International Journal of Technology and Applied Science (IJTAS)
E-ISSN: 2230-9004 ● Website: www.ijtas.com ● Email: editor@ijtas.com

PHYTOREMEDIATION OF CHROMIUM USING CO-CULTIVATOR PLANT

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Abstract:
The present study revealed the impact of various concentrations of chromium on the growth of *Amarathus viridis* L. and *Mirabilis jalapa* L. The results showed that the morphometric characters such as root length, shoot length, leaf area, fresh weight and dry weight of *Amarathus viridis* L. were significantly decreased with increasing concentration of heavy metal, but those characters were increased in *Amarathus viridis* L. when it was cultivated together with *Mirabilis jalapa* L. than the *Amarathus viridis* L. grown individually. Thus, from the above findings, it is clear that the plant *Mirabilis jalapa* L. acted as hyper-accumulator and because of the phytoextraction capability of this hyper-accumulator, the experimental plant, *Amarathus viridis* L. (hypo-accumulator) may grow well in metal stressed environment.

Key words: Heavy metals, chromium, *Amarathus viridis* L. and *Mirabilis jalapa* L.

INTRODUCTION

In recent years, heavy metals-induced soil pollution has increased due to the widespread usage of chromium (Cr) in chemical industries. The release of Cr into the environment has reached its peak causing hazardous environmental pollution. Heavy metal-induced soil pollution is one of the most important abiotic stresses acting the dynamic stages of plant growth and development. In severe cases, it can kill the plants and their derivatives and thereby pose a potential threat to human food safety. Heavy metal contamination of soils is a major environmental problem worldwide (CI, 2011) and phytoextraction has emerged as a potential cost-effective and environmentally sustainable approach for removing toxic metals from soils (Eriksson *et al*., 1997). Phytoextraction and phytoremediation have been applied successfully for cleaning – up soils contaminated with metals from tannery sludge.

Chromium (Cr) is the seventh element on earth that has various valence states of oxidation (Iyaka, 2009), including trivalent (Cr^{3+}) and hexavalent nature (Cr^{6+}). Trivalent chromium is present in its most stable and common valence level. Trivalent chromium is an essential trace element for the human body and plays an important role in the regulation of blood sugar (Zhang *et al*., 2019), and excessive chromium has an adverse effect on plant photosynthesis and nutrient absorption (Cai *et al*., 2019). In addition, chromium accumulated in plants enters the human body through the food chain, causing dermatitis, bronchitis, tuberculosis, and so on. It also increases the risk of cancer in humans. The toxicity of hexavalent chromium is 100 times more than that of trivalent chromium with serious toxicity (Qianqian *et al*., 2022), and its high chemical activity, which occurs with the rapid development and use of chemicals in a wide range of applications, is more easily absorbed by plants, resulting in its accelerated release into the environment.

The remediation of soil contaminated with heavy metals involves two main aspects of consolidation and activation. The earlier remediation methods were mainly based on excavation and landfill, but their sustainability was not viable, especially with the advancement of science and technology. Further, the treatment of heavy metal soil pollution has now evolved into the use of chemical soil stabilization (Hamon *et al*., 2002), soil leaching (Huang and Keller, 2020), electrodynamic restoration (Zhang M. *et al*., 2019), and the use of plant ecological restoration (Lian *et al*., 2018). The earlier methods were relatively expensive and labor intensive; however, the chemicals released during the process can cause secondary pollution of the soil environment. Using plants for heavy metal remediation is relatively inexpensive, and also has good economic and environmental benefits, which is the key direction for future development and exploitation.

The relationship between soil and water pollution and the uptake of metals by plants is determined by many chemical and physical factors of the soil and the physiological functions of the plants. Soils contaminated with trace elements of metals can pose a threat, both directly; due to the negative impact of metals on plant growth and yield, and indirectly; due to entering the human food chain with a potential negative impact on humans. A drop in yields of just a few percent could ultimately lead to significant losses in production and income. Some food importers are now specifying the acceptable maximum contents of metals in food, which might limit the possibility for the farmers to export their contaminated crops (Bjuhr 2007), (Liu *et al*. 2013).

Phytoremediation is an environmentally friendly, cost-effective, in-situ process that uses living plants to reduce, remove, or immobilize hazardous contaminants from soil and ground water and render them innocuous. It is a financially possible and feasible ways in regards to the remediation of our current circumstance without presenting any new pollutants. Here, we summarize some of studies carried out regarding phytoremediation of chromium metal using hyper-accumulator plant.

MATERIALS AND METHODS

Seeds of *Amarathus viridis L and Mirabilis jalapa* L were procured from the local seed centers, Sivagangai, Sivagangai Distirct, Tamil Nadu. The plant *Mirabilis jalapa* L was chosen as hyper-accumulator and as co-cultivator for *Amarathus viridis L* in this study. The effect of various concentrations of chromium sulphate on the morphometric characters were analyzed on the selected plants. The experimental soil for raising the cultivars was prepared by mixing red soil, black soil and sandy soil in the ratio of 1 : 1 : 1. The prepared soil was sterilized by solar sterilization method (Handiseni *et al*., 2010) for 5 days. *Mirabilis jalapa* L and *Amarathus viridis L.* seeds of 10 numbers each were sown together in all the pots for the experimental purpose. The heavy metal of chromium sulphate was treated in the experimental plants with different concentrations viz., 2 mM, 4 mM, 6 mM, 8 mM and 10 mM (w/v) in five replicates. The aqueous solutions of chromium sulphate were applied in the pots after the development of first leaves in the seedlings. Then the plants were watered with the individual concentration of metal on every alternate days. A set of plants without heavy metal treatment was maintained as control and on 35th day growth parameters were analyzed in all pots including the control (Arts and Marks 1971), (Abdul baki and Anderson 1973).

Phytoremediation treatment: Co-cultivation of the hypo-accumulator and the hyper-accumulator

Amarathus viridis L. (hypo-accumulator) and *Mirabilis jalapa* L. (hyper-accumulator) seeds were sown together uniformly in all the pots. Appropriate amount of chromium sulphate was given separately for the experimental plants with different concentrations
(2 mM, 4 mM, 6 mM, 8 mM and 10 mM (w/v)) in 5 replicates. Then the plants were watered with the respective concentration of metals on every alternate day and on 35th day growth parameters were analyzed.

RESULTS AND DISCUSSION

The effect of chromium sulphate on the morphometric characteristics viz., root length, shoot length and leaf area of study plants has been presented in Table 1 and the effect on fresh weight and dry weight in Table 2. The root length, shoot length, leaf area, fresh weight and dry weight of *Amarathus viridis L.* were reduced with the increasing concentrations of the metal when comparing to the control but they were increased in *Amarathus viridis L* ., when it was co-cultivated with *Mirabilis jalapa* L. (Table 1 and Table 2).

Heavy metals either retard the growth of the whole plant or plant parts (Shaq and Iqbal 2006), (Shanker *et al*. 2005). Chromium stress has a tingling effect at low concentrations, however inhibits the growth of most plant seedlings at high concentrations. Low chromium concentrations can increase the net rate of photosynthesis and promote plant growth by promoting the electron transport activity of PSII, which can increase the proportion of medulla and outer skin tissue in the root and promote root and root hair growth. A high concentration of chromium hinders water transport, reduces transpiration, affects the uptake of minerals by the root system, and disrupts enzymatic reactions in the plant body, resulting in short stature of the plant, yellowing, and dropping of the leaves, as well as a significant reduction in leaf area and biomass. It was reported that high chromium concentrations could cause permanent plasma wall detachment and water loss in plant tissues (Madrid, 2010; Singh *et al.*, 2021; Srivastava *et al.*, 2021) and irreversible damage to mitochondria, resulting in decreased respiration and even plant cell death. It was also found that high chromium concentrations caused abnormal stomatal conductivity, reduced intercellular space, and reduced growth and yield of plants (Rath and Das, 2021). The plant parts typically the roots which have direct contact with the tainted soils show fast and delicate changes in their development. Huge impacts of number of metals (Cu, Ni, Pb, Cd, Zn, Al, Hg, Cr, As, Fe) on root growth varied and the root system usually becoming malformed with short curved side roots (Wong and Bradshaw 1982). In this work, reduction in plant height might be mainly due to the reduced root and shoot growth and furthermore, lesser supplements and water transport to the above parts of the plant. Similarly it was accounted that Cr transport to the upper parts of the plant can have a direct effect on cell elongation of shoot adding to the decrease in plant stature (Shanker *et al*. 2005).

The uptake of heavy metals in plants affects the normal metabolism of plants. To reduce its toxic effects, plants also excrete organic acids, with a high affinity for heavy metals to combine with and to form chelates, which can be divided into external and internal chelation (Oliveira, 2012; Liu *et al*., 2018). External chelation refers to plants that secrete organic acids through their roots into the rhizosphere that combine with metal ions to alter their mobility and solubility in the medium (Zeng *et al*., 2008). Increasing and prolonging the treatment time of chromium content in the culture medium increased the secretion of organic acids such as oxalic acid, citric acid, and malic acid, which promoted the absorption of chromium ions by rice. The exogenous application of citric acid to duckweed (*Lemna minor)* also promoted the absorption of chromium ions by rice (Sallah-Ud-Din *et al*., 2017).

As an industrially widespread heavy metal, chromium is one of the main sources of environmental pollution. Its high toxicity is closely related to its rapid permeability through biofilm and subsequent interaction with proteins and nucleic acids in plants. For some plants, the effects of chromium from the point of view of independent seed germination, external performance, aboveground and root growth, internal regulatory genes, and the change in the protein, show that the plant's defense against heavy metal chromium absorption, resistance, and detoxification mechanism are very complicated and that there are major differences between different plants in their response to chromium stress. In addition, previous studies have shown that chromium also has some effect on the absorption of other heavy metals (Turner and Rust, 1971b; Moral *et al.*, 1996)

The use of hyper-accumulators, in soils contaminated with heavy metals to restore ecological function is quite common. Hyper-accumulator plants refer to plants capable of growing in metal-rich soils and whose absorption of heavy metal ions from the soil is more than 100 times that of ordinary plants, and whose leaf-to-root ratio of heavy metal content is >1 (Baker *et al.*, 2000). For chromium, the plant absorption threshold should be 300 μg·g −1 (Garcíahernández *et al*., 1998; Van Der Ent *et al*., 2013). Current studies have identified that plant such as *Prosopis laevigata* (Buendía-González *et al.*, 2010; Gill *et al.*, 2016), can be hyper-accumulator of chromium. However, existing hyper-accumulator plants have low biomass and economic benefits, so they cannot be used in a variety of practical remediation projects.

Heavy metals uptake using phytoremediation process, seems to be a prosperous way to remediate heavy metals contaminated environment. It has benefits compared to the other normally utilized ordinary technologies. A few components must be considered to achieve a superior of remediation result. The most significant factor is a suitable plant which can be utilized to take-up the heavy metals. Indeed, the phytoremediation strategy is by all accounts truly outstanding however, has a few restrictions. More research should be directed to limit this restriction to apply this procedure successfully.

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Table 1: Effect of Chromium sulphate on the morphometric characteristics of hyper-accumulator (*Mirabilis jalapa* L.) and hypo-accumulator (*Amarathus viridis* L.)

Metal concentration	Root length (cm)			Shoot length (cm)			Leaf area (cm2)		
	Chromium stress on ***Amarathus viridis* L.**	After co-cultivation		Chromium stress on ***Amarathus viridis* L.**	After co-cultivation		Chromium stress on ***Amarathus viridis* L**	After co-cultivation	
		***Amarathus viridis* L.**	*Mirabilis jalapa* L.		***Amarathus viridis* L.**	*Mirabilis jalapa* L.		***Amarathus viridis* L**	*Mirabilis jalapa* L.
Control	7.51±0.05	8.2±0.04	6.2±0.03	18.23±0.45	20.73±0.56	16.32±0.54	10.26±0.11	10.75±0.03	8.9±0.04
2mM	7.23±0.03	8.4±0.02	5.92±0.75	17.9±0.056	19.87±0.31	15.34±0.65	10.12±0.23	10.25±0.04	8.5±0.36
4mM	6.92±0.02	7.8±0.04	5.3±0.041	16.23±0.05	18.43±0.56	14.43±0.21	9.43±0.02	10.12±0.14	8.2±0.03
6mM	6.2±0.03	7.5±0.04	4.9±0.072	15.9±0.067	17.54±0.43	13.45±0.56	9.12±0.008	9.97±0.086	7.8±0.06
8mM	5.35±0.02	5.65±0.03	4.2±0.43	14.51±0.03	16.08±0.78	12.29±0.67	8.23±0.026	9.29±0.978	7.3±0.010
10mM	4.98±0.02	5.23±0.05	3.7±0.78	13.25±0.06	14.65±0.11	11.45±0.78	7.78±0.022	9.01±0.045	6.782±0.01

All the values are the averages of five observations. Mean±Standard error

International Journal of Technology and Applied Science (IJTAS)
E-ISSN: 2230-9004 ● Website: www.ijtas.com ● Email: editor@ijtas.com

Table 2: Effect of Chromium sulphate on the morphometric characteristics of hyper-accumulator (*Mirabilis jalapa* L.) and hypo-accumulator (*Amarathus viridis L.*)

Metal concentration	Fresh weight (gm)			Dry weight (gm)		
	Chromium stress on ***Amarathus viridis L.***	After Co-cultivation		Chromium stress on ***Amarathus viridis L.***	After Co-cultivation	
		Amarathus viridis L.	*Mirabilis jalapa* L.		***Amarathus viridis L.***	*Mirabilis jalapa* L.
Control	5.6±0.045	6.2±0.034	7.8±0.341	1.23±0.023	2.23±0.034	3.96±0.33
2mM	4.95±0.11	5.34±0.23	6.8 ±045	0.92±0.034	1.96±0.045	3.45±0.04
4mM	3.23±0.034	5.02±0.98	6.20±0.76	0.86±0.067	1.56±0.098	3.01±0.045
6mM	2.87±0.023	4.98±0.23	5.69±0.34	0.75±0.056	1.23±0.034	2.8±0.034
8mM	2.08±0.034	3.89±0.34	4.98±0.32	0.45±0.045	0.93±0.054	2.2±0.045
10mM	0.87±0.05	1.9±0.098	2.5±0.45	0.12±0.056	0.33±0.078	2.8±0.098

All the values are the averages of five observations. Mean±Standard error