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Heavy Metals in Healthy Water Samples Used by Newborn in Al-Najaf Governorate, Iraq

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Author Contributions

each authors Ban Hussein Ali and Ali Abid Abojassim subscribes to conceiving and designing the analysis; Collected the data; Contributing data or analysis tools; Performed the analysis; Writing the paper.

ORIGINAL STUDY

Heavy Metals in Healthy Water Samples Used by Newborn in Al-Najaf Governorate, Iraq

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Abstract

Water is essential for life, so monitoring water quality is important. This research focuses on measuring heavy metals (Lead, Pb; Cadmium, Cd; Chromium, Cr (in 14 healthy water samples of two types; bottled water and filtered water used by newborn children in Al-Najaf governorate, Iraq. Atomic absorption spectroscopy (AA-7000, Japan) was used to conduct the experimental analysis. Also, health risks parameters (non-carcinogenic and carcinogenic) due to Pd, Cd, and Cr concentrations were calculated. The results of Pb, Cd, and Cr concentrations in mg/L for bottled water samples were ranged 0.03–0.83, 0.01–0.69, and 0.01–0.76, respectively, while the range values for filter water samples were 0.06–4.54, 0.08–1.2, and 0.62–1.62, respectively. Statically using ANOVA-test confirmed, that the result is no significant at p > 0.05, where the average values of Pb, Cd, and Cr in bottled water samples were lower than in filter water samples. Accordingly, the results of Pb, Cd, and Cr concentrations in healthiest water samples for two types of Al-Najaf governorate, were larger than safe limit that were recommended by several organizations and commissions, including the WHO, USEPA, ECE, and Iraq. Meanwhile, the results of non-cancer and cancer health risks were within safe limits according to EPA and WHO.

Keywords: Heavy metals, Health water, Bottle water, Newborn, Cancer, Iraq

1. Introduction

he natural toxins (heavy metal) were released into the air and water when people moved to cities and businesses and farms grew, which individuals inhale through contaminated air, consume contaminated water, and consume contaminated food [1]. Also, natural sources, such as the earth's crust, are where these elements enter humans. Erosion then transports them to water and air [2]. Heavy metals are chemicals that living things need in certain amounts, but when they get too high, they become harmful and hurt the environment. Heavy metal pollution is a problem that has gotten a lot of attention around the world [3-9]. The harmful effects of these minerals are most noticeable in very low amounts that don't break down [10,11] Water is the most important thing for life. Although 70 % of the Earth's surface is water, only 0.3 % of that water can be used and is safe for everyday use [10]. It is

impossible to list all the good things that water does for us, but it also picks up pollutants from the environment and then pollutes it. Pollution is any change in the basic properties of a natural element that leads to many health problems [1]. Heavy metal poisoning of water is caused by many natural and human actions. Heavy metals get into water from many industrial sources, such as the steel industry, the food processing industry, the plastics industry, metal works, leather tanning, and more [12].

Milk is important for babies and kids because it's nutritious. It has Carbohydrates, proteins, fats, and some heavy elements that kids need to grow and for cells and organs to do their jobs [13]. Instead of breast milk, babies are often given canned water or water that has been cooked to make milk. Toxic substances like lead (Pb), Cadmium (Cd) and Chromium (Cr) may be in this water. Heavy metals like Pb, Cd, and Cr can leach into drinking water from household plumbing and service lines, mining

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operations, petroleum refineries, electronics manufacturers, municipal waste disposal, cement plants, and natural mineral deposits. These substances are harmful to babies and have a big effect on their lives. Pb is one of the most dangerous metals for people. It can mess up the nervous system, lead to abortions in pregnant women, high blood pressure, kidney damage, kids' nervous systems shrinking, and kids not paying attention [1]. Cd is very bad for living things' health because it can cause high blood pressure, cancer, and infertility. It can also damage the heart muscle and cause breathing problems and osteoporosis [10]. Animals and plants are at a high risk of getting sick from Cr, which can hurt the kidneys, liver, lungs, skin, and even cause lung cancer [10]. These factors led to the selection of Pb, Cr and Cd as research targets. Monitoring the concentrations of these substances in water is essential for safeguarding ecological and human health due to their detrimental effects and long-lasting presence. Iraq's Water sources have significantly deteriorated in recent times due to wars. In the last twenty years, Iraq has faced serious and dangerous pollution in the natural environment, such as air pollution, soil pollution, and water pollution. The country is now going through a decline and deterioration related to the quality of water because of the multiple sources of pollution with no strategies to develop and strengthen the foundations for providing clean water. The water sources in Iraq represented by drinking water, rivers, and groundwater are polluted [14]. The pollution problems of rivers and water by heavy metals are considered the most prominent environmental problems. Many studies worldwide have studied the concentrations of heavy metals in healthy drinking water [6,15-20].

Heavy metals pose a particular threat to human children. Moreover, the presence and concentration of heavy metals in water are of particular concern and importance due to their potential toxicity and detrimental effects on both human health and the environment. Therefore, the aim of the current work is to measure Pb, Cd, and Cr concentrations in healthy water samples that are used by newborns of Al-Najaf governorate. The study is done by using atomic absorption spectroscopy, also, there are many non-cancer (CDI_{nca}, HQ, and HI) and cancer (CDI_{ca}, CR, and TCR) health risks were calculated.

2. Materials and methods

2.1. Area of study

In the South West Region of Iraq, AL Najaf Province is situated, which is represented by a latitude (29o50'00" N-32o21'00" N) and by a longitude (42o50'00" E-45o44'00" E) covering an area of (11281153.88) donuts, From the north it is bounded by the provinces of Babel and Karbala, and from the east it is bounded by the provinces of Qadsia and Muthna from the south and southwest by the Kingdom of Saudi Arabia [21].

2.2. Water samples

14 samples of healthy water were collected from Al-Najaf governorate duration from 1/10/2024 to 1/11/2024 as shown in Table 1. Initial quality control properties for samples water in the present study like packaging, labeling, expiry, etc., were performed. Water samples were divided into groups according to use by the mother of a newborn; bottles of water and 7 filters of water. Bottled of water were collected from markets that were used by children only. Filters of water (home desalination/purification devices (were collected from some locations in

Table 1. Information about the water samples from the study area.

No.	Sample type	Sample name or location	Sample code	Production date	Expiration date
1	Bottled water (BH)	Kawthar kids	BH1	1/09/2024	1/12/2024
2		Mina kids	BH2	3/09/2024	3/12/2024
3		Lojin	ВН3	2/09/2024	2/12/2024
4		Kids life	BH4	5/09/2024	5/12/2024
5		Mazi baby	BH5	7/09/2024	7/12/2024
6		Dora keelage	BH6	4/09/2024	4/12/2024
7		Slemani	BH7	10/09/2024	10/12/2024
8	Filter water (FH)	Mutanabbi	FH1	11/09/2024	11/12/2024
9		Old city	FH2	8/09/2024	8/12/2024
10		Salam	FH3	14/09/2024	14/12/2024
11		Abbasiya	FH4	9/09/2024	9/12/2024
12		Al-Mishkhab	FH5	12/09/2024	12/12/2024
13		Nasr	FH6	13/09/2024	13/12/2024
14		Askari	FH7	6/09/2024	6/12/2024

the Al-Najaf governorate and were used by different age groups including children (from 1 day to 2 year). The study samples for bottles of water were selected based on their availability in local markets, while filters of water samples for the areas indicated in Table 1 were based on their geographical distribution which includes all parts of the governorate.

2.3. Water preparation and digestion

2 ml water (Bottled water and Filter water) are mixed with 10 ml of deionized water, 2 ml of HNO_3 [22], and 1 ml of H_2O_2 into a volumetric flask with a volume of 150 ml. After that, 25 ml of deionized water is added to the mixture to get the samples ready for digestion. Water samples were first digested using heating digested at 200 °C for 1 h. After being broken down, all of the samples were left to cool at room temperature. 50 ml of deionized water was added to the samples, and filter paper $(0.45~\mu m)$ was used to remove impurities [23].

2.4. Atomic absorption spectrometer

Pb, Cd, and Cr concentrations were measured using the AA-7000 (SHIMADZU model) at wavelengths of 283.3 nm, 228.8 nm, and 357.9 nm, respectively, with a deuterium background corrector (BGC-D), used in this work. The determination of Pb, Cd, and Cr in cheese samples was done by using an air/acetylene flame (Air-C2H2). The value of slit width was 0.7 nm and the value of lamp current for Pb and Cr elements was 10 mA, while for the Cd element it was 8 mA. The LOD

(BGC-D2) for lead, cadmium, and chromium were (0.03-0.1, 0.002-0.008, and 0.005-0.02) ppm; the LOQ (BGC-D2) were (0.1-0.4, 0.007-0.03, and 0.02-0.07) ppm, respectively.

2.5. Calculation of health risk assessments

In the present work, health risk assessments such as CDI (Chronic Daily Intake), HQ (Hazard Quotient), HI (Hazard Index), CR (Cancer Risk), and TCR (Total Cancer Risk) were estimated due to exposure to heavy metals (Pb, Cd, and Cr) for newborns ingesting healthy water which included non-carcinogenic and carcinogenic analysis as follow [24–27].

$$CDI\left(\frac{mg}{L}.d^{-1}\right) = \frac{Cs \times IR \times EF \times ED \times CF}{BW \times AT} \tag{1}$$

where CDI is "Chronic Daily Intake", IR is "ingestion rate" of newborn water ($268.2 \pm 250.3 \text{ ml/d}$) [1], EF is "exposure frequency", ED is "exposure duration", CF is "conversion factor", BW is "body mas" which given for newborn (5.7 kg) [28], and AT is "averaging time".

$$HQ = \frac{CDI_{nca}}{RFD_O} \tag{2}$$

where, CDI_{nca} is non-carcinogenic of chronic Daily Intake which calculated by Eq. (1) when AT is equal 365 \times 30 d, and RFDo is "chronic reference dose of the toxicant" (Pb = 0.004 mg/kg. d⁻¹, Cd = 0.001 mg/kg. d⁻¹, and Cr = 0.003 mg/kg. d⁻¹) [29].

Table 2. Results o	f hearni	metals	for water	samnles	in the	current studu
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No.	Sample code	Concentration (mg/L)			
		Pb	Cd	Cr	
1	BH1	0.03	0.69	0.05	
2	BH2	0.58	0.69	0.76	
3	ВН3	0.10	0.02	0.09	
4	BH4	0.12	0.01	0.03	
5	BH5	0.23	0.02	0.01	
6	BH6	0.52	0.03	0.10	
7	BH7	0.83	0.02	0.07	
8	FH1	1.58	0.33	1.19	
9	FH2	0.09	0.08	1.19	
10	FH3	4.54	0.91	0.62	
11	FH4	1.58	0.64	1.26	
12	FH5	0.06	0.82	1.48	
13	FH6	0.84	1.09	1.62	
14	FH7	2.69	1.22	0.98	
Average \pm S.E. (sample 1–7)		0.34 ± 0.10	0.21 ± 0.11	0.16 ± 0.09	
Average ± S.E. (sample 8–14)		1.62 ± 0.55	0.73 ± 0.14	1.19 ± 0.11	
Average \pm S.E.		0.99 ± 0.33	0.47 ± 0.11	0.68 ± 0.15	

$$HI = \sum_{1}^{K} \frac{CDI_{k}}{RFD_{k}} \tag{3}$$

The values of HQ and HI due to heavy metals must be below the safe limit that equals one, according to EPA [30].

$$CR = CDI_{ca} \times SF \tag{4}$$

where, CDI_{ca} is carcinogenic of chronic Daily Intake which calculated by Eq. (1) when AT is equal 365×70 d, and SF is "slope factor" (Pb = 0.0085 mg/kg. d⁻¹, Cd = 6.7 mg/kg. d⁻¹, and Cr = 0.5 mg/kg. d⁻¹) [31].

$$TCR = \sum_{1}^{k} CDI_{k} \times SF_{k} \tag{5}$$

3. Results and discussion

The statistical analyses of the data were conducted using IBM SPSS Statistics software, especially (version 27 for the Windows) operating system. Single-factor Analysis of Variance (ANOVA) method was employed to conduct a comparative analysis among several groups of water samples and three heavy metals in the present study (Pb, Cd, and Cr). Finally, Pearson correlation coefficients were applied to examine the association between heavy metals concentrations. The analyses encompass the average and standard error (Av.±S.E.), including significant differences (P < 0.01). The researchers established a significance level of 0.05 for this investigation.

3.1. Heavy metals results

Table 2 shows healthy water samples of Pb, Cd, and Cr concentrations. The range values of Pb, Cd, and Cr concentrations (mg/L or ppm) in bottled water samples were 0.03-0.83, 0.01-0.69, and 0.01-0.76, respectively, with average values of 0.34 ± 0.10 , 0.21 ± 0.11 , and 0.16 ± 0.09 . Whereas, the range values of Pb, Cd, and Cr concentrations in filter water samples were 0.06-4.54, 0.08-1.22, and 0.62-1.62, respectively, with average values of 1.62 ± 0.55 , 0.73 ± 0.14 , and 1.19 ± 0.11 . While, the average values of Pb, Cd, and Cr concentrations for all samples were 0.99 \pm 0.33, 0.47 \pm 0.11, and 0.68 ± 0.15 , respectively. Fig. 1 shows the percentage values of all water samples (bottled and filter) in the present work. From Fig. 1, the maximum percentage of Pb, Cd, and Cr concentrations were 33 % in FH3 (filter in Salam region), 17 % in FH6(filter in Nasr

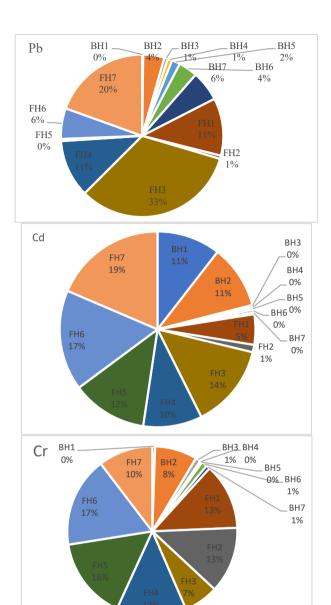


Fig. 1. The percentage value of heavy metals in water samples in the present study.

region), and 17 % in FH6(filter in Nasr region), respectively.

Fig. 2 shows the difference in the average of heavy metals in water samples between bottled and filtered. The average of all heavy metals (Pb, Cd, and Cr) in filtered samples is higher than in bottled samples. This may be due to pollution of tap water that is used in the filters of houses. As well as, heavy metals may leach into tap water supplies through pipes, corrosion of household plumbing fixtures, and fittings. Heavy metal can be introduced into the Iraqi water through several points, point and non-point sources including the leather industry, coal

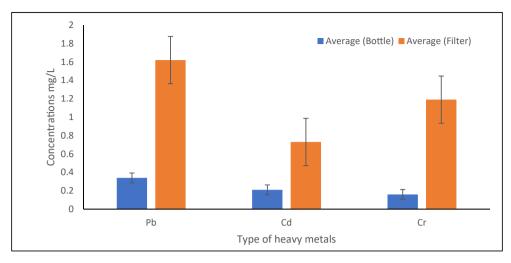


Fig. 2. Comparison of the average of Pb, Cd, and Cr in water samples between the bottled and filter.

mining, agricultural activity, and domestic waste. The results show significant (p < 0.05) differences in the average of heavy metals (Pb, Cd, Cr) in bottled and filtered water. Fig. 3 shows the comparison between the average values of Pb, Cd, and Cr in water samples in the present study with a world limit recommended by WHO [32], USEPA [33], ECE [34], and IQs (Iraq standards) [35]. This study shows that the average of Pb, Cd, and Cr was higher than the all-world limit. This is due to pollution in the main water used to make bottled and filtered water. where heavy metals can get into water through underground flow and seepage and runoff from the

surface. Heavy metal poisoning, liver, kidney, and intestine harm, anemia, and even cancer can happen to people who eat a lot of heavy metals [10].

The box plots for (Pb, Cd, and Cr) in Fig. 4. From Fig. 4, the medium of Pb concentration lies towards the lower whisker and has one sample (FH3) is an outlier within the distribution. Meanwhile, the median of Cd and Cr in these boxes lies towards the top whisker. This indicates a skewness in the distributions, and the results tend to be low values. The increase in this sample FH3 can be attributed to several reasons, industrialization, treatment facilities, insufficient water water

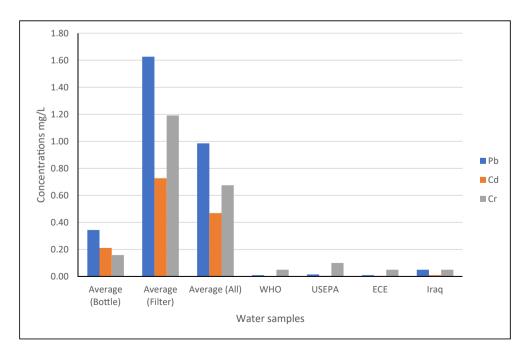


Fig. 3. Comparison of the average of heavy metals in water samples between the samples of the present study and safe limit.

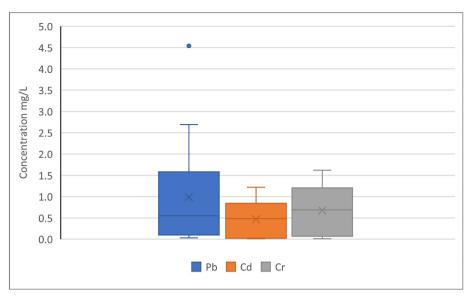


Fig. 4. Box plot of Pb, Cd, and Cr concentrations in all water samples under study.

supplies, agricultural activities, sources of tap water, and natural factors like weathering, and rock abrasion.

3.2. Health risk assessment

Table 3 displays the results of non-carcinogen parameters (CDI_{nca}, HQ, and HI) due to Pb, Cd, and Cr concentrations for water samples in the present study. The average values of CDI_{nca} (unit μ g/L. d⁻¹ or mg/L. d⁻¹ \times 10⁻³) for Pb, Cd, and Cr concentrations were 0.044 \pm 0.014, 0.021 \pm 0.005, and 0.021 \pm 0.005, respectively. The average values of HQ of Pb, Cd, and Cr concentrations were 0.011 \pm 0.003, 0.021 \pm 0.005, and 0.010 \pm 0.002, respectively. At the same time, as presented in Table 3, the results of HI vary from 0.002 in sample

BH4 (Kids life) to 0.102 in sample FH3 (Salam), with an average of 0.042 \pm 0.009.

The carcinogen parameters of newborns from healthy water samples can be found through calculated CDIca, CR, and TCR shown in Table 4. So, Table 4 shows that the average values of CDIca are due to Pb, Cd, and Cr concentrations in unit μ g/L. d⁻¹ were 0.019 \pm 0.006, 0.009 \pm 0.002, and 0.013 \pm 0.003, respectively. While, the average value of CR was 0.164 \pm 0.05 for Pb concentrations, 60.71 \pm 14.86 for Cd concentrations, and 6.52 \pm 1.50 for Cr concentrations. Also, the results of ECR, as presented in Table 4 ranged from 1.65 in the sample BH4 (Kids Life) to 167.64 in the sample FH7 (Askari), with an average value of 67.40 \pm 15.86.

From Tables 3 and 4, as well as Fig. 5, it can be noted that the results of CDI (nca and ca) received

Table 3. Results of non-carcinogen parameters for water samples in the current study.

No.	Sample code	CDI_{nca} (µg/L. d ⁻¹)			HQ			HI
		Pb	Cd	Cr	Pb	Cd	Cr	
1	BH1	0.002	0.0312	0.002	0.0004	0.0312	0.0008	0.032
2	BH2	0.026	0.0312	0.034	0.0066	0.0312	0.0115	0.049
3	ВН3	0.005	0.0007	0.004	0.0011	0.0007	0.0014	0.003
4	BH4	0.005	0.0005	0.002	0.0013	0.0005	0.0005	0.002
5	BH5	0.010	0.0009	0.001	0.0026	0.0009	0.0002	0.004
6	BH6	0.024	0.0014	0.005	0.0059	0.0014	0.0016	0.009
7	BH7	0.037	0.0008	0.003	0.0094	0.0008	0.0011	0.011
8	FH1	0.071	0.0149	0.054	0.0178	0.0149	0.0179	0.051
9	FH2	0.004	0.0035	0.054	0.0011	0.0035	0.0179	0.022
10	FH3	0.205	0.0410	0.028	0.0512	0.0410	0.0093	0.102
11	FH4	0.071	0.0288	0.057	0.0178	0.0288	0.0190	0.066
12	FH5	0.003	0.0370	0.067	0.0006	0.0370	0.0222	0.060
13	FH6	0.038	0.0492	0.073	0.0094	0.0492	0.0243	0.083
14	FH7	0.121	0.0549	0.044	0.0303	0.0549	0.0147	0.100
Avea	rge ± S.E.	0.044 ± 0.014	0.021 ± 0.005	0.021 ± 0.005	0.011 ± 0.003	0.021 ± 0.005	0.010 ± 0.002	0.042 ± 0.009

No.	Sample code	imple code CDI_{ca} (µg/L. d ⁻¹)			$CR \times 10^{-6}$			$TCR \times 10^{-6}$
		Pb	Cd	Cr	Pb	Cd	Cr	_
1	BH1	0.001	0.0134	0.0010	0.01	89.72	0.50	90.23
2	BH2	0.011	0.0134	0.0147	0.10	89.72	7.37	97.18
3	ВН3	0.002	0.0003	0.0017	0.02	1.94	0.87	2.83
4	BH4	0.002	0.0002	0.0007	0.02	1.30	0.33	1.65
5	BH5	0.004	0.0004	0.0002	0.04	2.59	0.12	2.75
6	BH6	0.010	0.0006	0.0020	0.09	3.89	1.01	4.98
7	BH7	0.016	0.0004	0.0014	0.14	2.43	0.68	3.24
8	FH1	0.030	0.0064	0.0230	0.26	42.78	11.50	54.54
9	FH2	0.002	0.0015	0.0230	0.02	9.92	11.50	21.44
10	FH3	0.088	0.0176	0.0120	0.75	117.87	5.99	124.60
11	FH4	0.030	0.0123	0.0244	0.26	82.67	12.19	95.12
12	FH5	0.001	0.0158	0.0285	0.01	106.13	14.26	120.40
13	FH6	0.016	0.0211	0.0313	0.14	141.33	15.64	157.11
14	FH7	0.052	0.0235	0.0189	0.44	157.76	9.44	167.64

 0.013 ± 0.003

 0.164 ± 0.05

Table 4. Results carcinogen parameters for water samples in the current study.

by Pb, Cd, and Cr concentrations for all healthy water samples were within the safe limit (Pb = 0.004 mg/kg. d^{-1} , Cd = 0.001 mg/kg. d^{-1} , and Cr = 0.003 mg/kg. d^{-1}) [29]. Also, the values of HQ and HI in the present samples of the study were smaller than the safe limit of 1, as established globally by EPA [30], Fig. 6 shows the comparison of the results of HI and safe limit. On the other hand, the range of safe limit of cancer risk (CR) and total cancer risk (TCR) for heavy metals according to US EPA reports was from 10^{-6} to 10^{-4} [36,37]. Consequently, the values of the CR in Table 4 for Pb were within the safe limit, while for Cd and Cr in most water samples were high. Moreover, according to

 0.019 ± 0.006

 0.009 ± 0.002

Avearge ± S.E.

Fig. 7, the results of TCR are safe, except for four samples (FH3, FH5, FH6, and FH7) which exceed the maximum value of safe limit. Persistent exposure to Pb, Cd, and Cr in drinking water above permissible levels poses significant health risks, including increased incidences of skin, lung, bladder, and kidney cancers.

 6.52 ± 1.50

 67.40 ± 15.86

 60.71 ± 14.86

Generally, it compared the results of the present study with studies in other regions based on several factors, including healthy drinking water and measuring the same heavy elements, in addition to the diversity of countries, as well as local studies. Therefore, Table 5 compares the comparison of Pd, Cd, and Cr concentrations in the health of drinking

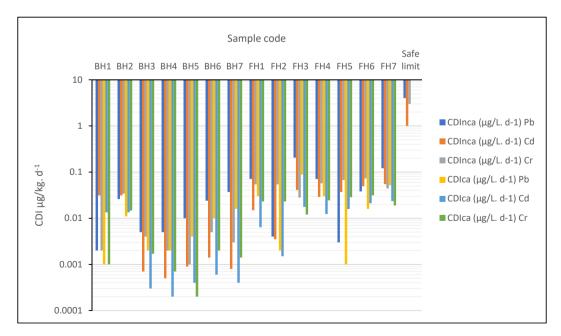


Fig. 5. Comparison of CDI (nca and ca) between the samples of the present study and safe limit.

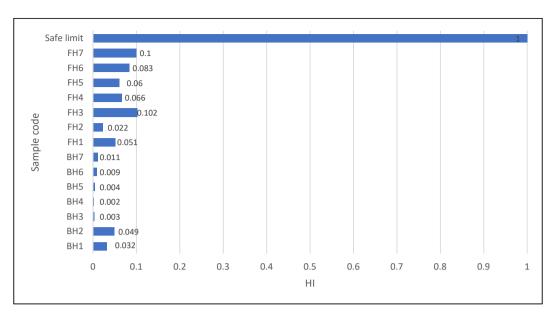


Fig. 6. Comparison of HI in water samples between the samples of the present study and safe limit.

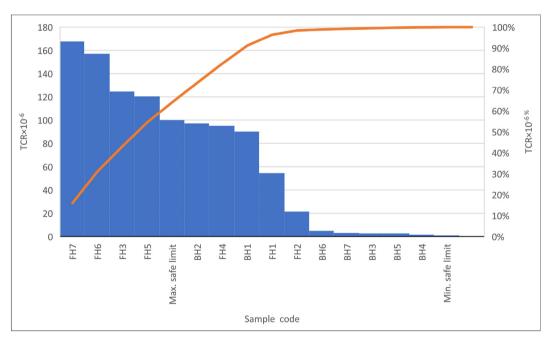


Fig. 7. Comparison of TCR in water samples between the samples of the present study and safe limit.

Table 5. Comparison of Pb, Cd, and Cr in healthy drinking water with previous studies.

No.	Country	Concentr	References		
		Pb	Cd	Cr	
1	Iran	0.0032	0.00043	0.00501	[24]
2	Egypt	0.00058	0.00045	_	[38]
3	Ghana	0.000272	0.0000199	_	[39]
4	Ethiopia	1.347	0.017	0.146	[40]
5	Pakistan	0.01	0.0036	0.130	[41]
6	Iraq (Baghdad)	0.5870	ND	0.0374	[42]
7	Iraq (Najaf)	0.99	0.47	0.68	Present work

water in previous studies. So, it was observed that the average values of Pb, Cd, and Cr in the presence of healthy drinking water are relatively high compared to the countries listed in Table 5.

Table 6. Correlation and p-value between Pb, Cd and Cr in all samples in the present study.

Heavy metals	Pb	Cd	Cr	P-value
Pb	1.00	0.53	0.21	0.289
Cd	0.53	1.00	0.62	
Cr	0.21	0.62	1.00	

Statically, using ANOVA -test to calculate the correlation and p-value between three heavy metals (Pb, Cd, and Cr), as shown in Table 6. So, from Table 6, it is noted that the result is not significant at p < 0.05.

4. Conclusion

The present work estimated the concentrations of Pb, Cd, and Cr and it is healthy risk parameters (non-carcinogenic and carcinogenic) in two types of healthy drinking water; bottled and filtered used by newborns in Al-Najaf Governorate, Iraq. The concentrations of Pb, Cd, and Cr in all water samples in the present study were higher than the drinking water standards by WHO, USEPA, ECE, and IQs. The results of non-carcinogenic were low risk. While carcinogenic parameters for 4 samples were high risk. Therefore, it can be concluded that most samples of healthy drinking water in Al-Najaf Governorate are not safe. So, remedial action and control methods are required in this community. Finally, through the present study and according to the obtained results and the conclusions, important recommendations can be outlined such as the need to routinely monitor the concentration of heavy metals in all types of water for Najaf governorate and other governorates. As well as study the quality of drinking water before it hits the consumer's tap to ensure optimized drinking water pumping.

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Conflict of interest

There is no conflict-of-interest statement.

Ethical approval

All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors.

Data availability

Data generated or analyzed during this study are included in this published article.

Author contributions

Each authors Ban Hussein Ali and Ali Abid Abojassim subscribes to conceiving and designing the analysis; Collected the data; Contributing data or analysis tools; Performed the analysis; Writing the paper.

Consent for publication

It has been taken informed consent from Volunteers for this study.

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