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Impact of waste dumps of mines on plants in genomic & bio molecules level

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Abstract

The prime aim of this study was to analyze the effects of mine waste dumps on plants namely *J. curcas*and*G. hirsutum*, applied to phytoremediation process on waste dumps of magnesite and bauxite mines. The waste dumps of the mines contain impermissible limits of heavy metals Cd (2070 & 1060) and Pb (443 & 742.6 mg/kg^{-1}), it's followed by Zn (1141 & 827.5) and Cr (69.96 & 553.7 in magnesite and bauxite mine respectively). The phytochemical and macro biomolecule analysis showed deviation among the plants in chlorophyll, protein and carbohydrate content. These plants possess fine phytoremediation potentiality on both mine soil. A random amplified polymorphic DNA (RAPD) technique was exhibited to detect genotoxin-induced DNA damage of plants from heavy metal contaminated soil (mines) during phytoremediation. The changes in DNA profiles included variation in band intensity, presence or absence of certain bands and even appearance of new bands in RAPD profiles with universal 20 operon 10-base pair primers (OPA serious), with 60% – 70% GC content and they showed significant changes happened in plants applied for phytoremediation process on mine waste. The dendrogram reports are clearly reported the DNA variation in the plants (mutation). The results showed that RAPD analysis could be a useful tool for detection of genotoxic effects of heavy metal (Cd) toxicity on plants from mine soil.

Keywords; Mining, waste dumps, toxic metals, Plants, DNA Profile, Dendrogram.

Introduction

There are a large number of sites worldwide polluted with trace elements as a result of human activities. The mining industry has produced a significant legacy of polluted and degraded soils [1].In the developing countries heavy metal pollution becomes serious problematic, causing by mining, mineral, smelting and tannery activities [2]. The physicochemical properties of heavy metals contaminated soils (waste dumps of mining) tend to inhibit plant growth [3]. Further the metal pollution diminishes the quality and quantity of soil biota and trim down the quality of the atmosphere, water bodies, threatens the health of higher animals and plants. For long term remediation, metal tolerant species are commonly used for re-vegetation of degraded land [4]. Finding the optimum plant species for remediation of a determined soil will be the main point controlling the success of the process, as well as the selection of adequate soil amendments which would improve soil conditions allowing plant survival and growth.

There are some limitation occur still to elucidate the physiology, biochemistry and metal hyper accumulation in plants [5].For the effective phytoremediation process the plant species should be non-edible and which can be grown abundantly in large scale on wastelands and that should give economic to country. The biomass of the plant which is used for remediation process is an important factor in considering the phytoremediation efficiency of the plants [6].

The major phytochemicals like chlorophyll is the principal pigment in plants, converting light energy to chemical energy (photosynthesis) for effective growth of plants and high yield of biomass [7]. By gravely considering all the options mention above, finally we have elected the *Jatrophacurcas*&*Gossypiumhirsutum*for this experiment to study phytoremediation efficiency by conducting a greenhouse experiment on waste dumps of magnesite and bauxite mines and impact of metal restrain of mine soil on plants in genomic (Randomly Amplified Polymorphic DNA-RAPD analysis) & bio molecules (chlorophylls, carbohydrates and protein) level.

Materials and methods

Sources of soil samples and site description

Soil samples were collected from magnesite (Latitude 11.7376301 & Longitude 78.1363106) and bauxite mines (Latitude 11.8160139 & Longitude 78.223353) in Salem districts, Tamil Nadu, India. These two mining industries are opencast type; the bauxite mining is located in peak of Shervaroy hill, the magnesite mine located in the foot region of the hill. The geological condition of the hill and foothill is red soil, loamy and lateritic in nature. The area is made up of Archaean crystalline rock like amphibolites, leptynites, garnetiferous granites and charnockites. Bauxite and magnesite are the chief mineral resources. The mean annual rainfall is 1638 mm at the upper hills and 850 mm at the foothills. The temperature ranges from 13 to 29°C on the peaks, and 25 to 40°C at the foothills. The plant *Chionanthusramiflorus* and *Ligustrumperrottetii*, are dominant top of the hills (bauxite) and *Azadirachtaindica* and *Acacia niloticaor*

Acacia arabica are dominant in the foothills region (magnesite). The agriculture farm soil was considered as control soil. The collected soil were mixed in large containers and air-dried at room temperature and then the samples were sieved [8].

Physicochemical and metal analysis

The pH of soil samples were determined by using glass electrodes in the supernatant solution prepared by 5g of soil dissolved in 12.5 ml of distilled water and leaves it for 5-10 min (3:1 ratio) to allow ionic exchange to reach equilibrium. The Electrical conductivity (EC) was determined and the N, P, K, $CaCl_2$ and texture was analyzed as per the methods of soil testing laboratory, Department of Agriculture, Govt. of Tamil nadu, India. High Analytical grade reagents were used for this study. The total heavy metal content in the soil samples was analyzed by an acid digestion method [9]. The digested liquid was filtered through Whatman No. 0.5 filter paper and the heavy metal contents of filtrate were analyzed by using inductively coupled plasma-optical emission spectrometry (ICP-OES, Perkin-Elmer, USA). Procedural blanks and standard solutions were prepared and included for analytical quality control to assure the accuracy and reproducibility of the results and the hard chemicals used for this study was Hi- Media chemicals (Hi-Media Pvt. Lid. Mumbai, India).

Test plant and Green house Experiment

The air-dried soil samples were taken in polyethylene bags (2kg). Seeds of *J. curcas*and*G. hirsutum*(from Tamil Nadu Agricultural University-TNAU) were sown in polyethylene bags. The whole experiment was carried out in a greenhouse condition. The moisture content of each pot was maintained at 75% water holding capacity by weighing the pots two times per week [10] and it was maintained the bags were watered with 50 ml of deionised water and it retained under the greenhouse with natural light and temperature (30°C) for eight weeks.

Bio molecule analysis

Chlorophyll analysis (*a* and *b*)

The total chlorophyll content (*a*&*b*) was recorded by using a spectrophotometer (UV-double beam spectrophotometer, Spectronic) at two different wavelengths (647 and 664 nm) as per the modified protocol [11].

Macro biomolecules analysis

The leaf samples (0.2g) were taken from the mid shoots of each plant, was used for analyzing the bio molecules (carbohydrate and protein), and based on the modified method [12].

Plant Harvest for metal analysis

The shoots and roots of each plant were harvested and washed thoroughly with deionised water and rinsed with 0.1N HCl for 30 seconds and again washed with deionised distilled water. The metal content of the dried test plants were determined by acid digestion method, as per the method of [13], and digest was analyzed by using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES, Perkin-Elmer, USA).

Plant genomic DNA isolation and optimization

Total genomic DNA from young leaves of *J. curcas*&*G. hirsutum* from waste dumps of mines (magnesite and bauxite) and control soil, was extracted by following the standard CTAB method with minor modifications [14]. DNA concentration and purity was determined by intensities of the band when compared with the lkb Lambda DNA marker (used to determine the concentration) on 0.8% agarose gel electrophoresis.

Screening of RAPD Primers

Twenty primers from the Operon 10 mers (Universal Medox Operon primer Kit A serious: OPA 01 to OPA 20) with 60% – 70% GC content was used RAPD analysis (Table 1).

RAPD Amplification and optimization

The genomic DNA of *J. curcas* and *G. hirsutum* were amplified using RAPD-PCR. The amplifications were carried out in a MyGeneTM series Peltier thermal cycler, Model MG 25+ (Long Gene Scientific instruments Co., Ltd). The various volume of samples (2,3,5,10,20,30,50,75 and 100µl of DNA templates), $MgCl_2$ (1, 2, 3, 4, and 5 mM), dNTPs (0.1, 0.2, 0.3 and 0.4 mM); primer (1, 1.5, 2, 2.5, 3, 3.5, 4.0, 4.5 and 5.0 µl), Taq DNA polymerase (0.1, 0.5 and 1 unit) and the total reaction volumes (14, 16, 18 and 20 µl) were tested. The thermo cycler was programmed for an initial denaturation step of various min (2, 3, 4 and 5) at 94°C, followed by various number of cycles: 25, 30, 35, 40, 45 and 50 cycles, and at various annealing temperature (45, 50, 55, 60, 65 and 70°C) for 1 min, extension was carried out at 72°C for 1 min and final extension at 72°C for 7 min and hold temperature of 4°C at the end. The amplified PCR products were electrophoresed on 1% (w/v) agarose gels (stained with ethidiumbromide 0.5 µg/ml) in 1X TBE Buffer at 50 V for 3 hours. Gels with amplification fragments were visualized and photographed under UV light. The intact 1kb Lambda DNA (DNA) was used as a marker throughout study.

Table 1

What is this table stand for please mention it here

S.NO	Primers	Nucleotide sequence
1	OPA-04	5'AATCGGGCTG3'
2	OPA-13	5'CAGCACCCAC3'
3	OPA-18	5'AGGTGACCGT3'
4	OPA-07	5'GGTGACGCAG3'
5	OPA-01	5'TTCGAGCCAG3'
6	OPA-02	5'GTGAGGCGTC3'
7	OPA-03	5'GGGGGTCTTT3'
8	OPA-04	5'CCGCATCTAC3'
9	OPA-05	5'GATGACCGCC3'
10	OPA-06	5'GAACGGACTC3'
11	OPA-07	5'GTCCCGACGA3'
12	OPA-08	5'TGGACCGGTG3'
13	OPA-09	5'CTCACCGTCC3'
14	OPA-10	5'TGTCTGGGTG3'
15	OPA-05	5'TGTTCCACGG3'
16	OPA-07	5'CAGCGACAAG3'
17	OPA-16	5'TCTCCGCCCT3'
18	OPA-18	5'AATGCGGGAG3
19	OPA-11	5'GTCCACTGTG3'
20	OPA-02	5'CACAGCTGCC3'

Genetic Variation analysis – Dendrogram

The amplified DNA was photographed and the image was transferred to total lab software. The genetic variation was analyzed according to the presence and absence of DNA bands after amplification. This variation was analyzed by Hierarchical Cluster Analysis according to disc coefficient method.

Results and discussions

Heavy metal analysis

The results of physicochemical parameters of mine soil showed that the acidic (5.41) and alkaline (8.19) pH in bauxite and magnesite soil respectively. The low pH of the tailings may be due to the weathering of the sulphide minerals, and the alkaline values may be due to the reaction with carbonates. The parent material of each site has an important influence on the pH of the soil-mine waste materials. Reported as the high pH can result in significant loss of N by volatilization, since NH tends to convert to NH_3 gas, it diffuses from alkaline soil to the atmosphere [14].

Stated that the physical characteristics of contaminated soil are also important for the selection of remediating plants. There was no significant variation in the values of the Electric conductivity (EC 0.1 µs/cm) and hardness (absence) on both mine and control soil, but the high values were reported [15] on metal contaminated soil. The concentrations of nitrogen (32.26 and 46 kg/hc) and potassium (19.33 and 27 kg/hc) in magnesite and bauxite soil too low than permissible limits except phosphorus (11 and 14.66 kg/hc). Both mine soil texture was sandy- clay- loamy (SCL) in nature. Almost the fine physicochemical parameters were observed in the control soil (Table 2).

The highest concentrations (average) of Cd (1811.66 and 911.53 mg/kg^{-1}) and Pb (428.33 and 802.66 mg/kg^{-1}) were observed, in waste dump of magnesite and bauxite mine it followed by Zn (1097.33, and 728.8 mg/kg^{-1}) and Cr (55.22 and 377.5 mg/kg^{-1}), tiny amount of Hg (18.33 mg/kg^{-1}) was detected in bauxite soil, these were cross permissible limits. The mine surrounded agricultural soils (control) also posses significantly some amount of Cd (37.98 mg/kg^{-1}) and Pb (416.79 mg/kg^{-1}). Apart from Cd, Pb, Cr and Hg on both mine sites, further it also had typical quantity of Cu (59 & 80.96), Fe (2207.33 & 2207.33) Ca (4548 & 64.99), Zn (1097.33 &728. 8), Mg (5338.66 & 2305.33) and Mn (3139.66 & 7111) in waste dumps of both mines (Table 2). Most of the metals exist within the permissible limits to the Environmental Quality Standards, except Cd and Pb. These results were supported by Ang, & [16], they reported as the ex-mines contain four potentially toxic elements (PTEs) of heavy metals (Pb, Cd, As and Hg).

Table 2

Physicochemical and metal characteristics of waste dumps of magnesite mine

S.No	Physicochemical/Metals	Magnesite soil	Bauxite soil	Control soil	Permissible limit
		Average of triplicates of three sites			
1	pH	8.19	5.41	7.10	6-8
2	Temperature	30^{o}C	25^{o}C	30^{o}C	-
3	EC (dsm^{-1})	0.1	0.1	1	0.1-1
4	$Cacl_2$	-	-	Nil	-
5	Texture	-	-	RLL	-
6	N (Kg/acre)	*32.66	*46	*75	114-180
7	P (Kg/acre)	**11	**14.66	8	4.6-9
8	K (Kg/acre)	*19.33	*27	51	49-113
9	Ca (mg/kg^{-1})	4548	64.99	2089.23	52000
10	Mg	5338.66	2305.33	4274.12	9000
11	Cd	1811.66	911.53	37.98	2-6
12	Cu	59	80.96	95.42	100
13	Fe	2207.33	2207.33	1802.3	129000
14	Zn	1097.33	728.8	659.31	300-600
15	Cr	55.22	377.5	102.48	1000
16	Mn	3139.66	7111	4614.69	1000
17	Pb	428.33	802.86	416.79	200
18	Hg	-	18.33	-	NA

SCL: Sand-Clay-Loamy soil, **RLL**: Red, Loamy and Lateritic. *****lower than permissible limits, ******higher than the permissible limits. **NA**: Not Available. The values are average of the mean of triplicates. The permissible limit for serial number 1-8 adopted from Tamil Nadu soil testing laboratory and 9-17 (in ppm)

Bio molecules analysis

The chlorophylls (*a* and *b*) content of plants from magnesite and bauxite soils were nearly similar to control soil (5.32 & 8.76 mg/g^{-1} and 4.52 & 8.27 mg/g^{-1} in *G. hirsutum* and *J. curcas*from magnesite and bauxite soil respectively) (Table 3). The plants (*J. curcas* and *G. hirsutum*) from magnesite soil were posses similar/higher amount of carbohydrate (175 & 98 mg/g^{-1}) and protein (396 & 362 mg/g^{-1}) compared to plants from control soil. But the plants (*J. curcas* and *G. hirsutum*) from bauxite soil were posses poor quantity of carbohydrate (97 & 24 mg/g^{-1}) and protein (352 & 84 mg/g^{-1}) than control plants. These variations may be due to the metal stress, pH and moisture holding nature of mine soil it enhances the plants metabolic mechanisms. These overall results are correlated with the result of [16] [17], they reported that the higher concentration of heavy metals containing environment (Cd) decrease the nutritive value of plants (bean).

Table 3

Bio molecules of plants from waste dumps of mines and control

Soil	Chlorophyll (*a* and *b*) in mg/g^{-1}		Carbohydrate in mg/g^{-1}		Protein in mg/g^{-1}	
	G.hirsutum	*J. curcas*	*G.hirsutum*	*J. curcas*	*G.hirsutum*	*J. curcas*
Magnesite	5.32	8.76	98	175	362	396
Bauxite	4.52	8.27	24	97	84	352
Control	3.44	8.81	92	182	296	378

Phytoremediation potentiality of the plants

The metal absorbance potentialities of these plants were listed as roots and shoot wise in table 4. The *J.curcas* and *G.hirsutum* effectively absorb the metals (Cd, Cr, Pb, Mn and Hg) from the metal pools of waste dumps of mines (magnesite and bauxite). The metal stress and physicochemical parameters (pH, EC, texture, NPK, etc.,) of the mine sites were highly enhanced the plants to take the metals. Compared to the other metals less amount of Hg was accumulated only in the shoot region of *J.curcas* (18 mg/kg^{-1}) and *G.hirsutu* (47 mg/kg^{-1}), this indicates that these plants posses the translocation efficiency (transfer of metals from root to shoot). This study was partially Stated that the high metal tolerant index of the plant (*J. curcas*) was observed from the metal contaminated soils. Fotiadis, et al. fromDepartment of Agriculture, University of Thessaly, Greece, studied the metal tolerant and the metalsorption efficiency of *G. hirsutum*, and they reported as *G. hirsutum* hasefficiency for metal tolerance and metalsorption for Cd (80 mg/kg). [18] also described fine in situ heavy metal remediation efficiency poses plants and [19] made experiment by using plants on natural metal contaminated soil and with synthetic metal contaminated soil and he concluded that plants are partially suitable for metal remediation process.

Table 4
Metal absorbance potentiality of plants on mines (mg/kg^{-1})

Name of the metals	*G. hirsutum*				*J. curcas*				Control			
	Magnesite		Bauxite		Magnesite		Bauxite		*G. hirsutum*		*J. curcas*	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Cd	28	36	52	28	92	41	33	138	7	20	19	10
Cr	342	82	314	51	446	129	376	163	110	37	89	45
Pb	207	325	467	289	1041	405	282	612	71	104	370	87
Mn	426	297	354	123	2771	774	994	907	129	75	1908	195
Hg	0	0	0	47	0	5	0	18	-	-	-	-

Note: Mentioned values are mean of triplicates

DNA isolation and metal impact analysis - RAPD

The high quality, low-polysaccharide genomic DNA from *J. curcas*and *G. hirsutum* taken from magnesite, bauxite and control (phytoremediation) was observed in agarose gel electrophoresis and the bands were observed in the range of 1800 to 2200 bp (Fig.1). [19] described as the presence of oxidizing agents (polyphenols), found in many plant species, can reduce the yield and purity by binding covalently to the extracted DNA making it useless for most research applications.

RAPD profiles detect alterations in genomic DNA with the use of arbitrarily primed PCR reactions and clearly showed promise in the detection of pollutant-induced DNA effects in the genome of *J.curcas* and *G.hirsutum.* The RAPD profiles showed substantial differences between both plants from the magnesite and bauxite plants, with changes in the number, size and intensity of the bands. The changes in DNA caused by genotoxic chemicals may be monitored using different biomarker assays both at the biochemical and molecular level [20]. The approximate size range of the RAPD products of the both plants from magnesite and bauxite was 400–3800 base pairs.

A total of 38 amplified bands were observed, out of 38 bands, 32 bands showed the changes as compared to their controls. These results were supported by the result of [21]. The red lines in figure 2 & 3 indicate the absence and/or presence of new bands in the test plant by compared with control plant. The main observation of metal impact on plant's genome was the difference in the intensities of bands, the appearance of some new bands and absence of bands. The clear genome level metal impacts were observed in the dendrogram analysis (Fig. 4), these results indicated that the *G. hirsutum* shows genome variable at 9 to 12 nucleotide sequence range and *J. curcas* shows variation at 5 to 10 nucleotide range. The overall results show that there were high genome level impacts were observed in both plants from mine soil when it's compared with plants from the garden soil.

This result indicates these genome impacts may be raised by Cd, as high quantity of Cd was found in the both mine soil than Pb. Besides high amount of Pb and low amount of Cd also present in control soil, but there was no genotoxicity observed in control plants. This indicates that the high concentration of Cd in mine soil is might be induced genomtoxicity in these plants.

In support of this point described that the plant species (*Allium/Vicia*) respond differently to exposure of the same metal depending on genetic structures.Further the bio molecule results indicated that these plants are flexible in nature to survive in the normal sites to metal polluted sites.

However the genotoxic effect noticed on plant by Cd, the biomolecules and yield of the plants were amendable, since the concentration of residual metals (Mn, Cu, Fe) in mine soil was amendable. This point was interrelated with micronutrients such as Iron (Fe), Manganese (Mn) and Copper (Cu) are essential metals for plant growthand yield.

The changes observed in the DNA profiles such as modifications in band intensity and loss of bands may be due to the changes in oligonucleotide priming sites mainly due to genomic rearrangements, and less likely to point mutations or DNA damage in the primer binding sites or the presence of DNA photoproducts which can block or reduce the polymerization of DNA in the PCR reaction [22].In many studies, Cd has been reported as a genotoxic metal plant [23] [24].

In several studies performed by many researchers, has determined as the Cd enhanced the effects of mutation [25] on plants. In addition of this, recently has concluded that the Cd induced genotoxicity in Maize by using RAPD technique. Furthermore, similar results with respect to metal toxicity changes in genetic pattern were also reported in *Arabidopsis thaliana* when exposed to Pb, Mn, Cd etc. The changes in DNA profiles under heavy metal stress were also assessed in *Daphnia magna*, *Hordeumvulgare*and *Phaseolus vulgaris* [26] [27] . Lead, copper and cadmium affect the DNA of *Sileneparadoxa* and barley plants showed the similar type of response as it was observed in the present study [28].

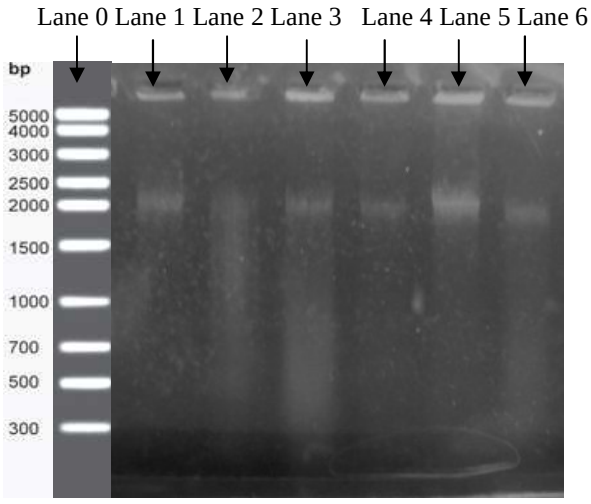


Figure 1: Lanes 0-6 represents the agarose gel (0.8%) of DNA isolated from 2 plants from magnesite, bauxite and garden viz. Lane 0: lamda molecular marker DNA; Lane 1: *G. hirsutum* from garden soil; Lane 2: *J. curcas* from garden soil; Lane 3: *J. curcas* from magnesite soil; Lane 4: *J. curcas* from bauxite soil; Lane 5: *G. hirsutum* from magnesite soil; Lane 6: *G. hirsutum* from bauxite soil.

Fig. 1: Plants genomic DNA on agarose gel

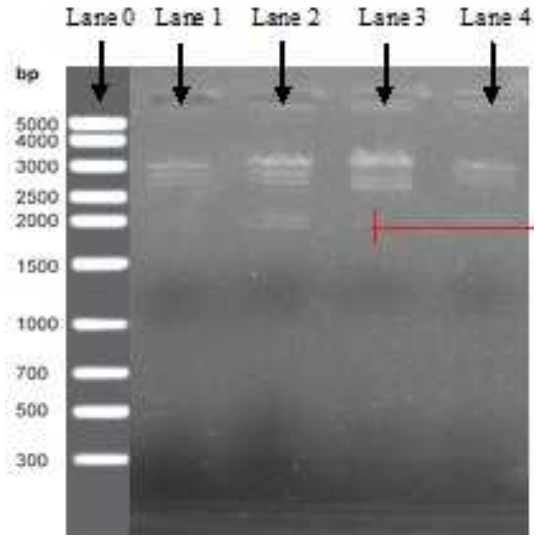


Fig. 2: PCR- amplified plants genome

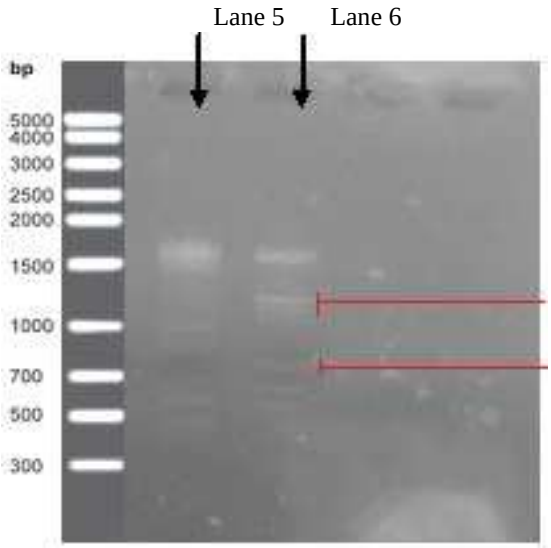
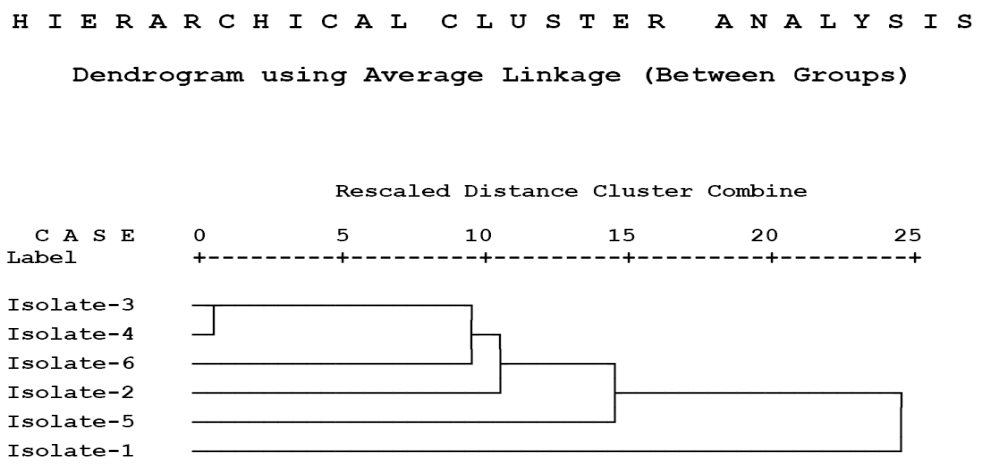


Fig. 3: PCR- amplified plants genome

Figure 2 & 3 Representative gel fraction of RAPD-PCR amplification products of plant genomic DNA (16 µl). Amplification products were fractionated in an 8% agarose gel. Lane 0 represents a molecular marker (Lambda DNA *Hind*III/*EcoRI* double digest) Lanes 1-6 represents the RAPD- PCR products amplified from genomic DNA of 2 plants from 3 sites. Red lines indicate absence and presence of new bands.



Isolate 1: *G. hirsutum* from garden soil;Isolate 2: *J. curcas* from garden soil; Isolate 3: *J. curcas* from magnesite soil; Isolate 4: *J. curcas* from bauxite soil; Isolate 5: *G. hirsutum* from magnesite soil; Isolate 6: *G. hirsutum* from bauxite soil. The distance between the cluster combine is 5 nucleotide sequences.

Figure 4(what is this figure stand for please mention it)

Conclusion

The presented results showed that the plants *J.curcas* and *G.hirsutum* applied to phytoremediation process on metal containing waste dumps of magnesite and bauxite mines, shows good phytoremediation efficiency. The DNA polymorphisms (RAPD analysis) were observed on those plants it may be the toxicity of Cd. Although these plants effectively grown on the mine soil, in support of remaining micro elements (Fe, Cu, Mn, etc.) and physicochemical properties of soil, It can be applied as a suitable biomarker assay for the detection of genotoxic effects of heavy metal contamination on plants.

Molecular characterization of these markers would be able to indicate that such primers could amplify heavy metal induced changes in plant genome and thus have wide applicability in toxicological study. Finally, further research is required to better understand the potential and limitations of the RAPD assay for the detection of DNA damage and mutations at a specific sequence induced by the heavy metal stress.

References

[1]Carrillo Gonzalez R, Gonzalez-Chavez M.C.A. Metal accumulation in wild plants surrounding mining wastes. doi:10.1016/j.envpol.2006.01.006.2005

[2] Wang H, Kimberley MO, Schlegelmilch M. Biosolids derived nitrogen mineralization and transformation in forest soils. J Environ Qual, Vol. 32, pp. 1851–1856. 2001.

[3]Sopper W.E. Municipal Sludge Use in Land Reclamation. Lewis Publishers, CRC Press, INC., Berlin. 1993.

[4]Lan C.Y, Shu W.S, Wong M.H. Revegetation of lead/zink mine tailing at shaoguan, Guangdon Province, China: phytotoxicity of the tailing. In: Wise, D.L. (Ed.), Global Environmental Biotechnology. Elsevier Science, pp. 119–130. 1997.

[5]Khan. A.G, Kuek .C, Chaudhry. T.M, Khoo. C.S, Hayes W.J. Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere ,Vol.41, pp.197-207. 2000.

[6]Ciura J, Poniedziałek M, Sękara A, Jędrszczyk, E. The Possibility of Using Plants as Metal Phytoremediants. Polish J Envir Stu.,Vol. 14,pp.17-22. 2005.

[7]Jeffrey, S.W, Mantoura, R.F.C. Development of pigment methods for oceanography: SCOR-supported Working Groups and objectives. In Phytoplankton pigments in oceanography: guidelines to modern methods, pp.19–36.1997.

[8]Black B.C, Evans D.D, White J.I, Ensminger L.E, Clark F.E. Methods of Soil Analysis, Amer. Soc. Agron. Inc. Madison, Wisconsin USA. 1982.

[9]Michalak, A. Phenolic Compounds and Their Antioxidant Activity in Plants Growing under Heavy Metal Stress. Polish J of Environ Stud. Vol.15, No.4:pp. 523-530. 2006.

[10] Mathiyazhagan N, Natarajan D. Bioremediation on effluents from Magnesite and Bauxite mines using Thiobacillus Spp and Pseudomonas Spp. J Bioremed Biodegrad ,vol. 2 ,pp.115. doi:10.4172/2155-6199.1000115. 2011.

[11] Doong R.L, MacDonald, G.E. Shilling ,D.G. Effect of fluridone on chlorophyll,carotenoid and anthocyanin content of Hydrilla. J of Aqua Plant Managvol.Vol.31,pp. 55–59. 1993.

[12] Conte, C. Mutti, I. Puglisi, P. Ferrarini, A. Regina, G. Maestri, E. Marmiroli, N.DNA fingerprinting analysis by a PCR based method for monitoring the genotoxic effects of heavy metals pollution. Chemosphere, Vol. 37,pp. 2739–2749. 1998

[13] Jones J.R. Wolf, B. Mills, H.A. Plant Analysis Handbook. Micro-Macro Publishing House, Moscow, Russia. 1991.

[14] McGrath, S.P, Cunliffe, C.H. A simplified method for the extraction of the metals Fe, Zn, Cu, Ni, Cd, Pb, Ni, Cr, Co, and Mn from soils and sewage sludge. J.Sci. Food and Agric. Vol.36, pp.794- 798. 1985.

[15] Patil, M D. Biradar, D.P. Patil, V.C, Janagoudar, B.S, Nadaf, H. L. Analysis of Genetic Diversity of Cotton Genotypes using RAPD PCR Technique. Karnataka J.Agric. Sci., Vol.20, No. 2 :pp. 215-217. 2007.

[16] Mengel, K. Kirkby, E.A. Principles of Plant Nutrition. International Potash Institute: Bern.1982.

[17] Ang, L.H. Ang, T.B. Site toxicity of tin tailings and its implications on land use in Peninsular Malaysia. In P 116-123, Proceedings of MSTC.pp.23-25. 1998.

[18] Azmat, R. Akhter, Y. Talat, R. Uddin, F. The inhibition of bean plant metabolism by Cd metal and atrazine: I the effect of atrazine with Cd Metal on growth, photosynthesis, nutritional level and rhizophere of soil. Biotechno., Vol. 4, pp. 238-242. 2005.

[19] Kumar, G.P. Yadav, S.K. Thawale, P.R. Singh, S.K. Juwarkar, A.A . Growth of Jatrophacurcas on heavy metal contaminated soil amended with industrial wastes and Azotobacter – A greenhouse study. BioresTechno. Vol. 99, pp.2078–2082. 2008.

[20] Baker, A.J.M. McGrath S.P. Sidoli, C.M.D. Reeves, R.D . The possibility of in situ heavy metal decontamination of polluted soils using plants of metal-accumulating plantsResourConservRecyc, Vol.11, pp. 41- 49. 1994.

[21] Lombi, E. Zhao, F.J.Dunham S.J. McGrath, S.P. Phytoremediation of heavy metal contaminated soils: Natural hyperaccumulation versus chemically enhancedphytoextraction J Environ Qual, Vol.30, pp. 1919-1926. 2001.

[22] Porebski, S. Baily, L.G.Baum, B.R. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant MolBiolRep. ,Vol. 15, pp. 8-15. 1997.

[23] Savva, D. Use of DNA fingerprinting to detect genotoxic effects. Ecotoxi Environ Safe , Vol. 41, pp.103– 106. 1998.

[24] Nelson, J.R, Lawerence, C.W. Hinkle, D.C. Thyminethymine dimmer bypass by yeast DNA-polymerase-zeta. Science, Vol. 272,pp. 1646–1649. 1996.

[25] Fatur, T. Lah, T.T, Filipic, M . Cadmium inhibits repair of UV-, methylmethanesulfonate- and N-methyl-N-nitrosourea-induced DNA damage in Chinese hamster ovary cells. Mutat Res., Vol. 529, pp. 109–116. 2003.

[26] Liu, W.Li, P.J. Qi, X.M, Zhou, Q.X. Zheng, L. Sun, T.H, Yang, Y.S .DNA changes in barley (Hordeumvulgare) seedlings induced by cadmium pollution using RAPD analysis. Chemosphere,vol. 61, pp.158–167. 2005.

[27] Enan, M.R. Application of random amplified polymorphic DNA to detect genotoxic effect of heavy metals. Biotechno App Bioche, Vol.43, pp. 147–154. 2006.

[28] Fojtova, M. Kovarik, A. Genotoxic effect of cadmium is associated with apoptotic changes in tobacco cells. Plant Cell Environ, Vol.23, pp..531–537. 2000.