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Estimation of Lead and Cadmium in the Hair and Nails of Petrol Station Workers

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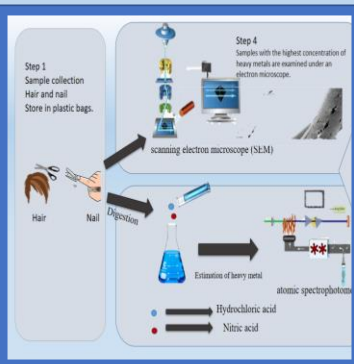
Abstract: Lead and cadmium are among the most dangerous heavy metals for human health, as several studies have proven the effect of these elements on the nervous system, kidneys, liver, and respiratory and reproductive systems. According to the recommendations of the World Health Organization, human hair and nails have been identified as important biomarkers to assess exposure to potentially harmful elements. The purpose of this research was to determine the levels of lead and cadmium in the hair and nail samples of petrol station workers in Al-Ramadi-Iraq. A total of 20 male petrol station workers and 20 males working in offices were recruited for the study. Hair and nail samples were collected from all participants and stored in plastic tubes for analysis. Histological examination using SEM was conducted on the hair and nail samples. The results revealed that the levels of lead and cadmium in the hair and nail samples were significantly elevated compared to control values, with mean values of (14.01 mg kg − 1) for lead and (5.96 mg kg − 1) for cadmium in hair, and (7.99 mg kg − 1) for lead and (3.19 mg kg − 1) for cadmium in nails. There was a significant positive correlation ($P < 0.05$) between the levels of lead in the workers' hair and nails and work duration (0.450162, 0.487173), also between concentration of Pb in the hair of workers and concentration of Pb and Cd in the nail of workers (0.660219,0.574807), as well as between concentration of Pb in the nail of workers and concentration of Cd in the nail of workers (0.881698).



Keywords: Heavy metals, SEM, Hair, Nail, Histological changes.

INTRODUCTION

Lead and cadmium are toxic elements. Both metals cause adverse health effects in humans, and their widespread presence in the human environment comes from anthropogenic activities [1]. Ingested or inhaled large amounts of heavy metals could cause adverse impacts on the human body, including the brain, liver, lungs, and bones and each metal causes different effects [2]. These metals are part of the earth's crust but have got to a stage where they are now considered environmental pollutants caused by different activities such as industries, farming, and mining [3]. Metals have contaminated the soil, water, air, and dust over recent years as a result of the advancement in industrialization and urbanization processes [4].

The presence of heavy metals in various organisms is mainly due to their persistence and hence bioaccumulation in food chains and living organisms [5–8].

Iraqi crude petroleum contains various heavy metals, including copper, cadmium, and lead, which are incorporated to enhance performance [9]. The use of lead and cadmium in petrol was responsible for substantial human lead and cadmium exposure, with millions of tons added globally [10]. Lead products are commonly used as anti-knock agents in petroleum-based products, which raises environmental lead levels. In Baghdad, lead concentrations in gasoline and fuel oil were determined to be within international limits, with leaded gasoline having the highest level at 105 mg/L and unleaded gasoline having the lowest at 3.1 mg/L [11]. In contrast, diesel fuels have been found to contain approximately 3.316 ppm of cadmium in raw samples [12]. A study conducted in Baghdad found lead concentrations in diesel fuel ranged from 1.85 to 2.05 mg/L [11]. There the Workers who work at petrol stations are very likely to breathe heavy metals because of fumes resulting from evaporated fuel and emissions from the vehicles. This has proved to be the case, especially when drivers rev their engines as they wait in line. Various industries emit toxins such as heavy metals and chemicals in the air, and dust particles picked up from the ground are often recycled back into the air as well. These pollutants can move from one place to another through wind and thus cause pollution of ecosystems [13]. The workplace environment is significant because people have direct contact with chemical agents with which exposure is possible [14–16]. Toxic substances are released as spray or dust they can pass through the respiratory system or contact with the skin consequently making it necessary to determine toxic elements in human tissue [17–19]. Peripheral neuropathy is also associated with long-term exposure to heavy metals including cadmium, lead, and arsenic metals. These metals have been shown to affect tiny nerve fibers and temperature-dependent pain thresholds [20].

Biological monitoring is widely utilized, particularly using human biomarkers, to screen for, identify, and assess metal

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exposures and related dangers. Hair, nails, blood, and urine samples have been traditionally utilized for testing heavy metal levels [21, 22]. When it comes to identifying heavy metal exposure, hair and nails are very helpful bioindicators [23]. Over the last thirty years, there has been considerable interest in the determination of trace elements in hair and nails in biomedical and environmental research. These measures used in assessing the nutritional status, diagnosing diseases, and determining environmental exposures are still under scientific consideration due to differing beliefs about their precision [24, 25].

Hair samples are still more ideal than blood and other biohumates for evaluating trace metals because hair analysis is easier, more effective and less vulnerability to environmental pollutants than other biomarkers [26–28]. In our region, we sought to investigate heavy metal exposure among workers in petrol stations. The study was therefore geared towards establishing the correlation between the working conditions in the petrol stations and the degrees of toxicity levels of the heavy metals traced on the hair and nails of the workers. The impact of the working environment and length of service on the accumulation of toxic heavy metals in the body samples of the workers was compared in the study.

MATERIALS AND METHODS

Sample Collection

Hair and nail samples were gathered from 20 male workers at petrol stations; samples were collected from 8 petrol stations located at different places in Al-Ramadi city. The names and locations of petrol stations are shown in Fig 1., and 20 samples from an unpolluted workplace (office, college, house) from different sites in Al-Ramadi city, Iraq. Other Data collected from the participants included age, daily hours, and the duration of work. The participants are aged 16 to 60 years. Hair Scalp samples were collected by cutting 2 cm of the scalp with sterilized stainless-steel scissors . The hair samples were then placed in the plastic pouches until subjected to analysis. Fingernail clippings were obtained from the workers' hands and feet after they washed their hands and feet with medicated soap and water to eliminate any metallic interference and subsequently, dried them using a sterile towel or tissue to eliminate external deposits and then placed in the plastic pouches until subjected to analysis.

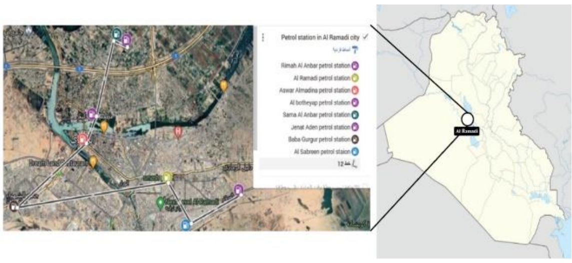

Figure (1): Location map of petrol stations that were selected for hair and nail sampling from workers.

Sample preparation

Samples of and scalp hair fingernails were cleaned with no-ionic detergent, Triton X 100, then CH3COCH3 (acetone), and finally deionized water. After washing, fingernails and scalp hair samples were dried in an electric oven at 60°C and stored in polyethylene bags for analysis. According to the International Atomic Energy Agency [29,20], this method has proven to be one of the most effective washing procedures for removing exterior trace elements while retaining internal.

Digestion of Hair and nail Samples

(1 g) of hair were measured and placed in a test tube. Also, (1 g) of nails was measured and placed in another test tube The hair and nail samples were combined with 10 ml of Nitric acid and hydrochloric acid in a 1:3 ratio, for digestion. The mixture was further mixed in the flask until homogenized and then it is placed in the microwave (Dill, Germany) where power (1050 w), temperature (150 c), and time (20 min), are installed. Digestion was followed by rinsing the container with distilled water until it was devoid of the sample residues. Then, it was filtered with filter papers until it reached a size of 50 ml [31].

Estimation of Pb, and Cd levels in hair and nail samples

Lead and Cadmium concentrations were estimated using an Atomic Absorption Spectrophotometer AAS (Phoenix-986-USA). In this process, the flame conditions were made as per Pb, and Cd-specific manufacturer conditions. These elements in their standard solutions were first applied in the calibration of the AAS before application in the analysis of the samples. Three different concentrations of standards for each element. Where a standard solution of 1000 mg/L prepared for each element by dissolving the required weight in a liter of suitable solvent. The absorbance of these standards was then measured by the instrument to automatically generate calibration curves using the instrument's software. The concentrations of each element were determined from the standard curves using the following formula:

- [Sample concentration= Read concentration × dilution factor]
- In all sample masses of Lead and Cadmium were presented as (mg kg − 1).

Scanning electron microscopy procedure

The most highly contaminated samples from the workers were chosen for microscopic examination after determining the levels of heavy metals in their hair and nails.

To prepare the human scalp hair samples for examination, they were attached to carbon conductive tape, placed on aluminum stubs, and coated with platinum using a sputter coating machine in a high vacuum environment sputter coating machine utilizing platinum as a metal target with a thickness of 10–20 nm [32]. The Scanning electron microscopy (Model: JSM 6390 LA, JEOL) at 15–20 kV was then used for scanning electron microscopy to analyze multiple sections of the hair shafts for uniformity and damage assessment according to a five-grade scale developed by Kim [33].

Grade (0) in the grading system represents virgin intact hair with a regular overlay of the cuticle. Grade [1] indicates an irregular overlay of the cuticle without cracks or holes. Grade [2] shows severe lift-up of the cuticle with cracks or holes but without exposure to the cortex. Grade [3] reflects partial exposure of the cortex. Grade [4] represents the complete disappearance of the cortex.

Statistical analysis

The data was analyzed using IBM SPSS Statistics(Statistical Package for the Social Sciences) 21 software for statistical analysis. The findings were presented through the use of mean and standard deviation. The concentrations of toxic elements in the fingernails and scalp hair of petrol station workers and control volunteers were compared using Microsoft Excel in Office 365 to calculate the T-test. It was determined that there wasn't a normal distribution, a value of $p < 0.05$ was considered to be significant. Pearson correlation was used to identify connections between the levels of heavy metals in (hair, and nails) and the length of time spent working. A significance level of p≤0.05 was utilized.

RESULTS

Lead and Cadmium contents in hair and nail sample

Table 1 shows the concentration of toxic metal (Pb, and Cd) in fingernails and scalp hair samples of 20 unpolluted workplace site volunteers (control) It was found that the concentration ranges of Pd and Cd in the hair of unpolluted workplace site volunteers were .6.01 _13.92,0.11– 0.44. In contrast, the concentration ranges of Pd and Cd in the nails of unpolluted workplace site volunteers were .7.01_ 13.01, 0.28– 0.63.

According to Table 2, the concentrations for workers of petrol station groups were found in the range (2.54–35.0) for Pb in hair (3.00_ 18.00) for Pb in nails, and (1.00– 12.5) for Cd in hair (1.24– 18.00) for Cd in nails.

From Table 3. The average concentration of Pb was (14.00 in the hair of workers at the petrol stations and (7.99) in the nails. The average concentration of Cd was (5.96) in the hair of petrol station workers and (3.91) in the nails. The average concentration of Pb and Cd in the hair and nails of volunteers from an uncontaminated workplace site were in sequence (9.82), (10.22), (0.26), (0.40).

A comparison of the concentrations of toxic and heavy elements in scalp hair and nails of petrol station workers and control volunteers was carried out using Microsoft Excel for Office 365 to calculate the T-test. It was determined that there wasn't a normal distribution, a value of $p < 0.05$ was considered significant.

The data on unpolluted workplace sites and petrol station workers were compared. In general, it was noted that the concentrations of Pb and Cd were higher in petrol station workers' hair and nail samples than in control samples ($p < 0.05$), and this is in agreement with similar studies conducted in Iraq [34].

Table (1): The concentration of heavy metals in hair and nails (mg kg − 1) of unpolluted workplace site (control).

Sample	Age	Hair		Nail	
		Pb	Cd	Pb	Cd
1	51	10.1	0.4	12.9	0.51
2	35	13	0.21	10.38	0.33
3	41	10.88	0.21	9.4	0.31
4	20	6.6	0.18	10.51	0.42
5	42	8.1	0.31	12.21	0.41
6	35	11.30	0.21	9.41	0.32
7	38	9.33	0.44	12.1	0.63
8	25	9.01	0.21	7.01	0.30
9	42	8.02	0.4	10.01	0.51
10	50	12.01	0.23	9.11	0.42
11	41	10.02	0.11	12	0.28
12	44	11.11	0.22	8.5	0.40
13	35	9.04	0.25	10.04	0.40
14	53	9.12	0.4	13.01	0.61
15	55	13.92	0.31	10	0.49
16	35	6.01	0.33	8.61	0.48
17	40	8.1	0.22	9.9	0.29
18	29	11	0.2	8.59	0.29
19	22	9.05	0.25	12	0.33
20	29	10.77	0.21	8.88	0.28

Table (2): The concentration of heavy metals in petrol station workers' hair and nails (mg kg − 1) and their age, daily work hours, and working duration.

Sample	Age year	Work Duration (month)	Daily Hours	Hair		Nail	
				Pb	Cd	Pb	Cd
1	22	200	12	34.50	12.25	17.95	7.83
2	24	100	12	20.06	1.00	10.25	3.87
3	16	12	12	10.40	5.10	12.46	5.16
4	31	60	12	2.54	10.9	6.43	2.88
5	32	8	12	2.56	5.47	3.03	1.24
6	61	144	12	26.36	7.36	4.75	2.50
7	56	72	9	17.57	5.52	10.70	5.04
8	52	240	8	14.30	6.25	4.70	2.00
9	25	24	12	6.66	2.60	4.05	3.53
10	26	24	8	4.00	2.10	5.22	3.67
11	39	240	12	35.00	12.3	18.00	8.00
12	38	276	12	20.10	1.00	10.30	4.00
13	38	264	12	10.40	5.10	13.00	5.20
14	37	180	12	3.00	11.00	6.00	3.00
15	31	72	12	3.00	6.00	3.00	1.24
16	18	36	12	26.40	7.80	5.00	3.00
17	24	60	12	18.00	6.00	11.00	5.00
18	29	96	12	14.30	6.25	5.00	2.00
19	35	60	12	7.00	3.10	4.10	5.00
20	26	84	5	4.00	2.00	5.00	4.10

Table (3): Descriptive statistics of the heavy metal concentration found in hair and nail of workers.

	Statistics	Age	Hair		Nail	
			Pb	Cd	Pb	Cd
Polluted site (petrol station)	Mean	33.00	14.00	5.96	7.99	3.91
	Median	31.00	12.35	5.76	5.60	3.77
	Std-deviation	12.06	10.52	3.52	4.66	1.85
	Minimum	16.00	2.54	1.00	3.00	1.24
	Maximum	61.00	35.00	12.30	18.00	8.00
Unpolluted site (Control	Mean	38.1	9.82	0.26	10.22	0.40
	Median	39	9.675	0.225	10.00	0.4
	Std-deviation	9.904	1.996	0.088	1.654	0.10
	Minimum	20	6.01	0.11	7.01	0.28
	Maximum	55	13.92	0.44	13.01	0.63

Correlation between heavy metal in (hair, nail) and work duration

The Pearson's Correlation Coefficient was conducted to detect if there were correlation between metal concentration in worker's hair and nail, and other Variables (age, daily hour and work duration). The results of the statistical analysis demonstrated a significant positive correlation ($P < 0.05$) between the work duration of employees and the level of lead (Pb) found in their hair and nails (0.450162, 0.487173) ,also there was a significant positive correlation ($P < 0.05$) between concentration of Pb in the hair of workers and concentration of Pb and Cd in the nail of workers(0.660219,0.574807) , as well as between concentration of Pb in the nail of workers and concentration of Cd in the nail of workers (0.881698) as indicated by Table (4), and Figures 2 and 3.

Table (4): Pearson's Correlation Coefficient between metal concentration in worker's hair and nail, and other Variables (age, daily hour and work duration).

			hair		nail	
	Age	work duration	Pb	Cd	Pb	Cd
Age	1					
work duration	0.452694	1				
Pb	0.135789	0.450162	1			
Cd	0.1127	0.259676	0.372844	1		
Pb	-0.09001	0.487173	0.660219	0.363863	1	
Cd	-0.13838	0.328403	0.574807	0.26376	0.881698	1

Histopathological observations

This research suggests that exposure to heavy metals such as lead and cadmium may be damaging to the nails as well as the hair shaft as illustrated in Figures 4, 5, 6, 7, 8, and 9.

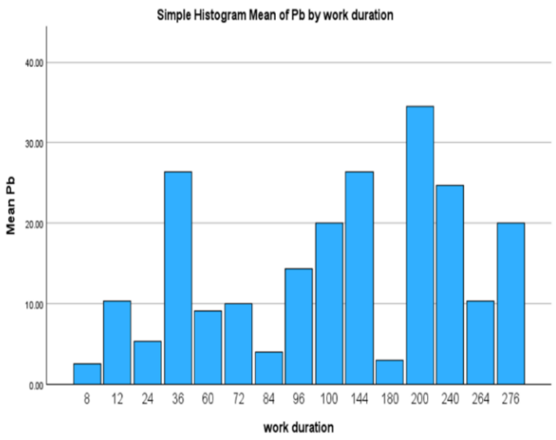

Figure (2): Correlation between lead levels in hair and work duration.

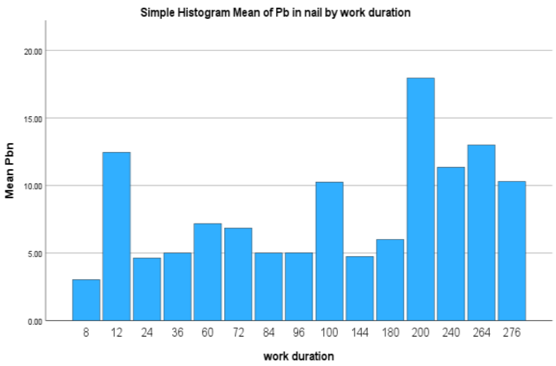

Figure (3): Correlation between lead levels in nail and work duration (Pbn: mean of Lead in nail).

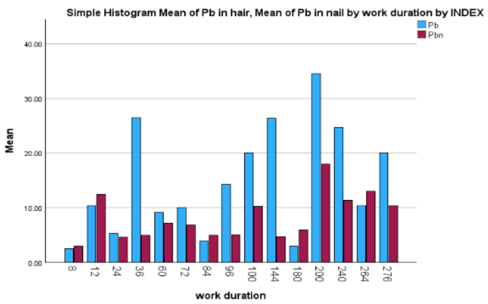

Figure (4): Correlation between lead levels in (nail, hair) and work duration.

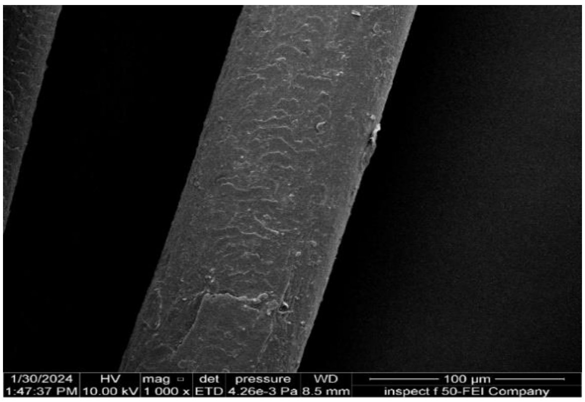

Figure (5): Scanning electron microscopy A Grade 0 (control) – Virgin intact hair with a regular overlay of the cuticle. 100 microns, x 1000, 10 kv**.**

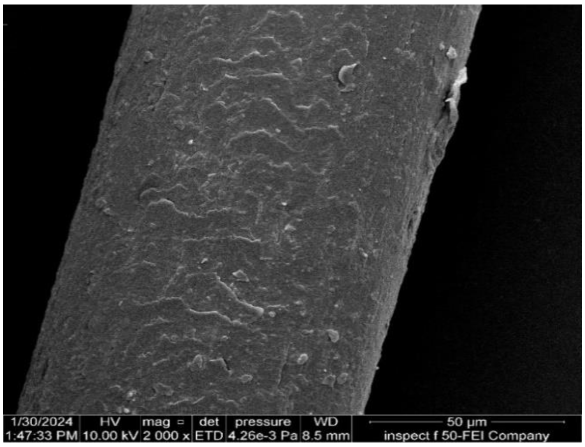

Figure (6): Scanning electron microscopy Grade 1 – Irregular overlay of the cuticle without cracks or holes. 50 microns, ×2000, 10 Kv.

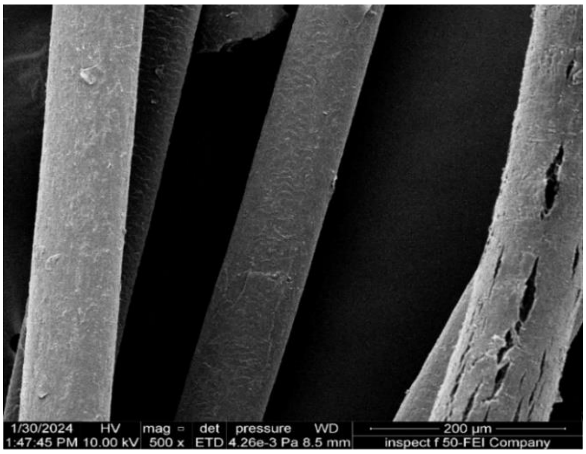

Figure (7): Scanning electron microscopy Grade 2 – Severe lift-up of the cuticle with cracks or holes (blue arrow) but without cortex exposure. 200 micron, ×500, 10 Kv.

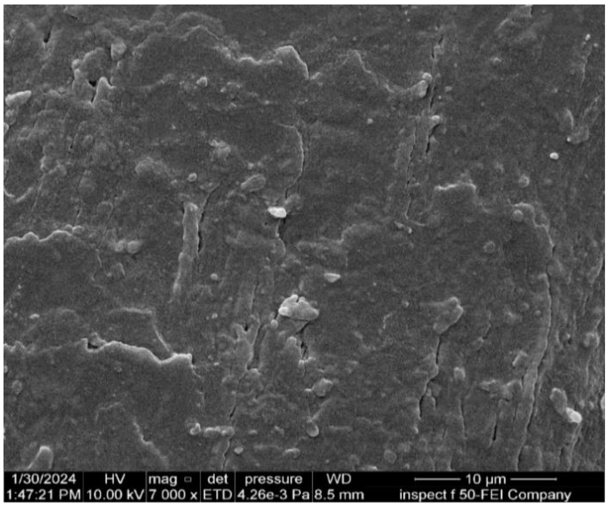

Figure (8): Scanning electron microscopy Grade 3: Partial exposure of cortex, 10 micron, x 7000, 10 kv.

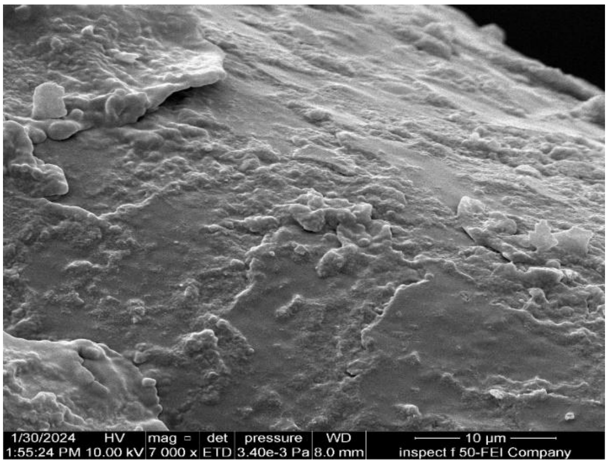

Figure (9): Scanning electron microscopy Grade 4: Complete disappearance of cortex. 10 micron, x7000, 10 kv A B.

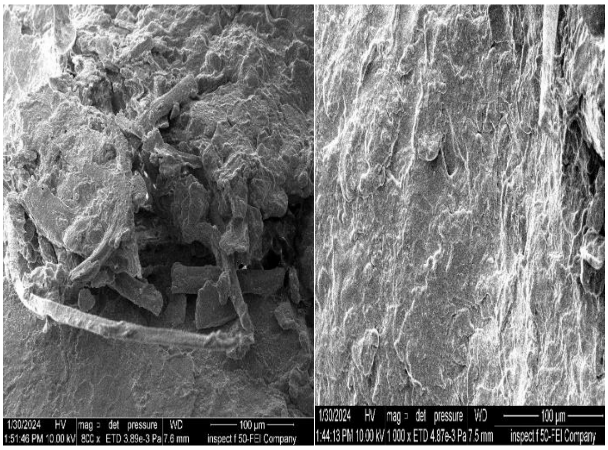

Figure (10): SEM of affected nail. (A) healthy human nail is uniform, composed of flat, overlapping slate-like sheets oriented in the plane of the nail, (B) Affected dorsal human nail appears to be rough, with globular domains (blue arrow) due to exposure to heavy metal) 100-micron, x 1000, 10 kv

DISCUSSION

Every workplace possesses distinct attributes and inherent hazards for employees. The main focus of the ongoing investigation was on the employees working at the Al-Ramadi petrol stations. The main purpose of the current analysis was to assess the levels of metals (specifically Cadmium and Lead) found in the nails and hair of petrol station workers. This study highlighted the hazards of exposure to toxic and heavy metals in highly contaminated environments, where employees come into direct contact with various pollutants. Health risk hazards by metals have become a common challenge within workplaces across the globe. In this context, the use of hair and nail analysis to measure toxic and heavy metal levels has been supported by the WHO, due to the link between environmental exposure and the concentrations of these metals in these biological samples. Hair is commonly used as a biomarker for heavy metals in the human body [35–38]. World Health Organization (WHO) has recommended that Human hair and nails as bio-indicators for environmental pollution with heavy metals [39]. Generally, hair analysis can be seen as a rather reliable approach to the assessment of heavy metals impact and its results can be used as the biomarkers of environmental/occupational exposure to metals. Nails can serve as biomarkers for heavy metal exposure [40–42]. Studies have shown that the concentration of heavy metals in nails can reflect exposure levels and potential health risks [43, 44].

The concentration of lead (Pb) in human hair and nail samples is elevated, as indicated in Table 2. Upon comparing the average concentration of the current study on lead and Cadmium levels in hair and nail samples with the average concentration of control values found in Table 3, it is evident that lead and Cadmium average concentrations are considerably higher in hair and nail samples from workers than control group this is consistent with other studies conducted in Iraq in the Baghdad [45] and Basra [46] regions. There are many possible reasons why workers are exposed to lead. Despite the ban on lead as an additive in consumer fuel for cars by the USA and the European Union, many countries in the Middle East, such as Iraq, still use lead in petrol [47]. A study in Baghdad found lead levels in leaded gasoline reaching up to 105 mg/L, while unleaded gasoline had a much lower concentration of 3.1 mg/L [10]. Also research in the Kurdistan region revealed lead concentrations in gasoline ranging from 0.016 to 0.18 gm/L, with tetraethyl lead (TEL) and tetramethyl lead (TML) also detected [48]. Workers at gasoline stations are often exposed to benzene and gasoline as part of their daily duties. The presence of lead at the workplace is due to emissions in the form of tiny particles that are breathed in through the lungs, as well as through skin contact and ingestion [49].

Petrol station workers have high levels of cadmium in their hair and nail samples. When compared to control values, all samples showed significantly elevated levels of cadmium.

Occupational exposure is likely the main source of cadmium in the hair of petrol station workers. Workers employed in industries such as roadway workshops, carriage workshops, battery factories, and plants using cadmium-containing dyes have been shown to have higher cadmium levels in the hair than the normal population [50]. Those living near these industrial areas, including petrol station workers, may also have increased cadmium levels in their hair due to environmental pollution from cadmium emissions [51]. Environmental exposure is considered a major risk factor as cadmium, a dangerous heavy metal, can be found in the air, water, and soil, and can enter the body through inhalation, ingestion, or skin contact [52]. Additionally, smoking has been linked to higher cadmium levels in nails [53]. Recent studies have pointed out significant heavy metal pollution in the area [54–56].

Correlation between lead levels in hair and work duration

The study also evidences a positive significant relationship between the work duration and the level of lead detected in hair and nails also between concentration of Pb in the hair of workers and concentration of Pb and Cd in the nail of workers, also there was a significant positive correlation ($P < 0.05$) between concentration of Pb in the nail of workers and concentration of Cd in the nail of workers. That indicating their possible common variations or sources causing increasing concentratin of Pb, and Cd in the hair and nails of the workers. This pathophysiological connection may be a result of accumulated build-up of lead in these tissues from the environment. Lead is one of the metallic elements and is present in aerosols, water, and foods. Hair and nails are preferable biomarkers of long-term lead exposure because hair and nails are less likely to undergo the same changes that alter the concentrations of trace metals during their incorporation into tissues. The results of the present study are consistent with previous research [57, 58]. Studies have shown that extended hours at work are associated with raised lead concentrations in hair. This research suggests that hair and nails are the biological markers, signalling the detection of the contamination and providing information regarding long-term contact with the lead.

Scanning electron microscopy (SEM)

The SEM operates under magnifications between 1 μm to 1 nm with better resolution and depth of focus compared to optical microscopes. It has the capability determining of the components on the surface through energy-dispersive X-ray analysis [58]. Many of research prove how toxic compounds such as lead or cadmium are toxic to the hair and nails. A particular study found that changes in hair texture, such as breakage, roughness, and tearing, occurred in samples treated with various physical (straightening) and chemical (bleaching, coloring, waving) processes [32]. According to the SEM results regarding hair grades the following information can be extracted about the cuticle Although it has an irregular layering the cuticle in Grade 1 is almost entirely preserved and there are no holes present on it and neither any fissures are observed (Fig. 6). The most severe type of hair loss is the complete loss of the cortex observed through SE microscope (Fig. 9) (grade 4) Long term exposure to the pollutants and severe environmental agents such as heavy metals weaken the hair structure gradually. The cortex, and even more the cuticle, can dissolve from its entirety when it comes in contact with strong mineral acids, alkalis, or hazardous industrial chemicals.

Heavy metals ' effect on lipids and proteins alters the smooth outer layer known as the hair cuticle. Such chemical conversations might cause localized brittleness and erosion that when observed on skin definitely looks like there are holes or gaps, but cortexes are very much present. Cuts and abrasion of the skin's cuticle are particularly worsened whereby the person is prone to handling heavy metals usually found in industries [59]. This indicates that there is a low level of structural damage, which may be caused by small outside stimuli such as environmental pollution exposure. This area of the hair is still functional as a protective sheath covering the deeper sections. On the other hand, significant fragmentary damage like fracture implies a lift-up of the cuticle. It could be suggested that the damage to the hair shaft observed in our study participants is not severe enough to cause noticeable changes that can be seen under light microscopy or dermoscopy. This shows how essential it is to apply scanning electron microscopy, or SEM, for detecting early signs of minor hair shaft damage. SEM is usually considered a useful extra instrument to examine abnormalities in hair shafts [60]. In their normal state, the nail plate which is a laminate structure composed of flat overlapping slates like keratin scales arranged in the direction of the terminal plane of the nail is uniform and has a planar surface. Power, elasticity, and shield are the advantages the people can get thanks to such well-organized staking. Due to their adherence, the keratinized cells have a more polished surface and a definite mechanical support. Lead is a heavy metal preferred especially sulfhydryl groups of keratins such as cysteine, cadmium closely follows lead and mercury is the last one. Metal-protein interactions follow and disturb the flat as a whole. Chronic effect of heavy metals causes a pathological change in the structure and function of tissues of nail bed leading to irregular aggregation of keratin because of interference of normal cellular metabolism leading to improper formation of keratin sheets due to rough patches [61, 62].

CONCLUSIONS

It could be inferred that increased amounts of heavy metals in hair and nail samples of petrol station workers of Al-Ramadi city parallel results observed in other parts of the globe. They recommend that further research should be done, which includes blood and urine examination to assess the level of toxins in the body, especially in special districts of Al-Ramadi city. Occupational health and safety standards should be observed to ensure petrol station employees avoid exposure to heavy metals, therefore protecting themselves.

DISCLOSURE STATEMENT

- **Ethics approval and consent to participate:** All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki
- **Consent for publication:** Not applicable
- **Availability of data and materials:** The raw data required to reproduce these findings are available in the body and illustrations of this manuscript.

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