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RESEARCH ARTICLE

Evaluation of Trace Lead, Chromium and Cobalt Level in the Urine Samples of Industrial Workers

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Abstract

For clinical reasons, and in order to control minimise the effects on the health of individuals exposed to toxic elements, it is most important to monitor concentrations of trace elements in biological materials. Such an examination was achieved for urine samples of workers with different occupations and of different ages. Concentrations of lead, chromium and cobalt were examined in forty urine samples using the flame atomic absorption spectroscopy (F-AAS) technique. The average concentrations of Co, Cr and Pb were 0.0069 to 0.0484 ppm, 0.1056 to 0.9645 ppm and 0.1188 to 0.5067 ppm, respectively. A low detection limit for Co was registered compared to the Pb and Cr. This study has shown that Cr concentrations in the urine samples of people who work in oil refineries were higher than for people working in other vocations. Concentrations of trace Co, Cr and Pb in the urine of people aged 30-40 years were larger than the concentration found for individuals in other age groups. The T-test, F-test and ANOVA have been used to examine the accuracy and precision of results and compare them with those published in the literature (literature review results).

Keywords: *Lead, Chromium, Cobalt, Urine, FAAS.*

Introduction

It is very important to monitor the concentrations of trace elements such as Pb, Cr, Cd, Ni, Hg, Co and Cu in the human body because an increasing concentration of these elements will ultimately have serious effects on human health. Urine is commonly used an indicator of past exposure, where the presence of high concentrations of Pb, As, Cr, Ni, Cu and Co indicates that the individual in question has been exposed to a source of these elements for a long period of time.

Increasing concentrations of the above in blood are an indication of acute current exposure [1]. The existence of Pb in the blood is an indicator of likely exposure to a source of contamination due to lead binding to red blood cells, while presence of Cd in the blood is a marker of industrial exposure [1-3]. Some elements such as Cu, Co, Cr and Mn can be normally detected in the human body due to these metals being required for a large number of enzymes to function properly [4]. In biochemistry, trace elements termed "micronutrients", are small amounts of the chemical species required to allow for proper growth, development, and physiology of the organism [4, 5]; however, increasing the concentrations of these species can result in a serious risk to health. Thus, this encouraged the researchers to monitor the concentrations of trace elements in the human's body, especially in people who have jobs where exposure to toxic trace elements is more likely.

Several methods can be used to measure the concentrations of trace elements in biological materials [4, 6, 7]. Inductively coupled plasma–atomic emission spectroscopy (ICP-AES) has been widely used in the determination of trace elements [4, 8]. Moreover, inductively coupled plasma mass spectrometry (ICP-MS) has been used to monitor the concentration of trace elements, where these techniques can be used to detect very low concentrations of elements, have wide dynamic ranges and the capability for rapid multielemental determination [9-15]. There are advantages to using the ICP technique which are its high sensitivity, high accuracy, can be examined a wide range of sample types and small amount of sample are needed [16, 21].

However, interferences in the ionization can occur in this technique due to high numbers of electrons already present in the plasma suppressing further ionisation and thus providing a steady equilibrium between the numbers of atoms and ions. Trace elements in biological materials can be examined using atomic absorption spectrometry (AAS), which is one of the most widely used techniques in the determination of the concentrations of elements [22, 23]. In this study, trace element concentrations of Co, Cr and Pb were investigated using flame atomic absorption spectroscopy (F-AAS). The study was conducted using forty urine samples from people with different careers (oil refineries, welding, casting alloys, fuel terminals and dyeing).

Materials and Methods

Standard solutions of 1000 ppm Co, Cr, and Pb were prepared in a solution of 70% HNO_3, 50% H_2O_2 and 3 M HCl (BDH). Concentrations of Pd, Cr and Co in the urine samples were measured using an atomic absorption spectrometer (Atomic Absorption - AA-7000- Shimadzu-Japan). According the manufacture, the parameters for the AAS instrument should be adjusted for each experiment, as listed in Table 1.

Table 1: Instrumental operating parameters for each trace element

Elements	Parameters				
	Wavelength (nm)	Slit width (nm)	Burner height (mm)	Fuel gas flow (l/min)	Oxidant and Fuel
Co	240.7	0.2	7	2.2	Air - acetylene
Cr	357.9	0.5	9	2.8	Air - acetylene
Pb	217.0	0.5	7	2.0	Air - acetylene

Sampling

Urine samples were collected from people with different careers (dyeing, welding, plumbing, and working in oil refineries and fuel terminals). In order to prevent their decay, the samples were stored in new polyethylene bottles (120 ml) and then placed in a fridge at 0-5°C. The urine samples were treated as follows: a mixture of 12 ml H_2O_2 and 6 ml of conc.

HNO_3 were added to the 60 ml urine sample and then accurately measured into a 100 ml beaker, which was then placed on a hot plate. The samples were then gently heated until their amber colour disappeared, and the residual fluid then evaporated almost to dryness. 2.5 ml of conc. nitric acid was then added, and the liquid once again evaporated to dryness. This process was repeated until a white ash was formed. In the final step, the residue was dissolved in 2.5 ml of 3.0 M of HCl and then diluted to 30 mL with deionized water [22, 24, 27].

Result and Discussion

After the urine samples had been digested as above, the concentrations of trace elements (Pb, Cr and Co) were investigated for each of the forty samples, the results for which are reported in Table 2. The samples were collected from people aged 20 to 60 years and in different jobs, but all of whom were male. The results were obtained using the F-AAS Method.

Table 2: Data results of trace element in urine sample by FAAS.

ID	Sex	Age (years)	Conc. of Cr (mgl^{-1})	Conc. of Pb (mgl^{-1})	Conc. of Co (mgl^{-1})
A001	Male	55	0.4737	0.3503	0.0069
A002	Male	37	0.1056	0.1626	--
A003	Male	28	0.6032	0.3628	0.0231
A004	Male	26	0.8077	0.3253	0.0392
A005	Male	27	0.6032	0.2127	0.0277
A006	Male	50	0.4056	0.3003	0.0427
A007	Male	23	0.2420	0.2002	0.0288
A008	Male	22	0.2147	0.1501	--
A009	Male	60	0.3987	0.1689	0.0231
A010	Male	49	0.2760	0.2752	--
A011	Male	28	0.3578	0.1939	0.038

A012	Male	29	0.3442	0.2440	--
A013	Male	53	0.3374	0.2314	--
A014	Male	45	0.2897	0.2502	--
A015	Male	32	0.5282	0.4316	--
A016	Male	25	0.3919	0.1939	0.0392
A017	Male	27	0.6918	0.1751	0.0081
A018	Male	26	0.3442	0.3253	0.0081
A019	Male	35	0.4192	0.1188	0.0023
A020	Male	45	0.4056	0.1814	0.0219
A021	Male	24	0.9645	0.2940	0.0069
A022	Male	22	0.3442	0.1001	0.0081
A023	Male	23	0.2965	0.1439	0.0104
A024	Male	42	0.3510	0.2127	0.0150
A025	Male	35	0.4260	0.3065	--
A026	Male	25	0.4942	0.1626	0.0173
A027	Male	38	0.0579	0.4879	0.0484
A028	Male	55	0.2624	0.2752	--
A029	Male	47	0.0784	0.0375	0.0291
A030	Male	45	0.1943	0.0938	--
A031	Male	38	0.6237	0.1751	--
A032	Male	48	0.6100	0.4316	--
A033	Male	41	0.3306	0.2690	0.0438
A034	Male	34	0.4056	0.2877	0.0415
A035	Male	55	0.1602	0.1314	0.0334
A036	Male	53	0.1670	0.3190	--
A037	Male	22	0.0920	0.3378	--
A038	Male	28	0.6509	0.7756	--
A039	Male	43	0.5282	0.3315	--
H040	Male	33	0.4805	0.5067	0.0461

Table 3 shows the average concentrations of Co, Cr and Pb in the urine samples of people from the different professions mentioned above. It clears from Table 3 that the average concentrations of Cr in almost all the samples are higher than the concentrations of Pb and Co. A low detection limit of Co was registered in the urine samples. It was found that concentrations of Co in the urine of workers of dyers was greater than those for individuals in other professions, while the average concentration of Cr in welders was greater than for other professions, which may be because welders deal extensively with alloys containing chromium [28]. From table 3 it can be seen that the percentage of Pb in the urine samples of oil refinery workers was higher than in other lines of work; again, this is not surprising because they deal with gasoil which is often treated with lead-based species to enhance its properties [29]. It can be summarised from the data that Co concentrations lie approximately between 0.025 to 0.039 ppm, which is in agreement with literature values classified as ultra-trace elements in urine samples [1, 9, 26, 30].

Table 3: Mean Concentrations of Co, Cr and Pb in urine samples for people with different jobs

Type of job	**Mean ±SD**		
	Co (ppm)	Cr (ppm)	Pb (ppm)
Oil refineries (A)	0.0288285± 0.01710 n = 7	0.431 ± 0.1901255 n = 15	0.2922 ± 0.1044361 n = 15
Welding (B)	0.025 ± 0.0139076 n = 6	0.4975 ± 0.2284337 n = 10	0.2727 ± 0.0972265 n = 10
Casting of alloys (C)	0.029966 ± 0.00751 n = 5	0.29784 ± 0.0779026 n = 5	0.1976 ± 0.0477549 n = 5
Terminals to provide fuel (D)	0.03745 ± 0.005727 n = 2	0.2829 ± 0.1735240 n = 5	0.2095 ± 0.11052079 n = 5
Dyeing (E)	0.0392 ± 0.00000 n = 5	0.40418 ± 0.077159 n = 5	0.2602 ± 0.1212796 n = 5
Total	0.02925 ± 0.013360 n = 25	0.41973 ± 0.1848177 n = 40	0.26462 ± 0.0995602 n = 40

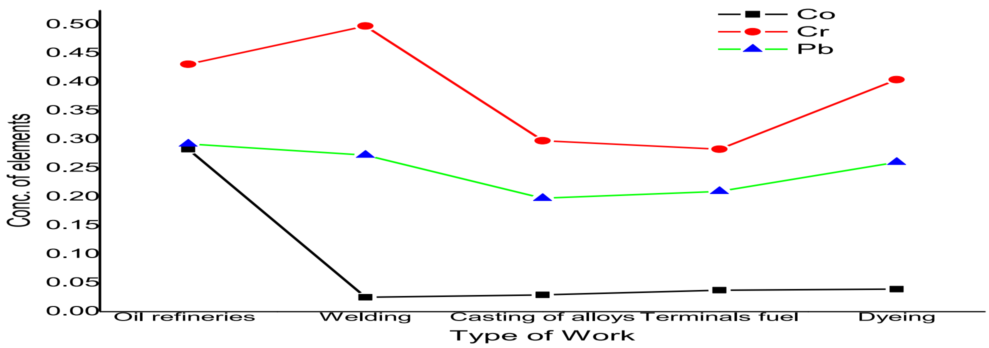

Fig.1: Type of occupation versus trace element concentrations for Co, Cr and Pb

From Fig. 1, it can be seen that the concentrations of Cr and Pb are linked in a given line of work. For example, when the concentration of Cr increased in the urine samples of welders, their average concentration of Pb increased as well. In general, the concentrations of the metals are greater than the concentrations of the same metals measured in urine samples of people who did not work in industrial fields.

Concentrations of Co, Cr and Pb in urine have been determined in other studies [1, 9, 26, 30]. The study results are shown in Table 4. Thus, the average concentrations of Co, Cr, and Pb recorded in this work were compared to literature results (Table 4). It was found that the detection limits of Co, Cr and Pb in urine samples in this work are less than those shown in Table 4. This may be due to type of employment of the people who were selected for the test and their associated potential exposure to contamination. Concentration ranges for trace elements remain unknown for a significant number of people, though the literature gives some guidance when comparing the concentrations of trace elements in individuals. The average concentrations of trace elements in the human body can be affected by a number of factors.

For example, urine samples collected from non-smokers gives different results compared to those collected from smokers [31]. Age can also affect the concentration ranges of trace elements in urine, where in this work the detection limits of Co, Cr and Pb were found to differ with age [32]. Furthermore, there can be trace elements deficiencies depending on the nature of food consumed [33].

Table 4: Literature results for detection limits of trace elements in urine samples

Elements	Concentration	Reference
Co	0.0353 mg l^{-1}	1
	0.18 mg l^{-1}	9
	0.030-0.040 mg l^{-1}	26
	0.002-0.135 mg l^{-1}	30
Cr	0.0355 mg l^{-1}	1
	0.157 mg l^{-1}	9
	0.001 mg l^{-1}	26
	0.0004-0.050 mg l^{-1}	30
Pb	0.0241 mg l^{-1}	11
	0.0011 mg l^{-1}	12
	0.012-0.030 mg l^{-1}	14

Statistical Analysis of Data

In the treatment of the data, significance tests such as ANOVA, pair t-test and f-test were used in this study. This has been carried out by distinguishing between the experimental results and the results of the literature survey. Here, the pair t-test was used to estimate the p-value at a confidence level of 95%, the results of which are reported in table 5. From this, it may be noted that the value of the t-calculated values were higher than the t-critical values for each of the trace elements. Thus, a significant difference between the observed data and the literature data was found. This may be due to workers having greater exposure to certain sources of contamination and also could be related to a lack of commitment to safety and security regulations, where this might ultimately increase the concentration of such elements in the urine of affected workers.

Table 5: Results of the average concentration treatment by T-test

Element	t-calculated $t = \bar{y} - \mu \dfrac{\sqrt{n}}{\sigma_{n-1}}$	t-critical	p-value
Co	9.512	2.101	0.00001
Cr	13.806	2.030	0.00001
Pb	15.889	2.028	0.00001

To be more certain, the ANOVA test was applied due to its greater dependability. In this test, the overall experimental results were compared with the overall concentration of these elements reported in the literature. The results in Table 6 indicate a significant difference between the measured concentrations of the trace elements in this study with those published in the literature. It may be noted that the p-value was higher than 0.05, indicating a significant difference between the two sets of results. Thus, the experimental value can be more acceptable and null hypothesis is more agreed.

Table 6: One –way ANOVA test results for the correlation of elements

Metal	Total Conc. $\mu g\ ml^{-1}$ by FAAS	Quoted value $\mu g\ ml^{-1}$	$d.f_{between}$ $d.f_{within}$ $d.f_{total}$	$SS_{between}$ SS_{within} SS_{total}	$MS_{between}$ MS_{within}	$F = \dfrac{MS_{between}}{MS_{within}}$	P-value
Co	0.0292	0.00017	2	0.0387	0.0193	0.47127	0.66377
Cr	0.4197	0.00018	3	0.1231	0.041		
Pb	0.2646	0.00110	5	0.1618			

Here, the f-test method was used to examine the correlation between pairs of elements, as shown in Table 7. From this, a high correlation between the concentrations of Cr and Pb was observed. This indicates that contamination by Cr and Pb runs in parallel.

Table 7: F- test for correlation effect between elements

Element ratio	F-calculate $F = \dfrac{\sigma^2}{\sigma^2}$	F-critical value	P- value
Co : Cr	200.88	1.89862196	0.00001
Co : Pb	58.235	1.90734592	0.00001
Cr : Pb	3.4494	1.75229897	0.00019

Trace Element Concentrations in Urine for Different Age Groups

In this study, age was selected as a parameter by which to study the average concentrations of trace elements in urine. An average concentration of Co, Cr and Pb in the urine of people aged between 20 to60 is shown in Table 8. It was found that the detection limit of Co in the samples of people aged 30-40 years is about 0.0349 ppm, which is the highest of all the age groups. The same was found for Pb, where the highest concentrations of Pb were detected (0.03096 ppm) in the urine of people aged between 30 and 40 years. Table 8 shows that high concentrations of Cr were recorded in the urine samples of people aged between 20 and 30.

Table 8: Detection limits of trace elements in urine for people of different ages

Age (years)	Mean of concentration ($\mu g.ml^{-1}$)		
	Co	**Cr**	**Pb**
20-30	0.0252	0.4900	0.2372
30-40	0.0349	0.4269	0.3096
40-50	0.0311	0.3731	0.2788
50-60	0.0330	0.3150	0.2537

Fig. 2 shows average concentrations of Pb, Co and Cr in the urine of people in different age groups. The highest concentrations of Cr were reordered in the urine samples of people aged between 20 and 30. However, low concentrations of Cr were detected in the urine samples of people aged 50-60. In Figure 2, no clear change can be observed in the concentration of Co in the urine samples.

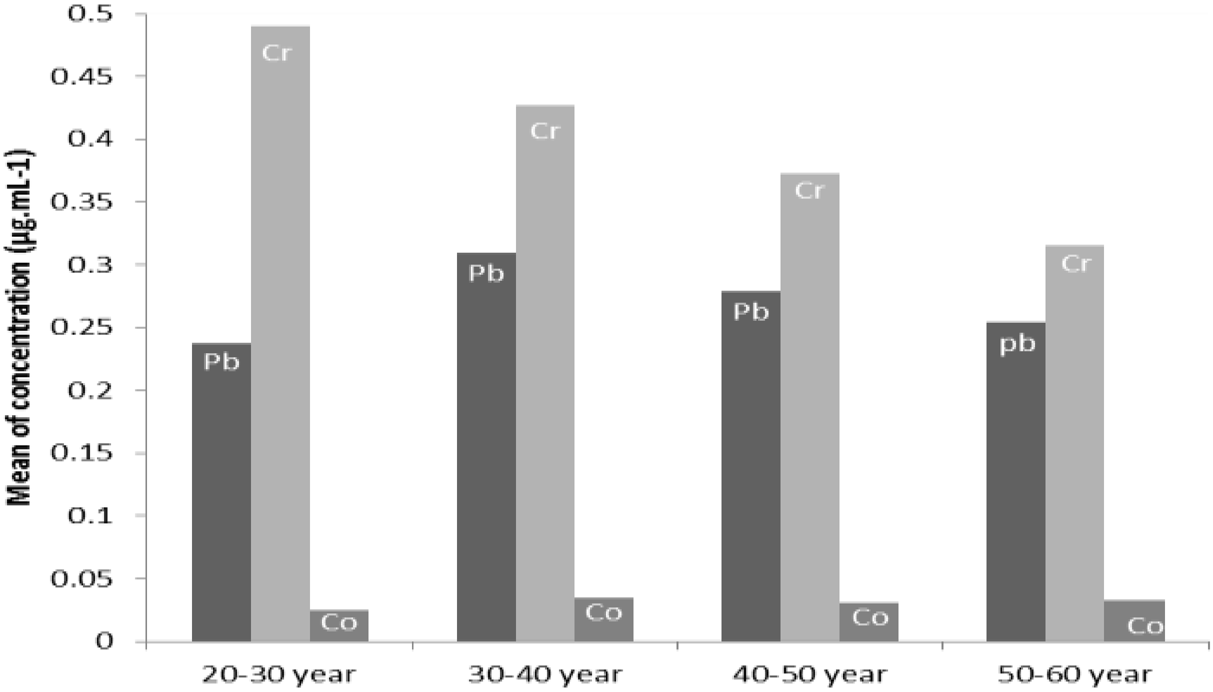

Fig 2: Trace element concentrations in the urine of people of different ages

Conclusion

Concentrations of trace elements (Co, Cr and Pb) in the urine samples of people living in Iraq and having different occupations and different ages were investigated using F-AAS. This method was found to be accurate, fast, highly selective and highly sensitive. This study observed that the detection limits of Pb in the urine samples of people working in oil refineries were higher than for people working as welders, in alloy casting and in dyeing. No significant correlation was observed between average concentrations of Co, Cr and Pb in this study and other studies. The concentrations of Co and Pb in the urine of people aged 20-30 years was lower than those aged 30 to 40 years, while the highest average concentrations of Co, Pb and Cr were recorded in the urine samples of people aged between 30 and 40. It was concluded from statistical analysis that, the experimental value can be more acceptable and null hypothesis is more agreed.

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