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Evaluation of phytochelatin mediated heavy metal (HM) uptake efficiency of wheatgrass plant from heavy metal contaminated soil

Tirodkar Tanuja V[1]*, Ambre Snehal J[1], Bhike Vaishnavi V[1], Ghag Vignesh V[1]

[1]Department of Biotechnology, Kelkar Education Trust's V.G. Vaze College of Arts, Science and Commerce, Mulund East, Mumbai-400081, India.
*Corresponding author Email: tirodkartanuja@gmail.com

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ABSTRACT

Phytoextraction - form of phytoremediation involves growth of tolerant plants in contaminated soils; plants take up large amounts of heavy metals from the soil, depleting it of the target pollutants and translocating them to the aerial parts of the plant. In the present study phytochelatin synthesis in Wheatgrass (Triticum aestivum) was used as a measure for phytoremediation of heavy metal (Cr, Pb, Cd) contaminated soil for a period of 14 days. Phytochelatin (PCs) – small metal binding polypeptides are synthesized non-translationally from reduced glutathione by enzyme phytochelatin synthase in hyperaccumulating plants only when exposed to heavy metal toxicity which translocate contaminants in plant vacuoles making them inert. Wheat grass is a hyperaccumulator species for Cr, Cd, Pb, Zn etc. So, PC content in Wheatgrass plant exposed to varying concentration of heavy metals was assessed by Ellman's Test Protocol and changes in factors influencing phytoextraction like plant growth rate, biomass, phytotoxicity were observed alongside to study the efficiency of phytoremediation. An increase in PC content was observed in the order Control < Cr < Pb <Cd. With the plant exposed to Cd producing maximum phytochelatin. Gradual decrease in plant growth rate and biomass content was observed after tenure of 14 days.

Keywords: Wheatgrass plant, Heavy metals, Phytochelatin, Phytoremediation.

INTRODUCTION

Modern lifestyle and industrialization have led to many environmental problems (Lal, et al., 2019) . Generation of different types of waste, and its dumping without proper treatment have led to the accumulation of pollutants in the environment (Qadir, et al., 2018). Unlike organic pollutants, heavy metal (HM)s once introduced into the environment cannot be biodegraded (Rajeshwari & Sailaja, 2014). Heavy metals conventionally defined as elements with metallic properties and an atomic number >20 are micronutrients necessary for plant growth, such as Zn, Cu, Mn, Ni, and Co, while others have unknown biological function, such as Cd, Pb, and Hg (Lasat, 2000).

Toxic heavy metals such as Pb, Co, Cr, Cd can be differentiated from other pollutants, since they cannot be bio-degraded but can be accumulated in living organisms, thus causing various diseases and disorders even in relatively lower concentrations (Pehlivan & Ozkan, 2009). Therefore, proper set of processes to treat the contaminated soil or water are to be followed before they can be brought to common use. Conventionally, remediation of heavy-metal-contaminated soils involves either onsite management or excavation and subsequent disposal to a landfill site. This method of disposal solely shifts the contamination problem elsewhere along with the hazards associated with transportation of contaminated soil and migration of contaminants from landfill into an adjacent environment. Soil washing for removing contaminated soil is an alternative way to excavation and disposal to landfill. This method is very costly and produces a residue rich in heavy metals, which will require further treatment· Moreover, these physio-chemical technologies used for soil remediation render the land usage as a medium for plant growth, as they remove all biological activities (Gaur & Adholeya, 2004). The effective treatments are quite costly and lead to the production of undesirable side products, incomplete destruction of target pollutants, high energy and time input, soil structure variation and alteration (Jadia & Fulekar, 2008). In order to overcome the shortcomings of conventional methods, phytoremediation is introduced into remediation field (Gaskin, et al., 2008). This cost-effective plant-based approach to remediation takes advantage of the remarkable ability of plants to concentrate elements and compounds from the environment and to metabolize various molecules in their tissues. Toxic heavy metals and organic pollutants are the major targets for phytoremediation (Salt, et al., 1998).

Phytoremediation is a bioremediation process that uses various types of plants to remove, transfer, stabilize, and/or destroy contaminants in the soil and groundwater. Phytoextraction-form of phytoremediation involves growth of tolerant plants in contaminated soils; the plant takes up large amounts of heavy metals from the soil, depleting it of the target pollutants and translocating them to the aerial parts of the plant, specifically into vacuoles (Rafati, et al., 2011). The efficiency of phytoextraction depends on characteristics of soil and contaminants (Thangavel & Subbhuraam, 2004) Grasses are more preferred for phytoextraction because of their densely spread network of roots with extensive surface in soil for uptake, high growth rate, more adaptability to environmental stress and high biomass (Kulakowl & Erickson, 2000; Paschke et al., 2009). The current study reports phytochelatin mediated heavy metal uptake by Indian wheatgrass *(Triticum Aestivum)* in soil spiked with Cr, Pb and Cd heavy metals in varying concentration and changes in factors influencing phytoextraction like plant growth rate, biomass, phytotoxicity to study its efficiency.

Phytochelatin (PC) synthesis is one of the responses exhibited by plants exposed to HMs, and it is a useful biomarker, reflecting plant's actual exposure to excess internal HM content (Emamverdian, et al., 2015). Indian wheatgrass plant is the plant of primary importance chosen for this research. It is a monocot plant whose stems contain various cells (parenchyma and sclerenchyma) that aid in the capacity of the plant to withstand stress factors. Wheat plants may also be grown throughout the year I.e., even in colder seasons which suggest that these plants can withstand harsh environmental conditions (Bidlack & Jansky, 2011).

MATERIALS AND METHODS

Pot experimental setup was designed to facilitate phytoextraction of heavy metal spiked soil by wheatgrass plant for a period of 14 days. During the tenure plants were observed for morphological changes and growth rate, after exposure to externally spiked heavy metal contaminated soil the plants were checked for their phytochelatin content as a measure for evaluating phytoremediation.

Plant cultivation:

Materials

Soil: Soil was obtained from a local nursery in Mulund, Mumbai (India). The soil was sieved with a 0.5cm sieve before analysis.

Plant Sample: Wheat (*Triticum aestivum*) grains were acquired from a local supermarket of variety MP Sihore wheat. Wheat grains (*Triticum aestivum*) were washed thoroughly and were kept for germination for 2 -3 days in moist cotton cloth at room temperature.

Heavy metal salt solution: $Pb(NO_3)_2$, $K_2Cr_2O_7$, $CdCl_2$.

Method:

Experimental Set-up (Athar & Ahmad , 2002): Experiment pots were set-up containing 800gms of soil incorporated with varying concentrations of heavy metals (as per the table given below), along with a control (No heavy metals added).

The addition of heavy meals was followed by stabilization of soil.

Table No. 1 Amount of heavy metal added to the soil at various dose levels

METALS	0.5 x	1 x	2 x
Pb (Lead) $Pb(NO_3)_2$	75ppm 119.9mg/L	150ppm 239.86mg/L	300ppm 472.7mg/L
Cr (Chromium) $K_2Cr_2O_7$	30ppm 84.8mg/L	60ppm 169.6mg/L	120ppm 339.2mg/L
Cd (Cadmium) $CdCl_2$	5ppm 8.16mg/L	10ppm 16.33mg/L	20ppm 32.66mg/L

Uniformly germinated wheat seeds were selected and sown in the stabilized soil. The plants were watered daily for 14 days.

Soil analysis

The pH of the soil was checked by pH strip and the soil was analyzed for organic matter content by Walkley–Black method (Allison & Black, 1965).

Phytochelatin Content:

Phytochelatin = Non protein thiol – Reduced Glutathione (GSH)

Spectrophotometric method is routinely used to determine glutathione levels in cells. The spectrophotometric assay involves oxidation of GSH by the sulfhydryl reagent 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB) to form the yellow derivative 5′-thio-2-nitrobenzoic acid (TNB), measurable at 412 nm

Materials: Chemicals: 10mM DTNB stock, 5% Sulfosalicylic acid, 1M Tris. HCl 7.4 pH, 0.5Mm EDTA, 100Mm Phosphate buffer, Standard Reduced Glutathione, Cysteine Hydrochloride.

10mM DTNB stock: 0.04g DTNB in 10 ml DMSO (dimethyl sulfoxide)

1mM DTNB: 1ml 10Mm DTNB in 9ml 5% Sulfosalicylic acid (SSA)

0.5 mM EDTA: 0.0816g EDTA in 80 ml D/W water.

100 mM PBS: 1.549g $Na_2HPO_4.H_20$ + 0.583g $NaH_2PO_4.H_2O$ in 80ml D/W water.

Method:

Ellman's Protocol (Garg & Kaur, 2012):

1) Reduced Glutathione content:

- 0.5g of fresh wheatgrass leaves were homogenized in 2ml of 5% sulfosalicylic acid.
- The homogenate was centrifuged at 10000rpm for 10min.
- 0.5ml of supernatant + 0.6ml of 100mM phosphate buffer (pH-7) + 40microlitre of DTNB.
- After 2 min the absorbance was read at 412nm on the UV Vis spectrophotometer.

2) Total Non-protein thiols:

- 100 microliters of supernatant + 0.5ml reaction buffer +0.5ml DTNB.
- Mix well and keep it for 10mins.
- Absorbance was read at 412nm using UV-Vis spectrophotometer after 10mins.
- Blank was set with the same reaction except supernatant replaced by D/W.

Graph was plotted for GSH as well as TNP - SH against their respective standards and their concentration in plant extract was estimated.

Phytotoxicity:

Phytotoxicity is the capacity of a compound (plant part) to cause temporary or long-lasting damage to the plant part in response to environmental stress.

Method:

To calculate the percentage phytotoxicity the shoot lengths were measured after a 10 days period. Random sampling method was followed and 5 leaves from each concentration of heavy metal and Control were analyzed for deformations (Meier, 2001)

(Deformations cover any morphological changes in the plant or plant part deviating it from the control. It includes curling, rolling, stunting or elongation, change in size or volume).

$$\% \, Phytotoxicity = \frac{Shoot\ of\ control - Shoot\ of\ plant\ exposed\ to\ heavy\ metal}{Shoot\ of\ control} X100$$

RESULTS

Soil analysis:

The pH of the soil was measured with help of pH paper was found to be 7.3pH.

Soil organic analysis was done with the help of Walkley – Black method using endpoint from purple to brilliant green.

The percentage organic matter in the procured soil sample was calculated to be 18.93%C per gram of soil and % of carbon in the soil sample was 11.01%.

Phytochelatin content

Phytochelatin (PC) content formed during Phytoextraction of heavy metals contaminated soil was estimated by Ellman's Assay.

Concentration of Reduced Glutathione (GSH) and Non protein thiol (TNP-SH) was estimated by plotting a graph of absorbance at 412nm against concentration of each respectively.

Phytochelatin content was seen to be in order of Control<Cr<Pb<Cd.

Phytotoxicity: -

The wheatgrass plants subjected to phytoextraction of heavy metals showed some morphological differences. After a period of 10 days the plants were analyzed for phytotoxicity and the differences in results were obtained which are depicted in Table 3.

Gradually from the 10th day the volume of the leaves was observed to decrease. The leaves started wilting.

Table 2: Phytochelatin content produced due to phytoextraction of heavy metals

Plant shoot	(GSH)O.D. at 412nm	GSH in (mM) Acc.to graph	TNP-SH O.D. at 412nm	TNP-SH in (mM)	PC
Control	0.163	0.226	0.272	0.5	0.274
Cr x 0.5	0.234	0.326	0.199	0.37	0.044
Cr x 1	0.149	0.206	0.269	0.49	0.284
Cr x 2	0.116	0.159	0.173	0.29	0.14
Pb x 0.5	0.125	0.17	0.216	0.4	0.23
Pb x 1	0.072	0.09	0.299	0.56	0.44
Pb x 2	0.068	0.08	0.230	0.41	0.32
Cd x 0.5	0.194	0.270	0.383	0.72	0.45
Cd x 1	0.377	0.528	0.432	0.82	0.42
Cd x 2	0.178	0.247	0.233	0.42	0.19

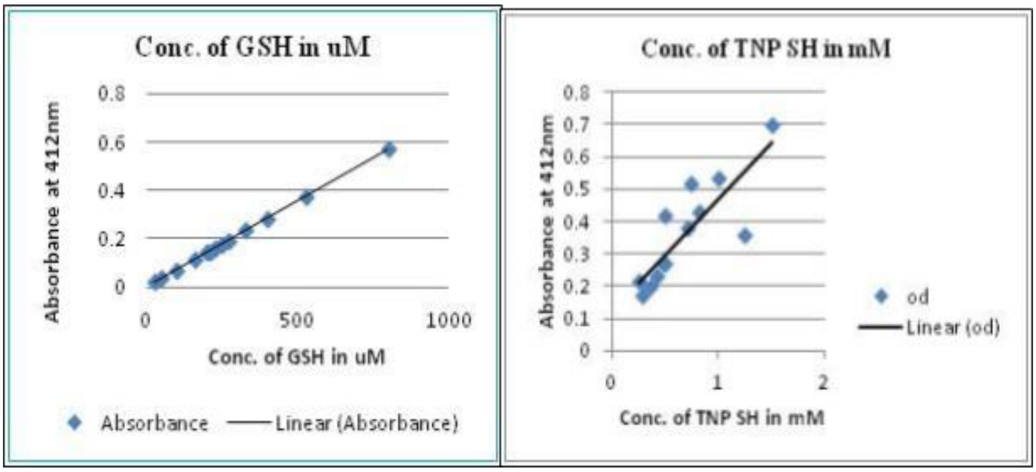

Fig. 1 and Fig. 2 Graph showing concentrations of GSH and TNP SH respectively.

Table 3: % phytotoxicity at varying metal contamination concentration

Plant shoot	Length of shoot (in cm.)	%Phytotoxicity
Control	20	-
Cr x 0.5	18.9	5.5
Cr x 1	18	10
Cr x 2	17.8	11
Pb x 0.5	19	5.2
Pb x 1	18	10
Pb x 2	17.5	12.5
Cd x 0.5	18.7	6.5
Cd x 1	18.3	8.5
Cd x 2	17	15

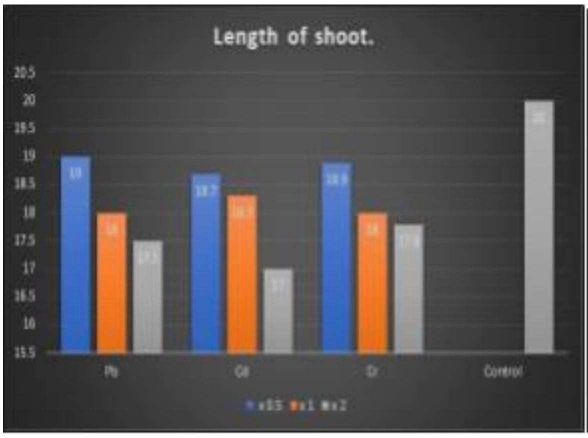

Fig 3: Graph showing length of shoot.

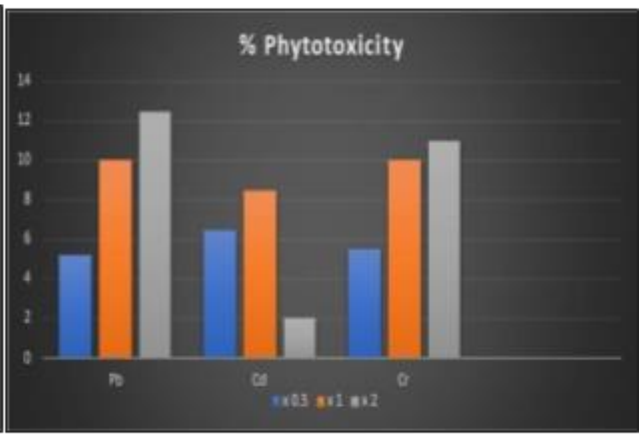

Fig 4: Graph showing % Phytotoxicity.

DISCUSSION

Morphological changes analyzed over the period of 14 days revealed that there was a decrease in the volume of plant shoot as the concentration of heavy metal was amplified. Over the time of exposure, thinning and wilting of leaves was observed, along with steady decline in shoot volume, Cd showed the greatest inhibition of volume as compared to control. After 11th day plants exposed to heavy metals irrespective of their concentration displayed discoloration, yellowing, and stunting of growth, which concur with the work of (Ouzounidou, et al., 1997) The study further followed Phytochelatin production in the wheatgrass plants exposed to heavy metals as a measure to evaluate phytoremediation capability. The precipitous induction of PCs occurs inside cells as result of the varying levels of multiple types of HMs where PCs via sulfhydryl and carboxyl groups can attach to some HM cations and anions such as Cd, Cu, Cr, Ag, Zn, Pb, Ni, and Ar. Phytochelatin production in the experimental plants was observed to be in the order Control < Cr < Pb < Cd. Cd2+ ions are found to be the most effective stimulator of PCs synthesis where they are 4 to 6-fold stronger in inducing PCs than any other metal, this might be the reason for high PC content in Cd exposed plants. (Emamverdian, et al., 2015) With increase in the heavy metal concentration, phytochelatin content showed a trend wherein it is low in the beginning, then rises eventually and lowers at the highest concentration of heavy metal. The decrease in phytochelatin content in the plant exposed to highest concentration of heavy metal may be a consequence of ongoing phytochelatin production which would have risen if exposed for an extended time period or due to decrease in available biomass to bioaccumulate heavy metals (Emamverdian, et al., 2015; Hentz & McComb, 2015). Because a similar order of sequence was observed for phytotoxicity Control < Cr < Pb < Cd which would have possibly decreased the rate of sequestration of higher concentration of heavy metals. In agricultural practice, plants cultivated on heavy metal contaminated soils are often not stressed to the extent of severe growth reduction. In such situations only increased metal levels in the plant are observed. Whether under less extreme conditions PCs are induced in detectable concentrations. For such conditions the usefulness of PC as biomarkers for heavy metal stress of plants particularly depend on (i) the sensitivity of techniques to detect PC in plant tissues at very low levels and (ii) the contribution of micro-nutrients to induction of PC synthesis (Keltjens & Beusichem, 1998). Therefore, for determining the exact heavy metal concentration sequestered by wheatgrass plant ICP – MS analysis needs to be carried out.

CONCLUSION

The experiment suggests that Indian wheatgrass *(Triticum aestivum)* could sequester up to 60ppm Chromium (Cr), 150ppm Lead (Pb), and 10ppm Cadmium (Cd) since a linear phytochelatin production graph was obtained up to these levels. All the plants survived the stress condition but the plant growth rate and uptake were affected, the plants demonstrated tolerance to chromium, lead and cadmium contamination which may have aided to their colonization in a heavy metal contaminated environment. It appeared that total thiols which represented phytochelatin synthesis can partially explain the tolerance and uptake of these metals by wheatgrass plant (Emamverdian, et al., 2015) The variation in the accumulation and resultant phytochelatin production could be due to the difference in the uptake mechanism of different heavy metals by wheatgrass plant (Emamverdian, et al., 2015). This specific range of heavy metal concentration is usually found in soils contaminated by extreme overuse of pesticides or near Municipal sewage sludges. Thus, identifying the potential of Indian wheatgrass for phytoremediation of pesticide overused fields or near agricultural farms alongside railway tracks. The current study focused on evaluation of phytochelatin mediated heavy metal uptake of Chromium (Cr), Lead (Pb), and Cadmium (Cd) by wheatgrass plant, further analysis of the factors affecting uptake of heavy metals in wheat grass plant, its effect on phytochelatin content, and its efficacy in phytoremediation need to be studied.

Conflicts of interest: The authors stated that no conflicts of interest.

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