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Effects of Henna milling on spirometry parameters, fractional exhaled nitric oxide and respiratory symptoms: A cross-sectional study

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ABSTRACT

Respiratory diseases as a consequence of occupational hazardous exposure are common in many workers. Henna milling is grinding of red henna (*Lawsonia Intermis*) plant roots to produce a colorizing powder used mainly for cosmetic purposes. This processing takes place only in limited parts of the world in small workshops. This is probably the main reason for absence of any report about respiratory diseases related to this industry in the literature. Because Yazd is one of the main producers and exporters of henna in the world, we conducted the present study to turn attentions toward such industry. Another driving force for this study was rarity of respiratory complaints among workers of this industry in 25 years of experiences of one of the authors (M.R). Thus at present study we measured respiratory symptoms, spirometric parameters as well as Fractional Exhaled Nitric Oxide (FE_{NO}) among henna workers. This was a cross-sectional study of 45 workers of all eleven small traditional henna mills in Yazd, central Iran. Researchers went to all of these workshops and gathered study data from interviews, spirometry and FE_{NO} measurements in place. Spirometry and FE_{NO} measured during a work shift by a trained technician. Interview results showed that acute work-related symptoms were not so common. Furthermore, pulmonary function test results showed that FEV_1/FVC, FEV_1, FVC and FE_{NO} were most of the times at their normal predicted limits. At the next stage, correlations measured between any pulmonary function abnormality and study predictors (e.g. age, smoking, exposure levels and duration) which showed no significant correlations. Paradoxically, adjustment for smoking level revealed no additional effect on spirometric values or FE_{NO}. Based on present study results, no significant negative effect of henna milling on respiratory symptoms, spirometric values and FE_{NO} could be shown despite heavy dust exposures.

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1. Introduction

Occupational exposure to some mineral or organic dusts could cause respiratory diseases. The incidence and severity of induced disease depend on many factors, including level of exposure, susceptibility of the host and also other contributing factors like atopy and concurrent smoking status (1). Furthermore, it is well known that occupational diseases could be more severe and frequent when preventive efforts are lacking.

Milling of henna shrub produces a colorizing powder, known as red henna '*Lawsonia inermis*', used primarily for cosmetic purposes (e.g. as hair dye). This industry needs a hot and extremely dry climate and therefore, is limited to a few countries across the world. These countries usually are in the North Africa and the Middle East. In Middle East, Iran is one of the main producers of henna and in Iran, it is mainly produced in Yazd province. Due to rarity of this industry worldwide, no study could be found throughout the literature on the respiratory effects of chronic exposure to dusts raised by henna milling. This study conducted to turn attentions toward such an exposure.

Another driving force for performing such a study was the rarity of respiratory complaints among workers of this industry in 25 years pulmonary disease management experiences of one of the authors (M.R) in Yazd. Henna milling industry in Iran is mainly traditional with a low level of work hygiene. Huge amounts of dust, ineffective pollution controls (e.g. poor ventilation) and lack of personal preventive efforts are prominent features of this industry. In the present study, we measured respiratory symptoms, spirometric parameters as well as Fractional Exhaled Nitric Oxide (FE_{NO}) among henna workers.

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2. Material and Methods:

This study is a cross-sectional survey of the 45 current (and two former) male workers in small traditional henna mills in Yazd, central Iran, have done in autumn 2014. These 45 workers were tracked down and accumulated from 11 of 12 workshops in Yazd. The twelfth workshop denied to participate in the study (probably due to insurance problems). Researchers did their best to take a detailed written informed consent for interviewing and performing spirometry and measurement of FE_{NO} but in 26 instances, we obliged to take it orally due to workers' inability to read and write. One of the authors (M.H.) interviewed all the workers prior to tests. Both Consents and interviews included questions about occupational, smoking and past medical (especially pulmonary) history. Current work-related respiratory symptoms that were improved after moving away from mills were also questioned. Exclusion criteria for spirometry were vertebral column or thoracic cage abnormalities, neuromuscular diseases, heart failure, chest trauma, malignancy and abdominal surgery in previous three months and for FE_{NO} measurement were history of enalapril, corticosteroids, trinitroglycerin (TNG) and sildenafil consumption in last three months. There were also questions about continuous versus intermittent henna occupation and duration of leave periods and alternate occupations meanwhile.

Exposure levels grading: Workers had exposure in an everyday manner because they had no weekends, except a few short holidays during a year. Based on mean hours of exposure during a single workday and distances of workplaces from milling area (which had the most severe pollution) we divided exposures to four levels. Salesclerks were farthest from milling area and had less than an hour of exposure with henna milling during a typical workday and therefore, received a 'very low' grade of exposure. 'Packing-only', 'packing and milling intermittently' and 'milling-only' workers received a 'low', 'moderate' and 'high' grades of exposure, respectively. 'Milling-only' workers must have a minimum of six hours of exposure with milling during a workday and 'packing and milling intermittently' group had between one and six hours of exposures with milling. It must be mentioned that most of the workers, worked for more than ten hours during a typical workday. Packing takes place in an environment near but physically distinct from milling area; and milling workplace is polluted by a lot of dust particles raised by working of mills (Fig. 1).



Fig. 1 Henna packing and milling workplaces with a huge amount of dust pollution. a shows packing workplace and b deposition of henna powder on flat surfaces in this place in a period of about three months. c and d show modern, e and f traditional milling workplaces and traversing sun beams through them and very heavy pollution in these areas.

Smoking level grading: Cigarette smokers were whom that smoked at least 100 cigarettes in their lifetime, otherwise they were considered as never-smokers. Current smokers reported regular use of cigarettes within the past year, otherwise considered as ex-smokers. Pack/years is the number of cigarettes smoked per day/20 × years smoked. We also asked workers if they had smoked water-pipe or opium in their lifelong (even though the amount can't be classified to the best of our knowledge). Workers who mentioned such smoking habits were classified as 'any smoker' group in the statistical analysis.

FE_{NO} measurement and spirometry

FE_{NO} measurement and spirometry, were conducted by a trained technician using NObreath device (Bedfont Scientific Ltd, Kent, England) and Spirolab III (MIR Corporation, Rome, Italy), respectively. Test results were interpreted by authors as monitoring tests, according to American Thoracic Society and European Respiratory Society guidelines (2-6). According to these guidelines, presence of airway inflammation should be considered 'likely' if FE_{NO} is more than 50, 'unlikely' if less than 25 and 'probable' if it is between 25 and 50. Tests were carried out at the workplace in four days, each in the middle day of four consecutive weeks, started at about 11 AM after at least three hours of exposure with henna, for all current workers. Workers were asked not to smoke during the test day. Two former workers were invited to the clinic by phone, and tests were carried out at same hour of the day. FE_{NO} was done prior to spirometry to prevent a decline in FE_{NO} and reported as mean of three subsequent maneuvers with a rest period between each of them. FE_{NO} was performed with a 50 ml/s flow rate. Spirometry was done in standing position. Only forced vital capacity (FVC) maneuver performed. Each worker had three to eight spirometry attempts (with a rest period between each) until at least three acceptable maneuvers were attained. Global Lung Function Initiative (GLI) reference values were used to calculate percent predicted and Z-scores for forced expiratory volume in one second (FEV_1)/FVC, FEV_1 and FVC (7). Type of ethnic group was considered Oriental (Middle East) which is treated as Caucasians and didn't need any correction.

Statistical analysis

Statistical analysis was done by using SPSS software for Windows (Release 24.0, SPSS Inc., Chicago, U.S.), in 44 current henna milling workers. Contingency tables and descriptive statistics were used to explore any differences in respiratory symptoms, spirometry and FE_{NO} values based on different exposure levels. Spearman's rank-order correlation was run between continuous and ordinal predictors and outcomes to explore for probable associations. A point-biserial correlation was used between dichotomous predictors and continuous outcomes. An ANCOVA was done to determine if there is any effect of exposure levels on the FEV_1/FVC after controlling for exposure years. A multiple regression was performed to predict continuous outcomes from exposure years and levels, and history of continuously milling, other occupations and smoking. Two-tailed p-values considered as statistically significant at the level of 0.05 and very significant at 0.01.

3. Result and Discussion

All of the 45 current workers of the eleven henna mills (each had one to six workers) attended the interview. Also FE_{NO} measurement and spirometry performed in all of them. The twelfth mill with six workers refused to incorporate (maybe due to insurance problems).

One of the worker's spirometric data was outlier and excluded from analyses. He was a non-smoker 50 years-old Afghan who worked for only four years in high exposure level of the henna mill. Previously, he was working in fan belt production for six years and in construction sites for ten years. His FEV_1/FVC, FEV_1 and FVC were 64.6%, 48.6%, and 75.2% predicted values (Z-score: -3.64, -3.69 and -1.87), respectively. His FE_{NO} was 7.7, which made airway inflammation unlikely. Thus, his severe obstructive ventilatory impairment assumed to be related to other reasons, including his other occupations rather than henna exposure.

We also had access to two retired workers whom we just reported them as cases and excluded them from analysis due to mentioned reasons. One was a non-smoker 66 years-old native man with 40 years of moderate exposure. He has a severe accident during milling, which led to thoracic cage and lumbar vertebral fractures. He remembered some coughs and rhinorrhea during working years. Surprisingly, his FEV_1/FVC, FEV_1 and

FVC were 72.5%, 98.19%, and 135.7% predicted values (Z-score: -2.57, -.12 and 2.32), respectively. His FE_{NO} was 10.7, which made airway inflammation unlikely. Because thoracic cage trauma was one of our exclusion criteria, we excluded him from study. The second worker was a non-smoker 78 years-old native man with 54 years of moderate exposure. He mentioned productive cough, phlegm, dyspnea and chest tightness during working years, which persisted until now. He also reported a strong childhood and familial history of asthma, which was also was one of our exclusion criteria. His FEV_1/FVC, FEV_1 and FVC were 54.6%, 29.1% and 53.2% predicted values (Z-score: -3.53, -3.57 and -2.82), respectively. His FE_{NO} was 18.3, which made airway inflammation unlikely.

Thirty-six of the remaining 44 current workers were Iranian, and eight were Afghan. None of the workers complained of previous pulmonary problems. Twenty workers had other occupations with possible negative impact on the respiratory system. Seven agricultural, seven construction, two bakery, one with history of each of the; chemical fertilizer production, crystallized sugar production, welding, carpenter and truck driver working history.

Descriptive statistics are presented in Table 1 and 2.

In spite of smoking history in 14 workers (nine cigarette and five water-pipe smokers), surprisingly spirometry showed that only one of 44 workers had low FEV_1/FVC of 76.5% predicted value (Z-score = -2.19), which was below the lower limit normal (LLN: 5th percentile, at z-score of -1.645). His FEV_1 was 63% predicted value (Z-score = -2.22) which was suggestive of a moderate obstructive ventilatory impairment. This worker had 53 years of moderate exposure. This finding suggests a very low incidence of airflow limitation, even lower than general population.

There were also three workers with restrictive ventilatory impairment. Respectively, they had 18, three and five years of high, high and moderate exposure with FEV_1/FVC of 104.9%, 103.7% and 103.6% (Z-scores of .71, .50 and .49) and FVC of 73.3%, 79 and 80% (Z-scores of -2.21, -1.82 and -1.74) predicted.

FE_{NO} measurement showed that airway inflammation is probable in six and likely in three workers (none of the workers have asthma like symptoms) (Table 2).

The most frequent respiratory symptom was rhinorrhea in 43.2% (19 workers), followed by sneezing in 34.1% (15 workers) (Table 2).

Spearman's rank-order correlation (Table 3) showed no correlation between continuous or ordinal predictors and outcome variables except in two instances. First, a correlation between exposure level and FEV_1 Z-score ($r_s(40) = -.314$, $p = .038$) and secondly between years of other occupations and airway inflammation ($r_s(40) = .420$, $p = .005$).

Point-biserial correlation (Table 3) showed no correlation between dichotomous predictors and continuous outcomes. We also conducted a sensitivity analysis (after excluding outliers) for some variables, but results did not show any meaningful change from original values. So we can't draw different conclusions, except in two instances. There are statistically significant correlations between continuous henna occupation and FVC percent predicted and Z-score ($r_{pb}(42) = .335$, $p = .030$, and $r_{pb}(42) = .342$, $p = .027$, respectively). Workers who milled henna continuously had higher FVC than whom leaved henna industry intermittently (mean ± standard deviation: 97.0 ± 13.5 versus 85.1 ± 7.0 and -0.23 ± 1.12 versus -1.24 ± 0.61, respectively). continuous henna occupation accounted for 11.2 % and 11.7 % of variability in FVC percent predicted and Z-score, respectively.

ANCOVA statistics (Table 4) showed that after adjustment for exposure years, there wasn't any statistically significant difference in FEV_1/FVC according to different exposure levels, ($F(3, 39) = 1.934$, $p = .140$, partial $\eta^2 = .130$ for its percent predicted and $F(3, 39) = 1.545$, $p = .218$, partial $\eta^2 = .106$ for its Z-score). (Standardized residuals for the Exposure levels and for the overall models were normally distributed just for FEV_1/FVC, and not for other spirometric parameters and FE_{NO} logarithm. However, considering the test robustness to this assumption revealed no significant results for other outcomes (data are not shown)).

The multiple regression models (Table 5) could not predict outcomes at any statistically significant levels. In most instances, putting variables into the model could not produce statistically significant change in prediction rules. There were six exceptions to this: Exposure years added statistically significantly to the prediction of FEV_1/FVC percent predicted and FEV_1 percent predicted and Z-score; Similarly, for Low exposure to FEV_1/FVC percent predicted and FE_{NO} logarithm, and Moderate exposure to FE_{NO} logarithm (Table 6).

Discussion:

Respiratory abnormalities such as workers' complaint, decline in spirometric parameters or increase in FE_{NO} were not so common in henna workers despite their heavy dust exposures. There were only four workers with spirometric abnormalities and nine which their FE_{NO} levels suggested airway inflammation. Rhinorrhea was the most common respiratory complaint (Table 2). Surprisingly, there were no additional effects of smoking on spirometric parameters or FE_{NO}.

As in every cross-sectional occupational study, healthy worker effect (HWE) should also be considered (8, 9). This effect is more probable if findings are negative (health outcomes are not deteriorated) and/or in physically demanding occupations, which both were existed in this study. HWE can be more important in older workers; conversely, most of the present study workers were young. HWE consisted of three categories which some of them may play a role in the present study as a source of bias.

First, selection bias claims good health for being employed (Healthy Hire Effect) and more important to remain in the workforce (Healthy Worker Survivor Effect). It is well known that non-healthy workers have no interest in employment in high dust pollution industries; however, in Iran rare employment chances may oblige workers to accept any available job. These problems are even more evident for Afghan workers with much fewer chances for employment. On the other hand, the possibility that remaining current workers are healthier than former workers could be correct in the present study sample. So, in this study, maybe better-than-average health status is important for remaining in the workforce, but not for employment. Unfortunately, there was no way to reduce this bias.

Second, information bias may occur due to comparison of workers with general population but there are no other alternative references available. On the other hand, this obligatory approach will provide sufficient statistical power, which is not the case for other alternatives. Another way to minimize HWE is performing a sensitivity analysis to compare individuals with high and low exposure levels. These comparisons revealed no prominent effect of henna exposure levels on measured outcomes.

Third, confounding bias talks about health-related behaviors. These can be prohibiting smoking in working hours, improved access to healthcare, routine disease screening and more gym access after employment (Beneficial Effect of Work). Nevertheless, these confounders were not a concern for us, as employers not even offer these to henna workers.

In addition, incorporation of history of other occupations into the statistical analysis did not show any consistent association between this parameter and spirometric values, as well as FE_{NO}. This can suggest the presence of HWE in this group of henna workers.

Individuals who are resistant to the pulmonary destructive effects of smoking are also more likely to be immune to the effects of occupational hazardous exposures, referred to as 'healthy smoker effect' (10). Statistical analysis revealed no correlation between smoking status and spirometry values, as well as FE_{NO}. This can suggest the presence of healthy smoker effect in this group of henna workers.

Usually all workers encouraged to use a face mask, but most of them refused to take such an occupational precaution. As a prophylaxis, workers just tried to remain as far from milling areas as possible. Therefore, we decided not to ask about protective measures. However, during the study data collection period, we observed that workers who use masks more regularly were current smokers. This can show smokers concerns about their health, which lead to take more precautions relative to non-smokers. This finding may lead to a bias and therefore, detailed data were not shown or analyzed.

Unfortunately, there was no other study about effects of henna milling on respiratory system, which may be due to rarity of this industry. Effect of the main component of henna known as '*Lawsone*' also was not studied in the respiratory field. There are only four case reports about six hairdressers (beauticians) who used henna as hair dye and experienced some respiratory symptoms. These are considered to be related to hypersensitivity to red henna (*Lawsonia inermis*) as a severe occupational hazard. Positive skin prick tests and specific IgE in patients' sera for

henna were found in two and three of the them, respectively (11-14). There was also an herbal shop worker who complained of more severe respiratory symptoms than other cases. Positive skin prick test and specific IgE for black henna (*Indigofera argentea*) were positive in her. Contrary to previous cases, diagnosis of occupational asthma due to black henna was made for her rather than more commonly used red henna. (15)

There were also a number of case reports of severe skin allergic symptoms (e.g. contact dermatitis), and even anaphylactic reactions to henna. These occurred following application of henna to the skin as temporary tattoo and to hair as dye. These cases were seen in both occupational and non-occupational settings (hairdressers and henna users). These reactions are more related to black henna, rather than red henna and could be mostly related to Paraphenylenediamine (PPD) as a strong sensitizing aromatic amine present in black but not red henna (16).

Some limitations prevented this study to achieve stronger conclusions. The most important but unavoidable one was limited number of workers. This study achieved a participation rate of 88%, which suggests that findings are representative.

It is predictable that some workers want to acquire probable benefits form their employer by exaggerating respiratory symptoms.

It was probable that some workers may exaggerate their symptoms to acquire gain, but fortunately the prevalence of symptoms in the present study was not so high. On the other hand, this also prevents us to assess some symptoms, which are well known to be related to pulmonary diseases (e.g. wheezing in asthma). Furthermore, this issue may even contribute some bias in the measured symptoms.

Henna workers had irregular working shifts and holidays. 37 workers were living in the workshop area. There was a low answer rate to telephone calls for follow-up measurements. These problems prevented accessibility to lung function changes across-shift, after weekends or even over a defined time period (e.g. three months). Therefore, a cross-sectional design was mandatory.

Besides the exposure years, we obliged to use ranking exposure levels rather than quantitative measurements of dust pollution (air sampling in workplace). These problems prevented making more accurate analyses and conclusions. In fact, air sampling was performed by an external institution in three mills in one day. Inhalable (inspirable) dust was 18.33 and 20 mg/m^3 in two separated mills, and respirable dust was 10 mg/m^3 in another one. However, that institute could not guaranties accuracy of their devices and researchers decided to omit their data.

4. Conclusion

This was a small study which showed no excessive respiratory abnormalities in male workers in traditional henna mills. Furthermore, pulmonary functions were mostly preserved in spite of very large dust pollution exposures and smoking. Further studies are needed to seek for unknown factor(s) and examining each ingredient present in the henna milling process (e.g. sunflower seeds '*helianthus annuus*' - used as its oil).

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Table 1 Demographic data, exposure and smoking status, other occupations duration, spirometry and FE_{NO} values in four exposure levels and total of 44 current henna workers.

variable		Exposure level: Very Low (N = 5) Mean	Very Low SD	Low (N = 8) Mean	Low SD	Moderate (N = 16) Mean	Moderate SD	High (N = 15) Mean	High SD	Total (N = 44) Mean	Total SD
Age (years)		48	6.6	23	3.8	39	15.4	28	8.2	33	13.4
Height (cm)		177	5.9	166	7.0	170	7.6	170	5.9	170	7.2
Weight (Kg)		75	3.9	58	9.3	64	4.5	65	8.1	65	8.0
Exposure duration (years)		27	5.2	7	2.8	20	17.6	7	4.9	14	13.2
Other occupations duration (years) [a]				1		6	5.2	5	4.2	5	4.4
Pack/Years smoked [b]		10	11.0	5		12	15.4	4	4.9	9	11.0
FEV_1/FVC	Raw	81.1	2.3	83.4	7.5	79.6	7.7	82.0	4.9	81.3	6.3
	% Predicted	102.1	3.5	97.4	8.1	97.0	7.3	97.4	5.4	97.8	6.5
	Z-score	0.27	0.44	-0.29	1.10	-0.31	0.81	-0.32	0.70	-0.24	0.80
FEV_1	% Predicted	95.3	11.0	100.7	11.6	94.3	14.9	89.2	9.8	93.8	12.6
	Z-score	-0.38	0.82	0.07	1.00	-0.38	1.09	-0.91	0.84	-0.48	1.00
FVC	% Predicted	93.0	8.8	103.3	13.3	96.8	13.8	91.7	13.9	95.8	13.5
	Z-score	-0.55	0.67	0.28	1.14	-0.22	1.11	-0.72	1.18	-0.34	1.13
FE_{NO}	Raw	25	13.4	9	4.6	19	28.1	22	19.2	19 [c]	21.0
	Logarithm	1.35	0.21	0.91	0.23	0.97	0.51	1.21	0.34	1.09	0.41

SD: standard deviation

[a] There are total of 20 workers with history of other occupations and the groups' frequencies are 0, 1, 7 and 12, accordingly.

[b] There are total of 9 workers with history of cigarette smoking and the groups' frequencies are 2, 1, 4 and 2, accordingly.

[c] FE_{NO} geometric mean in total: 12.162692

Note: Complete form of this table including median, minimum, and maximum is available as Appendix Table 1.

Table 2 Henna and other occupations history, smoking status, airway inflammation and respiratory symptoms in four exposure levels and total of 44 current henna workers.

Variable			Exposure Level: Very Low	Low	Moderate	High	Total Count	Total %	Level
Henna occupation history		Intermittent	0	1	5	3	9	20.5%	
		Continuous	5	7	11	12	35	79.5%	
Other occupations history		No	5	7	9	3	24	54.5%	
		Yes	0	1	7	12	20	45.5%	
smoking: Any		No	3	6	11	10	30	68.2%	
		Yes	2	2	5	5	14	31.8%	
smoking: Current cigarette		No	4	8	14	14	40	90.9%	
		Yes	1	0	2	1	4	9.1%	
smoking: Ex-cigarette		No	4	7	14	14	39	88.6%	
		Yes	1	1	2	1	5	11.4%	
smoking: Water-pipe		No	5	7	15	12	39	88.6%	
		Yes	0	1	1	3	5	11.4%	
smoking: Opium		No	5	8	14	15	42	95.5%	
		Yes	0	0	2	0	2	4.5%	
Airway Inflammation [a]	Unlikely		4	8	12	11	35	79.5%	
	Probable		1	0	2	3	6	13.6%	
	Likely		0	0	2	1	3	6.8%	
respiratory Symptom: Dry cough		No	5	7	13	12	37	84.1%	
		Yes	0	1	3	3	7	15.9%	
respiratory Symptom: Productive cough		No	5	8	13	6	32	72.7%	
		Yes	0	0	3	9	12	27.3%	
respiratory Symptom: Phlegm		No	5	6	12	10	33	75.0%	
		Yes	0	2	4	5	11	25.0%	
respiratory Symptom: Dyspnea		No	5	8	13	9	35	79.5%	
		Yes	0	0	3	6	9	20.5%	
respiratory Symptom: Chest tightness		No	5	8	13	12	38	86.4%	
		Yes	0	0	3	3	6	13.6%	
respiratory Symptom: Rhinorrhea		No	5	5	8	7	25	56.8%	
		Yes	0	3	8	8	19	43.2%	
respiratory Symptom: Sneezing		No	4	6	11	8	29	65.9%	
		Yes	1	2	5	7	15	34.1%	
			5	8	16	15	44		

[a] Presence of airway inflammation is considered 'likely' if FENO is more than 50, 'unlikely' if less than 25 and 'probable' if it is between 25 and 50.

Note: Complete form of this table including percentage within level and variable is available as Appendix Table 2.

Table 3 Correlations between predictors (Age, exposure and smoking status, other occupations and milling history) and spirometry and FE_{NO} values in 44 current henna workers.

Predictor (Number)			FEV_1/FVC		FEV_1		FVC		FE_{NO}	
			Predicted Percentage	Z-score	Predicted Percentage	Z-score	Predicted Percentage	Z-score	Log	Airway Inflammation
Age (44)			-0.045	-0.038	-0.106	-0.086	-0.070	-0.016	0.229	0.120
Exposure level (44)			-0.155	-0.155	-0.292	-.314*	-0.254	-0.286	0.085	0.161
Exposure Duration (44)			0.027	0.039	-0.012	0.001	-0.012	0.044	-0.007	-0.157
Other Occupations Duration (20)			0.124	0.138	0.089	0.103	0.026	-0.011	0.247	0.358
Pack/Years smoked (9)			0.159	0.159	-0.109	-0.109	-0.142	-0.142	-0.184	
		Group	No Yes	No Yes	No Yes	No Yes	No Yes	No Yes	No Yes	No Yes
smoking	Current cigarette (No=40, Yes=4)		-0.140	-0.143	-0.165	-0.149	-0.085	-0.065	-0.126	Raw FE_{NO}
	Descriptive statistics	Mean	98.1 94.9	-0.21 -0.60	94.5 87.4	-0.43 -0.94	96.2 92.2	-0.31 -0.57	1.10 0.93	19.8 10.8
		SD	6.5 7.2	0.79 0.92	12.2 16.6	0.97 1.26	12.9 20.8	1.09 1.67	0.41 0.40	21.8 7.3
	Ex- cigarette (No=39, Yes=5)		0.183	0.174	-0.030	-0.043	-0.112	-0.116	-0.157	
	Descriptive statistics	Mean	97.3 101.1	-0.29 0.14	94.0 92.8	-0.46 -0.60	96.3 91.6	-0.29 -0.70	1.11 0.91	20.0 10.9
		SD	6.7 4.5	0.82 0.59	12.7 13.5	1.00 1.05	13.7 12.5	1.14 1.02	0.41 0.38	21.9 8.9
	Water-pipe (No=39, Yes=5)		0.087	0.075	-0.057	-0.071	-0.091	-0.096	0.051	
	Descriptive statistics	Mean	97.6 99.3	-0.27 -0.08	94.1 91.9	-0.45 -0.67	96.2 92.4	-0.30 -0.64	1.08 1.14	19.2 17.1
		SD	6.8 4.0	0.83 0.55	13.0 9.4	1.02 0.82	13.8 11.9	1.15 1.03	0.42 0.34	22.1 10.4
	Continuous henna occupation (No=9, Yes=35)		-0.030	-0.009	0.175	0.190	0.183	0.186	-0.040	
	Descriptive statistics	Mean	98.1 97.7	-0.23 -0.25	89.6 95.0	-0.85 -0.38	91.0 97.0	-0.75 -0.23	1.12 1.08	25.5 17.3
		SD	4.1 7.1	0.55 0.86	11.4 12.8	0.96 1.00	13.1 13.5	1.12 1.12	0.51 0.38	34.8 16.1
	Other occupations history (No=24, Yes=20)			-0.256	-0.276	-0.282	-0.159	-0.169	0.273	
	Descriptive statistics	Mean	99.2 96.0	-0.06 -0.47	97.0 90.1	-0.22 -0.78	97.8 93.5	-0.17 -0.54	0.99 1.21	13.1 26.1
		SD	6.6 6.1	0.87 0.66	11.9 12.7	0.95 0.98	14.0 12.9	1.16 1.07	0.35 0.44	10.7 27.6

***** Correlation is significant at the 0.05 level (2-tailed).
Note: Outliers were included in calculation of descriptive statistics.
Note: Complete form of this table including sensitivity analysis is available as Appendix Table 3.

Table 4 ANCOVA Descriptive Statistics for Impact of exposure levels on the FEV_1/FVC after controlling for exposure years in 44 current henna workers.

			Unadjusted		Adjusted estimates	
		Exposure Level (No.)	Mean	SD	Mean	SD
FEV_1/FVC	% predicted	Very Low (5)	102.1	3.5	104.6	3.0
		Low (8)	97.4	8.1	96.1	2.3
		Moderate (16)	97.0	7.3	98.1	1.6
		High (15)	97.4	5.4	96.1	1.7
		Total (44)	97.8	6.5		
	Z-score	Very Low (5)	0.27	0.44	0.52	0.38
		Low (8)	-0.29	1.10	-0.42	0.29
		Moderate (16)	-0.31	0.81	-0.21	0.21
		High (15)	-0.32	0.70	-0.45	0.22
		Total (44)	-0.24	0.80		

Note: Covariates appearing in the model are evaluated at exposure years = 14.00.

Table 5 Multiple regression models in 44 current henna workers.

Outcomes		R	R Square	Adjusted R Square	F	Sig.
FEV_1/FVC	% predicted	0.488	0.238	0.090	1.608	0.165
	Z-score	0.432	0.187	0.029	1.180	0.338
FEV_1	% predicted	0.531	0.282	0.143	2.023	0.079
	Z-score	0.510	0.260	0.117	1.811	0.115
FVC	% predicted	0.435	0.189	0.032	1.200	0.328
	Z-score	0.428	0.183	0.025	1.155	0.352
FE_{NO} Logarithm		0.487	0.237	0.089	1.601	0.167

Table 6 Multiple regression coefficients for prediction of spirometry and FE_{NO} from exposure years and levels, and history of continuous milling, another occupations and smoking in 44 current henna workers.

Outcome		Predictor	Unstandardized coefficients		Standardized coefficients	Sig.
			B	Std. Error	Beta	
FEV_1/FVC [a]	% predicted	(Constant)	108.016	4.196		0.000
		Exposure duration *	-0.206	0.093	-0.418	0.032
		Low exposure *	-8.142	3.960	-0.486	0.047
		Moderate exposure	-5.101	3.418	-0.380	0.144
		High exposure	-5.786	4.129	-0.425	0.170
		Continuous henna occupation	-1.159	2.647	-0.072	0.664
		Other occupations history	-3.857	2.469	-0.297	0.127
		Any smoking	1.963	2.093	0.142	0.355
	Z-score	(Constant)	0.863	0.531		0.112
		Exposure duration	-0.020	0.012	-0.335	0.093
		Low exposure	-0.887	0.501	-0.433	0.085
		Moderate exposure	-0.554	0.432	-0.337	0.208
		High exposure	-0.632	0.522	-0.379	0.234
		Continuous henna occupation	-0.122	0.335	-0.062	0.718
		Other occupations history	-0.460	0.312	-0.290	0.149
		Any smoking	0.188	0.265	0.111	0.483
FEV_1	% predicted	(Constant)	100.122	7.848		0.000
		Exposure duration *	-0.458	0.173	-0.482	0.012
		Low exposure	-2.478	7.407	-0.077	0.740
		Moderate exposure	-1.147	6.394	-0.044	0.859
		High exposure	-11.908	7.723	-0.454	0.132
		Continuous henna occupation	7.472	4.951	0.242	0.140
		Other occupations history	-2.142	4.618	-0.086	0.645
		Any smoking	0.289	3.915	0.011	0.942
	Z-score	(Constant)	-0.192	0.630		0.762
		Exposure duration *	-0.029	0.014	-0.385	0.044
		Low exposure	-0.044	0.595	-0.017	0.942
		Moderate exposure	0.028	0.513	0.014	0.957
		High exposure	-0.880	0.620	-0.424	0.164
		Continuous henna occupation	0.612	0.397	0.251	0.133
		Other occupations history	-0.131	0.371	-0.066	0.726
		Any smoking	-0.032	0.314	-0.015	0.919
FVC	% predicted	(Constant)	92.618	8.969		0.000
		Exposure duration	-0.287	0.198	-0.281	0.156
		Low exposure	5.393	8.465	0.155	0.528
		Moderate exposure	3.963	7.307	0.142	0.591
		High exposure	-5.873	8.826	-0.208	0.510
		Continuous henna occupation	8.672	5.658	0.261	0.134
		Other occupations history	0.751	5.277	0.028	0.888
		Any smoking	-1.404	4.474	-0.049	0.756
	Z-score	(Constant)	-0.676	0.750		0.373
		Exposure duration	-0.020	0.017	-0.230	0.245
		Low exposure	0.506	0.707	0.175	0.479
		Moderate exposure	0.365	0.611	0.158	0.554
		High exposure	-0.467	0.738	-0.199	0.531
		Continuous henna occupation	0.707	0.473	0.256	0.143
		Other occupations history	0.063	0.441	0.028	0.888
		Any smoking	-0.140	0.374	-0.058	0.711
FE_{NO} Logarithm		(Constant)	1.476	0.261		0.000
		Exposure duration	-0.005	0.006	-0.173	0.363
		Low exposure *	-0.582	0.246	-0.559	0.024
		Moderate exposure *	-0.529	0.213	-0.634	0.018
		High exposure	-0.454	0.257	-0.535	0.086
		Continuous henna occupation	0.028	0.165	0.028	0.866
		Other occupations history	0.263	0.154	0.326	0.096
		Any smoking	-0.015	0.130	-0.017	0.912

* predictions is significant at the 0.05 level (2-tailed).

[a] There were no studentized deleted residuals greater than ±3 standard deviations, except for just one worker. These are 3.08 and 3.52 for his FEV_1/FVC predicted percentage and Z-score, respectively. Sensitivity analysis without these outliers revealed same results; therefore, these were included in the data report.

Note: Original exposure levels with four levels converted to four dichotomous variables by dummy coding. The 'very low exposure' category was considered as reference. Other three categories were entered the model.

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Appendix Table 1 Demographic data, exposure and smoking status, other occupations duration, spirometry and FE_{NO} values in four exposure levels and total of 44 current henna workers.

		Exposure level																				Total (N = 44)				
		Very Low (N = 5)					Low (N = 8)					Moderate (N = 16)					High (N = 15)									
variable		Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max
Age (years)		48	48	6.6	40	58	23	24	3.8	17	27	39	38	15.4	19	68	28	25	8.2	22	54	33	29	13.4	17	68
Height (cm)		177	178	5.9	168	183	166	166	7.0	158	176	170	169	7.6	158	182	170	170	5.9	158	181	170	170	7.2	158	183
Weight (Kg)		75	75	3.9	70	81	58	59	9.3	50	78	64	65	4.5	55	70	65	66	8.1	50	78	65	65	8.0	50	81
Exposure duration (years)		27	30	5.2	20	32	7	7	2.8	3	11	20	12	17.6	4	53	7	6	4.9	2	18	14	9	13.2	2	53
Other occupations duration (years) [a]							1	1		1	1	6	4	5.2	1	15	5	5	4.2	1	15	5	4	4.4	1	15
Pack/Years smoked [b]		10	10	11.0	3	18	5	5		5	5	12	5	15.4	4	35	4	4	4.9	0	7	9	5	11.0	0	35
FEV_1/FVC	Raw	81.1	82.1	2.3	77.9	83.4	83.4	80.8	7.5	74.5	98.8	79.6	79.7	7.7	59.3	91.8	82.0	82.2	4.9	72.7	88.7	81.3	80.8	6.3	59.3	98.8
	% Predicted	102.1	100.7	3.5	98.2	106.5	97.4	96.0	8.1	87.7	113.4	97.0	98.0	7.3	76.5	107.3	97.4	96.8	5.4	87.4	105.6	97.8	98.1	6.5	76.5	113.4
	Z-score	0.27	0.10	0.44	-0.23	0.79	-0.29	-0.53	1.10	-1.48	1.96	-0.31	-0.25	0.81	-2.19	1.03	-0.32	-0.42	0.70	-1.57	0.78	-0.24	-0.25	0.80	-2.19	1.96
FEV_1	% Predicted	95.3	91.5	11.0	83.5	111.8	100.7	100.5	11.6	83.8	118.1	94.3	94.6	14.9	62.9	120.3	89.2	85.2	9.8	77.0	110.2	93.8	92.7	12.6	62.9	120.3
	Z-score	-0.38	-0.67	0.82	-1.24	0.85	0.07	0.05	1.00	-1.35	1.56	-0.38	-0.40	1.09	-2.22	1.73	-0.91	-1.26	0.84	-1.83	0.91	-0.48	-0.58	1.00	-2.22	1.73
FVC	% Predicted	93.0	88.9	8.8	84.8	104.7	103.3	97.8	13.3	86.6	128.6	96.8	94.8	13.8	76.4	122.7	91.7	85.8	13.9	73.3	117.8	95.8	94.3	13.5	73.3	128.6
	Z-score	-0.55	-0.90	0.67	-1.14	0.33	0.28	-0.19	1.14	-1.14	2.46	-0.22	-0.38	1.11	-1.74	1.90	-0.72	-1.23	1.18	-2.21	1.49	-0.34	-0.47	1.13	-2.21	2.46
FE_{NO}	Raw	25	22	13.4	12	48	9	9	4.6	3	18	19	6	28.1	2	109	22	13	19.2	5	74	19 [c]	12	21.0	2	109
	Logarithm	1.35	1.34	0.21	1.09	1.68	0.91	0.94	0.23	0.52	1.26	0.97	0.80	0.51	0.37	2.04	1.21	1.12	0.34	0.70	1.87	1.09	1.06	0.41	0.37	2.04

SD: standard deviation, Min: minimum, Max: maximum

[a] There are total of 20 workers with history of other occupations and the groups' frequencies are 0, 1, 7 and 12, accordingly.

[b] There are total of 9 workers with history of cigarette smoking and the groups' frequencies are 2, 1, 4 and 2, accordingly.

[c] FE_{NO} geometric mean in total: 12.162692

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Appendix Table 2 Henna and other occupations history, smoking status, airway inflammation and respiratory symptoms in four exposure levels and total of 44 current henna workers.

Variable			Exposure Level												Total	
			Very Low			Low			Moderate			High				
			Count	% Level	% variable	Count	% Level	% variable	Count	% Level	% variable	Count	% Level	% variable	Count	% Level
Henna occupation history		Intermittent	0	0.0%	0.0%	1	12.5%	11.1%	5	31.3%	55.6%	3	20.0%	33.3%	9	20.5%
		Continuous	5	100.0%	14.3%	7	87.5%	20.0%	11	68.8%	31.4%	12	80.0%	34.3%	35	79.5%
Other occupations history		No	5	100.0%	20.8%	7	87.5%	29.2%	9	56.3%	37.5%	3	20.0%	12.5%	24	54.5%
		Yes	0	0.0%	0.0%	1	12.5%	5.0%	7	43.8%	35.0%	12	80.0%	60.0%	20	45.5%
smoking	Any	No	3	60.0%	10.0%	6	75.0%	20.0%	11	68.8%	36.7%	10	66.7%	33.3%	30	68.2%
		Yes	2	40.0%	14.3%	2	25.0%	14.3%	5	31.3%	35.7%	5	33.3%	35.7%	14	31.8%
	Current cigarette	No	4	80.0%	10.0%	8	100.0%	20.0%	14	87.5%	35.0%	14	93.3%	35.0%	40	90.9%
		Yes	1	20.0%	25.0%	0	0.0%	0.0%	2	12.5%	50.0%	1	6.7%	25.0%	4	9.1%
	Ex-cigarette	No	4	80.0%	10.3%	7	87.5%	17.9%	14	87.5%	35.9%	14	93.3%	35.9%	39	88.6%
		Yes	1	20.0%	20.0%	1	12.5%	20.0%	2	12.5%	40.0%	1	6.7%	20.0%	5	11.4%
	Water-pipe	No	5	100.0%	12.8%	7	87.5%	17.9%	15	93.8%	38.5%	12	80.0%	30.8%	39	88.6%
		Yes	0	0.0%	0.0%	1	12.5%	20.0%	1	6.3%	20.0%	3	20.0%	60.0%	5	11.4%
	Opium	No	5	100.0%	11.9%	8	100.0%	19.0%	14	87.5%	33.3%	15	100.0%	35.7%	42	95.5%
		Yes	0	0.0%	0.0%	0	0.0%	0.0%	2	12.5%	100.0%	0	0.0%	0.0%	2	4.5%
Airway Inflammation [a]		Unlikely	4	80.0%	11.4%	8	100.0%	22.9%	12	75.0%	34.3%	11	73.3%	31.4%	35	79.5%
		Probable	1	20.0%	16.7%	0	0.0%	0.0%	2	12.5%	33.3%	3	20.0%	50.0%	6	13.6%
		Likely	0	0.0%	0.0%	0	0.0%	0.0%	2	12.5%	66.7%	1	6.7%	33.3%	3	6.8%
respiratory Symptom	Dry cough	No	5	100.0%	13.5%	7	87.5%	18.9%	13	81.3%	35.1%	12	80.0%	32.4%	37	84.1%
		Yes	0	0.0%	0.0%	1	12.5%	14.3%	3	18.8%	42.9%	3	20.0%	42.9%	7	15.9%
	Productive cough	No	5	100.0%	15.6%	8	100.0%	25.0%	13	81.3%	40.6%	6	40.0%	18.8%	32	72.7%
		Yes	0	0.0%	0.0%	0	0.0%	0.0%	3	18.8%	25.0%	9	60.0%	75.0%	12	27.3%
	Phlegm	No	5	100.0%	15.2%	6	75.0%	18.2%	12	75.0%	36.4%	10	66.7%	30.3%	33	75.0%
		Yes	0	0.0%	0.0%	2	25.0%	18.2%	4	25.0%	36.4%	5	33.3%	45.5%	11	25.0%
	Dyspnea	No	5	100.0%	14.3%	8	100.0%	22.9%	13	81.3%	37.1%	9	60.0%	25.7%	35	79.5%
		Yes	0	0.0%	0.0%	0	0.0%	0.0%	3	18.8%	33.3%	6	40.0%	66.7%	9	20.5%
	Chest tightness	No	5	100.0%	13.2%	8	100.0%	21.1%	13	81.3%	34.2%	12	80.0%	31.6%	38	86.4%
		Yes	0	0.0%	0.0%	0	0.0%	0.0%	3	18.8%	50.0%	3	20.0%	50.0%	6	13.6%
	Rhinorrhea	No	5	100.0%	20.0%	5	62.5%	20.0%	8	50.0%	32.0%	7	46.7%	28.0%	25	56.8%
		Yes	0	0.0%	0.0%	3	37.5%	15.8%	8	50.0%	42.1%	8	53.3%	42.1%	19	43.2%
	Sneezing	No	4	80.0%	13.8%	6	75.0%	20.7%	11	68.8%	37.9%	8	53.3%	27.6%	29	65.9%
		Yes	1	20.0%	6.7%	2	25.0%	13.3%	5	31.3%	33.3%	7	46.7%	46.7%	15	34.1%
			5		11.4%	8		18.2%	16		36.4%	15		34.1%	44	

[a] Presence of airway inflammation is considered 'likely' if FE_{NO} is more than 50, 'unlikely' if less than 25 and 'probable' if it is between 25 and 50.

Appendix Table 3 Correlations between predictors (Age, exposure and smoking status, other occupations and milling history) and spirometry and FE_{NO} values in 44 current henna workers.

		FEV_1/FVC				FEV_1				FVC				FE_{NO}			
Predictor (Number)		Predicted Percentage		Z-score		Predicted Percentage		Z-score		Predicted Percentage		Z-score		Log		Airway Inflammation	
Age (44)		-0.045		-0.038		-0.106		-0.086		-0.070		-0.016		0.229		0.120	
Exposure level (44)		-0.155		-0.155		-0.292		-.314*		-0.254		-0.286		0.085		0.161	
Exposure Duration (44)		0.027		0.039		-0.012		0.001		-0.012		0.044		-0.007		-0.157	
Other Occupations Duration (20)		0.124		0.138		0.089		0.103		0.026		-0.011		0.247		0.358	
Pack/Years smoked (9)		0.159		0.159		-0.109		-0.109		-0.142		-0.142		-0.184			
	Group	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
smoking	Current cigarette (No=40, Yes=4)		-0.140		-0.143		-0.165		-0.149		-0.085		-0.065		-0.126		
	Sensitivity analysis		-0.187		-0.170											Raw FE_{NO}	
	Descriptive statistics Mean	98.1	94.9	-0.21	-0.60	94.5	87.4	-0.43	-0.94	96.2	92.2	-0.31	-0.57	1.10	0.93	19.8	10.8
	Descriptive statistics SD	6.5	7.2	0.79	0.92	12.2	16.6	0.97	1.26	12.9	20.8	1.09	1.67	0.41	0.40	21.8	7.3
	Ex- cigarette (No=39, Yes=5)		0.183		0.174		-0.030		-0.043		-0.112		-0.116		-0.157		
	Sensitivity analysis		0.224		0.218												
	Descriptive statistics Mean	97.3	101.1	-0.29	0.14	94.0	92.8	-0.46	-0.60	96.3	91.6	-0.29	-0.70	1.11	0.91	20.0	10.9
	Descriptive statistics SD	6.7	4.5	0.82	0.59	12.7	13.5	1.00	1.05	13.7	12.5	1.14	1.02	0.41	0.38	21.9	8.9
	Water-pipe (No=39, Yes=5)		0.087		0.075		-0.057		-0.071		-0.091		-0.096		0.051		
	Sensitivity analysis		0.069		0.109		-0.075		-0.158		-0.186		-0.192		0.145		
	Descriptive statistics Mean	97.6	99.3	-0.27	-0.08	94.1	91.9	-0.45	-0.67	96.2	92.4	-0.30	-0.64	1.08	1.14	19.2	17.1
	Descriptive statistics SD	6.8	4.0	0.83	0.55	13.0	9.4	1.02	0.82	13.8	11.9	1.15	1.03	0.42	0.34	22.1	10.4
	Continuous henna occupation (No=9, Yes=35)		-0.030		-0.009		0.175		0.190		0.183		0.186		-0.040		
	Sensitivity analysis		0.011		-0.046		0.252		0.274		.335*		.342*				
	Descriptive statistics Mean	98.1	97.7	-0.23	-0.25	89.6	95.0	-0.85	-0.38	91.0	97.0	-0.75	-0.23	1.12	1.08	25.5	17.3
	Descriptive statistics SD	4.1	7.1	0.55	0.86	11.4	12.8	0.96	1.00	13.1	13.5	1.12	1.12	0.51	0.38	34.8	16.1
	Other occupations history (No=24, Yes=20)				-0.256		-0.276		-0.282		-0.159		-0.169		0.273		
	Sensitivity analysis				-0.211												
	Descriptive statistics Mean	99.2	96.0	-0.06	-0.47	97.0	90.1	-0.22	-0.78	97.8	93.5	-0.17	-0.54	0.99	1.21	13.1	26.1
	Descriptive statistics SD	6.6	6.1	0.87	0.66	11.9	12.7	0.95	0.98	14.0	12.9	1.16	1.07	0.35	0.44	10.7	27.6

***** Correlation is significant at the 0.05 level (2-tailed).

Note: Outliers were included in calculation of descriptive statistics.