Cr AND Ni SIMULTANEOUS PHYTOTOXICITY AND MUTAGENICITY ASSAY

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Abstract: For genotoxicity study simultaneous phytotoxicity and mutagenicity assay with *Vicia sativa* L. var. Klára was used. For phytotoxicity the following rank orders of growth inhibition can be arranged: for roots: Ni(II) > Cr(VI) > Cr(III); for shoots: Ni(II) > Cr(VI) ≥ Cr (III). For mutagenicity assay root tips of *V. sativa* were used and chromosome aberrations were determined at least in 500-anatelophases. All tested metals exerted in *V. sativa* a significant increase of chromosomal aberration rate in applied concentrations. Maximum of aberrations invoked Cr(VI) and the rank order of aberrations fall was: Cr(VI) > Ni(II) > Cr(III). Genotoxic effects of metals were determined by analysis of micronuclei frequency in the pollen tetrads of *Tradescantia* plants. None of tested metal significantly stimulated micronuclei frequency and genotoxic effect was decreased in order: Cr(VI) ≥ Ni(II) > Cr(III).

Key words: chromium, nickel, *Vicia sativa*, phytotoxicity, chromosomal aberation assay, *Tradescantia* micronucleus assay

1. Introduction

Vascular plants have been found to be highly effective for recognizing and predicting metal stress in the environment (growth inhibition, reduction of biomass production, changes in water absorption and translocation) (SHANKER *et al*., 2005; SZÁRAZOVÁ *et al.*, 2008). For genotoxicity studies are plants highly responsible and sensitive. Their beneficial interest is that seeds and pollen grains can be easy storage away and offer cheap, relative easy and accurate toxicological assessment (KRISTEN, 1997). By their ability to accumulate toxic substances, they indicate metal presence in the environment even in very low concentration (CHANDRA, 2004).

Contamination of soil and water by Cr and Ni are of particular recent concern. The impact of Cr contamination on the physiology of plants depends on the metallic species responsible for its mobilization, uptake and toxicity in the plant system (BENNICELLI *et al*., 2004). While Cr is not considered an essential element for plant nutrition (SHARMA *et al*., 1995), Ni is classified an essential trace element (BROWN *et al.,* 1987); and although it is found everywhere in the environment, it usually occurs only in trace amounts.

2. Materials and methods

Simultaneous phytotoxicity and mutagenicity assay was carried out on plant species *Vicia sativa* L. var. Klára according to MIADOKOVÁ *et al*. (2005). After 24 h of soaking at 25 °C in distilled water or solution with metal concentration equal to IC_{50} value the seeds of *V. sativa* were allowed to germinate in Petri dishes (diameter = 18.5

cm) with filter paper soaked with the same concentration of tested metal as that used for soaking. Phytotoxicity was assayed after 72 h of the dark cultivation in the thermostat at 25 °C by the same way as described previously SVETKOVÁ and FARGAŠOVÁ (2007) for *S. alba*. The roots and shoots of *V. sativa* seedlings were measured and the growth inhibition percentage was assessed. The seedling roots used for chromosome and genome mutability evaluation were fixed and permanent slides were prepared by the Feulgen method. Chromosome aberrations were determined at least in 500-anatelophases. For statistic analysis the Student's t-test was used.

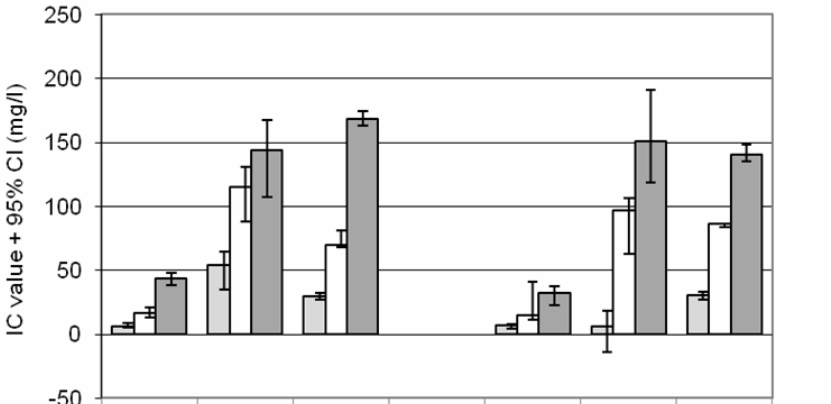
The procedures for maintaining the *Tradescantia* plants and for analyzing micronuclei frequency in the tetrads have been described by MIŠÍK *et al*. (2006; 2007). *Tradescancia paludosa* clone 03 was standardly cultivated at the Department of Botany, Faculty of Natural Sciences, Comenius University in Bratislava. Inflorescence were harvested at the 8-10-buds stage and immersed into 500 ml of tested metal solutions (100 mg/l CrO_3 and $NiCl_2$, 1000 mg/l $Cr(NO_3)_3$) for 12 h. As control tap water was used. The 24 h reconvalescence, during which inflorescence peduncles were dipped in 500 ml of tap water, succeeded to 12 h exposure. Then the buds were fixed for 24 h in ethanol : acetic acid (3 : 1). The fixed material was stored in 70% ethanol. Slides were prepared from the fixed material using the aceto-carmine squash technique. Micronuclei were scored in the early tetrad stages of pollen mother cells. In the present study, 15 to 20 inflorescences comprised a sample. Three hundred tetrads were scored from each of five slides prepared from a treatment sample for a total of 1,500 tetrads per plot. Data were recorded as the number of micronuclei (MCN) per 100 tetrads. A change of frequency of MCN/100 tetrads was considered statistically significant (at $P < 0.05$) if the difference between the mean of the control population and the mean of the treated population was at least twice as large as the standard error of the difference between the two means (MA *et al*., 1994; MIŠÍK *et al*., 2007).

For samples of tested metals $NiCl_2.6H_2O$, $Cr(NO_3)_3.9H_2O$ and $CrCO_3$ of analytical grade p.a. were obtained from Lachema, Brno, Czech Republic.

All experiments for growth inhibition were set up in a completely randomized design with three replicates. Chronic toxicity was assessed as inhibition of roots and shoots growth and results were evaluated by Gryck-Haustein method and IC_{25}, IC_{50} and IC_{75} concentrations were determined. The results were statistically evaluated by using Toxicity program.

3. Results and discussion

Toxic effects of heavy metals, mainly during chronic exposure, are not visible immediately hence ecotoxicological studies request also assessment of genotoxicity. Genotoxicological effect is developed then the concentration is low order than that for fytotoxicity effect (MIČIETA and MURÍN, 1998). For phytotoxicity and clastogenicity study *V. sativa* seedlings were used. Phytotoxicity was determined through IC_{25}, IC_{50} and IC_{75} values and for roots and shoots the strongest inhibitory effect had Ni(II) (Fig. 1). No significant differences were confirmed between Cr(III) and Cr(VI) adverse effects on *V. sativa* shoot growth. On the basis of these values, and their statistical evaluation, metals can be arranged in the following rank orders of inhibition: for roots: Ni(II) > Cr(VI) > Cr(III); for shoots: Ni(II) > Cr(VI) ≥ Cr (III).



	Root			Shoot		
IC25	6.21	53.78	28.94	6.77	6.19	30.58
IC50	16.46	114.41	69.69	14.62	96.49	85.96
IC75	43.61	143.38	167.79	31.59	150.44	140.11

Fig. 1. IC_{25}, IC_{50} and IC_{75} values and their 95% confidence intervals (CI) (ml/l) for *Vicia sativa* L. after 72 h application; the mean of three determinations with a standard deviation 6% or less

For mutagenicity assay root tips of *V. sativa* were used and chromosome aberrations were determined at least in 500-anatelophases. All tested metals exerted in *V. sativa* a significant increase of chromosomal aberration rate in applied concentrations (Table 1). From all tested metals Cr(VI) invoked maximum of aberrations in anatelophase cells. The rank order of aberrations fall was: Cr(VI) > Ni(II) > Cr(III).

Table 1. Potential clastogenicity evaluation of Cr and Ni in *Vicia sativa* L. (n = 500)

Metal	Metal concentration (mg/l)		Number of aberrations ± SD	Percentage of aberrations ± SD
	Ni	Cr		
Control	<0.07	<0.01	7 ± 0.69	2.33 ± 0.23
Ni(II)	16.46 ± 2.16		10 ± 0.75 **	3.33 ± 0.25 **
Cr(III		114.41 ± 13.36	8 ± 0.75 *	2.67 ± 0.25 *
Cr(VI)		69.69 ± 8.66	13 ± 0.69 **	4.33 ± 0.23 **

SD – standard deviation; ** significant differences in comparison with control at P <0.01; * significant differences in comparison with control at P <0.05; control – sterile distilled water

Genetic variation in susceptibility to environmental agents and metals can be considered as differences in metabolism of these agents in various organisms (OMENN, 1991). In addition, DNA target size and DNA content are also important in determining genotoxic hazard of metals. As described KOVALCHUK *et al.* (1998) and CHAUHAN *et al.* (1998) genotoxicity can be obtained as a result of multipolar anaphase and c-mitose or damage of protein synthesis in the presence of DNA

toxicant. Simultaneous toxicity and clastogenity of wastes with Cr and Ni content was also confirmed for *V. sati*va by MIADOKOVÁ *et al*. (1999) and for *V. faba* and *Allium cepa* by Chandra et al. (2004; 2005). Chromozomal fragments and bridges created in Cr(VI) presence indicated as introduced QUIAN (2004) that CrO_3 affecting DNA structure and conformation.

Table 2. Micronuclei frequency in the *Tradescantia* pollen tetrads after treatment with Cr and Ni solutions (n = 1,500)

Metal	Metal concentration (mg/l)		Number of micronuclues ± SD	Percentage of micronucleus ± SD
	Ni	Cr		
Control	<0.07	<0.01	43 ± 13.74	2.89 ± 0.92
Ni(II)	24.71 ± 0.25		59 ± 17.48	3.93 ± 1.17
Cr(III		130.00 ± 1.30	47 ± 16.61	3.13 ± 1.11
Cr(VI)		52.00 ± 0.52	60 ± 15.98	4.00 ± 1.07

SD – standard deviation

For determination of Cr and Ni genotoxic effects was also used analysis of micronuclei frequency in the pollen tetrads of *Tradescantia* plants. As it is evident from Table 2 none of tested metal significantly stimulated, in comparison with the control, micronuclei frequency and genotoxic effect was decreased in order: Cr(VI) ≥ Ni(II) > Cr(III). *Tradescantia* micronucleus test (Trad-MCN) belongs together with *Allium cepa* L. and *Vicia faba* L. tests with root tips to most frequently used genotoxicity tests on plants (MAJER *et al.*, 2005) and it is very popular now for *in situ* biomonitoring of air pollution (MIŠÍK *et al*., 2006; 2007). Results obtained during our genotoxicity tests are in good agreement with those introduced by KNASMÜLLER *et al.* (1998) when CrO_3, $CrCl_3$ and $NiCl_2$ up to concentration 10 mM did not evoke genotoxic effects. The same conclusion also introduced MAJER *et al*. (2005) for Cr(III). Higher genotoxicity of Cr(VI) than Cr(III) determined during our experiments also described NĚMEČEK *et al*. (2002). ROSSMAN (1995) introduced that molecular mechanism of DNA damage by Cr(VI) involve induction of DNA-DNA and DNA-protein cross-links and genotoxic effect can be also increased by reactive oxygen produced during intracellular reduction. For Ni(II) no genotoxic effects confirmed for bacteria ROSSMAN (1995) and PATIERNO and COSTA (1987) introduced that mutations after Ni applications are also the result of DNA damage and DNA-protein cross-links formation.

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