Monitoring of Lawsone, *p*-phenylenediamine and heavy metals in commercial temporary black henna tattoos sold in Turkey

Ayca Aktas Sukuroglu[1,2], Dilek Battal[1,3,4] and Sema Burgaz[2]

[1]*Faculty of Pharmacy, Department of Toxicology, Mersin University, 33169, Mersin, Turkey,* [2]*Faculty of Pharmacy, Department of Toxicology, Gazi University, 06330, Ankara, Turkey,* [3]*Advanced Technology Education Research and Application Center, Mersin University, 33343, Mersin, Turkey, and* [4]*Faculty of Pharmacy, Department of Toxicology, Near East University, 99138, Nicosia, TRNC*

doi:10.1111/cod.12702

Summary

Background. Henna has a very low allergic potential, and severe allergic contact dermatitis is mainly caused by *p*-phenylenediamine (PPD), which is added to temporary black 'henna tattoos', and potentially also by some heavy metals.
Objective. To determine the presence of, and quantify, Lawsone, PPD and heavy metal contaminants (cobalt, nickel, lead, and chromium) in commercial temporary black henna tattoo mixtures (n = 25) sold in Turkey.
Methods. Lawsone and PPD concentrations were analysed with high-performance liquid chromatography, and heavy metal quantification was performed with inductively coupled plasma mass spectrometry.
Results. PPD was found in all 25 black henna tattoo samples purchased from tattoo shops; levels varied between 3.37% and 51.6%. Lawsone was detected (0.002–88.2%) in 21 of the 25 temporary black henna tattoo samples analysed. Heavy metal contaminant levels were 0.44–3.11 ppm for Co, 1.13–2.20 ppm for Ni, 1.59–17.7 ppm for Pb, and 35.0–76.9 ppm for Cr.
Conclusions. Our results suggest that commercial temporary black henna mixtures containing PPD levels up to 51.6% pose a risk of contact sensitization and severe allergic contact dermatitis among users. It is important to identify both the additives and metallic contaminants of black henna tattoo products; the significance of metal contaminants has still to be assessed.

Key words: contact dermatitis; heavy metal contaminants; Lawsone; *p*-phenylenediamine; temporary black henna tattoos.

Henna is a member of the Lythraceae family. The dried leaves of the plant are crushed to a powder to produce a pigment with the main ingredient Lawsone [2-hydroxy-1,4-naphthoquinone (HNQ); CAS no. 83-72-7]. Henna has been used as a hair dye, and is also applied to the hands and feet as an expression of body art in Arab, Indian and Turkish cultures (1–4). In the past 10 years, natural henna has been mixed with additives such as *p*-phenylenediamine (PPD), coffee or black tea, lemon juice, eucalyptus and clove or mustard oil to obtain a darker colour in the applications, and the new mode of this application, called 'temporary black henna tattoo', has become very popular in holiday resort areas. Although pure henna is relatively safe, with only a few reports of allergic reactions, black henna tattoos have been increasingly reported as a cause of allergic skin reactions (5–11), because PPD is known as a potent

Correspondence: Dilek Battal, Mersin University, Yenisehir Campus, Faculty of Pharmacy, Department of Toxicology, 33169, Yenisehir, Mersin, Turkey. Tel: +90 324 341 28 15/3819; Fax: +90 324 341 30 20. E-mail: diakunal@yahoo.com

Conflicts of interest: The authors have no conflicts of interests to declare. Funding: This study was supported by Mersin University Department of Scientific Research Projects (Grant number: BAP-SBE EMBB (AA) 2013-3YL).

Accepted for publication 5 September 2016

contact allergen (12, 13). Although PPD is limited in hair dye to 2% calculated as free base (14), it has still been found in unregulated temporary black henna products at alarming levels ranging up to 64%. Moreover, positive reactions to cobalt (Co) and nickel (Ni) have been observed in a patient with allergic contact dermatitis caused by henna (15).

PPD has been categorized as an allergen by the Scientific Committee on Consumer Products and Food and Drug Administration (FDA). Although PPD levels in hair dyes have been regulated in Turkey, there is no regulation for temporary henna tattoos (16–18). The aim of this study was to quantify the levels of HNQ, PPD and heavy metals such as Co, Ni, as well as chromium (Cr) and lead (Pb) contaminants, in commercial temporary black henna tattoo products.

Materials and Methods

Chemicals and reagents

Chemicals were obtained as follows: the PPD standard, purity 99%, from Sigma-Aldrich (Steinheim, Germany); the Lawsone standard (HNQ), purity 97%, from Sigma-Aldrich (Madrid, Spain); methanol, high-performance liquid chromatography (HPLC) grade, from Scharlau Chemie (Barcelona, Spain); glacial acetic acid, purity 99.5%, nitric acid, purity 70%, and hydrochloric acid, purity 37%, from Fluka (Madrid, Spain); and analytical-grade ammonium acetate, purity 99.5%, trimethylamine, purity 99.5%, and hydrogen peroxide solution for ultratrace analysis, purity 35%, from Sigma-Aldrich (Steinheim, Germany). Water was obtained from a Milli-Q™ system (Millipore, Bedford, MA, USA).

Sample collection and storage

A total of 25 commercial temporary black henna tattoo dyes used for tattooing in five different cities (Adana, City 1; Ankara, City 2; Izmir, City 3; Istanbul, City 4; and Mersin, City 5) in Turkey were collected between June and August 2013. The powder samples were bought from 15 different tattoo shops, where tattoo artists prepare the black henna tattoo mixtures themselves. They explained that black henna tattoo mixtures had been prepared by crushing the henna stone into powder and adding Indian henna powder sold as dark hair dye obtained from sellers of herbs. Özkaya et al. (19) have previously proposed that commercially available solid material sold as 'henna stone' in our local market contains high levels of PPD (up to 91%) (20, 21). Samples were collected in glass containers and kept at room temperature under dark and non-humid conditions until analysis. Analyses were completed within ~1 month after collection.

PPD and HNQ analysis

Instrumentation. Identification and quantification of the target compounds (PPD and HNQ) were performed with an Agilent 1200 Series with an HPLC system equipped with an isocratic pump (Agilent 1200 Series System, Santa Clara, CA, USA), a quaternary pump, a low carry-over autosampler, a thermostatted column compartment, and a diode array detector.

Sample preparation. The same sample preparation procedure was used for the determination of PPD and HNQ levels in collected tattoo samples by HPLC. Each of the collected samples (0.1 g) was weighed into a 10-ml volumetric flask by use of an analytical balance, diluted with water, and sonicated for 30 min. The supernatant was filtered with a 0.45-µm syringe filter, and 1 ml of prepared sample was placed into a vial with a screw top.

Chromatographic parameters. PPD levels were analysed by modifying a method developed for PPD analysis by Shao et al. (22). Briefly, PPD was separated on a μ-Bondapak C18 column (10 µm × 3.9 mm × 300 mm; Waters, Milford, MA, USA). PPD analysis was performed under isocratic conditions. The mobile phase was prepared with a methanol/water solution (90:10). Aqueous solutions were prepared with 1.54 g of NH_4Ac and 0.01% triethylamine in water. The pH was adjusted to 5.2 with acetic acid. The parameters were as follows: flow, 1.5 ml/min; column temperature, 25°C; wavelength, 254 nm; and injection volume; 20 µl. Quantification of PPD was based on a calibration curve obtained after the addition of known amounts of PPD to the water (5, 10, 15, 20 and 25 ng/ml). The correlation curve showed a linear tendency, with correlation coefficients (r^2) of 0.9997. HNQ levels were analysed by modifying a method developed and validated for HNQ analysis by Babula et al. (23). Briefly, HNQ was separated on a a reverse-phase Zorbax C18-AAA column (150 mm × 4.6 mm, 3.5 µl; Agilent). HNQ analysis was also performed under isocratic condition. Spectra were registered in the range of 190–400 nm with λ_{max} at 280 nm. The mobile phase was prepared with 0.1 mol/l acetic acid/methanol (35:65). The parameters were as follows: flow, 0.8 ml/min; column temperature, 40°C; wavelength, 280 nm; injection volume, 5 µl. Quantification of HNQ was based on a calibration curve obtained after the addition of known amounts of HNQ to the water (25, 50, 100, 150 and 200 ng/ml). The calibration curve showed a good linear tendency, with correlation coefficients (r^2) of 0.9999.

Table 1. Limits of detection (LODs) and limits of quantification (LOQs), and the linear range

Sample	Linear range (ng/ml)	LOD (ng/ml)	LOQ (ng/ml)
PPD	5–25	0.05	0.16
HNQ	25–200	0.02	0.07

LOD corresponds to a signal-to-noise ratio (S/N) of approximately 3:1. LOQ corresponds to an S/N of approximately 10:1.
HNQ, 2-hydroxy-1,4-naphthoquinone; PPD, *p*-phenylenediamine.

Method validation. The limits of detection (LODs) and limits of quantification (LOQs), considered as signal-to-noise ratios of three and 10 times the background chromatographic noise, respectively, following IUPAC recommendations (22), were determined. The LODs and LOQs, and the linear range, are shown in Table 1. The resulting calibration plots, regression data and correlation coefficients are shown in Figures S1 and S2. The extraction recovery rates of PPD and HNQ were determined by comparing the peak heights of PPD and HNQ after spiking with those of the corresponding PPD and HNQ standards. The recovery rates of PPD and HNQ were found to be approximately 99.71% and 99.04%, respectively. The precision and accuracy of the method were determined according to intra-day and inter-day assay variance. The relative standard deviations (%) were 0.26 and 0.32 for PPD and HNQ, respectively; accuracies were 99.71% for PPD and 99.04% for HNQ. These results are shown in Table 2 (24).

Heavy metal analysis

Instrumentation. The inductively coupled plasma mass spectrometry (ICP-MS) instrument used was an Agilent 7500ce Octopole Reaction System with 99.99% helium from Agilent Technologies (Tokyo, Japan), consisting of an ICP source with a plasma-shielded torch (grounded metal plate), an octapole reaction system operated in radiofrequency (RF)-only mode, and a quadrupole mass analyser with a secondary electron multiplier operating in dual mode (i.e. either a pulse-counting mode or analogue mode, depending on the ion intensity). The operating conditions were as follows: nebulizer type, concentric nebulizer; nebulizer gas (argon) flow rate, 0.9 l/min; auxiliary gas (argon) flow rate, 0.14 l/min; plasma gas (argon) flow rate, 15 l/min; reaction gas (helium) flow rate, 0.14 l/min; spray chamber (S/C) temperature, 2°C; and ICP RF power, 1500 W. The argon gas utilized was of spectral purity (99.998%). Validation parameters for the ICP-MS method are shown in Table 3.

Sample preparation. Two different sample preparation methods (A and B) were used for the determination of heavy metal levels in original samples of temporary black henna tattoos, and to quantify the levels of heavy metals passing from the original sampled temporary black henna to water. For the first sample preparation method (A), a sample (0.1 g) was taken in 300-mm-long Pyrex glass digestion tubes (Foss, MN, USA). The powder sample was then prepared with 6 ml of HCl (37%), 2 ml of HNO_3 (70%), and 2.0 ml of H_2O_2, and digested by use of a heating block. The temperature was increased gradually, with heating for 10 min up to a temperature of 180°C, and heating for 20 min at 180°C. The digestion was completed in approximately 20–30 min, as indicated by the appearance of an approximately 5.0-ml yield volume. The mixture was left to cool, and the contents of the tubes were transferred to 50-ml polypropylene volumetric tubes and then diluted 1:5 with ultrapure deionized water (25–27). We used water to extract heavy metals from the temporary black henna samples for the second sample preparation method (B). A 0.4-g sample was added to a 15-ml Falcon tube with pure water to make 10 ml of solution. The solution was heated in an 80–90°C water bath for 30 min. After being cooled, it was sonicated for 15 min, centrifuged at 2599 g for 6 min, and then filtered with a 0.45-μm syringe filter. Samples were diluted 1:5 with water, and analysed with ICP-MS (15). Heavy metal levels in tattoo samples and the levels passing from tattoos to water were expressed as ppm and ppb, respectively. The LODs and LOQs for heavy metals analysed with method A were 0.02 and 0.0008 ppm for Co, 0.0068 and 0.007 ppm for Ni, 0.25 and 0.0007 ppm for Pb, and 0.02 and 0.0008 ppm for Ni, respectively; those for heavy metals analysed with method B were 0.06 and 0.002 ppb for Co, 0.002 and 0.003 ppb for Ni, 0.83 and 0.003 ppb for Pb, and 0.07 and 0.05 ppb for Cr, respectively.

Results

The HPLC methods modified from the study of Babula et al. (23) and Shao et al. (22) were used to analyse 25 temporary henna tattoos for PPD and HNQ. Chromatograms of PPD and HNQ are shown in Figures S1 and S2, respectively. Retention times of PPD and HNQ were 3.41 and 2.82 min, respectively.

All of the temporary black henna tattoo samples used by tattoo artists contained PPD at levels between 3.37% and 51.6% (Table 4). HNQ was detected in 21 of the 25 commercial temporary black henna tattoo samples at levels ranging from 0.002% to 88.2%, whereas four temporary black henna tattoo samples did not contain HNQ (Table 5).

Metal contaminant levels determined with method A were 0.44–3.11 ppm for Co, 1.13–2.20 ppm for Ni,

Table 2. Validation parameters of the high-performance liquid chromatography method

Sample	Mean ($n = 10$)	Rec.* (%)	RSD (%)	Repeatability	Reproducibility	Uncertainty of measurement
HNQ (1.0 ng/ml)	1.01 ± 0.01	99.04	0.32	0.002	0.004	0.058
PPD (5.0 ng/ml)	4.98 ± 0.02	99.71	0.26	0.009	0.019	0.035

HNQ, 2-hydroxy-1,4-naphthoquinone; PPD, *p*-phenylenediamine; RSD, relative standard deviation.
*Rec.: recovery, accuracy.

Table 3. Validation parameters of the inductively coupled plasma mass spectrometry method

Sample	Method type	Calibration range (ng/ml)	Determination coefficient (R^2)	Rec.* (%)	RSD (%) (n = 10)
Co	A	0–50	0.9997	93.4	0.56
	B			103.6	0.04
Ni	A	0–50	1	98.7	0.432
	B			98.6	0.054
Pb	A	0–50	0.9998	105.4	1.22
	B			95.4	0.01
Cr	A	0–50	1	101.5	0.60
	B			98.6	0.03

RSD, relative standard deviation.
*Rec.: recovery, accuracy.

1.59–17.7 for Pb, and 35.0–76.9 ppm for Cr; levels passing from the tattoos to water determined with method B were 0.15–0.18 ppb for Co, 0.32–0.42 ppb for Ni, 0.55–0.67 ppb for Pb, and 0.13–0.38 ppb for Cr. The heavy metal levels are summarized in Table 6.

Discussion

In this study, we found PPD levels varying between 3.37% and 51.6% in all 25 black henna tattoo samples purchased from tattoo shops. HNQ was detected (0.002–88.2%) in 21 of the 25 commercial temporary black henna tattoo samples analysed; only four temporary black henna tattoo samples did not contain HNQ. We analysed heavy metal contaminants such as Cr, Co, Ni and Pb in black henna tattoo samples. Two different sample preparation methods (A and B) were used in order to determine the heavy metal levels of original samples of temporary black henna tattoos, and the level passing from the original sampled temporary black henna to water in our study. The latter approach (method B) is considered to reflect actual skin exposure because the the tattoo products were prepared with water. Metal contaminant levels determined with method A were found to be 0.44–3.11 ppm for Co, 1.13–2.20 ppm for Ni, 1.59–17.7 for Pb, and 35.0–76.9 ppm for Cr; according to method B, levels passing from the tattoos to water were 0.15–0.18 ppb for Co, 0.32–0.42 ppb for Ni, 0.55–0.67 ppb for Pb, and 0.13–0.38 ppb for Cr. On the basis of the overall mean levels, the mean heavy metal levels in henna tatto mixtures were in the following decreasing order: Cr > Pb > Ni > Co.

It is known that the dyeing component of henna, HNQ, is relatively well tolerated (1). However, there are some reports of adverse health effects of topical application of henna in glucose-6-phosphate dehydrogenase-deficient persons (16). The addition of PPD, a well-known extremely potent contact allergen, massively increases the risk of allergic contact dermatitis being caused by black henna tattoo mixtures, and a vast number of cases have been reported in several countries (e.g. 16–18) as well as in Turkey (28, 29). It is also known that patients who have applied black henna tattoos and have become sensitized to PPD will then cross-react with other para-amino compounds available in hair dyes (30, 31) or dyes used in textiles (32), or, of course, a PPD-based oxidative hair dye.

European regulations and our national regulations allow a maximum level of 2% PPD calculated as free base in hair dyes, whereas there is no limit in the United States. However, the FDA prohibits the use of PPD for direct skin applications (14, 33, 34). The Scientific Committee on Consumer Safety (SCCS) of the European Commission has recently considered the use of *Lawsonia inermis* (henna) as a hair dye with a maximum level of Lawsone of 1.4% as safe (SCCS/1511/13). The presence of other species or other constituents is not allowed in products named as henna. However, the traditional and currently increasing use of henna as a body paint has not yet been assessed (35).

The importation of temporary tattoos containing PPD or with inappropriate labelling has been banned in the United States, Canada, New Zealand, and Australia (18).

Table 4. *p*-Phenylenediamine levels (wt %) in commercial black henna samples*

Sample	City 1	City 2	City 3	City 4	City 5
Sample 1	8.64 ± 0.76	16.54 ± 0.56	41.86 ± 1.57	12.9 ± 1.41	25.45 ± 1.43
Sample 2	18.47 ± 1.41	32.27 ± 1.41	24.92 ± 2.13	31.47 ± 1.55	7.36 ± 1.41
Sample 3	14.44 ± 1.55	23.31 ± 1.55	29.36 ± 1.49	51.59 ± 0.14	27.15 ± 1.41
Sample 4	14.36 ± 0.28	13.04 ± 1.40	41.99 ± 1.41	14.34 ± 1.56	5.37 ± 1.55
Sample 5	5.88 ± 1.69	17.66 ± 1.41	28.16 ± 0.71	3.37 ± 1.41	27.17 ± 1.41

*Data are presented as the mean ± standard deviation of triplicate experiments.

Table 5. 2-Hydroxy-1,4-naphthoquinone levels (wt %) in commercial black henna samples*

Sample	City 1	City 2	City 3	City 4	City 5
Sample 1	0.04 ± 0.10	0.17 ± 0.001	ND	0.02 ± 0.001	0.079 ± 0.001
Sample 2	0.043 ± 0.001	0.12 ± 0.001	0.002 ± 0.01	0.07 ± 0.00	88.2 ± 0.577
Sample 3	0.011 ± 0.001	0.14 ± 0.405	11.26 ± 0.00001	0.08 ± 0.007	0.12 ± 0.001
Sample 4	ND	0.63 ± 0.00	ND	0.051 ± 0.01	13.94 ± 0.208
Sample 5	ND	0.22 ± 0.001	0.027 ± 0.0001	0.045 ± 0.00	0.046 ± 0.001

ND, not detected.
*Data are presented as the mean ± standard deviation of triplicate experiments.

However, at present, there is no regulatory statute on the practice of application of henna tattoos by tattoo artists in Turkey (33) or in most other countries (18).

The use of most metals as active ingredients in cosmetic products is not allowed in most countries, owing to their toxicity. According to Annex II of Directive 76/768/EEC, metals such as antimony, arsenic, cadmium, Cr, Co, mercury, Ni and Pb are banned from use in cosmetics, because they are considered to be hazardous (36). Nevertheless, these substances may still be present in cosmetics as contaminants in trace amounts. Thus, from the perspective of public health, it was considered important to analyse HNQ, PPD and heavy metals such as Co, Ni, Cr and Pb in commercial temporary black henna tattoo mixtures collected from tattoo shops in Turkey.

As shown in Table 4, there are considerable differences between PPD levels in commercial black henna tattoo preparations. The highest level of PPD found in our study (51.59%) was higher than that reported in the studies of Brancaccio et al. (15.7%) (37), Kang and Lee (2.35%) (15), and Al-Suwaidi and Ahmed (29.5%) (38), but lower than that in the Almeida et al. study (64%) (39). Özkaya et al. have found PPD levels ranging between 89.99% and 90.90% in samples called 'henna stone' bought from Turkish sellers of herbs (19). In our study, PPD levels in all of the commercial black henna samples analysed were (much) higher than the permitted level of PPD (2%) in hair dye products established by the European and our national regulations (14, 33). This finding is in accordance with the above-mentioned studies.

In general, it is known that henna (*L. inermis*) contains HNQ at levels of 0.5–2% (34). As shown in Table 5, HNQ levels were below the LOD by up to 0.63% in 22 of the 25 commercial temporary black henna tattoo samples, indicating that 88% of the samples were almost free of HNQ in our study. El-Shaer et al. (40) found HNQ levels of 0.004–0.608% in commercial henna powders, which is similar to our results. Almeida et al. (39) found HNQ at levels of 0.21–2.27% in commercial henna samples. These authors also found that only one of the three products used by tattoo artists contained HNQ (0.21–0.35%) (39). Gallo et al. reported that henna raw material comes from several countries, and that investigation of the constituents is often lacking. Thus, origin, purity and preparation techniques as principal features for the quality and safety of henna are often unknown. It has also been stated that commercial products called 'henna' can contain other natural dyes (41). In our study, HNQ levels in three tattoo samples were much higher (11%, 13.94%, and 88.2%) than in the other samples that we analysed (Table 5), as well as in the other two studies cited (36, 37). It seems that synthetic HNQ may have been added to these tattoo samples as an adulterant to enhance its activity, as suggested by Gallo et al. (41).

Regarding the heavy metal contaminants, Pb and Cr levels, but not Ni levels, in the tattoo samples analysed with sample preparation method A in our study were higher than those in the henna samples used in the SCCS assessment (Table 6). The levels of heavy metal impurities such as Pb, Cr and Ni were 1.04, 9.4 and 8.06 ppm, respectively, in the safety assessment of *L. inermis* (henna) as a hair dye by the SCCS (35). Comparable results were found regarding Pb levels in other studies using a similar sample preparation procedure (26, 27). Moreover, the Co

Table 6. Heavy metal levels in black henna tattoos determined with methods A and B*

	Co		Ni		Pb		Cr	
Sample	A (ppm)	B (ppb)	A (ppm)	B (ppb)	A (ppm)	B (ppb)	A (ppm)	B (ppb)
City 1 (n = 5)	1.89 ± 0.58	0.18 ± 0.02	1.40 ± 0.35	0.42 ± 0.13	9.16 ± 2.07	0.67 ± 0.2	52.29 ± 5.81	0.23 ± 0.06
City 2 (n = 5)	3.11 ± 1.35	0.17 ± 0.01	2.20 ± 0.82	0.40 ± 0.11	17.7 ± 10.24	0.58 ± 0.05	76.9 ± 21.83	0.22 ± 0.03
City 3 (n = 5)	1.04 ± 0.001	0.15 ± 0.01	1.13 ± 0.17	0.42 ± 0.14	7.08 ± 0.76	0.64 ± 0.06	55.25 ± 2.13	0.38 ± 0.24
City 4 (n = 5)	0.74 ± 0.61	0.15 ± 0.02	2.10 ± 1.17	0.33 ± 0.17	6.69 ± 0.8	0.58 ± 0.06	66.8 ± 19.04	0.13 ± 0.07
City 5 (n = 5)	0.44 ± 0.35	0.15 ± 0.02	1.70 ± 0.91	0.32 ± 0.25	1.59 ± 0.49	0.55 ± 0.09	35.0 ± 47.42	0.18 ± 0.06

*Data are presented as the mean ± standard deviation of triplicate experiments for each sample.

and Ni levels in our henna tattoo samples determined with the two sample preparation methods were lower than those in the samples analysed by Kang and Lee (15).

For cosmetic colour additives, a maximum Pb level of 20 μg/g has been established by the FDA (34). As can be seen in Table 6, Pb levels in all samples determined with two sample preparation methods were below this limit. These data are consistent with the results of Kaličanin and Velimirović (42). However, Jallad et al. have found Pb levels ranging from 2.29 to 65.98 ppm in green, red and black henna samples (43). Considering the fact that there is prolonged skin contact in the course of henna tattoo application, it is important to monitor and control the heavy metal contaminant levels in these products. Basketter et al. (44), on the basis of quantitative risk assessment, have suggested that Ni, Cr and Co levels not exceeding 1 ppm in consumer products have a low risk for the induction of dermal sensitization. As shown in Table 6, Ni, Cr and Co levels determined with sample preparation method A, but not with method B, exceeded 1 ppm in almost all of the tattoo samples. We suggest that the data obtained with sample preparation method A are more appropriate, taking into account the worst-case principle in risk assessment. Our results suggest that commercial temporary black henna mixtures containing PPD up to 51.6% as well as heavy metal contaminants increase the risk of allergic contact sensitization and dermatitis among users. The scientific literature shows that the popularity of henna usage for body art has gone along with widespread adulteration with PPD of commercial henna products (45).

In conclusion, quality control measures for henna tattoo products by a declaration of the origin of henna raw material and a declaration of additives such as PPD and other synthetic dyes and metallic contaminants with appropriate labelling are needed. It will be important to monitor the HNQ levels, to identify both the additives and the metallic contaminants of black henna tattoo products. Awareness of both consumers and tattoo artists concerning the risks involved in the use of temporary black henna products should be raised.

Acknowledgements

This study was supported by Mersin University Department of Scientific Research Projects [Grant number: BAP-SBE EMBB (AA) 2013-3YL].

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Chromatograms of PPD from sample 1 (City 5).
Figure S2. Chromatograms of HNQ from sample 1 (City 1).

References

1 Kazandjieva J, Grozdev I, Tsankov N. Temporary henna tattoos. *Clin Dermatol* 2007: **25**: 383–387.
2 van Braak H. *Natürliches Henna*: Niedernhausen, Falken Verlag, 2001.
3 Raboobee F. Sensitisation from PPD in temporary henna tattoos and subsequent severe allergic contact dermatitis from hair dye. *Curr Allergy Clin Immunol* 2004: **17**: 195.
4 Le Coz C J. Allergic contact dermatitis caused by skin painting pseudotattooing with black henna, a mixture of henna and p-phenylenediamine and its derivatives. *Arch Dermatol* 2000: **136**: 1515–1517.
5 Majoie I M, Bruynzeel D P. Occupational immediate-type hypersensitivity to henna in a hairdresser. *Am J Contact Dermatitis* 1996: **7**: 38–40.
6 Wöhrl S, Hemmer W, Focke M et al. Hypopigmentation after non-permanent henna tattoo. *J Eur Acad Dermatol Venereol* 2001: **15**: 470–472.

7 Carsia Ortiz J C, Terron M, Bellido J. Contact allergy to henna. *Int Arch Allergy Immunol* 1997: **114**: 298–299.
8 Wantke F, Götz M, Jarisch R. Contact dermatitis due to henna, solvent red 1 and solvent red 3. A case report. *Contact Dermatitis* 1992: **27**: 346–347.
9 Nigam P K, Saxena A K. Allergic contact dermatitis from henna. *Contact Dermatitis* 1988: **18**: 55–56.
10 Cupta B N, Mathur A K, Agarwal C, Singh A. Contact sensitivity to henna. *Contact Dermatitis* 1986: **15**: 303–304.
11 Diepgen T L, Naldi L, Bruze M et al. Prevalence of contact allergy to p-phenylenediamine in the European general population. *J Invest Dermatol* 2016: **136**: 409–415.
12 Wakelin S H, Creamer D, Rycroft R J et al. Contact dermatitis from paraphenylenediamine used as a skin paint. *Contact Dermatitis* 1998: **39**: 92–93.
13 Chung W H, Chang Y C, Yang L J et al. Clinicopathologic features of skin reactions to temporary tattoos and analysis of possible causes. *Arch Dermatol* 2002: **138**: 88–92.
14 de Groot A C. Side-effects of henna and semi-permanent 'black henna' tattoos: a full review. *Contact Dermatitis* 2013: **69**: 1–25.
15 Kang I J, Lee M H. Quantification of para-phenylenediamine and heavy metals in henna dye. *Contact Dermatitis* 2006: **55**: 26–29.
16 Raupp P, Hassan J A, Varughese M, Kristiansson B. Henna causes life threatening haemolysis in glucose-6-phosphate dehydrogenase deficiency. *Arch Dis Child* 2001: **85**: 411–412.
17 Kind F, Scherer K, Bircher A J. Contact dermatitis to para-phenylenediamine in hair dye following sensitization to black henna tattoos an ongoing problem. *J Dtsch Dermatol Ges* 2012: **10**: 572–577.
18 Marcoux D, Couture-Trudel P M, Riboulet-Delmas G, Sasseville D. Sensitization para-phenylenediamine from a streetside temporary tattoo. *Pediatr Dermatol* 2002: **19**: 498–502.
19 Özkaya E, Yazganoglu K D, Arda A et al. The 'henna stone' myth. *Indian J Dermatol Venereol Leprol* 2013: **79**: 254–256.
20 Özkaya E, Yazganoğlu K D. Henna stone: a lesser-known solid material from which to obtain black henna paste. *Contact Dermatitis* 2013: **69**: 386: doi:10.1111/cod.12132.
21 Özkaya E, Topkarci Z. Airborne allergic contact dermatitis caused by a henna stone. *Contact Dermatitis* 2016: **75**: 191–192: doi:10.1111/cod.12599.
22 Shao B, Xu X, Yan J, Fu X. Quantitative determination of commercial oxidation hair dyes by reversed-phase HPLC. *J Liq Chromatogr Relat Technol* 2001: **24**: 241–249.
23 Babula P, Mikelova R, Potesil D et al. Simultaneous detemination of 1,4-naftoquinone, lawsone, juglone and plumbagin by liquid chromatography with UV detection. *Biomed Pap* 2005: **149** (Suppl. 1): 25–28.
24 Thompson M, Ellison S L R, Wood R. Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report). *Appl Chem* 2002: **74**: 835–855.
25 Kuria N, Sharon H, Flegal A R. Determination of lead in vinegar by ICP-MS and GFAAS: evaluation of different sample preparation procedures. *Talanta* 2004: **24**: 258–263.
26 Nouioui M A, Mahjoubi S, Ghorbel A et al. Health risk assessment of heavy metals in traditional cosmetics sold in Tunisian local markets. *Int Sch Res Notices* 2016: 1–12: doi:10.1155/2016/6296458.
27 Ibrahim S Y, Fawzi M M, Saad M G, Rahman S M A. Determination of heavy metals and other toxic ingredients in henna (Lawsonia inermis). *J Environ Anal Toxicol* 2016: **6**: 3: doi:10.4172/2161-0525.1000364.
28 Onder M, Atahan C A, Oztaş P, Oztaş M O. Temporary henna tattoo reactions in children. *Int J Dermatol* 2001: **40**: 577–579.
29 Gokalp H, Kaya K. Angioedema-like allergic contact dermatitis related to black henna. *Dermatol Online J* 2014: **20**: pii: doj_21549.
30 Saunders H, O'Brien T, Nixon R. Textile dye allergic contact dermatitis following paraphenylenediamine sensitization from a temporary tattoo. *Australas J Dermatol* 2004: **45**: 229–231.
31 Teixeira M, de Wachter L, Ronsyn E, Goossens A. Contact allergy to paraphenylenediamine in a permanent eyelash dye. *Contact Dermatitis* 2006: **55**: 92–94.
32 Boschnakow A, Treudler R, Lieps D et al. Temporary tattooing with henna induces contact allergy to textile dyes. *J Dtsch Dermatol Ges* 2003: **1**: 962–964.
33 T.C. Sağlık Bakanlığı. Kozmetik Yönetmeliği, Sayı: 26317, 12.10.2006. Available at: http://www.saglik.gov.tr/TR/belge/1-472/kozmetik-yonetmeligi.html (last accessed 03 June 2016).
34 US Food and Drug Administration. Cosmetics. Available at: http://www.fda.gov/cosmetics/productsingredients/products/ucm108569.html (last accessed 03 June 2016).
35 European Commission, Scientific Committee on Consumer Safety (SCCS). Opinion on Lawsonia inermis (Henna)COLIPA no. C169, SCCS/1511/13, 19 September 2013.
36 Chukwujindu M A, Bassey F I, Tesi G O et al. Safety evaluation of metal exposure from commonly used moisturizing and skin-lightening creams in Nigeria. *Regul Toxicol Pharmacol* 2015: **71**: 484–490.
37 Brancaccio R R, Brown L H, Chang Y T et al. Identification and quantification of para-phenylenediamine in a temporary black henna tattoo. *Am J Contact Dermat* 2002: **13**: 15–18.
38 Al-Suwaidi A, Ahmed H. Determination of para-phenylenediamine (PPD) in henna in the United Arab Emirates. *Int J Environ Res Public Health* 2010: **7**: 1681–1693.
39 Almeida P J, Borrego L, Pulido-Melian E, Gonzalez-Diaz O. Quantification of p-phenylenediamine and 2-hydroxy-1,4-naphthoquinone in henna tattoos. *Contact Dermatitis* 2011: **66**: 33–37.
40 El-Shaer N S, Badr J M, Aboul-Ela M A, Gohar Y M. Determination of lawsone in henna powders by high performance thin layer chromatography. *J Sep Sci* 2007: **30**: 3311–3315.
41 Gallo F R, Multari G, Palazzino G et al. Henna through the centuries: a quick HPTLC analysis proposal to check henna identity. *Br J Pharmacol* 2014: **24**: 133–140.
42 Kaličanin B, Velimirović D. A study of the possible harmful effects of cosmetic beauty products on human health. *Biol Trace Elem Res* 2016: **170**: 476–484.
43 Jallad K N, Espada-Jallad C. Lead exposure from the use of Lawsonia inermis (henna) in temporary paint-on-tattooing and hair dying. *Sci Total Environ* 2008: **397**: 244–250.
44 Basketter D A, Angelini G, Ingber A et al. Nickel, chromium and cobalt in consumer products: revisiting safe levels in the new millennium. *Contact Dermatitis* 2003: **49**: 1–7.
45 Gallo F R, Badoni Semwal R, Semwal D K et al. Lawsonia inermis L. (henna): ethnobotanical, phytochemical and pharmacological aspects. *J Ethnopharmacol* 2014: **155**: 80–103.