treatment options, soil and contaminant characteristics and degree of contamination.

II. METHODS AND MATERIAL

2.1 LEAD TOXICITY

Lead (Pb) is a bluish-grey metal naturally found in traces in the Earth's crust. It was also known as plumbum, lead metal, and pigment metal. Various industrial processes that involve the use of lead were as mining, smelting, manufacture of pesticides and fertilizers, dumping of municipal sewage and the burning of fossil fuels that contain a lead additive. Many commercial products such as paints, ceramic glazes, television glass, ammunition, batteries, medical equipment (i.e., x-ray shields, fetal monitors), and electrical equipment also contain lead. Lead has been listed as a potential carcinogen in the EPA Toxic Release Inventory TRI). Inhalation and ingestion are the two routes of exposure of lead. It accumulates in the body organs (i.e., brain), which may lead to poisoning or even death. The gastrointestinal tract, kidneys, and central nervous system are also affected by the presence of lead. Children exposed to lead are at risk for impaired development, lower IQ, shortened attention span, hyperactivity, and mental deterioration, with children under the age of six being at a more substantial risk. Adults usually experience decreased reaction time, loss of memory, nausea, insomnia, anorexia, and weakness of the joints when exposed to lead [1]. It shows half-life of more than 1000 years [2]. Pb has been shown to accumulate in plants from several sources including soil but the reports on accumulation of the Pb within plants are variable [3]. Pb is often found in the cytoplasm of cells associated with electron-dense precipitates localized in membranous inclusions, vesicles or organelles [4].

Some workers also used tissue culture to develop plants with acid soil, heavy metal tolerance, potentially for hard-rock mine land reclamation. They tissue cultured local native plants which grow directly on acid in heavy metal contaminated soil [5]. According to Pauline M. Doran [10], 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 infinitely and are available on demand. No commercial plant nurseries or tissue culture labs provide any heavy metal accumulating locally adapted tissue cultured plants[6] [7].

Some researchers [8], carried out several experiments to study heavy metal tolerance in tissue cultures or whole plants of *S. grandiflora*, the callus was grown and maintained on modified Murashige and Skoog, 1962 medium supplemented with growth hormones. Heavy metal such as lead was added to the culture medium at different concentrations as a contaminant. Effect of these heavy metals on percentage of survival and shoot length was also assessed [9].

A. Establishment of *Lantana camara* in Tissue Culture

Small tender twigs of *Lantana camara* were collected from contaminated sites, cut into 0.5-1.0cm nodal and shoot tip segments and used as explants for the induction of multiple shoots.

B. Sterilization of Explant

Twigs of Lantana camera cut into 0.5-1.0 cm from the nodal segments and used as explants for the induction of multiple shoots. Similarly shoot tip region was also excised from the twig. Firstly explants were washed thoroughly under running tap water for 15-20 min. Then surface sterilization was done by antifungal agent (Bavistine), soap solution and different concentrations of $HgCl_2$ solution and washed thrice with sterile distilled water in the laminar air flow chamber.

C. Culture Medium and Conditions for Plant Regeneration

After sterilization of explants, explants were inoculated in culture bottles aseptically. For inoculation explants were transferred to large sterile glass petri plate or glass plate with the help of sterile forceps under strict aseptic conditions. Here the explants were further trimmed and extra outer leaves were removed to make them in suitable sizes. Trimming can be done and leaves were removed with sterile scalpel blade. After vertically inoculating the explants in culture bottle the mouth of bottle is quick flamed and bottles are tightly capped and mouths of the bottles was properly sealed to avoid entry of external air. After proper labelling, clearly mentioning media code, date of inoculation etc. the bottles were transferred to growth room.

The basic nutrient medium with different concentration of growth hormones viz. Cytokinins, Auxins etc. used in MS medium (1962). The pH of the medium is adjusted between 5.5-5.8 by using 0.1 N NaOH or 0.1 N HCI before sterilization.

Under a laminar flow cabinet explants were inoculated aseptically on MS (Murashige and Skoog, 1962) medium supplemented with various concentrations of 6-Benzyl amino purine (BAP) alone or in combinations with naphthalene acetic acid (NAA) and kinetin (KIN). All media were adjusted to pH 5.8, and 0.8% agar and $30gl^{-1}$ sucrose were added. About 15ml of the medium

were dispensed in each culture bottle and sealed with plastic cover before autoclaving at 121ºC for 15min under pressure of 15 Psi. The media were left to cool as slant in the culture room until use. All cultures were maintained at 16 hr light of 1000 lux using fluorescent lamps at (25 ± 2) ºC. Results were observed at regular intervals and data were collected from three independent experiments and presented as average.

D. Shoot Induction Experiment

For these studies the nodal explants and shoot tip explants were inoculated on Murashige and Skoog (1962) basal medium supplemented with cytokinins like BAP and kinetin KN, in the concentration of (0.1-0.7) mg/l alone or in combination with other cytokinins of each, containing sucrose 30 g and gelled with agar 4 g/l. In addition auxins like IAA or NAA (0.1-0.7 mg/l) were used for promoting the shoot induction.

E. Incubation

Incubation of culture bottles is carried out in incubation room under cool white fluorescent light (200-300 flux) of 16 hours photoperiod and 8 hours dark period at a temperature of $25+3^0$C and relative humidity of 50-60%. The cultures were observed after one week.

2.2 SCREENING OF *LANTANA CAMARA* ON LEAD SUPPLEMENTED M. S. MEDIA

For screening experiment, *In vitro* culture of *Lantana camara* on heavy metals supplemented M. S. medium was carried out in controlled conditions. Plantlets established on control were transferred on different concentrations of heavy metal contaminated Medium for growth. The primary objective of this study is to assess the effects of lead in developing cultures of *Lantana camara in vitro*. The study was designed to investigate the impact of Lead on growth and morphology of the plant.

The culture medium was agar solidified M.S. basal medium supplemented with 2 % sucrose (w/v). The pH of the media was adjusted to 5.8 prior to autoclaving. Stock solution of Lead chloride ($PbCl_2$) was prepared and filter sterilized. Suitable aliquots of filter sterilized solution of metal were added aseptically to attain final concentrations of 0.1 mg/l to 50 mg/l. The media were distributed in sterilized culture bottles.

The *in vitro* cultured plants tested for heavy metal tolerance and accumulation. This will be achieved by increasing the heavy metal concentration in media. The specific toxic effect on plants will also be studied. The growth parameters are optimized for better growth and accumulation of heavy metals heavy metals.

In screening experiments, total stem length was measured of surviving plantlets. Survival and mortality rate of plants were calculated by rating the plants grown in contaminated medium in comparison to uncontaminated control medium.

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 as accumulator to toxic metal ions.

After about 20- 25 days of incubation the initiated plants were taken out the culture bottles 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.

III. RESULTS AND DISCUSSION

In vitro culture establishment of *Lantana camara* was done by standardization and formulation of tissue culture medium. M S medium with different concentrations of plant growth regulators such as BAP, NAA and KN alone or in combinations were tested for initiation of culture of *Lantana camara* from nodal and shoot tip explants. These explants were surface sterilized before inoculation. The percent survival rate and shoot length was represented in Table 2.

In vitro studies conducted revealed that the response of the explants of *Lantana camara* was very delayed and slow. Majority of the response in culture was dependent upon the age of explants and season of collection. Shoots excised from the plant shows better results during the month August to February were more vigorous and responded more with less contamination. The time period of the treatment of surface sterilizing agent 0.1% $HgCl_2$ was standardized from 3-4 minutes on which 80% explants were sterilized. The explants

dehydrated and blackens when treated with 70% ethanol solution may be due to the softness of shoots or excretion of phenolic compounds in media.

Table 1 had shown that the initiation response of *Lantana camara* was better in the combinations of different growth factors such as BAP, KN and NAA than individual plant growth regulator. Figure 1 had shown the effect of PGR on percentage initiation of culture and according to the data media for initiation containing 0.5 mg/l BAP, 0.5 mg/l KN and 0.5 mg/l NAA had shown the highest percentage i.e. 55 % of initiation of culture of Lantana camara.

At each trail of combinations of PGRs Average shoot length of the initiated shoot was also considered for evaluation of growth of culture. Figure 1 had shown that average shoot length of *Lantana camara* was highest at 0.5 mg/l BAP, 0.5 mg/l KN and 0.5 mg/l NAA. The inoculated cultures were maintained at 25^0C and 15-16 hours photoperiod. After 6 to 7 days bud breaking from the axillary and apical meristem was observed. The best response was observed on medium supplemented with combination of BAP, NAA and Kinetin. The elongation of shoot was observed up to 3.7 cm only in stable culture.

TABLE 1
IN-VITRO ESTABLISHMENT OF *LANTANA CAMARA* IN CONTROL

Media Code MSI*	MS + Growth Regulators (mg/l)			% of Intiation	Average Shoot Length (cm)
	BAP	NAA	KN		
MSI 1	0.1	-	-	-	-
MSI 2	0.5	-	-	-	-
MSI 3	1	-	-	20	0.5
MSI 4	2	-	-	35	1.05
MSI 5	0.5	0.1	-	40	1.51
MSI 6	0.5	0.5	-	45	1.42
MSI 7	1	0.5	-	40	1.52
MSI 8	2	0.5	-	40	1.07
MSI 9	0.1	0.5	0.5	50	1.01
MSI 10	0.5	0.5	0.5	55	2.16
MSI 11	1	0.5	0.5	50	2.21
MSI 12	2	0.5	0.5	40	2.43
MSI 13	0.5	-	0.5	20	1.33
MSI 14	1	-	0.5	35	1.16
MSI 15	2	-	0.5	40	2.67

* MSI – MS Media for Initiation

3.1 EFFECT OF LEAD ON IN-VITRO CULTURES OF LANTANA CAMARA

For selection of *Lantana camara* as an accumulator plant for Lead. The established cultures were inoculated on MS medium supplemented with 0.5 BAP, NAA and Kinetin and also the addition of Lead at concentrations 0.1-50 mg/l. 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 formed and grown on a medium with increasing concentrations of lead. Table 2 shows that the increased external Lead concentration considerably decreased the shoot length and vigorous growth of Lantana camara.

TABLE 2
EFFECT OF DIFFERENT CONCENTRATIONS OF LEAD ON % SURVIVAL OF LANTANA CAMARA

0.5 MS + PbSO2 (mg/l)	% of Survival*	Average Shoot Length* (cm)	Average No. of Shoots*
0.1	75	2.5	1-2
0.2	74	2.52	1-2
0.3	72	2.54	1-2
0.5	65	2.57	1-2
1	60	2.60	1-2
5	60	2.61	1-2
10	58	2.63	1-2
15	55	2.64	1
20	54	2.68	1
25	50	2.69	1
30	50	2.71	1
35	45	2.72	1
40	45	2.72	1
45	40	2.72	1
50	40	2.72	-
SD (±)	11.61	0.08	
SE (±)	3	0.02	

* Values are mean of 3 replicates

To assess the effect of increasing concentrations of Lead on Lantana camara, Lead treatments were done. The observations were tabulated in Table 2 summarized the effect of Lead treatments on Lantana camara. As the heavy metal concentration increases growth of Lantana reduces. On the other side, accumulation capability culture has shown the survival up to 50 mg/l Lead in

culture medium showing its phytoremediation potential. Upon increasing the concentrations of Pb in medium, the percentage survival rate was decreased in the culture. Shoot length was also affected by higher concentrations of Pb in the medium. The proportion of growth in shoot length was decreases at the higher concentration of lead in the medium. Number of shoots was decreased due to necrosis and browning during sub culturing in varying concentrations.

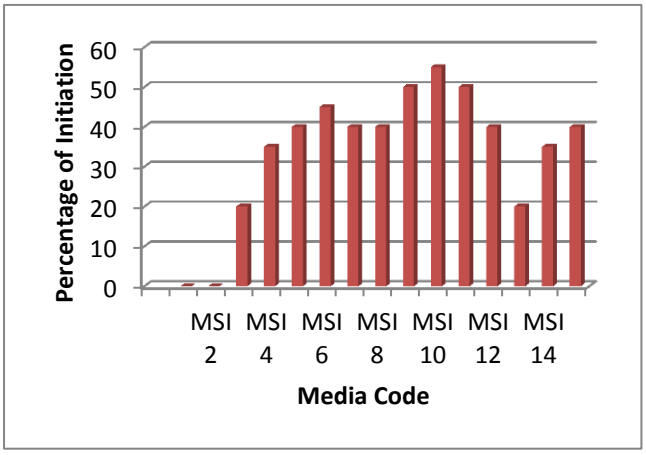

Figure 1: Effect of PGR Concentrations on % of Initiation of Lantana camara

IV. CONCLUSION

The expected outcome of the proposed research work will be to identify the best suitable plant species for phytoremediation at specific contaminated site according to the type of heavy metal pollution. Contaminated sites that require remediation often contain a complex mixture of heavy metals and organic contaminants. Yet, little is known about phytoremediation systems capable of remediating mixtures of contaminants.

The results obtained and thereafter the methods developed from the research will be of direct benefit to scientific communities and remediation specialists who will use it in the real-world conditions towards achieving a greener environment. Several conclusions were reached from analyses carried out on soil samples collected in the field, and plant and heavy metal stress *in vitro* cultures.

V. REFERENCES

[1] Environment Writer: 2000. "Lead (Pb) Chemical Backgrounder", Available [Online]: http://www.nsc.org/ehc/ew/chems/lead.htm.

[2] Merrington, G., 1995. "Historic Metalliferous Mine Sites: A Major Source of Heavy Metal Contamination", Land Contamination and Reclamation, 3, 3, 173-179.

[3] Al Salman IM and Abdul Aziz, M. 2002. "Lead bio-accumulation in Baghdad city, Iraq". Jordan J Appl Sci 4: 6–11.s.

[4] Sinha P, Dube BK, Sriastava P, Chatterjee, C. 2006. "Alteration in uptake and translocation of essential nutrients in cabbage by excess lead", Chemosphere, 65: 651–656.

[5] Sandra King S, King, Jennings S, Neuman D, 2009. "Using plant tissue culture to develop plants with acid soil, heavy metal tolerance, potentially useful for hard-rock mine land reclamation", National Meeting of the American Society of Mining & Reclamation, Revitalizing the Environment: Proven Solutions and Innovative Approaches, R.I. Barnhisel (Ed.); ASMR, 3134; KY 40502.

[6] Majerus, M. 2003. "New Native Pre-Varietal Germplasm Releases for the Northern Great Plains and Intermountain Region", pp. 733-748. In: Proceedings of the 2003 National Meeting of the American Society of Mining and Reclamation and the 9th Billings Land Reclamation Symposium, Billings, MT June 3-6, 2003. CD ROM. Published by the ASMR, 3134 Montavesta Road, Lexington, KY 40502.

[7] Majerus, S. and M.E. Majerus. 2008. "Notice of Release of Opportunity Germplasm Nevada Bluegrass Selected Class of Natural Germplasm", USDANRCS; Montana State University, Bozeman, MT; University of Wyoming, Laramie, WT; Deer Lodge Valley Conservation District, Deer Lodge, MT. pp 1- 28.

[8] Kadhim M. Ibrahim and Shaimaa A. Yousir, 2009. "In vivo and *in vitro* studies on heavy metal tolerance in Sesbania grandiflora L.", Biotechnology Research Center (special edition) Vol. 3-No. 2.

[9] Waoo A.A., Khare S., Ganguly S., 2014. "Extraction and Analysis of Heavy Metals from Soil and Plants in the Industrial Area Govindpura, Bhopal, in Journal of Environment and Human, 2373-8332, 158-164, 1(2).

[10] Paulin M. Doran, 2008. "Applications of plant tissue cultures in phytoremediation research: Incentives and limitations", Wiley Interscience (www.interscience.wiley.com). DOI 10.1002/bit22280.