COMPARATIVE TISSUE CULTURE STUDIES ON *LANTANA CAMARA* AND *DATURA INOXIA* AT HEAVY METAL CONTAMINATED SITE AND PHYTOREMEDIATION APPROACH AT INDUSTRIALLY CONTAMINATED SITES

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ABSTRACT

Contaminated soils pose a major environmental and human health concern; phytoremediation technology provides solution to this critical problem. Bhopal, the city of lakes have currently facing the enormous amount of industrial pollution mainly at the industrial area due to direct dumping of industrial wastes at nearby soil. The research work mainly concentrates on investigation of heavy metal pollution at industrial area and the soil analysis results revealed that soil was heavily contaminated with chromium and lead. To eliminate the contamination of heavy metals phytoremediation approach is most suitable therefore botanical survey was carried out and Datura inoxia and Lantana camara were selected for phytoremediation purpose due to their maximum density at industrially contaminated area. These plants were tested in vitro for their accumulation properties by establishing them on MS media.

In recent days understanding of the contamination at a particular site is a prerequisite for protection of human health and environment. Phytoremediation is an area of central thrust and it has the capacity to use the native plants for removing the contamination. The use of native plants for phytoremediation purpose is an eco green approach and it also sustains the balance of ecosystem. This research paper explores the new possibilities to remediate soils polluted by heavy metals. Phytoremediation still required more efforts to be applicable India. Thus research must be done for seeking the native plants having phytoremediation potential and the kind of pollution in existing areas so that there were no any types of hazardous effects of pollution on human being.

By eliminating the effects of micro flora and translocation barriers, a closer approximation to the intrinsic capacity of particular plant species or cultivars removed from the effects of microbial activity.

KEYWORDS

Heavy Metals, Human health, In vitro, Low-cost, Soil Pollution, Phytoremediation, Tissue culture.

1. INTRODUCTION

Toxic heavy metals are biomagnified through the food chain. They contaminate the environment by altering soil properties its biomass, fertility, and crop yields and thus the human health (Varsha Mudgal et al., 2010). Heavy metals are generally defined as they are the elements with metal characteristics (conductivity, ductility, stability, and ligand specificity, etc.) and having atomic number >20. Metals are the natural constituents found in soil. Most common heavy metals contaminants are Cd, Cr, Ni, Hg, Pb, Cu and Zn.

Natural composition of soil with heavy metals, do not have hazardous effect on animal or plants. Some manmade activities like sewage sludge, fertilizers, and the direct discharge of domestic and industrial wastes in land and in air were the main source of higher concentration of these metals.

Decontamination of heavy metals polluted soil could be possible using some physico-chemicals processes like ion-exchange, precipitation, evaporation, reverse osmosis, and chemical reduction. However, these processes were costly and required external resources. (Nooshin Karimi., 2013). According to nature of the contaminant the remediation process was selected, like different organic compounds can be treated by thermal desorption, soil washing, incineration and some land filling (Singh and Jain, 2003). In case of heavy metal pollution, phytoremediation is the best technology.

Planted vegetation on contaminated sites such as industrial dumpsites, landfills, and mine areas play a significant role in maintaining aesthetic sense, controlling erosion and removing contaminants like heavy metals (Nagendran et al., 2006). Phytoremediation is a term generally used to for the mechanisms by which living plants can change the soil's chemical composition where they are growing. In simple word it is the use of plants to recover soil pollution. Phytoremediation is an eco friendly, cheap, and environmentally sound technique for recovery of heavy metal polluted sites (Cummingham et al, 1997). The word phytoremediation is coined from the Greek prefix "phyto" means the plant, and the Latin suffix "remedium" means to clean or restore (Cunningham et al., 1997). Phytoremediation was actually represents the diverse collection of plant-based technologies that uses native plants or genetically improved plants for decontamination of the environment (Flathman et al, 1998). Essentially these phytoremediation technologies have the potential of low-cost remediation (Ensley, 2000).

Industrial activities results in contamination of soil and spoil the vegetation cover. Phytoremediation plays the important role on such polluted lands for its ecological and sanitary significance (Antonkiewicz & Jasiewicz, 2002). Proper management of plants having phytoremediation potential on such areas may significantly restore the natural environment. Plants having phytoremediational potential have the ability to alter the rhizosphere effect, and abiotic factors such as soil type, water, pH, organic content, and nutrients. These factors create hydraulic barrier and capture the pollutant. However, despite the enormous potential of in situ treatment and availability of literature on the phytoremediation of diverse range of contaminants, there is very little to no awareness and implementation of this technology in India.

Techniques of Plant tissue culture like callus culture, cell suspension culture, and hairy root culture are applied frequently in phytoremediation research as model plant systems. Tissue culture proved to be a most powerful tool which has the potential to grow millions of plantlets from a single cell under in culture conditions, and to study the physiological mechanisms found in the plant cells under stressful conditions. Plant tissue culture and genetic engineering have opened new aspects in plant improvement. In vitro screening and selection of the plant cell for increased tolerance, accumulation or resistance. In this way screened tissue cultured plants could be used for transplantation onto polluted sites. Tissue culture plants have several advantages:

- Plants are genetically identical, i.e. clone, and will have the same characteristics, e.g. heavy metal tolerance, as the parent material.
- They can be very precise in location, and proliferated from plants exist on that specific location.
- Large number of plantlets can be generated in short period of time
- Tissue cultured plants were very quick to mature

No commercial plant nurseries or tissue culture labs provide any type of plant for phytoremediation purpose. Plant tissue culture technology was proved to be more adventitious than the use of whole plant or natural plant production. Because in vitro plant cultures are genetically similar, improved in case of desirable characters and free from microbial contamination.

Native plant species used for the phytoremediation process must generate large biomass and they can capable of accumulating high concentration of the toxic metal. (Chaney, 1997).There were only few studies about in vitro culture of Datura and Lantana, but these plants are having enormous potential of heavy metal accumulation. Therefore, taking in to consideration, the phytoremediation importance, it is necessary to study the effective tissue culture protocols for it. Datura constitutes a genus of nine species of vespertine flowering plants of the Solanaceae family. Its common name was Angel's Trumpets. Other plant *Lantana camara* is a native of West Africa and tropical Americas. It is commonly known as sauge, red sage, yellow sage, and lakana. Lantana is an aromatic shrub having quadrangular stems and prickles on it.

2. METHODOLOGY

Bhopal is having tremendous industrial development. Govindpura is one of the industrial areas in Bhopal. This area has hazardous waste due to dumping of industrial garbage, such dumping of waste in to soil results in incorporation of toxic compounds like, heavy metals organics and radioactive compounds. Mainly this site contains high concentrations of heavy metals. For the remediation of this site the plants having majority at this site were selected. The site represents majority of *Lantana camara* and *Datura inoxia* plants.

2.1. Collection of explants and surface sterilization

For in vitro studies explants were collected from the industrial area Govindpura in the form of small, fresh twigs of *Lantana camara* and *Datura inoxia*. From the twigs 0.5-1.0cm nodal segments and shoot tips were isolated and used as explants for the induction of culture. Firstly the explants were washed thoroughly under running tap water for 15 min and then were surface-sterilized by antifungal agent Bavistin and also treated with 0.1% $HgCl_2$ solution for 10 min. and then by washing with double sterilized distilled water for 10-15 Minutes.

2.2. Culture Medium and Conditions for Plant Regeneration

MS (Murashige and Skoog) medium with varying concentrations of 6- Benzyl amino purine (BAP), naphthalene acetic acid (NAA) and kinetin (KIN) was used for initiation of cultures. The explants were inoculated in aseptic condition with the help of laminar hood. Different combinations of media were adjusted to pH 5.8, and 0.8% agar and 30g/lit sucrose were added. The culture bottles containing media were sealed autoclaved at temperature121ºC for 15-20 minutes and under pressure of 15 lbs/inches^2. Maintenance of cultures was done at 16 hours

photoperiod of 1000 lux intensity at 25 ± 2ºC. At regular intervals results were noted and average readings were considered.

2.3. Establishment of in vitro culture of *Lantana camara* and *Datura inoxia* and Effects of Basal Medium Strength on Multiple Shoots Induction

In vitro culture studies were determined on the basis of standardization of protocol for establishment of the cultures of Lantana and Daturaby using various concentrations of MS media, salts and their combination were supplemented with 0.5mg/L BAP with 0.5mg/l NAA.

2.4. Initiation of Shoot by using Nodal and Apical Meristem

Murashige and Skoog basal medium fortified with cytokinins like BAP and kinetin KIN, by the concentration of (0.5-2.0) mg/l alone or in combination with other cytokinins of each, containing sucrose 30g and gelled with agar 4g/l was used for inoculation of the nodal and shoot tip explants. On the other hand auxins like IAA or NAA (0.1-1.0 mg/l) were also used for promoting the shoot induction.

2.5. Medium Used in Shoot Initiation

- Medium 1 MS + 0.1 mg/l BAP
- Medium 2 MS + 0.3 mg/l BAP
- Medium 3 MS + 0.5 mg/l BAP
- Medium 4 MS + 1.0 mg/l BAP
- Medium 5 MS + 0.1 mg/l KIN
- Medium 6 MS + 0.3 mg/l KIN
- Medium 7 MS + 0.5 mg/l BAP + 0.5 mg/l KIN
- Medium 8 MS + 1.0 mg/l BAP + 0.5 mg/l KIN

2.6. Heavy Metal Analysis from Soil at Industrially Contaminated Site

The study area was waste sites at industrial area Govindpura in Bhopal which receives enormous amount of heavy metal pollution. Randomly 3 samples were collected from the overall contaminated area following proper soil sampling protocol.

2.6.1. Sample Collection

Soil samples were collected at each sampling point among the selected three contaminated sites by using instrument called *khurpi*. They were taken from approximately 10 to 20 cm depth (Recatalá et al. 2002 and 2004).

2.6.2. Sample Preparation

Pre processing of samples were done by air drying them at ambient temperature and they were sieved to pass a mesh sieve of 2-mm. Soil properties were related to control the mobile nature and bio available forms of heavy metals (Adriano 2001).

2.6.3. Sample Analysis

The samples after drying were subjected to a cool extraction with 0.5M HNO_3 for 30 minutes. Heavy metals concentrations such as concentrations of Lead (Pb), Cadmium (Cd), Nickel (Ni),

and Chromium (Cr) were determined by atomic absorption spectrophotometer (Bada and Raji., 2010).

Table 1: Concentrations of Heavy Metals in Different Soil Samples at Industrial Sites

Soil Samples	Chromium (mg/kg)	Lead (mg/kg)	Cadmium (mg/kg)	Nickel (mg/kg)
Soil Sample-1	140	199	59	37
Soil Sample-2	159	180	70	38
Soil Sample-3	201	258	62	32

3. RESULTS AND OBSERVATIONS

Extraction of heavy metals from soil at industrial area revealed that the soil of industrial area was highly polluted with toxic concentrations. The heavy metal concentrations in the sediment from sampling sites are shown in Table 1. In soil samples, the increasing order of heavy metal concentration at contaminated site was Ni < Cd < Cr < Pb. Among the analyzed heavy metals, Pb had shown the highest concentrations in all the sampling sites, while Cd had shown the lowest concentrations. Soil analysis in the industrial area was characterized by the high values of lead and Cr. The chromium was found in very high concentration at the study area. The analysis had shown that the industrial area was at a high risk of Cr pollution.

Plant tissue cultures have many important technical advantages in case of phytoremediation. Because by using plant tissue cultures, fast growing, genetically similar and stress tolerant or pollutant tolerable plantlets can be produced in large amount in short period and such plants were used for phytoremediation purpose.

The present paper describes standard protocol for large scale production of plantlets through *Datura inoxia* and *Lantana camara*, the plants having phytoremediation potential and this method will be useful for the in vitro studies on heavy metal accumulation and tolerance mechnism. So that large number of improved plantlets can be grown and used for phytoremediation at industrially contaminated site. The response of *Datura inoxia* and *Lantana camara* for different concentrations and combinations were shown in Table 2.

Plant tissue cultures have many important technical advantages in case of phytoremediation. Because in vitro plant cultures give fast growing genetically similar and pollutant tolerable plantlets in large amount in very little time period. Thus they can be easily used for removing soil pollution. Government and industry must be committed to phytoremediation program.

Comparative analysis of tissue culture studies revealed that Datura was more responsive and fast growing plant in vitro but Lantana was quite sensitive and slow growing plant. Both plants were shown good results on Medium 3 MS + 0.5 mg/l BAP.

4. FUTURE WORK

- Extraction and stabilization of heavy metals at contaminated soils
- Analysis of uptake and sequestration of Heavy metals and study of metabolic products involved in it.
- Rhizosphere studies of each plants and identification of microbial flora of rhizosphere.

Table 2: Effect of Plant Growth Hormones on *Lantana camara* and *Datura inoxia.*

Plants	Plant Growth Regulators mg/l	Nodal Explants			Shoot Tip Explants		
		Response of Initiation in %	No. of Shoots in Culture	Avg. Length of Shoots (cm)	Response of Initiation in %	No. of Shoots in Culture	Avg. Length of Shoots (cm)
Datura inoxia	**BAP**						
	0.3	10	12	2.1	20	13	2.3
	0.5	90	25	4.2	75	15	3.2
	0.7	15	12	2.4	20	10	2.4
	Kinetin						
	0.3	11	10	1	12	11	1
	0.5	65	20	3.2	60	14	4.1
	0.7	13	10	2.2	15	9	2.5
	NAA						
	0.3	5	7	1	10	9	1
	0.5	70	25	3.1	60	45	3.4
	0.7	10	8	2.3	5	10	2
Lantana Camara	**BAP**						
	0.3	5	6	2.2	4	8	1
	0.5	60	12	4.1	75	12	3.2
	0.7	10	5	2.5	20	6	2.3
	Kinetin						
	0.3	8	4	2.4	10	9	1
	0.5	55	6	3.2	60	12	2.6
	0.7	20	5	2.6	9	6	1
	NAA						
	0.3	5	6	2	12	8	1
	0.5	60	25	3.3	60	45	3.2
	0.7	5	8	2.4	10	10	2

5. CONCLUSION

Environmental contamination was the great ecological hazard and proved to be threat to human being. Phytoremediation has the potential of protecting human life, ecological balance and original beauty of the planet. Phytoremediation of pollutants is a topic of central thrust on plant physiology. Thus, understanding of natural habitat of the degrading plant population and selective property of accumulation of specific heavy metal can be help to manage a cost-effective, and eco friendly plan for decontamination of industrially polluted site.

In coming future, it may possible that the metal rich plant material will safely harvested and removed from the site without any widespread excavation, removal cost and loss of top soil concerned with traditional remediation practices. The genomics can accelerate the discovery of genes that code key characters, allowing their modification.

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