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Toxic effect of different lead concentrations on *in-vitro* culture of *Datura inoxia*

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Abstract

Metals are the part of natural constituents of soil but industrial activities such as mining and smelting of ores, electroplating work, gas exhaust, energy and fuel production, fertilizer and pesticide application, and municipal waste results in enormous amount of increase in their natural concentrations in soil. Hazardous compounds or chemicals such as heavy metals, oils and battery metals from industries and organic solvents are major soil pollutants. These substances get deposited to the soils of the neighboring area and pollute them by changing the chemical and biological properties of soil of that area. The aim of this research was to identify some interesting accumulators which may associate an important biomass production with an effective heavy metal accumulation, absorption and translocation and their tissue culture approach. This research is mainly focused on the ability of some native plants to accumulate and tolerate high concentrations of heavy metals which are often associated in polluted areas. It has been found that *Datura inoxia* has the capacity to accumulate the heavy metals in its part. The *in vitro* culture of *Datura inoxia* shows better response in Lead supplementing M. S. Media. Although lead is a toxic heavy metal for plants, but *Datura inoxia* shows good response at higher concentration..

Keywords: Accumulator, Heavy metal, *In vitro* culture, Soil, *Datura inoxia*.

Introduction

Heavy metals such as lead (Pb), chromium (Cr), cadmium (Cd) and nickel (Ni), includes a group of inorganic chemical hazards at contaminated sites. Soils contamination occurred by the accumulation of heavy metals by emissions from the rapidly expanding industrial areas, mining, dumping of heavy metal wastes, sewage sludge, paints, fertilizers, pesticides, wastewater irrigation, animal manures, coal combustion residues, atmospheric deposition and spillage of chemicals. The disturbance and acceleration of nature's geochemical cycle of metals by anthropogenic activities results in accumulation of one or more heavy metals above normal values proven to be high enough to cause risks to entire ecosystem including human health.

Heavy metals disrupt the physiology as well as morphology of plants and thereby affect plant growth and biomass production. Some plant species have the ability to grow and develop in metal rich soils such as in the vicinity of mines or at industrial area. These plants can be explored to clean up heavy metal contaminated sites.[1]

Remediation of heavy metal contaminated soil is yet a thrust research area today. Heavy metals proved to be very hazardous contaminants because they are mostly non-biodegradable, their mobilization depends on physio-chemical properties of soil and they are very toxic even at low concentrations. Selection of a suitable remediation procedure depend upon the factors like location, accessibility to the site, size, history of site, treatment options, soil and contaminant characteristics and degree of contamination.[2]

Metal uptake by plants depends on the bioavailability of the metal in the water phase, and it is depends on the metal retention time, and its interaction with other elements. Moreover, upon

binding to the soil, the pH, redox potential, and organic matter content will all affect the tendency of the metal to exist in ionic and in available form. Plants may affect the soil composition due their ability to lower the pH and oxygenate the sediment, which affects the metal availability, increase in the heavy metals bioavailability by the addition of biodegradable physicochemical factors such as micronutrients and chelating agents.[3]

Lead Toxicity

Lead is a bluish-white, soft, elastic, and lustrous metal. It is poor electric conductor and very resistant to corrosion. Lead is one of the most persistent heavy metal; showing half-life of more than 1000 years.[4] Natural soil concentrations are mainly less than 20 mg kg"; low level contamination results in to 30-100 mg/kg. The major sources of lead contamination are mining and smelting, manures, pesticides, sewage, and vehicle exhausts. Generally Lead occurs naturally in the soil. Mostly lead concentrations that are found in the environment are due to the human activities. Lead is burned in car engines and lead salts (chlorines, bromines, oxides) will originate. Lead is a major constituent of the lead-acid battery used extensively in car batteries and also used as a coloring element in ceramic glazes and as projectiles in some candles. It is used as electrodes in the process of electrolysis and also used in organ pipes. One if its major uses are in the glass of television screens and computers. It is also used in solders, cables, sheeting, bearings, and as weight in sport equipment. It resides naturally in the surface soil horizons with the organic fraction. In the soil solution, it occurs as cationic and neutral inorganic species, some organic complexes may also exist.[5]

Lead toxicity leads to several ill effects. It causes disruption of the biosynthesis of haemoglobin resulting in anemia, rise in blood pressure, miscarriages and kidney damage, nervous systems and brain damage etc. Wild and domestic animals can ingest lead while grazing. They also experience the same kind of effects as people who are exposed to lead. Lead can enter a fetus through the placenta and causes damage to the nervous system and the brains of unborn children.

Plant tissue culture is a key tool in phytoremediation research. Tissue cultures also propose experimental convenience, high speed as compared with studies on whole plant systems. Here intrinsic capacity of plant to detoxify the pollutants can be studied by eliminating rhizospehere effect. But it cannot be the whole replacement for studies on soil-cultivated plants. The technique of plant tissue culture can be used in study of metal tolerance of a plant by exposing it in culture media containing known quantities of the specific heavy metal. Plants identified and screened by this method can then be tested for phytoremediation at polluted land. This technique also has the potential to study the effect of metal on whole plants.

Datura inoxia is an annual shrubby plant that typically reaches a height of 0.6 to 1.5 metres. It is widely distributed as a member of wild solanaceous plants in Sudan called as Devil's apple. This local name indicates that the plant generally induces narcotic or toxic effects on grazing animals, and thus they have been used for a long time by traditional herbalists for different purposes.[6, 7] The *in vitro* screening of *Datura inoxia* was carried out by using Lead supplementing M S Media.

Material and Methods

Several studies have been conducted to evaluate the effects of different heavy metal concentrations on living plants. Numerous *in vitro* experiments have focused on the effects of high concentrations of heavy metals on the regeneration of plants like accumulator, tolerant or sensitive to industrial pollution. Selection of plants under natural conditions of environmental pollution or *in vitro* may result in the selection of clones accumulator to toxic metal ions.

After about 20- 25 days of incubation the initiated plants were taken out the test tube with a clean and sterilized forceps in the laminar flow hood also the medium adhered to the plants was removed, broken or brownish leaves were excised from the plants and were taken to the culture bottles containing autoclaved semi-solid media supplemented with individual heavy metal of varying concentrations for screening the effect of heavy metals on *in vitro* plantlets.

Then the bottles were incubated in the culture room under the standard conditions of temperature like (25± 2°C) for 16/8 hrs of day/night break under the cool white fluorescent light of average 2500 lux.

Effect of Lead on *in vitro* Cultures of *Datura inoxia*

Culture initiation, establishment of culture and selection of Lead tolerant plant lets were performed on MS medium supplemented with 0.5 BAP, NAA and KN and supplemented with Lead at concentrations 0.1 – 50 mg/lit. Thus the cultures were exposed to a selection pressure by toxic metal for several months to increase the accumulation capacity during *in vitro* cultivation.

The cultures were inoculated and grown on a medium containing increasing concentrations of Lead, whereas such lead concentration was already lethal for the tissue cultures. Increased external Pb concentration considerably decreased the shoot length and vigorous growth also, whereas more than 90% of response was shown by culture of Datura on control medium.

Results and Discussion

Screening of *Datura inoxia* was carried out for studying the effect of Lead concentrations by using MS media with 0.5 BAP, 0.5 NAA, 0.5 KN gives the established cultures of *Datura inoxia*. To study the growth behavior of *Datura inoxia*, 0.5 Murshige and Skooge's (0.5 BAP+0.5 NAA+0.5 KN) medium with increasing concentrations of lead was given in various treatments. For these trails already established cultures of *Datura inoxia* was taken and inoculated on Lead supplemented medium.

Table 1 had shown that as the heavy metal concentration increases growth of *Datura inoxia* reduces but due to accumulation capability culture have shown the survival up to 45 mg/l lead concentration. As the lead concentration increases the percentage survival rate was decreased in the culture. Shoot length was also affected by higher concentrations of lead in the medium. The proportion of growth in shoot length was decreases at the higher concentration of lead in the medium. Above tabulated data revealed that *Datura inoxia* has in average 50 percentage of survival in all lead concentration of 50 mg/l. Figure 1 shows the cultures of *Datura inoxia* on Lead supplemented media.

Table 1: Effect of Various Concentrations of Lead on % Survival of *Datura inoxia*

0.5 MS + $PbSO_2$ (mg/l)	**% of Survival***	**Average Shoot Length***	**Average No. of Shoots***
0.1	98	**3.54**	**2-3**
0.2	90	3.65	2
0.3	82	3.66	2
0.5	75	3.68	1-2
1	75	3.72	1-2
5	70	3.76	1-2
10	70	3.81	1-2
15	60	3.83	1
20	55	3.85	1
25	54	3.87	1
30	50	3.9	1
35	50	3.98	1
40	45	4	1
45	35	4	1
50	-	-	-
SD (±)	**24.18**	**0.99**	
SE (±)	**6.24**	**0.26**	

* Values are mean of 3 replicates

Figure 1: Effect of Lead Treatements on Shoots

Conclusion

The overall response of Datura showed its good phytoremediation capacity. Generally this potential of Datura is due its accumulation mechanisms of different heavy metals. *Datura inoxia in vitro* culture was established in artificial MS Medium contaminated with Lead. As the concentrations of heavy metals increases the Percentage of survival of cultured *in vitro* plants examined for Average shoot length, reduced plant biomass, browning of culture and reduced the inoculant's capacity to promote plant growth. Overall study shown that percentage survival and growth of the plant retarded at higher concentration of lead. With the help of tissue culture experiments the potential of plant for metal accumulation can be studied easily.

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