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DETERMINATION OF PARA-PHENYLENEDIAMINE IN HENNA COSMETIC PRODUCTS IN BENGHAZI

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ABSTRACT

Red or natural henna is a very popular cosmetic preparation used in Libya as part of many traditions. Black henna is another type of cosmetic products and it may contain paraphenylene-diamine (PPD), it is chemical substance which can stain the skin black quickly, but can cause toxic reactions such as severe allergic reactions . **the aim of our study** to detect the level of PPD in various cosmetic preparations. **Method:** ten samples were collected from different sites in Benghazi city during august 2022 and they analyzed quantivily and qualititvilty for detection PPD level in the sample using Gas chromatography–mass spectrometer (GC-MS). **Results**: Seven samples were containing PPD with a large variations in their abundance (from **2.33 to 37.04 %) and the rest three** samples were free from PPD. The level of PPD in six (6) samples were higher than allowed maximum concentration of PPD in hair dyes as specified by European Union EU, which is 6.0% however the EU banned the use of PPD directly on the skin. **Conclusion**: The presence of PPD in black henna will definitely lead to many subsequent problems such as allergic skin reactions and may cause toxicity to organs such as the heart and kidneys and may lead to death if the patient is not treated quickly.

Keywords: henna, black henna, para-Phenylenediamine **,** toxicity

INTRODUCTION :

Red Henna or natural henna is a flowering plant (*Lawsonia inermis*, family Lythraceae) or shrub native to tropical and subtropical regions of Africa, and Southern Asia (**Fig. 1**). Henna contains a burgundy dye molecule, lawsone (2-hydroxy-1,4-naphthoquinone) **[1, 2**] (**Fig. 2**).

Fig. 1: Leaves of *Lawsonia inermis* [3]

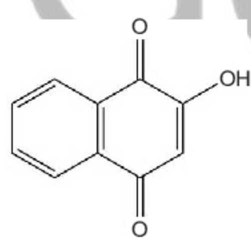

Fig. 2: Chemical structure of lawsone [4]

Lawsone molecule has the ability to bind with proteins, and consequently has been widely used in body art to dye skin, hair and fingernails, and to dye silk, leather, and wool. Henna body art is made by applying henna paste to the skin. Henna paste is prepared by drying the henna leaves and grinding them to powder, and then this powder is mixed with oil or water to form the paste. When this henna paste is applied to the skin the dye (lawsone) migrates from the paste to the outermost layer of the skin; more lawsone will migrate if the paste is left on the skin for a longer time, thus creating a red-brown stain [**4].**

Henna is used since antiquity to dye skin, hair, fingernails, leather and wool. The name is also used for preparations used from the plant, and for the art of temporary tattooing based on these dyes. Additionally, the name is misused for other skin and hair dyes, such as black henna or neutral henna, which do not derive from the plant. Black henna powder may be derived from indigo, or it may also contain unlisted dyes and chemicals that called Black henna [**5]**.

Black henna may contain paraphenylene-diamine (PPD), which can stain the skin black quickly, but can cause severe allergic reactions. Allergic reactions to PPD include rashes, contact dermatitis, itching, blisters, scarring and potentially harmful systemic effects [6].

para-Phenylenediamine (PPD) is an aromatic amine compound; it is derivative of aniline (**Fig**. **3**), its chemical formula is $C_6H_8N_2$ and its molecular weight is 108.15 g/mol.[2] PPD is a colorless, slightly pink, gray or yellow crystalline solid powder (**Fig. 4**). On oxidation, usually through exposure to air, it turns red, brown, then finally black. PPD is described as a black mineral from the banks of Nile River [**5**].

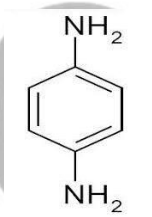

Fig. 3: *para*-Phenylenediamine chemical structure [6].

Fig. 4: Crystal form of PPD

PPD is widely used in many products such as a hair dye, textiles, leather and temporary tattoo, and in photochemical measurements[4]**.** It is primarily used as an ingredient of oxidative hair coloring products at a maximal concentration of 4.0%, however, after mixing in a 1:1 ratio with hydrogen peroxide prior to use this concentration will be 2% at the time of application to the hair[**7].**

PPD is a widely used in the Middle East, parts of Africa, India, and Pakistan in blend with Lawsonia alba leaves (henna) or as a substitute to dye the hair [**8].** In Libya, It is used in combination with natural henna for tattooing hands and feet with henna to give black color in a short time in traditional and during local and social festival. It was found to be toxic and there are some reports from these countries showing its toxicity on different systems of the body. The consumers use this product because its price is less expensive than cosmetic preparations**.**

PPD poisoning is amongst one of the emerging causes of poisoning in many countries like in Libya. It is a constituent of many cosmetic products such as Black henna marked with many trade names and hair dye formulation. PPD is crushed, mixed with henna and used as hair dye for enhancing its color or used for skin tattoos [**9].**

Dermal exposure study of environmentally available chemicals is a new initiative in the field of toxicology research. This is particularly more relevant in case of chemicals that find its way into human system through the skin[4]**.**Individuals may be occupationally exposed to PPD during its manufacture or use, and the exposure may occur through inhalation, skin and/or eye contact, and ingestion [**5,10].**

Prolonged skin exposure to PPD can lead to toxicity. PPD is well known as a skin irritant and sensitizer, and allergic contact dermatitis may appear following exposure to PPD in hair dye and skin tattoo pastes [**9].** PPD exposure has been shown to increase in the production of reactive oxygen species (ROS), lipid peroxidation (LPO), the collapse of mitochondrial membrane potential (MMP), and the release of cytochrome c in skin fibroblast cells [**11]**. PPD exposure has been shown to downregulate Claudin-1 (CLDN-1), *CLDN8*, *CLDN11*, CXADR-like membrane protein *(CLMP)*, occludin *(OCLN)* proteins that are important in keeping the integrity of skin barrier [**12]**. Moreover, following PPD exposure, HaCaT human keratinocyte cells' gene expression profiles showed changes in genes, and database analysis highlighted many biological processes and pathways that were likely connected with these differentially expressed genes [**13].** Furthermore, PPD exposure has been shown to induce hepatotoxicity manifested by increased liver enzymes such as aspartate transaminase (AST), serum alanine transaminase (ALT) & serum alkaline phosphatase (ALP) in albino rats and DNA damage in liver cells [**14].**

Overall, determination the levels of PPD in cosmetic products is important in order to prevent black henna toxicity. By targeting these pathways, researchers hope to improve the safety of skin tattoo pastes and reduce potential harm caused by prolonged exposure to this chemical.

MATERIALS AND METHOD.

Samples:

The analyzes were carried out on ten (10) mixed samples of henna, seven (7) samples were marketed as black henna and three (3) samples were marked as red henna. All henna samples were collected in august 2022 from different stores inside Benghazi (Badriya market, Al-Salmani area, Al-Berka area).

Materials:

Solvents were taken by Nawah Laboratories (solvents produced by Oxford-Laboratory reagent) in Egypt. All analyzes were performed in Nawah laboratories (Nawah scientific Egypt).

Method

The samples were analyzed in September 2022. The chemical composition of samples were analyzed using Gas chromatography–mass spectrometer (GC-MS) in Nawah scientific center, Egypt.

RESULTS AND DISCUSSION

In recent years, there are an increasing number of the studies about poisoning of PPD-containing cosmetic products such as hair dyes and black henna in developing countries [**15]** and recently, interest in it has begun in more developed countries. It is now possible to obtain direct evidence of PPD containing products poisoning situation in adults with suicidal intentions, As well as children who are exposed to contact with PPD, people who use it for cosmetic purposes, and workers who come into contact with it during industry or while working in beauty salons [**16]** .

Many cases of contact allergy had been reported in many countries like in Egypt and Sudan after Local application of black henna on skin. In Egypt, many cases were reported with eytherma and inflammation after application black henna on skin (Picture 1) **[17]**. In Libya, many cases of toxicities were reported after the use of black henna with the same signs.

A **B**

Picture 1: Erythema, vesicles(A) , and inflammation(B) on the skin of two patients after black henna application on skin in Egypt

A variety of analytical methods were used for determination of PPD in cosmetic products as gas chromatography coupled mass spectrometry (GC/MS) [**18,19]**, inductively coupled plasma mass spectrometry (ICP/MS)**,** high-performance liquid chromatography (HPLC), and capillary electrophoresis (CE)**.** In our study, GC-MS is the method used for detection of PPD in all samples qualitatively and quantitatively. The advantage of using GC-MS determines PPD and its derivatives [**20,21]**.

The figures from 5 to 14 show the chromatograms of samples from 1 to 10 which determine the abundance of different components in the samples. The retention time (RT) of PPD in samples was between 2.53 to 2.63 min, and the abundance of PPD was between 2.33 to 37.04%. All samples mentioned as black henna(No. 1,2,3,4,5,6 and 10) were containing PPD but with a large variations in their abundance while the other samples (No. 7,8 and 9) were free from PPD and these types were mentioned as Red Henna, As shown in Table No. 1 and Fig. 5.

Sample No	**RT**	**% PPD**
1	**2.52**	**36.56**
2	**2.62**	**13.94**
3	**2.62**	**8.66**

4	**2.55**	**30.31**
5	**2.60**	**37.04**
6	**2.63**	**2.33**
7	**--**	**ND**
8	**--**	**ND**
9	**--**	**ND**
10	**2.61**	**6.60**

Table 1: Concentrations of PPD in different samples (ND=not detected)

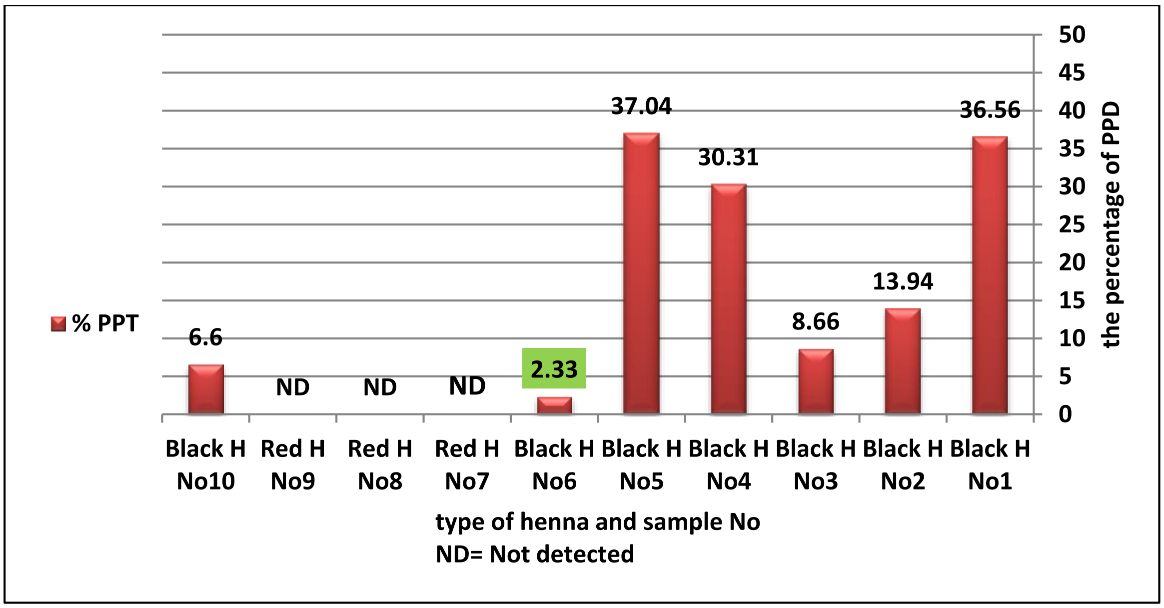

Fig. 5: The percentages of PPD in different samples of Henna

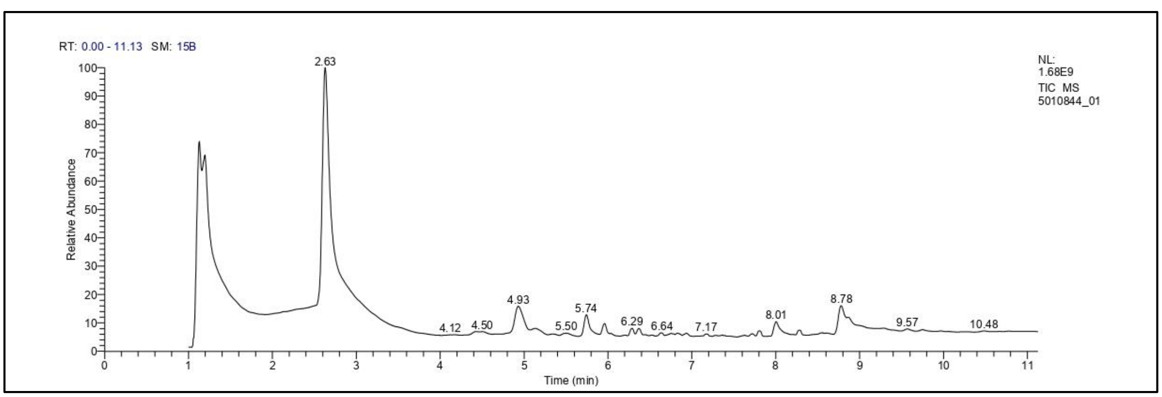

Figure 6 : the gas chromatogram for Sample No 1

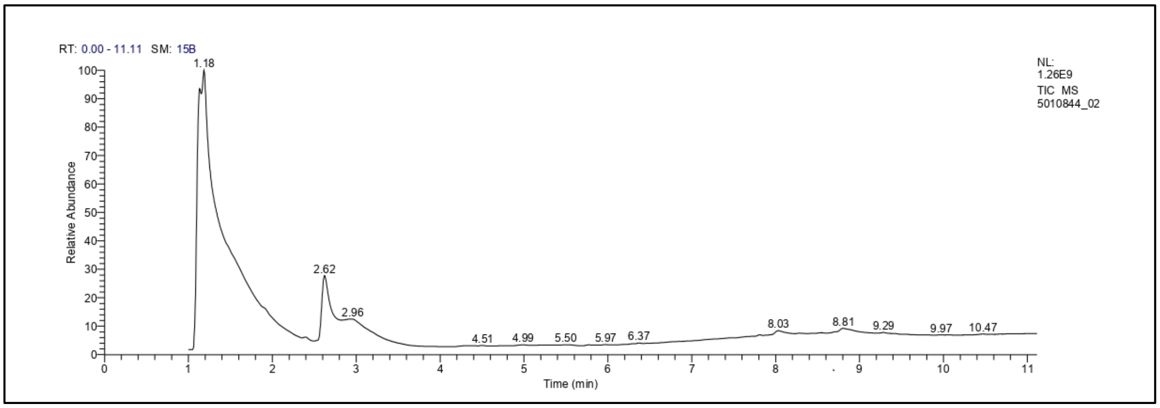

Fig. 7: the gas chromatogram for Sample No 2

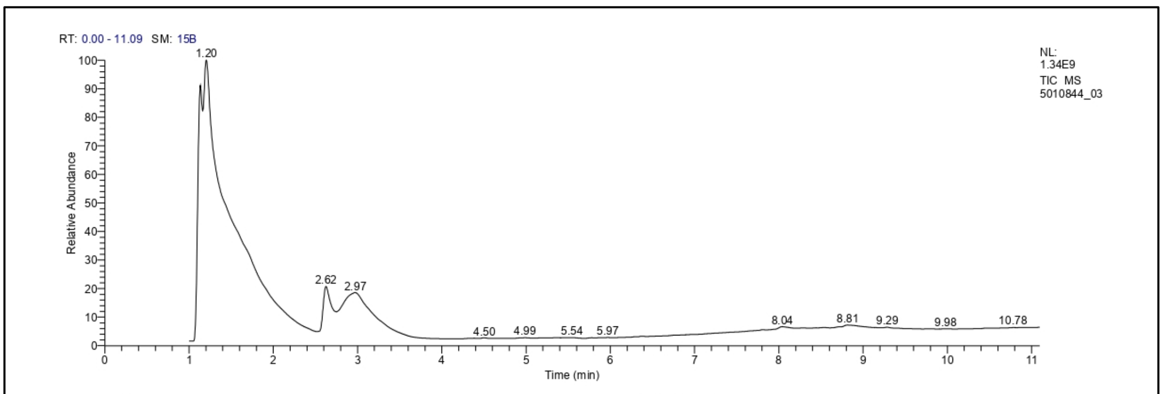

Fig. 8: the gas chromatogram for Sample No 3

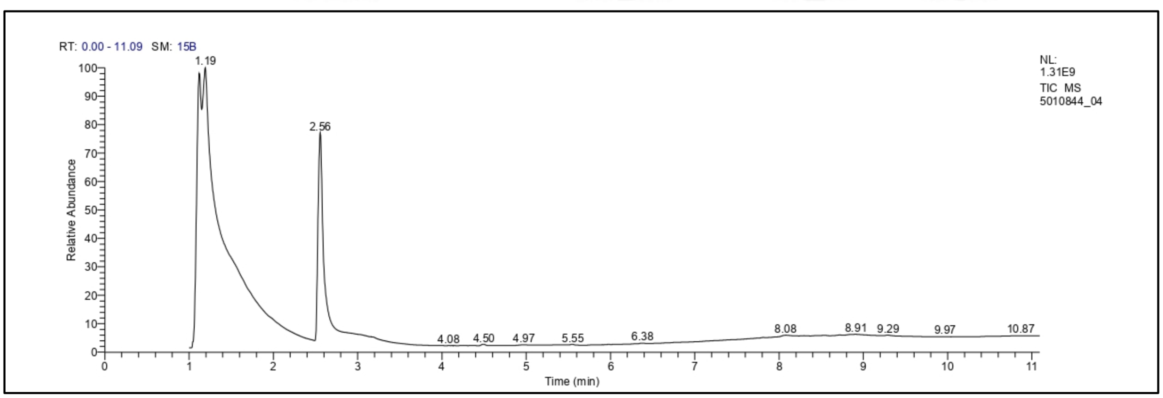

Fig. 9: the gas chromatogram for Sample No 4

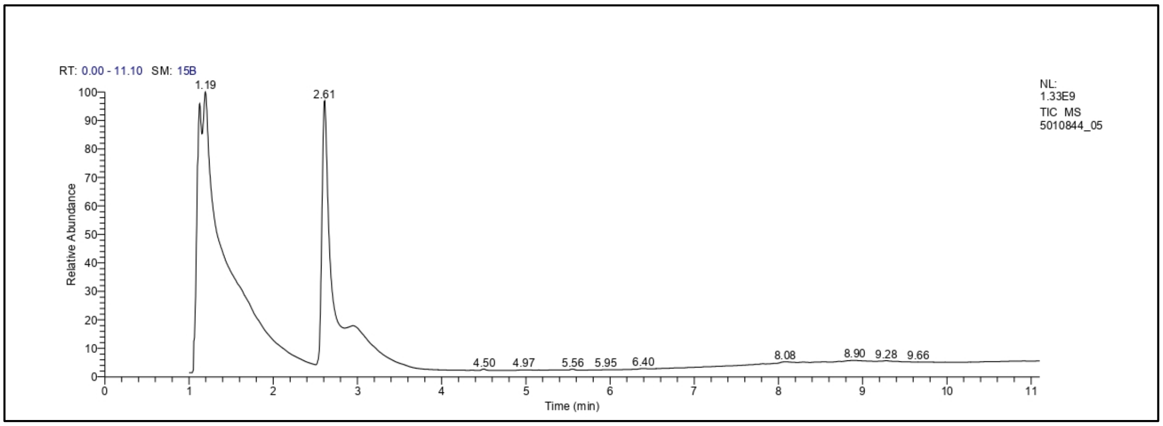

Fig. 10: the gas chromatogram for Sample No 5

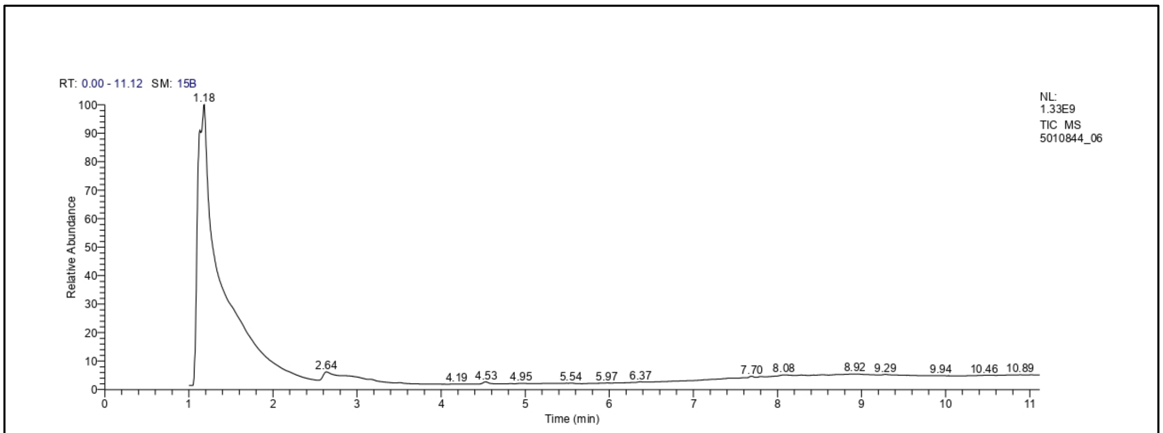

Fig. 11: the gas chromatogram for Sample No 6

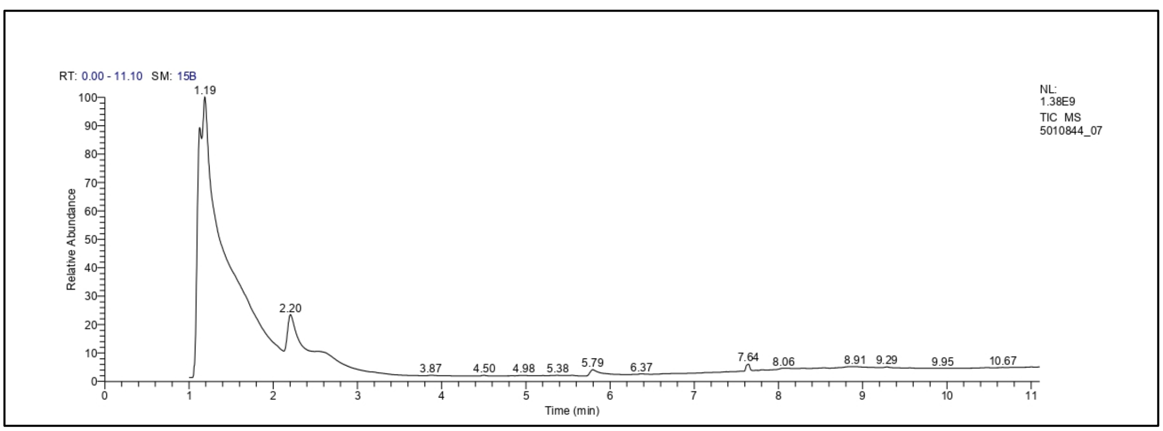

Fig. 12: the gas chromatogram for Sample No 7

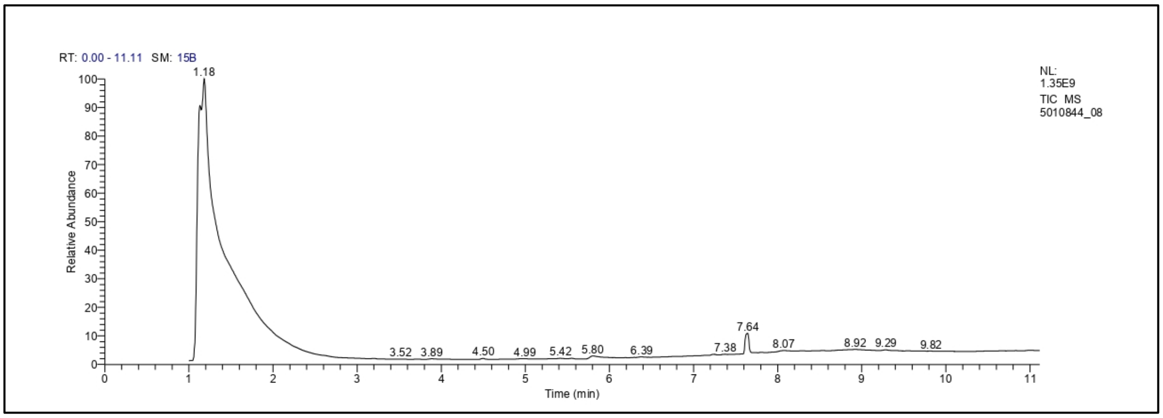

Fig. 13: the gas chromatogram for Sample No 8

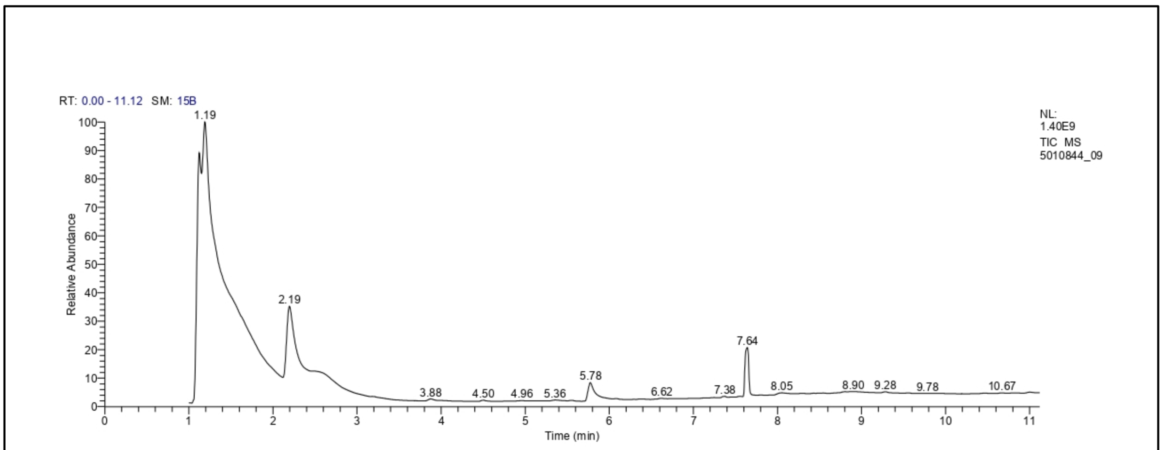

Fig. 14: the gas chromatogram for Sample No 9

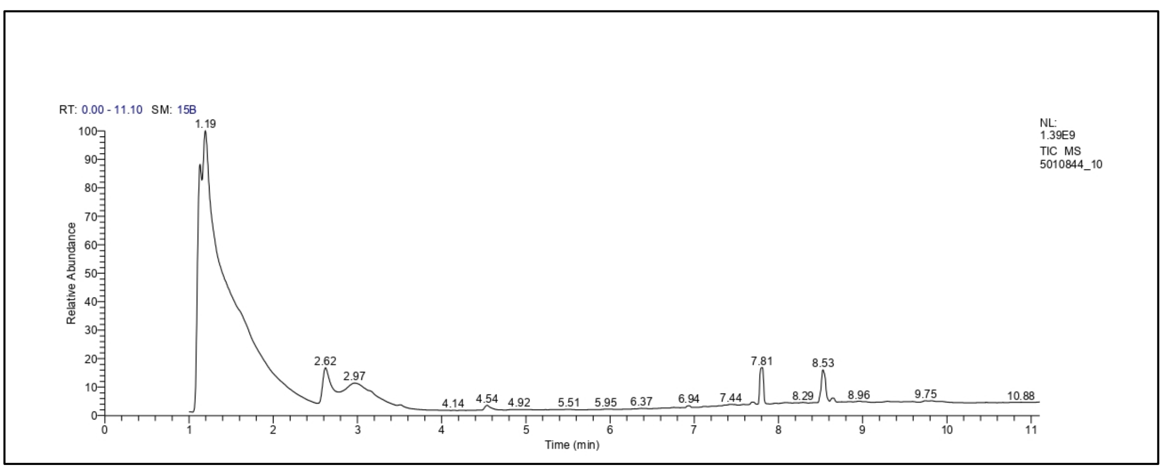

Fig. 15: the gas chromatogram for Sample No: 10

The lowest concentration of PPD was in sample No 6 (2.33%) and The highest concentrations were in samples No.1 and 5 (36.56% and 37.04 respectively). In our study, the level of PPD in samples No 2,3 4 and 10 (13.94, 8.66 30.31 and 6.6.0 respectively) were higher than allowed maximum concentration in hair dyes and skin tattoo products as specified by European Union, which is 6.0% , and this finding is consistent with other researchers who found the level of PPD in henna tattoos was much higher than that found in hair color. However, the European Union has banned the use of PPD directly on the skin, eyelashes or eyebrows, and Food and Drug administration (FDA) has defined PPD as "Coal-tar hair dyes", prohibited the use of PPD directly on the skin [**22]**.

Our overall results showed that the concentrations of PPD in our samples were higher than the concentrations determined by other researchers in (0.4%-29.5%)[7], where the concentrations of PPD was ranged between 11.2% and 26.9%..

CONCLUSION

The presence of PPD poisoning in black henna has been proven beyond doubt, although the commercial and private interests of the beneficiaries may try to hide this fact, either by denying this fact or claiming that the percentages of PPD are small and have no effect.

According to what we have come to know, the presence of black henna that contains PPD is an important reason for the toxicity and sometimes it the arrival of a deadly poison.

The presence of PPD in black henna will definitely lead to this substance reaching the skin, which will result in many subsequent problems for many organs such as the heart and kidneys and may lead to death if the patient is not treated quickly.

Not including black henna as a product to which international standards of quality must be applied in third world countries, greatly contributed to the passage of these toxins without direct control by governments.

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DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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