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Phytoremediation of Pb and Ni Contaminated Soils Using *Catharanthus roseus* (L.)

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Abstract:
Phytoremediation is an emerging technology that uses various plants to degrade, extract, contain or immobilize contaminants from soil and water. Plants can help clean up many kinds of pollution including metals, pesticides, explosives and oil. This technology has been receiving attention as an innovative, cost-effective alternative to the more established treatment methods used at hazardous waste sites. Contaminated soils and waters pose a major environmental and human health problem. Oil refineries and chemical plants produce billions of gallons of contaminated wastewater each year. Soils may become contaminated by the accumulation of heavy metals and metalloids through emissions from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline and paints, land application of fertilizers, animal manures, sewage sludge, pesticides, waste water irrigation, coal combustion residues, spillage of petrochemicals and atmospheric deposition. In the present study *Catharanthus roseus* has been used for lead and nickel phytoremediation. Pot experiments were conducted using aqueous solutions of lead and nickel. The two metal solutions were added to the pots for a period of 60 days on alternate days. Accumulations of the heavy metals were analyzed after 20, 40 and 60 days in leaves, stem and roots by AAS. The results showed that lead and nickel highly accumulated by the roots than stem and leaves. It was concluded that the plant species was a good accumulator of lead and nickel.

Keywords: Accumulation, Bioconcentration factor, Metals, Lead, Nickel, Phytoremediation, Translocation factor.

1.0 Introduction
Metals are natural components in soil. Some of these metals are micronutrients necessary for plant growth, such as Zn, Cu, Mn and Co, while others have unknown biological function, such as Cd, Pb, Ni, Cr, As and Hg (Gaur and Adholeya 2004; Lasat, 2000). Many species of plants have been successful in absorbing contaminants such as lead, cadmium, chromium, arsenic, and various radionuclides from soils. Toxic heavy metals such as Pb, Co, Cd, Ni, As, Cr are accumulated in the plants and animals. Since they are not biodegraded thus causing various diseases and disorders even in relatively lower concentrations (Pehlivan *et al.,* 2009). Heavy metals are the major environmental contaminants and pose a severe threat to human and animal health by their long-term persistence in the environment (Gisbert *et al.,* 2003; Halim *et al.,* 2003). Heavy metals constitute an ill-defined group of inorganic chemical hazards, and those most commonly found at contaminated sites are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg) and nickel (Ni) (GWRTAC, 1997). Heavy metals, with soil residence times of thousands of years, pose numerous health dangers to higher organisms. They also effect on plant growth, ground cover and have a negative impact on soil microflora (Roy *et al*., 2005). For instance, lead (Pb) has a soil-retention time of 150–5,000 years and was reported to maintain a high concentration for as long as 150 years after application of sludge to the soil (Nandakumar *et al.,* 1995).

The problems of ecosystems are increasing with the advancement in technology. The elevated level of lead and other heavy metals, e.g. cadmium, chromium and mercury, in the local water streams is a major concern to public health. Soils contaminated by the accumulation of heavy metals and metalloids through emissions from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline and paints, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, spillage of petrochemicals, and atmospheric deposition (Khan *et al*., 2008; Zhang *et al*., 2010). Plants have evolved highly specific and very efficient mechanisms to obtain essential micronutrients from the environment, even when present at low ppm levels. Plant roots, aided by plant-produced chelating agents and plant induced pH changes and redox reactions, are able to solubilize and take up micronutrients. Plants have also evolved highly specific mechanisms to translocate and store micronutrients. These same mechanisms are also involved in the uptake, translocation, and storage of toxic elements, whose chemical properties simulate those of essential elements. Thus, micronutrient uptake mechanisms are of great interest to phytoremediation (USDE 1994). Some metals with unknown biological function (Cd, Cr, Pb, Co, Ag, Se and Hg) are also accumulated (Cho-Ruk *et al.,* 2006). Contaminated soils and waters pose a major environmental and human health problem, which may be partially solved by the emerging phytoremediation technology (Salt *et al*., 1998). Phytoremediation is the use of vegetation for in situ treatment of contaminated soils, sediments, and water. It is applicable at sites containing organic, nutrient, or metal pollutants that can be accessed by the roots of plants and sequestered, degraded, immobilized, or metabolized in place. The generic term "phytoremediation"consists of the Greek prefix phyto (plant), attached to the Latin root remedium (to correct or remove an evil) (USEPA 2000; Erakhrumen and Agbontalor, 2007). Heavymetals uptake, by plants using phytoremediation technology, seems to be a prosperous way to remediate heavymetals contaminated environment. (Bieby *et al.,* 2011). Techniques used for removal of heavy metals, like chemical precipitation, lime coagulation, ion exchange, reverse osmosis and solvent extraction are expensive and non-environmental friendly.

According to Sinha *et al.,* (2004) the plants act both as "accumulators" and "excluders". Accumulators survive despite concentrating contaminants in their aerial tissues. They biodegrade or biotransform the contaminants into inert forms in their tissues. The excluders restrict contaminant uptake into their biomass. Plants to be used for phytoextraction should have: (a) tolerance to high concentrations metals, (b) high metal-accumulation capability, (c) heavy biomass, (d) ability to grow fast and a (e) profuse root system. The success of phytoextraction depends especially on the plant's ability (a) to accumulate biomass rapidly, and (b) to store large quantities of the uptaken metals in the shoot tissue (Blaylock *et al.,* 1997; McGrath, 1998; Blaylock and Huang, 2000). Pb is not an essential nutrient for plants, majority of lead is easily taken up by plants from the soil and accumulated in root while only a small fraction was translocated upward to the shoots (Patra *et al.,* 2004). Pb affects several metabolic activities in different cell compartments. The effect of Pb depends on concentration, type of soil, soil properties and plant species. Pb toxicity leads to decreases germination per cent, length and dry mass of root and shoots (Munzuroglu and Geckil, 2002), disturbed mineral nutrition (Paivoke, 2002), reduction in cell division (Eun *et al.,* 2000).

Nickel has been classified among the essential micro nutrients and remains associated with some metallo-enzymes, but Ni is toxic at elevated concentrations in plants (Srivastava *et al*., 2005). In plants Ni is responsible for chlorosis, yellowing and necrosis of leaves, deformation of plant parts, stunted growth and generation of free radicals (Halliwell and Gutteridge, 1999). In the environment, Ni is found primarily combined with oxygen (oxides) or sulphur (sulfides) (Ministry of the Environment, 2001). The present study used *Catharanthus roseus* a non-edible plant for phytoremediation of Nickel and Lead from the contaminated soils.

Phytoremediation is currently divided into the following areas: (1) phytoextraction use hyperaccumulators to remove metals or organics from soil by concentrating them in above soil tissue, (2) phytodegradation use of plants and their associated microorganisms to degrade organic pollutants, (3) rhizofiltration use of plant roots to absorb and adsorb pollutants, mainly toxic heavy metals, from water and aqueous waste streams, (4) phytostabilization use plants to reduce the bioavailability of pollutants in the environment, and (5) phytovolatilization use plants to volatilize pollutants and remove them from air. Based on the phytoremediation techniques listed above, we can see that plants have abilities of cleaning waste elements in different ways, and phytoaccumulation is the most widely studied topic. Plants that can grow on metalliferous soils without suffering phytotoxic effects and accumulate extraordinarily high amounts of heavy metals in the aerial organs are described as hyperaccumulators. To date, there are more than 450 hyperaccumulation species, accounting only for less than 0.2% of all known species. (Rascioa and Navari-Izzo, 2011).

2.0 Materials and methods:

2.1: Experimental plant description:

Catharanthus roseus (Periwinkle) is a species of Catharanthus genus and Apocynaceae family native to Madagascar. Synonyms include *Vinca rosea* (the basionym), *Ammocallis rosea*, and *Lochnera rosea*; other English names occasionally used include Cape Periwinkle, Rose Periwinkle, Rosy Periwinkle, and "Old-maid". It is also widely cultivated and is naturalized in subtropical and tropical areas of the world. As an ornamental plant, it is appreciated for its hardiness in dry and nutritionally deficient conditions, popular in subtropical gardens where temperatures never fall below 5°C to 7°C, and as a warm-season bedding plant in temperate gardens. It is noted for its long flowering period, throughout the year in tropical conditions, and from spring to late autumn, in warm temperate climates. Full sun and well-drained soil are preferred. Numerous cultivars have been selected, for variation in flower colour (white, mauve, peach, scarlet and reddish-orange), and also for tolerance of cooler growing conditions in temperate regions. Notable cultivars include 'Albus' (white flowers), 'Grape Cooler' (rose-pink; cool-tolerant), the Ocellatus Group (various colours), and 'Peppermint Cooler' (white with a red centre; cool-tolerant). (Huxley, 1992). It is an evergreen sub-shrub or herbaceous plant. The flowers are white to dark pink with a darker red centre. The fruit is a pair of follicles 2–4 cm long and 3 mm broad. As an ornamental plant, it is appreciated for its hardiness in dry and nutritionally deficient conditions, popular in subtropical gardens. It is noted for its long flowering period, throughout the year in tropical conditions. Numerous cultivars have been selected, for variation in flower colour (white, mauve, peach, scarlet and reddish-orange), and also for tolerance of cooler growing conditions in temperate regions. (Gamble, 2008).

Photo: Photograph of *Catharanthus roseus*, the experimental plant

2.2: Sample collection and metal analysis:

Catharanthus roseus plants were grown in pots filled with garden soil. The seedlings were collected from the uncontaminated soils. All the selected seedlings were of uniform size and free of any disease symptoms. Nickel and Lead were selected for the study , the uptake was estimated in root, stem and leaves for every 20 days for a total period of 60 days. In addition a set of control blank experimental pots was also maintained. The metal solutions prepared by dissolving in distilled water to prepare stock solution of 1000 ppm for each metal. The calibration curves for each metal were also prepared. A blank reading was taken to incorporate necessary correction factor. The heavy metal solutions of 5mg/L was prepared from the stock and administered to the plants and care was taken to avoid leaching of water from the pots. The metal uptake was estimated once in every 20 days. The sample plants were removed from the pots and washed under a stream of water and then with distilled water. The collected plants were air dried, then placed in a dehydrator for 2-3 days and then oven dried for four hours at 100 ºc. The dried samples of the plant were powdered and stored in polyethylene bags. The powdered samples were subjected to acid digestion. 1gm of the powdered plant material were weighed in separate digestion flasks and digested with HNO_3 and HCl in the ratio of 3:1. The digestion on hot plate at 110ºc for 3-4 hours or continued till a clean solution was obtained. After filtering with Whatman No. 42 filter paper the filtrate was analyzed for the metal contents in AAS. (Simarzdu 6800).

3.0 Results and Discussion

In the present investigation, *Catharanthus roseus* plant accumulated both the metals. By 20th day Lead content was high in roots and low in leaves. While in stem it was 67.31 mg/kg biomass. There was no change in lead accumulation in leaf after 40th day. Stem concentration increased to 68.09 mg/kg and root concentration was increased to 88.74 mg/kg biomass much change observed in the roots (Table 1). In the 60th day only minimum change was observed in leaf, stem and root. Finally, after the total experimental period it was concluded that root accumulation was higher compared to stem and leaves.

Table 1: Total accumulation of Lead (mg/kg) in *Catharanthus roseus* during the experimental period

Plant part	Control	20th day	40th day	60th day	Total Accumulation
Leaf	24.03±0.41	24.53±0.15	24.5±0.16	24.95±0.08	0.92
Stem	60.69±0.16	67.31±0.18	68.09±0.08	69.49±0.17	8.8
Root	21.47±0.16	84.32±0.15	88.74±0.08	88.81±0.17	67.34
Total Accumulation	106.19	176.16	181.33	183.33	77.06

Fig 1: Accumulation of lead in the experimental plants

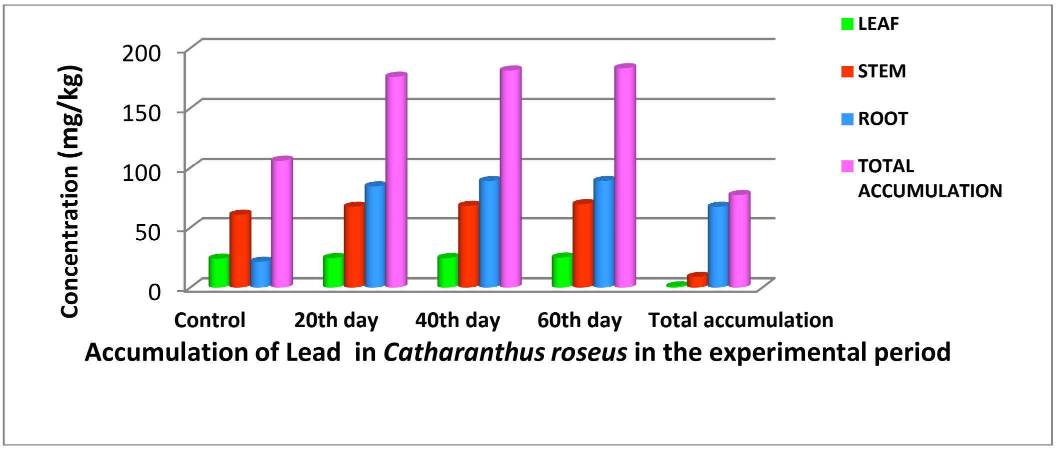
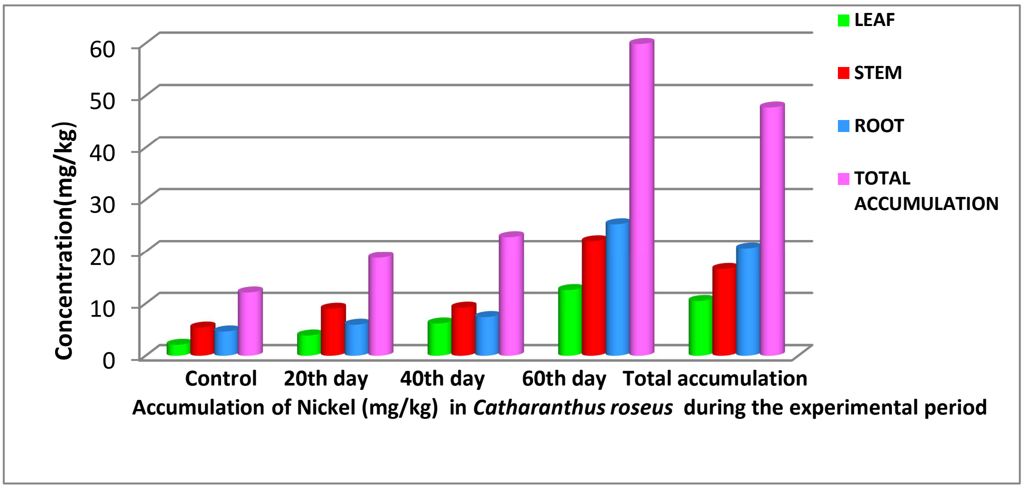


Table 2: Total accumulation of Nickel (mg/kg) in *Catharanthus roseus* during the experimental period

Plant part	Control	20th day	40th day	60th day	Total Accumulation
Leaf	2.09±0.18	3.87±0.15	6.18±0.16	12.58±0.08	10.49
Stem	5.39±0.49	9±0.18	9.21±0.08	22.02±0.17	16.63
Root	4.65±0.16	5.94±0.15	7.41±0.08	25.28±0.17	20.63
Total Accumulation	12.12	18.82	22.8	59.87	47.75

Fig 2: Accumulation of nickel in the experimental plants



In the 20th day Nickel accumulation was observed that it was increased than the control plants. In this period high accumulation was observed in the stem part than roots and leaves. After 40th day root, stem and leaf accumulations was increased and high accumulation difference was observed in the leaf part. After 60th day high accumulation was observed in the leaf, stem and root almost doubled than the 40th day accumulations, a high accumulation difference observed in the root part. (Table 2). After the experimental period nickel accumulated in the order roots > stem > leaves.

Bioconcentration factor (BCF) and Translocation factor (TF):

The Bioconcentration Factor (BCF) of metals was used to determine the quantity of heavy metals that is absorbed by the plant from the soil. This is an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil (Ghosh and Singh, 2005a) and is calculated using the formula:

$$BCF = \frac{\text{Metal concentration in plant tissue}}{\text{Initial concentration of metal in substrate (soil)}}$$

The higher the BCF value the more suitable is the plant for phytoextraction (Blaylock et al., 1997). BCF Values > 2 were regarded as high values. To evaluate the potential of plants for phytoextraction the translocation factor (TF) was used. This ratio is an indication of the ability of the plant to translocate metals from the roots to the aerial parts of the plant (Marchiol et al., 2004). It is represented by the ratio:

$$TF = \frac{\text{Metal concentration (stems + leaves)}}{\text{Metal concentration (roots)}}$$

Metals that are accumulated by plants and largely stored in the roots of plants are indicated by TF values < 1 with values > 1 indicating that the metals are stored in the stems and leaves. Determination of hyperaccumulator and excluder plant species is based on strict criteria. A plant is classified as a hyperaccumulator for heavy metal (s) when it meets four criteria; (a) shoot/root quotient (level of heavy metal in the shoot divide by level of heavy metal in the root) > 1, (b) extraction coefficient (level of heavy metal in the shoot divide by total level of heavy metal in the soil) > 1; extraction coefficient gives the proportion of total heavy metal in the soil which is taken up by the plant shoot/aerial part of the plant (Harrison and Chirgawi 1989, Rotkittikhun *et al*. 2006), (c) higher levels of heavy metals of 10 – 500 times the levels in normal plants (uncontaminated plants) according to Allen (1989) (d) more than 1000g/g of copper, lead, nickel, chromium; or more than 100g/g of cadmium or more than 10000g/g of zinc (Shen and Liu 1998, Ginocchio and Baker 2004, , Rotkittikhun *et al*. 2006). Furthermore, a plant which has high levels of heavy metals in the roots but with shoot/root quotients less than 1 is classified as a heavy metal excluder (Boularbah *et al*. 2006). According to Baker and Walker (1990) an indicator plant species is the one of which the levels of heavy metals in the tissues are similar to those in the surrounding environment; soil.

The rate of metal translocation from the root to the shoot may depend on metal concentration in the root (Hardiman *et al.,* 1984). The movement of the heavy metal from the polluted sediments into the roots of the plant and the ability to translocate the metals from roots to aerial parts were assessed correspondingly by means of Bioconcentration Factor (BCF) and the Translocation Factor (TF). Bioconcentration factor is an index of the ability of plant to accumulate a particular metal with respect to its concentration in the sediment (Ghosh and Singh, 2005). Bioconcentration factor (BCF) was calculated as a ratio of concentration of heavy metal in plant roots to that of soil. Translocation factor is the ratio of metal concentration in the shoots to the roots. The ability of plants to tolerate and accumulate heavy metals is useful for phytoextraction and phytostabilization purpose (Yoon *et al.,* 2006). Plants with both bioconcentration factors and translocation factors greater than one (TF and BCF> 1) have the potential to be used in phytoextraction. The higher the BCF value higher the suitability of the plant for phytoextraction (Blaylock *et al*., 1997). This ratio is an indication of the ability of the plant to translocate metals from the roots to the aerial parts of the plant (Marchiol *et al*., 2004). Based on the results bioconcentration factor and translocation factor values were calculated. Bioconcentration factor was calculated using soil lead and nickel background concentrations. Lead and Nickel backgrouned concentration was 9.47mg/kg and 8.43mg/kg respectively. Lead bioconcentration factor was 7.1 and translocation factor was 0.14. Nickel bioconcentration factor value was 2.44 and translocation factor value was 1.31. Based on these values the plant species was highly accumulated these metals in the roots part, finally it was very useful for phytostabilization.

4.0 Conclusion:

1. Heavymetals uptake by plants using phytoremediation technology seems to be a prosperous way to remediate heavymetals contaminated environment. Phytoremediation is a fast developing field, sustainable and inexpensive process.
2. Fast growing plants with high biomass and good metal uptake ability are needed. In most of the contaminated sites hardy, tolerant, weed species exist and phytoremediation through these and other non-edible species can restrict the contaminant from being introduced into the food web.
3. In the present study *Catharanthus roseus*, a non edible, shrub species aesthetically pleasent with beautiful flowers. Finally it was concluded that the plant species highly accumulated lead than nickel. Based on the bioconcentration factor and

translocation factor values the plant species was a good accumulator of these two metals.

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