr) разных валентностей в условиях небольших искусственных экосистем — микрокосмов. Определялись эмиссия диоксида углерода (CO₂) и оксидов азота (NO_x) из почвы и системы почва–растение, морфометрия и содержание

углерода в побегах фасоли обыкновенной (*Phaseolus vulgaris* L.). Моделированные грунтовые воды одних микрокосмов содержали Cr(III), а других – Cr(VI). Установлено, что влияние Cr(VI) снизило интенсивность эмиссии CO₂ (дыхание) и повысило интенсивность эмиссии NO_x в почвенных и почвенно-растительных микрокосмах, а интенсивность эмиссии исследованных газов не претерпела статистически достоверных изменений под действием Cr(III). С помощью корреляционного анализа была обнаружена обратная связь между эмиссиями CO₂ и NO_x. Интенсивность эмиссии исследованных газов из почвы без растений оказалась выше, чем из почвы с растительным покровом. Этот факт еще раз указывает на то, что почва, лишенная растительного покрова, является дополнительным источником эмиссии парниковых газов. Морфометрия и накопление углерода побегами *P. vulgaris* не претерпели статистически достоверных изменений под действием Cr(VI), тогда как под действием Cr(III) снизились рост листьев в длину и накопление углерода побегами фасоли. Изменения исследованных параметров свидетельствуют об актуальности проблемы загрязнения почвы и грунтовых вод соединениями как Cr(VI), так и Cr(III).

Ключевые слова: загрязнение соединениями хрома, экофизиологические параметры, микрокосмные модели, система почва–растение, *Phaseolus vulgaris* L.

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