Water Air Soil Pollut (2014) 225:1933
DOI 10.1007/s11270-014-1933-x

Assessment of the Cytogenetic Damage Induced by Chromium Short-Term Exposure in Root Tip Meristems of Barley Seedlings

Elena Truta · Cosmin Mihai · Daniela Gherghel · Gabriela Vochita

Received: 30 October 2013 / Accepted: 10 March 2014 / Published online: 26 March 2014

Abstract Heavy metals are determinant factors in increasing environmental pollution, and chromium is considered to be of highest concern because of its genotoxicity in microorganisms, animals, and humans. Relatively few studies are focused on the injury induced in plant genetic material. Therefore, the main objective of this work was to evaluate the extent of the cytogenetic damage induced in root meristems of barley (*Hordeum vulgare* L.) after short-term seed exposure to 10, 100, 250, and 500 μM $K_2Cr_2O_7$ (Cr(VI) concentration is 1.04, 10.39, 25.99, and 51.99 μg ml^{-1}) and 10, 100, 250, and 500 μM $CrCl_3$ (Cr(III) concentration is 0.52, 5.19, 12.99, and 25.99 μg ml^{-1}). Chromium genotoxic potential was proved by significant increases in the rates of the ana-telophase chromosomal aberrations (1.3–2.3 times higher for $K_2Cr_2O_7$ and 1.7–2.2 times higher for $CrCl_3$, as compared to the control; $p<0.05$, $p<0.01$) and of metaphase disturbances (5.0–7.5 times more numerous in chromium-treated groups than in control; $p<0.001$). The pattern of the chromosomal aberrations is constituted by chromatid bridges, complex aberrations, lagging, and vagrant chromosomes, while the abnormal metaphases are c-like metaphases, sticky metaphases, and metaphases with chromosomes expulsed from equatorial plate. The mitotic indices and the growth of the barley plantlets in the early ontogeny were stimulated by chromium. The changes induced in the frequency of division stages mainly consisted in prophase and telophase accumulation and diminution of metaphase and anaphase proportion.

Keywords Aneugenic effects · Chromosome aberrations · Clastogenic action · Genotoxicity · Heavy metals

E. Truta (✉) · D. Gherghel · G. Vochita
Department of Experimental and Applied Biology, NIRDBS, Institute of Biological Research,
47 Lascar Catargi Street, 700107 Iasi, Romania
e-mail: trutaelena@yahoo.com

C. Mihai
Interdisciplinary Research Department, Field Sciences, "Al. I. Cuza" University,
54 Lascar Catargi Street, 700107 Iasi, Romania

1 Introduction

Environmental pollution by heavy metals underwent a marked increase in the last decades due to their continuous release and accumulation in the terrestrial and aquatic ecosystems. Such metals can accumulate in the plants growing on contaminated soils; they then enter the food chain through consumption of plant material and finally affect the health of animal and human systems. The heavy metals are toxic even at low doses because they are not biodegradable (Migid et al. 2007). Arsenic, cadmium, cobalt, chromium, copper, mercury, manganese, nickel, lead, tin, and thallium are considered by the European Union to be of the highest concern (Santos and Rodriguez-Gomez 2012).

Chromium, widely present in environment and in various products or compounds with which people come in contact (photographic films, leather in shoes, toner in photocopiers, cosmetics, vitamins), became a serious environmental pollutant. It is one of the most abundant elements in the Earth's crust (100–300 μg g^{-1}) (Cervantes et al. 2001; Castro et al. 2007), but the principal causes of the increasing chromium pollution are the specific activities developed in certain industrial and manufacturing sectors (metallurgy, electroplating, production of chromium-pigment-based paints and of fungicides, leather tanning, wood preservation, chemical industry, paper production) in which chromium compounds are used (Cervantes et al. 2001; Ali et al. 2004; Ozdener et al. 2011).

In normal soils, chromium ranges from 5 to 3,000 μg g^{-1} (Skeffington et al. 1976) or 4,000 μg g^{-1} (Dayan and Paine 2001), but extremely contaminated surface soils were found in proximity of chromium smelter heaps where the metal concentration exceeded 10,000 μg g^{-1} (Kabata-Pendias and Pendias 2001). Its presence in agricultural soils can be attributed to the use of organic wastes as fertilizers and of waste waters for irrigation or to the high chromium amount included in some phosphate fertilizers (Kabata-Pendias and Pendias 2001; Oliveira 2012). In Romania, the chromium amount in podzols and sandy soils is 12–86 μg g^{-1} dry weight, in loamy and clay soils is 19–73 μg g^{-1} dry weight, whereas kastanozems and brown soils contain 15–67 μg g^{-1} dry weight (Rauta et al. 1987). As concerns the atmospheric heavy metal deposition, Romania had the highest concentration of cadmium, chromium, and zinc among eastern European countries which participate in biomonitoring studies by moss surveys (Blum 2007). Despite the fact that the exploitation of the rich sources of heavy metals has been reduced or abandoned, the level of these elements in Romanian soils is still high and the risk for population health via consumption of the vegetables grown in old mining areas is proven by several studies (Harmanescu et al. 2011).

A number of international guidelines establish the maximum accepted levels for heavy metals in soils and agricultural purpose waters. For example, the regulatory limits on heavy metals applied to soils stipulate a maximum concentration of chromium in sludge of 3,000 mg kg^{-1} (USDA-NRCS 2000) but, despite the specific measures carried out in many countries, it seems impossible to completely stop the environmental chromium accumulation which in excess affects the living systems by inducing the chromosome damage in plants and increasing the carcinogenic risk in humans (Oliveira 2012).

Chromium is an essential element for humans and animals, but not for plants and microorganisms (Cervantes et al. 2001; Singh et al. 2013) although it was proved that at low concentrations (0.05–1.00 mg l^{-1}), this heavy metal promoted plant growth (Zou et al. 2006; Oliveira 2012). Although chromium exists in several oxidation states, the most stable and common are the trivalent and hexavalent forms which have very different chemical and hence biological properties (Dayan and Paine 2001). The hexavalent chromium is several times more toxic than the less mobile trivalent form (Cervantes et al. 2001; Jamal et al. 2006; Pohren et al. 2013). The presence of hexavalent chromium over safe levels in edible plants represents a potential hazard to animals and humans (Oliveira 2012), existing sufficient evidence in humans which allowed its introduction in the group I of carcinogens (IARC 1990). Concentrations of hexavalent chromium above 30 mg kg^{-1} and of trivalent chromium above 10,000 mg kg^{-1} may cause adverse health effects in humans (USEPA 2006).

Chromium was genotoxic in some prokaryotes (Petrilli and De Flora 1977), lower eukaryotes such as fission yeast (Papp et al. 2011), insects (Michailova et al. 2001), fish species (Yadav and Trivedi 2006; Normann et al. 2008; Velma and Tchounwou 2010), rodents (Seoane and Dulout 1999; Balansky et al. 2000), and humans, where especially hexavalent chromium compounds have mutagenic and carcinogenic properties (Benova et al. 2010; Maeng et al. 2004; Patlolla et al. 2009; Halasova et al. 2012). There are also many studies on negative effects determined by chromium in plant metabolism (Cervantes et al. 2001; Ali et al. 2004; Shanker et al. 2005; Jamal et al. 2006). The stress induced by hexavalent chromium in plants such as rice and maize is analyzed inclusively at transcriptomic, proteomic, and metabolomic levels (Dubey et al. 2010; Wang et al. 2013) but, unlike other heavy metals, relatively few information is available on chromium-induced effects on plant genetic material (Villalobos-Pietrini et al. 1986; Rodriguez-Gomez 2011; Srivastava and Jain 2011; Mishra et al. 2012). The reported range of phytotoxic concentrations is variable: 18–24 μg g^{-1} dry weight in tobacco, 4–8 μg g^{-1} dry weight in corn, 10 μg g^{-1} dry weight in barley, 10–

100 μg g^{-1} dry weight in rice, and 1–2 μg g^{-1} dry weight in some sensitive plants (Kabata-Pendias and Pendias 2001). According to Akinci and Akinci (2010), the critical level of chromium in soil solutions ranges between 1 and 5 μg g^{-1} dry weight for many plants, whereas Sinha et al. (2005) noted a larger interval of chromium concentrations before appearance of toxicity symptoms in plant tissues, depending on species, from about 5 μg g^{-1} dry weight (barley, corn, oats, citrus) to 175 μg g^{-1} dry weight (tobacco).

Sometimes, even at values significantly lower than the maximum levels accepted by the legislation of some countries, chromium induced genotoxic effects in several plant and animal systems (Depault et al. 2006; Matsumoto et al. 2006; Yadav and Trivedi 2006; El-Yamani et al. 2011). Even trivalent chromium, although less toxic, can produce serious injuries to living tissues—in some cases it has more toxic effects than hexavalent chromium, but at higher concentrations than this (Cervantes et al. 2001; Gardea-Torresdey et al. 2005). It is constitutive part in some nutritional supplements for humans and animals, but several researchers have recently raised the question of the long-term use risk of these trivalent chromium-containing supplements because it was proved that they exert genotoxic action under certain conditions (Eastmond et al. 2008).

In view of these, it is necessary to extend the evaluation of chromium genotoxicity on a larger spectrum of plant species. In this study, barley (*Hordeum vulgare* L.) was selected as experimental biological material not only because of its great economical, edible, medicinal, and pharmacological importance, but also because it has a chromosome set constituted by a small number of large chromosomes ($2n=14$) suitable for the analysis of the damage amplitude in the genetic material, expressed in chromosome aberrations and disturbances of the mitotic cycle.

The main objective of this study is to evaluate and to provide new data on the genotoxic potential of chromium, provided as $K_2Cr_2O_7$ and $CrCl_3$, by quantifying the rates of the ana-telophase chromosome aberrations and of metaphase disturbances induced in root tips of barley seedlings after short-term seed exposure to chromium and by analyzing the chromium-induced effects on mitotic index and on the frequency of division stages. A second objective of this paper was to analyze the influence of chromium on length growth in the early ontogeny of barley plantlets.

2 Materials and Methods

2.1 Plant Material and Treatment Conditions

Seeds of *Hordeum vulgare* L. cv. Madalin (Center for Agricultural Research and Development Secuieni—Neamt, Romania) were utilized. Chromium was provided as potassium dichromate [Cr(VI)], $K_2Cr_2O_7$ (CAS registry number 7778-50-9, molecular weight 294.185 g/mol) and chromium chloride [Cr(III)], $CrCl_3.6H_2O$ (Merck, Darmstadt; CAS registry number 10060-12-5, molecular weight 266.45 g/mol). Barley seeds were immersed for 3 h in each of the aqueous solutions of 10, 100, 250, and 500 μM $K_2Cr_2O_7$, corresponding to a chromium concentration of 1.04, 10.39, 25.99, and 51.99 μg ml^{-1} and in each of the solutions of 10, 100, 250, and 500 μM $CrCl_3.6H_2O$, corresponding to a chromium concentration of 0.52, 5.19, 12.99, and 25.99 μg ml^{-1}. Controls were prepared in distilled water. After chromium treatment and several rinses with running tap water, the seeds (50 per variant) were placed on moistened filter paper, in covered glass Petri dishes, and incubated in the dark, at 20 °C, until germination took place. The germinated seeds were then maintained at a photoperiod of 16 h/8 h (light/dark), at 23±1 °C, in order to determine the growth in length of the barley plantlets in early ontogeny.

2.2 Preparation and Analysis of Slides

For the cytogenetic examination, barley roots (15–20 mm in length) were fixed for 24 h in alcohol/acetic acid (3:1, *v*/*v*), at room temperature, then they were washed, transferred to 70 % ethyl alcohol, and stored at 4 °C. The plant material was hydrolyzed for 20 min in 37 % HCl/distilled water (1:1, *v*/*v*), and then stained in modified charbol-fuchsin solution (Gamborg and Wetter 1975). Three rootlets/germinated seed were mounted on a slide and squashed in a drop of 45 % acid acetic, under a coverslip. Five slides/variant and 10 fields/slide were analyzed using Novex light microscope to score the cell cycle phases (interphases, prophases, metaphases, anaphases, and telophases) and the chromosome aberrations. The best preparations were photographed using Nikon Eclipse 600 microscope, with Nikon Cool Pix 950 digital camera (1,600×1,200 dpi) at ×100 objective.

2.3 Cytogenetic Parameters

The mitotic index (MI%) and the frequencies of the division stages (prophase index (PI%), metaphase index (MeI%), anaphase index (AI%), telophase index (TI%)) were calculated according to Eqs. 1 and 2, respectively:

$$\mathrm{MI}(\%) = \frac{\text{total dividing cells}}{\text{total analyzed cells}} \times 100 \quad (1)$$

$$\text{Phase index}(\%) = \frac{\text{cells in respective mitotic phase}}{\text{total dividing cells}} \times 100 \quad (2)$$

To calculate the frequency of the ana-telophase chromosomal aberrations ($CA_{A\text{-}T}$%) and the rate of the metaphase disturbances (M_{abn}%), the Eqs. 3 and 4 were used:

$$CA_{A\text{-}T}(\%) = \frac{\text{chromosomal aberrations in ana-telophase}}{\text{total dividing cells}} \times 100 \quad (3)$$

$$M_{abn}(\%) = \frac{\text{total abnormalities in metaphases}}{\text{total dividing cells}} \times 100 \quad (4)$$

The quantitative results are based on at least 6,500 cells counted in each experimental variant, including controls.

2.4 Statistical Analysis

The results are expressed as the mean values and standard error of the means (± SE) for all groups of investigated parameters. The estimation of the increase/decrease (±) rate was based on Eq. 5:

$$\pm\text{rate}(\%) = \left(1 - \frac{x}{y}\right) \times 100, \quad (5)$$

where y is the average value detected in the control and x is one of each treated samples (Liu et al. 2005). Statistical analysis was carried out according to Student's t test for each parameter in order to establish the differences between the treated variants and the controls. The differences were considered as significant at levels of $*p<0.05$, $**p<0.01$, and $***p<0.001$. The Microsoft Office Excel 2003 software of Windows XP operating system was used to calculate and to graphically represent the statistical parameters.

3 Results and Discussion

3.1 Chromium Influence on Growth of Young Barley Plantlets

Both $K_2Cr_2O_7$ and $CrCl_3$ generally promoted the growth of barley seedlings in the early ontogeny (Fig. 1). Statistical analysis revealed that the stimulative action of the two chromium-containing compounds on shoot length was significantly higher in younger plantlets ($p<0.001$ for 100 and 500 μM $K_2Cr_2O_7$; $p<0.01$ in the variants treated with 10, 100, and 500 μM $CrCl_3$). In older plantlets, only $K_2Cr_2O_7$ at 500 μM concentration determined significant differences as compared to the untreated control ($p<0.05$). In 9-day-old plantlets, chromium at 250 μM concentration inhibited the growth for both compounds, but not in a significant manner (inhibition rate was −3.79 %, for $K_2Cr_2O_7$, and −2.18 %, for $CrCl_3$, respectively).

In literature, both inhibitory and stimulatory effects of chromium on plant growth have been reported, depending on experimental coordinates. Low chromium concentrations (0.05–1.00 mg L^{-1}) promoted plant growth in bean (Bonet et al. 1991), maize (Mengel and Kirkby 2001), and green amaranth (Zou et al. 2006), whereas other works evidenced inhibitive effect of the heavy metal to wheat growth (Jamal et al 2006).

Jun et al. (2009) noted the differentiated behavior of the plant growth to chromium exposure depending on species. At levels significantly higher than those tested in our experiment (above 145 mg kg^{-1} Cr), chromium suppressed the growth of pea plantlets (Bishnoi et al. 1993), while Castro et al. (2007) observed the stimulation of *Arabidopsis thaliana* seedling growth at concentrations of chromium lower than 100 μM and the inhibition of growth at levels of hexavalent chromium higher than 200 μM correlated to reductions in mitotic index.

3.2 Chromium Influence on Division Indices

Chromium also exerted stimulative action on cell division. Both chromium-containing compounds induced increases in mitotic index (MI%) in all treated variants (Table 1, Fig. 2). The stimulatory rate ranged between 20.36 and 42.52 % in the variants exposed to $K_2Cr_2O_7$ and from 33.24 to 36.85 % in the variants treated with $CrCl_3$. There were significant differences between MI% of the control and treatment groups: $p<0.05$, for

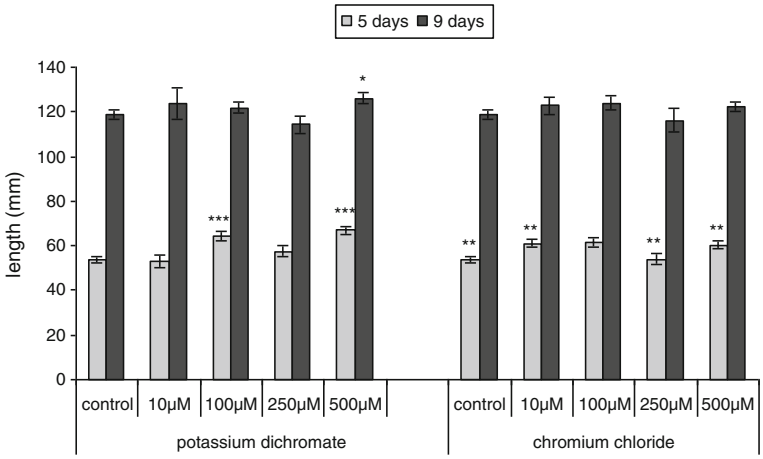

Fig. 1 Growth of the barley plantlets in early ontogeny, after seed exposure to different concentrations of $K_2Cr_2O_7$ and $CrCl_3$. *Vertical bars* represent the standard errors of the means. *Asterisks* indicate the levels of significance in the differences between the control and the treatments: *$p<0.05$, **$p<0.01$, and ***$p<0.001$

100 μM $K_2Cr_2O_7$ and $p<0.01$, for the other treatment groups, except for 500 μM.

The highest tested concentration induced a nonsignificant increase in the frequency of dividing cells (4.67 ±0.44 %, for $K_2Cr_2O_7$, and 4.12±0.20, for $CrCl_3$, as compared to the control, 3.88±0.18 %). The results did not show a linear dose–response relationship.

Chromium influence on the frequency of division stages mainly consisted of prophase and telophase accumulation and diminution of metaphase and anaphase proportion in almost all chromium-treated variants (Table 1). $CrCl_3$ induced significant increase ($p<0.05$) of prophases at concentration of 250 μm (39.51±2.37 % as compared to 29.27±2.28 %, for the control), while 10 μm $K_2Cr_2O_7$ significantly lowered the anaphase frequency ($p<0.05$), in this case, the decrease rate being −38.5 % as compared to the control.

A variable profile of MI% induced by chromium treatment was reported in literature, depending on tested compound, plant species, heavy metal concentration, oxidation state, and exposure duration. So, in green amaranth, at the maximum tested concentration (10^{-3} M), hexavalent chromium determined a marked decline of MI% as compared to the control (Zou et al. 2006), whereas in *Vicia faba*, increases were observed in MI% after the treatment with $K_2Cr_2O_7$ (Qian 2004). In this species, other authors (Mishra et al. 2012) established that the exposure to chromium trioxide resulted in values significantly lower than the control in longer treatments (6, 12 h), but it was stimulative in short-term treatments (4 h).

In onion root meristems, chromium nitrate lowered MI% and modified the proportion of division phases (Liu et al. 1992; Glinska et al. 2007). Concerning the frequency of division stages consisting in prophase and telophase accumulation, our results are in agreement with those reported in blackgram (Chidambaram et al. 2009) and sugarcane (Mishra et al. 2012).

3.3 Chromium-Induced Disturbances in Mitotic Ana-Telophases and Metaphases

As Fig. 3 shows, the profile of the rates of total chromosome aberrations ($CA_{A\text{-}T}$%) induced by chromium in ana-telophases of root meristems of barley seedlings is relatively similar for $K_2Cr_2O_7$ and $CrCl_3$, namely, $CA_{A\text{-}T}$% registered an ascendant trend from 10 to 250 μM, followed by a decline at the maximum tested concentration, although the level still maintained over the average control values at 500 μM (Fig. 3). Significant differences in the percentage of chromosomal aberrations were observed in chromium-treated variants as compared to the control average value (6.83±1.25 %).

The exposure to $K_2Cr_2O_7$ induced increments by 1.3–2.3 times in $CA_{A\text{-}T}$%—the differences between treatment groups and control were found significant ($p<0.01$, $p<0.05$) at concentrations of 100, 250, and 500 μM. In the variants treated with $CrCl_3$, $CA_{A\text{-}T}$% average values surpassed 1.7–2.2 times the control. This increase was significant at 100 and 250 μM ($p<0.05$).

The chromosome bridges involving one or more chromosomes (Fig. 4c, d) represented the prevalent category among ana-telophase aberrations, for both chromium-containing compounds. Their frequency in the treated groups was 1.4–2.1 times higher than control. The complex aberrations such as bridges+lagging

Table 1 Behavior of the genetic indices in barley root meristems after seed exposure to different concentrations of $K_2Cr_2O_7$ and $CrCl_3$

Variant	Concentration (μM)	Total analyzed cells	MI% $\bar{x}\pm$ SE	Indices of mitotic phases $\bar{x}\pm$ SE			
				PI%	MeI%	AI%	TI%
Control	0	9,694	3.88±0.18	29.27±2.28	27.42±3.06	20.84±1.97	22.44±2.80
$K_2Cr_2O_7$	10	8,554	5.31±0.40**	32.26±3.52	26.50±2.60	12.81±1.34*	28.41±1.29
	100	8,000	4.74±0.23*	30.27±3.13	22.85±3.67	17.40±0.76	29.48±1.83
	250	7,024	5.53±0.33**	33.42±2.63	25.01±0.98	21.16±2.28	20.41±2.19
	500	7,864	4.67±0.44	35.90±4.30	21.50±3.57	17.75±3.26	24.83±2.81
$CrCl_3$	10	7,798	5.29±0.30**	31.17±1.29	26.40±1.38	16.40±1.16	26.03±2.95
	100	6,701	5.17±0.34**	34.95±1.47	27.38±2.90	16.09±1.36	21.57±1.71
	250	6,603	5.31±0.31**	39.51±2.37*	22.56±1.65	20.35±2.17	17.58±1.25
	500	7,759	4.12±0.20	34.72±1.92	20.74±2.10	16.43±1.87	28.11±0.60

$\bar{x}\pm$ SE represents means±standard errors of the means

*$p<0.05$, significant differences from the control; **$p<0.01$, significant differences from the control

chromosomes (Fig. 4g), multipolarity+bridges+vagrant chromosomes (Fig. 4h), etc. occupy the second place as frequency, followed by lagging chromosomes (Fig. 4e), registered in higher percentage at the concentrations of 100 and 250 μM, for both compounds. In addition to these aberrations, vagrant chromosomes and multipolar ana-telophases (Fig. 4f) were observed, but in smaller proportion.

As concerns the micronuclei (Fig. 5), they were sporadically observed in this study and not in all chromium-treated variants. Their presence was noted in 500 μM $K_2Cr_2O_7$ (Fig. 5b) and in 250 μM $CrCl_3$ (Fig. 5c).

They can result from an aneugenic (mitotic spindle malfunctioning) or a clastogenic event (chromosome breakage) induced by the stressor (Medeiros et al. 2003; Attia 2011). Unlike our results, several authors reported substantially increased frequencies of micronuclei after chromium exposure in many plant species—*Tradescantia* (Villalobos-Pietrini et al. 1986), *V. faba* (Qian 2004; Zhang et al. 2009), and blackgram (Chidambaram et al. 2009) as well as in animals (Balansky et al. 2000). In onion root meristems, Eleftheriou et al. (2012) observed that micronuclei frequency was higher after long treatments with chromium (48, 72 h), not in short exposure as in our work.

Both $K_2Cr_2O_7$ and $CrCl_3$ increased significantly the frequency of metaphase disturbances (M_{abn}%) in barley root meristems at all tested concentrations (Fig. 3). Their percentage was by ~5.0–7.5 times higher than in

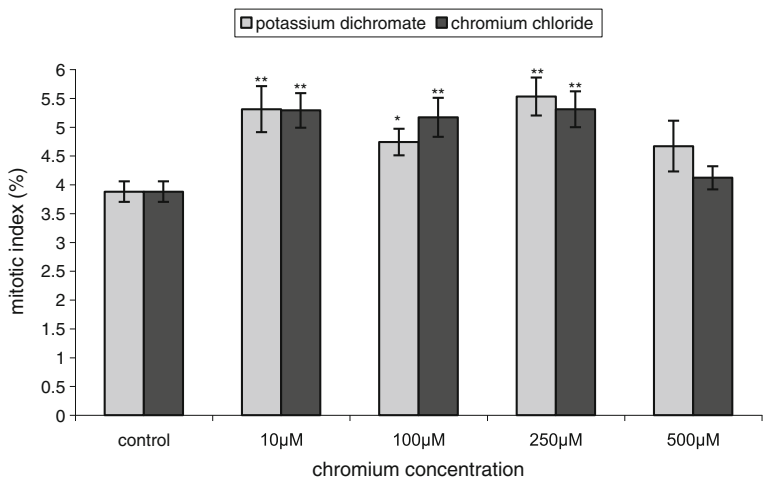

Fig. 2 Evolution of MI% in barley root meristems after exposure to chromium. *Vertical bars* represent the standard errors of the means. *Asterisks* indicate the levels of significance in the differences between the control and the treatments: *$p<0.05$ and **$p<0.01$

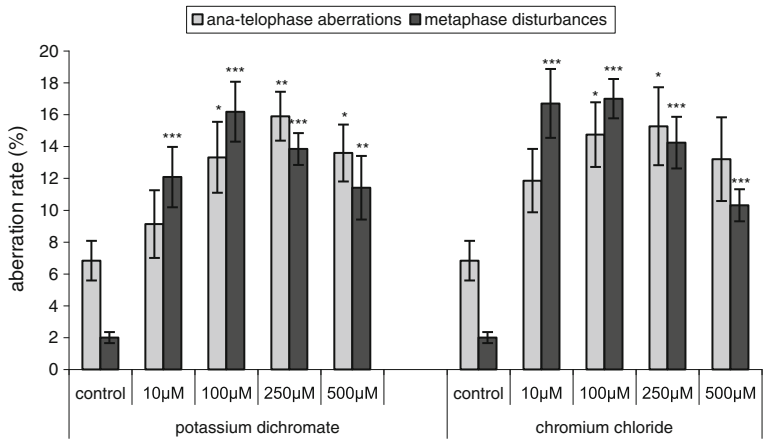

Fig. 3 Frequency of the ana-telophase chromosome aberrations and metaphase disturbances in barley root meristems after seed short-term exposure to different concentrations of $K_2Cr_2O_7$ and $CrCl_3$. *Vertical bars* represent the standard errors of the means. *Asterisks* indicate the levels of significance in the differences between the control and the treatments: *$p<0.05$, **$p<0.01$, and ***$p<0.001$

control. So, the difference was $p<0.05$ in 500 μM $K_2Cr_2O_7$, but $p<0.001$ in the other treatments groups, as compared to the control (2.00±0.35 %). The main categories of abnormal metaphases were c-like metaphases (Fig. 6b) which surpassed the control value in all chromium-treated variants. The most numerous were present in 100 and 250 μM $CrCl_3$ (8.63 and 6.84 %, respectively) and in 250 μM $K_2Cr_2O_7$ (5.64 %), as compared to the control (0.70 %). The occurrence of this type of configurations indicates an effect similar to that colchicine-induced, which consists in thick and shorter chromosomes blocked at metaphase and scattered throughout the cell as result of the damaged mitotic spindle. This action, proved for other heavy metals such as lead or cadmium (Wierzbicka 1999; Liu et al. 2003), was also previously confirmed for chromium in other works (Liu et al. 1992; Glinska et al. 2007).

A substantial increase in the number of metaphases with sticky chromosomes (Fig. 6c) was observed in all chromium-treated variants. Stickiness reflects the high toxicity of the tested compound on genetic material and can be attributed to the action of the heavy metal on chromosome DNA with occurrence of depolymerizations, condensation, or entanglement of interchromosomal chromatin fibers and subsequent formation of subchromatid connections between chromosomes (Yildiz et al. 2009). Metaphases with chromosomes expulsed from equatorial plate (Fig. 6d) were present in lesser extent than c-metaphases and stickiness.

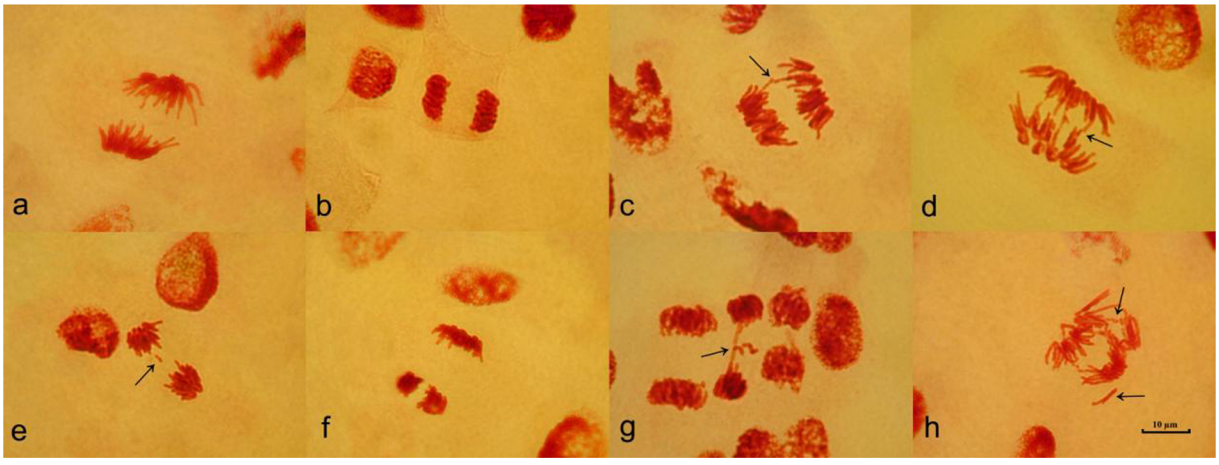

Fig. 4 Normal and aberrant ana-telophases in barley root meristems after chromium treatment. **a** Normal anaphase. **b** Normal telophase. **c** Anaphase with one chromatid bridge, 100 μM $CrCl_3$. **d** Anaphase with multiple chromatid bridges, 10 μM $CrCl_3$. **e** Anaphase with lagging chromosome, 250 μM $K_2Cr_2O_7$. **f** Tripolar telophase, 250 μM $CrCl_3$. **g**, **h** Complex aberrations (**g** telophase with chromatid bridge and lagging chromosomes, 250 μM $K_2Cr_2O_7$; **h** tripolar anaphase with chromatid bridges and vagrants, 250 μM $CrCl_3$). *Scale bar*=10 μm

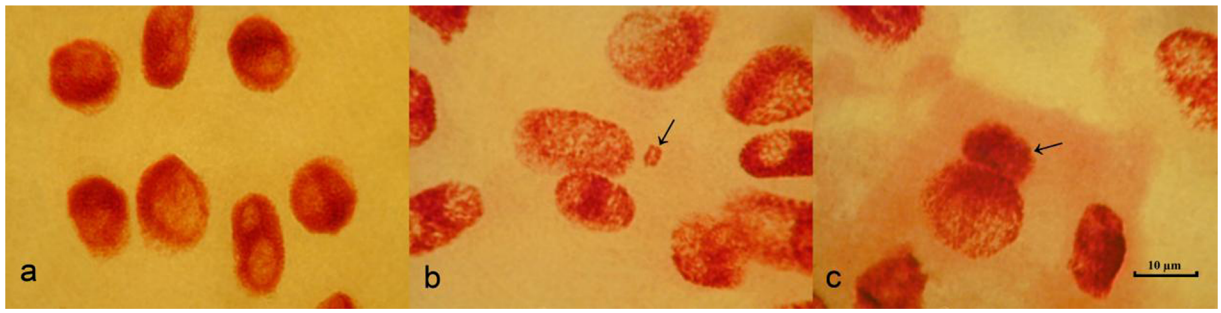

Fig. 5 Micronuclei in barley root meristems after chromium treatments. **a** Normal interphases. **b** Small micronucleus, 500 µM $K_2Cr_2O_7$. **c** Large micronucleus, 250 µM $CrCl_3$. *Scale bar*=10 µm

Concerning the dose–effect relationship, the results herein did not show a linear relation between chromium concentration and the aberration rates. The data reported in literature on the amplitude of cytogenetic damage induced in plants depending on chromium concentration are variable. In some short-term treatments (1–3 h), no relation was found between the chromium trioxide concentration and proportion of the chromosome aberrations recorded in *V. faba* root meristems (Gomez-Arroyo et al. 1987), whereas in blackgram and sugarcane, the percentage of chromosome abnormalities increased with the increase of chromium concentration (Chidambaram et al. 2009; Srivastava and Jain 2011). In other research using *Vicia* bioassay, the occurrence of different types of chromosomal abnormalities observed after chromium trioxide treatment did not show any consistent pattern (Mishra et al. 2012). In literature, numerous situations of nonmonotonic and nonlinear relationships between dose and the analyzed endpoints have been in fact reported, especially at lower concentrations of a chemical stressor, and this fact must not be ignored (Vandenberg et al. 2012), although it is still looking for plausible explanations.

The high number of the chromosomal aberrations identified in chromium-treated variants could be the consequence of the heavy metal immobilization and accumulation in barley roots (Ali et al. 2004), as it was observed in many other plant species (Cervantes et al. 2001; Jamal et al. 2006; Scoccianti et al. 2006; Santos and Rodriguez-Gomez 2012). According to Zayed and Terry (2003), >99 % of the absorbed chromium is retained in the roots, a low translocation from root to plant aerial parts being evidenced (Singh et al. 2013). Rodriguez-Gomez (2011) considers that the damage induced by chromium on genetic material in root cells is larger than that observed in leaf cells because of the direct contact between the root, which acts as a barrier against chromium translocation, and the heavy metal. In our study, the genotoxic action of chromium is supported by the high levels of $CA_{A\text{-}T}$% and M_{abn}%. The potential of chromium, either trivalent or hexavalent, to produce lesions in genetic material was evidenced in many plant species, but the results existing in literature are sometimes conflicting, depending on plant species, tested chromium compound, concentration range, duration of exposure, etc. (Gomez-Arroyo et al. 1987; Liu et al. 1992; Matsumoto et al. 2006; Zou et al. 2006; Glinska et al. 2007; Chidambaram et al. 2009; Srivastava and Jain 2011; Mishra et al. 2012).

The mechanisms of the genotoxicity and mutagenicity induced by chromium are not yet entirely known (Matsumoto et al. 2006; Rodriguez-Gomez 2011; Santos and Rodriguez-Gomez 2012), but most of the scientists sustain that the formation of reactive intermediates and generation of oxidative stress is the main mechanism

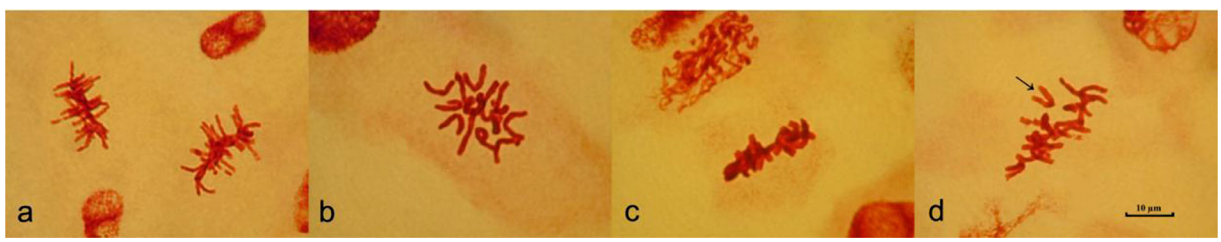

Fig. 6 Metaphases in barley root meristems after chromium treatment. **a** Normal metaphases. **b** C-metaphase, 100 µM $CrCl_3$. **c** Sticky metaphase, 100 µM $K_2Cr_2O_7$. **d** Metaphase with chromosome expulsed from equatorial plate, 100 µM $K_2Cr_2O_7$. *Scale bar*=10 µm

involved in DNA damage (Medeiros et al. 2003; Patlolla et al. 2009; Labra et al. 2010; Wang et al. 2013) and in occurrence of chromosomal aberrations (Shanker et al. 2005; Velma and Tchounwou 2010; Halasova et al. 2012). Changes in the activity of enzymes for antioxidative protection (superoxide dismutase, catalase, and peroxidase) under chromium stress have also been observed in barley seedlings in relation to heavy metal concentration and plantlet age (Olteanu et al. 2012). Therefore, the chromosome aberrations can be the result of either clastogenic or aneugenic action of chromium (Pohren et al. 2013). The clastogenic action implies chromosome structural damage during division, while the aneugenic effects are due to the noxious action on the fibers of cell division spindle, with repercussions in its assembly and functioning, or on the centromere structure, so disturbing the normal migration of the chromosomes to the cell poles (Parry et al. 2002; Attia 2011). Recently, Eleftheriou et al. (2012) established that the chromium-induced chromosome aberrations in *Allium cepa* L. were in relation with the changes in the organization of cytoskeleton microtubules (considered as main subcellular target for chromium) during mitotic division.

Some recent works, focused on chromium-induced DNA damage, provided supplementary information, but without elucidate the intimate mechanisms of this process. Two synthetic studies (Nickens et al. 2010; Tabrez et al. 2014) conclude that DNA adducts, DNA strand breaks, DNA-protein crosslinks, oxidized bases, abasic sites, and DNA inter and intrastrand crosslinks are the most abundant genetic lesions produced by Cr(VI), these modifications being considered to be primary causes of genotoxicity and mutagenicity of hexavalent chromium. Also, Khorsandi and Rabbani-Chadegani (2013) state that chromium oxide genotoxicity arises from DNA structural and conformational changes caused by chromium binding to both phosphate groups and base pairs of nuclear DNA. These modifications have negative repercussions on DNA function, replication, translation, and transfer of genetic information. Chromium can also lead to aberrant cell cycle checkpoints or to disturbances in regulation of DNA repair mechanisms so promoting genomic instability and favouring carcinogenesis (Tabrez et al. 2014). It is also important to recognize the role of nonmutagenic, but heritable, epigenetic alterations in chromium-induced genotoxicity and mutagenicity (Nickens et al. 2010).

Barley Madalin cultivar is recommended for malt and beer production, also for animal fodder; other cultivars are used to produce certain distilled beverages or as components of various health foods. Therefore, this major cereal—the fourth most important crop in the world—constitutes an important link in food chain and the results obtained in this study must be taken into consideration. The continuous increase of the pollution by heavy metals, including chromium, is a serious concern, and every positive result on their genotoxic, mutagenic, and carcinogenic potential must be considered as a warning signal about the risks of the uncontrolled release of the heavy metals in environment because they finally affect the plant, animal, and human systems.

4 Conclusions

This study provides useful information about genotoxic potential of chromium, a heavy metal which became a serious threat to environmental safety. Both potassium dichromate and chromium chloride generally stimulated the growth of barley seedlings in the early ontogeny. The mitotic index was significantly increased after short-term exposure to potassium dichromate and chromium chloride. Potassium dichromate and chromium chloride increased frequency of chromosomal aberrations and mitotic abnormalities, thus showing both clastogenic and aneugenic activity. In view of its overall sensitivity, the Barley test can be recommended as a useful cytogenetic assay to study the cytotoxic and genotoxic effects of heavy metals. We consider that further research should be carried out to clarify the intimate mechanisms of chromium action in living organisms and to estimate not only the extent of the injury induced on genetic material but also the repercussions of this damage on phenotype traits.

Acknowledgments This work was supported by BIODIV research project (PN 09-36-301/2010-2011) from the National Program for Research and Development in Romania.

References

Akinci, I. E., & Akinci, S. (2010). Effect of chromium toxicity on germination and early seedling growth in melon (*Cucumis melo* L.). *African Journal of Biotechnology, 9*, 4589–4594.

Ali, N. A., Ater, M., Sunahara, G. I., & Robidou, P. Y. (2004). Phytotoxicity and bioaccumulation of copper and chromium using barley (*Hordeum vulgare* L.) in spiked artificial and

natural forest soils. *Ecotoxicology and Environmental Safety, 57*, 363–374. doi:10.1016/S0147-6513(03)00074-5.

Attia, S. M. (2011). Comparative aneugenicity of doxorubicin and its derivative idarubicin using fluorescence *in situ* hybridization techniques. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 715*(1–2), 79–87. doi:10.1016/j.mrfmmm.2011.07.012.

Balansky, R. M., D'Agostini, F., Izzotti, A., & De Flora, S. (2000). Less than additive interaction between cigarette smoke and chromium (VI) in inducing clastogenic damage in rodents. *Carcinogenesis, 21*(9), 1677–1682. doi:10.1093/carcin/21.9.1677.

Benova, D., Hadjidekova, V., Hristova, R., Nikolova, T., Boulanova, M., Georgieva, I., Grigorova, M., Popov, T., Panev, T., Georgieva, R., Natarajan, A. T., Darroudi, F., & Nilsson, R. (2010). Cytogenetic effects of hexavalent chromium in Bulgarian chromium platers. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 514*, 29–38. doi:10.1016/S1383-5718(01)00320-5.

Bishnoi, N. R., Dua, A., Gupta, V. K., & Sawhney, S. K. (1993). Effect of chromium on seed germination, seedling growth and yield of peas. *Agriculture, Ecosystems & Environment, 47*(1), 47–57. doi:10.1016/0167-8809(93)90135-C.

Blum, O. (2007). Atmospheric heavy metal deposition in Romania and neighboring countries: comparative evaluation on the basis of all European moss monitoring. In R. N. Hull, C. H. Barbu, & N. Goncharova (Eds.), *Proceedings on the NATO advanced research workshop on strategies to enhance environmental security in transition countries (6-9 September 2006, Sibiu, Romania)* (pp. 369–386). The Netherlands: Springer.

Bonet, A., Poschenrieder, C., & Barcelo, J. (1991). Chromium III-iron interaction in Fe-deficient and Fe-sufficient bean plants. I. Growth and nutrient content. *Journal of Plant Nutrition, 14*, 403–414. doi:10.1080/01904169109364211.

Castro, R. O., Trujillo, M. M., Bucio, J. L., Cervantes, C., & Dubrovsky, J. (2007). Effects of dichromate on growth and root system architecture of *Arabidopsis thaliana* seedlings. *Plant Science, 172*, 684–691. doi:10.1016/j.plantsci.2006.11.004.

Cervantes, C., Campos-Garcia, J., Devars, S., Gutierrez-Corona, F., Loza-Tavera, H., Torres-Guzman, J. C., & Moreno-Sanchez, R. (2001). Interactions of chromium with microorganisms and plants. *FEMS Microbiology Reviews, 25*, 335–347. doi:10.1016/S0168-6445(01)00057-2.

Chidambaram, A., Sundaramoorthy, P., Murugan, A., Ganesh, K. S., & Baskaran, L. (2009). Chromium induced cytotoxicity in blackgram (*Vigna mungo* L.). *Iranian Journal of Environmental Health Science & Engineering, 6*, 17–22.

Dayan, A. D., & Paine, A. J. (2001). Mechanisms of chromium toxicity, carcinogenicity and allergenicity: review of the literature from 1985. *Human & Experimental Toxicology, 20*, 439–451. doi:10.1191/096032701682693062.

Depault, F., Cojocaru, M., Fortin, F., Chackrabarti, S., & Lemieux, N. (2006). Genotoxic effects of chromium(VI) and cadmium(II) in human blood lymphocytes using the electron microscopy in situ end-labelling (EM-ISEL) assay. *Toxicology In Vitro, 20*, 513–518. doi:10.1016/j.tiv.2005.09.003.

Dubey, S., Misra, P., Dwivedi, S., Chatterjee, S., Bag, S., Mantri, S., Asif, M., Rai, A., Kumar, S., Shri, M., Tripathi, P., Tripathi, R., Trivedi, P., Chakrabarty, D., & Tuli, R. (2010). Transcriptomic and metabolomic shifts in rice roots in response to Cr(VI) stress. *BMC Genomics, 11*, 648. doi:10.1186/1471-2164-11-648.

Eastmond, D. A., MacGregor, J. T., & Slesinski, R. S. (2008). Trivalent chromium assessing the genotoxic risk of an essential trace element and widely used human and animal nutritional supplement. *Critical Reviews in Toxicology, 38*, 173–190. doi:10.1080/10408440701845401.

Eleftheriou, E. P., Adamakis, I. D. S., & Melissa, P. (2012). Effects of hexavalent chromium on microtubule organization, ER distribution and callose deposition in root tip cells of *Allium cepa* L. *Protoplasma, 249*, 401–416. doi:10.1007/s00709-011-0292-3.

El-Yamani, N., Zuniga, L., Stoyanova, E., Creus, A., & Marcos, R. (2011). Chromium-induced genotoxicity and interference in human lymphoblastoid cell (TK6) repair process. *Journal of Toxicology and Environmental Health. Part A, 74*(15–16), 1030–1036. doi:10.1080/15287394.2011.582282.

US Environmental Protection Agency (2006). Innovative technology verification report. XRF technologies for measuring trace elements in soil and sediment. EPA/540/R-06/003. Office of Research and Development, Washington. http://www.clu-in.org/conf/tio/xrf_082808/cd/EPA-ORD-Innovative-Technology-Verification-Reports-Feb-2006/NitonXLi700.pdf. Accessed 29 Oct 2013.

Gamborg, O. L., & Wetter, L. R. (1975). *Plant tissue culture methods*. Saskatoon: Prairie Regional Laboratory, National Research Council.

Gardea-Torresdey, J. L., De la Rosa, G., Peralta-Videa, J. R., Contesa, M., Cruz-Jimenez, G., & Cano-Aguilera, I. (2005). Differential uptake and transport of trivalent and hexavalent chromium by tumbleweed (*Salsola kali*). *Archives of Environmental Contamination and Toxicology, 48*, 225–232. doi:10.1007/s00244-003-0162-x.

Glinska, S., Bartczak, M., Oleksiak, S., Wolska, A., Gabara, B., Posmyk, M., & Janas, K. (2007). Effects of anthocyanin-rich extract from red cabbage leaves on meristematic cells of *Allium cepa* L. roots treated with heavy metals. *Ecotoxicology and Environmental Safety, 68*(3), 343–350. doi:10.1016/j.ecoenv.2007.02.004.

Gomez-Arroyo, S., Vellagomez, L., & Villalobos-Petrini, R. (1987). Effects induced by chromium trioxide on root tip chromosomes of *Vicia faba*. *Revista Internacional de Contaminacion Ambiental, 3*(1), 55–61.

Halasova, E., Matakova, T., Musak, L., Polakova, V., Letkova, L., Dobrota, D., & Vodicka, P. (2012). Evaluating chromosomal damage in workers exposed to hexavalent chromium and the modulating role of polymorphisms of DNA repair genes. *International Archives of Occupational and Environmental Health, 85*, 473–481. doi:10.1007/s00420-011-0684-x.

Harmanescu, M., Alda, L. M., Bordean, D. M., Gogoasa, I., & Gergen, I. (2011). Heavy metals health risk assessment for population *via* consumption of vegetables grown in old mining area; a case study: Banat County, Romania. *Chemistry Central Journal, 5*, 64. doi:10.1186/1752-153X-5-64.

International Agency for Research on Cancer. (1990). Chromium and chromium compounds. In: *IARC monographs on the evaluation of carcinogenic risks to humans—Chromium, nickel and welding* (pp. 49–214). Lyon, France: IARC Scientific Publications.

Jamal, S. N., Iqbal, M. Z., & Athar, M. (2006). Phytotoxic effect of aluminum and chromium on the germination and early

growth of wheat (*Triticum aestivum*) varieties Anmol and Kiran. *International Journal of Environmental Science & Technology, 3*(4), 411–416. doi:10.1007/BF03325950.

Jun, R., Ling, T., & Guanghua, Z. (2009). Effects of chromium on seed germination, root elongation and coleoptile growth in six pulses. *International Journal of Environmental Science & Technology, 6*(4), 571–578.

Kabata-Pendias, A., & Pendias, H. (2001). *Trace elements in soils and plants* (3rd ed., pp. 266–273). Boca-Raton: CRC Press.

Khorsandi, K., & Rabbani-Chadegani, A. (2013). Studies on the genotoxic effect of chromium oxide (Cr VI): interaction with deoxyribonucleic acid in solution. *Mutation Research/ Genetic Toxicology and Environmental Mutagenesis, 750*(1–2), 105–110. doi:10.1016/j.mrgentox.2012.10.002.

Labra, M., De Mattia, F., Bernasconi, M., Bertacchi, D., Grassi, F., Bruni, I., & Citterio, S. (2010). The combined toxic and genotoxic effects of chromium and volatile organic contaminants to *Pseudokirchneriella subcapitata. Water, Air, & Soil Pollution, 213*, 57–70. doi:10.1007/s11270-010-0367-3.

Liu, D., Jiang, W., & Li, M. (1992). Effects of trivalent and hexavalent chromium on root growth and cell division of *Allium cepa. Hereditas, 117*, 23–29. doi:10.1111/j.1601-5223.1992.tb00003.x.

Liu, D., Jiang, W., & Gao, X. (2003). Effects of cadmium on root growth, cell division and nucleoli in root tip cells of garlic. *Biologia Plantarum, 47*(1), 79–83. doi:10.1023/A:1027384932338.

Liu, W., Li, P., Qi, X., Zhou, Q., Zheng, L., Sun, T., & Yang, Y. (2005). DNA changes in barley (*Hordeum vulgare*) seedlings induced by cadmium pollution using RAPD analysis. *Chemosphere, 61*, 158–167. doi:10.1016/j. chemosphere . 2005.02.078.

Maeng, S. H., Chung, H. W., Kim, K. J., Lee, B. M., Shin, Y. C., Kim, S. J., & Yu, I. J. (2004). Chromosome aberration and lipid peroxidation in chromium-exposed workers. *Biomarkers, 9*(6), 418–434. doi:10.1080/13547500400022200.

Matsumoto, S. T., Mantovani, M. S., Malagutti, M. I. A., Dias, A. L., Fonseca, I. C., & Marin-Morales, M. A. (2006). Genotoxicity and mutagenicity of water contaminated with tannery effluents, as evaluated by the micronucleus test and comet assay using the fish *Oreochromis niloticus* and chromosome aberrations in onion root-tips. *Genetics and Molecular Biology, 29*(1), 148–158. doi:10.1590/S1415-47572006000100028.

Medeiros, M. G., Rodrigues, A. S., Batoreu, M. C., Laires, A., Rueff, J., & Zhitkovich, A. (2003). Elevated levels of DNA–protein crosslinks and micronuclei in peripheral lymphocytes of tannery workers exposed to trivalent chromium. *Mutagenesis, 18*(1), 19–24. doi:10.1093/mutage/18.1.19.

Mengel, K., & Kirkby, E. A. (2001). *Principles of plant nutrition* (5th ed.). The Netherlands: Kluwer Academic Publishers, Springer-Science+Business Media, BV.

Michailova, P., Petrova, N., Sella, G., Bovero, S., Ramella, L., Regoli, F., & Zelano, V. (2001). Genotoxic effects of chromium on polytene chromosomes of *Chironomus riparius* Meigen 1804 (Diptera, Chironomidae). *Caryologia, 54*(1), 59–71. doi:10.1080/00087114.2001.10589213.

Migid, H. M. A., Azab, Y. A., & Ibrahim, W. M. (2007). Use of plant genotoxicity bioassay for the evaluation of efficiency of algal biofilters in bioremediation of toxic industrial effluent. *Ecotoxicology and Environmental Safety, 66*(1), 57–64. doi:10.1016/j.ecoenv.2005.10.011.

Mishra, S. N., Abha, A., & Jha, A. M. (2012). Genotoxicity testing of chromium trioxide—a study using *Vicia* bioassay. *Journal of Phytology, 4*(5), 37–41.

Nickens, K. P., Patierno, S. R., & Ceryak, S. (2010). Chromium genotoxicity: a double-edged sword. *Chemico-Biological Interactions, 188*(2), 276–288. doi:10.1016/j.cbi.2010.04.018.

Normann, C. A. B. M., Moreira, J. C. F., & Cardoso, V. V. (2008). Micronuclei in red blood cells of armored catfish *Hypostomus plecostomus* exposed to potassium dichromate. *African Journal of Biotechnology, 7*(7), 893–896.

Oliveira, H. (2012). Chromium as an environmental pollutant: insights on induced plant toxicity. *Journal of Botany.* doi:10.1155/2012/375843.

Olteanu, Z., Oprica, L., Truta, E., Zamfirache, M. M., & Rosu, C. M. (2012). Changes induced by two chromium-containing compounds in antioxidative response, soluble protein level and amylase activity in barley seedlings. *Analele Stiintifice ale Universitatii "Al. I. Cuza" Iasi, Sect. Genetica si Biologie Moleculara, 13*(3), 41–46.

Ozdener, Y., Aydin, B. K., Aygun, F., & Yurekli, F. (2011). Effect of hexavalent chromium on the growth and physiological and biochemical parameters on *Brassica oleracea* L. var. *acephala* DC. *Acta Biologica Hungarica, 62*(4), 463–476. doi:10.1556/ABiol.62.2011.4.11.

Papp, G., Nagy, G., Pocsi, I., Pesti, M., & Banfalvi, G. (2011). Genotoxic chromatin changes in *Schizosaccharomyces pombe* induced by hexavalent chromium (CrVI) ions. In G. Banfalvi (Ed.), *Cellular effects of heavy metals* (pp. 179–193). Netherlands: Springer.

Parry, E. M., Parry, J. M., Corso, C., Doherty, A., Haddad, F., Hermine, T. F., Johnson, G., Kayany, M., Quick, E., Warr, T., & Williamson, J. (2002). Detection and characterization of mechanisms of action of aneugenic chemicals. *Mutagenesis, 17*(6), 509–521. doi:10.1093/mutage/17.6.509.

Patlolla, A. K., Barnes, C., Hackett, D., & Tchounwou, P. B. (2009). Potassium dichromate induced cytotoxicity, genotoxicity and oxidative stress in human liver carcinoma (HepG2) cells. *International Journal of Environmental Research and Public Health, 6*(2), 643–653. doi:10.3390/ijerph6020643.

Petrilli, F. L., & De Flora, S. (1977). Toxicity and mutagenicity of hexavalent chromium on *Salmonella typhimurium. Applied and Environmental Microbiology, 33*(4), 805–809.

Pohren, R. S., da Costa, T. C., & Ferrão Vargas, V. M. (2013). Investigation of sensitivity of the *Allium cepa* test as an alert system to evaluate the genotoxic potential of soil contaminated by heavy metals. *Water, Air, & Soil Pollution, 224*, 1460–1469. doi:10.1007/s11270-013-1460-1.

Qian, X. W. (2004). Study on teratogenic effect of potassium dichromate on *Vicia faba* root tip cells. *Yi Chuan, 26*, 337–342. doi:10.1631/jzus.2004.1570 (abstract in English, article in Chinese).

Rauta, C., Carstea, S., & Mihailescu, A. (1987). Influence of some pollutants on agricultural soils in Romania. *Archiwum Ochrony Srodowiska, 1*(2), 33–37.

Rodriguez-Gomez, E. (2011). *Genotoxicity and cytotoxicity of Cr(VI) and Pb^{2+} in Pisum sativum*. PhD thesis, 177 pages. Portugal: University of Aveiro.

Santos, C., & Rodriguez-Gomez, E. (2012). Review on some emerging endpoints of chromium (VI) and lead phytotoxicity. In: J. K. Mworia (Ed.), *Botany* (pp. 61–82). InTech.

Scoccianti, V., Crinelli, R., Tirillini, B., Mancinelli, V., & Speranza, A. (2006). Uptake and toxicity of Cr(III) in celery seedlings. *Chemosphere, 64*(10), 1695–1703. doi:10.1016/j.chemosphere.2006.01.005.

Seoane, A. I., & Dulout, F. N. (1999). Contribution to the validation of the anaphase-telophase test: aneugenic and clastogenic effects of cadmium sulfate, potassium dichromate and nickel chloride in Chinese hamster ovary cells. *Genetics and Molecular Biology, 22*(4), 551–555. doi:10.1590/S1415-47571999000400015.

Shanker, A. K., Cervantes, C., Loza-Tavera, H., & Avudainayagam, S. (2005). Chromium toxicity in plants. *Environment International, 31*(5), 739–753. doi:10.1016/j.envint.2005.02.003.

Singh, H. P., Mahajan, P., Kaur, S., Batish, D. R., & Kohli, R. K. (2013). Chromium toxicity and tolerance in plants. *Environmental Chemistry Letters, 11*, 229–254. doi:10.1007/s10311-013-0407-5.

Sinha, P., Dube, B. K., & Chatterjee, C. (2005). Amelioration of chromium phytotoxicity in spinach by withdrawal of chromium or iron application through different modes. *Plant Science, 169*(4), 641–646. doi:10.1016/j.plantsci.2004.07.003.

Skeffington, R. A., Shewry, P. R., & Peterson, P. J. (1976). The uptake and transport of chromium by barley seedlings (*Hordeum vulgare* L.). *Planta (Berlin), 132*, 209–214.

Srivastava, S., & Jain, R. (2011). *In situ* monitoring of chromium cytotoxicity in sugarcane. *Journal of Environmental Biology, 32*, 759–763.

Tabrez, S., Priyadarshini, M., Priyamvada, S., Khan, M. S., Arivarasu, N. A., & Zaidi, S. K. (2014). Gene-environment interactions in heavy metal and pesticide carcinogenesis. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 760*, 1–9. doi:10.1016/j.mrgentox.2013.11.002.

US Department of Agriculture, Natural Resources Conservation Service. (2000). Heavy metal soil contamination. Soil quality. USDA, NRCS Urban technical note 3. http://soils.usda.gov/sqi/management/files/sq_utn_3.pdf. Accessed 29 Oct 2013.

Vandenberg, L. N., Colborn, T., Hayes, T. B., Heindel, J. J., Jacobs, D. R., Lee, D.-H., Shioda, T., Soto, A. M., vom Saal, F. S., Welshons, W. V., Zoeller, R. T., & Myers, J. P. (2012). Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocrine Reviews, 33*(3), 378–455. doi:10.1210/er.2011-1050.

Velma, V., & Tchounwou, P. B. (2010). Chromium-induced biochemical, genotoxic and histopathologic effects in liver and kidney of gold-fish *Carassius auratus. Mutation Research, 698*(1–2), 43–51. doi:10.1016/j.mrgentox.2010.03.014.

Villalobos-Pietrini, R., Gómez-Arroyo, S., Márquez, A. R. F., & Cisneros, A. (1986). *Tradescantia*—micronucleus test on potassium dichromate. *Revista Internacional de Contaminación Ambiental, 2*, 63–70.

Wang, R., Gao, F., Guo, B. Q., Huang, J. C., Wang, L., & Zhou, Y. J. (2013). Short-term chromium-stress-induced alterations in the maize leaf proteome. *International Journal of Molecular Sciences, 14*(6), 11125–11144. doi:10.3390/ijms140611125.

Wierzbicka, M. (1999). The effect of lead on the cell cycle in the root meristem of *Allium cepa L. Protoplasma, 207*(3), 186–194. doi:10.1007/BF01282999.

Yadav, K. K., & Trivedi, S. P. (2006). Evaluation of genotoxic potential of chromium (VI) in *Channa punctata* fish in terms of chromosomal aberrations. *Asian Pacific Journal of Cancer Prevention, 7*(3), 472–476.

Yildiz, M., Cigerci, I. H., Konuk, M., Fidan, A. F., & Terzi, H. (2009). Determination of genotoxic effects of copper sulphate and cobalt chloride in *Allium cepa* root cells by chromosome aberration and comet assays. *Chemosphere, 75*(7), 934–938. doi:10.1016/j.chemosphere.2009.01.023.

Zayed, A. M., & Terry, N. (2003). Chromium in the environment: factors affecting biological remediation. *Plant and Soil, 249*(1), 139–156. doi:10.1023/A:1022504826342.

Zhang, J., Zhang, D., Yong, L., & Ling, Z. (2009). Micronucleus kinetics induced by chromium trioxide in *Vicia faba* root tip cells. In: *Proceedings of the 3rd International Conference on Bioinformatics and Biomedical Engineering* (pp. 1–3). Beijing, China, 11–16 June 2009.

Zou, J. H., Wang, M., Jiang, W. S., & Liu, D. H. (2006). Effects of hexavalent chromium (VI) on root growth and cell division in root tip cells of *Amaranthus viridis* L. *Pakistan Journal of Botany, 38*, 673–681.