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Phytotoxic effect of hexavalent chromium on germination and seedling growth of seeds of different plant species

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Abstract Hexavalent chromium is toxic to many plants at high levels. However, it may be accumulated at low levels and since its accumulation is biomagnified at different trophic levels through food chain, this may affect human health.
The aim of this work was to evaluate the effect of different concentrations of hexavalent chromium Cr(VI) (0, 100, 200 and 300 mg/L) on seed germination, root and shoot growth of different plant species: seeds of lentils, soya bean, haricot bean, fenugreek, sesames, wheat, and linseed and to elucidate the toxicity effect of Cr(VI). This study showed that germination and viability of seeds were negatively affected by elevated chromium concentration. Response of seedlings to chromium was more noticeable than that of seed germination; this event is based on the impermeability of seed coats and selectivity of embryos against chromium. Since Cr(VI) did not get detoxified the inhibition of roots and shoots length may be attributed to its accumulation and may causes health risks through consumption of plant material. Although, there was a significant difference in the degree of tolerance of Cr(VI) among plant species. Linseed was the least tolerant to Cr(VI), and Soya bean was the most tolerant.

Key words: Hexavalent chromium, toxicity, germination, seedling growth, tolerance.

Introduction

Hexavalent chromium used extensively worldwide in various industrial activities is therefore considered a serious environmental pollutant and poses a threat to human health. Its presence in agricultural soils can be attributed to the use of organic wastes as fertilizer and the use of waste water for irrigation (Pillay *et al*., 2003). It is the case of the region of Fez (Morocco), where tanneries among others industrial activities are the most polluting industries in the region. Effluents produced daily in tanneries are simply discharged into

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nearby watercourses without any treatment. Consequently, considerable amounts of toxic chemicals used in the tanning process, find their way into natural waters. Sebou River, the primary source of water in the region, is used for a variety of purposes (i.e. drinking, agriculture, industry, recreation) in spite of its degraded quality (Koukal *et al*., 2004).

Chromium like others heavy metals do not degrade biologically, its remains stable for several months in the soil without changing its oxidation state. Cr (VI) is accumulated by plants and its accumulation is biomagnified at different trophic levels through food chain (Kotas and Stasicka, 2000; Rogival *et al*., 2007).

High levels of metals in soil can be phytotoxic. Toxicity of Cr to plants depends on its valence state: hexavalent chromium Cr(VI) is highly toxic and mobile whereas trivalent chromium Cr(III) is less toxic (Oliveira, 2012). Toxic effects of Cr on plant growth and development include alterations in the germination process as well as in the growth of roots, stems and leaves, which may affect total dry matter production and yield (Shanker *et al.*, 2005). The phytotoxic levels of Cr in most plants seem to limit its accumulation in the food chain. Because most plants have low Cr concentrations, even when grown on Cr rich soils, the food chain is well protected against Cr toxicity.

Chromium interferes with several metabolic processes, causing toxicity to plants as exhibited by reduced seed germination or early seedling development (Sharma *et al*., 1995), root growth and biomass, chlorosis, photosynthetic impairing and finally, plant death (Scoccianti *et al*., 2006).

The seeds germination begins with the crucial stage of water absorption by the seed. Numerous are the environmental constraints which can compromise the capacity of seeds to germinate; salinity (Abari *et al*., 2011; Kazemi *et al*. 2012), water stress (Jajarmi, 2009), inadequate humidity and temperature (Ravikumar *et al*., 2002). However, rare are the works which studied the impact of the stress by heavy metals on the seed germination. The inhibition of this process seems to depend, however, on the kind of metal and on its concentration, on the exposure time of seeds and on the botanical species, even on the variety and on the seed, in particular the nature of its integuments (Munzuroglu *et al*., 2002). Since seed germination is the first physiological process affected by Cr(VI), the ability of a seed to germinate in a medium containing Cr(VI) would be indicative of its level of tolerance to this metal (Peralta *et al.*, 2001). Also, there are different researches which show that early growth stages of seedling are very important indicators in determining toxicity impacts of heavy metals like chromium in plants (Sharma *et al*., 1995; Pandey and Pandey, 2008). The highest risk for human health is when plants develop tolerance mechanisms against metals and when those plants are incorporated into the food chain. Chromium may reach human beings either through polluted drinking water sources or through the food chain and its accumulation in higher concentration may lead to cancer and associated health hazards.

In this study, phytotoxic effect of different Cr(VI) concentration was tested on various seeds: lentils, soya bean, wheat, fenugreek, sesames, haricot bean and linseed. Parameters studied to be affected by Cr(VI) treatment were germination and seedling growth (root and shoot length). Tolerance of plants to Cr(VI) was evaluated by calculating the relative degree of toxicity.

Materials and methods

As a biological material, we used uniform seeds of Leguminosae: fenugreek (*Trigonella foenum-graecum)*, lentil (*Lens culinaris)*, soya bean (*Glycine max)* and haricot bean (*Phaseolus vulgaris)*, Gramineae: wheat (*Triticum* spp) and sesames (*Sesamum indicum)* and Linaceae: linseed or flax seed (*Linum usitatissimum)*. The phytotoxic effect of hexavalent chromium on the germination and early growth of these seeds was performed in Petri plates. Solutions of 0.7% agar in distilled water were prepared, after autoclaving (121 °C, 20 min), chromium was added (a stock solution of $K_2Cr_2O_7$ was sterilized by filtration and used as a Cr(VI) source) to obtain concentrations of 100, 200 and 300 mg Cr(VI)/L and distributed in sterilized Petri plates. In control, potassium chromate was not added.

For surface-sterilizing plant material, isopropyl alcohol is a powerful sterilizing agent but also extremely phytotoxic. Therefore, plant material was typically exposed to 70% ethanol for only 2 minutes, and immediately rinsed twice with distilled and sterilized water.

A rate of 20 uniform sized seeds were selected and sown at equal distance in a Petri dish, in triplicate for each treatment. The plates were incubated at 30 °C in the dark, and germination percentage was calculated by counting the number of germinated seeds on the seventh day. The effect of chromium on the root and shoot growth was studied by measuring the length.

Germination percentage (%)

Germinated seeds were counted according to the seedling evaluation procedure in the Handbook of Association of Official Seed Analysts (AOSA, 1983). The number of germinated seeds was recorded. Seven days after germination, the germination percentage (Gp) was calculated using the formula below for each replication of the treatment (Tanveer *et al*., 2010).

$$\mathrm{Gp} = \frac{\text{Germinated seed}}{\text{Total seed}} \times 100$$

Relative toxicity and tolerance (%)

Degree of relative toxicity of Cr (VI) on seed germination or seedling growth of cultivars was calculated by comparing with control. The relative degree of toxicity was calculated by the following formula.

$$\text{Relative toxicity } = \frac{X - Y}{X} \times 100$$

Where,
X= Germination percentage or seedling growth at particular time interval.
Y= Germination percentage or seedling growth in treatment at same time interval.

Results and discussions

Effect of chromium stress on seed germination

Table 1, shows that the germination of all seeds tested occurred at all chromium treatments from 0.0 mg/L to 300 mg/L with variations depending on Cr(VI) concentration and plant species. Indeed, Inhibition of 5, 15 and 20 % of lentil seeds was observed respectively for 100, 200 and 300 mg Cr(VI)/L. For soya bean and sesames, we can observe that there was no inhibition with 100 and 200 mg/L and only 5% and 10 % inhibition respectively at 300 mg/L.

Germination of fenugreek and haricot bean seeds was not affected by a concentration of 100 mg/L Cr(VI), but showed 20 and 10% inhibition by 200 mg/L Cr(VI) and 25 and 20% inhibition by 300 mg/L Cr(VI). Germination of linseed, in comparison with other seeds was the most affected by chromium concentrations: 20, 25 and 30% germination inhibition for 100, 200 and 300 mg Cr(VI)/L. Besides, germination of wheat seeds was not inhibited by 100 mg/L chromium treatment and slightly inhibited by 200 and 300 mg/L Cr(VI).

Similar result was obtained by Jamal *et al*. (2006) with two wheat (*Triticum aestivum*) varieties Anmol and Kiran treated with chromium. This metal has been reported not to inhibit germination but impair the growth of new roots and seedling establishment (Rellén-Álvarez *et al*., 2006). The concentration of 40 mg/L Cr(VI) inhibited significantly seed germination of Alfalfa plant (*Medicago Sativa*) by 54% (Aydinalp and Marinova, 2009).

Peralta *et al*. (2001) also found that 40 mg/L of Cr(VI) reduced by 23% the ability of seeds of lucerne (*Medicago sativa* cv. Malone) to germinate and grow in the contaminated medium. Reductions of 32–57% in sugarcane bud germination were observed with 20 and 80 mg/L Cr, respectively (Jain *et al*., 2000).

Metabolic alterations by Cr exposure have also been described in plants either by a direct effect on enzymes or other metabolites or by its ability to generate reactive oxygen species which may cause oxidative stress (Shanker *et al*, 2005).

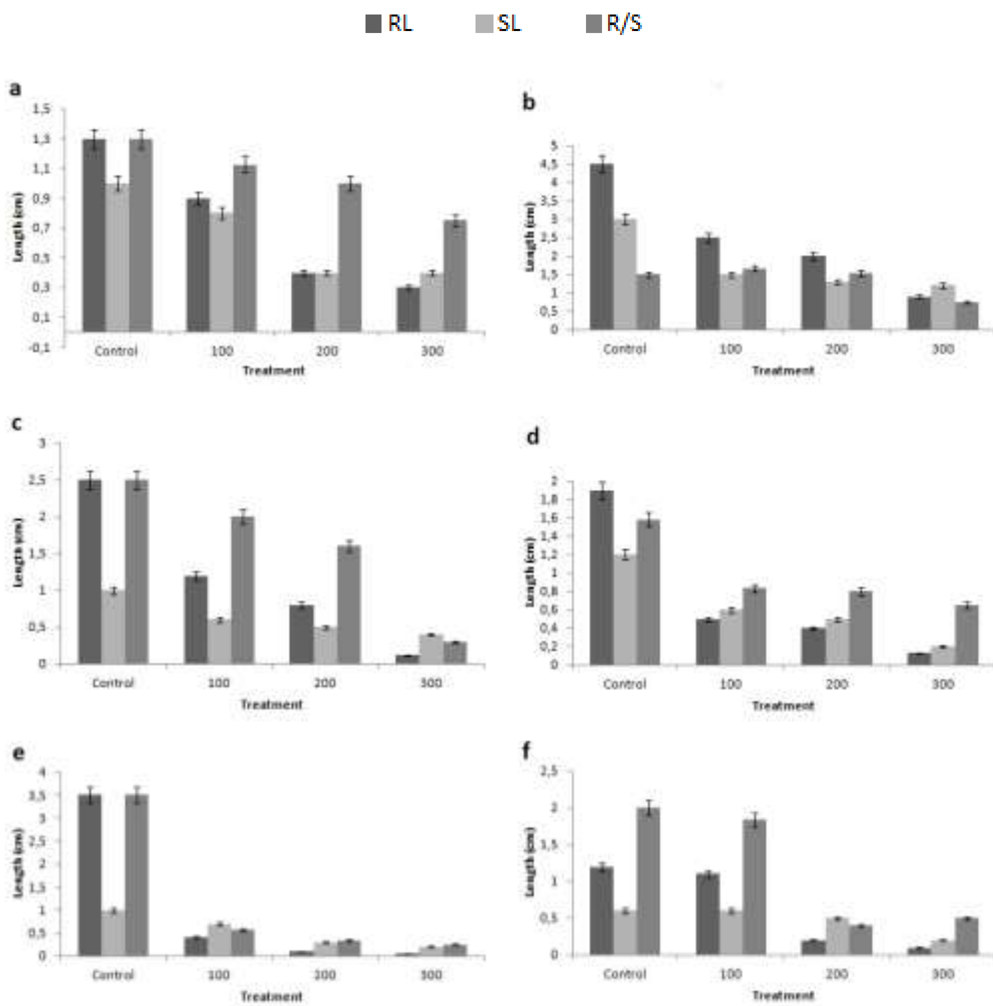
Shanker *et al.* (2005) in his Review article have reported that the reduced germination of seeds under Cr stress could be a depressive effect of Cr on the activity of amylases and on the subsequent transport of sugars to the embryo axes. On the contrary, Protease activity increases with the Cr treatment, which could also contribute to the reduction in germination of Cr-treated seeds.

Table 1. Effect of different concentrations of Cr (VI) on germination percentage of various seeds.

Treatment:	**Germination rate of Seeds (%)**						
(Cr(VI) added mg/L)	Lenses	Soya bean	Wheat	Fenugreek	Sesames	Haricot bean	Linseed
0 (Control)	90	100	85	100	100	100	77
100	85	100	85	100	100	100	57
200	75	100	80	80	100	90	52
300	70	95	80	75	90	80	47

Effect of chromium on seedling growth

Fig 1 shows that the control plants grow tall than any other chromium treated plants. In addition, more the concentration of chromium increases from 100 to 300 mg/L Cr(VI) more the inhibiting effect on the growth of all the seeds, represented by the roots and shoots length increase. The inhibition affects both root and shoot growth in comparison with control, but the inhibition effect of Cr(VI) was more pronounced in root length than shoot length of all seeds. In other words, root to shoot length ratio decrease with increasing Cr(VI) concentration. This inhibitive effect was accompanied with significant morphological changes in root. In fact, roots were shorter and brownish and presented less number of roots hairs in chromium-treated plants in contrast to the control, in which thin, elongated roots were formed. Similarly, both root length and shoot length of *Arachis hypogea* were found to be affected by the increasing concentrations of Cr(VI) (Rajalakshmi *et al*., 2010). Gyawali and Lekhak (2006) have also reported that root growth was comparatively more inhibited than shoot of rice (*Oryza Sativa* L.) cultivars. In the same context, Abdul Ghani (2011) have reported that increasing concentrations of chromium caused significant reduction in root length and shoot length of *brassica juncea* L.. Root dry weight and shoot dry weight were also significantly decreased at 10, 20, 30 and 40 mg/L of chromium as compared to control. Bishoni (1993) reported that Cr(VI) did not affect the percentage germination but suppressed the growth of radical and plumule, significantly and its effect was more pronounced on roots than on the shoots.



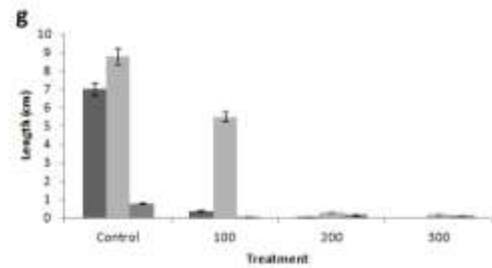


Fig. 1. Effect of chromium on root length (RL) and shoot length (SL) and root to shoot length ratio (R/S) of lentils (a), soya Bean (b), wheat (c), fenugreek (d), sesames (e), haricot bean (f) and linseed (g).

Arduini *et al*. (2006) have reported that root growth of miscanthus was less affected than shoot growth, but root morphology changed drastically. Also, Samantary (2002) have reported that the development of lateral roots and root number was affected by Cr exposure. Moreover, roots of *Zea mays* L. treated with Cr(VI) were shorter and brownish and presented less number of roots hairs (Mallick *et al.*, 2010). These observations are in agreement with that obtained in this work with all seeds tested.

Decrease in root growth in presence of Cr(VI) can be explained by inhibition of root cell division and/or elongation, which might have occurred as a result of tissue collapse and consequent incapacity of the roots to absorb water and nutrients from the medium (Barcel ́o *et al.*, 1985). The decrease in plant height could be due to the reduced root growth and consequent decreased nutrients and water transport to the higher parts of the plant. Moreover, Cr transport to the aerial part of the plant can directly impact cellular metabolism of shoots contributing to the reduction in plant height (Oliveira, 2012).

Seeds tolerance to Cr(VI)

The tolerance of the different seeds was calculated on the basis of the relative degree of toxicity from the different parameters: germination and root and shoot length (Figs 2 and 3). A general scenario of Cr (IV) associated inhibition of germination and growth may be as follows:

- Relative degree of toxicity on seed germination:
Soya < Sesames < Wheat < Haricot bean < Lenses < Fenugreek < Linseed

- Relative degree of toxicity on shoot length:
Haricot bean < Wheat < Soya < Fenugreek < Lenses < Sesames < Linseed

- Relative degree of toxicity on root length:
Soya < Wheat < Lenses < Fenugreek < Haricot bean < Sesames < Linseed

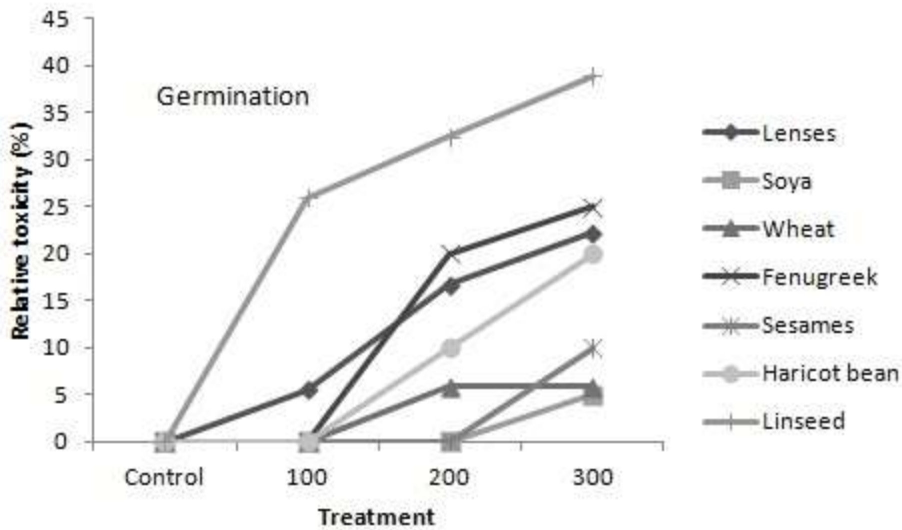
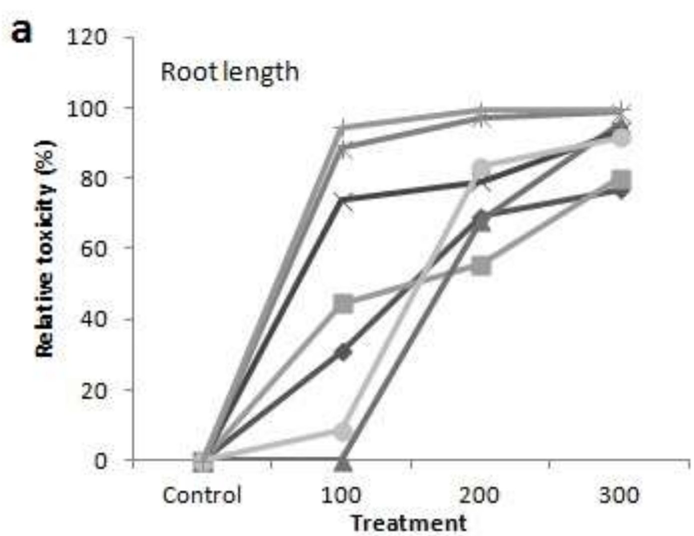


Fig. 2. Relative degree of toxicity of Cr (VI) on seed germination.



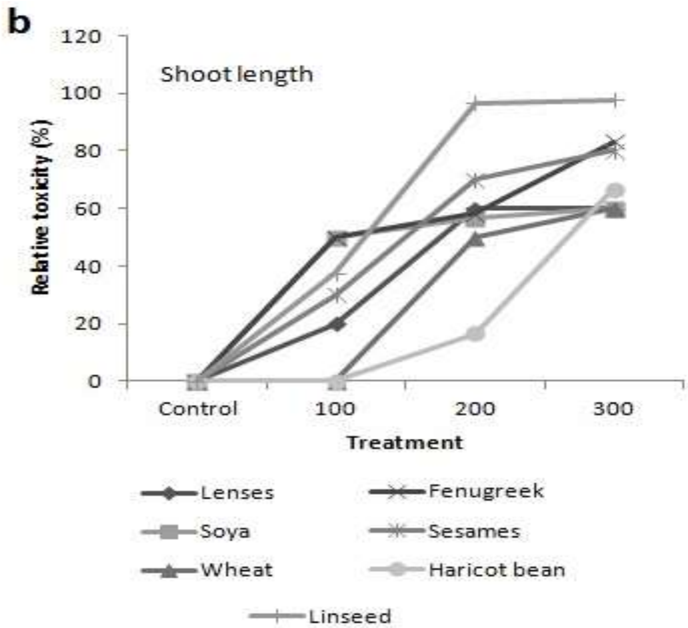


Fig. 3. Relative degree of toxicity of Cr (VI) on root (a) and shoot (b) growth of seeding.

Consequently, from the above results, it was evaluated that haricot bean was the most tolerant with the minimum relative degree of toxicity in most of all the growth parameters. Similarly Linseed was the most sensitive which showed the maximum relative degree of toxicity in all the growth parameters (Fig 4).

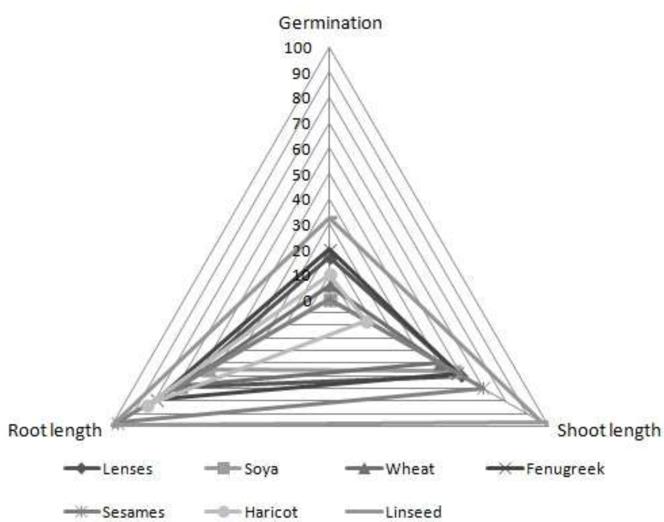


Fig. 4. Relative degree of Cr (VI) toxicity on seeds germination, root and shoot growth.

Since seed germination is the first physiological process affected by Cr, the ability of a seed to germinate in a medium containing Cr would be indicative of its level of tolerance to this metal (Peralta *et al*., 2001). Akinci and Akinci (2010) suggested that germination can be used practically and cheaply for selection of tolerant species or varieties to chromium during the early seedling stages. Plant species have different responses to heavy metal pollution of soils. Although it may exist a relationship between heavy metal accumulation and plants tolerance, many plant species grow on contaminated soils and yet do not accumulate metals.

Soil ecosystems throughout the world have been contaminated with heavy metals by various human activities, and movement of metals up the food chain has become a human health hazard (Naidu et al., 1996). The rapid increase in population together with the unplanned disposal of effluent from tanneries and textile industries have increased the threat of soil pollution by the water-soluble, highly mobile and very toxic Cr(VI), formed by oxidation of Cr(III), in several sites around the world (Khan, 2001).

In Fez, leather tanneries were releasing in 1997 up to 360 kg of chromium per day that reached rivers and streams, contaminating precious water resources. The effluents discharged by the tanneries and other industries into the sewer without any treatment have contaminated the Oued Fez, which is released into the Oued Sebou downstream, making this river one of the most polluted in Morocco. In spite of its degraded quality, it is used for a variety of purposes (i.e. drinking, agriculture, industry, recreation, watering animals). Moreover, Sebou River is one of the regions with the most important agricultural vocation in Morocco (ABH, 2011). In this study, the most problematic pollutant discharged by tanneries, chromium, was tested on germination and growth of some of cultivated plants. We demonstrate that, chromium affect growth of seedlings, consequently, affect the agricultural yield. What is more, it’s possible accumulation into plant materials cause health problems espetially that its accumulation is biomagnified at different trophic levels through food chain.

Conclusion

The increase in Cr(VI) concentration causes inhibition of germination and seedling growth represented by root and shoot length. Inhibition effect of Cr(VI) was more pronounced in root length than shoot length of all seeds and was accompanied with morphological changes in root. Haricot bean was the most tolerant with the minimum relative degree of toxicity in most of all the growth parameters. Similarly Linseed was the most sensitive which showed the maximum relative degree of toxicity. This work has a special interest in the awareness of the danger of resistance of some plants to heavy metals and their risk of biomagnification in the trophic chain.

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