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# Health Effects of Environmental Pollutants on Workers in the Libyan Plastic Factories, Part B: Based on Molecular Levels Characteristics

Suliman O. Alayeb*

Department of Environmental Science, Faculty of Sciences, Alasmarya Islamic University, Zliten, Libya.

***E-mail:** solimanomar430@gmail.com

# الآثار الصحية للملوثات البيئية على العاملين في مصانع البلاستيك الليبية
# الجزء ب: بناءً على خصائص المستويات الجزيئية

سليمان عمر العائب*

قسم علم البيئة، كلية العلوم، الجامعة الأسمرية الإسلامية، زليتن، ليبيا.

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## Abstract

This study aimed to disclose the reasons for the health damage and heritable genetic mutations among workers in plastic manufactories due to negative environmental impacts. Moreover, this study may constitute an initial database that expresses the negative impact on the environment in the plastics factories that may have a negative impact on workers. To study the effects of long-term exposure to plastic and its solvent vapors during plastic manifesting on the blood DNA contents, blood samples were withdrawn from volunteers working in a plastics factory in Kasr Alakhyar city, Libya. Six different samples were collected from Workers exposed to plastic vapor for different periods as follows, control (did not expose to plastic vapor), one-year exposure, three years' exposure, six years' exposure, seven years' exposure, and twelve years' exposure.

Blood samples were collected and subjected to molecular analysis using randomly amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR). Molecular analyses were performed to assess the genotoxicity effect of plastic vapor on the molecular level. On the molecular level, the DNA of the six aforementioned volunteers was extracted and subjected to RAPD analysis. The results showed several differences in the banding patterns was recorded between the control volunteer and volunteer and those who exposed to plastic and its solvents vapor for different exposure periods. These changes in the banding patterns suggested that a modification in the genomic DNA occurred in form of modifications in the nitrogen bases of the genomic DNA as a response to the exposure to plastic vapor for a different time exposure.

***Keywords**: DNA, Genetic mutation, Molecular analysis, Plastic factory.*

## الملخص

تهدف هذه الدراسة إلى الكشف عن الأسباب التي أدت إلى أضرار صحية وراثية مثل الطفرات الوراثية بين العاملين في المصانع البلاستيكية بسبب الآثار البيئية السلبية. وعلاوة على ذلك، يمكن أن تشكل هذه الدراسة قاعدة بيانات أولية عن التأثير السلبي على البيئة في مصانع البلاستيك التي قد يكون لها أثر سلبي على العمال. لدراسة آثار التعرض على المدى الطويل لأبخرة البلاستيك والمذيبات الداخلة في تصنيعها والذي يظهر على محتويات DNA في الدم، تم سحب عينات دم من المتطوعين الذين يعملون في مصنع البلاستيك في مدينة قصر الأخيار، ليبيا. تم جمع ست عينات مختلفة خمسة منهم

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من عاملين بالمصنع ممن يتعرضوا لبخار البلاستيك لفترات مختلفة على النحو التالي، الكنترول (لم تتعرض لبخار البلاستيك)، التعرض لسنة واحدة، ثلاث سنوات من التعرض، التعرض لست سنوات، سبع سنوات واثنتي عشرة سنة من التعرض للأبخرة.
كما تم جمع عينات الدم وإجراء التحليل الجزيئي باستخدام الحمض النووي متعدد الأشكال تضخيم عشوائيا (RAPD). أجريت التحليلات الجزيئية لتقييم أثر السمية الجينية الناتجة عن التعرض لأبخرة البلاستيك على المستوى الجزيئي. على المستوى الجزيئي، تم عزل الحمض النووي DNA من المتطوعين المذكورة آنفا الستة وقد خضعت لتحليل RAPD. وأظهرت النتائج حدوث العديد من الاختلافات في السلوك التحزمي بين المتطوع الكنترول والمتطوعين الذين تعرضوا للمواد البلاستيكية وبخار المذيبات لها لفترات تعرض مختلفة. دلت هذه التغييرات في السلوك التحزمى على أن التعديل في الحمض النووي الجيني قد حدثت في شكل تعديلات في القواعد النيتروجينية في الحمض النووي DNA كاستجابة للتعرض لبخار البلاستيك لفترات تعرض زمنية مختلفة.

**الكلمات الدالة**: الحمض النووي، الطفرات الجينية، التحليل الجزيئي، مصنع البلاستيك.

## 1. Introduction

Hundreds of thousands of people worldwide live or work in close proximity to plastic mills. Integrated plastic products generate chemical pollution such as plastic and other polymers containing compounds that can induce genetic damage (Alayeb, 2019; Legzdins *et al*., 1995; and Williams *et al*., 1990). Previous investigations demonstrated elevated DNA mutation rates near plastic mills but could not determine the importance of airborne or aquatic routes of contaminant exposure, or eliminate possible confounding factors such as nutritional status and disease burden (Manikkam *et al*., 2012; Somers *et al*., 2002; and Yauk & Quinn, 1996). To address these issues experimentally, laboratory mice were exposed in situ to ambient air in a polluted industrial area near plastic mills. Heritable mutation frequency at tandem-repeat DNA loci in mice exposed 1 km downwind from two integrated plastic mills was 1.5- to 2.0-fold elevated compared with those at a reference site 30 km away. This statistically significant elevation was due primarily to an increase in mutations inherited through the paternal germ line. The results indicate that human and wildlife populations in proximity to integrated plastic mills may be at risk of developing germ line mutations more frequently because of the inhalation of airborne chemical mutagens (Somers *et al*., 2002).

This study aimed to disclose the reasons for the health damage and heritable genetic mutations among workers in plastic manufactories due to negative environmental impacts. Moreover, this study may constitute an initial database express the negative impact to the environment in the plastics factories that may have a negative impact on workers.

## 2. Materials and Methods

To study the effects of long term exposure to plastic and its solvents vapors during plastic manifesting on the blood protein contents, blood samples were withdrawn using 10 mL syringe from volunteers working in plastic factory in Kasr Alakhyar city, Libya. Six different samples were collected from Workers exposed to plastic vapor for different periods as follow, control (did not exposed to plastic vapor), one year exposure, three years exposure, six years exposure, seven years exposure and twelve years exposure.

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Blood samples were collected in plyprobline tubes coated with EDTA to avoid the agglutination of blood and prepared for molecular analysis using randomly amplified polymorphic DNA (RAPD) and inter simple sequences repeats (ISSR). Molecular analyses were performed to assess the genotoxicity effect of plastic vapor on the molecular level.

### 2.1. Assessment of genotoxicity on the molecular level

The aforementioned six samples of the volunteers' blood were collected and subjected for molecular analysis using randomly amplified polymorphic DNA (RAPD).Peripheral blood leukocytes are a main source of animal genomic DNA, but sample collection is difficult as blood must be withdrawn from the animal. Blood contains a range of compounds like proteins, lipids, white blood cells, red blood cells, platelets, and plasma, which can contaminate the DNA sample. The primary contaminant of animal DNA extracted from blood samples is heme, the non-protein component of hemoglobin.

#### *2.1.1. Genomic DNA extraction*

Total DNA was extracted from one mL of blood sample using a Bioflux Kit (from china) as described in the kit manual.

#### *2.1.2. Polymerase chain reaction (PCR) conditions*

PCR- RAPD reactions were conducted according to Williams *et al*. (1990) using nine arbitrary 10-mer primers (Operon Technologies, Inc.), as shown in Table (1). The reaction conditions were optimized and the mixtures were prepared (25 µL total volumes) consisting of the following;

2.5 µL of dNTPs (8 mM mix), 0.2 µL of Taq DNA polymerase (5 U/µL), 2.5 µL of 10X buffer with 15 mM $MgCl_2$, 1.0 µl of Primer (10mM), 1.0 µL of Template DNA (10-50 ng/µL), 3.0 µL of MgCl2, and 16.3 µL $H_2O$ (dd).

Amplification was carried out in Strategene Robocycler Gradient 96, which was programmed for 40 cycles as follows:

Denaturation (one cycle) 94 $^oC$ for 4 min., (40 cycles) of the following sequence 94 $^oC$ for 1 min. and 30 sec., 36 $^oC$ for1 min. and 30 sec., 72 $^oC$ for 2 min. and 30 sec., then extension (one cycle) 72 $^oC$ for 7 min.

**Table 1.** List of Operon primers for RAPD and their nucleotide sequences

| Primer | Sequence | Primer | Sequence |
|---|---|---|---|
| Op A12 | TCGGCGATAG | Op L15 | AAGAGAGGGG |
| Op C02 | GTGAGGCGTC | Op L17 | AGCCTGAGCC |
| Op C04 | CCGCATCTAC | Op q16 | AGTGCAGCCA |
| Op E12 | TTATCGCCCC | Op q18 | AGGCTGGGTG |
| Op E19 | ACGGCGTATG | ///////// | ////////////// |

#### *2.1.3. Gel electrophoresis*

Agarose (1.2 %) ultra-pure (GIBCO-BRL) was used according to Sambrook *et al*. (1989) for resolving the PCR products one Kb plus DNA Ladder 1µg /µL (Invitrogen) was used.

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***TAE buffer (50x)***

| | |
|---|---|
| Tris | 242 g |
| Glacial acetic acid | 57.1 mL |
| EDTA | 37.2 g |
| Double distilled water | up to 1 L |

### *2.1.4. Gel preparation*

Agarose gel was prepared according to Sambrook *et al*. (1989) as follow:

| | |
|---|---|
| Agarose | 1.2 g |
| TAE buffer (1x) | 100 mL |
| Ethidium bromide (10µg /µl) | 1.5 µL |

***Loading buffer (6x)***

0.25 g Fumasine Red was dissolved in 100 mL of sucrose (80%).

### *2.1.5. Sample preparation*

| | |
|---|---|
| PCR- product | 20 µL |
| Loading buffer (6x) | 5 µL |

The run was performed for one hour at 100 Volt using Biometra gel electrophoresis submarine (20 cm×10 cm). Bands were detected on UV- Transilluminator and photographed by gel documentation system (Biometra Bio Doc Analyzer-2000).
The polymerase chain reaction products were resolved on T.A.E. agarose gels and recorded as (1) for the present bands and (0) for the absent ones.

## 3. Results and Discussions

Molecular analysis using randomly amplified polymorphic DNA (RAPD) were performed. DNA of the six volunteer's blood under investigation were extracted and subjected for molecular analysis using randomly amplified polymorphic DNA (RAPD) with nine ten-mer random primers, six out of the used primers only which produced scorable banding patterns. This technique was used to investigate whoever the exposure to plastic and its solvents vapors affect DNA or not. The six primers which have scorable banding patterns produced total number of 76bands, with average of 12.67 bands per primer. Out of the 76 produced bands, 44 monomorphic bands and 32 polymorphic bands. Sequences of some of these polymorphic bands seems to be modified as response for the exposure to plastic or its solvents vapors during the manifesting processes.

### 3.1. Primer Operon A-12

The results of primer (OP A-12) are shown in Figure (1) and Table (2). This primer resulted in kindly polymorphic products with the six blood samples under investigation. The PCR products using this primer resulted in a total number of ten bands with molecular sizes ranged from 320 to 1125 bp. All of the ten produced bands using this primer were polymorphic bands among the six blood samples under investigation.

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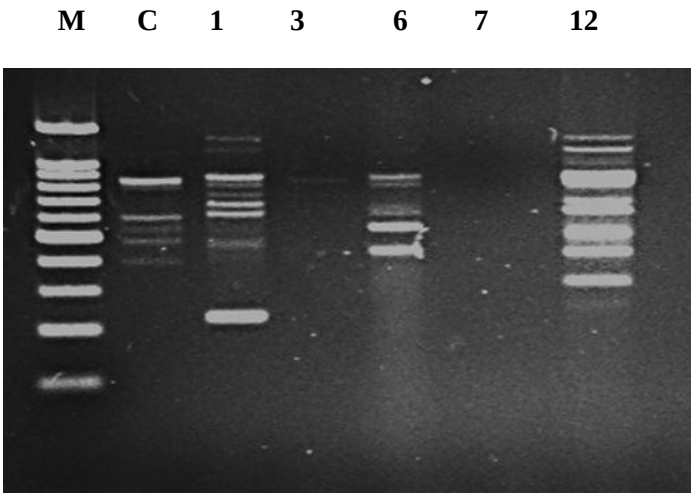


**Figure 1.** Banding patterns of the six blood samples using RAPD-PCR primer (OP A-12) represented different exposure periods for plastic and its solvents vapors during manifesting processes

**Table 2.** Screening of RAPD banding patterns in the six blood samples using RAPD-PCR primer (OP A-12) represented different exposure periods for plastic and its solvents vapors during manifesting processes*

| Bp | control | 1 year | 3 years | 6 years | 7 years | 12 years |
|---|---|---|---|---|---|---|
| 1125 | 0 | 1 | 0 | 0 | 0 | 1 |
| 1010 | 0 | 1 | 0 | 0 | 0 | 1 |
| 825 | 0 | 0 | 0 | 0 | 0 | 1 |
| 800 | 0 | 1 | 0 | 1 | 0 | 1 |
| 710 | 1 | 1 | 0 | 1 | 0 | 1 |
| 640 | 1 | 1 | 0 | 1 | 0 | 1 |
| 580 | 1 | 1 | 1 | 1 | 0 | 1 |
| 500 | 1 | 0 | 0 | 0 | 0 | 0 |
| 390 | 0 | 0 | 0 | 0 | 0 | 1 |
| 320 | 0 | 1 | 0 | 0 | 0 | 0 |

* (1) presence, (0) absence.

The results also showed that band with molecular size of 500 bp were presented only in control (volunteer never exposed to plastic or its solvents vapors) and it was absent in the blood samples of the exposed volunteers for the different periods under investigation.

On the other hand, two bands were developed with length of 825 and 390 bp were present only in the blood sample of withdrawn from volunteer exposed to plastic or its solvents vapors for 12 years. These three aforementioned bands reflected a kind of trending modifications in the genomic DNA of the volunteers exposed to plastic or its solvents vapors for different lengths if compared with the banding patterns of the blood sample which withdrawn from control volunteer. These changes in the banding patterns between the blood samples withdrawn from the exposed volunteers and the blood samples of the non-exposed volunteer could be due to modification in the nitrogen bases unless the primer could not

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recognized the complement sequences and that is avoid the DNA amplification using polymerase chain reaction (PCR).

### 3.2. Primer Operon C-02

The results of primer (OP C-02) are shown in Figure (2) and Table (3). This primer resulted in kindly polymorphic products with the six blood samples under investigation. The PCR products using this primer resulted in a total number of fifteen bands with molecular sizes ranged from 370 to 1675 bp. eight bands out of the fifteen produced bands using this primer were monomorphic bands with lengths of 890, 820, 715, 680, 600, 540, 480, and 370 bp among the six blood samples under investigation.

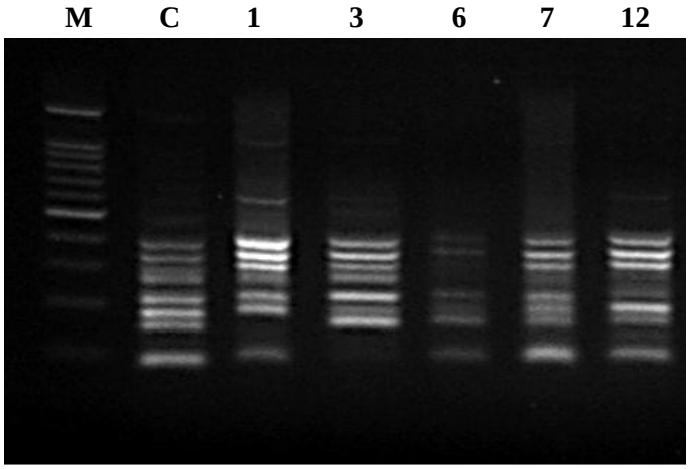


**Figure 2.** Banding patterns of the six blood samples using RAPD-PCR primer (OP C-02) represented different exposure periods for plastic and its solvents vapors during manifesting processes

**Table 3.** Screening of RAPD banding patterns in the six blood samples using RAPD-PCR primer (OP C-02) represented different exposure periods for plastic and its solvents vapors during manifesting processes*

| Bp | control | 1 year | 3 years | 6 years | 7 years | 12 years |
|---|---|---|---|---|---|---|
| 1675 | 1 | 0 | 0 | 0 | 0 | 0 |
| 1490 | 0 | 1 | 1 | 1 | 1 | 1 |
| 1125 | 0 | 1 | 1 | 1 | 1 | 1 |
| 1090 | 1 | 0 | 0 | 0 | 0 | 0 |
| 1015 | 0 | 1 | 1 | 1 | 1 | 1 |
| 980 | 1 | 1 | 0 | 0 | 0 | 0 |
| 890 | 1 | 1 | 1 | 1 | 1 | 1 |
| 820 | 1 | 1 | 1 | 1 | 1 | 1 |
| 715 | 1 | 1 | 1 | 1 | 1 | 1 |
| 680 | 1 | 1 | 1 | 1 | 1 | 1 |
| 600 | 1 | 1 | 1 | 1 | 1 | 1 |
| 540 | 1 | 1 | 1 | 1 | 1 | 1 |
| 480 | 1 | 1 | 1 | 1 | 1 | 1 |
| 420 | 0 | 0 | 0 | 0 | 0 | 1 |
| 370 | 1 | 1 | 1 | 1 | 1 | 1 |

* (1) presence, (0) absence.

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The results also showed that the bands with molecular sizes of 1675 and 1090 bp were presented only in control (volunteer never exposed to plastic or its solvents vapors) but they were absent in the blood samples of the exposed volunteers for the different periods under investigation.

On the other hand, four bands were developed with length of 1490, 1125, 1015 and 420 bp were absent only in the blood sample of withdrawn from volunteer who never exposed to plastic or its solvents vapors (control) while they present in the blood samples of all the volunteers exposed to plastic or its solvents vapors from one year to 12 years exposure. Moreover, one band with length of 980 bp was present only in the blood samples of control and one year exposure volunteers but it was absent in the blood samples of the volunteers exposed to plastic or its solvents vapors for a periods of 3, 6, 7 and 12 years.

These seven aforementioned bands reflected a kind of trending modifications in the genomic DNA of the volunteers exposed to plastic or its solvents vapors for different lengths if compared with the banding patterns of the blood sample which withdrawn from control volunteer. These changes in the banding patterns between the blood samples withdrawn from the exposed volunteers and the blood samples of the non-exposed volunteer could be due to modification in the nitrogen bases unless the primer could not recognized the complement sequences and that is avoid the DNA amplification using polymerase chain reaction (PCR).

### 3.3. Primer Operon C-04

The results of primer (OP C-04) are shown in Figure (3) and Table (4). This primer resulted in kindly polymorphic products with the six blood samples under investigation. The PCR products using this primer resulted in a total number of seventeen bands with molecular sizes ranged from 80 to 1340 bp. seven bands out of the seventeen produced bands using this primer were monomorphic bands with lengths of 1340, 1270, 1210, 930, 860, 780 and 615 bp among the six blood samples under investigation.

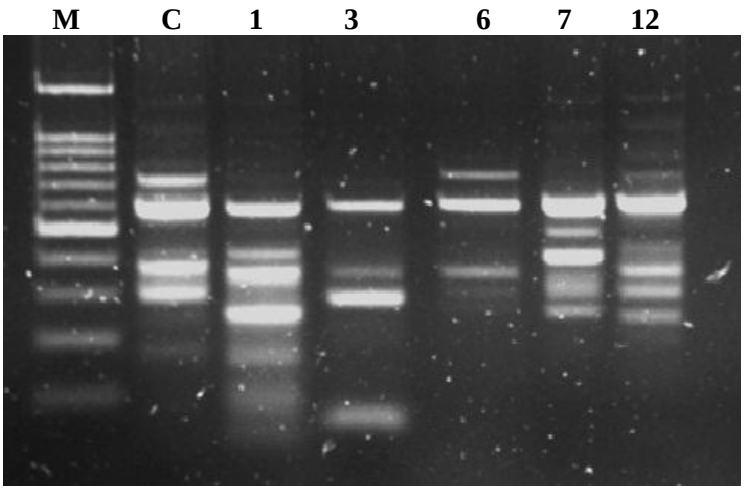


**Figure 3.** Banding patterns of the six blood samples using RAPD-PCR primer (OP C-04) represented different exposure periods for plastic and its solvents vapors during manifesting processes

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**Table 4.** Screening of RAPD banding patterns in the six blood samples using RAPD-PCR primer (OP C-04) represented different exposure periods for plastic and its solvents vapors during manifesting processes*

| Bp | control | 1 year | 3 years | 6 years | 7 years | 12 years |
|---|---|---|---|---|---|---|
| 1340 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1270 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1210 | 1 | 1 | 1 | 1 | 1 | 1 |
| 930 | 1 | 1 | 1 | 1 | 1 | 1 |
| 860 | 1 | 1 | 1 | 1 | 1 | 1 |
| 780 | 1 | 1 | 1 | 1 | 1 | 1 |
| 615 | 1 | 1 | 1 | 1 | 1 | 1 |
| 510 | 0 | 0 | 0 | 0 | 1 | 1 |
| 470 | 1 | 1 | 1 | 0 | 1 | 1 |
| 430 | 1 | 0 | 0 | 0 | 0 | 0 |
| 390 | 0 | 0 | 0 | 0 | 1 | 1 |
| 280 | 0 | 1 | 1 | 1 | 1 | 1 |
| 240 | 1 | 0 | 0 | 0 | 0 | 0 |
| 200 | 0 | 1 | 0 | 0 | 0 | 0 |
| 170 | 0 | 1 | 1 | 0 | 0 | 0 |
| 135 | 0 | 1 | 0 | 0 | 0 | 0 |
| 80 | 0 | 0 | 1 | 0 | 0 | 0 |

* (1) presence, (0) absence.

The results also showed that the bands with molecular sizes of 430 and 240 bp were presented only in control (volunteer never exposed to plastic or its solvents vapors) but they were absent in the blood samples of the exposed volunteers for the different periods under investigation.

On the other hand, two bands were developed with length of 510 and 390 bp were absent only in the blood sample of withdrawn from volunteer who exposed to plastic or its solvents vapors for 7 and 12 years while they were absent in the blood samples of all the volunteers exposed to plastic or its solvents vapors from one year to 6 years exposure and control. Moreover, one band with length of 280 bp was absent only in the blood samples of control while it was present in blood samples withdrawn from volunteers exposed to plastic or its solvent vapors for periods of 1, 3, 6, 7 and 12 years. These five aforementioned bands reflected a kind of trending modifications in the genomic DNA of the volunteers exposed to plastic or its solvents vapors for different lengths if compared with the banding patterns of the blood sample which withdrawn from control volunteer. These changes in the banding patterns between the blood samples withdrawn from the exposed volunteers and the blood samples of the non-exposed volunteer could be due to modification in the nitrogen bases unless the primer could not recognized the complement sequences and that is avoid the DNA amplification using polymerase chain reaction (PCR).

### 3.4. Primer Operon E-19

The results of primer (OP E-19) are shown in Figure (4) and Table (5). This primer resulted in kindly polymorphic products with the six blood samples under investigation. The PCR

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products using this primer resulted in a total number of eleven bands with molecular sizes ranged from 260 to 910 bp. nine bands out of the eleven produced bands using this primer were monomorphic bands with lengths of 910, 870, 800, 680, 600, 510, 440, 310 and 260 bp among the six blood samples under investigation.

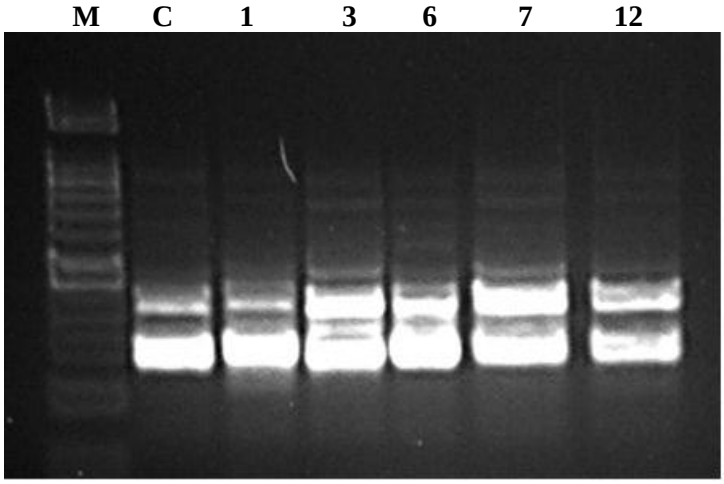


**Figure 4.** Banding patterns of the six blood samples using RAPD-PCR primer (OP E-19) represented different exposure periods for plastic and its solvents vapors during manifesting processes

**Table 5.** Screening of RAPD banding patterns in the six blood samples using RAPD-PCR primer (OP E-19) represented different exposure periods for plastic and its solvents vapors during manifesting processes

| Bp | control | 1 year | 3 years | 6 years | 7 years | 12 years |
|---|---|---|---|---|---|---|
| 910 | 1 | 1 | 1 | 1 | 1 | 1 |
| 870 | 1 | 1 | 1 | 1 | 1 | 1 |
| 800 | 1 | 1 | 1 | 1 | 1 | 1 |
| 730 | 0 | 0 | 1 | 1 | 1 | 1 |
| 680 | 1 | 1 | 1 | 1 | 1 | 1 |
| 600 | 1 | 1 | 1 | 1 | 1 | 1 |
| 510 | 1 | 1 | 1 | 1 | 1 | 1 |
| 440 | 1 | 1 | 1 | 1 | 1 | 1 |
| 380 | 0 | 0 | 1 | 1 | 1 | 1 |
| 310 | 1 | 1 | 1 | 1 | 1 | 1 |
| 260 | 1 | 1 | 1 | 1 | 1 | 1 |

* (1) presence, (0) absence.

The results also showed that two bands with molecular sizes of 730 and 380 bp were absent only in control (volunteer never exposed to plastic or its solvents vapors) and one year exposure but they were present in the blood samples of the exposed volunteers for the periods of 3, 6, 7 and 12 years. These two aforementioned bands reflected a kind of trending modifications in the genomic DNA of the volunteers exposed to plastic or its solvents vapors for different lengths if compared with the banding patterns of the blood sample which withdrawn from control volunteer. These changes in the banding patterns between the blood samples withdrawn from the exposed volunteers and the blood samples of the non-exposed

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volunteer could be due to modification in the nitrogen bases unless the primer could not recognized the complement sequences and that is avoid the DNA amplification using polymerase chain reaction (PCR).

### 3.5. Primer Operon Q-16

The results of primer (OP Q-16) are shown in Figure (5) and Table (6). This primer resulted in kindly polymorphic products with the six blood samples under investigation. The PCR products using this primer resulted in a total number of thirteen bands with molecular sizes ranged from 150 to 1790 bp. Eleven bands out of the thirteen produced bands using this primer were monomorphic bands with lengths of 1790, 1710, 1580, 1270, 1120, 980, 760, 615, 480, 330 and 150 bp among the six blood samples under investigation.

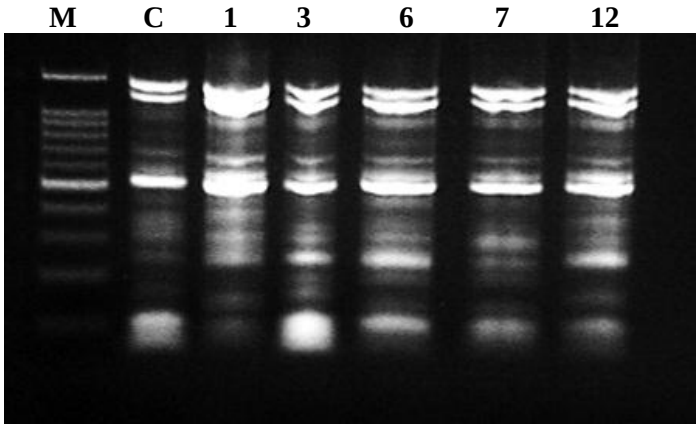


**Figure 5.** Banding patterns of the six blood samples using RAPD-PCR primer (OP Q-16) represented different exposure periods for plastic and its solvents vapors during manifesting processes

**Table 6.** Screening of RAPD banding patterns in the six blood samples using RAPD-PCR primer (OP Q-16) represented different exposure periods for plastic and its solvents vapors during manifesting processes

| Bp | control | 1 year | 3 years | 6 years | 7 years | 12 years |
|---|---|---|---|---|---|---|
| 1790 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1710 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1580 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1270 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1120 | 1 | 1 | 1 | 1 | 1 | 1 |
| 980 | 1 | 1 | 1 | 1 | 1 | 1 |
| 950 | 1 | 1 | 0 | 0 | 0 | 0 |
| 760 | 1 | 1 | 1 | 1 | 1 | 1 |
| 615 | 1 | 1 | 1 | 1 | 1 | 1 |
| 480 | 1 | 1 | 1 | 1 | 1 | 1 |
| 330 | 1 | 1 | 1 | 1 | 1 | 1 |
| 190 | 0 | 0 | 1 | 1 | 1 | 1 |
| 150 | 1 | 1 | 1 | 1 | 1 | 1 |

* (1) presence, (0) absence.

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The results also showed that a band with molecular size of 950 bp was present only in control (volunteer never exposed to plastic or its solvents vapors) and one year exposure but it was absent in the blood samples of the exposed volunteers for the periods of 3, 6, 7 and 12 years.

On the other hand, a band with molecular size of 190 bp was absent only in control (volunteer never exposed to plastic or its solvents vapors) and one year exposure but it was developed in the blood samples of the exposed volunteers for the periods of 3, 6, 7 and 12 years. These two aforementioned bands reflected a kind of trending modifications in the genomic DNA of the volunteers exposed to plastic or its solvents vapors for different lengths if compared with the banding patterns of the blood sample which withdrawn from control volunteer.These changes in the banding patterns between the blood samples withdrawn from the exposed volunteers and the blood samples of the non-exposed volunteer could be due to modification in the nitrogen bases unless the primer could not recognized the complement sequences and that is avoid the DNA amplification using polymerase chain reaction (PCR).

### 3.6. Primer Operon Q-18

The results of primer (OP Q-18) are shown in Figure (6) and Table (7). This primer resulted in kindly polymorphic products with the six blood samples under investigation. The PCR products using this primer resulted in a total number of ten bands with molecular sizes ranged from 75 to 1630 bp. Nine bands out of the ten produced bands using this primer were monomorphic bands with lengths of 1630, 615, 570, 500, 430, 350, 225, 160 and 75 bp among the six blood samples under investigation.

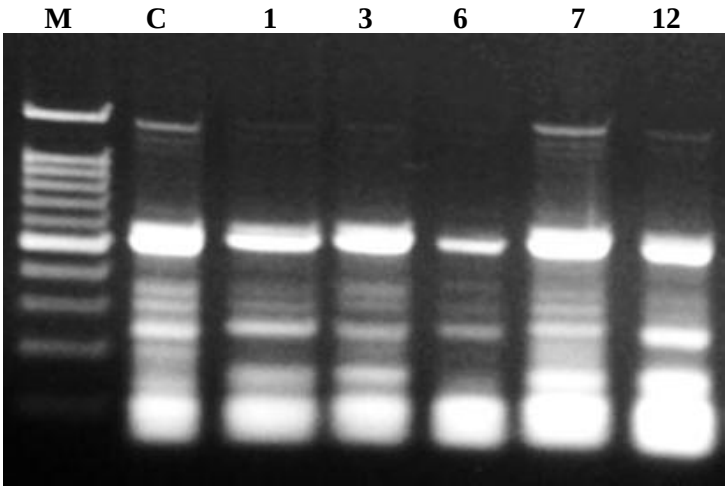


**Figure 6.** Banding patterns of the six blood samples using RAPD-PCR primer (OP Q-18) represented different exposure periods for plastic and its solvents vapors during manifesting processes

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**Table 7.** Screening of RAPD banding patterns in the six blood samples using RAPD-PCR primer (OP Q-18) represented different exposure periods for plastic and its solvents vapors during manifesting processes*

| Bp | control | 1 year | 3 years | 6 years | 7 years | 12 years |
|---|---|---|---|---|---|---|
| 1630 | 1 | 1 | 1 | 1 | 1 | 1 |
| 690 | 1 | 0 | 0 | 0 | 0 | 0 |
| 615 | 1 | 1 | 1 | 1 | 1 | 1 |
| 570 | 1 | 1 | 1 | 1 | 1 | 1 |
| 500 | 1 | 1 | 1 | 1 | 1 | 1 |
| 430 | 1 | 1 | 1 | 1 | 1 | 1 |
| 350 | 1 | 1 | 1 | 1 | 1 | 1 |
| 225 | 1 | 1 | 1 | 1 | 1 | 1 |
| 160 | 1 | 1 | 1 | 1 | 1 | 1 |
| 75 | 1 | 1 | 1 | 1 | 1 | 1 |

* (1) presence, (0) absence.

The results also showed that a band with molecular size of 690 bp was present only in control (volunteer never exposed to plastic or its solvents vapors) but it was absent in the blood samples of the exposed volunteers for the periods of 1, 3, 6, 7 and 12 years. This aforementioned band reflected a kind of trending modifications in the genomic DNA of the volunteers exposed to plastic or its solvents vapors for different length of periods if compared with the banding patterns of the blood sample which withdrawn from control volunteer. These changes in the banding patterns between the blood samples withdrawn from the exposed volunteers and the blood samples of the non-exposed volunteer could be due to modification in the nitrogen bases unless the primer could not recognized the complement sequences and that is avoid the DNA amplification using polymerase chain reaction (PCR).

In general the six primers which produced several differences in the banding patterns between the genomic DNA of the volunteer who never exposed to plastic or its solvents vapor (control) and the genomic DNA of the volunteers exposed to plastic or its solvents vapor for different period lengths during plastic manifesting. These results were in agreement with the findings of Wu *et al*. (2010) who stated that maternal exposure to DEHP was shown to increase DNA methylation and expression levels of DNA methyltransferases in mouse testis. Fetal testis was a main target for DEHP as evidenced in testicular dysgenesis syndrome due to a reduction in insulin-like hormone 3 (INSL3) expressions and testosterone production Also confirmed by Kundakovic *et al.* (2011) who reported that molecular mechanisms that underlie the long-lasting effects of BPA and phthalates continue to be elucidated, and they likely involve disruption of epigenetic programming of gene expression during development. It will be important to determine whether epigenetic markers in more accessible tissues correlate with epigenetic markers in target tissues. So, their results strongly imply that exposures to endocrine-disrupting chemicals (EDCs) may have cumulative adverse effects on future generations and that these effects could be mediated through epigenetic mechanisms.

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## 4. Conclusion

The study showed several differences in the banding patterns was recorded between the control volunteer and volunteer and those who exposed to plastic and its solvents vapor for different exposure periods. These changes in the banding patterns suggested that a modification in the genomic DNA occurred in form of modifications in the nitrogen bases of the genomic DNA as a response to the exposure to plastic vapor for a different time exposure.

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