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PHYTOREMEDIATION POTENTIAL OF *CATHARANTHUS ROSEUS* L. AND EFFECTS OF LEAD (Pb) TOXICITY ON ITS MORPHO-ANATOMICAL FEATURES

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Abstract

This research work was conducted to explore some morpho-anatomical features of periwinkle (*Catharanthusroseus* L.) that are affected by lead toxicity. Pb pollution is a common problem in Lahore and its surroundings. Untreated industrial waste is disposed in the rivers and canals which causes high levels of Pb in waters and soils in Pakistan. An economically and medicinally important plant species was exposed to different concentrations of Pb $(NO_3)_2$ and various morpho-anatomical parameters were checked in order to observe the accumulation and the extent of toxicity of this heavy metal in treated plants. The parameters included root, shoot and leaf anatomies, chlorophyll estimation, and changes in structures of tissues. Atomic Absorption Spetroscopy was also done to observe the estimated amounts of metal accumulated in different tissues of treated plants. Results showed that the applications of higher concentrations of Pb caused reduction in sizes of cells, especially in the vascular tissues of roots and stems; this was an indication that Pb was accumulated in the treated plants. It was also observed that the accumulation of this metal was higher in roots than the aerial parts. Hence, the treated plant species appeared to be affected by exposure to higher concentrations of Pb without any considerable harm to its anatomy and physiology. Periwinkle, a medicinally and ornamentally important plant, can be a potential accumulator of Pb and can be used in excluding toxic metals from the contaminated soils although further research is required in this regard.

Key words: Lead, Periwinkle, Accumulation, Toxicity, Tissues, Bioaccumulator.

Introduction

Heavy metals pollution is a serious problem in many developing countries including Pakistan. It is causing environmental deterioration and affecting the entire ecosystem. Heavy metals are naturally occurring elements. About forty elements fall into this category and are mostly found in rocks (Sharma & Agarwal, 2005). Their multiple applications have increased their distribution in the environment, raising their potential harm for flora and fauna (Tchounwou *et al*., 2012). Pb and other heavy metals affect the developmental stage of plants which causes reduction in growth and metabolic activities resulting in low quality and quantity of fruits. They hinder the biochemical pathways that are crucial for plant development (Khan *et al*., 2016). Heavy metals may also reduce the number of branches and leaf area (Bhatti *et al*., 2017). However, some plants may accumulate these metals and convert them into non-toxic form. These plants can be used for phytoremediation which is an emerging technology that makes use of plants to remove pollutants from the environment. Phytoremediation techniques can minimize environmental disruption, maintain soil fertility, are cost effective and environment friendly. In some cases it is the only way to remove soil and water pollutants which are produced because of human activities. Plants can remove these pollutants either through phytostabilization, phytodegradation, rhizofiltration or phytoextration. There are two basic strategies of phytoremediation of heavy metals: accumulation and exclusion. In accumulation, metals can be concentrated in plant parts from low or high background levels while in elimination, metals are differentially taken up and transported by the plant (Baker, 2008). Other ways of detoxification or elimination of these toxins include compartmentation in vacuole and chelation of metals in cytosol (Hall, 2001). The movement of metal from soil to root and then to shoot is phytoextraction. Research shows that chemically assisted phytoextraction can be a feasible technology for phytoremediation (Araújo do Nascimento & Xing, 2006).

Hyperaccumulators are those plant species which have the ability to uptake metals at higher rates and translocate them to roots and aerial parts (Khan *et al.,* 2016). These plants are especially important in phytoremediation as they accumulate the pollutants even at higher concentrations than their environments. Some research has been conducted on phytoremediation abilities of herbaceous plants in Pakistan; one such study suggests that medicinally important plants such as *Calotropis procera* and *Peristrophe bicaliculata* have the ability to store Pb in higher amounts (Begum *et al*., 2017) but not most of the plants studied have been declared as potential hyperaccumulators. *C. roseus* might have the ability to accumulate Pb with minute anatomical and physiological changes in the plant (Pandey *et al*., 2007). This plant is also relatively resistant to other heavy metals such as Ni and can be used as a phytoremediator against many pollutants at the same time (Arefifard *et al*., 2014). The concentration of accumulated Pb was found to be more in roots than the aerial parts of the *C. Roseus*. (Subhashini & Swami, 2013). *C. roseus* may be one of the most suitable plants to accumulate Pb from contaminated soils.

Heavy metals have the largest availability in soil and aquatic ecosystems as compared to the atmosphere. The natural way of entry of these metals is through marine salts and they are introduced through agricultural and mining activities. Pb enters the atmosphere during the combustion of petrol and eventually enters the food chains (Tyler, 1972). It is used in battery making, metallurgy and in building construction (Lees & Langois, 1994). It is available abundantly in the soils; it enters the ecosystem primarily by uptake of plants and causes abnormal cell growth. It traverses through food cycle and causes nervous disorders in humans and other animals (Khan & Frankland, 1983). It is relatively fixed and immobile in soils (Broyer *et al*., 1972), this reduces the availability for absorption by plants. However, no extensive research has been done regarding the movement or the pathways of heavy metals in the plant system. Pb accumulation is associated with the ability of plants to restrict Pb to the cell walls and activation of antioxidant defense system (Sharma & Dubey, 2005). Plants used for phytoremediation, especially the species used against heavy metals, require further processing. The plant material is concentrated by composting or ashing and then used to recycle the metal or is discarded in a landfill (Pilon-Smits & Freeman, 2006). Proper discarding of plant material is mandatory after its use as a phytoremediator to eliminate any further threats to environment. The aim of this study was to identify the phytoremediation potential of periwinkle to eradicate heavy metals present in soil and water bodies in Lahore and surrounding regions. Cleansing the environment through the usage of periwinkle will not only help the scientists, agriculturists and farmers reduce the amounts of heavy metals but it is also of ornamental importance and can be planted anywhere in the city from roadsides, parks to lawns. If proven to be a potential hyperaccumulator, the easily accessible periwinkle species can greatly reduce the ever-increasing amounts of heavy metals, especially Pb, and create better conditions for crops and other important plants to grow.

Materials and Methods

In order to find out the effects of Pb toxicity in periwinkle, some morpho-anatomical features were studied and compared with control (tap water) group. Chlorophyll estimation was done through spectrophotometry for both control and experimental groups. Atomic absorption spectroscopy was conducted to estimate the amount of accumulated Pb in experimental groups and the estimated amounts were compared with the control group. Anatomical sections were prepared by hand-sectioning; cell sizes were then measured and compared with those of the control group. All these parameters were tested in order to explore the potential of periwinkle to withstand Pb toxicity.

Following methodologies were used to meet the objectives of this research:

Sample collection and experiments: *Catharanthus roseus* plants of almost equal size were collected from the botanical garden of Forman Christian College (A Chartered University), placed in beakers (1L capacity) and marked according to their treatments. Each beaker contained five-six fully grown plants. The solutions in the beakers were replaced at regular intervals (every week) and were maintained under the natural conditions of light, temperature and humidity. Different concentrations of $Pb(NO_3)_2$ applied individually were 20 ppm $Pb(NO_3)_2$, 35 ppm $Pb(NO_3)_2$ and 50 ppm $Pb(NO_3)_2$. A separate group of plants was kept in tap-water as control group.

Morphology: Data regarding the external morphology was recorded on monthly basis. Length of plants and leaf texture was observed in control as well as treated plants. Leaf epidermis was also observed under light microscope to study the changes in stomata.

After two months of growth in different gradients of $Pb(NO_3)_2$ solutions, slides were prepared of the transverse and longitudinal sections of roots and stems and were viewed and photographed under light microscope (MT5300H – Meiji Techno, Japan). All the observations were taken at 10X and 40X power of light microscope, however; the sizes of cells were studied at 10X. The length and diameter of cells were recorded with the help of ocular micrometer. The data obtained was converted into micron meters (μm) with the help of stage micrometer. Parameters evaluated in the transection were the diameter of cortical cells and vascular cells of roots. Diameters of vascular bundles, cortical cells and pith cells in stem cross sections were also studied. The micrometry of leaves comprised of the evaluation of diameters of upper and lower collenchymas, xylem and phloem cells.

Chlorophyll Estimation: Chlorophyll is soluble in acetone. When the sample is macerated in acetone, optical density of the extract was measured at 645 nm and 663 nm. Leaves of *C. roseus* were weighed (measuring 3 cm^2) and were cut into small pieces. They were crushed in mortar with 5 mL of 80% acetone for 4 to 5 minutes until the paste was produced. Rinsing was done using 1 mL of 80% acetone. Acetone was added to make final volume up to 10 mL total in test tube. Then, a sample measuring 3 mL was added from top of the sample into the curvet for spectrometry. First the wavelength was set at 663 nm and the readings for 80% acetone were made as zero. Then the absorbance of sample was recorded through spectrophotometry (Arnon, 1949).

Chlorophyll 'a' (mg/g) = [(12.7 × A663) – (2.6 × A645)] × milliliters of acetone / milligrams of leaf tissue

Chlorophyll 'b' (mg/g) = [(22.9 × A645) – (4.68 × A663)] × milliliters of acetone / milligrams of leaf tissue

Total chlorophyll (mg/g) = Chl 'a' + Chl 'b' (Arnon, 1949)

Atomic absorption spectroscopy (AAS): Atomic absorption spectroscopy was done for roots and stem tissues by digesting 0.10 grams of plant material in a mixture of 25 mL of concentrated nitric acid and 10 mL of concentrated sulphuric acid under a fume hood. Sample was heated (with watch glass over the beaker) and mixture was added slowly to approximately 50 mL of distilled water in a 100 mL volumetric flask. Beaker was rinsed several times to ensure that all material is transferred and was diluted to the mark with distilled water. All readings were recorded through atomic absorption spectrometer. Fifteen mL of each sample material was tested by flame atomic absorption spectrometer (Hseu, 2003).

Results and Discussion

Plants treated with Pb showed wilting and yellowing of leaves after a month. Excessive shedding was observed in lower leaves, especially in the groups treated with highly concentrated solutions. It was also observed that the control group showed lesser shedding as compared to the experimental group. The yellowing of leaves and wilting of the plants may be a sign of Pb toxicity. There were no noticeable changes in stomata and the plants of experimental group were functioning well even after exposing them to $Pb(NO_3)_2$, this is a sign of resistance to Pb. Some previous research has also shown that *C. roseus* shows high resistance against Pb (Pandey *et al*., 2007). This work is another step towards indicating that periwinkle may have the potential to withstand higher concentrations of Pb.

Change in cell sizes is one of the most important parameters to measure heavy metal resistance. There was a noteable decrease in sizes of xylem and phloem cells of roots and stems in plants treated with $Pb(NO_3)_2$ as compared to the cell sizes in control group (Figs. 1-3). The decrease was significant, especially in the plants which were given 50 ppm $Pb(NO_3)_2$. This decrease might be an indication of Pb toxicity and since the aerial parts (vascular tissue in stem) showed decrease in average sizes, the plant can be thought of as an accumulator of Pb. The accumulation of Pb causes reduction in growth of roots (Sharma & Dubey, 2005). Our results corroborate with the previous research conducted in this field. However, there was no significant decrease in cell sizes in cortical region. The cross sections of leaves of treated plants showed that all cells in the leaves including upper and lower collenchymas, and vascular tissues showed a significant decrease in sizes (Figs. 1 & 2). These results are an important demonstration of Pb accumulation in aerial parts of the treated plants.

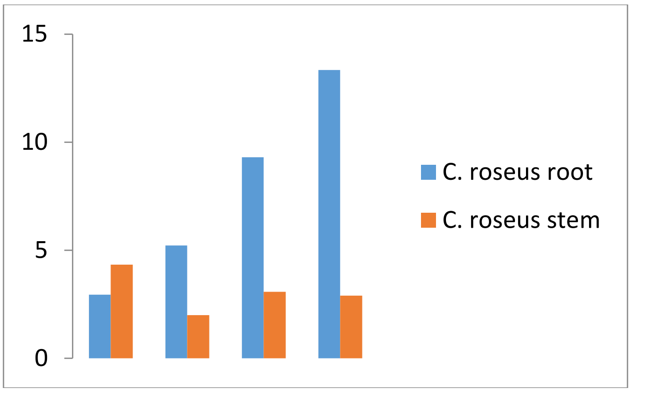

Fig. 3. Amount of Pb (Mg/L) in *C. roseus* tissues through AAS.

Table 1. Effects of doses of $Pb(NO_3)_2$ on the amounts of chlorophyll 'a' and 'b' in *C. roseus*.

Treatments of $Pb(NO_3)_2$ (ppm)	Amount of chlorophyll (mg/g)	
	Chl. 'a'	Chl. 'b'
0 (control)	40.00	194.55
20	34.99	295.83
35	25.55	129.93
50	29.32	139.90

Table 2. Effects of doses of $Pb(NO_3)_2$ on cell sizes in transverse sections of *C. roseus* roots.

Treatments of $Pb(NO_3)_2$ (ppm)	Average diameter (µm)		
	Xylem cells	Phloem cells	Cortex cells
0 (control)	416	160	384
20	320	144	315
35	324	128	320
50	208	96	352

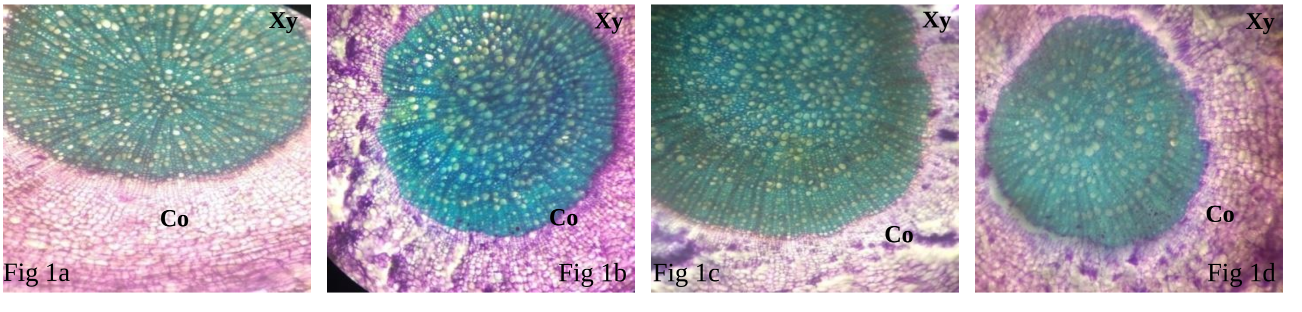

Fig. 1a. T.S. of *C. roseus* control root at 40X. (Fig 1b): T.S. at 40X of *C. roseus* root treated with 20 ppm $Pb(NO_3)_2$. (Fig 1c): T.S. (40X) of *C. roseus* root treated with 35 pm $Pb(NO_3)_2$. (Fig 1d): T.S. (40X) of *C. roseus* root treated with 50 ppm $Pb(NO_3)_2$. Cortex (Co), Xylem (Xy).

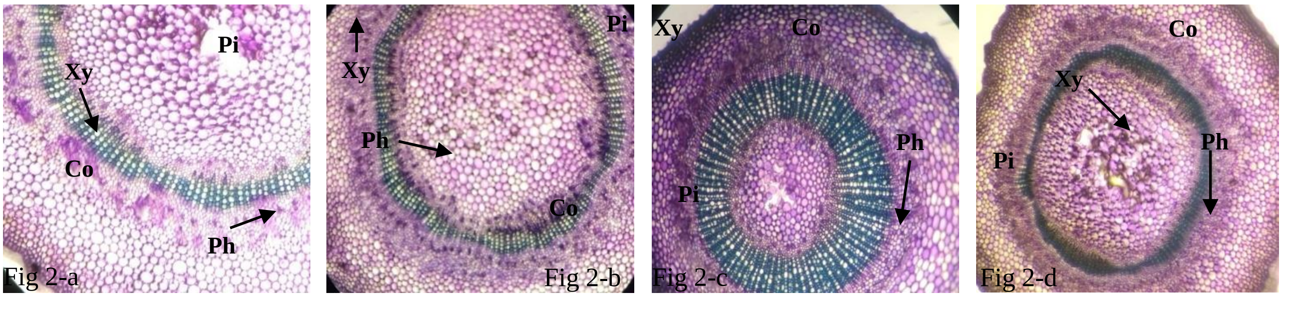

Fig. 2-a. T.S. of *C. roseus* control stem at the magnification of 40X. (Fig 2-b): T.S. (40X) of *C. roseus* stem treated with 20 ppm $Pb(NO_3)_2$. (Fig 2-c): T.S. of *C. roseus* stem treated with 35 ppm $Pb(NO_3)_2$ at 40X magnification. (Fig 2-d): T.S. of *C. roseus* stem treated with 50 ppm $Pb(NO_3)_2$ at 40X. Cortex (Co), Pith (Pi), Phloem (Ph), Xylem (Xy).

There was a notable decrease in the amounts of Chlorophyll 'a' and 'b' in treated plants, especially at higher concentrations of Pb (Table 1).This reduction might be another indication of Pb toxicity and since the plants were functioning properly after the experiment, it can be said that the plants were somewhat resistant to this toxic salt. Atomic Absorption Spectroscopy (AAS) results further supported the supposed reason behind reduction in the amounts of chlorophylls. AAS of the treated plants showed an increase in the amount of Pb present in the stem and root tissue (Table 2 & 3). This furthers that Pb reached the aerial parts and the plant was able to accumulate it. These results correspond with some previous research which suggests that this plant is an excellent accumulator of Pb and can be used for phytoremediation (Pandey *et al*., 2007). It has been reported that Pb tends to accumulate more in roots as compared to the shoot system (Subhashini& Swami, 2013). The work shows that the amount of Pb in root tissue was higher than that in stem tissue (Tables 2 & 3); this suggests that the plant has a potential to be used to remove Pb from contaminated soil. This plant is an important species as it is a commonly found and already being used as an ornamental in floriculture in Lahore and surrounding areas. This plant holds a great medicinal importance as it is a source of over 100 alkaloids including vincristin, vinblastin and serpentin which hold considerable importance in chemotherapy (Pandey *et al*., 2007). *C. roseus* can be an excellent indicator or accumulator of Pb with other important benefits, although extensive research is to be done in this field to prove the potential of periwinkle as a potential accumulator.

Table 3. Effects of doses of $Pb(NO_3)_2$ on cell sizes in transverse sections of *C. roseus* stems.

Treatments of $Pb(NO_3)_2$ (ppm)	Average Diameter (µm)			
	xylem cells	phloem cells	pith cells	cortex cells
0 (control)	245	384	392	392
20	129	194	347	323
35	194	160	452	371
50	196	194	473	359

Table 4. Effects of doses of $Pb(NO_3)_2$ on cell sizes in transverse sections of *C. roseus* leaves.

Treatments of $Pb(NO_3)_2$ (ppm)	Average Diameter (µm)			
	xylem cells	phloem cells	pith cells	cortex cells
0 (control)	245	384	392	392
20	129	194	347	323
35	194	160	452	371
50	196	194	473	359

Conclusion and future perspectives: Accumulation of Pb in *C. roseus* suggests its potential role in removal of Pb from the water.Anatomical parameters further support this idea as there was a significant decrease in cell sizes of aerial parts of the treated plants. Atomic Absorption Spectroscopy of tissues and chlorophyll analysis further supported the hypothesis that Pb can be accumulated and translocated by periwinkle as it reached the aerial parts. However, role of *C. roseus* in phytoremediation can be studied further at molecular level or through studying metabolic pathways to declare the role of this plant as a potential accumulator along with being medicinally and ornamentally important.

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