REMOVAL OF ZINC BY NATIVE ACCUMULATOR PLANTS

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ABSTRACT

Phytoremediation is the natural process in which the plants are used to bio-accumulate, degrade contaminants in soils, water, (or) air. This study involves the phytoremediation of zinc metal in the soil using native hyper accumulator plants such as Amaranthus dubius and Calendula officinalis. In this experiment both plants were studied for their ability to uptake zinc from contaminated soil. Zinc chloride was used for the preparation of synthetic contaminated soil and various concentrations of zinc solutions were introduced in the experimental pots (25ppm, 50ppm, 75ppm and 100ppm) for a 40days of cultivation period. In the study, the various physiochemical properties of soil were analyzed and the accumulation ability of Zn ions in Amaranthus dubius and Calendula officinalis were detected on 20th and 40th days of experiment by using Atomic Absorption Spectrophotometer (AAS) after the acid digestion method. From the observations made, it is clearly seen that, as the concentration of the element increases in the soil, the efficiency or the ability of the plant to uptake the element decreases. This condition is same for both the plants. 25ppm is assessed as the optimum concentration here in these cases. Comparing the 20th and 40th day of both the specimen plants, there is a peculiarity that, both the plants exhibit higher efficiency on the 40th day than that of the 20th day. Hence it is considered as the optimum cultivation period. Individually, Calendula officinalis shows higher removal efficiency than that Amaranthus dubius on the 20th and 40th day observations.

Keyword: - *Phytoremediation, hyper accumulator plants, contaminated soil, and AAS*

1. INTRODUCTION

Nowadays life has become easier with the help of technology, industries, machineries, .etc. But as the residual outcome of such development, land, water resources, are contaminated by the pollutants generated by industrial, military, and agricultural activities. Ignorance and lack of vision cause this hazard. The buildup of toxic pollutants (metals, radio nuclides and organic contaminants in soil, surface water and ground water) not only affects natural resources but also causes a major strain on ecosystems. The methods usually adopted to solve this contamination issues such as 'pump and treat' and 'dig and pump' are expensive and of limited potential and usually applicable only at small areas. These conventional approaches to remediation often make the soil infertile and unsuitable for agriculture and other uses by destroying the microenvironment too. Hence environmentally sound technologies must be adopted for solving such issues. Phytoremediation is a cost-effective plant-based approach of remediation that takes advantage of the ability of plants to concentrate elements and compounds from the environment. Hyper accumulators (natural ability of certain plants) to bioaccumulation, degrade, or render harmless contaminants in soils, water, or air.

Toxic heavy metals and organic pollutants are the major targets for phytoremediation. Knowledge of the physiological and molecular mechanisms of phytoremediation began to emerge in recent years together with biological and engineering strategies designed to optimize and improve phytoremediation. In addition, several field trials confirmed the feasibility of using plants for environmental cleanup [2]

Over the past 20 years, this technology has become increasingly popular and has been employed at sites with soils contaminated with heavy metals. While it has the advantage that environmental concerns may be treated in situ; one major disadvantage of phytoremediation is that it requires a long-term commitment, as the process is dependent on a plant's ability to grow and thrive in an environment that is not ideal for normal plant growth. Heavy metals are conventionally defined as elements with metallic properties (ductility, conductivity, stability as cations, ligand

specificity, etc.) and an atomic number >20. The most common heavy metal contaminants are Cd, Cr, Cu, Hg, Pb, and Zn. Metals are natural components in soil. Contamination, however, has resulted from industrial activities such as mining and smelting of metalliferous ores, electroplating, gas exhaust, energy and fuel production, fertilizer and pesticide application, and generation of municipal waste. High levels of metals in soil can be phytotoxic. Poor plant growth and soil cover caused by metal toxicity can lead to metal mobilization in runoff water and subsequent deposition into nearby bodies of water. Furthermore, bare soil is more susceptible to wind erosion and spreading of contamination by airborne dust. In such situations, the immediate goal of remediation is to reclaim the site by establishing a vegetative cover to minimize soil erosion and pollution spread [3].

This study deals with the phytoremediation of zinc from the soil by using native hyper accumulator plants (Amaranthus dubius and Calendula officinalis). Hence compare the phytoremediation potential of both the plants with respect to various concentrations of zinc and number of days. Also find out the optimum values of metal concentration and contact time. The specific objectives of present study are to identify the optimum concentration and time for the removal of zinc by Amaranthus dubius and Calendula officinalis and to compare the phytoremediation potential of both plants.

2. METHODOLOGY

2.1 Soil preparation and analysis

Soils were collected from 15 to 30cm depth of ground surface and air dried under shade for 3days. Equal quantities (5kg) of soil were filled in all pots. Soils were crushed, sieved (using 2mm sieve) and made ready to analyze various physiochemical properties (Table 1). A triplicate of metal salt concentration was also prepared.

2.2 Physical and chemical analysis

Determination of pH: Weighed 20 gm of 2.0 mm air dried soil into a 100ml beaker. Added 50 ml of distilled water and stirred with a glass rod thoroughly for about 5 minutes and kept for half an hour. Standardized the glass electrode using standard buffer of pH = 7 and calibrated with the buffer pH = 4 or pH = 9.2. Dipped the electrodes of pH meter in the beakers containing the soil water suspension and the pH of soil was measured.

Electrical Conductivity: Calibrated the conductivity cell with the help of standard KCL solution and determined the cell constant. The soil water suspension of 20 gm: 50 ml ratio prepared for the determination of pH was used for conductivity measurements. After the calibration, dipped the conductivity cell in the supernatant liquid of the soil water suspension and measured the conductivity of test solution in proper conductance range.

Moisture content: Placed the moist soil sample in a moisture box and weighed it immediately. Placed the box with lid off in an oven (105^oC) and dried the soil to a constant weight. Removed the sample from the oven, and placed the box in the desiccators until it is cooled. Weighed it and also determined the mass of the empty moisture box and determined the mass of the moisture.

$$\% \text{ of soil moisture} = \frac{(\text{Initial Wt of soil} - \text{oven dried Wt soil}) \times 100}{\text{Initial weight of soil}}$$

Determination of Organic Carbon: Walkley – Black Method was used. Weighed 1gm. of 0.5 mm sieved soil into dry 500 ml conical flask. Added 10 ml of $K_2Cr_2O_7$ into the flask with pipette and swirled. Allowed the reaction to proceed for 30 min on asbestos sheet to avoid burning of table due to release of intense heat due to reaction of sulphuric acid. Added slowly 200 ml of distilled water, 10 ml of concentrated orthophosphoric acid and add about 0.2 gm NaF and allowed the sample to stand for 1.5 hrs. The titration end point is clear in a cooled solution. Just before titration add 1 ml ferroin indicator into the conical flask. Titrated the excess $K_2Cr_2O_7$ with 0.5 N ferrous ammonium sulphate till the colour flashes from yellowish green to greenish and finally brownish red at the end point. Simultaneously blank test also measured without soil

$$\% \text{ Organic carbon} = \frac{(B - S) \times N \times 0.003 \times 100}{\text{Wt. of soil (oven dry)}}$$

Particle size distribution (pipette analysis): took 50 gm of air dry soil (passed through 2 mm sieve) in 500 ml bottle. Added 100ml above dispersion solution in 50gm soil in 500ml plastic bottle. Shook a set of sample bottles at regular intervals for half an hour on shaking machine for preparing homogeneous solution. Transferred above soil sample solution to 1000 ml glass measuring cylinder and made solution 1000 ml by adding water. As per International approved system, shook the sample solution for 30sec. Depending on the solution temperature and sedimentation chart, first pipetting is done with 50ml pipette at 10cm depth. In first pipetting, 50 ml solution sucked and transferred in 60 ml china dish. This sample solution contained mixed of clay and silt particles. Depending on the solution temperature and sedimentation chart, second pipetting is done with 50 ml pipette at 10 cm depth. In second pipetting 50ml solution sucked and transferred in 60ml china dish. This solution contained clay particles in soil sample. Transferred remaining soil solution in 1 lit measuring cylinder by using 0.02 mm sieve and washed the material through the sieve using jet of water. Sand particles on sieve are collected in china dish. Transfer pipetted solution in 3 dishes and dry overnight in an oven at 105°C, Cool in desiccators and weighed quickly. The weight of fine sand determined by deducting the weight of clay, silt and coarse sand particle from 100.

Determination of Total Lead in Soil: 1g of dry soil (passed through 2mm sieve) was weighed into 50ml beakers, followed by addition of 10ml of HNO_3 and 5ml of distilled water. The digestion on hot plate at 110°c for 2 hours or continued till a clean solution was obtained and kept for cooling. Then the solution was digested with H2SO4 till a white precipitate occurs, and filtered with Whatman No. 42 filter paper and made up to 50ml solution. The solution was analyzed for the metal contents in AAS.

Determination of Cation Exchange Capacity (CEC):
Determination of Exchangeable bases (Ca, Mg, K, Na, Mn) Neutral ammonium acetate method was used. 5g of air-dried soil was weighed into sample bottle; 60ml of 1N ammonium acetate solution was added and shaken using an orbital shaker for 2 1/2hours. The clear supernatant was filtered into a 100ml measuring cylinder. Another 30ml of ammonium acetate solution was added to the soil and shook for 30 minutes. The supernatant was also filtered into the same cylinder. Ammonium acetate was added to make up to 100 ml. K, Ca and sodium were determined using a flame photometer while Mg and Mn were determined using Atomic Absorption Spectrophotometer
Determination of Exchangeable Acidity (Al and H) Titrimetry was used. The soil was first extracted with 1N KCl. This was carried out by weighing 5g of the air- dried soil (passed through 2mm sieve) into sample bottle, 90ml of 1N KCl was added and shaken for 2hours using an orbital shaker. The supernatant was filtered. 25ml of extract was pipetted into a conical flask and 100ml of distilled water was added 4 drops of phenolphthalein indicator was added and the solution was titrated with 0.05 N NaOH was added to bring the colour back to the colourless state and 10 ml of NaF solution was added to observe any colour change and the reading taken.

2.3 Preparation of zinc solution

Stock solution of zinc concentration (1000 mg/L) was prepared by diluting 2.085 gm of zinc chloride solution in a 1000 ml of distilled water. Later it was diluted to get the test solutions of varying concentrations of 25, 50, 75 and 100ppm.

2.4 Phytoremediation experiment

Seeds were collected from a local agricultural agency at Mukkam and allowed to grow for 12days. Prepared zinc solutions (25, 50, 75 and 100 ppm) are applied on experimental pots. Metal analysis was conducted on 20th and 40th days of experiment.

2.4 Plant harvesting and metal analysis

Plants harvesting took place on 20th and 40th day of experiment. The collected plant samples were washed in water to eliminate dust, dirt and they were again washed with deionized water after that they air dried for 1day. Each plant sample was oven-dried at 100°C for 24 hours. The accumulation ability of Zn ions in Amaranthus dubius and Calendula officinalis were detected by Atomic Absorption Spectrophotometer (AAS) after the acid digestion method. In AAS, wave length of 213.9nm, slit 0.2 nm and flame nitrous oxide-acetylene was used for detection of Zn from plant. In acid digestion method, 1g of dry matter was weighed into 50ml beakers, followed by addition of 10ml of HNO_3 and 5ml of distilled water. The digestion on hot plate at 110°c for 2 hours or continued till a clean solution was obtained and kept for cooling. Then the solution was digested with H_2SO_4 till a white precipitate

occurs, and filtered with Whatman No. 42 filter paper and made up to 50ml solution. The solution was analyzed for the metal contents in AAS.

3. RESULTS AND DISCUSSION

Native accumulator plants Amaranthus dubius   and Calendula officinalis were used as hyper accumulator plants in the present study to remove zinc from the soil. The results of this study are discussed in the following section. Table 1 shows the various physical and chemical properties of the soil selected for the experiment.

3.1 Accumulation ability of Calendula officinalis plant upon Zn exposure:

The growth of Calendula officinalis plant on exposure to various concentrations (25ppm, 50ppm, 75ppm and 100ppm) of zinc was observed. It was found that the Calendula officinalis plant has high potential at the low concentrations of the Zn metal. From the table 2, for 20th day of the accumulation of zinc by plant, it can be seen that 25 ppm is the optimum concentration. The respective %uptake of the plant is 48.20. When the concentration reaches 100, the %uptake falls to 17.43. Analyzing the table we can see that as the concentration increases, the efficiency of the plan decreases. Taking a look at the table 3, of 40th day, for the initial concentration level, the respective %uptake is 57.18 and for the 100ppm concentration, the %uptake is 20.19. That shows, as the time period passes from 20th day to the 40th day, the %uptake increases for the respective concentration levels. Then, from the bar diagram, it can be seen that for the optimum concentration level, the 40th day is showing considerably large difference than that of the 20th day. But when it comes to the 100 ppm, the difference is a bit lower. But in all the ways, the 40th day exhibits the larger difference from that of the 20th day's readings means the efficiency increases.
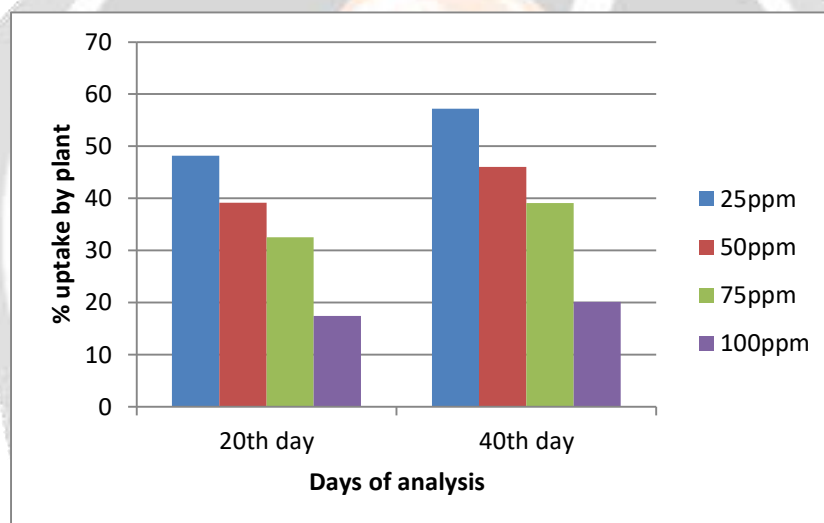
Table -1: Physiochemical properties of soil

Sl No	Properties	Result
1	pH of soil	5.8
2	Electrical Conductivity	0.64 mho/m
3	Moisture content	16.64%
4	Particle size distribution • Clay • Silt • Sand	22.03% 15.01% 62.96%
5	Organic carbon	0.75%
6	T_{zn}	0.072mg/kg
7	Cation exchange capacity	7cmol/kg

Table -2: Accumulation of Zinc by *Calendula officinalis* on 20th day

Initial concentration in soil (ppm)	Concentration of metal in plant (ppm) 20th day	% uptake by plant (%)
25	12.05	48.20
50	19.58	39.16
75	24.41	32.55
100	17.43	17.43

Table -3: Accumulation of Zinc by *Calendula officinalis* on 40th day

Initial concentration in soil (ppm)	Concentration of metal in plant (ppm) 40th day	% uptake by plant (%)
25	14.29	57.18 %
50	23.02	46.04 %
75	29.34	39.12 %
100	20.19	20.19 %

Chart -1: Accumulation of Zinc in Calendula officinalis during the experimental period

3.2 Accumulation ability of Amaranthus dubius plant upon Zn exposure

The growth of Amaranthus dubius plant on exposure to various concentrations (25ppm, 50ppm, 75ppm and 100ppm) of zinc was observed. Secondly, considering the 20th day of the accumulation of zinc by Amaranthus dubius, for the optimum concentration the %uptake is 41.69 and for the 100ppm it becomes 13.82. The same plant exhibits some changes on the 40th day such as, for the optimum concentration the %uptake increases into 49.01 and for 100ppm concentration the uptake is 14.40. Same as the previous specimen this plan also losses its efficiency as the concentration increases and same time, as the time period increases the %uptake property increases for the plant. From the respective bar diagram, comparatively a large difference can be noticed. The 40th day's readings exhibit considerable differences than the 20th day's observations.

Table -4: Accumulation of Zinc by *Amaranthus dubius* on 20th day

Initial concentration (ppm)	Concentration of metal in plant (ppm) 20th day	% uptake by plant (%)
25	10.42	41.69
50	16.91	33.83
75	22.08	29.44
100	13.82	13.82

Table -5: Accumulation of Zinc by *Amaranthus dubius* on 40th day

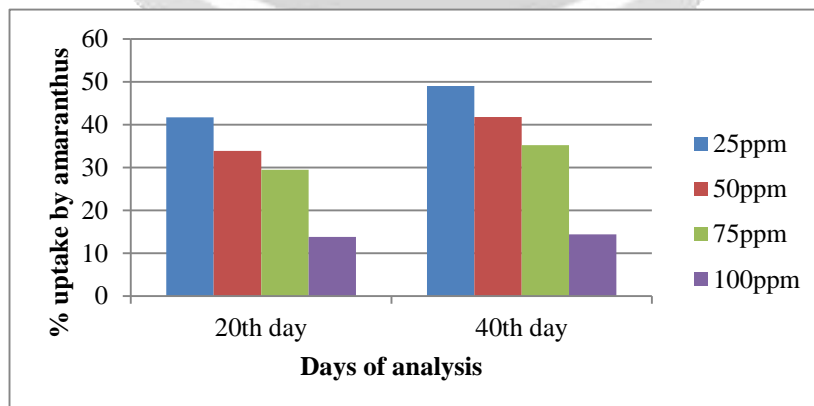| Initial concentration (ppm)	Concentration of metal in plant (ppm) 40th day	% uptake by plant (%)
25	12.25	49.01
50	20.87	41.73
75	26.41	35.21
100	14.40	14.40

Chart -1: Accumulation of Zinc in Calendula officinalis during the experimental period

CONCLUSIONS

This study involves the phytoremediation of zinc metal in the soil using native hyper accumulator plants such as *Amaranthus dubius and Calendula officinalis* .From the observations made, it is clearly seen that, as the concentration of the element increases in the soil, the efficiency or the ability of the plant to uptake the element decreases. This condition is same for both the plants. 25ppm is assessed as the optimum concentration here in these cases. Comparing the 20th and 40th day of both the specimen plants, there is a peculiarity that, both the plants exhibit higher efficiency on the 40th day than that of the 20th day. Hence it is considered as the optimum cultivation period. Individually, *Calendula officinalis* shows higher removal efficiency than that *Amaranthus dubius* on the 20th and 40th day observations.

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