# Graviperceptional changes in the roots of cadmium-treated soybean seedlings

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**Plant roots are the main organs through which water, nutrients and pollutants enter the plant body. The roots of soybean plants exhibited absence of root caps and damaged roots when treated with heavy metal cadmium beyond 20 μM. Normally, the roots perceive gravity and grow towards it; but, with heavy metal exposure they become gravi-irresponsive and start growing against the gravity and law of nature. As the graviperception sensors lie in the root cap, roots lose directional growth in the absence of root caps and keep growing in the direction of hilum positioning inside the substratum. So far, no study has reported that heavy metals can cause loss of graviperception in the roots of plants.**

**Keywords:** Cadmium, graviperception, heavy metals, root, soybean.

ENVIRONMENTAL pollution has become a big menace to the human race these days. Heavy metals are causing widespread contamination of soil and groundwater resources. Their continuous inflow into the terrestrial food chain via agricultural crops is a serious concern[1]. Cadmium (Cd) is one such heavy metal which enters the environment through human activities[2]. Cd disturbs the cellular redox environment of the root causing oxidative stress[3]. Cd-induced generation of reactive oxygen species (ROS) inhibits plant growth and suppresses the root system severely[4]. Cd interferes with water uptake and mineral nutrients through the root system[5] and affects major physiological processes such as transpiration, stomatal conductance and exchange of gases[6] leading to a significant rise in the leaf temperature[7]. In most of the species, a large proportion of Cd gets deposited in the roots and only a small fraction is translocated to upper portions. Many reports indicate induction of metabolic and physiological changes by toxicity of this metal and adaptation by tolerant species. Most of the environmental parameters vary in quality and intensity with the notable exception of gravity which remains basically the same in value as on Earth[8]. Not much information is available specifying the recognized situation that links the signalling pathway with initial stimuli to long-term biological responses damaging the root system. We have aimed to study the rhizogenetic growth profile of seedlings exposed to Cd stress and the long-term effect of Cd on agricultural crops grown in heavy metal polluted soils.

Soybean (*Glycine max* (L.) Merr. cultivar PK-416) seeds were procured from the Plant Breeding Department, Punjab Agricultural University, Ludhiana, India. The plants were grown in acid-washed sand in the laboratory using small plastic pots (capacity: 500 g sand) and were supplied with different doses of Cd from the beginning of the trial along with nutrient (+N) medium[9]. In the second set, soybean seeds were sown and allowed to germinate in washed sand for 12 days and then the seedlings were shifted to hydroponic cultures kept under controlled conditions (temperature 25 ± 2°C, R.H. 80–90%, photoperiod 16/8 h). The hydroponic bottles (capacity 500 ml, Tarson make) contained 500 ml of 1/20 nutrient (+N) solution. The plants were allowed to acclimatize for 3 days before Cd (4, 8, 12, 16 and 20 μM) treatment. This solution was replaced every third day so as to maintain the required level of Cd in the solution culture. The rooting data was recorded after 15 days of growth. Three to five replicates were selected for each sampled parameter. Standard growth formulae were used for determining tolerance index[10], phytotoxicity index[11], vigour index (VI)[12] and relative root length (RRL) ratio[13] in studies related to growth and morphology. All the results were analysed statistically using one-way analysis of variance (ANOVA) with the help of software BioStat Professional Package Release 5.2.5.0 (AnalystSoft, Robust Business Solutions, Vancouver, Canada).

In the plants grown in washed sand with Cd application from the beginning, the worst affected plant parts are roots, which always lie in direct contact with the soil. There was decrease in root length ranging from 6% to 95% with respect to control plants in different Cd concentrations (Table 1, Figure 1). The shoot system was also affected adversely but a little lesser in comparison to the root system. It decreased in length from 2% to 74% of the normal shoot height of control plants. There was an abnormal increase in shoot/root ratio especially above 20 μM of Cd treatment and it reached 5.63 (486% higher than the normal seedlings) at 120 μM of Cd phytotoxicity. Water content in the shoot as well as roots decreased under different Cd levels. A decrease of 8–15% in water content was noticed in higher Cd concentrations. The VI of soybean seedlings reduced to 96% in highest Cd concentration as compared to the control. The tolerance index decreased considerably from 94 in 1 μM to just 5 in 120 μM of Cd treatment. Similarly, phytotoxicity index increased from 6 in 1 μM to as high as 95 in 120 μM of Cd application. At higher Cd treatment levels, i.e. above 20 μM, a change in the behaviour of radicles and roots was noticed which was quite abnormal. The seeds when treated with 20 μM and above concentrations of Cd, roots of some seedlings started growing in the upward direction against gravity, a pattern totally opposite to the normal growth of a root system. Thus, out of

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**Table 1.** Effect of Cd (μM) on root parameters of soybean grown in washed sand and Cd applied from the beginning

| Treatment | Control | Cd 1 | Cd 3 | Cd 5 | Cd 8 | Cd 10 | Cd 13 | Cd 16 | Cd 20 | Cd 25 | Cd 32 | Cd 36 | Cd 40 | Cd 60 | Cd 80 | Cd 100 | Cd 120 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Root length (cm) | 17.6$^{a}$ ± 0.26 | 16.5$^{b}$ ± 0.19 | 15.4$^{c}$ ± 0.20 | 14.9$^{c}$ ± 0.15 | 14.6$^{c}$ ± 0.12 | 13.2$^{d}$ ± 0.12 | 11.0$^{e}$ ± 0.09 | 9.9$^{f}$ ± 0.13 | 7.0$^{g}$ ± 0.06 | 5.4$^{h}$ ± 0.03 | 3.6$^{i}$ ± 0.10 | 2.4$^{j}$ ± 0.06 | 1.5$^{k}$ ± 0.06 | 1.4$^{k}$ ± 0.07 | 1.2$^{k}$ ± 0.07 | 1.0$^{kl}$ ± 0.06 | 0.8$^{l}$ ± 0.06 |
| Shoot length (cm) | 17.0$^{a}$ ± 0.15 | 16.7$^{a}$ ± 0.51 | 15.8$^{b}$ ± 0.29 | 15.3$^{b}$ ± 0.18 | 15.1$^{bc}$ ± 0.03 | 14.6$^{c}$ ± 0.14 | 13.9$^{cd}$ ± 0.10 | 13.2$^{d}$ ± 0.15 | 12.2$^{e}$ ± 0.12 | 10.7$^{f}$ ± 0.03 | 9.7$^{g}$ ± 0.09 | 8.2$^{h}$ ± 0.09 | 7.7$^{hi}$ ± 0.10 | 6.9$^{i}$ ± 0.12 | 5.9$^{j}$ ± 0.09 | 5.2$^{jk}$ ± 0.09 | 4.5$^{kl}$ ± 0.07 |
| Shoot/root ratio | 0.97$^{a}$ ± 0.01 | 1.01$^{a}$ ± 0.02 | 1.02$^{a}$ ± 0.01 | 1.03$^{a}$ ± 0.01 | 1.03$^{a}$ ± 0.01 | 1.11$^{a}$ ± 0.01 | 1.27$^{a}$ ± 0.01 | 1.33$^{a}$ ± 0.01 | 1.74$^{ab}$ ± 0.01 | 1.99$^{ab}$ ± 0.01 | 2.71$^{ab}$ ± 0.06 | 3.43$^{b}$ ± 0.05 | 5.13$^{bc}$ ± 0.14 | 4.85$^{bc}$ ± 0.15 | 4.78$^{bc}$ ± 0.20 | 5.19$^{bc}$ ± 0.21 | 5.63$^{c}$ ± 0.35 |
| Water content in roots | 92.15$^{a}$ ± 0.331 | 92.78$^{a}$ ± 0.402 | 92.73$^{a}$ ± 0.185 | 92.20$^{a}$ ± 0.213 | 92.48$^{a}$ ± 0.404 | 90.65$^{ab}$ ± 0.316 | 90.66$^{ab}$ ± 0.248 | 90.24$^{ab}$ ± 0.170 | 89.13$^{b}$ ± 0.271 | 89.37$^{b}$ ± 0.211 | 89.51$^{b}$ ± 0.170 | 86.41$^{bc}$ ± 0.530 | 87.17$^{bc}$ ± 0.653 | 84.75$^{bc}$ ± 0.486 | 85.15$^{bc}$ ± 1.081 | 80.60$^{c}$ ± 0.758 | 78.29$^{c}$ ± 0.645 |
| Vigour index of seedlings | 1615$^{a}$ ± 14.52 | 1299$^{b}$ ± 40.03 | 1168$^{c}$ ± 15.38 | 1088$^{d}$ ± 13.12 | 1024$^{de}$ ± 2.35 | 978$^{e}$ ± 9.71 | 886$^{f}$ ± 6.38 | 801$^{g}$ ± 9.29 | 702$^{h}$ ± 6.61 | 602$^{i}$ ± 1.85 | 461$^{j}$ ± 4.22 | 331$^{k}$ ± 3.58 | 255$^{l}$ ± 3.35 | 167$^{m}$ ± 2.94 | 18$^{mn}$ ± 11.82 | 88$^{n}$ ± 1.55 | 58$^{n}$ ± 0.91 |
| Tolerance index | – | 93.92 | 87.67 | 84.43 | 83.13 | 74.77 | 62.27 | 56.42 | 39.77 | 30.45 | 20.45 | 13.64 | 8.52 | 8.13 | 6.99 | 5.68 | 4.55 |
| Phytotoxicity index | – | 6.08 | 12.33 | 15.57 | 16.88 | 25.23 | 37.73 | 43.58 | 60.23 | 69.55 | 79.55 | 86.36 | 91.48 | 91.88 | 93.01 | 94.32 | 95.45 |

Values are mean ± standard errors, $n = 3$. Different superscript letters on mean values along the rows indicate significant differences within $P < 0.05$ according to Tukey's HSD range test.

curiosity, a new experiment was designed to study the effect of Cd on gravi-sensitivity and perception of the root system. The soybean seeds (3 pots with 30 seeds for each treatment) were grown in small plastic pots in washed sand. They were supplied with nutrient solution and Cd from the beginning. The soybean seeds which are epigeous in nature were sown in such a way that the radicle should first emerge out and then bend down towards gravity. Seed emergence was 93% in control but decreased considerably to 20% in highest Cd concentration used (Table 2). In control and plants treated with low Cd concentrations (up to 16 μM), all the seedlings exhibited normal positive geotropic behaviour of their roots. However, at higher concentrations (more than 20 μM), radicles/roots lost the power of graviperception (Figures 2 and 3).

In the hydroponic cultures containing 1/20 dilution of Minchin and Pate[9] nutrient medium and different Cd concentrations, there was an increase of 84% in root length over the initial (starting point, 9.57 ± 0.536 cm) root length in control plants, but this increase was just 23% and 6% in 4 and 8 μM Cd (Table 3). At concentrations more than 12 μM of Cd, there was a decrease in size rather than increase over the initial value. The number of secondary roots also decreased considerably from 53 to 14 in different Cd concentration levels. The number of secondary roots was less than the number (43.00 ± 1.15) present at the start of the hydroponic culture experiment. This indicates that higher Cd concentrations caused degeneration of the existing secondary roots which was visibly evident. The number of secondary roots on the 1st centimetre of the root also decreased with increasing Cd concentrations. In this experiment, the RRL ratio was found to decrease from 28 in 4 μM of Cd to –45 in 20 μM. The root tolerance index decreased while phytotoxicity index increased. It was noticed that plants grown in hydroponics were more prone to Cd toxicity (tolerance index 34 and phytotoxicity index 66 at 20 μM Cd) than those grown in washed sand (tolerance index 40 and phytotoxicity index 60).

This study revealed that root system of soybean plants was severely damaged by Cd toxicity. The root length,

**Figure 1.** Effect of cadmium (control, 10, 20, 40, 60, 80, 100, 120 μM) on roots (15 DAT).

**Table 2.** Effect of Cd treatment on graviperception in roots of plants grown in washed sand and cadmium applied from the beginning

| Treatments | Total seed emergence (%) | Normal gravi-responsive roots (%) | Abnormal gravi-irresponsive roots (%) |
|---|---|---|---|
| Control | 93 | 93 | 0 |
| Cd 4 μM | 70 | 70 | 0 |
| Cd 8 μM | 67 | 67 | 0 |
| Cd 12 μM | 63 | 63 | 0 |
| Cd 16 μM | 60 | 60 | 0 |
| Cd 20 μM | 57 | 54 | 3 |
| Cd 24 μM | 56 | 53 | 3 |
| Cd 28 μM | 54 | 47 | 7 |
| Cd 32 μM | 47 | 40 | 7 |
| Cd 36 μM | 40 | 33 | 7 |
| Cd 40 μM | 37 | 27 | 10 |
| Cd 60 μM | 23 | 0 | 23 |
| Cd 80 μM | 20 | 0 | 20 |

**Figure 2.** Cadmium (60 μM Cd) treatment 15 DAT (Cd applied from the beginning) showing damaged root tips and radicles growing upwards in four seedlings and one plant growing normally.

**Figure 3.** Cadmium (80 μM Cd) treatment 15 DAT (Cd applied from the beginning) showing damaged root tips and radicles growing upward.

**Table 3.** Effect of Cd on root morphological data of soybean grown in hydroponic culture

| Parameters | Control | Cd 4 μM | Cd 8 μM | Cd 12 μM | Cd 16 μM | Cd 20 μM |
|---|---|---|---|---|---|---|
| Root length (cm) | $17.57^{a} \pm 0.23$ | $11.77^{c} \pm 0.18$ | $10.17^{ei} \pm 0.20$ | $9.13^{gi} \pm 0.20$ | $6.47^{hj} \pm 0.15$ | $5.97^{j} \pm 0.12$ |
| No. of secondary roots | $53.67^{a} \pm 1.20$ | $34.67^{b} \pm 1.45$ | $27.67^{df} \pm 1.45$ | $20.00^{ef} \pm 0.58$ | $15.6^{eg} \pm 0.33$ | $13.6^{g} \pm 0.88$ |
| Longest secondary root | $8.73^{a} \pm 0.15$ | $5.50^{bh} \pm 0.12$ | $4.73^{dh} \pm 0.23$ | $4.67^{d} \pm 0.09$ | $3.93^{eg} \pm 0.12$ | $3.73^{g} \pm 0.18$ |
| Total secondary roots on 1st cm | $18.33^{a} \pm 0.89$ | $11.67^{b} \pm 0.33$ | $6.67^{d} \pm 0.67$ | $6.33^{d} \pm 0.33$ | $6.33^{d} \pm 0.33$ | $5.67^{d} \pm 0.33$ |
| Relative root length ratio | – | 27.50 | 7.50 | –5.50 | –38.75 | –45.00 |
| Tolerance index | – | 66.99 | 57.88 | 51.96 | 36.82 | 33.98 |
| Phytotoxicity index | – | 33.01 | 42.12 | 48.04 | 63.18 | 66.02 |

Values are mean ± standard errors, $n = 3$. Different superscript letters on mean values along the rows indicate significant differences within $P < 0.05$ according to Tukey's HSD range test.

number of secondary roots, especially their number at 1st centimetre of the root and tolerance index of affected plants, decreased with an increase in Cd stress level. This leads to nearly five-fold increase in shoot/root ratio in the highest Cd treatment and negative relative root length ratio. The growth inhibition of seedlings can be attributed to non-mobilization of food reserves and inhibition of activities of hydrolytic enzymes[14]. The lower water content reported in the roots of seedlings also points to the factors which may be involved in the non-activation of protein and enzyme systems. The higher shoot/root ratio, indicative of decreased root length in heavy metal treated plants along with damage to the root tip and disruption of membrane functions[15], results in non-establishment of seedlings on the substratum and their inability to absorb water, and thus causing reduced water content of these plants. All these factors directly or indirectly are responsible for reduced VI and increased phytotoxicity index. The reduction in root length, lateral root development and biomass accumulation can be attributed to phytotoxicity induction by Cd through various mechanisms and subsequent cell death as indicated by necrotic areas on the root. These results are in agreement with the reports of earlier studies related to different heavy metals in different crops[16–18].

Root and its growth are important characteristics in crop production. Water absorption and nutrient uptake are the vital processes limited by any change in root morphology, size and architecture caused by environmental constraints. Prolific root growth provides many advantages to the plant system helping improved crop productivity[19]. Development and architecture of the plant roots are regulated by phytohormones. Synthesis of cytokinins (CK) in the root cap promotes cytokinesis, vascular cambium sensitivity, vascular differentiation and root apical dominance, while auxin (IAA) synthesized in the young shoot organs promotes root development and induces vascular differentiation[20].

Loss of gravity perception by root tip was the most unusual feature of Cd-treated seedlings especially beyond 20 μM of treatment. They become gravi-irresponsive and start growing against the gravity and law of nature. To the best of our knowledge, no such report in the literature indicates such behaviour of roots. To understand this, a deeper look into the mechanisms of gravity perception is desirable. Plant roots perceive gravity and grow towards it. Many models explain phenomenon such as sedimentation of statoliths in gravity-sensing cells called amyloplasts or statocytes present in the columella region of the root cap which help the root in gravity perception and its response[21]. An interactive role of amyloplasts with distal complex of endoplasmic reticulum in gravity perception in the primary roots of *Lepidium* was proposed[22]. According to Moore and Evans[23], starch-dense amyloplast mediates the role of $Ca^{2+}$ and calmodulin in activating graviresponses. This model also stressed upon IAA playing a significant role in graviresponse movements. The inhibitory role of cytokinins on root elongation, gravity perception and consequent bending movements in horizontally growing roots was reported by Aloni *et al.*[20]. Multiple pathways may modulate gravity signal transduction within the root tip[24].

In our findings, roots of soybean plants treated with high concentrations of Cd exhibited absence of root caps and damaged, brittle and crinkled roots. As the machinery for graviperception lies in the root cap and all these roots lacked root tips, roots became irresponsive in the absence of gravisensing mechanism. In this case, if the hilum was positioned upwards inside the substratum, damaged radicle emerged on the upper side and kept growing in that direction only and ultimately the seedling died. On the other hand, in case of control seedlings, radicle grows normally, may have emerged in any direction, perceived gravity and grows downward in its direction. These findings have grave implications for the farmers in the form of less and poorly established seedlings and reduced crop yields in the Cd-contaminated agricultural fields.

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# Geochemical characterization of Neoproterozoic heavy oil from Rajasthan, India: implications for future exploration of hydrocarbons

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**The discovery of heavy oil in the Bikaner–Nagaur basin of Rajasthan in western India in reservoirs of Neoproterozoic age during the early nineties was one of the most significant events in the history of oil and gas exploration in India. Recently, discovery of heavy oil in the Punam Structure in the same basin has reconfirmed the hydrocarbon potential of the basin and has regenerated tremendous interest in exploration activities. Another significant factor that enhances exploration interest and hydrocarbon prospectivity of this basin relates to the fact that the Indian Mesoproterozoic and Neoproterozoic sedimentary basins share similar tectonic settings and depositional environments as their producing counterparts elsewhere (Oman, China, Siberian Platform, North Africa, Australia and so on). In this study, geochemical characterization of the heavy oil from Punam-X has been carried out for determining the source, maturity and extent of biodegradation using established biomarker ratios. Organic geochemical studies on the heavy oil from Punam-X indicate that the oil was generated in an anoxic hypersaline environment from marine clastic source rock. The oil is found to be generated from early mature source rock. Gas chromatograph analysis of the oil shows that it has also undergone some degree of biodegradation. Various similarities have been found between the Punam-X oil and other heavy oils of similar age found in Oman, Pakistan and in the adjoining areas in the Bikaner–Nagaur basin.**

**Keywords:** Bikaner–Nagaur, Infra-Cambrian, neoproterozoic, petroleum geochemistry, Rajasthan basin.

UNCONVENTIONAL oil and gas, including heavy oil is widely acknowledged as an important component of global petroleum resource. Current estimates indicate that these resources are several orders of magnitude more abundant than conventional oil. During the early nineties, the exploration for hydrocarbons in Bikaner–Nagaur basin in Rajasthan by Oil India Limited resulted in the discovery of heavy oil in Baghewala-1 well within Jodhpur sandstone of Neoproterozoic age. This was the first discovery of oil in rocks of late Proterozoic age in Indian sedimentary basins. Consequently, there was an increased interest in Baghewala and adjoining areas of Bikaner–Nagaur basin

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