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ARACHIS BIOASSAY FOR SOIL CONTAMINATED WITH HEXAVALENT CHROMIUM

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Abstract

Heavy metals evoke multiple direct and indirect effects on plant growth and affect many physiological functions. Negative impacts include inhibition of seed germination, reduction in plant growth and yield and metabolic disturbances evaluated in terms of altered biochemicals. *In vitro* and pot culture studies revealed that there is a significant uptake of Chromium by natural growth conditions. There was also a strong negative correlation between the concentration of Cr(VI) and the biomass production in *A. hypogea*. The control plant grows tall than any other Chromium treated plant. The paper wig method (Whattman filter paper number 3) was found to be effective in screening the Cr(VI) absorption. Interestingly, Chromium in mild concentrations is promoting the cellular elongation resulting in the longest root of seedlings reared in the 1mM hexavalent Chromium. Chromium when supplemented with the selected bioinoculants to the experimental formulations showed promising results in the plant growth and development than the control seedlings. Both pot cultured plants and *in vitro* raised plants were found to contain 375.80 ppm (per 5g) and 47.06 ppm (per 5g) of Chromium respectively in the Atomic Absortion Spectrometric quantifications. Further experiments are underway to study the biological effects and accumulation of absorbed Cr(VI) in this economically important legume.

Keywords: Chromium, Cr(VI), Heavy metal toxicity, Bioaccumulation, *Arachis hypogea*

Introduction

The ubiquity of heavy metals in the environment results in the introduction of high amounts of toxic metals into the food chain from various sources. The heavy metals commonly found in the environment include Cu, Zn, Ni, Pb, Cd, Co, Hg, Cr and As. Some of these metals act as micronutrients at small concentration in living organism for their normal physiological activities; however accumulation is toxic to most life forms. The toxicity of heavy metals is mainly attributed to their ability in binding with enzymes which in turn brings forth alteration of catalytic functions and inactivation [1, 2]. Heavy metals also bind strongly to oxygen, nitrogen and sulphur atoms that often interrupt the spatial configuration of biomolecules [3].

Chromium is used in several industries such as metal finishing, petroleum refining, iron and steel industries, leather tanning, inorganic chemicals production, textile manufacturing and pulp producing, electroplating and mine tailings [4]. The leather industry is the major cause for the high influx of chromium to the biosphere, accounting for 40% of the total industrial use [4]. In India, about 2000 to 32,000 tons of elemental chromium annually escapes into the environment from tanning industries [5]. The toxicity of chromium is highly dependent on its oxidation state. Cr(III) species are less toxic and less mobile, with very low solubility at all pH levels above 5.5 [5].

Reports are available on inhibitory effects of Chromium on growth and metabolism of many plant species like mosses, rice, pea, wheat, etc. in relation to oxidative stress. Accumulation of Cr(VI) by plants can reduce growth, induce chlorosis in young leaves, reduce pigment content, alter enzymatic function, damage root cells and cause ultrastructural modifications of the chloroplast and cell membrane [6, 7, 8, 9,10]. Roots accumulate several magnitudes higher chromium than shoots. The excess soluble salts in the root causes osmotic stress resulting in the disturbance of the plant water relation, uptake and utilization of essential nutrients. At the cellular level, both Cr(VI) and Cr(III) are toxic to plants. Cr(VI) is a strong oxidizing agent and causes severe damage to cell membranes [11]. Chromium (VI) is toxic to plants because of its ability to form complexes with nucleic acids, proteins and organic compounds.

In vitro trials offer a wide scope in understanding the mechanism and dynamics of plant growth and development as we can control, examine and manipulate the desirable variables involved. Literature is available for different scientific enquiries of developmental, physiological, biochemical and molecular evidences have been carried out *in vitro*. This present study is an earnest attempt to investigate

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the biological effects of Cr (VI) on plants of *A. hypogea, in vitro* and *in vivo* and aims to evaluate the morphogenetic changes during seedling growth to predict the levels of Cr(VI) in aqueous extracts of soil polluted with hexavalent chromium.

Materials and Methods

Screening of Seedling growth in tannery effluent soil

Soils were collected from tannery effluent infested fields in the environs of Shanmuga tanning factory, Gundur, Tiruchrappalli district. The soil samples were dried in hot air oven at 80^{o}C for 48 hrs then tyntallized using standard procedure. Dried soil samples were homogenized with clean dry pestle and mortar. Then the samples were sieved with a metal mesh of ~2mm size. The following formulations were used to fill paper cups of 100 ml capacity to raise *A. hypogea* seedlings. The soil samples were collected from three sites viz., soil from agriculture land near the effluent stream (Soil 1), bottom soil of the effluent stream (Soil 2), and Garden soil with rich compost (Soil 3). The Table – 1 indicates the various formulations of the experimental soil mixtures and their ratios. Commercially available bioinoculants were mixed in different combinations.

Table – 1. Soil mixtures and their ratios

S.No.	Experiment code	Soil 1 (g)	Soil 2 (g)	Soil 3 (g)	Bioinoculant (g)
1.	Exp-1	0	100	-	-
2.	Exp-2	25	75	-	-
3.	Exp-3	50	50	-	-
4.	Exp-4	75	25	-	-
5.	Exp-5	100	0	-	-
6.	Exp-6	-	0	100	-
7.	Exp-7	-	25	75	-
8.	Exp-8	-	50	50	-
9.	Exp-9	-	75	25	-
10.	Exp-10	-	100	0	-
11.	Exp-11	0	-	100	-
12.	Exp-12	25	-	75	-
13.	Exp-13	50	-	50	-
14.	Exp-14	75	-	25	-
15.	Exp-15	100	-	0	-
16.	Exp-16	0	100	-	
17.	Exp-17	25	75	-	10
18.	Exp-18	50	50	-	10
19.	Exp-19	75	25	-	10
20.	Exp-20	100	0	-	10
21.	Exp-21	-	0	100	10
22.	Exp-22	-	25	75	10
23.	Exp-23	-	50	50	10
24.	Exp-24	-	75	25	10
25.	Exp-25	-	100	0	10
26.	Exp-26	0	-	100	10
27.	Exp-27	25	-	75	10
28.	Exp-28	50	-	50	10
29.	Exp-29	75	-	25	10
30.	Exp-30	100	-	0	10

After 40 days of seedling growth both *in vitro* and paper cups they were plucked out and washed in running tap water and distilled water. Then air dried and then oven dried for 48 hrs at 80^{o}C. The powder was digested with concentrated HNO_3. Heavy metal analysis was carried out for the quantification of Cr(VI)

by Atomic Absorption Spectrophotometer (Analyst 400/HGA900/AS800 Perkin Elmer) at Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Thanjavur – 613 402.

In vitro assay for chromium toxicity

Fruits of *Arachis hypogea* L. cv VR-2 procured from Anbil Dharmalingam College of Agriculture, Tiruchirappalli were used as explant. MS liquid medium (half strength) was used for the present work. Inoculation was carried out aseptically. A stock solution of 0.5M was prepared. By dilution of 0.5 M stock, different levels of chromium concentration (0.5, 1, 2, 3and 5mM) had been incorporated into half strength MS liquid medium under aseptic conditions. Seeds were inoculated over a 'M' shaped paper wig made by sterilized Whatman No2 filter paper (Figure – 1). After inoculation, cultures were moved to the incubation room, where the temperature hovered around 25±2^{0}c and an 18 hrs illumination of 1000 lux intensity was provided to cultures with cool, white fluorescent lamps.

Results

Both the root system and shoot system responded to the gradient of the Cr(VI) concentrations. There was a strong negative correlation between the concentration of Cr(VI) and the biomass production in *A. hypogea* seedlings. The paper wig method was effective in screening the Cr(VI) absorption. There was a visual evidence of the color change in the paper wig evidenced by the capillary movement. This proves that the control plant obtained the nutrients in the same way. The paper wig was strong only when prepared with Whattman filter paper number 3 (Plate 2). The plant height is negatively correlated with the concentration of the Cr(VI) as evidenced by the Table-1. Both root length and shoot length and root length are found to be affected by the increasing concentrations of Cr(VI).

Figure – 1

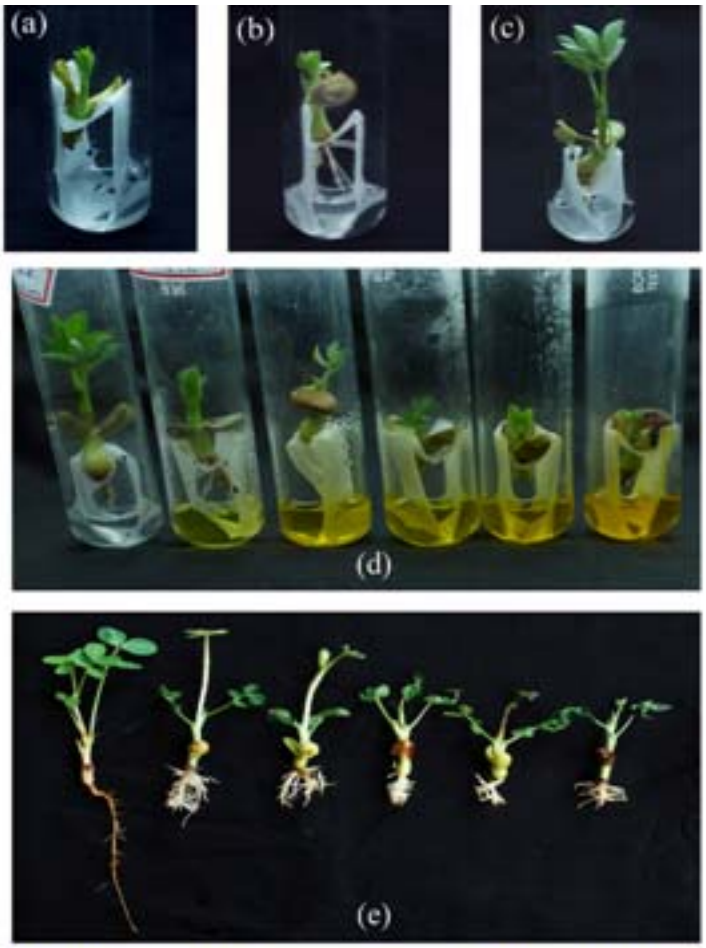


Figure (a), (b) & (c) *In vitro* germination of seedlings of *A. hypogea* on paper wigs dipped on MS basal medium.
Figure (d) Effect of concentration of Cr(VI) on development of seedlings.
Figure (e) Morphology of seedlings grown on a gradient of Cr(VI) concentrations.

The high Cr(VI) concentrations not only affect the terminal bud growth and development but also the nodal branch formation as well the endogenously originated secondary branches of roots. This is evidenced by the reduced number of branches and number of leaves in Table 1. The cellular elongation in the roots is responsible for the length of roots. Mild concentrations like 1 mM Cr(VI) promotes the cellular elongation resulting in the longest root of seedlings. Regarding the fresh weight of the shoots there is a normal distribution of fresh weight in correlation with the increasing Cr(VI). Pot culture studies revealed that there is significant uptake of the Chromium by natural growth conditions also. In comparison with the experimental formulations without bio-inoculants, the experimental formulations with bioinoculants showed efficient growth. Regarding the Chromium accumulation in plants, both the *in vitro* and pot cultured plants showed 375.80 ppm and 47.06 ppm respectively in the Atomic Absorption Spectrophotometer quantifications.

Figure – 2

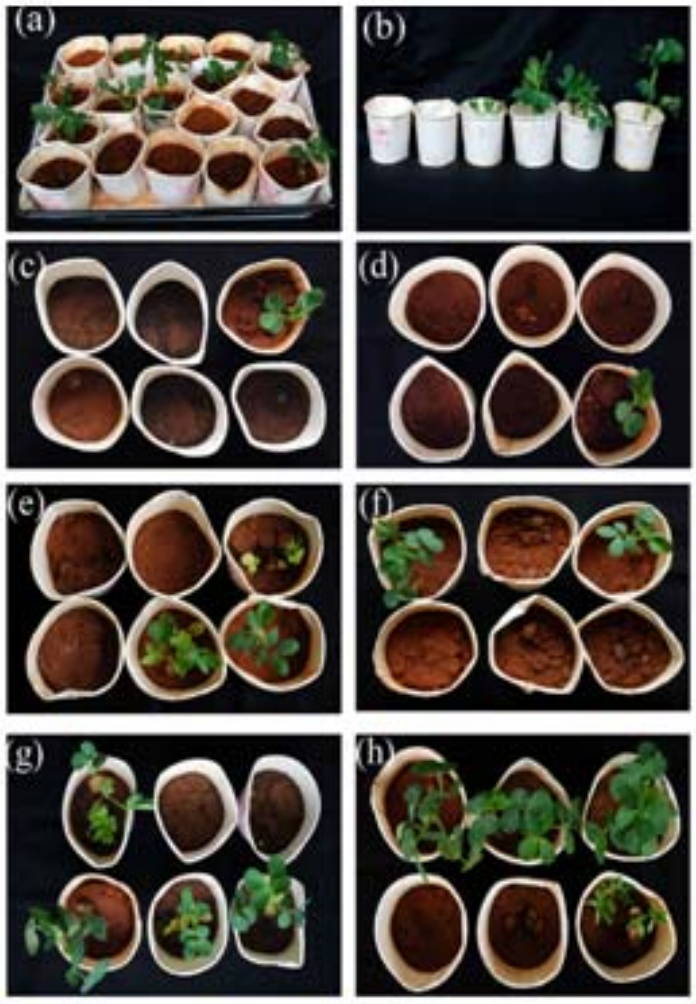


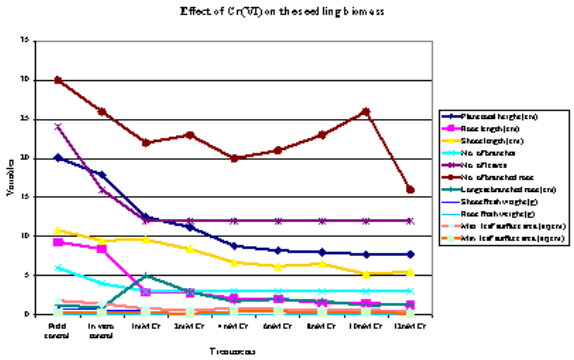
Figure (a) Experimental set up **(b)** *Ex vitro* germination of seedlings of *A. hypogea* on paper cups with normal garden soil with added gradient of Cr(VI) concentrations.
Figure (c), (d) & (e) Seedling germination on various ratios of effluent soil without bioinoculants.
Figure (f), (g) & (h) Seedling germination on various ratios of effluent soil with bioinoculants showing relatively good germination and growth.

Table 2 Chromium inflicted morphometric characters in in vitro and pot cultures of *Arachis hypogea* L.

Parameters	Control		1mM		2mM		4mM		6mM		8mM		10mM		12mM	
	F	I	F	I	F	I	F	I	F	I	F	I	F	I	F	I
Plant total height (cm)	20.1	17.9	12.5	15.2	11.2	13.1	8.8	6.4	8.2	6.3	8.0	5.4	7.7	4.3	7.7	3.2
Root length (cm)	9.3	8.4	2.9	4.4	2.8	3.2	2.1	1.6	2.0	1.2	1.5	0.8	1.5	0.6	1.2	0.4
Shoot length (cm)	10.8	9.5	9.6	10.2	8.4	9.8	6.7	5.4	6.2	4.3	6.5	3.4	5.2	2.2	5.5	1.6
No. of branches	6	4	3	6	3	5	3	3	3	2	3	1	3	1	3	-
No. of leaves	24	16	12	16	12	16	12	11	12	8	12	6	12	4	12	2
No. of branched root	30	26	22	28	23	25	20	22	21	18	23	16	26	14	16	12
Longest branched root (cm)	1.2	0.9	5.0	3.0	2.9	4.1	1.7	1.4	2.0	1.6	1.7	1.2	1.2	0.8	1.3	0.4
Shoot fresh weight (g)	0.764	0.612	0.629	0.812	0.684	0.801	0.659	0.541	0.664	0.521	0.591	0.042	0.427	0.312	0.464	0.210
Root fresh weight (g)	0.063	0.047	0.059	0.041	0.055	0.041	0.052	0.041	0.046	0.031	0.042	0.031	0.026	0.012	0.018	-
Max. leaf surface area (sq cm)	1.8	1.4	0.7	0.9	0.6	0.5	0.8	0.7	0.7	0.5	0.6	0.4	0.6	0.3	0.4	0.2
Min. leaf surface area (sq cm)	0.3	0.3	0.24	0.41	0.15	0.21	0.4	0.3	0.4	0.3	0.24	0.1	0.35	0.15	0.12	0.12

F – Field grown plant ; I – *In vitro* reared plant

Figure 3 Chromium inflicted morphometric characters *in vitro* cultures of *A. hypogea*



Discussion

The present study indicates that the chromium inhibited the plant growth and development at higher concentrations (4 mM to 12 mM) both *in vitro* and in pot cultures. Chromium toxicity deleteriously affects the percentage germination, root growth, shoot growth, that has been widely reported [8, 11]. Seedling adapted well on half-strength MS medium augmented with lower chromium concentration (0.5mM & 1.0 mM). Table 2 reveals the morphometric evaluations such as percentage germination, root-shoot growth fresh and dry weight of both root and shoot systems of mild concentrations of chromium did not deviate much from the control. Figure 1 (e) shows the chromium in mild concentrations are promoting the cellular elongation resulting in the longest root of seedlings raised in the 1mM hexavalent Chromium. The observations are corroborating with our previously reported [12] fact that chromium supplemented at extremely low concentrations even enhanced greening of callus and culture tissue in selected treatments.

Threshold for inflicting injuiries in field studies and pot experiments is significantly higher than what is required for soil less *in vitro* system [13]. The results of

the present investigation of chromium-invoked inquiries were comparatively lesser in pot cultures than in *in vitro* that accords with the elemental property of Cr(VI) [14, 15]. And interestingly, the pot cultures supplemented with bioinoculants showed no significant growth change even when chromium is presented at slightly higher concentrations (4 mM to 8 mM) and this may be due to the intervention of the bioinoculant with the chromium absorption and transport.

Figure 1(a, b, c, d) presents the novel technique of employing 'M' shaped paper wig that emerged in this course of study. The colour development from straw yellow to orange yellow confirmed the idea of chromium absorption via capillary movement that emphasizes the effect of chromium in water relations and mineral uptake. Turner and Rust in the year 1971 [15] reported wilting of various plant species due to chromium toxicity that coincides with the present results. The seedlings both *in vitro* and pot cultures showed the signs of wilting that includes drooping leaf and leaves with minimized surface area. However it was not true with the pot cultures bestowed with bioinoculants (Figure 2: f, g, h). Evaluating the effects of Cr(VI), and the paper wig method provides a new avenue of screening the cultivar varieties for Cr(VI) tolerance [16].

This study confirms that *in vitro* assays are valuable for its greater flexibility and precision, found place in many applied frontiers of agriculture, horticulture and biotechnology. Further experiments can help to study the biological accumulation of absorbed Cr(VI) in edible parts of *A. hypogea* economically important legume of the tropical world.

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