Published in final edited form as:

Environ Sci Technol. 2011 February 1; 45(3): 1082–1087. doi:10.1021/es102647w.

Kinetin increases chromium absorption, modulates its distribution, and changes the activity of catalase and ascorbate peroxidase in Mexican Palo Verde

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Abstract

This report shows, for the first time, the effectiveness of the phytohormone kinetin (KN) in increasing Cr translocation from roots to stems in Mexican Palo Verde. Fifteen-day-old seedlings, germinated in soil spiked with Cr(III) and (VI) at 60 and 10 mg kg⁻¹, respectively, were watered every other day for 30 days with a KN solution at 250 μM. Samples were analyzed for catalase (CAT) and ascorbate peroxidase (APOX) activities, Cr concentration, and Cr distribution in tissues. Results showed that KN reduced CAT but increased APOX in the roots of Cr(VI)-treated plants. In the leaves, KN reduced both CAT and APOX in Cr(III) but not in Cr(VI)-treated plants. However, KN increased total Cr concentration in roots, stems, and leaves by 45%, 103%, and 72%, respectively, compared to Cr(III) alone. For Cr(VI), KN increased Cr concentrations in roots, stems, and leaves, respectively, by 53%, 129%, and 168%, compared to Cr(VI) alone. The electron probe microanalyzer results showed that Cr was mainly located at the cortex section in the root, and Cr distribution was essentially homogenous in stems. However, proven through X-ray images, Cr(VI)-treated roots and stems had more Cr accumulation than Cr(III) counterparts. KN increased the Cr translocation from roots to stems.

Keywords

Chromium; Mexican Palo Verde; Phytoremediation; Electron Probe Microanalyzer; ICP-OES

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Supporting Information Available

Additional supportive data are provided for the physico-chemical properties of the soil used in this research (Table 1SI), translocation factors (TF) for Cr (Table 2 SI), seedlings growth (Figure 1 SI), and a cross-section back scattered electron image of Mexican Palo Verde seedlings (Figure 2 SI). This information is available free of charge via the Internet at <http://pubs.acs.org/>.

1. Introduction

Excess chromium (Cr) around industrial zones or dumping sites is a significant problem in several areas of the United States and other countries. It is documented elsewhere that metallic [Cr(0)], trivalent [Cr(III)], and hexavalent [Cr(VI)] are the most stable forms of Cr (1). Cr(III) is an essential trace element in the metabolism of mammals, but excess Cr(III) may cause toxicity. Conversely, Cr(VI) exhibits a strong oxidizing activity and is a known carcinogen (1). Cr(VI) is considered an important controlled contaminant by most countries (1, 2). Chemical, physical, and biological processes are employed to remediate chromium contamination (3-5). However, a number of papers have depicted the disadvantages of the current techniques and the advantages of the emergent green technique, phytoremediation (6). For an efficient application of phytoextraction, plants must accumulate an excess of the target elements in the aboveground parts. However, as defense mechanisms, some elements are stored mostly in roots, hindering their remediation through phytoextraction. Lead (7) and Cr (1) are among the elements with low translocation in plants. Researchers have tried to increase the translocation of Pb from roots to leaves by using phytohormones. For example, Lopez et al. (7, 8) found that kinetin (KN), coupled with EDTA, significantly increased the concentration of Pb in alfalfa (*Medicago sativa* L.) leaves. Also, Tassi et al. (9) demonstrated that exogenous KN facilitates the phytoextraction of Zn and Pb in *Helianthus annuus*. Other studies have demonstrated that transition elements alter the metabolism of reactive oxygen species (ROS). Superoxide radical ($O_2^{\cdot-}$), singlet oxygen (1O_2), hydroxyl radical ($OH^{\cdot}$), and hydrogen peroxide (H_2O_2) are ROS and are important indicators of a plant's stress. It is well known that transition elements like Cr can induce stress, forming ROS molecules in plant cell organelles (10). It is also known that the stress can be monitored and quantified by the activity of antioxidant enzymes such as catalase (CAT 1.11.1.6) and ascorbate peroxidase (APOX 1.11.1.11) (11). The effects of phytohormones such as indole-3-acetic acid (IAA), KN, and gibberellic acid on the reduction of ROS molecules have been studied in Pb-stressed alfalfa plants (8). Wang et al. (12) studied Pb accumulation and antioxidant response (reduction of ROS) in maize (*Zea mays* L.) seedlings under the effect of IAA.

Parkinsonia aculeata (Fabaceae) is native to the southwestern United States and northern Mexico, where it is commonly known as Mexican Palo Verde (MPV) (13). Previous studies have shown that MPV is able to reduce Cr(VI) to the less toxic Cr(III), as well as predict the impact of Cr species on MPV's macromolecular components (14, 15). However, there are no reports on either the uptake of Cr by MPV in the presence of phytohormones or the Cr distribution and antioxidant response of MPV under Cr(III) and Cr(VI) stress.

The objectives of this study were to address these questions and to determine MPV's ability to grow in Cr-contaminated soil. It is well known that KN stimulates cell division, leaf expansion, and chlorophyll synthesis, among others (7-9). In addition, exogenous KN increases plant transpiration (9) and metal phytoextraction. Thus it is hypothesized that KN can increase the movement and accumulation of Cr in the shoots of MPV. For these studies, an electron probe microanalyzer (EPMA) was used to map the Cr distribution in MPV tissues. Previous reports have shown the efficiency of the EPMA to map Cr in *Prosopis* (2)

and *Brassica juncea* (16). Cr uptake was measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES). CAT and APOX were determined by standard assays.

2. Methodology

This research was performed in two stages. The first stage was an exploratory experiment set to determine the total Cr uptake of MPV in soil with different concentrations of KN. The second stage was the evaluation of effects of the KN on antioxidant enzymes and of the Cr distribution in plant tissues.

2.1 Seed and Soil Treatment

MPV seeds were collected in El Paso, TX from a region with no reports on Cr contamination. All seeds were immersed in concentrated sulfuric acid for 3 h, rinsed and immersed in deionized water (DI) for 24 h. Seeds were sown in top soil (30 cm) taken from a place in El Paso with no report of metal contamination. The soil was previously dried for 48 h at 60 °C and sieved through a 2 mm mesh. The physio-chemical properties of the soil are described in Table S1 (17). The soil used was treated with 60 mg kg⁻¹ Cr(III) or 10 mg kg⁻¹ Cr(VI). These Cr concentrations were previously determined as the concentrations that stressed MPV without deterring its growth (15).

2.2 Plant Growth and Treatments

In the first stage, the response of MPV to Cr(III) or Cr(VI) in the presence of KN at different concentrations was determined. Results from Cr(VI) and Cr(III) were not compared because the Cr ions were used at different concentrations. For this exploratory part, 400 g of soil were placed in 9 general purpose plastic pots. Four were contaminated with Cr(III) [from a solution of Cr(NO₃)₃, pH 5.0 ± 0.1], 4 with Cr(VI) [from a solution of K₂CrO₄, pH 5.3 ± 0.1], and 1 was set as control (no Cr). Cr(III)-treated pots were allowed to sit for 15 days to permit the absorption and equilibration of Cr(III) in the soil prior to sowing (18). Cr(VI)-treated pots were sown with the MPV seeds the same day as the treatment's application since the Cr(VI) is reduced to Cr(III) swiftly in the environment (19). Each pot was planted with 15 MPV seeds and watered with a modified Hoagland nutrient solution (20). After germination, only 9 similar seedlings were allowed to grow in each pot. Fifteen days after seeding, DI and KN solutions were added to the soil. KN was used at 0, 100, 250, and 500 µM. Each pot was watered with 20 mL of DI or KN solution every other day. Plants were treated for 30 days before harvesting and were a total of 45 days old. Upon harvest, samples of three plants from each pot were considered as a replicate (three replicates/treatment). For plant size, each plant was measured from the main root apex to the crown and from the crown to the main shoot apex.

For the second stage of this research, plants were treated with 250 µM KN. This concentration was based on the previous experimental stage. The second series of experiments entailed the determination of CAT and APOX activities on plant tissues by standard assays, and the determination of the Cr distribution in MPV tissues by EPMA. For these experiments, 18 pots each filled with 1800 g of clean soil were used for the following treatments (three pots per treatment): universal control (only nutrient solution), control plus

250 μM KN solution, Cr(III) (60 mg kg^{-1}), Cr(III) (60 mg kg^{-1}) with 250 μM KN, Cr(VI) (10 mg kg^{-1}), and Cr(VI) (10 mg kg^{-1}) with 250 μM KN. As in the first stage, soil containing Cr(III) was allowed to sit for 15 days before seed planting. Thirty MPV seeds were sown in each pot and enriched with the modified Hoagland solution. Every other day they were watered with 90 mL of DI. To have enough biomass for all the analyses, after germination, weak seedlings were eliminated from each pot and 20 seedlings per pot were grown for further analyses. Fifteen days after seeding, all pots were watered every other day with 90 mL of either DI or KN solution.

For both series of experiments, pots were set in a room with controlled temperature (25 ± 2 °C), light/dark cycle of 12/12 h, and light intensity of $53 \text{ mol m}^{-2}\text{s}^{-1}$. Plants were harvested after 30 days of treatment application.

2.3 Determination of Cr Concentration in Plant tissue

For Cr concentration in tissues, plants were previously immersed for about 10 seconds in 0.01M HNO_3 , rinsed with DI, separated into roots, stems, and leaves, and oven dried at 60 °C for 72 h. After weighing, the dry samples were digested in a microwave oven (CEM MarsX, Mathews, NC) with 3 mL trace pure, concentrated HNO_3 (SCP Science, NY) and then diluted to 25 mL with double DI. Total Cr concentrations in digested samples of roots, stems, and leaves were determined by ICP-OES (Optima 4300 DV, Perkin Elmer, Shelton, CT).

2.4 Determination of Catalase Activity (CAT)

The activity of catalase in MPV was evaluated using a published process (21) with slight modification on sample preparation. For each treatment, 0.100 g of roots, stems, and leaves of MPV were homogenized with 900 L of 25 mM phosphate buffer (pH 7.4). Homogenates were centrifuged for 5 minutes at 14000 rpm on an Eppendorf centrifuge (Eppendorf AG, model 5417R, Hamburg, Germany). 50 L of the supernatant fraction and 950 L of 10 mM H_2O_2 were placed in a quartz cuvette. CAT activity was determined by monitoring the decrease of absorbance at 240 nm, as a consequence of H_2O_2 consumption, using a Cary 50 UV-Visible spectrophotometer (Varian, Palo Alto, CA). The extinction coefficient of H_2O_2 was $23.53 \text{ mM}^{-1} \text{ cm}^{-1}$. The enzyme activity was expressed as nmol of $\text{H}_2\text{O}_2 \text{ min}^{-1}$ per mg of decomposed protein. A standard of bovine serum albumin was used and the protein content from the extracted sample was determined by Bradford assay.

2.5 Determination of Ascorbate Peroxidase Activity (APOX)

The activity of ascorbate peroxidase in MPV was determined according to a process by Murguía et al. (22) with minor revision. Extracts of roots, stems, and leaves of MPV were prepared using the same procedure as that of the CAT assay. After centrifugation, 100 μL of the supernatant fraction, 886 μL of 0.1 M phosphate buffer (pH 7.4), 10 μL of 17 mM H_2O_2 , and 4 μL of 25 mM ascorbate solution were placed in a quartz cuvette. APOX activity was determined by measuring the decrease of absorbance at 265 nm using a Cary 50 UV-Visible spectrophotometer.

2.6 Electron Microprobe Analysis

For Electron microprobe analysis, plants were washed with 0.01M HNO₃, rinsed with DI, separated into roots and stems, and cryosectioned by using a minotome Plus™ (Triangle Biomedical Sciences, Durham, NC). The roots and stems were dissected into about 3 mm lengths, and then frozen onto sample holders enclosed by Tissue Tek (Sakura Finetek, Inc., Torrance, CA) at -40 °C. Roots and stems were cut; the 10 µm thick, frozen samples were thaw-mounted onto microscope slides (Kevley Technologies, Indianapolis, IN). An Ernest carbon evaporator (F. Fullam Incorporated, Latham, NY) was used to coat the carbon onto the slides' surface. The Cameca SX50 EPMA (Cameca S.A. Courbevoie Cedex, France) has four wavelength dispersive detectors and a state-of-the-art Rontec solid state energy dispersive detector. The probe has LIF, PET, TAP, and PC1 analyzing crystals, which can analyze most of the elements (from F to U) with detection limits as low as 100 ppm. The operating software of the SX50 is SX RAY N50 on Solaris 2. The back scattered electron image (BSE) and X-ray fluorescence mapping of roots and stems were acquired at a 15 keV accelerating voltage, 100 nA beam current, 5 µm beam size, and 20 s peak counting time. The scanning size was set based on the size of each sample.

2.7 Statistical Analysis

Data of total Cr concentrations, CAT, and APOX activities were analyzed with one-way analysis of variance using SPSS software, version 12.0 (SPSS, Chicago, IL). Significant differences between treatments were detected using the Tukey-HSD test. References to significant differences between treatment means were based on a probability of $p < 0.05$, unless otherwise stated.

3. Results and Discussion

3.1 Effect of Cr(III) or Cr(VI) Coupled with KN on MPV Growth

Compared to control plants, as seen in Figure 1A SI, 60 mg kg⁻¹ Cr(III) alone and Cr(III) coupled with different concentrations of KN did not produce toxicity symptoms or phenotypic changes such as necrosis and chlorosis. On the other hand, as seen in Figure 1B SI, MPV exhibited necrosis, chlorosis, and stunting under Cr(VI) treatments. The quantitative data (Figure 1C SI) showed that none of the Cr(III) treatments affected root elongation. Meanwhile, the shoots were longer (13.5 cm) at 100 µM KN and shorter (8.5 cm) at 500 µM KN, compared to Cr(III) treatment alone (11.5 cm). For the series of Cr(VI)-treated soil, as seen at Figure 1D SI, the length of the root was between 1.5 and 2 cm in all treatments (no significant difference). The shoot length (average of 5 cm) was similar for all treatments of Cr(VI) alone. This result, in agreement with Lopez et al. (23) and Tanimoto (24), showed that KN did not have a significant effect on the elongation of the roots and shoots.

3.2 Effect of KN on Total Cr Uptake by MPV

The total Cr concentration in roots, stems, and leaves of MPV exposed to 60 mg kg⁻¹ Cr(III) or 10 mg kg⁻¹ Cr(VI) at different concentrations of KN are shown in Figure 1. As seen in Figure 1(A-B), the total Cr accumulation in roots, stems, and leaves increased at all KN concentrations. In all cases, Cr accumulation significantly increased compared to Cr(III) or

Cr(VI) alone, except in roots treated with Cr(III) and 100 μM KN, and in leaves treated with Cr(III) and 500 μM KN. Plants treated with Cr(III) plus 250 μM KN increased their total Cr concentration in roots, stems, and leaves by 45%, 103%, and 72%, respectively, compared to Cr(III) alone. For Cr(VI) with 250 μM KN, the total Cr concentration in roots, stems, and leaves increased by 53%, 129%, and 168%, respectively, compared to Cr(VI) alone. Moreover, as seen in Table 2 SI, the highest translocation factors (TF) were obtained at 250 μM KN for the Cr(III) treated plants and at 500 μM KN for Cr(VI) treated plants. Based on the overall results, 250 μM KN had induced the best effect for Cr absorption and was the concentration selected for the next experimental stage. Kinetin promotes cell division (25) and increases the number of microtubules (26).

The increase in Cr accumulation by plants could be related to a stimulation of cell division promoted by KN that not always includes plant elongation. KN has been also associated to an increase in transpiration that improves the uptake of water and dissolved contaminants (9). In duckweed plants, KN increased the production and changed the quality of proteins (27). In MPV, previous results showed that Cr increased protein production in cortex and xylem (15). Such cases might also be responsible for the high increases of Cr concentration in MPV treated with KN. Previous studies have shown that in MPV, the Cr ions are in an octahedral conformation bound to six oxygen atoms. In addition, the XAS data showed an absence of Cr(VI) in shoots and leaves due to reduction to Cr(III) in the roots (14). Most likely, this is the main Cr(VI) detoxification mechanism in MPV.

3.3 Effect of KN on CAT

The activity of CAT in roots, stems, and leaves of MPV treated with Cr(III) at 60 mg kg^{-1} or Cr(VI) at 10 mg kg^{-1} plus different KN concentrations is shown in Figure 2A. As seen in this figure, the response to treatments was different in every plant organ. For instance, none of the Cr(III) treatments or KN changed CAT activity in roots; all of them reduced CAT in stems, while KN, alone or combined with Cr(III), reduced CAT in leaves compared to the control and Cr(III) treatment. Moreover, in leaves, CAT activity in plants treated with Cr(III) alone reached 40 $\mu\text{mol mg}^{-1}\text{min}^{-1} \text{H}_2\text{O}_2$ (similar to control), but in plants treated with Cr(III) plus KN, CAT was less than 10 $\mu\text{mol mg}^{-1}\text{min}^{-1} \text{H}_2\text{O}_2$.

As expected, Cr(VI) produced a significant increase on CAT activity in MPV. As seen in Figure 2A, only in roots, the addition of KN reduced CAT activity generated by Cr(VI). However, the reduction was not enough to reach the activity observed in the control. The increase in CAT activity in Cr(VI) treated roots is attributed to a defending response to oxidative stress produced by Cr(VI) (Figure 2A). However, as seen in this figure, CAT activity was statistically reduced by KN, which means that KN alleviates the toxicity of Cr(VI) in MPV roots. As explaining above, this was seen only in roots, corroborating previous XAS results that did not show Cr(VI) in stems and leaves. It has been reported that heavy metals increase CAT activity as a result of ROS formation inside plant tissues (28, 29). Shanker et al. (30) concluded that the increase in antioxidant enzymes activity could be a response to the generation of the superoxide radical by Cr-induced blockage of the electron transport chain in the mitochondria. As an adaptive mechanism, antioxidative enzymes, such as CAT, are correspondingly induced for coping with ROS. However, in the

present study, CAT activity in roots treated with KN alone at 250 μ M, Cr(III) alone, and Cr(III) with 250 μ M KN showed no significant difference compared to control roots, indicating that Cr(III) has no toxicity on the MPV root. The decreasing activity of CAT in stems and leaves might be due to the inhibitory effect of Cr ions on the enzyme system itself (30). It has also been predicted that Cr(III) can decrease the availability of Fe in protoporphyrins, confining heme biosynthesis, which causes a decrease in CAT activity in Cr(III)-stressed plants (31). In other plants like alfalfa, KN increases the activity of CAT to reduce Pb stress (32).

3.4 Effect of KN on APOX

The APOX activity in roots, stems, and leaves of MPV under the investigated treatments is shown in Figure 2B. As seen in this figure, the mere addition of KN at 250 μ M significantly increased APOX activity in MPV roots and stems but reduced it in leaves. This could happen due to the cell division promotion and radial enlargement of the cells produced by KN (33). The addition of KN also increased ($p < 0.05$ at root level) APOX activity for Cr(VI)-treated plants. As shown in Figure 1B, Cr(VI) plus KN treated plants had a higher amount of Cr in tissues, which suggests that APOX has a fundamental role for Cr detoxification in MPV. Chromium(VI) has been reported to raise lipid peroxidation, resulting in ROS formation (29). APOX could be correspondingly induced for coping with ROS. The decrease in APOX activity by Cr(VI) in leaves could result from the attack caused by metal ion-induced oxygen species (21). The decrease in the activity of APOX could be due to a decline in enzyme synthesis, or a change in the assembly of enzyme subunits under Cr stress (12, 30). Also, it could be due to the negative effect by Cr on the heme biosynthesis, which led to the reduction of APOX activity in plants (31).

3.5 Effect of Treatments on Chromium distribution in MPV

The EPMA, BSE, and X-ray fluorescence mapping images for control plants and plants treated with KN are shown in Figure 2 SI; while the images corresponding to Cr treatments in MPV roots and stems are shown in Figures 3 and 4. The intensity of the spots shown on the X-ray mapping images corresponds to the concentration of Cr in tissues.

Figure 2 SI shows the cross-section BSE image of the roots and stems of MPV under control and 250 μ M KN treatments. As seen in Figures 2C and D SI, the tissue structure of the 250 μ M KN-treated root and stem appeared more compact compared to the control plants (Fig 2A and B SI) since the KN promotes cell division (25) and an increase in the number of microtubes (26).

Figure 3 show the longitudinal BSE and X-ray image of roots and stems treated with 60 mg kg^{-1} Cr(III) alone and Cr(III) with KN at 250 μ M. As shown in the X-ray mapping for the roots' images (Figures 3B and 3F), most of the Cr is located in the cortex section. Previous results have already shown that Cr(III) produced an increase in root and stem cortex lignin in MPV, which is proposed as a Cr storage compound (15). Bluskov et al. (16) reported that the sites for Cr localization are in epidermal and cortical cells in the roots of *Brassica juncea* (Indian mustard). It had also been reported that Cr in *Leersia hexandra* Swartz was preferentially stored in the cell walls of the roots (34). Generally, epidermal and cortical

cells in roots contain higher amounts of metals than phloem and xylem, due to the Casparian strip, which is the barrier inside the endodermis (35). However, as seen in Figures 3D and 3H, the Cr distribution within the stem was essentially homogenous. Moreover, comparing Figures 3B and 3F to Figures 3D and 3H, it can be seen that there was higher deposition of Cr in roots than in stems since the X-ray image of the roots was brighter and showed a higher intensity compared to stems. These images confirmed the higher Cr accumulation in roots than in stems for Cr(III) treatments. This result is also in agreement with the ICP-OES results (Figure 1A) that showed total Cr concentrations of 695 and 1010 mg kg⁻¹ DW in roots, and 51 and 104 mg kg⁻¹ DW in stems for the treatments of Cr(III) alone and Cr(III) with KN, respectively. Although the X-ray mapping shows low Cr concentration in stems, Figure 3H shows that Cr(III)-KN treated plants had a higher Cr concentration compared to plants treated with Cr(III) alone. This result proves that the phytohormone KN increased Cr translocation from roots to stems in MPV.

In general, the Cr distribution in MPV under Cr(VI) treatment showed similar trends as in the Cr(III)-treated plants. Figure 4 show the longitudinal BSE and X-ray images of roots and stems grown in soil containing 10 mg kg⁻¹ Cr(VI) alone or Cr(VI) with KN at 250 µM. As in the case of Cr(III)-treated plants, the distribution of Cr in roots was mostly located in the cortex section (Figures 4B and 4F). However, as seen in Figures 4D and 4H, the Cr distribution in the stem was essentially homogenous with less density of Cr compared to the root. The X-ray images shown in Figure 4 also show that the roots and stems of Cr(VI)-treated plants had more Cr accumulation than the corresponding part in Cr(III)-treated plants. The ICP results (Figure 1B) corroborated these results: the total Cr concentrations were 2927 and 4465 mg kg⁻¹ DW in roots, and 373 and 852 mg kg⁻¹ DW in stems for the treatments of 10 mg kg⁻¹ Cr(VI) alone and Cr(VI) with KN, respectively. Comparing Figures 4D and 4H, it can be seen that KN increased the translocation of Cr from roots to stems in Cr(VI)-treated MPV plants. KN could have caused the increase in protein content and cell division, which might be responsible for the increase of Cr uptake in MPV (25, 27).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This material is based upon work supported by the National Science Foundation and the Environmental Protection Agency under Cooperative Agreement Number EF 0830117. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation or the Environmental Protection Agency. J. Gardea also acknowledges the USDA grant # 2008-38422-19138, the LERR and STARs programs of the UT System, the Toxicology Unit of the BBRC (NIH NCRR Grant # 2G12RR008124-16A1), the NSF Grant # CHE-0840525, and the Dudley family for the Endowed Research Professorship in Chemistry.

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Brief

Kinetin increases chromium absorption and distribution in Mexican Palo Verde
(*Parkinsonia aculeata*)

Figure 1.

Total Cr concentrations in roots, stems, and leaves of Mexican Palo Verde grown in soil for 45 days with (A) Cr(III) at 60 mg kg⁻¹ or (B) Cr(VI) at 10 mg kg⁻¹ and treated for 30 days with kinetin at 0, 100, 250 or 500 μM. Error bars represent SE. Lowercase letters stand for significant differences at $p < 0.05$.

Figure 2.

(A) Catalase and (B) ascorbate peroxidase activities in roots, stems, and leaves of 45 day old Mexican Palo Verde seedlings grown in soil containing Cr(III) at 60 mg kg⁻¹ or Cr(VI) at 10 mg kg⁻¹ and kinetin at 0 or 250 μM. Error bars represent SE. Lowercase letters stand for significant differences at $p < 0.05$.

Figure 3.

Back scattered electron image (BSE) and Cr X-ray mapping of roots and stems of Mexican Palo Verde grown for 45 days in soil containing Cr(III) at 60 mg kg^{-1} . The bottom row of figures shows images of the same Cr(III) treatment plus $250 \text{ }\mu\text{M}$ of kinetin for 30 days. From top left to right, longitudinal view (A) BSE of root, (B) Cr X-ray mapping of root, (C) BSE of stem, and (D) Cr X-ray mapping of stem. From bottom left to right, longitudinal view (E) BSE of root, (F) Cr X-ray mapping of root, (G) BSE of stem, and (H) Cr X-ray mapping of stem. The intensity of the spots shown on the X-ray mapping images corresponds to the relative concentration of Cr in tissues.

Figure 4.

Back scattered electron image (BSE) and Cr X-ray mapping of roots and stems of Mexican Palo Verde grown for 45 days in soil containing Cr(VI) at 10 mg kg^{-1} . The bottom row of figures shows images of the same Cr(VI) treatment plus $250 \mu\text{M}$ of kinetin for 30 days. From top left to right, longitudinal view (A) BSE of root, (B) Cr X-ray mapping of root, (C) BSE of stem, and (D) Cr X-ray mapping of stem. From bottom left to right, longitudinal view (E) BSE of root, (F) Cr X-ray mapping of root, (G) BSE of stem, and (H) Cr X-ray mapping of stem. The intensity of the spots shown on the X-ray mapping images corresponds to the relative concentration of Cr in tissues.