e-ISSN: 2320-0189
p-ISSN: 2347-2308

Research and Reviews: Journal of Botanical Sciences

A Case Study of Native Plants at Industrially Contaminated Area in Bhopal and Their Tissue Culture Approach for Phytoremediation.

Ashwini A Waoo[1]*, Swati Khare[2], and Sujata Ganguly[1].

[1]Research Scholar, Govt. M.V.M., Bhopal, Madhya Pradesh, India.
[2]Dept. of Biotechnology, Institute for Excellence in Higher Education (IEHE), Bhopal, Madhya Pradesh, India.

Research Article

Received: 17/07/2014
Revised : 13/08/2014
Accepted: 20/08/2014

***For Correspondence**

Research Scholar, Govt. M.V.M., Bhopal, India.

Keywords: Heavy Metals, In-vitro, Micro propagation, Phytoremediation, Plant Tissue Culture, Pollutants, Soil pollution.

ABSTRACT

In phytoremediation techniques,experiments were done with normal soil-grown or hydroponically grown plants. In recent days, more and more effort is directed toward research to understand and improve the performance of plants in remediation technologies, the number of results obtained with the help of in vitro plant cell and tissue cultures is rapidly increasing.This review focuses on the report of various phytoremediation technologies, paying special attention to removal of pollutants from soil and the application of in vitro systems for basic research in the role of plants for the remediation of contaminated sites, and in the improvement of their effectiveness. Present study was conducted in Govind pura industrial area having heavy metal polluted soil. Extensive botanical survey was carried out in that area for selection of heavy metal accumulated plants, which can be used for phytoremediation of soil. 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 is a powerful tool that gives the possibility to grow millions of cells under in vitro conditions, and to understand physiological information about the behavior of the plant cells under stress conditions. Plant tissue culture and molecular genetics have opened new avenues in plant improvement. Screening and selection at the plant cell level has established plant clones with increased tolerance or resistance in salt, heat, cold, drought, disease, insects, heavy metals and herbicides. Plant cell and tissue culture is considered an important tool for fundamental studies that provide information about the plant-contaminant interactions, it also helps to forecast plant cell responses towards environmental pollutants, and redesign the plants with improved characteristics for phytoremediation. Plant tissue culture is used to study the interactions among plants and pollutants under in vitro conditions.

INTRODUCTION

Soils are critical environment where rock, air and water interface. Consequently, they are subjected to a number of pollutants due to different anthropogenic activities (Industrial, agricultural, transport etc.). The industrial activity accelerates pollution of the biosphere; especially the soil. Recently soil pollution is receiving extensive public attention since the magnitude of this problem is growing rapidly.

Environmental pollution with metals and xenobiotics is a worldwide problem, and the progress of phytoremediation technologies for the plant-based clean-up of contaminated soils is therefore of significant interest. Contaminated soils and waters pose a major environmental and human health related problems, which might be solved by the emergent phytoremediation technology. This economical and

plant-based methodology takes advantage of the remarkable ability of plants to concentrate elements and compounds from the environment and to metabolize various molecules in their tissues [1].

Toxic heavy metals and organic pollutants are the major targets for phytoremediation. Now days, facts of the physiological and molecular mechanisms of phytoremediation began to emerge together with biological and engineering strategies designed to optimize and improve phytoremediation. Soils normally contain low levels of heavy metals. Excessive levels can be hazardous to man, animals and plants. Metals, radio nuclides and other inorganic contaminants are among the most prevalent forms of environmental pollutants and their remediation in soils and in the sediments is a very difficult task [2].

Sources of anthropogenic metal contamination include smelting of electroplating, energy and fuel production, metalliferous ore, the application of fertilizers, gas exhaust, and municipal waste to land, and industrial manufacturing. Heavy metal contamination of the biosphere has increased sharply and poses major environmental and human health problems worldwide.

Traditional technologies for removal of pollutants can be successful in particular situations, but these technologies are having high cost [3]. There must be a vigorous effort to search and develop more cost-effective techniques to remediate contaminated soils, therefore more attention is concentrating on advanced biological methods like phytoremediation, offering the use of plants to sequester, extract and purify contaminants [4] [5].

Phytoremediation is the application of live green plants for removal of pollutants from polluted soil, air, and water sediments. Specifically engineered plants are used in this technology. Phytoremediation is cost effective, artistically attractive method of remediating sites where there is low to modest levels of contamination [6].

The present study aimed to find a suitable plants species for use in cleaning up the soil in industrial regions. In this work we were studied crop species that were found at contaminated area such as *Daturainoxia, Calotropisprocera, Ricinuscommunis*and *Lantana camara*, the uptake of both plants on contaminated soil were analyzed by determining the levels of heavy metals in plants and its capacity to grow in in - vitro condition.

METHODOLOGY

Phytoremediation consists of four different plant-based technologies each having a different mechanism of action for the remediation of soil polluted with heavy metals or water. These technology comprise: rhizofiltration, involving the use of plants to remediate aquatic environments; phytostabilization, involving the use of plants to stabilize polluted soil; phytovolatilization, involving the use of plants to extract some metals from soil and then releasing them into the atmosphere by volatilization; and phytoextraction, involving the use of plants to absorb metals among the soil and their translocation in the harvestable shoots for accumulation [7]. Plants also have some capacity to decrease the threats of pollutants [8].

Processes of Phytoremediation

Natural phenomenons carried out by plants were the base of phytoremediation technology such as:

- Metals and certain organic compounds uptake
- These chemicals accumulate and processed via lignification, mineralization and volatilization,
- Complex organic molecules broken down into simpler molecules by the enzymes
- Capture of groundwater and utilization for plant processes

Plant tissue culture is a convenient laboratory tool for phytoremediation studies. The forms of tissue culture most frequently employed are cell suspensions and hairy roots. Once established, these in vitro cultures can be propagated indefinitely and are available on demand. In contrast, whole plants grown either in soil or hydroponic systems have a limited lifespan and each individual plant needs to be replaced and reestablished after each experiment. Therefore, the time required to carry out experimental investigations may be substantially reduced using plant tissue cultures rather than whole plants [9].

Locally adapted tissue cultured plants could be used for seed increase or 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.
- Multiplication can be rapid, 4 to 8X every 4 to 6 weeks,
- Plants may be very quick to mature
- Many thousands of plants can be cultured in a very small space.

Plant Tissue Culture Protocol

No commercial plant nurseries or tissue culture labs provide any type of plant for phytoremediation purpose. Plant tissue cultures also offer important technical advantages compared with whole plants. Because in vitro plant cultures are grown and maintained free from microbial contamination.Plant Tissue Culture involved following protocol:

- To standardize a micro-propagation protocol
- To standardize the protocol of sterilization.
- Standardization of suitable media (Shown in Table.1) for aseptic culture initiation, establishment and multiplication the following parameters: shoot length, number of shoots and number of nodes.
- To standardize an appropriate media for rooting and
- A suitable protocol for acclimatization.
- Toxicity tolerance studies on plants.
- Screening of Tolerant plants.
- Hardening of tolerant plants.

The basal medium is formulated so that it provides all of the compounds needed for plant growth and proliferation, it also includes some chemical compounds which might be made by an whole plant, but an isolated piece of plant tissue may not able to produce it. Figure 1 shows the tissue culture room having various culture bottles in controlled light, temperature and humidity conditions.

The culture medium contains 95% water, macro- and micronutrients, vitamins, amino acids, sugars. The nutrients in the media are used by the plant cells as building blocks for the synthesis of catalytic activators which are used in enzymatic reactions or organic molecules. The macronutrients are essential in millimolar (mM) quantities while micronutrients are needed in much lower (micromolar, µM) concentrations. Vitamins are organic substances that are parts of enzymes or cofactors for essential metabolic reactions. Sugar is used as energy source for in vitro growth and development as most plant cultures are unable to photosynthesize effectively for a variety of reasons. Murashige&Skoog (1962) medium (MS) is the most suitable and commonly used basic tissue culture medium for plant regeneration as shown in Table 1.

Figure 1: Photograph of In-vitro Cultured Plantlets

Micro Propagation: Micro propagation differs from all other conventional propagation methods in that aseptic conditions are essential to achieve success. The process of micro-propagation can be divided into four stages:

- *Initiation Stage*: A piece of plant tissue (called an explant) is (a) cut from the plant, (b) disinfested (removal of surface contaminants), and (c) placed on a medium. A medium typically contains sucrose,

mineral salts, and agar as a solidifying agent. After that the next step is to achieve an aseptic culture without contamination of any other microorganisms.

- *Multiplication Stage:* Growing explants can be induced to produce vegetative shoots by including a plant growth regulator cytokine that promotes shoot formation from growing plant cells.
- *Rooting or Pre-Plant Stage:* Growing shoots can be induced to produce adventitious roots by including plant growth regulators auxin that stimulates root formation. An auxin is commonly not necessary for easy rooting plants and many commercial labs will skip this step.
- *Acclimatization:* A growing, rooted shoot can be removed from tissue culture and then established in soil. During this moisture must be gradually reduced over time because tissue-cultured plants are extremely susceptible to wilting.

After this stage tolerant plants having the phytoremediation potential were obtained and used for treatment of contaminated sites. Stable in vitro plantlets of study plants found at industrially contaminated area, Govindpura, are shown in Figure 2.

Table 1: Composition of Media used for in Vitro Culture

Constituents	Murashige and Skoog (MS, 1962) mg/l
A. Inorganic	
Macronutrients	
$MgSO_4$. $7H_2O$	370
KH_2PO_4	170
$NaH_2PO_4.H_3O$	.....
KNO_3	1900
NH_4NO_3	1650
$CaCl_2.2H_2O$	440
$NH_4H_2PO_4$	.....
Micronutrients	
H_3BO_3	6.2
$MnSO_4.4\ H_2O$	22.3
$ZnSO_4.7\ H_2O$	8.6
$Na_2MoO_4.2\ H_2O$	0.25
$CuSO_4.5\ H_2O$	0.025
$CoCl_2.6\ H_2O$	0.025
KI	0.83
$FeSO_4.7H_2O$	27.8
Na_2EDTA	37.3
B. Organic	
Thiamine HCl	0.1
Pyridoxine HCl	0.5
Nicotinic Acid	0.5
Myo-inositol	100
Glycine	2.0
Sucrose	30

Phytoremediation typically involves the exposure of plants to pollutants in the environment over relatively long periods of time [11]. Accordingly, stable genetic transformation to produce recombinant plants is generally a more appropriate approach than the use of transient expression systems such as plant viral vectors. Plant tissue culture is a necessary step in most techniques used to incorporate foreign genes into the nuclear genomes of plants. After transformation, plant cell cultures also play an important role in the selection of successful transformants and to confirm that enhanced phenotypic traits have been acquired before the time-consuming process of plant regeneration is carried out [10].

e-ISSN: 2320-0189
p-ISSN: 2347-2308

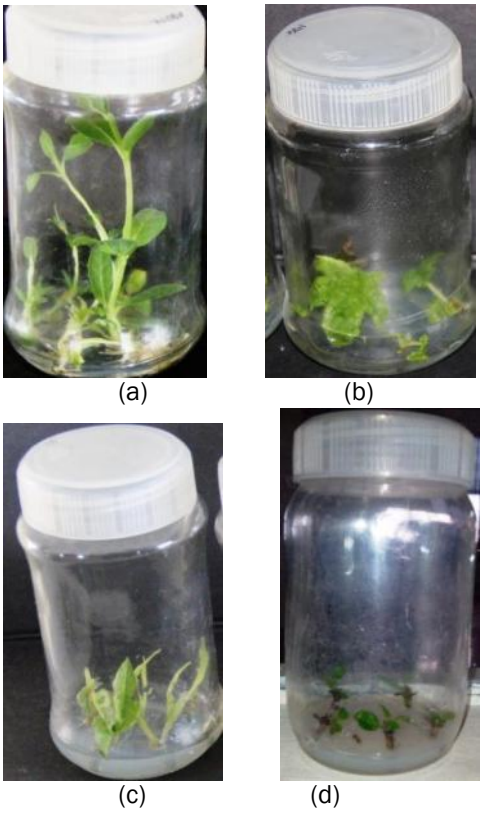

Figure 2: Tissue Culture of Heavy Metal Tolerant Plants
(a) Calotropisprocera (b) Ricinuscommunis
(c) Daturainoxia (d) Lantana camara

RESULT AND CONCLUSION

The importance of biodiversity (below and above ground) is increasingly considered for the cleanup of the metal contaminated ecosystems. This area is evolving as a revolutionary area of research gaining commercial significance in the contemporary field of environmental biotechnology.Tissue cultures offer more than just experimental convenience and speed compared with whole plant systems. By eliminating the effects of micro flora and translocation barriers, a closer approximation to the intrinsic capacity of plant cells for detoxification of pollutants can be obtained.

Tissue culture of locally adopted plants at industrially contaminated area was producing the genetically similar plantlets, which can be used in phytoremediation purpose.

Plant tissue cultures are a powerful tool in phytoremediation research as model systems to identify the biochemical responses of plant cells to environmental contaminants, the metabolic capabilities of plant tissues, and the reaction products formed. Plant tissue culture offers the opportunity to examine the genetic endowment or intrinsic enzymatic capacity of particular plant species or cultivars removed from the effects of microbial activity. Much further work is needed in this area to enhance our understanding of the complex biotransformation mechanisms and pathways employed by plants to detoxify organic pollutants.

Nomenclature

- MS – Murashige and Skoogs
- BAP – Benzyl Amino Purine
- NAA- Naphthalene Acetic acid

ACKNOWLEDGEMENT

For this research work I would like to thank to my Supervisor Dr. Swati Khare, Professor, Institute for Excellence in Higher Education (IEHE), Bhopal and Co- Supervisor Dr. ShujataGanguly, Professor, M.V.M.

College, Bhopal (M.P.), for their complete support and guidance.I also thankful to Dr. Shagufta Khan, Director, Growtips Biotech Training Institute, Bhopal for her support in my research work. Along with this, I would like to thanks to Dr. AabhaGargava, Principal, Govt. M.V.M. Bhopal to provide me resources in M.V.M. college.

REFERENCES

1. Raskin, I. and Ensley, Phytoremediation of toxic metals: using plants to clean up the environment, B.D. eds, New York, John Wiley and Sons, 2000, 352 p. ISBN0-47- 119254-6.
2. Prabha K. Padmavathiamma& Loretta Y. Li, Phytoremediation Technology: Hyper-accumulation Metals in Plants, *Springer Science* + Business Media B.V. May 2007
3. Francis U UmeoguajuNnamdiAzikiwe, Conventional and new ways of remediating soils polluted with heavy metals, (2009) Dept of Applied biochemistry, University. Awka. Nigeria. (Review article).
4. Chhotu D. Jadia, Madhusudan H. Fulekar, Phytoremediation: The Application Of Vermicompost To Remove Zinc, Cadmium, Copper, Nickel And Lead By Sunflower Plant, Environmental Biotechnology Laboratory, Department of Life Sciences, University of Mumbai, Santacruz (E), Mumbai – 400098
5. T. Macek, M. Macková, J. Ká, Exploitation of plants for the removal of organics in environmental remediation, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague, Czech Republic
6. Raskin, I., Plant genetic engineering may help with environmental cleanup, Proceedings of the National Academy of Sciences of the United States of America, 1996,vol. 93, no. 8, p. 3164-3166
7. Erakhrumen, Andrew Agbontalor,Phytoremediation: An Environmentally Sound Technology for Pollution Prevention, Control and Remediation in Developing Countries, Department of Forest Resources Management, University of Ibadan, Ibadan, Nigeria. May, 2007
8. Cunningham, S.D.; Shann, J.R.; Crowley, D.E. and Anderson, T.A., Phytoremediation of contaminated water and soil, *Kruger, E.L.*
9. Ashwini A. Waoo, Dr. Swati Khare, Dr. SujataGanguli. In-vitro Culture of Latana camera from Nodal and Shoot-tip Explants in Phytoremediation Studies. Current Trends in Technology & Sciences. 2013;1(1):183-186.
10. Pauline M. Doran, Application of Plant Tissue Cultures in Phytoremediation Research: Incentives and Limitations , School of Biological Sciences and Department of Chemical Engineering,Monash University, VIC 3800, Australia; telephone: 61-3-9905-1373;
11. Anderson, T.A. and Coats, J.R., Phytoremediation of soil and water contaminants, eds, *ACS symposium series 664*. Washington, DC, American Chemical Society, 1997, p. 2-19.