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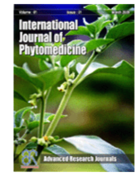

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Comparative evaluation of Nickel and Lead scavenging efficiency by *in-vivo* grown Plants using atomic absorption spectroscopy

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Abstract

Phytoextraction, the use of hyperaccumulator plant species to scavenge toxic heavy metals from contaminated soils are considered as an emerging technique for cost effective and environment friendly detoxification. The present study was conducted to evaluate and compare the scavenging efficiency of *in-vivo* grown climber plants, that is *Cvcitmis sativus, Armenian cucumber, Lufa acutangula, Momordica charantia descourt and Lagenaria siceraria* for the uptake of Nickel and lead using atomic absorption spectroscopy (AAS). Bioassays of field grown (*In-vivo*) plants were subjected to atomic absorption spectrophotometer for the analysis of scavenging efficiency of the aforementioned elements, after 98 days of growth in soils, contaminated with each of these, separately. The *in-vivo* grown climber plants absorbed much lesser amounts of Ni, that is, 0.79, 1.05, 0.45 and 1.61 ppm as compared to Pb plants which showed higher ranges of uptake, that is, 2.09, 10.39, 4.77 and 8.29 ppm, respectively. The metal uptake ratios were proportional to their concentrations in the contaminated soils. Maximum uptake for heavy metals was observed by the roots in all plants. Nodes also showed high heavy metal accumulation in the aerial region. Higher concentration of heavy metals in the soil showed a negative effect on their growth.

Keywords: Phytoremediation, in-vivo growth, atomic absorption, spectroscopy, hyperaccumulates.

Introduction

Phytoremediation is the most emerging field of environmental biotechnology. Most of the soil contaminants can be removed by many other physical methods but the heavy metal pollution of vast cultivated land areas are a serious threat to the agricultural biology. Land degradation is an important global issue for the 21st century because of its adverse impact on agricultural productivity, the environment, and its effect on food security and the quality of life [1]. The per capita arable land is decreasing steadily (0.22 ha per capita) due to increase population, land degradation and competition for non-agriculture land use [2,3].

An excess level of heavy metals are exposed into environment, for example by industrial waste and fertilizers causes serious concern in nature as they are non- biodegradable and accumulate at high levels. Heavy metal pollution is a global problem, although severity and levels of pollution differ from place to place. At least 20 metals are classified as toxic with half of them emitted into the environment that poses great risks to human health [4]. The common heavy metals like Cd, Pb, Co, Zn and Cr etc. are phytotoxic at both low concentration as well as very high concentration are detected in soil.

Phytoremediation broadly refers to the use of plants for environmental remediation [5]. The plant roots have natural ability to absorb the heavy metals of the soil, behaving as natural phytoremediates. Phytoremediation is a —Green technology that not only could rehabilitate contaminated soil. Planting creepers for phytoremediation of heavy metal contaminated soil could overcome phytoremediation limitations of time consumption as creepers has considerable potential use for it [6].

Some plants species act as hyper accumulators of the metals, depending upon their scavenging capacity and ability to deposit these metals in the different cellular compartments. These metals pass through the root cell membrane to the symplast, then metals could be passed to the vacuoles, (where their degradation occurs by enzymes) by membrane metal transporters, and could be deposited there with the help of special metal-binding proteins called metallothioneins. Heavy metals are supposed to replace other essential metals in pigments of the cellular structure, destroying their natural balance [7]. This technique is also useful to know the metal accumulation properties of each separate plant part, for example, Cd hyper-accumulation by roots of *Thlaspi caerulescens* [8].

Atomic absorption spectroscopy (AAS) is an alternative, simple and rapid technique for quantitative isolation from biological material. Therefore the main objective of the present study is to evaluate and compare the nickel and lead uptake by in vitro grown creepers to assess the effectiveness in removing pollutants from soil materials. The selected creepers is an herb belonging to the family Cucurbitaceae. It is cultivated as a vegetable crop. It was selected because it is fast-growing, resistant to pollution loads, produces high biomass and above all offers easy harvesting in a single growth period [9].

Materials and Methods

Experimental Plants

In this study, Cvcitmis sativus, Armenian cucumber, Lufa acutangula, Momordica charantia descourt and Lagenaria siceraria was used for phytoextraction of Pb, and Ni. The seeds of the plant were obtained from the local market.

Phytoextraction Experiments

Phytoextraction experiments were carried out in polythene bags. For this purpose, the soil was passed through a 2 mm sieve and 2.0 kg soil was put into each polythene bags. Polythene bags were divided into 100 groups at different concentration of metals for each plant i.e., Ni-contaminated, Pb-contaminated, and control (to which no metal was added). The metals were added to the soil in their powder form. The different concentration of metals added was 0.5, 1.0, 1.5, 2.0 mg metal per kg of soil. Ten seeds of *Cvcitmis sativus, Cyamopsis tetragonoloba, Lufa acutangula, Momordica charantia descourt and Lagenaria siceraria T. alexandrinum* were put into each polythene bags. For each metal two polythene bags were used (duplicate experiment). After germination, the seedlings were allowed to grow in the same until maturity. After 98 days, the mature plants were uprooted and separated into roots, stem and leaves. These parts i.e., roots, stem, and leaves were used for the analysis of accumulated heavy metals.

Analysis of Heavy Metals in Plants

Estimation of lead and nickel uptake by in-vitro method using AAS

The second step of the study was to estimate the, lead and nickel uptake by Cvcitmis sativus, Armenian cucumber, Lufa acutangula, Momordica charantia descourt and Lagenaria siceraria using AAS. All chemicals and reagents used in the study were of analytical grade and were used without further purification. Solutions were prepared in double distilled water.

Preparation of biomass

Elements in plants parts cannot be detected directly by AAS, so solutions for plants were prepared by wet digestion method and then samples were analyzed to determine the concentration of metal ions. After collecting root, stem and leaves of plant, they were washed with double distilled water and 1 % HCl to remove dust from plant. These root, stem and leaves were then dried in an oven at 80 C for 48 hours. The dried plants were then digested.

Method for digestion

The dried root, stem and leaves were digested according to Awofolu [1]: dried parts were weighed separately and 5.0 g was taken in a round bottom flask. The dried material was ashed in crucible muffle furnace at 500 C for 1 h. The residue was then wet digested by HCl/HNO_3 5 ml (1:3) and heated till dryness. After drying 5 ml of HNO3 was added in the same beaker and heated for 5 to 10 min (until the digest became clear). The volume was adjusted up to 50 ml with double distilled water and then was filtered (Whatman filter paper). The sample solutions were ready to be aspirated in AAS. These sample solutions of *in-vitro* grown root, stem and leaves were kept at 4 C with UV protection.

Calibration range

These samples were subjected to Z-5000 series polarized Zeeman Atomic Absorption Spectrophotometer. The calibration curve was obtained by running the standards of 0, 2, 4 and 8 ppm. A straight line was obtained between concentration and absorbance. All the points of the standard were tried to lie in the straight line, because the accuracy of the sample results is depends on exact absorbance of these standards.

Statistical Analysis

All the data were statistically analyzed following Steel and Torrie [10]. Each experiment was conducted in triplicate (n = 3). Results are shown as mean ± standard error. Experimental data were analyzed using statistical software SPSS 16.0. Heavy metal concentrations in control and experimental were compared using Paired-Samples T Test and One way ANNOVA and after comparing the results were authenticated by Wilcoxon Mann's whitney test.

Result and Discussion

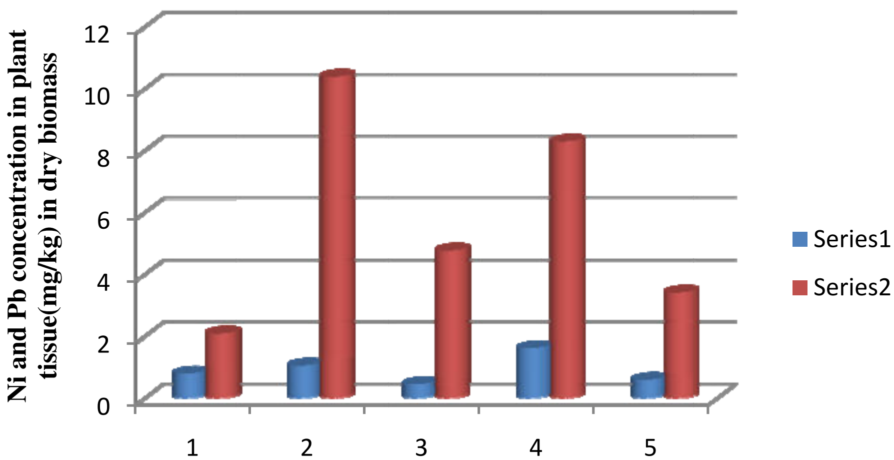
The hyperaccumulation of metals in various plant species has been extensively investigated and to date substantial progress has been made. It becomes clear that different mechanisms of metal accumulation, exclusion and compartmentation exist in various plant species [11].The data show that the concentrations of Pb and Ni in leaves, roots and stems increased with increasing Pb and Ni supply levels. The distributions of the metals in different plant parts decreased in the order: stem>leaf>root for Pb and leaf>stem>root for Ni. [12]

Table1. Concentration of Ni in the grown Climber plants, determined by AAS

S.No.	Climber Plants	Mean concentration of elements (ppm)
1.	*Cvcitmis Sativus*	0.79
2.	*Lagenaria Siceraria*	1.05
3.	*Lufa Acutangula*	0.45
4.	*Armenian Cucumber*	1.61
5.	*Momordica Charantia*	0.59

Table2.Concentration of Pb in the grown Climber plants, determined by AAS

S.No.	Climber Plants	Mean concentration of elements (ppm)
1.	*Cvcitmis Sativus*	2.09
2.	*Lagenaria Siceraria*	10.39
3.	*Lufa Acutangula*	4.77
4.	*Armenian Cucumber*	8.29
5.	*Momordica Charantia*	3.41



Nickel and Lead concentration added to soil (mg/kg)

This research work was to determine concentration of elements, that is, Nickel and lead in *in-vivo* grown plant tissues. The *in-vivo* grown climber plants absorbed much lesser amounts of Ni, that is, 0.79, 1.05, 0.45 and 1.61 ppm as compared to Pb plants which showed higher ranges of uptake, that is, 2.09, 10.39, 4.77 and 8.29 ppm, respectively, which was proportional to the metal concentration in the contaminated soil. One of the major factors influencing trace mineral uptake in plants is the composition of the soil. Fatima et al. also studied metal contents of *in vivo* grown *plants* and found that no metal was above the toxic limit [13]. Sahito et al. also studied the trace and essential elements of *Cvcitmis sativus, Armenian cucumber, Lufa acutangula, Momordica charantia descourt and Lagenaria siceraria* plants using atomic absorption spectroscopy technique [14]. The levels of essential elements were found high as compared to the levels of toxic elements. Lin et al. worked on effect of cadmium toxicity on nitrogen metabolism in leaves of *Solanum nigrum* as a newly found cadmium hyper accumulator [15]. They reported that hyper accumulators are ideal plant species used for phytoremediation of soils contaminated by heavy metals. A full understanding of metal tolerance mechanisms of hyperaccumulators will facilitate enhancing their phytoremediation efficiency [16]. During present study the concentrations of Nickel and Lead in *in-vivo* plant tissues, that is *Cvcitmis sativus, Armenian cucumber, Lufa acutangula, Momordica charantia descourt and Lagenaria siceraria* is predicted in Tables 1 and 2. Results show that all the above mentioned elements were in high concentration in field grown plant tissues. These results demonstrate that the composition of the soil plays an important role in mineral uptake of plants. This study also leads to the conclusion that *in-vivo* grown plants of *Cvcitmis sativus, Armenian cucumber, Lufa acutangula, Momordica charantia descourt and Lagenaria siceraria* can behave as natural scavengers if planted to the chemically polluted soils on large scale in future. The present piece of work fully supports our idea that purified *in-vivo* grown hyper accumulators, like *Cvcitmis sativus, Armenian cucumber, Lufa acutangula, Momordica charantia descourt and Lagenaria siceraria* can be used as the natural phytoremediates and heavy metal scavengers of the toxic elements for the treatment of contaminated and polluted agricultural lands on commercial scale.

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