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Heavy Metal induced Genotoxicity Detection by RAPD in Alligator Weed

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ABSTRACT

Indiscriminate use of heavy metals for rapid urbanization and industrial exploration is a pressing threat to ecosystem and human health. Among this Cd and Cr contamination are most dangerous as these metals directly enter into the food chain due to their higher solubility and mobility. Identification of a metal tolerant native plant species would be helpful to decontaminate Cd and Cr polluted land. Alligator weed [Alternanthera philoxeroides (Mart) . Griseb], is found to be the most widely distributed perennial stoloniferous herb in Cd, Cr contaminated areas in and around Kolkata.
To establish metal accumulation capacity of this species, the shoot and root metal content of the plant samples collected from different Cd, Cr contaminated areas were estimated. Plants were also grown under laboratory condition with external application of various concentrations of Cd and Cr individually and synergistically (0.5, 0.8, 1.0, 1.2, 1.5, 1.8 mM) to assess maximum metal tolerance potential. Laboratory grown plants treated with Cd and Cr, showed higher amount of metal accumulation in comparison to in situ plants. Hyperacuumulation property of this plant was recorded in 1mM metal concentration. The genotoxicity of these metals on alligator weed was established by RAPD method. Out of ten primers, four primers showed polymorphic bands due to DNA damage or modified bases in primer binding sites. Distinct variation in DNA banding profile between control and treated plants revealed the deleterious effect of Cd, Cr on this plant which was also supported by decreasing GTS% with increasing metal concentration.
Keywords: genotoxicity, cadmium, chromium, GTS, RAPD

1. INTRODUCTION

Cadmium and chromium pollution of soil and water is an alarming problem in urban and semi urban areas of India. Indiscriminate use of these metals in industries and agricultural fields contributes to the entry of these deadly metals into the foodchain. Being biologically non essential and having higher mobility, these two metals pose as extremely phytotoxic beyond the threshold value. According to Agency of Toxic Substances and Disease Registry (2007) [1] Cd and Cr occupy 7 th and 77 th position respectively in the list of most hazardous substances of the world. Mainly these contaminations are anthropogenic in nature. As these two metals have vast industrial uses (leather tanning, electroplating, mineral fertilizers, Ni- Cd battery production, paints used in glass and ceramics and soft drink), almost 65 % of industrial workers as well as normal population living in polluted areas are regularly exposed to the hazards of these two toxic metals [27]. Cd contamination of agricultural land occurs mainly through the irrigation water containing Cd, generated from nearby mines [25]. These sources may cause enhanced soil and crop Cd level, promoting its entry into the food chain [33, 36]. Therefore pollution of Cd is very significant from nutritional and environmental point of view [16]. Cr normally exists in two oxidation states in soils; trivalent Cr (III) and hexavalent (VI). Being more mobile, hexavalent Cr is extremely toxic to plants and animals in high doses and is responsible for inducing cancer and teratism, resulting liver and kidney damage. Cd with a long biological half life causes cancer of lung and prostate, kidney tubule damage, osteomalacia and fragility of bones [9]. Cd is capable of entering the food chain through uptake into plant tissues. Presence of excessive amount of Cd, Cr in soil and water causes a range of plant responses including leaf chlorosis, disturbance in nitrogen metabolism, photosynthesis and respiration [12], stunted growth and even death. The impact of Cr toxicity in plant depends on the metal speciation, that determines its mobility, subsequent uptake and resultant toxicity in plant [28, 29]. Plants reveal Cr toxicity in multiple levels, from reduced yield, through effects on leaf and root growth, to inhibition of enzyme activities and lastly mutagenesis. Cr is toxic to higher plants at 100 µM/ kg dry weight [11] and detrimental after crossing this level. In comparison to crop plants, indigenous weed plants usually display some inherent properties to hypertolerate, strong endurance and higher capacity to absorb heavy metals. Both Cd and Cr are also capable of inducing genotoxicity by binding with the functional sites of DNA.

Genotoxicity describes a deleterious action on cell's genetic material affecting its integrity. Cd and Cr induces DNA damage such as single stranded, double stranded breaks, modified bases, abasic sites, DNA protein crosslinks, oxidized bases and bulky adducts [3, 8, 21, 22, 10]. As genotoxicity of Cd is directly related to its effect on structure and function of DNA, it can be assayed or determined by a number of laboratory methods. In recent years several plant species have been used as bioindicators of genotoxicity [20] and various RAPD based (RFLP, RAPD, AFLP) tests have been developed to evaluate the toxicity of environmental contaminants [26]. Now RAPD has been successfully and extensively used as a sensitive and reliable method to detect Cd induced DNA damage such as mutational phenomenon, rearrangements, point mutation, small inserts or deletions of DNA and ploidy changes in cells of plant and animals [31, 4, 30]. Some researches on DNA alterations in plants induced by soil contamination stress have been reported worldwide [32, 13, 6]. This technique has been successfully used to detect DNA damage and other genotoxicity induced by various heavy metals [19, 5, 2].

Different Cd and Cr co-contaminated areas in and around Kolkata, India; with high population density; were surveyed and screened thoroughly for wildly grown native plants capable of accumulating these metals. Alligator weed (*Alternanthera philoxeroides*), a member of Amaranthaceae was found to be the most abundant with considerable amount of Cd, Cr in the above ground parts.

In the present work the metal accumulation capacity of Alligator weed was studied in *in situ* and *ex situ* condition under Cd and Cr stress and the genotoxic effect of these metals on this plant was studied by RAPD techniques. Alligator weed was chosen for two reasons. Firstly the plant was found to grow widely in various habitats; secondly, the plant was found to be most abundant in Cd and Cr contaminated areas where no other plants were to grow.

2. MATERIAL AND METHODS

2.1 Specimen

Alternanthera philoxeroides (Mart.) Griseb (Family- Amaranthaceae), common name: Alligator weed is a non woody perennial, stoloniferous herb, widely growing in rivers, lakes, ponds and irrigation canals, as well as many terrestrial habits. This fast growing weed has been found to grow profusely in soil of town and suburbans contaminated with heavy metals and in certain areas no vegetation other than this weed were found. Reproduction is predominantly through vegetative means. Individuals rarely produce seeds, but seeds are typically non – viable. Although the aquatic form of this species has been reported to withstand up to 30% salinity in flowing brackish water but studies related to tolerance to different heavy metals have not been undertaken.

2.2 Field Work

Seven experimental sites situated in periurban and urban areas of Kolkata metropolis were selected randomly. All these sites are localised in the close vicinity of industries that emit Cd and Cr in the environment contaminating soil and ground water. Such as factories of soft drink, leather tanning and Ni- Cd battery production unit etc. Some studied areas are co contaminated with both of these two metals. Soil of certain semi rural areas which are about 30 km away from the main city are heavily Cd-contaminated where dry cell batteries are recycled and scrap material is dumped in the open areas adjacent to the agricultural field. A brief description is given in Table 1.

Alligator weed is the most abundant herb in all those selected areas. Several individual plants were randomly collected from the sampling area were then mixed to give a composite whole plant sample. Plant samples were then divided into root and shoots, washed repeatedly with distilled water to remove adhering soil and dust. These were oven dried at 60º C for overnight and ground into fine powder for the determination of metal concentration. Soil samples were collected (3 replicas) from the sites at a depth of 15 cm. They were air dried for 7 days and was ground to pass through 1 mm nylon mesh. Plants collected from an uncontaminated site is considered as control plant and maintained in the botanical garden of the department.

2.3 Metal assay

Metal analysis of the plant and soil samples were carried out by acid digestion (3:1 conc. HNO_3 and conc. HCl v/v) of accurately 1 g of plant tissue and soil sample respectively. Metal concentration was measured by utilizing Flame atomic Absorption Spectrophotometer (Perkin Elmer) according to the APHA 1989.

2.4 Pot Culture Experiments
Seedlings of *A. philoxeroides* were collected from Cd and Cr contaminated areas during rainy seasons and potted in departmental garden. These seedlings were allowed to grow for one month then they were treated with Cd (cadmium chloride) and Cr (potassium dichromate) with individual concentrations of 0.5 mM, 0.8 mM, 1mM, 1.2 mM, 1.5 mM, 1.8 mM. At 2 mM conc of metals plant showed 90% chlorosis and permanent wilting. Physiological and biochemical parameters were measured after 10 days treatment. Plants without Cd and Cr stress are considered as control.

2.5 DNA extraction and RAPD analysis
Approximately 1 g leaf of control and treated samples as well as samples collected from different sites were collected and ground in liquid nitrogen. Total genomic DNA was extracted using modified CTAB method (Doyale 1991). The PCR amplification was carried out with Operon decamer random primers (OPA 1-10) and genomic DNA as the template. Out of ten primers two primers produce no bands (OPA 2, OPA 4). PCRs were performed in a reaction mixture of 25 µl containing 45-50 ng of genomic DNA dissolved in sterile distilled water, 10X PCR buffer (5 µl) containing 1.5 mM $MgCl_2$, 0.5 mM dNTPs , 2 µl of 10 µM primer and 1 U *Taq* DNA polymerase. The RAPD protocol consisted of an initial denaturing step of 3 min at 94ºC, followed by 35 cycles at 94ºC for 45 sec (denaturation), 37ºC for 1 min (annealing), and 72ºC for 1 min (extension) with an additional extension period of 7 min at 72ºC. All amplifications were carried out twice. The PCR amplification products were resolved electrophoretically in a 1.8% agarose gel using TAE buffer and Gene Ruler 1000 bp DNA ladder (Fermentas, Germany). All the PCR examinations were carried out by Applied Biosystems 2720 thermal cycler.

2.6 Estimation of Genomic template stability
GTS values are calculated according to the result of RAPD analysis. GTS implies a qualitative measure showing the obvious change to the number of RAPD profiles generated by the treated *A. philoxeroides*, in relation to profiles obtained from the control. GTS was calculated as

GTS=(1- a/n) × 100%, where a= RAPD polymorphic profile (total appeared and disappeared bands)

n = total number of bands in control.

2.7 Statistical analysis
The experiment was performed in completely randomized block design. All the experiments were carried out in triplicates. All datasets obtained from the experiments were subjected to one way analysis of variance (ANOVA) followed by Tukey's Multiple Range Test (TMRT) for multi comparisons of means. Significance level were compared at $p<0.05$. All results were expressed as means, with corresponding standard deviation.

3. RESULT AND DISCUSSION

Being located in the southern part of Bengal delta, without any Cd and Cr mine nearby, the chance of soil contamination of these experimental sites by mining is completely eliminated, and the possible sources of contamination is anthropogenic. The field study revealed that *A. Philoxeroides* grew well at all the selected contaminated sites where Cr, Cd concentration in the soil was much higher than 300 mg/kg and 3 mg/kg respectively, greatly exceeding the maximum allowable limits [15]. The present study recorded 1833.33 mg/kg of Cr in the soil from site II (Table 3) [23]. Till date the maximum amount of Cr level recorded from the soil in some industrial cities of India is 1220 mg/kg. Inspite of this Alligator weed grown in this region showed no sign of Cr toxicity.

Metal concentration of the plant and soil samples of the visited sites showed variable range of Cd and Cr content (table 3). Concentration of Cr ranges from 0.003 mg/kg soil (uncontaminated site) to a maximum of 1833.3 mg/kg soil (site II). Cr concentration is above the allowable limit (60-400 mg/kg soil) [15] in site I and II, while the other sites (III, IV, V, VI, VII) have lower Cr content. For Cd, the highest concentration was reported from site IV (44.82 mg/kg soil). Soil of uncontaminated site (site C) and site III and VI have Cd below detection level, whereas sites I, II, IV, V and VII have Cd concentration more than the allowable limit (0.5-3 mg/kg). Cr concentration of plants from different sites varied from 32 mg/kg to 343.03 mg/kg but no plant population accumulated Cr above 1000 mg/kg, the criterion assigned for a Cr hyperaccumulator plant species [7]. The Cr concentration in the roots was greater than those in the shoot except the plants of site I where the shoot Cr concentration was slightly higher than those of the roots. Cr is toxic to higher plant at 5.2

mg/kg dry weight [11]. Present investigation revealed that Cr concentration in the above ground part of the plant of site II reached 164.37 mg/kg without any visible Cr toxic symptoms. This record establishes the tolerance of *A. philoxeroides* to Cd and Cr. The Cd content in plants increase with the increase in metal concentration in soil and Cd accumulation in roots was higher than that in shoot indicating low mobility of Cd from the roots to shoots in this specific plant.

Heavy metal accumulation capacity of a higher plant is judged by its BCF (Bioconcentration factor) i.e. the ratio of metal concentration in the roots to that in the soil [37] and its ability to translocate metals from underground parts to the shoot is measured by TF (Translocation factor) i.e. ratio of metal concentration in the shoots to the roots. Plants collected from site I (area contaminated with tannery effluents) had TF values > 1 for Cr and < 1 for Cd. All other plant populations had low TF and BCF values with regard to the absorption of Cr and Cd indicating its limited capacity for accumulation and translocation of Cr and Cd.

As metal bioavailability and bioaccumulation is governed by several environmental factors, such as chemical speciation of the metal, pH, organic chelators, humic substances, presence of other metals and anions, ionic strength, and other prevailing electrochemical functions [17], metal uptake and bioaccumulation pattern may vary between *in situ* and *ex situ* study of same plant sample and same metals (Cd, Cr) concerned. Plants treated with 1mM and 1.2 mM Cr behaved as metal accumulator is evident by TF value (TF>1). Further increase in soil Cr concentration (1.5 mM, 1.8 mM), reduced metal uptake and accumulation capacity suggested that 1mM and 1.2 mM soil Cr concentration was the optimum tolerance level. Other than 1 mM and 1.2mM Cr treated plant, all other Cr treated plants showed better Cr accumulation in roots. The reason for greater accumulation of Cr in roots is due to accumulation of Cr in vacuoles of root cell, thus rendering it less toxic, which may be natural toxicity response of plant.

At 0.8 mM and 1 mM Cd treatment this plant satisfied both the criteria of hyperaccumulator for Cd, regarding metal accumulation (>100 mg/kg) and TF>1. But in all other concentrations root Cd content (Table 4) was higher than shoot making it suitable for phytoextraction rather than labeling this plant as Cd hyperaccumulator.

Linear correlation between soil metal content of field condition and plant metal content (Fig 1, 2) could not be drawn. Inspite of higher soil Cr content in experimental sites, plant took up little amount of Cr; while in laboratory condition Cr uptake was found to be more. Without any visible toxicity symptoms plants showed luxuriant growth in highly contaminated sites (site II for Cr, site IV for Cd). But only 2 mM Cd, Cr treatment became limiting for its *ex situ* growth. This might be due to the synergistic or antagonistic effect of some other metals that we have not been checked, which could ameliorate the toxic effect under natural habitat. When plant was treated in laboratory condition, better accumulation of both Cd and Cr were observed. As it is impossible to create the exact field condition in the laboratory, there was a disparity of the metal accumulation property of *A. philoxeroides* between *in situ* and *ex situ* condition. But the potential Cd, Cr tolerance of this weed is unquestionable.

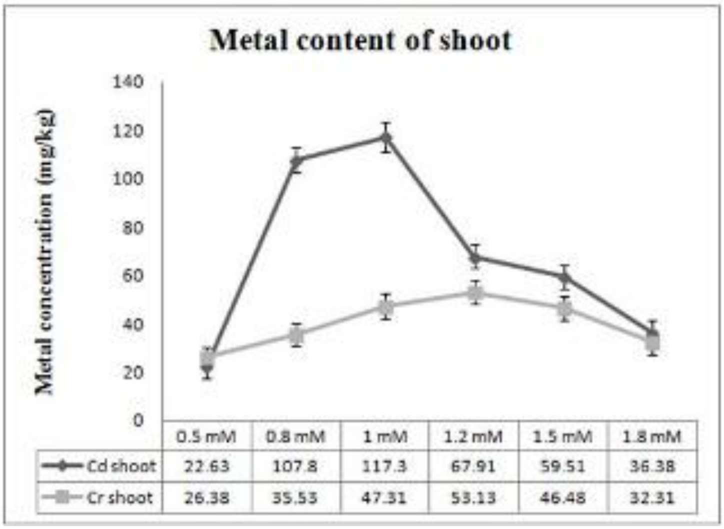

Fig 1: Accumulation of Cd and Cr content in the shoots of treated *A. philoxeroides*

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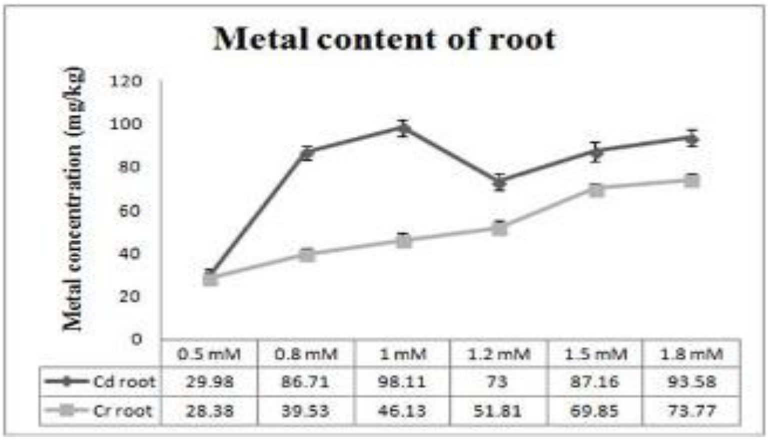

Fig 2: Accumulation of Cd, and Cr content in the roots of treated *A. philoxeroides*

RAPD profile of samples collected from the experimental sites showed no polymorphisms (Fig 3a-3g, except Fig 3h) indicating that the plants having no genetic variations. This observation is in conformation with previous reports [35] where it was revealed that variation in RAPD profile of *A. philoxeroides* due to ecotype variation is negligible. But when the plants were treated with Cd and Cr exogenously, polymorphism in RAPD banding pattern was observed, indicative of the genotoxicity of these metals. Most heavy metals damage cells by producing free radicals, which interact with cellular macromolecules (proteins, lipids and nucleic acid) and cause damages to them. Inpsite of being a Cd, Cr tolerant species, *A. philoxeroides* showed change in DNA banding pattern due to genotoxic effect of Cd and Cr. The use of RAPD analysis as a novel tool to detect DNA damage was well documented earlier. Out of ten primers we used, 2 could not amplify the genomic DNA, remaining 8 primers produced visible and reproducible bands. Only 2 primers; OPA 10 (Fig 3h) and OPA1 (Fig 3a) showed polymorphism among plants collected from contaminated sites. Plants of highest Cd polluted areas showed variation in banding pattern against OPA10 primer, evident from the appearance of extra bands compared to control plant (Fig 3h). RAPD carried out in treated plants with OPA7 (Fig 4e), OPA10 (Fig 4h) and OPA5 (Fig 4c) revealed appearance of new bands while OP A 3 (Fig 4b) resulted both appearance and loss of bands in *Alternanthera philoxeroides* exposed to higher concentration (1.5 and 1.8 mM) of Cd/Cr indicating their genotoxic effect on this plant. In all cases the polymorphism was due to the loss or gain of PCR fragments in treated plants compared to control. In this study the DNA damage was shown by RAPD profile via disappearance of previous bands or appearance of new bands. Disappearing bands are likely to be due to changes in oligonucleotide priming sites, originated from rearrangement, point mutation and DNA damage in primer binding sites induced by metal toxicity [13, 22, 14]. Appearance of new band is attributed to the creation of new priming sites. Changes in structure and sequence of DNA due to mutation or large deletion, brings two pre-existing annealing sites closure creating new priming site. Cd can induce single stranded and double stranded breakage, abasic site, modified bases, DNA-protein cross links, oxidized bases in organisms [34, 3, 18, 10]. Our result is in full accordance with the report of various workers [6, 2, 30, 24) regarding the use of RAPD for the assessment of genotoxic potential of Cd. The comparison among treated and control genome showed that RAPD can be used for evaluation of metal toxicity in plants. On the basis of this consideration, it could be suggested that RAPD is a promising technique for qualitative and quantitative analysis of genotoxicity of environmental pollutants. Though few reports are available regarding the RAPD mediated detection of Cr genotoxicity, but it could be suggested from our results that, the same procedure would be feasible for Cr also, just like Cd.

The genomic template stability (GTS%), is a qualitative measure used to detect gentoxicity of different agents and reflects the efficiency of DNA repair and replication. High GTS value indicates that the genome is less prone to alterations in its DNA, whereas low GTS value indicates greater chances of DNA alteration. In presence of efficient damage repair system the organism can show higher GTS value instead of high level of DNA damage, but high frequency of DNA damage can inhibit repair and replication [4]. In the present study, it is observed that, GTS value decreased with increasing concentrations of Cd and Cr (Table 5). The decrease

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was drastic in case of Cd, upto 1 mM concentration, GTS values were 94%, but at 1.5 mM concentration the vale decreased to 42.5%. At 1.8 mM concentration the value dipped to a lowest of 27.3 % indicating greater damage and subsequent loss of repair and replication mechanism of the damaged DNA.

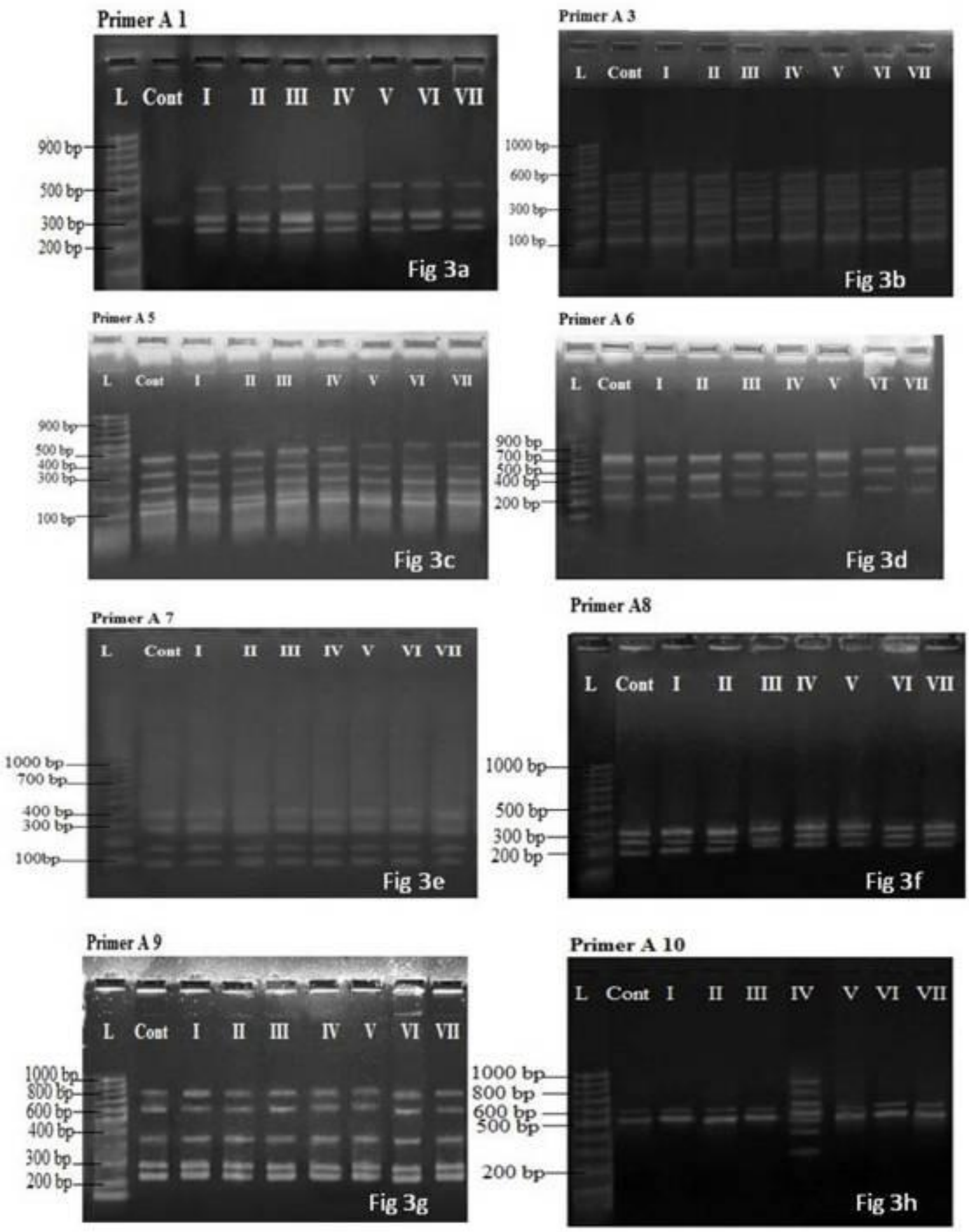

Fig 3: RAPD analysis of *A. philoxeroides* collected from contaminated sites, using primers OPA-01 (a), OPA-03 (b), OPA-05 (c), OPA-06 (d), OPA-07 (e), OPA-08 (f), OPA-09 (g), OPA-10 (h). (L: DNA ladder, I: Site I, II: Site II, III: Site III, IV: Site IV, V: Site V, VI: Site VI, VII: Site VII, VIII: Site VIII, C: Control)

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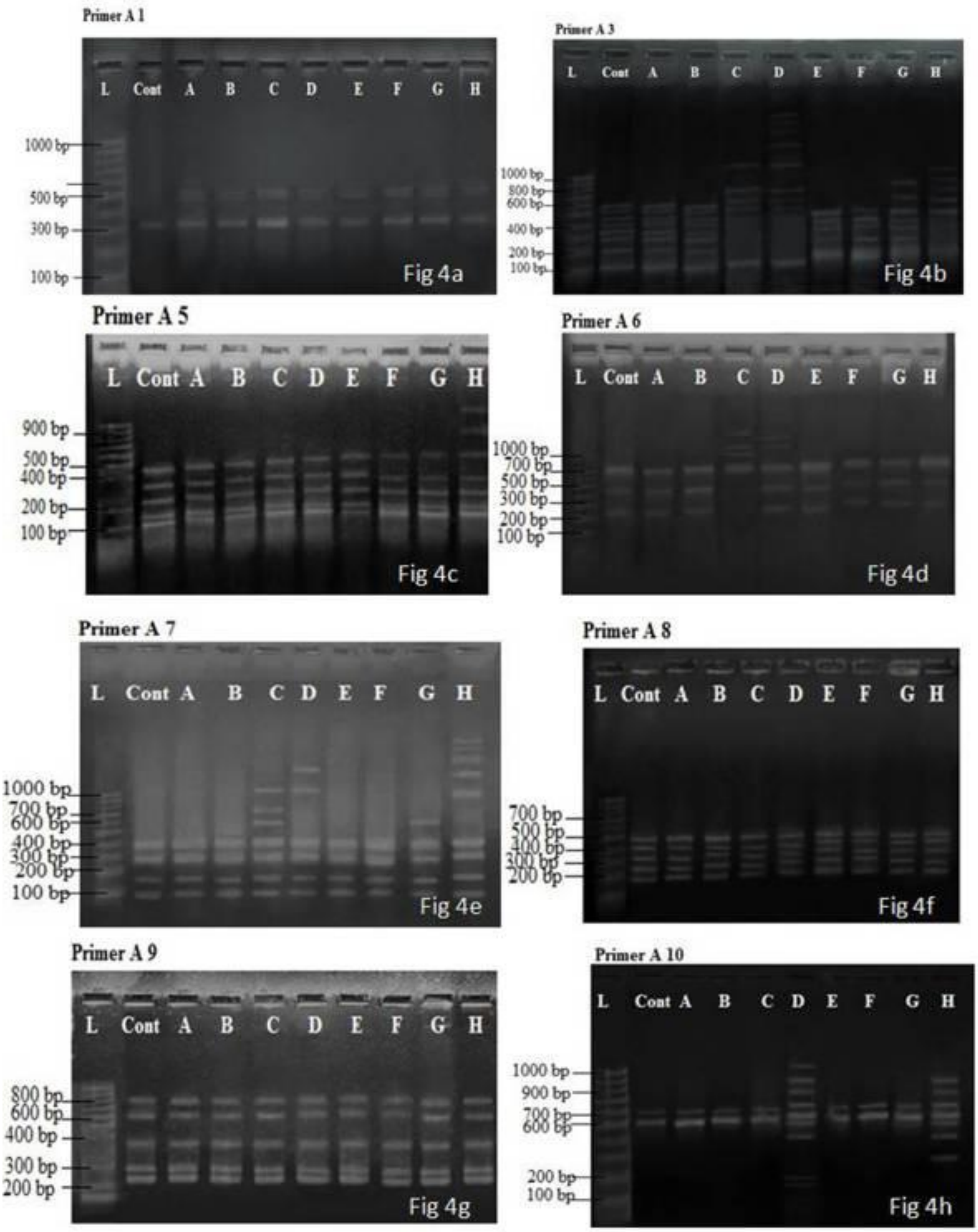

Fig 4: RAPD analysis of laboratory grown treated *A. philoxeroides* using primers; OPA-01 (a), OPA-03 (b), OPA-05 (c), OPA-06 (d), OPA-07 (e), OPA-08 (f), OPA-09 (g), OPA-10 (h).
Lane [A: 0.5 mM $CdCl_2$; B: 1 mM $CdCl_2$; C: 1.5 mM $CdCl_2$; D: 1.8 mM $CdCl_2$; E: 0.5 mM $K_2Cr_2O_7$; F: 1 mM $K_2Cr_2O_7$; G: 1.5 mM $K_2Cr_2O_7$; H: 1.8 mM $K_2Cr_2O_7$; Cont : Plant without treatment, L: DNA ladder]

In case of Cr treatment the lowest GTS value at 1.8 mM concentration. But unlike to Cd, at 0.5 mM and 1 mM concentration the GTS value were 85%, much lower than that of Cd indicating greater genotoxicity of Cr. From, the study this can be interpreted that plants grown in contaminated sites are adapted in such a way that they maintain their genome stability completely, only exception was observed in case of plants from site IV, that showed GTS value as 79% (Fig 3h).

Therefore, it is concluded that cadmium and chromium pose genotoxic effect and induce DNA damage in plants. This can be successfully detected by RAPD method. Inspite of having DNA alterations this alligator weed can survive in Cd, Cr contaminated areas and accumulate considerable amount of these two metals proving its metal tolerant capacity.

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Table 1: Description of experimental sites

Site Code	Sites	Character of site	Distance (Km) from city centre
I	Tiljala	Near E M Bye Pass Heavy traffic, tannery and garbage dumps.	7
II	Mathpukur	Near Science City, E M Bye pass, municipal sludges.	8
III	Shyamnagar	Industrial area near bank of Hooghly Heavy traffic, next to a large storage battery factory	34
IV	Habra	Rural residential area, less traffic, next to unorganized dry cell battery recycling unit.	60
V	Narendrapur	Semirural area, contaminated with the effluents of soft drink plant	20
VI	Kestopur	Semi rural residential area with huge population and traffic, heavily contaminated with municipal sewage sludges and household wastes	17
VII	Taratala Budgebudge	Rural industrial area contaminated with automobile pollution and soft drink plants	27

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Table 2: Nucleotide sequences of the ten OPA decamer primers used for RAPD analysis

Primers	Nucleotide sequences (5'-3')
OPA-01	CAGGCCCTTC
OPA-02	TGCCGAGCTG
OPA-03	AGTCAGCCAC
OPA -04	AATCGGGCTG
OPA -05	AGGGGTCTTG
OPA-06	GGTCCCTGAC
OPA-07	GAAACGGGTG
OPA-08	GTGACGTAGG
OP A-09	GGGTAACGCC
OP A -10	GTGATCGCAG

Table 3: Metal concentration of soil and different parts of *Alternanthera philoxeroides* collected from contaminated sites

SITES	Cr					Cd				
	Soil mg/kg	**Root mg/kg**	**Shoot mg/kg**	**TF**	**BCF**	**Soil mg/kg**	**Root mg/kg**	**Shoot mg/kg**	**TF**	**BCF**
C	0.003 ± .015	BDL	BDL	-----	-----	BDL	BDL	BDL	------	--------
I	883.7 ±1.22	107.3 ±0.62	135.27 ±0.66	1.26	0.121	8.1 ±0.36	5.185 ±0.42	3.6 ±0.4	0.7	0.63
II	* **1833.3** ±1.47	178.7 ±0.60	164.37 ±0.92	0.92	0.097	1.458 ±0.31	0.99 ±0.27	0.95 ±0.05	0.96	0.651
III	141.7 ±1.51	58.66 ±0.93	48.37 ±0.54	0.82	0.41	BDL	--	---	----	----
IV	64.4 ±0.79	36.33 ±0.75	23.55 ±0.47	0.64	0.56	** **44.82** ±0.88	28.96 ±0.48	18.6 ±0.36	0.64	0.64
V	40.58 ± 1.3	21.2 ± 0.75	18 ± 2.49	0.85	0.53	3.75 ± 2	1.75 ± 1.32	1.07 ± 0.1	0.61	0.47
VI	51.43 ± 2.54	33.75 ± 1.16	33.43 ± 1.02	0.99	0.65	BDL	---------	--------	-------	--------
VII	25.52 ± 0.7	18.3 ± 0.8	13.7 ± 0.36	0.748	0.72	3.01 ± 0.44	1.16 ± 0.26	0.93 ± 0.46	0.8	0.39

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BDL : Below detectable limit , *Maximum Cr content in soil, **Maximum Cd content in soil
The data represents means ± SD of three independent replicas.
MAL (Maximum allowable limit in soil) Cd- 1-3 mg/kg Cr : 30-400 mg/kg
According to European Commission Directorate of General Environment 2010.

Table 4: Metal concentration of soil and plants (Roots and Shoots) treated under laboratory condition

Conc. (mM)	**Cr**					**Cd**				
	Soil mg/kg	**Root mg/kg**	**Shoot mg/kg**	**TF**	**BCF**	**Soil mg/kg**	**Root mg/kg**	**Shoot mg/kg**	**TF**	**BCF**
Cont	0.033± 0.015	BDL	-----	-------	------	-------	-----	------	-----	-----
0.5	25.6 ± 3.2	28.38 ± 2.54	26.38 ± 1.27	0.93	1.11	56.3 ± 1.8	29.98 ± 1.31	22.63 ± 2.51	0.75	0.54
0.8	41.6 ± 2.8	39.53 ± 1.33	35.53 ± 0.63	0.898	0.95	90.0 ± 2.3	86.71 ±0.55	107.8 ±0.789	1.24	0.97
1.0	52.0 ± 1.1	46.13 ± 1.2	47.31 ±0.91	1.025	0.89	112.5 ± 1.67	98.11 ± 1.2	117.3 ±0.916	1.2	0.88
1.2	62.0 ± 2.2	51.81 ± 0.8	53.13 ± 1.0	1.02	0.84	135 ± 3.3	73 ± 2.17	67.91 ± 2.15	0.93	0.54
1.5	80.0 ± 1.98	69.85 ± 1.73	46.48 ± 1.3	0.665	0.88	168.7 ± 2.0	87.162 ± 1.56	59.51 ± 1.46	0.68	0.52
1.8	93.7 ± 1.2	73.77 ± 0.82	32.31 ± 1.0	0.505	0.79	202.34 ± 1.5	93.58 ± 0.97	36.38 ± 1.04	0.38	0.47

The data represents means ± SD of three independent replicas.

Table 5: Changes of total bands in control and polymorphic bands in treated plants and changes of GTS for all primers

Primer		Cd concentration (mM)								Cr concentration (mM)							
	Cont	0.5 Lane A		1.0 Lane B		1.5 Lane C		1.8 Lane D		0.5 Lane E		1.0 Lane F		1.5 Lane G		1.8 Lane H	
		a	b	A	b	a	b	a	b	A	b	A	b	a	b	a	b
OPA1	1	2	-	2	-	2	-	2	-	2	-	2	-	2	-	2	-
OPA3	8	-	-	-	-	3	6	7	5	-	3	-	3	2	3	4	6
OPA5	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-
OPA6	3	-	-	-	-	3	2	1	-	-	-	-	-	-	-	-	-
OPA7	4	-	-	-	-	3	-	2	-	-	-	-	-	1	-	5	-
OPA8	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OPA9	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OPA10	2	-	-	-	-	-	-	7	-	-	-	-	-	-	-	5	-
Total no of bands	33	2	-	2	-	11	8	19	5	2	3	2	3	5	3	18	6
a+b		2		2		19		24		5		5		8		24	
GTS %		94		94		42.5		27.3		85		85		75.8		27.3	

a: appearance of new bands, b: disappearance of control bands, a+b indicates polymorphic bands