Forensic Assessment of Genotoxic Effects of Lead Metal on leaf *Phragmites australis* Using Random Amplified Polymorphic DNA (RAPD-PCR) Markers

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Abstract

The influence lead metal on DNA integrity in leaf plant can measure by potential effects of pollutants accumulation metal, Pb in leaf plant *Phragmites australis* was subjected to RAPD analysis, have been found metal at concentrations of S1, S2,S3 (0.045 , 2.97, 0.93) Respectively. polymorphisms became evident as the presence and absence of DNA fragments in affected samples compared to effect samples. Five prime was selected OP-L05, OP-M14, OP-M20, OP-V09 and OP-M05 were used , the genomic DNA of leaf did not appear polymorphic band at OP-L05 except molecular weight 600bp and presence of polymorphic band ,OP-M20 show only polymorphic band at 850bp,OP-M05 did not show any polymorphisms and OP-M14, OP-V09 primers appeared polymorphisms when compare unexposed in control, this study confirmed RAPD markers a fast and simple technique can be used detection of forensic environmental.

Keywords: *Forensic environmental, RAPD-PCR, Lead , Phragmites australis.*

Introduction

Science is , developing at every moment, which has made the focus in recent times by researchers on the forensic science as a relatively new activity especially in the environmental field , newly defined as a strong method orientated mostly a technique driven an activity in the multidisciplinary of aquatic ecosystem within areas and surrounding it by plants or soil and sediments by survey after any incidents that occur . In the last years, the forensic science technology has advanced dramatically and has been very specialized are generally only performed in investigations of remarkable in genetic heterogeneity that affects the nature of organisms of trace evidence. Consequently, there is an opportunity for the application of analysis in the forensic examination of plants and organism accumulated in water or soil from a wider spectrum of forensic investigations as mentioned [1]. The Industrial crude waste, sewage water and crude oil residues without initial treatments pose environmental risks for the population due to the levels of contaminants released and represent a source of environmental liability due to their potential to accumulate and release large quantities of contaminants generated by the decomposition of pollutants. Effluents can carry microorganisms and metals through the water resources this is what researchers refer to [2]. Plants exposed to accumulate metals toxic concentrations and reveal a critical health risk to consumers by the food chain [3].High concentrations of lead have toxic effects on leaf plants ,the excess of metal is negatively affect the genotype plant [4]. Moreover, lead ions may bind to thylakoid membrane proteins with high concentrations and inhibition of electron flow and emergence of reactive oxygen forms by damaging the structures of photosynthetic thylakoid membrane [5]. The effects of metal on DNA occur directly or indirectly. These can be the destabilization of the double helical structure of DNA [6], mismatches of the bases on nucleic acids [7], single nuclease lesions on DNA, single DNA strand breaks, double DNA strand breaks and ion connections within strands and also degree of methylation of DNA [8,9].In brief, metals, even if indirectly, cause oxidative DNA damages and chromosomal abnormalities [10]. Advances and developments in molecular biology have provided new ways of detecting DNA damage on plants [11,12,13]. The detection of genotoxicity with DNA marker has many advantages, the explorations of random amplified

polymorphic DNA (RAPD) as genetic markers have improved the detection of DNA alterations after the influence of many genotoxic agents that affects plant growth and is considered an forensic environmental caused by pollution without any initial treatment [14].This technique using a single primer able to anneal and prime at multiple location throughout the genome can produce a spectrum of amplification products that are characteristics of the template DNA [15,16]. RAPD-PCR assay is one of the most reliably used techniques for detecting DNA damage as the amplification stops at the site of the damage. The changes occurring in RAPD profiles following genotoxic treatments include variation in band intensity as well as gain or loss of bands. This has been done through the analysis of band intensities and band gain or loss variation between exposed and non-exposed individuals [17].The organism is identified depending on a unique gene present in the organism. It takes set in three steps: denaturation, annealing and extension: each step is performed at an optimum temperature. The denaturation must be temperature higher than the melting point of the primers, but must not be too high as to cause loss of activity to the DNA polymerase. The annealing temperature is dependent on the temperature of the elongation step must be optimum for the polymerase activity and the properties of the primers [18].

As a new study of its kind in the role of plants and aquatic ecosystem in the forensic investigation , It was examine leaf *Phragmites australis* , that have occurred that have been selected for being one of the most environmentally damaging areas of crude oil, industrial waste , as well as a control to understand the concept , the important aims that identify the changes that had happened in the environmental from different sites to identification Pb element and confirm the availability for extraction DNA in the plant leaf *Phragmites australis* to detect molecular weight in the source of effectiveness the concentration as remarkable forensic by PCR.

Materials and Methods

Description and samplling

Three sites were selected on the basis have been damaged as evidence of the forensics environment in from the dominant type of plants which represented by S1=AL- Rashdia site as a control,S2= Midland oil company and as a crude oil waste and S3= AL-Musayib Babil as an electrical energy.

Collection samples and Preparation

Plant (*Phragmites australis*) (Class: Angiosperm, Family: Poaceae) (Linnaeus, 1758), were collected during January 2020. According to[19] all plant samples digest preparation to determine Pb element concentration .before that all samples must be converted to liquid by specific method of regulation and melting and used method validation was used as BAM Germany certified reference material with preparation of samples for analysis by Top wave analytic Jena type . Samples were examined by the Inductively Coupled Plasma Emission Spectrometry (ICP-ES). For all samples digestions, three replicates have been performed, calibration blanks of 2.0 mL deionized water are taken through the same digestion process. Detection limits for Pb metals in this study have calculated based on three times the standard deviation of the average of 5 blank measurements to one test. The evaluation of analytical results take place in one step by a computer program called smart analyzer. Results are given in unit mg/dm3 (ppm).

Genomic DNA isolation:

The DNA was extracted from the leaf of *Phragmites australis* plant by small-scale method using commercial kit (Bionner-Korea). The purity of DNA was measured dependent on optical density by using spectrophotometer, the DNA was detecting using agarose gel electrophoresis with ethidium bromide and visualized under UV light [20]

RAPD-PCR analysis:

In this study five of RAPD primers were used, the primers was synthase by (Bioneer-Korea) in lyophilized form and dissolved in sterile distilled water to get final conc. of (10pmol/ml) according to [21]. The primers and their sequences are listed in Table-1.

Table (1)The list of RAPD primer:

NO	Primer	Sequence
1	OP-L05	ACGCAGGCAC
2	OP-M14	AGGGTCGTTC
3	OP-M20	AGGTCTTGGG
4	OP-M05	GGGAACGTGT
5	OP-V02	AGTCACTCCC

Amplification of genomic DNA was performed with the master amplification reaction showed in Table-2.

Table (2) The master amplification Reaction:

Material	Final concentration	Volume for 1 tube
PCR pre mix	1x	5µI
Deionised D.W	——	11µI
Primer(10pmol/ µl)	10pmol /µl	2µI
DNA template	100ng	2Mi

RAPD – PCR premix (final reaction volume = 20 µI). No. of cycles = 40 cycles between initial denaturation and final extension, the following Table-3 shows the RAPD program Followed by a hold at 4°C [21]. Each PCR amplification reaction was repeated twice to ensure reproducibility the products analyzed by electrophoresis in 1.5% agars gels with 0.5µl stained ethidium bromide at 7vt/cm for 3hours [20].

Table (3):The optimum condition of detection gene.

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	3 min.	
2-	Denaturation -2	95°C	1min	40 cycle
3-	Annealing	36°C	1min	
4-	Extension-1	72°C	1 min	
5-	Extension -2	72°C	10 min.	

Results and Discussion

Lead Analysis (Pb) in leaf *Phragmites australis*

Results showed the presence of Lead concentration in plant but differ from area to other depending on the ability to absorb this element Consecutively in S2> S3 > S, the concentration of Lead in leaf *Phragmites australis* represented S1=0.045, S2=2.97 and S3=0.93respectively ,study area. that means a positive indicator of pollution in study areas . Through statistical analysis scored a significance between element and plants in a percentage of the corresponding (30% ,9%, 2%), Respectively, There was no significant correlation P-value (0.004) , this is consistent with some of the researchers in local studies [22] found Pb in *ceratophyllum demersum* between (0.3-1.9 ppm) and in *Phragmites australis* between (0.6-1.8 ppm) and [23]recorded the max concentration of lead in a plant (*C. demersum*) (0.23-2.01ppm).These indicate that aquatic plants are known in accumulating Pb from around its environment according to [24]. The rate of absorption of this element was a high , its being the most plants play an important role in circulating nutrients and trace metal in aquatic ecosystems according to[25]. They spread all over the world and due to their high capacity in uptake of nutrients and other pollutants from water treatment [26], the high concentration of Pb in leaf plant refers to uptake of inorganic complexes because of their higher surface area compared to their volume according to [26],Therefore, it may be considered one of the most harmful minerals and the extent the influence of the surrounding environment used it as a result of events and damage to plants . The normal level of lead element in the leaf tissues of mature plant growing on uncontaminated soil ranges from (0.01-0.1 ppm), It is toxic to the plant if it's between (3 ppm) [27]. In studied conducted in a mining area in Hamidan Province in the western part of Iran, the amount of lead in leaf plant *Typha domengensis* of the studied site was (2.33 ppm) [28], the amount as mining site in our study produce large amounts of lead pollutants.

Molecular identification by PCR:

A RAPD technique was applied to detect the genetic effects at the DNA level of *Phragmites australis* plant which exposed to metal. This study compared the effects occurring in the genomic DNA of *Phragmites australis* exposed to various levels of lead at concentration(2.97,0.93) ppm in plant of(S2andS3) comparison with the *Phragmites australis* in S1as control were less exposed to lead at concentration (0.045) ppm respectively .Five primers were utilized used for screening genomic DNA of *Phragmites australis* exposed and unexposed to heavy metal. Photo captures were used to calculate molecular weight to detect the bands in plant produced by the PCR reactions and compare them

to the size of the DNA ladder marker according to [29]. The results of the Rapid PCR were analyzed as shown in the statistical tables and according to the electrophoresis for each primer that (1) number symbolizes the presence of a band and (0) does not indicate the presence of a band. The variation in the number of bands amplified by different primers influenced by variable factors such as primer structure, template quantity and less number of annealing sites in the genome , the results were interpreted on the basis of [30].

Observed at OPL05 primer in Figure (1) when comparing plant samples (S2 and S3) with the control (S1) at the following molecular weights (1500 1250 850 ,400,350,300,200)bp no bands (could not amplify DNA) , This indicates that there has been no variation or the genetic material of the plant didn't affected by heavy metal or other pollutants.While at the molecular weight 600bp, been noted the presence of polymorphic band in the (2,3 plant sample) and their disappearance in control, this indicates the occurrence of a variation. And in (700,525)bp Monomorphic band (similarity). When comparing this study through this primer opl05 with [31] Which was explained the Assessment of genetic diversity among wheat where it was found that there were no bands at the following molecular weights from (100 to 500)bp, but at a molecular weight (600)pb, polymorphic band was observed when compared with the control, while [32] use OPL-05 for Assessment of genotoxic effects of copper on Cucumber Plant (*Cucumis sativus L.*) which is could not amplify the genomic DNA, gave extremely faint and ambiguous bands.

Figure (1) PCR product of primer OP-l05The product was electrophoresis on 2%agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. N: DNA ladder

The primer OP-M14 figure (2) observed in (polymorphic band) in all samples with control at all molecular weights except for (300pb), which did not show any band whether it was in control or other samples whilst at (500pb) Monomorphic band. This result disagree with [33]which used this primer (OP-M14)on (*Verticillium dahlia)* found polymorphic band between sample and control but monomorphic band were not included in the analysis.

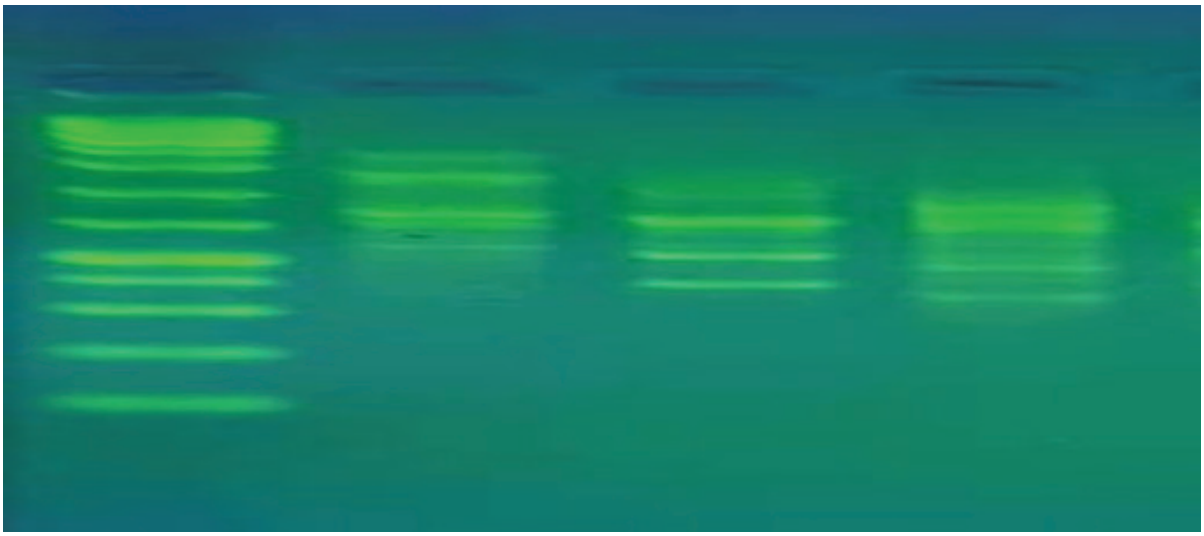

Figure (2) PCR product of primer OP-M14The product was electrophoresis on 2%agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. N: DNA ladder

In figure (3), the molecular weight(1250,675,500)bp of the primer (OP-M20) no bands appeared, while in another (1000,700,625 and400)bp There was monomorphic bands between the control and the affected samples ,and at(850) bp was polymorphic band compatible with [32] who was Assessment of genotoxic effects of copper on Cucumber Plant *(Cucumis)* used RAPD-PCR technique markers at primer (OP-M20), observed the presence of polymorphic band between the control and the copper treated samples at (400,500,600,700,800)bp so its RAPD profiles of OPM20 primer produced polymorphic bands with genomic DNA a various levels of lead metal tracers compared with control, such as the presence of new DNA bands which were not detectable in DNA of unexposed plants.

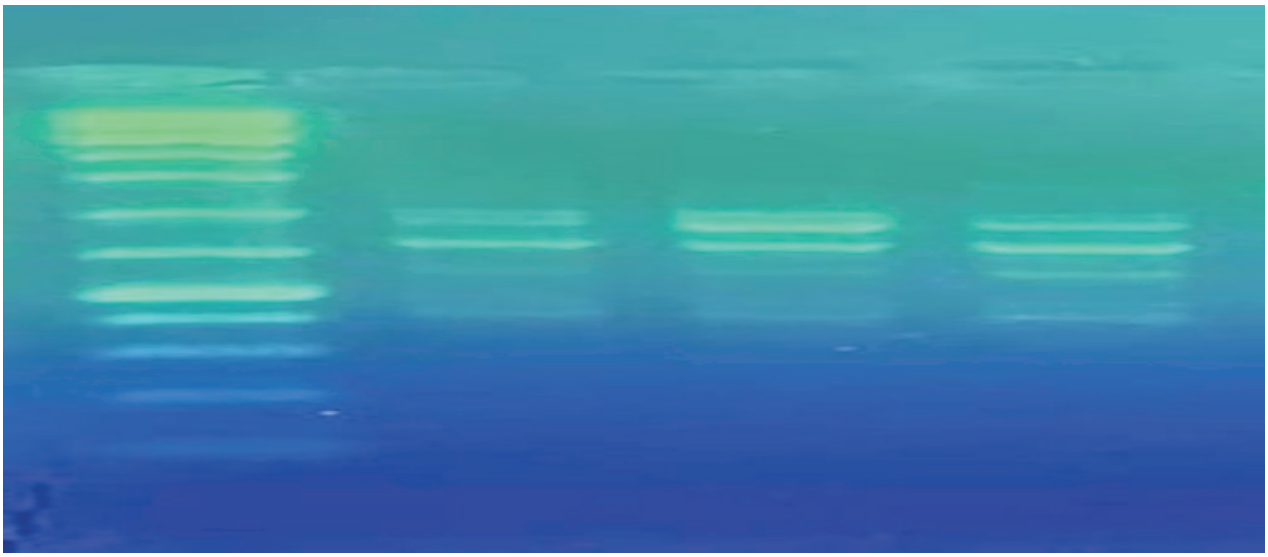

Figure (3) PCR product of primer OP-M20The product was electrophoresis on 2%agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. N: DNA ladder.

In *Phragmites australis* ,presence Monomorphic bands between the control and other samples of the plant at molecular weights (1500 and 950)bp at figure(4) genetic similarity and disappearance of band at most molecular weights, so absence of any variation at OP-M05. Hence, we explain that the genetic material of the control sample and other is not affected by any pollution in the area from which the samples are taken, This is not consistent with [34]Which was explained at primer (OP-M05) polymorphic band between control and study

plant at all molecular weight except 701 bp which shows Monomorphic band.

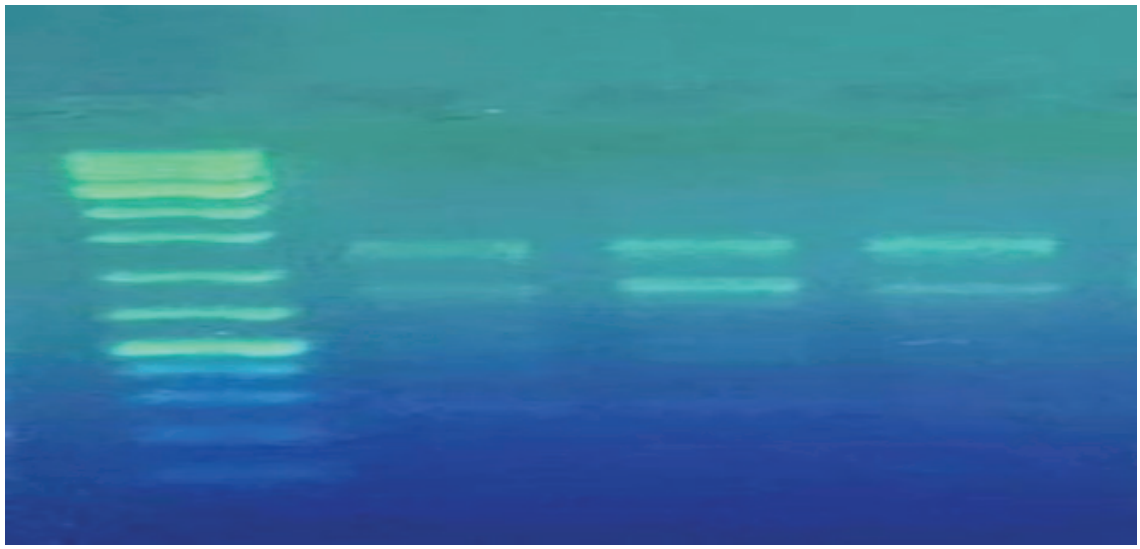

Figure (4) PCR product of primer OP-M05The product was electrophoresis on 2%agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. N: DNA ladder

The primer OP-V09 in figure (5), Monomorphic band (genetic similarity) was appear at the molecular weight of DNA marker (700,300)bp, and polymorphic band(variation) between control and studied sample at (1000, 900 ,600, 450)bp , should not DNA amplify in (1500,380). Comparison was made with [35] which arrived at 8 polymorphic band.

Figure (5) PCR product of primer OP-VO9 The product was electrophoresis on 2% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. N: DNA ladder (100).

In this study, RAPD analysis showed that there were detectable genetic changes when the genomic DNA of *Phragmites australis* plant was exposed to metal. Appearing bands may be the result of structural changes induced by lead adducts and/or by nongenotoxic events such as transposition, DNA amplification, and so on. Other changes in DNA patterns such as the variation in band intensities can be attributed principally to the presence of bulky adduct that potential block the PCR enzyme. Finally, the changes observed in RAPD patterns are likely to be due to the sum of all DNA alterations (e. g., adducts, mutations, rearrangements, structural changes) induced by heavy metal. In addition, dimmers can alter the structure of the DNA. If so, such structural changes are likely to have a significant effect on the kinetics of PCR events. New PCR products can be amplified because some sites become accessible to the primers after structural change or because the same mutations have occurred in the genome [36].

Conclusion

The metal caused genetic variation in *Phragmites australis* between control and exposed site of lead and metal, at least in two primers which leading to mutations in future. Therefore, biomarkers are necessary for detection heavy metals in this plant where it is growing .Moreover, RAPD were fast and simple technique for genotoxicity. The traceability of this measurement lead metal have a good correlation a high percentage of corresponding (30%) in S2 and Low Percentage of corresponding (0.04%) in S1 . These values instrument is a powerful tool that can be very effective in the validation of both the absence and presence of certain elements enter into the composition of crude oil or electrical energy site. In principle, this instrument could be employed to provide rapid in situ detection of the presence of toxic metals and it can be traced in cultivated plant species near the place of the event as has been done before from [37] have been used as a screening technique and are highly reliable in detecting samples and be applied with the rest elements contained in the C4 66% effective methodology needs to be verified and calibrated according to [38].

The molecular weight of the primer (OP-M20) , there was monomorphic bands between the control and the affected samples ,and at(850) bp was polymorphic band compatible , Thats assessment of genotoxic effects of RAPD-PCR technique markers at primer (OP-M20), observed the presence of polymorphic band between the control and the other sites , so its RAPD profiles of OPM20 primer produced polymorphic bands with genomic DNA a various levels of lead metal tracers compared with control, such as the presence of new DNA bands which were not detectable in DNA of unexposed plants. Results suggest that the biota of oil-polluted habitats may be experiencing increased mutation and the chlorophyll-deficiency has often been used as a sensitive genetic end point in plant mutation research due to increased accumulation of element

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