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Comparative Effect of Four Heavy Metals, Cd, Pb, Ni, and Cr, on *Datura inoxia* in Tissue Culture

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Abstract— **Global industrialization has badly effect on environment and the entire biospheres. This distraction of the ecosystem has a hazardous effect on health of human and other organisms. Efforts should be made for dense vegetation of heavy metal bioaccumulation plants at the contaminated soil so that they can remove pollution and also give eco-green pleasant sense. Purpose of this study was to emphasize the phytoremediation technique for the industrial area. In the present research, effects of increasing concentrations of heavy metals in soil, on species in vitro was investigated and results showed that Datura inoxia can tolerate higher concentrations of four toxic heavy metals like Pb, Cd, Cr and Ni indicating its phytoremediation potential. The study revealed that for each metal Datura inoxia had given better response in case of survival, which indicates its importance as a plant suitable for phytoremediation of heavy metal contaminated area.**

Keywords— **Biosphere, Bioaccumulation, Cadmium, Chromium, Ecosystem, Lead, Nickel, Phytoremediation etc.**

I. Introduction

Soil is a natural medium for the plant growth its physical and biological properties are very important, because it supports mechanically and biologically to the plant. In 2004, Nazarian et al. showed that the soil pH, organic carbon, organic matter, topographic factors of the region and moisture were important ecological factors. In the soil organic matter, coarse sand, calcium carbonate, pH and water holding capacity of soil affect the growth and development of plant. Due to industrial effluents sulphate and heavy metals were added to the soil and affected the plant growth adversely. Soil pollution at industrially affected areas has been studied and analyzed by many researches to investigate soil and plant interaction or pollutant and plant interaction. Soil analysis has also been used to detect the levels of metal pollution and its hazardous effect on ecosystem including human health (Maxwell, 1991; Wahab & Hashem, 1995).

At present, biome was entirely polluted by a variety of pollutants as a result of industrial activities and alters the normal biogeochemical cycling mechanisms. Many developed and developing countries utilizes bio resources for extraction of the metal from industrially polluted sites. These eco green technologies have gained extraordinary attention in last decades and in current commercialization process. Across the country, more than 125, mostly contaminated sites are present and more than 40% of chemical fertilizers leached into soil, 65 % or more Indian villages are facing pesticides risk. Heavy metal concentration found beyond permissible limits in around 13 states. These toxic heavy metals have contributed to a various hazardous effects on human health and ecosystem (Dembitsky, 2003) by the phenomenon of bio-magnification and bioaccumulation. On the basis of individual characteristics of metal, they are available in soil in various fractions such as in the form of free ions, soluble complexes in soil, adsorbed compounds by inorganic soil constituents, attached to soil organic matter, precipitates of pure or mixed solids or may be entrapped in structure of the silicate minerals etc. (Ann Mary Mathew, 2005).

Industrialization is centralized element of economic development and improved prospects for sophistication. But, industrialization has also resulted in to the input of a large number of toxic compounds that have been polluting the ecosystem. Furthermost of research regarding phytoremediation technologies has focused on the removal of pollutants like heavy metals and certain types of organic compounds.

Phytoremediation, it is the technique of using plants and their associated microbes from rhizospehere for reducing the soil pollution, it has proved to be a cost-effective, non-invasive or complementary technology for bioengineering-based remediation methods. Phytoremediation explores an integrated approach to overcome air, soil and water pollution along with global climate change and, also better aspects of human well-being. According to findings plant-based technologies should be an important part of a standard strategy to achieve the global demand of cleaner environment for human well-being (Weyens, et.al, (2015).

This technology is eco green and cost effective also. Previously, study was carried out to gather some information about heavy metals: lead, mercury and arsenic sources, effects and their treatment. The study was supported the advantages of this kind of technology for reducing heavy metal pollution (Tangahu et al, 2011). Toxic heavy metals like Lead, Cadmium etc. are more hazardous, because biodegradation of these metals cannot be possible, but can be accumulated in living organisms, thus causing various chronic diseases at low concentration also (Pehlivan, et al, 2009).

In present research, *Datura inoxia* plant was selected for the study due its majority in the industrially contaminated study area. Datura species are widely distributed across the world, in countries India, Caribbean Island, China U.S. Mexico and Africa (Burkill, 2000). *Datura inoxia* is one of the native plants found in majority at industrially contaminated area. This shows its high phytoremediation capacity. Datura, is a small genus of 9 species of vespertine flowering plants belonging to the family Solanaceae. They are also called as Angel's Trumpets, which are related to genus Brugmansia. Along with this plant, four metals were selected for the study according to pollution of nearby industries which will be discussed in next sections.

Cadmium is mostly used in batteries and electroplating, as stabilizers of plastics etc. Cadmium is known to be a toxic metal since from many years. In Japan, chronic cadmium indigestion is known as Itai-Itai disease. Cadmium exposure damages kidneys in humans. Bones and bone tissue can also be damaged by cadmium (Nishijo et al. 1996; Kasuya 2000). Cadmium Plant roots can easily absorb cadmium and then transport it to shoots (Toppi and Gabbrielli, 1999), which results in disorders in biophysiological processes, and then totally affects plant's general growth and development (Sgherri et al., 2002). Soil consists of the most stable oxidation states of chromium which are carcinogenic and mutagenic even at low concentrations.

Chromium contamination of agricultural areas It has also been reported that even the lower concentrations of chromium such as 72 ppm also showed inhibitory effect on plant growth and morphology (Sundaramoorthy et al., 2013).

Toxicity of nickel may have effect on number of biological and physiological processes in plants. In general wilting and leaf necrosis have been described as typical visible symptoms of nickel toxicity. Generally Nickel is present in uncontaminated soil in between 5-50 mg kg-1, and in the plants in between 0.4-3 mg kg-1 concentration (Prasad M., 2004). Nickel is an important cause of contact allergy. Nickel sulphide dust and fumes is believed to be carcinogenic.

Commercial products such as paints, television glass, ceramic glazes, batteries, medical equipments like i.e., x-ray shields, fetal monitors, and some of the electrical equipment are also contain lead. Lead is a potential carcinogen. Generally inhalation and ingestion are the general ways of exposure of lead. It accumulates in various body organs, which may lead to poisoning or even death (Environment Writer, 2000).

In 2009, Sandra King et al., uses tissue culture to develop plants with acid soil, heavy metal tolerance, potentially for hard-rocky mine soil reclamation. They carried out tissue culture of local plants which grow on acidic soil along with heavy metal contaminated soil. According to Pauline M. Doran, plant tissue culture is an appropriate laboratory tool for phytoremediation studies.

Root endophytes fortified with a metal-resistance or sequestration system can decrease metal phytotoxicity and enhance heavy metal accumulation in plants (Rajkumar, et. al, 2012). Phytoremediation studies was carried out by the f general weed plant Datura metel L. analyzing the different concentrations such as 0.5%, 1%, 1.5% and 2% on 30% of paper mill effluent on the parameters like growth and physiological characteristics of L. esculentum. Study had suggested that Datura metel L. is a suitable alternate for the remediation of paper mill effluent and relieved the experimental plants from stress (J. Biosci. Re, 2010).

II. Detail Methodology

Tissue culture studies on plants at contaminated sites provide a standard culture method in the form of in vitro establishment of nodal explants as well as shoot tip explants (Waoo et al. 2014). Thus *Datura inoxia* culture was established by standardizing the in- vitro parameters and composition of Murashige and Skooge's Medium. Establishment protocol for *Datura inoxia* was totally developed and then media was supplemented with increasing concentrations of four heavy metals. For screening experiment, in vitro culture of *Datura inoxia* on heavy metals supplemented M. S. medium was carried out. Plantlets established on control were transferred on increasing concentrations of heavy metal supplemented medium for further growth and study. The primary objective of this study is to assess the effects of Cd, Pb, Ni and Cr in developing cultures of *Datura inoxia* in vitro.

The study was focused on-

- The effect of Cd, Pb, Ni, Cr on the growth,
- Effect of these metals on structural morphology of the plant.

Media supplemented with individual heavy metal i.e. Chromium, Cadmium, Lead and Nickel of varying concentrations to study the relative impact of heavy metal concentration on in vitro cultures. Incubation of culture bottles was done at 25± 2°C for 16/8 hrs of photoperiod with the white fluorescent light of average 2500 lux intensity. Stock solution (100 mM) of Chromium Sulphate (CrSO4), Nickel Sulphate (NiSO4) and Cadmium Chloride (CdCl2) and Lead Sulphate (PbSO2) were prepared and filter sterilized for addition in media. Suitable aliquots of filter sterilized solution of four metals were added in vitro to attain increassing concentrations of 0.5, 1.0, 3.0, 5.0, 10, 15, 20, 25, 30, 35, 40, 45, 50 mg/l metal. M. S. Medium without metal was used as control.

III. Observations and Results

The effect of all treatments of increasing concentrations of four heavy metals was shown below in Table 1, considering average shoot length as a parameter

and parameter of percentage of survival was illustrated in Figure 1. For the assessment of effect of these heavy metals here percentage of survival of culture in various treatments of heavy metals was taken in to consideration during study. The tabulated data indicated that *Datura inoxia* has percentage of survival in all heavy metal concentration of 50 mg/l as up to 50%. The overall response of Datura exhibits its good phytoremediation capacity for studied heavy metals.

TABLE I
COMPARATIVE ANALYSIS OF AVERAGE SHOOT LENGTH OF *DATURA INOXIA* WITH 4 HEAVY METALS.

Treatments Number	Average Shoot-Length in cm			
	Cd	Pb	Ni	Cr
T1	3.5	3.54	3.5	3.8
T2	3.6	3.65	3.51	3.81
T3	3.61	3.66	3.53	3.83
T4	3.63	3.68	3.54	3.85
T5	3.65	3.72	3.55	3.87
T6	3.67	3.76	3.57	3.9
T7	3.71	3.81	3.6	3.91
T8	3.72	3.83	3.61	3.92
T9	3.73	3.85	3.63	3.96
T10	3.73	3.87	3.64	3.96
T11	3.73	3.9	3.66	3.97
T12	3.73	3.98	3.68	3.97
T13	3.75	4	3.68	3.97
T14	3.78	4	3.68	3.97
T15	3.78	-	3.68	-

*** T1-T15 is the number of heavy metal treatments for respective concentrations from 0.1 mg/l to 50 mg/l.**

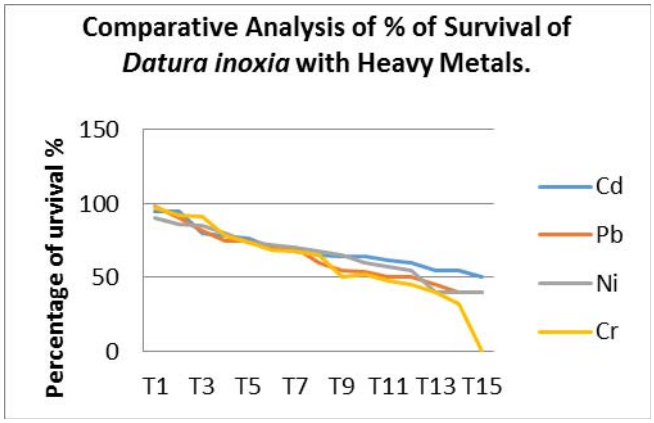

Fig. 1 Comparative Analysis of % of Survival of *Datura inoxia* with Heavy Metal Treatments

Fig. 2 Effect of Cadmium on Datura

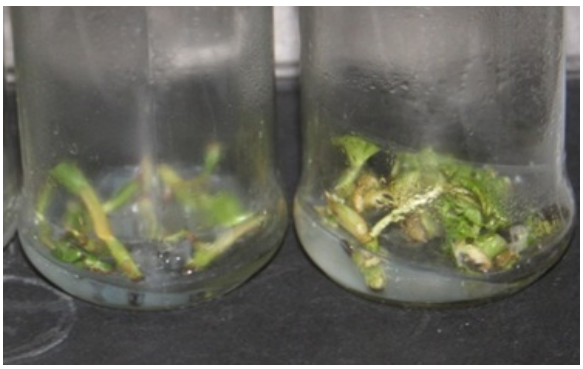

Fig. 3 Effect of Lead on Datura

Fig. 4 Effect of Nickel on Datura

Fig. 5 Effect of Chromium on Datura

IV. DISCUSSION

On industrially polluted lands, plantation plays immense significant bio ecological and hygienic roles (Antonkiewicz & Jasiewicz, 2002). Proper study and management of selected plants in polluted areas help to reduce level of pollution and ecological balance. This piece of research was carried out on *Datura inoxia* at contaminated sites. Belsky A J et al., (1989); suggested that several perennial grasses, shrubs and trees such as *Lantana Camara*, *Datura inoxia*, and several other plants are being used to remediate mine-tailing sites and suggested that these plants are suitable and effective for phytoremediation. Paul J. Jackson et al., (1984) studied on *Datura inoxia* cells from suspension cultures for their ability to grow and divide in lethal concentrations of cadmium. Richa Goel (2012) had developed a rapid and reproducible callus culture protocol for *Datura inoxia* using its germinated seedlings. Toxic and non-essential heavy metals are highly reactive and they interfere with the normal metabolism and results in toxicity effects on plants in the form of morphological and physiological changes. But despite this toxicity and oxidative stress, still most of the plants are hyperaccumulators of heavy metals. Thus present research compares the potential of *Datura inoxia* in the increasing concentration of four heavy metals. According to overall result *Datura inoxia* is the best species for phytoremediation purpose and can be used more in industrial area for soil remediation. One of the most important findings in this study was observed by comparing various chemical and biological soil parameters and evaluating the soil pollution at industrial area Govindpura. *Datura inoxia* also proved to be a potent plant species for heavy metal removal studies. The specialty of the plant lies in the fact that in vitro plants cultured on M. S. medium were able to survive on higher concentrations of heavy metals. The *Datura inoxia* comprise a native plant suitable for phytoremediation which ranked among the highest according to their heavy metal accumulation trials and the tissue culture studies than other plants in that area.

V. CONCLUSION

Datura inoxia species was screened for heavy metal accumulation and percentage of survival in artificial M. S. Medium contaminated with Pb, Cr, Cd and Ni. The Percentage of survival of cultured in vitro plants examined at increasing heavy metal concentrations, mainly for shoot length, plant biomass, culture browning and reduced plant growth, as shown in figure 2 to 5. Comparative data of all the testing on heavy metal containing media revealed that for each metal *Datura inoxia* had given better response which indicates its importance as a plant suitable for phytoremediation technology for heavy metal contaminated soil. *Datura inoxia* also proved to be a potent plant species for heavy metal removal studies. The specialty of the plant was that in vitro plants cultured on M. S. medium were able to survive on higher concentrations of heavy metals. The *Datura inoxia* represent an identical plant suitable for phytoremediation which ranked among the highest according to their heavy metal accumulation trials and the tissue culture studies.

In brief, Up till now, Phytoremediation is in its budding phase and thrust area, and many technical issues and problems are in association with it. Though results are encouraging and will suggest the further development in this area. Governmental and non-governmental Indian organizations are not taking any effort for use of phytoremediation on an industrial scale unlike U.S., Australia, and Europe. Recently, there is much interest in Indian universities and research institutes.

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