PHYTOREMEDIATIVE POTENTIAL OF IN VITRO GROWN *TROPAEOLUM MAJUS* L. FOR HEAVY METALS UPTAKE FROM THE TANNERIES CONTAMINATED SOILS OF KASUR

F. Khan,F. Khan, S. Shaheen and Z.K. Sial

Molecular Genetics and Biotechnology Lab, Dept of Botany, LCWU Lahore
E-mail of corresponding author: drfarah_khann@yahoo.com

ABSTRACT: The phytoremediative scavenging efficiency of in-vitro grown *Tropaeolummajus* (Garden nasturtium) for Chromium and Lead uptake was estimated and compared with that of field grown plants, using Atomic Absorption Spectroscopy. In vitro plantlets were grown on MS basal medium containing a combination of 2, 4-Dichlorophenoxyacetic acid (2,4-D)and 6-Benzylaminopurine (BAP) (2.0mg/L+1.0mg/L) under controlled physical factors. Both in- vivo and hardened in-vitroplants were shifted to contaminated soil of Tanneries at Kasur. After 60 days of growth in this contaminated soil, the plants bioassays were subjected to Atomic Absorption Spectroscopy for the estimation of heavy metals uptake. The in-vivo grown *Tropaeolummajus* absorbed much lesser amount of both the metals (2.65 and 1.42ppm) as compared to in-vitro grown plants which showed higher scavenging ranges (5.91 and 3.20 ppm) for Cr and Lead respectively. The in-vitro grown plants showed higher level of heavy metals scavenging potential as compared to their regular field grown counterparts. This increased level of phytoremediative efficiency of tissue cultured plants may have a practical application to clean the agricultural lands in future.

Keywords: Scavenging efficiency, Heavy metals, Atomic Absorption Spectroscopy, Phytoremediation.

INTRODUCTION

Phytoremediation is considered as the most emerging green technology to detoxify contaminated areas i.e. using the hands of nature to clean the nature. It can be used for both organic and inorganic pollutants, present in solid (soil) as well as liquid (water) substrates. The process of Phytoremediation of metals is being found as a potential environment friendly remediative solution to detoxify a large number of contaminated sites all around the world (Cutcheon*et al*., 2003).The conventional treatments of the sediments are no more suitable now due to their increasing cost. The plant roots phytoextract due to their natural ability to absorb the heavy metals of the soil. As natural processes are driven by solar energy, phytoremediation is on average ten folds cheaper than engineering-based remediation methods such as soil excavation, soil washing or burning, or pump-and-treat systems. Metals uptake using plants provides an environmental friendly solution of the soil pollution, which is a low cost, in-situ process, energized by solar energy (Archana*et al*., 2000). District Kasur of Punjab Province in Pakistan represents such a locality, which is badly polluted by tanning industry, due to deposition of its lethal chemicals in the local soils and canals, spoiling the land and biological life. This industry have given rise to serious environmental threats and complications specially in the countries lacking the implementation of environmental regulations. The shortage of funds and remote location of polluted areas are considered as the basic obstacles during their detoxification. There is a severe need of low energy green technologies applications e.g phytoremediation which can be used as an alternative.

Many plants species behave as hyper accumulators of the metals, depending upon their metal absorbing property. They have capacity to store these metals in the many different cells. These metals pass by the cellular membranes to inside the cell (symplast) from there they move to next destination which is plant vacuoles, It is place of enzymatic metal degradation which occur by membrane metal transporters, and are deposited there by some metal binding proteins (Manoko*et al*., 2006). Natural balance of molecules are destroyed when these heavy metals replace other essential metals in pigments inside the cell (Manios*et al*., 2003). The oxidative stress is also another major reason, especially for transition metals like Ferrous to ferric transition (Rivetta*et al*., 1997).

T*ropelummajus* L. of family Tropaeolaceae was selected as the experimental material during the present study. It is known as Indian Cress/Canary Bird Flower and most commonly garden nasturtium. A review of literature revealed that the plant has some good potential for tissue culture studies and also for natural phytoremediation (Aguiar*et al.,* 2006). This species is able to take up and accumulate in appreciable quantities of heavy metals like cadmium, copper, nickel, zinc, lead, hexavalent chromium, and selenium.

Plant tissue culture technique requires a controlled environment which is considered to be helpful for the estimation of several limiting factors. It is nowadays being used for evaluating and enhancing biotic stress to obtain variants with variable characters. This practice is also found to be beneficial for regenerated product to evaluate the heavy metal accumulation properties i.e. the removal of metals by different plant organs at different levels(Nedelkoska*et al*., 2000; Kartosentono*et al*., 2001).

Atomic Absorption Spectroscopy is a simple and rapid technique for quantitative and qualitative isolation of metals from soil rock and water even from vegetable material too (Rosselli*et al*., 2003). This process allows to avoid the organic matrix destruction stage, shortens the time, reduces cost, and minimizes hazards of loss and contamination. Therefore the main objective of the present study was to evaluate and compare the Chromium and Lead uptake by in vivo and in vitro grown *Tropaeolummajus* with the help of Atomic Absorption Spectroscopy (AAS) from the tanneries contaminated land near Kasur tanneries.

So the present investigation was formulated to evaluate the accumulation of Chromium and Lead in the plant body of *Tropaeolummajus*. It can also help to identify and compare the potential of plants to remove and remediate metals from the contaminated soil of tanneries. The research is also comprised of study about morphological and physiological responses of this plant under Cr and Pb stress.

We postulate that the hardened in vitro grown plants specially annual /biennial weeds may be used to scavenge the heavy metals from the polluted and contaminated soils due to their greater phytoremediative potential as compared to the regular field grown plants.

MATERIALS AND METHODS

Growth of *Tropaeolummajus* (both in vitro and in vivo):The certified seeds of *Tropaeolummajus*were sown and grown for 60 days in the regular soil of Lahore College for Women University, Lahore, Pakistan. The explants, taken from the field grown *Tropaeolummajus*were cultured and then subcultured on optimum culture medium containing different plant growth regulators (PGRs) for 8 weeks.MS (Mrashaige and Skoog, 1962) basal medium was used. Different PGRs i.e. 2, 4-D, BAP and combination of 2, 4-D and BAP were used. Sterilized explants were inoculated in MS basal medium supplanted with above mentioned PGRs.The best combination was further used for sub culturing after every 2 weeks.

Sugar contents were adjusted at 3% .Temperature was set at 25±2°C.Cultures were given a 16 hrs photoperiod and the pH of the medium was adjusted between 5.6-5.7.

After 60 days of in vitro growth, the rooted plantlets were gently removed from the culture tubes keeping the roots intact by using forceps with extreme care for avoiding any mechanical damage to the plantlets. These rooted plantlets were transplanted to small plastic bags containing a mixture of sterilized soil and sand, for hardening prior to their final transfer to the contaminated soil. These regenerated plantlets were then shifted to the contaminated soils of Kasur tanneries.

Estimation of Chromium and Lead uptake by the contaminated plants (in vivo and in vitro) using Atomic Absorption Spectroscopy: There was a comparative estimation of Chromium and Lead uptake by both types of *Tropaeolummajus* using Atomic Absorption Spectroscopy.

Samples were to be analyzed in solution form, so different dilutions were prepared .For making dilutions of solutions for plants before evaluating concentration of metal ions, wet digestion method was used, because elements in plants parts cannot be directly detected by atomic Absorption spectroscopy. Fresh leaves of both in vivo and in vitro plants were washed with double distilled water, dried and heated in an oven at 500°C for one hour. The ashed plant leaves were weighed separately and 5.0g of each type of leaves were wet digested in HCl/HNO_3 (mixed in 1:3 ratio) and heated till dryness. The volume was adjusted up to 50 ml with double distilled water and then was filtered. The sample solutions were then subjected to Atomic Absorption Spectrophotometer.

Three sets of each experiment were designed. The contamination percentage, percentage of callus bearing cultures and frequency of micro-propagated plants per explants were recorded. Mean deviation was calculated after using (Steel *et al*.,1997) following SPSS software (Levesque, 2007).

RESULTS AND DISCUSSION

A combination of 2, 4-Dichlorophenoxyacetic acid (2,4–D) and 6-Benzylaminopurine (BAP) (2.0mg/L+1.0mg/L) was proved to be the best for the in vitro growth of*Tropaeolummajus*L. The effect of different concentration of the 2, 4-D and BAP on in vitro growth of *Tropaeolummajus*in MS medium using different explants is given in table 1. Twenty-five cultures were inoculated for each type of explants and the best response was observed in nodal explants which gave maximum percentage of in vitro growth i.e. 87% in the medium containing 2, 4-D+ BAP (2.0+1.0 mg/L), whereas the minimum percentage i.e. 16% was found in 2, 4-D (1.0mg/L). These results are being supported by (Torres*et al.,*1992) who reported the micropropagation of *Tropaeolumtuberosum* depicting similar results.

Table1.Showing effect of different concentration of 2,4-D and BAP(mg/L) onin vitrogrowth of*Tropaeolummajus*using different explants.

Sr. no.	Explant used	2,4-D and BAP mg/L used	Number of Cultures inoculated	In Vitro growth/ cultures %age (mean)	In Vitro growth	LSD Value
i.	**Leaf**	1.0+1.0	25	32±0.51[cd]	++	1.21
		1.5+1.0	25	44±0.58c	++	
		2.0+1.0	**25**	**79±0.57[b]**	**+++**	
		2.5+1.0	25	29±0.34[a]	++	
ii.	**Node**	1.0+1.0	25	16±0.11[cd]	+	1.22
		1.5+1.0	25	51±0.39[c]	++	
		2.0+1.0	**25**	**87±0.41[b]**	**+++**	
		2.5+1.0	25	68±0.21[a]	++	
iii.	**Internode**	1.0+1.0	25	27±57[cd]	+	1.77
		1.5+1.0	25	16±0.58[c]	+	
		2.0+1.0	**25**	**64±0.31[b]**	**+++**	
		2.5+1.0	25	56±0.47[a]	+	
iv.	**Root**	1.0+1.0	25	02±0.99[cd]	+	1.96
		1.5+1.0	25	11±0.52[c]	+	
		2.0+1.0	**25**	**23±0.41[b]**	**+++**	
		2.5+1.0	25	02±0.32[a]	+	

±Standard error of the mean

The mean with different letters in each column are significantly different according to Duncan's multiple range tests (0.005p value)

Poor growth + Good Growth ++ Excellent Growth +++Regarding physical factors it was observed that a photoperiods of 16hrs light(3000 lux) and 8 hours dark showed 80% in vitro growth and zero hour photoperiod i.e., complete dark gave minimum, i.e.05% in vitro growth(Table 2). The maximum and minimum *i*nvitro growths, i.e 89% and 23% were seen at temperatures25±2°C and 20±2°C respectively.It was also noticed that *Tropaeolummajus* in liquid medium gave only 8% in vitro growth. Whereas, solidified medium (agar as solidifying agent) gave 80% in vitro growth for the plant (Table- 2). Most suitable pH value for in vitro growth was found to be 5.7 with 80% i.e. maximum in vitro growth percentage (Table- 3). While 30-35g sugar per liter of MS medium favoured maximum in vitro growth of the plants.(Sanatbi and Sharma2007) also reported the influence of pH, solidification and other physical factor on in-vitromicropropagtion of*Tropaeolummajus*. (Loaiza*et al.,* 2008) studied the effect of pH on thein -vitrogrowth of *some* cucurbitaceae family members. Asolidmedium with pH of 5.7-5.8 resulted in the maximum shoot proliferation rate, shoot length and leaves differentiation.It was reported by (Wielanek and Urbanek2006)that cutures of hairy roots of *T. majus* were obtained aftertransformation with *Agrobacterium rhizogenes* LBA 9402 (pRi 1855).The cultures were maintained on B5 medium containing 3% sucrose and 0.02% peptone from casein (pH 5.8), on theorbital shaker (110 rpm), in the dark, at 22°C.

Table 2.Showing effect of Agar Solidified medium and Photoperiod onin vitrogrowth of leafexplants of *Tropaeolummajus* in MS medium with 2, 4-D+2.0mg/L and BAP 1.0mg/L

Sr.No.	Physical factors	Maximum%In vitrogrowth (% mean)
1.	16 hrs Photoperiod(3000 lux)	**80±1.60**
2.	Agar SolidifiedMedium	**80±1.68**

Table 3: Showing effect of temperatures and pH onin vitrogrowth of leaf explants of*Tropaeolummajus*in MS medium with 2, 4-D+2.0mg/L and BAP 1.0mg/L

S.No	Physical factors	Ranges	Maximum%In vitro growth (% mean)
1.	pH	5.7	**80 ±1.21[a]**
2.	Temperature	25±2	**80±2.08[a]**

To determine the concentration of two heavy metals i.e; Chromium and Lead, both in field grown and in vitro grown plant tissues of *Tropaeolummajus,* after their shifting to the Kasur tanneries contaminated fields for 60 days, Atomic Absorption Spectroscopy technique was used. Field grown *Tropaeolummajus* gave Chromium and Lead uptake up to 2.65 and 1.42 ppm. Where as in in vitro grown *Tropaeolummajus* uptake for Chromium (Cr) and Lead uptake was found to be 5.91and 3.20 ppm respectively. Table 4 and 5 showed that Chromium and Lead, both were found in high quantities in invitrogrown plant tissues as compared toin vivogrown, which indicated thatthe composition of the media and soil type played an important role in mineral uptake of plants.

Table. 4. Showing concentration of Cr and Pd in in-vivo plant material of *T. majus* determined by Atomic Absorption Spectroscopy.

Selected plant	Plant tissue	Heavymetals	Metal uptake concentration(ppm)	%age Mean
Field grown *Tropaeolummajus*	LeafExplants	Cr	2.65 2.65 2.65	2.65±0.000
		Pb	1.42 1.42 1.42	1.42±0.000

Table. 5. Showing concentration of Cr and Pd in in-vitro plant material of *T. majus* determined by Atomic Absorption Spectroscopy

Selected plant	Plant tissue	Heavymetals	Metal uptake concentration(ppm)	%age Mean
Field grown*Tropaeolummajus*	LeafExplants	Cr	5.91 5.91 5.91	5.91±0.000
		Pb	3.20 3.20 3.20	3.20±0.000

In a study (Vijayarengan, and Lakshmanachary, 1994) reported that nickel uptake by the green grams showed a negative effect on their differential growth.

The uptake of Chromium and Lead was also reported by plant tissues ofmembers of Tropaeolaceae family (Archana*et al*., 2002). According to them the uptake concentrations of Chromium and Lead were also 3.54 ppm and 5.97ppm respectively. (Cardoso *et al.,* 2008) pointed out that trace mineral uptake by members of Rubiaceae family and the factors affecting them. (Stobart*et al.,* 2005) reported the deteriorating effects of Cd uptake on chlorophyll synthesis of Barley plants. The present work helped us to postulate the idea that in vitro grown plants can be a reasonable tool for practical implementation of phytoremediation and could behave better hyper accumulators of heavy metals as compared to their field grown counterparts. It was also postulated that the creeping weeds and annuals can scavenge the heavy metal contamination from a wider surface area and could be used as an effective phytoremediative tool infuture.

REFERENCES

Aguiar, E., G. Gomez, J. Aranda, J.D. Medina and K. Winter.Taxonomic significance of the epicuticular wax composition in species of the genus *Clusia* from Panama.Bioch. Syst. Ecol., 34(4): 319-326 (2006).

Archana, K. and S.L. Kothari. High efficiency adventitious shoot bud formation and plant regeneration from leaf explants of *Dianthus chinensis* L. Scientia Horticulture, 96: 205 – 212 (2002).

Cardoso, C.L., D.H.S. Silva, M.C.M. Young, I. Castro-Gamboa and V. da S. Bolzani.Indolemonoterpene alkaloids from *Chimarrhisturbinata* DC Prodr.: a contribution to the chemotaxonomic studies of the Rubiaceae family. Brazilian J. Pharm., 18(1): 26-29 (2008).

Kartosentono, S., A. Nuraida, G. Indrayanto and N. C. Zaini, Phytoremediation of Sr^{2+} and its influence on the growth, Ca^{2+} and solasodine content of shoot culture of *Solanumlaciniatum*. Biotechnology Letters, 12(2):153-155 (2007).

Levesque, R. SPSS Programming and Data Management:

A Guide for SPSS and SAS Users, Fourth Edition, SPSS Inc., Chicago III (2007).

Loaiza, J.C.M., L. Ernst, T. Beuerle, C. Theuring, C. L. Céspedes and T. Hartmann. .Pyrrolizidine alkaloids of the endemic Mexican genus Pittocaulon and assignment of stereoisomeric 1,2-saturated necine bases. Phytochemistry, 69(1): 154-161 (2008).

Lombardi, L and L. Sebastiani. Copper toxicity in *Prunuscerasifera*: growth and antioxidant enzymes responses of in vitro grown plants. Plant science, 168 (3): 797-802 (2004).

Manios, T., E. I. Stentiford and P. A. Millner. The effect of heavy metals accumulation on the chlorophyll concentration of *Thyphalatifolia* plants, growing in a substrate containing sewage sludge.Ecological Engineering, 2(1): 65-74 (2003).

Manoko, M.L.K., R.G. van den Berg, R.M.C. Feron, G.M. van der Weerden and C. Mariai.In vitro Cellular and Development Biology, 39: 500-504 (2007).

McCutcheon, S. C. and J. L. Schnoor, *Phytoremediation, Transformation andControl of Contaminants*. John Wiley-Interscience, New Jersey, 987 (2003).

Murashige, T and F. Skoog. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol plant, 15 (3): 473-479 (1962).

Nedelkoska, T. V. and P. M. Doran. Hyperaccumulation of cadmium by hairy roots of *Thlaspicaerulescens*. Biotechnology and Bioengineering, 67 (5): 607-615 (2000).

Rasselli A., L.G. Nair, K. Radhakrishnan, S. Seeni, G. M. Nair and P. Pushpangadan. Micropropagation of *Ulteriasalcifolia*, endemic and endangered ethnomedical plants of Western Ghats. Journal of Medicinal and Aromatic Plant Sciences, 25: 19 – 24 (2003).

Rivetta, A.; N. Negrini, and M. Cocucci. Involvement of Ca2+-calmodulin in Cd2+ toxicity during the early phases of radish (*RaphanussativusL.*) seed germination. Plant, Cell and Environment, 20 (5): 600-608 (1997).

Sanatbi, K., and G. J. Sharma. Micropropagation of *Capsicum frutescens*L. using axillary shoot explants. ScientiaHort, 113: 96-99 (2007).

Steel, R.G. D, J. H. Torrie and D. A. Dickey. Principles and Procedures of Statistics. biometrical approach. 3rd Ed., McGraw Hill Book Co, New York, USA (1997).

Stobart, A.K., W.T. Griffiths, I. Ameen-Bukhari and R. P. Sherwood.The effect of Cd on the biosynthesis of chlorophyll in leaves of barley. Physiol. Plant., 63: 293-298 (2005).

Torres, O,.M. Perea-Dallos andT. J. Fandiño.Micropropagation of Cubio (*Tropaeolumtuberosum*RandP).Biotechnology in Agriculture and Forestry.19:160-171(1992).

Vijayarengan, P. and A. S. Lakshmanachary.Differential nickel tolerance in greengram cultivars.Poll. Res. 13(3): 291-296 (1994).

Wielanek,M and H. Urbanek. Enhanced glucotropaeolin production in hairy root cultures of *Tropaeolummajus* L. by combining elicitation and precursor feeding.P lant Cell Tiss Organ Cult. 86:177–186 (2006).