



ASSESSING CHROMIUM STRESS ON THE PEANUT'S GROWTH USING MATHEMATICAL MODEL

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ABSTRACT

Heavy metals implicit deleterious effects to all living organisms owing to its unstable configuration. Hexavalent chromium, inevitable effluent of automobile industry and tanneries affect the growth and development of the peanut plant. On comparing the chromium stress on the Peanut plant's growth under in vitro and in vivo conditions, it was found that chromium at lower concentrations stimulated the growth of the peanut plant. The chromium accumulation was confined to the root than the shoot. Interestingly, the experimental results obtained were well goes with the Richard's model of plant growth. The results confirmed that Chromium (VI) influenced changes were development dependent in all the treated peanut plants were well goes with the Richard's model of plant growth. The results confirmed that Chromium (VI) influenced changes were development dependent in all the treated peanut plants.

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INTRODUCTION

Many heavy metals are bioavailable in soil at natural pH levels. This mobility makes it possible for heavy metals to be absorbed by plants in forest and agricultural soils (Guala *et al.*, 2010). The chemical reactions in soil systems involve complex and diverse sequences of phenomena (Lindsay, 1978). The presence of heavy metals in soils either as a result of natural processes or human activities may also imply similar reactions, substantiated by the fact that other metals may also bioavailable at neutral conditions (Guala *et al.*, 2010). Wang *et al.* (1991) have used a computer simulated model to evaluate the quenching role of possible organic and inorganic ligands of tobacco cells exposed to heavy metal.

The interaction between living organism and metallic ions in aqueous solution can be explained by involving biosorption and bioaccumulation mechanisms (Schmitt *et al.*, 2001). Biosorption takes place faster than the bioaccumulation. However, the metal bioaccumulation process may begin and increase after first process day. It should be noticed that the non-living biomass based metal removal kinetic models cannot be used to represent living biomass-based metal uptake data as there is a correlation between plant growth and metal uptake or concentration. However, the non-homogeneity of the plant tissue does not permit the biosorption to be uniform.

Espinoza *et al.* (2008) has proposed a non-structural kinetic modeling for phytoaccumulation of chromium ions in three floating aquatic macrophytes: *Salvinia auriculata*, *Pistia stratiotes* and *Eichornia crassipes* from a nutrient medium, obtaining different bioaccumulation rate. A mathematical model was suggested for a rapid assessment of the time and concentration parameters for the deployment of hyperaccumulator plants for phytoextraction purposes in *Alyssum bertolonii* Desv. (Gonnelli *et al.*, 2000). Investigations pertaining to the kinetics, equilibrium and thermodynamics in the biosorption of copper, cadmium and lead by *Acacia leucocephala* bark powder reported that the biosorption process of the metal ions followed pseudo-second-order model and the values obtained indicate that the biosorption process was exothermic and spontaneous (Munagapati *et al.*, 2010).

Karthika *et al.* (2010) observed that the mechanism of biosorption of copper from aqueous solutions using *Tridax procumbens* gave good fits for Freundlich and Langmuir models. A mathematical model for the biological removal of Cr(VI) is coupled with biomass growth under completely aerobic conditions was developed to accurately predict the transient concentration of Cr(VI) as a function of time in response to changes of the inlet Cr(VI) concentration in continuous systems of heterotrophic cells (Contreras *et al.*, 2011). Divya Chauhan and Nalini Sankararamakrishnan (2011) suggested Thomas model to evaluate the removal of hexavalent chromium from aqueous systems using fixed bed

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column. This paper aims to evaluate the chromium uptake by the peanut plant (*Arachis hypogaea* L.) using mathematical models under *in vitro* and *in vivo* conditions.

MATERIALS AND METHODS

Source plants

Seeds of *Arachishypogaea* L. cv VR-2 were procured from Anbil Dharmalingam college of Agriculture, Tiruchirappalli. The seeds were stored in dry containers in order to prevent fungal infestations. As the study involves both *in vitro* and pot culture, healthy seeds of peanut were segregated from the sample and stored separately for experiments.

Chromium treatment

A stock solution of 1mM was prepared from Potassium-dichromate ($K_2Cr_2O_7$) purchased from Merck. From the freshly prepared stock, different levels of chromium concentration (1, 2, 4, 6, 8, 10, 12 mM) had been incorporated into soil in the case of pot culture studies. Chromium was supplemented to the peanut plant by two methods, viz, (i) Directly adding freshly prepared $K_2Cr_2O_7$ solution with the soil and (ii) Foliar spray method. Foliar spray is given after 15 days of germination. For *in vitro* studies, $K_2Cr_2O_7$ solution in various concentrations is introduced into the MS medium after sterilization.

Construction of mathematical model

Data collection during growth at various chromium on concentrations

The chromium treated seedlings of *Arachis hypogaea* L. raised through *in vitro* culture were taken for sampling. Though the experiments were carried out in six different concentrations (1, 2, 4, 6, 8, 10, 12 mM); three selected concentrations (2, 6, 10 mM) were taken in the media composition containing MS medium fortified with BAP and GA for the purpose of mathematical modeling. From day 6 of culture, the sampling was carried out every 10 days for 3 months.

After harvesting, plant roots were rinsed with cool, double distilled water and then carefully submerged in Nitric acid 5 mM at 4°C for 15 minutes to remove the excess chromium adsorbed to the root hairs. Root and shoot lengths of 20 plants were measured; the plantlets were then divided into shoots and roots and dried at 80°C for 48 hours and subsequently weighed and decomposed by wet ashing with HNO_3 . The concentrations of chromium in the digests were determined by Atomic Absorption Spectrophotometer (Analyst 400/HGA900/AS800 Perkin Elmer) at Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Thanjavur, Tamilnadu, India.

Total root length was chosen as a measure of chromium tolerance, as root growth is often reported as more sensitive to the presence of metal toxins (Baker and Walker, 1989).

Selection of the mathematical model

Plant growth can be described in mathematical modeling through available quantitative data. The most widely used of such models is the Richards function (Causton and Venus, 1981), which is a modified logistic model. It has been used successfully to model vegetative growth on a broad scale, from single leaf to full crop. Further, it can be applied not only to dry weight but to any morphologically meaningful growth parameters such as length, volume etc. Like any logistic

growth model, it assumes that the specific growth rate is a function of the current living biomass, multiplied by a limiting factor that has the ecological role of carrying capacity.

Compared with the basic logistic curve, the Richards function has an additional parameter, in the form of a real number exponential, which is instrumental in tailoring the resulting sigmoidal growth curve to the characteristics of the particular plant being considered. Thus, the growth dynamics, considered as a continuous process, are described by the differential equation:

$$\frac{dX}{dt} = X \frac{r}{n} \left(1 - \frac{X^n}{K^n} \right) \quad (1)$$

Where in the present case X represents root length (cm), K is the carrying capacity (cm), which in this case represents the final root length, $r \in R (d^{-1})$ is the growth rate and $n \in R$ is the Richards exponent, adding flexibility to the dynamic response through modulation of the inflexion point. This basic growth equation, however, should be complemented with chromium absorption mechanism, for which the following dynamics was preferred to other factors:

$$\frac{dC}{dt} = K_c (C_{sat} - C) \quad (2)$$

Where, C is the internal chromium concentration (ppm/g dry weight), C_{sat} its saturation concentration and $K_c (d^{-1})$ is a mass transport co-efficient governing the rate of metal uptake. A conceptual justification of Equation (2) can be found in regarding the uptake mechanism as a diffusion process. In this regard, the equation is a special case of Fick's laws, where the mass transfer is governed by the concentration gradient. Similar equations are commonly used in modeling the diffusion of gas into the liquid phase (Britton, 1986; Holland and Anthony 1989).

It should be noted that the two Equations (1) and (2) are not directly related. This is a consequence of the fact that to a large extent growth is not affected by chromium uptake, so the two processes can develop independently from one another. The interaction between these two equations is part of a very complex dynamics, that has been explored by Agren and Bosatta (1996) and will be considered in order to make further progress in this field of research.

Model (1, 2) was calibrated with the experimental data using an optimized version of the flexible polyhedron search method. This algorithm, which is described in detail elsewhere (Marsili-Libelli, 1992) attempts to minimize the squared sum of errors between data and model response. The estimated parameters can then be obtained as

$$\hat{P} = \arg \min_P E(P), \text{ where:}$$

$$E(P) = \frac{1}{N - n_p} \left[\sum_{i=1}^N (X_{exp}(i) - X_m(i))^2 + \sum_{i=1}^N (C_{exp}(i) - C_m(i))^2 \right] \quad (3)$$

And the parameter vector $P \in R^n_p$ is defined as:

$$P = [r \ n \ K \ K_c \ C_{sat}]^T \quad (4)$$

Assessing Chromium Stress on the Peanut's Growth Using Mathematical Model

It should be stressed that fitting Model (1, 2) to the experimental data does not reduce to a mere linear regression. In fact, in this case a non-linear dynamic model is used, whereas linear regression can be applied only to algebraic linear models, which have no growth description capability.

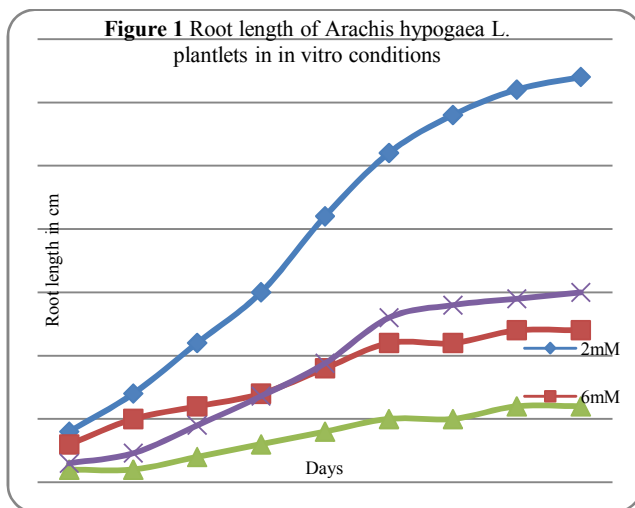
RESULTS AND DISCUSSION

Mathematical model for growth under chromium stress

The peanuts' root growth was used as a function of varying Cr concentrations in the culture medium is shown in Figure 1. The Richards model of Equations (1) and (2) was fitted to these growth data. During the 3-months experiment, the roots grew from day 10 and then reached a characteristic maximum length related to Cr concentration in the medium. It can be seen that the model could fit adequately each growth assay with differing parameter values, as shown in Table. The growth of the chromium treated plants was similar to the control plant till about 70 days. After this period, there was a statistically significant difference between the two curves and differing maximum values were reached. The other growth curves differed from the control during the entire growth period and reached lower maximum values. Data for shoot growth is not subjected to mathematical interpretation as they exhibit the same growth trend and the changes were more conspicuous in roots; however affected by larger relative errors caused by the smaller measured lengths.

Fitted Parameters' Values

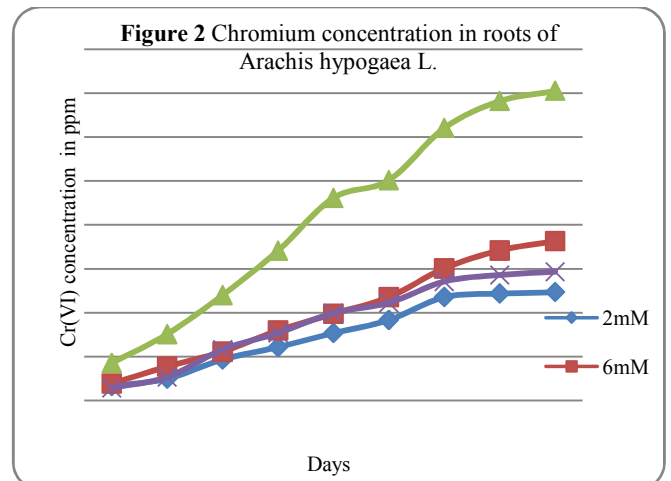
$K_2Cr_2O_7$ concentration	$r(d^{-1})$	$n(-)$	$K(cm)$	K_c	C_{sat}
Control	0.0564	0.0931	8.4	-	-
2mM	0.0832	1.7777	3.23	0.1031	121
6mM	0.0317	0.1566	1.20	0.0921	180
10mM	0.0279	0.0615	1.60	0.0888	350



Modeling the chromium uptake process

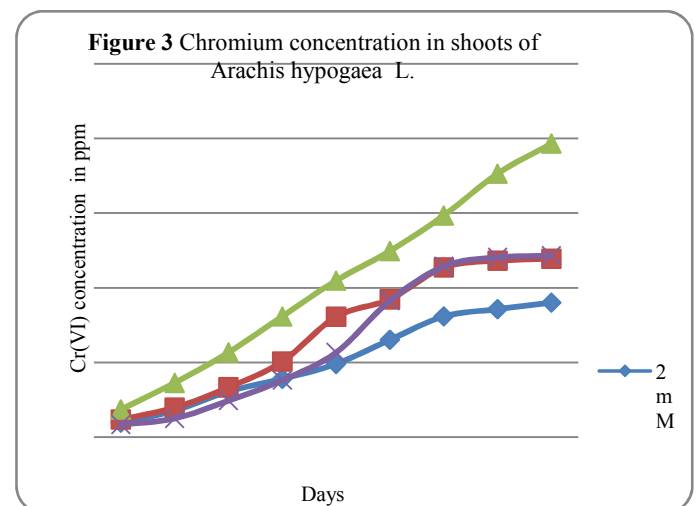
Figure 2 shows Cr(VI) concentration in the roots of *A. hypogaea* during various days of growth. Chromium concentration in the roots of plants treated with 10mM chromium reached saturation after 70 days of growth at about $310.32 \mu g g^{-1}$ dry weight. When the peanut was treated with 6mM Cr(VI), the root Cr(VI) was found to be almost the same value, but after 90 days of growth. In the 2mM chromium treated plants, root chromium concentrations increased during the growth period but not enough to reach the same saturation level as the other treatments. It was found that all growth data

sets could be fitted to a sigmoid curve produced by the Richards model (1).



The Cr(VI) concentrations in shoots of peanut plants exposed to different chromium concentrations is shown in Figure 12. The uptake curve for 10mM chromium treated plants achieved saturation levels in about 80 days of culturing and the maximum value reached was $176.36 \mu g g^{-1}$ dry weight. The other two treatments showed similar trends but achieved lower values: $85.75 \mu g g^{-1}$ dry weight for 2mM chromium and $118.12 \mu g g^{-1}$ dry weight for 6mM chromium treated plants respectively. After 50 days of growth when root and shoot development began to be significantly different for control and 10mM Cr(VI) treated peanut plants, the shoot and root Cr(VI) concentrations were found to be $104.73 \mu g g^{-1}$ dry weight and $230.76 \mu g g^{-1}$ dry weight respectively.

In the 2mM chromium concentrations, the accumulation of root chromium increased during the growth period; however, it does not attain the saturation level as the other treatments. It was found that all growth data sets could be fitted to a sigmoid curve produced by the Richards model.



The model explains why chromium uptake stops for a fixed time, that it is the accumulation level may depend on both the altered growth rate or biochemical changes and external chromium concentration. Higher chromium concentrations induce higher accumulation levels because the metal influx is higher. Thus, there is a two-factor relationship between internal and external chromium concentrations due to different chromium influx and different growth rates: This could explain

the non-linear proportionality obtained in the chromium treatments and the chromium saturation levels exist in the peanut.

DISCUSSION

Mathematical model constructed in the study suggests that there is a diminishing utility of chromium even as input at lower doses. At extremely low concentrations the element exerted a stimulatory influence. When the peanut plants were treated with chromium beyond concentrations of 6 mM the metallic element started evoking a negative influence. Further increase of chromium supply caused a proportionate reduction in all the growth parameters considered in this study.

Interestingly, if Cr(VI) was incremented continuously to the peanut plants, it is noticed that the treated plants gradually got adopted to chromium from supra optimal concentrations to very high dosage after a saturation period of 70 days. After the said number of days there could be no pronounced change in absorption as well as in the growth. Data obtained from the experimental investigation offers support to the view that Cr(VI) uptake is confined only to the active growth phase. In the 2 mM chromium concentrations, the accumulation of root chromium increased during the growth period; however, it does not reach the saturation level as the other treatments. It was found that the growth data picked from the study could be fitted to a sigmoid curve produced by the Richards model.

The model explains why chromium uptake stops for a fixed time. It can be deduced from the analysis of the model that the accumulation level may depend on both the altered growth rate or biochemical changes and external chromium concentration. Higher chromium concentrations induce higher accumulation levels because of the enhanced metal influx. The lower growth rates caused by the chromium at supra optimal doses and the diminishing trends in the uptake at these levels reflect a two-factor relationship between internal and external chromium concentrations. Due to different chromium influx, a non-linear proportionality of growth is inferred from chromium treatments at levels above the saturation point.

That chromium is not an inherent component of any biomolecule and does not any way limit the influence that the heavy metal can bring forth in the growth development of the plant. Observations made and data collected from the three different modes of chromium supplies offered to peanut plants in this study clearly reinforce the idea that hexavalent chromium can be both stimulatory and adverse in its effects depending on the dose supplied.

It is evident from the results of the study that Cr(VI) influenced changes are development dependent. As the responses of the selected species is differed not only in terms of dose supplied doses of chromium at the seed stage in pot and field studies., but the visible symptoms the growing plants evinced in the seedling and growing stages in responses to Cr(VI) supply at the said two conditions.

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