



Phytotoxicity of zinc, chromium (VI) and cadmium in purging nut (*Jatropha curcas*) seedlings grown in hydroponics

SOMEN ACHARYA¹, D K SHARMA² and H C JOSHI³

Indian Agricultural Research Institute, New Delhi 110 012

Received: 11 May 2011; Revised accepted: 23 April 2012

ABSTRACT

This study investigated physiological, morphological, biochemical and metal uptake patterns in purging nut (*Jatropha curcas* L.) seedlings exposed to zinc (Zn), chromium (Cr) and cadmium (Cd) in a hydroponic system. Uptake of metals in all parts of the seedlings was found to be highest for Zn, followed by Cd and Cr. Catalase (CAT) and superoxide dismutase (SOD) activities were increased on exposure to heavy metals, but 5 mM Cd and Cr significantly inhibited SOD activity compared to Zn. This may reflect damage to the plant due to lipid per-oxidation as shown by a decrease in the % membrane stability index at higher metal concentrations. With increase in concentration of all the heavy metals tested, there was a significant decrease in chlorophyll content, however there was a stimulatory effect at low Zn concentrations (up to 0.5 mM). Root length and surface area were both significantly decreased with increasing metal concentration.

Key words: Heavy metals, Hydroponics, *Jatropha curcas*, Oxidative stress, Phytotoxicity

Due to the energy crisis and escalation of petroleum prices, alternate energy sources are gaining importance, especially to the countries like India which consumes large amount of fossil fuels and rely heavily on import for the same (Dhyani *et al.* 2011). The concept of substituting bio-diesel produced from plantations on wastelands for conventional diesel fuel has gained widespread attention in India. In recent years, the Government of India, as well as some state governments, has expressed its support for bringing marginal lands, which cannot be used for food production, under cultivation for this purpose (Sharma *et al.* 2009). A large area in India is categorized as wasteland, e.g degraded land, saline soils etc. (India 2000). These wastelands can be brought under cultivation of biodiesel plants in general, and purging nut (*Jatropha curcas* L.) in particular, without any major hindrance. The Government of India has announced a 'National Policy on Biofuels' to meet the country's energy demand which emphasis on biodiesel production from non-edible oil seed like *J. curcas* in waste/degraded/marginal lands (Dhyani *et al.* 2011).

Heavy metal pollution is one of the most serious environmental problems and has been a subject of extensive research in recent years (Prasad *et al.* 2006). Heavy metal

contamination affects the biosphere in many places worldwide. Though some heavy metals are essential as micronutrients, uptake and accumulation of metals at higher concentrations can be cytotoxic in some plant species, causing structural and ultra-structural changes affecting the growth and physiology of the plants (Han *et al.* 2004). Oxidative stress caused by the toxic reactive oxygen species (ROS), which include the superoxide anion (O_2^-), hydroxyl radical (OH^\cdot) and hydrogen peroxide (H_2O_2), is one of the major damaging factors in plants exposed to environmental stress. Heavy metal toxicity in plants not only induces oxidative stress linked to oxidation of proteins and membrane lipids, but also causes alterations and damage of DNA (Fahad 2009). Most of the metal uptake studies have been conducted using seedlings or adult plants in soil with different amendments and different metal concentrations (Pulford *et al.* 2002). The present investigation was carried out to study the induction of phytotoxicity, metal uptake potential and oxidative stress in *J. curcas* seedlings subjected to heavy metal stress under hydroponic conditions.

MATERIALS AND METHODS

Seeds required for this experiment (2005–06) were collected from an experimental field at the Indian Agricultural Research Institute (IARI). The seeds were immersed in 3% v/v formaldehyde solution for 2 min. to avoid fungal contamination. Seeds were sown in plastic pots in sand culture and kept it there for about one month. The one-

¹ Scientist (e mail: someniari@gmail.com), Defence Institute of High Altitude Research (DIHAR), C/O 56 APO, Leh-Ladakh-901205 (J&K), India.

² Senior Scientist (e mail: dks_env@rediffmail.com), ³ Head (e mail: hcj_env@yahoo.com), Division of Environmental Sciences

month-old seedlings were taken to the laboratory for growing in hydroponics Hoagland's solution (Hoagland and Arnon 1950). The seedlings were kept in this solution for 15 days for acclimatization. Metal stress was given in the form of $ZnSO_4$, $Cd(NO_3)_2 \cdot 4H_2O$ and $K_2Cr_2O_7$ solutions at different concentrations (0.1 mM, 0.5 mM, 1.0 mM and 5.0 mM). After six weeks of treatment, plant materials were taken for metal uptake study, biochemical and enzyme estimation.

The following weights of the salts were dissolved in 1 l distilled water: 1.532 g K_2SO_4 , 2.4 g $MgSO_4$, 0.34 g KH_2PO_4 , 0.08 g KCl, 4.724 g $Ca(NO_3)_2$.

Micronutrient (Solution B + Solution C):

Solution B: (for one litre)

The following weights of the salts were dissolved in 1 l distilled water: 2.86 g Boric acid, 1.81 g $MnCl_2$, 0.22 g $ZnSO_4$, 0.08 g $CuSO_4$, 0.02g $H_2MoO_4 \cdot H_2O$.

Solution C: 0.5% ferric ammonium citrate solution.

If solution C is prepared along with solution B precipitation will occur, so these solutions were prepared separately. 100 ml solution A, 1ml solution B and 1 ml solution C were mixed and volume was made up to 1 litre with distilled water.

To determine Cd, Cr and Zn, the plants were separated into root, shoot and leaves then oven-dried at 70 °C for two days. The dried samples were then digested in 20 ml di-acid mixture (nitric and perchloric acids in a 9:4 ratio) in a conical flask in a heating block at 100°C until the solution became clear. The sample volume was raised to 50 ml by adding double distilled water. The concentrations of total heavy metal (Zn, Cd and Cr) in the digested plant samples (in roots, shoots and leaves) were measured by Atomic Absorption Spectrophotometry (Model No: AAS4141) using standard solution of Zn, Cd and Cr against unknown samples of plant materials.

Extraction and assay of enzymes was done by homogenizing the plant leaves in 0.1 M phosphate buffer (pH 6.8) in a pre-chilled mortar and pestle under cold conditions. The extract was centrifuged at 4 °C for 15 min. at 14 000 g in a cooling centrifuge. The supernatant was used for the assay of catalase (CAT) and superoxide dismutase (SOD) activity. CAT was assayed according to method described by Chance and Maehly (1955). 5 ml of the assay mixture of CAT comprised 3 ml of 0.1 M phosphate buffer (pH 6.8), 1 ml of 30 mM H_2O_2 and 1 ml of the enzyme extract. The reaction was stopped by adding 10 ml of 2% H_2SO_4 after 1 min incubation at 20 °C. The acidified reaction mixture was titrated against 0.01 M $KMnO_4$ to determine the quantity of H_2O_2 utilized by the enzyme.

Assay of SOD was done according to the method described by Beauchamp and Fridovich (1971). The SOD activity was estimated by recording the decrease in absorbance of formazan produced by superoxide and nitro-

blue tetrazolium by the enzyme. The assay mixture was prepared in test tubes and comprised methionine solution (200 mM), nitro-blue tetrazolium solution (1.125 mM), phosphate buffer (50 mM; pH 7.0), phosphate buffer (50 mM; pH 7.8) and riboflavin solution (75 µM). Riboflavin was added as the last component and the test tubes were shaken and placed 30 cm. below a light bank consisting of two 15-w fluorescent tubes. Then the reaction was started by switching on the light and was allowed to run for 10 min. Reaction was stopped by switching off the light and putting the test tubes into the dark immediately. A non-irradiated complete reaction mixture served as blank. Absorbance of the reaction mixture was measured at 560 nm. The total chlorophyll content was determined spectrophotometrically according to the DMSO method as described by Hiscox and Israelstam (1979).

The MSI was estimated using the method described by Sairam *et al.* (1997). Plant material (0.1 g) was taken in 10 ml of doubled distilled water in two sets. One set was subjected to 40° C for 30 min. and its conductivity was recorded using a conductivity meter (C_1). A second set was kept in a boiling water bath (100 °C) for 10 min. and its conductivity was recorded (C_2). The MSI was calculated using the following formula: $(MSI) = [1 - (C_1/C_2)] \times 100$. Leaf relative water content (RWC) was estimated according to Wheatherly (1950). Leaves of known fresh weight were taken and floated in distilled water in Petri-plates for about 4 hr. Then turgid weights of the samples were measured and the leaf samples were dried in an oven at 100 °C. RWC was calculated as follows: $RWC = [(fresh\ weight - dry\ weight) / (turgid\ weight - dry\ weight)] \times 100$.

The root morphology of *Jatropha* seedlings after harvesting from hydroponics were studied by Win-Rhizo, which is an image analysis software specifically designed for root measurement in different forms (total root length, average root diameter and number of root tips). Before analyzing the root morphology, the plant roots were washed free of soil and debris.

The data were analyzed through one-way analysis of variance (ANOVA) to determine the effect of treatments, and least significant difference (LSD) tests were performed to determine the statistical significance of the differences between means of treatments by Duncan's multiple range test at the 0.05 probability level ($P < 0.05$) using SPSS statistical software (SPSS for Windows, Release 12).

RESULTS AND DISCUSSION

Jatropha seedlings subjected to heavy metal stress in a hydroponics system showed signs of toxicity within one week in the case of 1.0 and 5.0 mM Cd and Cr, whereas plants grown in Zn solutions were more vigorous. Concentration of Zn in all plant parts (Fig 1) increased with increasing Zn in solution. The Cr concentration in roots increased with increasing solution concentration up to 1

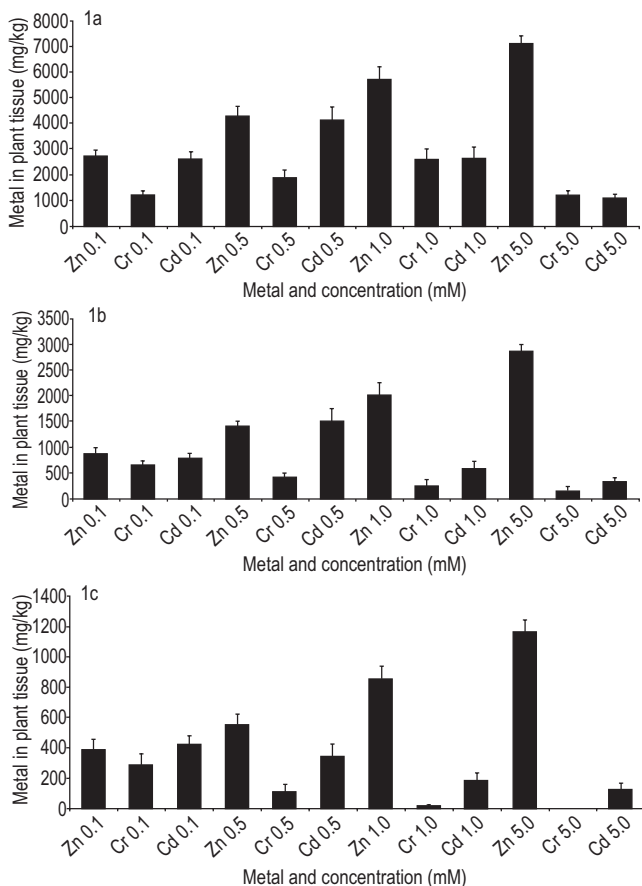


Fig 1 Metal accumulation in (a) roots, (b) shoots, and (c) leaves of *Jatropha curcas* after growing for six weeks in hydroponics under metal stress condition

mM, but declined at 5 mM Cr: in shoots and leaves however the Cr concentration decreased with increasing solution concentration. A similar pattern was seen with Cd; an increase in tissue concentration in roots and shoots up to 0.5 mM Cd in the growth medium, with a decrease at the two higher concentrations. The Cd concentration in leaf tissue progressively decreased going from 0.1 mM to 5.0 mM in solution. This metal uptake pattern into different plant parts at different concentrations of applied metal confirms the known greater toxicity of Cr and Cd compared to Zn. These two more toxic metals tend to be accumulated in root tissue in order to prevent translocation to the aerial parts of the plant, but beyond a certain critical concentration (1.0 mM Cr and 0.5 mM Cd in this case) they become toxic to the plant. This effect was particularly pronounced for Cr, with plants subjected to 1 mM and 5 mM solutions of Cr showing very little or no uptake into the shoots and leaves. The poor translocation of Cr from roots to shoots is a major hurdle in using plants and trees for phytoremediation. Pulford and Watson (2003) in a study with temperate trees confirmed that Cr was poorly taken up into the aerial tissues but was held predominantly in the root. Effect of variable levels of Cd on

fresh and dry biomass yield and Cd tolerance and accumulating capacity study was also evaluated with amaranthus cultivars under greenhouse condition (Varalakshmi and Ganeshamurthy 2009).

The activity of both CAT and SOD increased in *Jatropha* seedlings with increasing metal concentration in solution (Fig 2). Zn increased the activity of both enzymes at 1 mM and 5 mM, but there was not significant change in activity at 0.1 mM and 0.5 mM compared to the control treatment. Both Cr and Cd caused an increase in CAT and SOD activity up to a solution concentration of 1 mM, but the activity of both enzymes decreased when plants were subjected to solutions of 5 mM Cr and Cd. This effect was seen particularly with SOD (Fig 2b). Production of the antioxidant enzymes by plants like CAT and SOD increases in response to the production of ROS brought about by stress (Singh *et al.* 2010). The decrease of antioxidant enzyme activity can be explained by disturbed oxidation-reduction balance caused by a high level of ROS. Assimilation of toxic amounts of Cd and Cr in the plant from 5 mM solutions may have caused oxidative damage and inhibited plant growth at higher concentration.

Fig 3a shows the effect of heavy metals on the chlorophyll content of *J. curcas* seedlings grown in hydroponics. With increasing concentration of Cr and Cd there was a significant

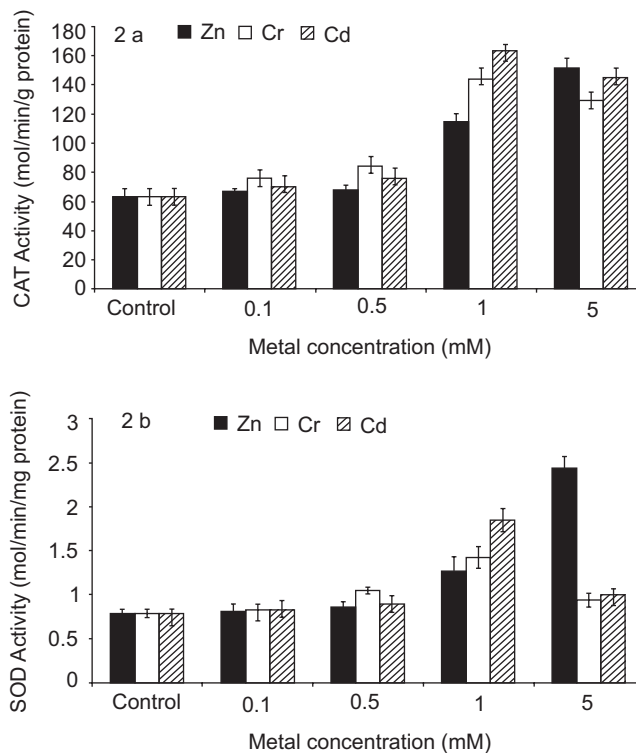


Fig 2 Effect of metal stress on the activity of (a) catalase (CAT), and (b) superoxide dismutase (SOD) of *Jatropha curcas* seedling after growing for six weeks in hydroponics under metal stress condition

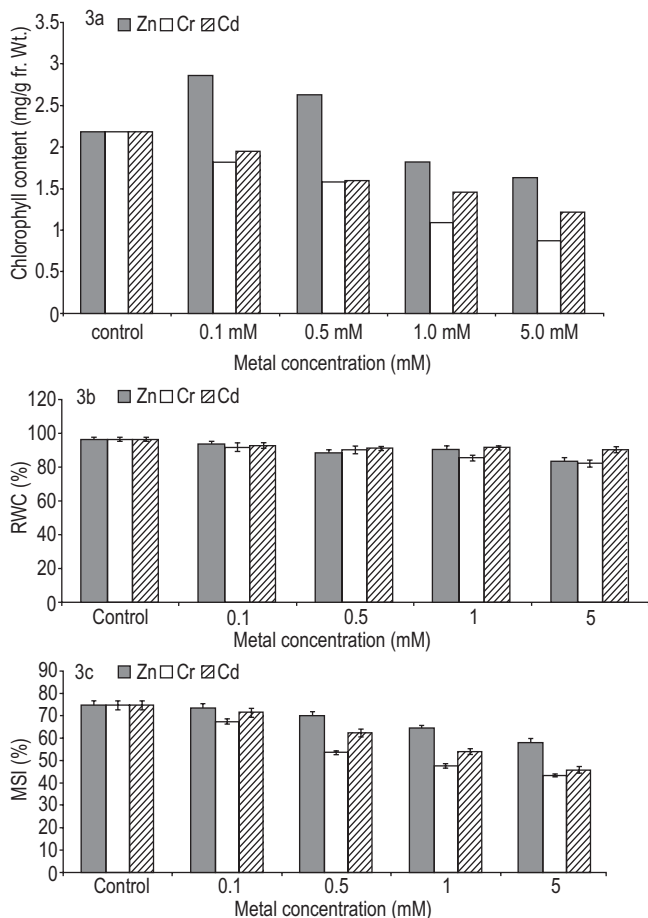


Fig 3 Influence of different concentrations of heavy metals on (a) chlorophyll content, (b) relative water content (RWC), and (c) membrane stability index (MSI) of *Jatropha curcas* seedling

decrease in chlorophyll content. However, 0.1 and 0.5 mM Zn stimulated chlorophyll content. Cd and Cr have been proven to be aggressive, oxidative damage inducing agents and effective competitors for essential metal cofactors participating in chlorophyll biosynthesis. Zn at high concentration also induces oxidative damage but at low concentrations it acts as stimulatory factor for chlorophyll production. The decline in chlorophyll content in plants exposed to heavy metals is believed to be due to inhibition of important enzymes, such as δ -aminolevulinic acid dehydratase and protochlorophyllide reductase associated with chlorophyll biosynthesis.

Leaf relative water content (RWC) and Membrane Stability Index (MSI) of *Jatropha* seedlings were also analyzed under different metal stress conditions (Figs 3b, c). The changes in RWC were small, there being only a slight decrease in metal-treated plants compared to the control. The greatest effect was found with Cr. The MSI value decreased with increasing metal concentration in solution, with the greatest effect caused by Cr and Cd. Under environmental

stresses plant membranes are subject to changes often associated with the increases in permeability and loss of integrity (Blokhina *et al.* 2003).

Total root length and root surface area of *Jatropha* seedlings showed significant differences under the lowest concentrations of all metal stress (0.5 mM of Cd, Cr and Zn) (Fig 4). The mean total root length for control plants was 224.4 cm. From Fig 4a, it is clearly shown that the Zn stressed plants significantly reduced the root length, but the values were not significantly different between all the concentrations. For Cd and Cr treatments, root length decreased with increasing metal concentration, with plants subjected to 5 mm Cd having the shortest roots (mean 93.23 cm). Fig 4b shows that for all metal stress conditions root surface area was decreased significantly. The severest effect was seen at the highest concentration of Cd (mean value 37.83 cm² against 67.96 cm² in control plants). The effect of metals on root length and surface area was in the order Cd>Cr>Zn. Prasad *et al.* (2001) reported that the order of metal toxicity to new root primordia in *Salix viminalis* is Cd>Cr>Pb, whereas root length was more affected by Cr than by other heavy metals studied. A greater impact of heavy metals was observed on root growth as compared to shoots, leading to a greater reduction in its length and fresh

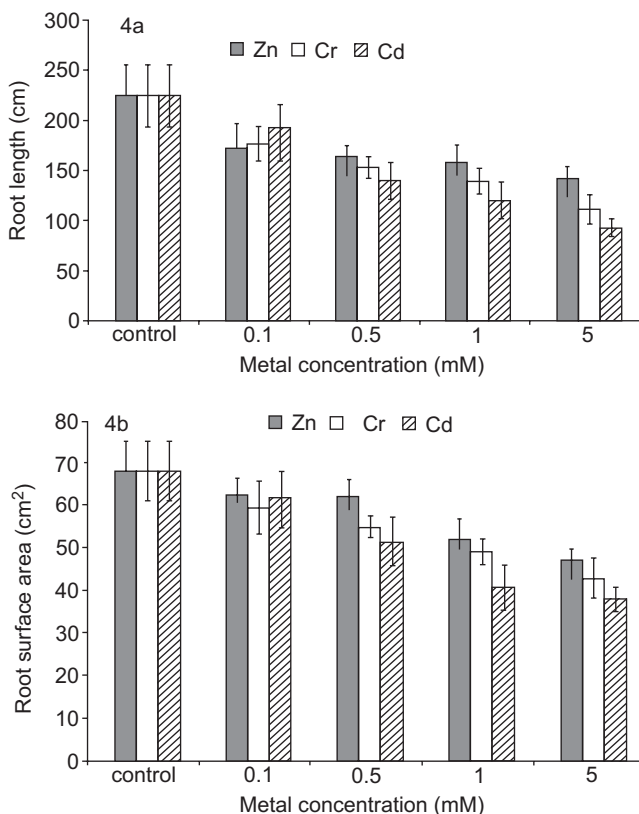


Fig 4 Influence of heavy metals on (a) root length, and (b) root surface area of *Jatropha curcas* seedlings measured by Winrhizo

weight. Under high concentrations of metal stress given, the reduction in root growth could be due to the direct contact of seedling roots with metals in the medium causing a collapse and subsequent inability of the roots to absorb water from the medium.

It can be concluded that imposition of heavy metal stress induces concentration dependent phytotoxicity and oxidative damage in *J. curcas*, as evidenced by metal uptake, biochemical changes and antioxidant activity. Although *Jatropha* has been considered to be a hardy plant species that could tolerate heavy metal contamination, these results show that Cd and Cr at high concentration in solution can cause visible plant injury symptoms due to stress. High concentrations of zinc resulted in less stress and therefore healthier plants, and so *Jatropha* may be grown in soils with a moderate degree of zinc contamination, but further studies under field conditions need to be performed in order to establish the maximum amount of Zn that the plants may tolerate.

REFERENCES

- Beauchamp C and Fridovich I. 1971. Superoxide dismutase: Improved assays and assay applicable to acrylamide gels. *Analytical Biochemistry* **44**: 276–87.
- Blokhina O, Virolainen E and Fagerstedt K V. 2003. Antioxidants, oxidative damage and oxygen deprivation stress. *Annals of Botany* **91**: 179–94.
- Chance B and Maehly A C. 1955. Assay of catalase and peroxidase. *Methods in Enzymology* **2**: 746–78.
- Dhyani S K, Kumar R V and Ahlawat S P. 2011. *Jatropha curcas*: a potential biodiesel crop and its current R&D status. *Indian Journal of Agricultural Sciences* **81** (4): 295–308.
- Fahad A Q. 2009. Toxicity of heavy metals and their molecular detection on *Phaseolus vulgaris* L. *Australian Journal of Basic and Applied Sciences* **3**(3): 3025–35.
- Han F X, Maruthi S B B, Monts D L and Su Y. 2004. Phytoavailability and toxicity of trivalent and hexavalent chromium to *Brassica juncea* L. Czern. *New Phytologist* **162**: 489–99.
- Hiscox J D and Israelstam G F. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany* **57**: 1332–4.
- Hoagland D R and Arnon D I. 1950. The water-culture method for growing plants without soil. *California Agricultural Experiment Station Circular No. 347*, 1–32.
- India. 2000. *Wasteland Atlas of India*. Department of land Resources and National Remote Sensing Agency, Ministry of Rural Development, Government of India, New Delhi.
- Prasad M N V, Greger M and Landberg T. 2001. *Acacia nilotica* L bark removes toxic elements from solution: corroboration from toxicity bioassay using *Salix viminalis* L in hydroponic system. *International Journal of Phytoremediation* **3**: 289–300.
- Prasad M N V, Sajwan K S and Naidu R. 2006. *Trace Elements in the Environment*. CRC Press, Boca Raton, Florida.
- Pulford I D, Riddell-Black D and Stewart C. 2002. Heavy metal uptake by willow clones from sewage sludge-treated soil: The potential for phytoremediation. *International Journal of Phytoremediation* **4**(1): 59–72.
- Pulford I D and Watson C. 2003. Phytoremediation of heavy metal-contaminated land by trees—a review. *Environment International* **29**: 529–40.
- Sairam R K, Deshmukh P S and Shukla D S. 1997. Tolerance to draught and temperature stress in relation to increased antioxidant enzyme activity in wheat. *Journal of Agronomy and Crop Science* **178**: 171–7.
- Sharma D K, Pandey A K and Lata. 2009. Use of *Jatropha curcas* hull biomass for bioactive compost production. *Biomass and Bioenergy* **33**: 159–62.
- Singh R, Tripathi R D, Dwivedi S, Kumar A, Trivedi P K and Charabarty D. 2010. Lead bioaccumulation potential of an aquatic macrophyte *Najas indica* are related to antioxidant system. *Bioresource Technology* **101**(9): 3025–32.
- Varalakshmi L R and Ganeshamurthy A N. 2009. Effect of cadmium on plant biomass and cadmium accumulation in amaranthus (*Amaranthus tricolor*) cultivars. *Indian Journal of Agricultural Sciences* **79** (11): 861–4.
- Wheatherly P E. 1950. Studies in the water relations of cotton plants: The field experiment of water deficit in leaves. *New Phytologist* **49**: 81–7.