

Concentrations of Pb, Ni and Cd in Urine and Tissue Samples of Southern Iraqi Cancer Patients

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ABSTRACT

Background: The assay of biological samples is the preferred method for monitoring the internal exposure of toxicological contaminants in human body. **Objective:** The objective of this study was to determine the concentrations of Pb, Ni and Cd in biological samples of Southern Iraqi cancer patients. Urine and soft tissue samples were taken from two groups of male and female participants: the cancer patients group and healthy group. The biological samples of the cancer patients group and healthy group were collected from three key Southern Iraqi governorates, namely: Basrah, Muthanna and Dhi-Qar. These governorates were centres of intensive military activities during the Gulf wars. **Results:** The levels of toxic elements (Pb, Ni and Cd) in urine samples of the cancer patients group are 0.435, 0.217 and 0.115 mg/l, respectively. Concentrations of (Pb, Ni and Cd) in kidney tissues of the cancer patients group are 2.21, 1.28 and 13.25 mg/l, respectively. Whilst the levels of the corresponding elements in breast tissue samples of the cancer patients group are 1.65, 1.03 and 0.68 mg/l, respectively. **Conclusion:** The results show that the levels of toxic elements in biological samples of the cancer patients group are significantly higher than those of the healthy group. These findings indicate an association between the contaminants of toxic elements in Southern Iraqi regions and the cancerous illnesses of these patients.

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INTRODUCTION

Pollution caused by heavy metals has negative effects on the individuals' health and the environmental balance. Metals such as lead (Pb), nickel (Ni) and cadmium (Cd) are regarded as the most toxic. Continuous exposure to low levels of these toxic elements may result in bioaccumulation and cause a wide variety of biological effects on human beings depending on the level and the duration of exposure (Mortada *et al.*, 2002; Vahter *et al.*, 2002; Hanns *et al.*, 2006; Godt *et al.*, 2006; Flora *et al.*, 2012).

These elements can be found in the workplace, drinking water, food and consumer products. The exposure to these elements is common in industry due to the use of these metals in a wide range of manufacturing processes. Man-made emission of these toxic elements exceeds emission from natural sources by twofold to tenfold (Waisberg *et al.*, 2003; Filipič *et al.*, 2006).

Toxic elements can enter into the human body through inhaling dust particles bearing toxic

elements or by ingesting water and food which are polluted by toxic elements. Toxic elements in the human body transfer through the bloodstream into the various organs of the body, causing several health problems for the exposed individuals (Hanns *et al.*, 2006; Godt *et al.*, 2006).

Many epidemiological studies consider these toxic elements as a possible human carcinogen. This classification is based on epidemiological studies in occupationally exposed workers and studies on animals (Denkhaus and Salnikow, 2002; Waisberg *et al.*, 2003; Strumylaite *et al.*, 2009).

Many authors have reported that the biological samples were considered as good indicators of environmental pollution. Therefore, using human tissues and urine as indicators of the environmental exposure to several trace elements have become a common indicator (Tariq *et al.*, 1995; Horng *et al.*, 2002).

The environment in Iraqi has been badly affected, and is suffering from the increasing toxic metal levels in a way affecting Iraqis' health and threatening the environment as a result of the

military events during the Gulf wars and the human activities (Al-Hamzawi *et al.*, 2013; Al-Hamzawi *et al.*, 2014a; Alaani *et al.*, 2011; Al-Sabbak *et al.*, 2012). Southern Iraq region contains many oil fields, so the petroleum and petrochemical industries are spread in that region. The petroleum and petrochemical industries are one of the sources for the chemical pollution in the environment of southern Iraq due to the oil refining operations and their uses in other industries.

In Iraq, the incidence of cancers that are registered annually by Iraqi Cancer Board involved an increase in the number of cancer cases that are recorded after the Gulf wars (Al-Hamzawi *et al.*, 2014b)

There are some areas in the southern of Iraq like (Basrah, Muthanna and Dhi-Qar) that have experienced a twofold to fivefold increase in reported cancers. Most of these cases involve damage to the lungs, kidney, bladder and skin. In addition, an increased incidence of stomach cancer in males and breast cancer in females has also been reported, as

well as an overall increase in leukemia cases (Al-Hamzawi *et al.*, 2014c; Al-Hamzawi *et al.*, 2014d).

The measurements of (Pb, Ni and Cd) concentrations in urine and tissue samples were carried out by inductively coupled plasma optical emission spectroscopy (ICP-OES).

Methodology:

Sample collection:

In this study, 60 urine and 18 tissue samples of volunteers, males and females, were taken from two groups, cancer patients group and healthy group. These samples were collected from three key southern Iraqi governorates, namely: Basrah, Muthanna and Dhi-Qar as shown in Figure 1. The volunteers from these groups had no previous history of occupational exposure to toxic elements. The participants completed a comprehensive questionnaire about demographic information such as age, gender, location, medical history and smoking habit. The average of the age of participants in this work was 33.73 ± 4.32 for the cancer patients group and 31.40 ± 4.32 for the healthy group.

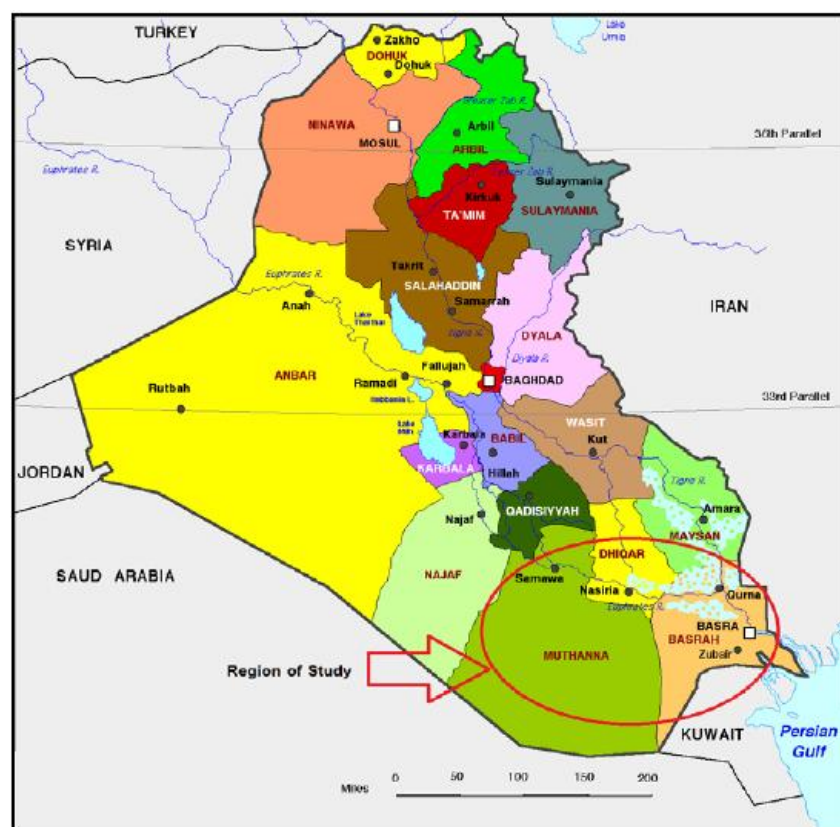


Fig.1: Map of Iraq showing the location of the three governorates involved in this study.

1.2 Experimental work:

The method to prepare the urine samples to determine their toxic elements concentrations was done as conducted by Lech and Lachowicz (2009) and Salih and Jaafar (2013). Urine samples with 8 ml were mineralized in 4 ml of nitric acid HNO_3 (65 %)

and 1 ml of hydrogen peroxide H_2O_2 (30 %). Samples of the soft tissues (kidney and breast) were cleaned using distilled water to remove the formalin liquid (conservator material). Following that, the tissues were dried at 37°C for 24 hours using an electric heating incubator. Thereafter, the samples

were ashed in an electric oven at 250°C for 8 hours. The powder tissue samples, weighing 0.5 g were mineralized in 4 ml of 65 % of nitric acid HNO_3 and 1 ml of 30 % of hydrogen peroxide H_2O_2 (Lech and Lachowicz, 2009; Kalisińska and Salicki, 2010).

Samples of biological materials were digested by using Microwave Reaction System MWRS (Anton Paar, Germany). Biological Samples of urine 8 ml and tissue 0.5 g with addition of 4 ml of nitric acid and 1 ml of hydrogen peroxide were transferred into a 125 ml pressure-resistant Teflon vessel. To remove the interfering matrix within the samples, the samples were first digested. The digested solutions were then diluted to a volume of 10 ml using de-ionized water. A blank sample which did not contain any biological sample was prepared to collect the background reading of the toxic elements. A blank sample was prepared by digesting 4 ml of nitric acid HNO_3 with 1 ml of hydrogen peroxide H_2O_2 . Then the mineralised samples were stored in a refrigerator at temperature of 4°C up to the time of analysis. The concentrations of (Pb, Ni and Cd) in urine and tissue samples were measured by using ICP-OES (PerkinElmer, USA). Calibration procedure was undertaken prior to using the ICP-OES to analyse the samples. Standard solutions used in calibration were prepared by diluting multi-elemental standard solutions of concentration of 100 mg/ml (PerkinElmer, USA).

Statistical analysis:

All the results that are obtained from all samples of the two groups were statistically analyzed using Statistical Package of the Social Sciences (SPSS, Version 21). The significance of the probability level (P) was estimated by Independent Sample *t* Test.

RESULTS AND DISCUSSION

Table 1 shows concentrations of toxic elements in urine samples of the cancer patients group and healthy group. For the patients group the concentrations of Pb range from 0.062 ± 0.015 mg/l to 0.91 ± 0.068 mg/l with a mean value of 0.453 ± 0.037 mg/l. The concentrations of Ni range from 0.052 ± 0.017 mg/l to 0.551 ± 0.034 mg/l with a mean value of 0.217 ± 0.021 mg/l. The concentrations of Cd in urine samples of these participants vary from 0.016 ± 0.009 mg/l to 0.39 ± 0.061 mg/l with a mean value of 0.115 ± 0.016 mg/l. From Table 1 the concentrations of Pb in urine of the healthy group range from 0.062 ± 0.008 mg/l to 0.746 ± 0.045 mg/l with an average value of 0.316 ± 0.034 mg/l. The levels of Ni vary from 0.033 ± 0.018 mg/l to 0.436 ± 0.023 mg/l with a mean value of 0.139 ± 0.014 mg/l. The levels of Cd in urine samples of the healthy group are in the range of 0.005 ± 0.001 mg/l to 0.153 ± 0.058 mg/l with an average value of 0.053 ± 0.007 mg/l. As shown in

Table 1 the mean value of Pb concentrations in urine samples of the cancer patients is significantly higher than that of the healthy group. Based on these mean values, the mean value of Pb contents of the cancer patients is 1.43 times higher than the healthy group. Independent Sample Test confirmed a significant difference in Pb concentrations in urine among cancer patients and healthy groups statistically ($P < 0.01$). The mean value of Ni concentrations in urine samples of the cancer patients group is 1.56 times higher than the healthy group. The difference of the Ni concentrations in urine samples among the cancer patients group and healthy groups is statistically significant ($P < 0.01$). The mean value of Cd concentrations in urine samples of the cancer patients group is 2.17 times higher than the healthy group. Independent Sample Test confirmed that the measured values of concentrations of Cd in urine samples of the cancer patients and healthy groups are significant ($P < 0.05$). These results suggest an association between the contamination of the toxic elements Pb, Ni and Cd in the environment of Southern Iraq and the cancerous diseases of the cancer patients in Southern Iraqi governorates Basrah, Muthanna and Dhi-Qar. The mean values of toxic elements in urine samples of the cancer patients and healthy groups in this study are higher than published values with other researchers (White and Sabbioni, 1998; Heidland and Köster, 2006; Zhifang *et al.*, 2008). This study indicates that the individuals living in the Southern Iraqi regions are exposed to high levels of toxic metals as a result of military events and human activities. Table 1 shows that the levels of Pb in urine samples is higher than that of Ni and Cd. The concentrations of toxic elements in urine samples of the present study can be arranged in the following sequence (from more available to less available): $\text{Pb} > \text{Ni} > \text{Cd}$. This can be attributed to the fact that the widespread use of Pb in the human activities led to the increase in the proportion of the human exposure to the element. This finding is in agreement with those of other researchers in other countries (Kristiansen *et al.*, 1997; Horng *et al.*, 2002).

To study the influence of the gender-biased in the accumulation of the toxic metals in human body, the concentrations of Pb, Ni and Cd in urine samples of the study groups as a function of gender are presented in Table 2. As shown in Table 2 the mean values of Pb contents in urine samples of males in the cancer patients group and healthy group are 0.584 ± 0.041 mg/l and 0.461 ± 0.038 mg/l, respectively. The average values of Ni concentrations in urine samples of males in the study groups are 0.259 ± 0.028 mg/l and 0.175 ± 0.024 mg/l, respectively. The mean values of Cd levels in urine samples of males in the study groups are 0.143 ± 0.024 mg/l and 0.074 ± 0.012 mg/l, respectively. Based on these mean values, the toxic metal concentrations are significantly higher in urine samples of males

compared to females, which can be caused by occupational exposure, and this finding is consistent

with the findings of other researchers (Sekhar *et al.*, 2006; Kristiansen *et al.*, 1997; Tariq *et al.*, 1995).

Table 1: Descriptive statistics of toxic element concentrations (mg/l) in urine samples of the study groups.

Element	Statistical values	Patients group n = 30	Healthy group n = 30
Pb	Min $\pm$ S.D	0.062 $\pm$ 0.015	0.062 $\pm$ 0.008
	Max $\pm$ S.D	0.91 $\pm$ 0.068	0.746 $\pm$ 0.045
	Mean $\pm$ Std. Error	0.453 $\pm$ 0.037	0.316 $\pm$ 0.034
	Std. Deviation	0.205	0.190
	P- Value	P < 0.01	
Ni	Min $\pm$ S.D	0.052 $\pm$ 0.017	0.033 $\pm$ 0.018
	Max $\pm$ S.D	0.551 $\pm$ 0.034	0.436 $\pm$ 0.023
	Mean $\pm$ Std. Error	0.217 $\pm$ 0.021	0.139 $\pm$ 0.014
	Std. Deviation	0.12	0.077
	P- Value	P < 0.01	
Cd	Min $\pm$ S.D	0.016 $\pm$ 0.009	0.005 $\pm$ 0.001
	Max $\pm$ S.D	0.39 $\pm$ 0.061	0.153 $\pm$ 0.058
	Mean $\pm$ Std. Error	0.115 $\pm$ 0.016	0.053 $\pm$ 0.007
	Std. Deviation	0.091	0.043
	P- Value	P < 0.05	

Table 2: Toxic element concentrations (mg/l) in urine samples of the study groups as function of gender.

Element	Statistical values	patients group		Healthy group	
		Male n = 18	Female n = 12	Male n = 14	Female n = 16
Pb	Mean $\pm$ Std. Error	0.548 $\pm$ 0.041	0.311 $\pm$ 0.048	0.461 $\pm$ 0.038	0.188 $\pm$ 0.030
	Std. Deviation	0.175	0.167	0.143	0.121
	P- Value	P < 0.01		P < 0.001	
Ni	Mean $\pm$ Std. Error	0.259 $\pm$ 0.028	0.154 $\pm$ 0.020	0.175 $\pm$ 0.024	0.109 $\pm$ 0.012
	Std. Deviation	0.121	0.071	0.091	0.048
	P- Value	P < 0.05		P < 0.05	
Cd	Mean $\pm$ Std. Error	0.143 $\pm$ 0.024	0.075 $\pm$ 0.016	0.074 $\pm$ 0.012	0.034 $\pm$ 0.007
	Std. Deviation	0.101	0.057	0.047	0.028
	P- Value	P < 0.05		P < 0.01	

The influence of age in the accumulation of the toxic elements in human body was studied and the results are shown in Figure 2. The ages of the healthy participants are divided into six age groups as: 1 – 10 y, 11 – 20 y, 21 – 30 y, 31 – 40 y, 41 – 50 y and up to 50 y. The results show that the concentrations of elements are found to be higher in the ages between 31 and 50 years. People in the age group of 31 – 50 years are found to be the most affected category as

shown by the high concentrations of toxic elements in their urine samples. The levels of Pb, Ni and Cd in persons of ages between 31 and 50 years are high which could be due to their relatively higher exposure (relative to other age groups) to the environment. This finding is consistent with previous reports (Sekhar *et al.*, 2006; Kristiansen *et al.*, 1997; Tariq *et al.*, 1995).

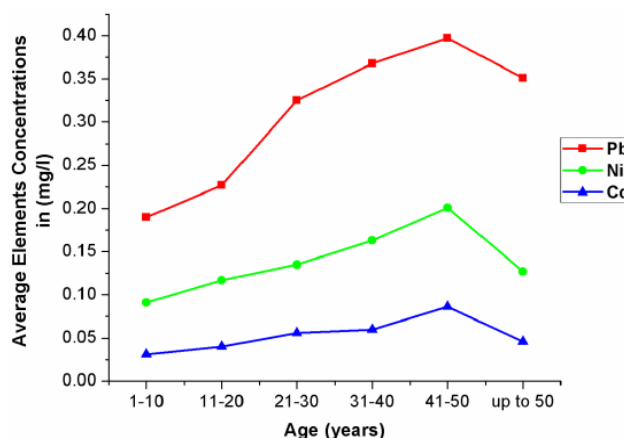


Fig. 2: Average values of elements concentrations in urine samples of the healthy group as a function of age

A significant relationship was observed between the participants' smoking habit with the accumulation of toxic elements in human body. The concentrations of Pb, Ni and Cd in urine samples of smokers are significantly higher than those of non-

smokers as shown in Figures 3 and 4. These results indicate that the smokers are exposed to high levels of toxic elements than the non-smokers. This can be attributed to the fact that the tobacco contains varying amounts of metals. A cigarette generally

contains 0.6 – 17 μg Pb, 4.2 – 7.5 μg Ni and 1 – 2 μg Cd. About 10% of this amount may be inhaled and enters to the human body via smoking (Özden *et al.*, 2007; Menden *et al.*, 1972). The causes for high concentrations of toxic elements in tobacco plants can be caused by the penetration of toxic metals from

soil to the tobacco plants; the use of chemical fertilizers and insecticides in tobacco fields; and these particles in air are accumulated on the leaves of tobacco plant and can also become a source of these toxic elements (Weaver *et al.*, 1996).

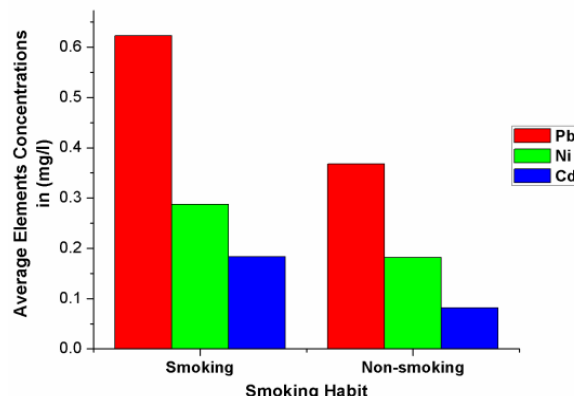


Fig. 3: Average values of elements concentrations in urine samples of the cancer patients group as a function of smoking habit.

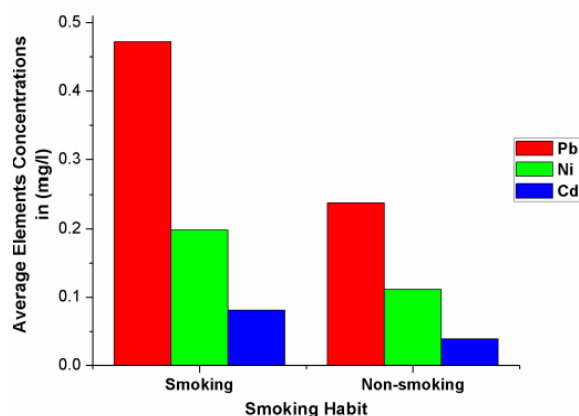


Fig. 4: Average values of elements concentrations in urine samples of the healthy group as a function of smoking habit.

The influence of the residential locations in the accumulation of toxic elements in the human body was studied. The mean values of the metals concentrations in urine samples of the cancer patients group and healthy group according to varied locations are shown in Figures 5 and 6. These figures show that Basrah province records the highest levels of the toxic element concentrations in urine samples than those of Muthanna and Dhi-Qar for both groups. This could be caused by the military activities of the Gulf wars and industrial wastes resulting from the petroleum and petrochemical industries deployed in Basrah governorate. Iraq is one of the third world countries facing alarming environmental problems. Motor vehicle exhausts, untreated sewage water, unchecked wastes of factories, the use of leaded gasoline and additionally the remnants of the Gulf wars are considered as the main sources of direct exposure of individuals to toxic metals.

The quantitative results of Pb, Ni and Cd concentrations in kidney tissues of the participants

are presented in Table 3. This table shows that the mean values of Pb concentrations in kidney tissues of the cancer patients group and healthy group are 2.21 ± 0.29 mg/l and 1.24 ± 0.25 mg/l, respectively. The mean values of Ni concentrations in kidney tissues of the cancer patients group and healthy group are 1.28 ± 0.16 mg/l and 0.75 ± 0.11 mg/l, respectively. The mean values of Cd concentrations in kidney tissues of the cancer patients group and healthy group are 13.25 ± 1.20 mg/l and 8.17 ± 0.59 mg/l, respectively. In Table 3 we notice that the cadmium records highest concentration of toxic elements in kidney tissues. This may be caused by the fact that the kidney is a critical organ for cadmium. This finding is in agreement with the findings of other researchers (Pasha *et al.*, 2008; Yaman, 2006). As seen in Table 3 the mean value of Pb concentrations in kidney tissues of the cancer patients group is significantly higher than that of the healthy group ($P < 0.05$). The mean value of Pb contents in kidney tissues of the cancer patients group is 1.78 times higher than the

healthy group. The mean value of Ni concentrations in kidney tissue samples of the cancer patients group is 1.7 times higher than that of the healthy group ($P <$

0.05). The mean value of Cd levels in kidney tissue samples of the cancer patients group is 1.62 times higher than that of the healthy group ($P < 0.01$).

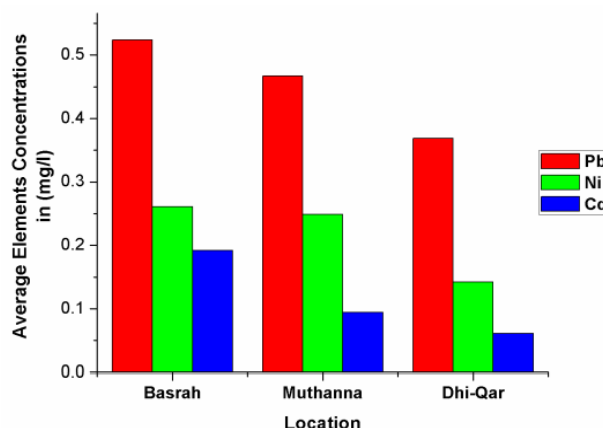


Fig. 5: Average values of elements concentrations in urine samples of the cancer patients group as a function of residential place.

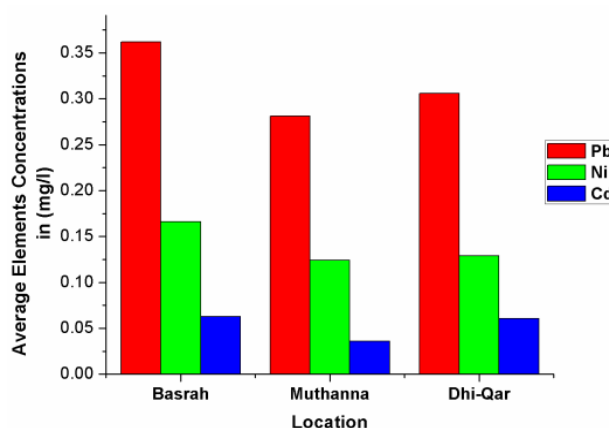


Fig. 6: Average values of elements concentrations in urine samples of the healthy group as a function of residential place.

Table 3: Descriptive statistics of toxic element concentrations (mg/l) in kidney tissues of the study groups.

Element	Statistical values	Patients group n = 6	Healthy group n = 3
Pb	Mean $\pm$ Std. Error	2.21 $\pm$ 0.29	1.24 $\pm$ 0.25
	Std. Deviation	0.71	0.44
	P- Value	P < 0.05	
Ni	Mean $\pm$ Std. Error	1.28 $\pm$ 0.16	0.75 $\pm$ 0.11
	Std. Deviation	0.41	0.20
	P- Value	P < 0.05	
Cd	Mean $\pm$ Std. Error	13.25 $\pm$ 1.20	8.17 $\pm$ 0.59
	Std. Deviation	2.94	1.02
	P- Value	P < 0.01	

Table 4 illustrates the concentrations of Pb, Ni and Cd in tissue samples of the participants. From Table 4 the mean values of Pb concentrations in breast tissues of the cancer patients group and healthy group are 1.65 ± 0.21 mg/l and 1.07 ± 0.26 mg/l, respectively. The average values of Ni concentrations in breast tissue samples of the cancer patients and healthy groups are 1.03 ± 0.11 mg/l and 0.69 ± 0.09 mg/l, respectively. Whilst the mean values of Cd contents in breast tissue samples of the

cancer patients and healthy groups are 0.68 ± 0.08 mg/l and 0.47 ± 0.07 mg/l, respectively. As shown in table 4 the mean values of Pb, Ni and Cd concentrations in breast tissues of the cancer patients group are significantly higher than those of the healthy group ($P < 0.05$). The mean value of Pb contents in breast tissues of the cancer patients group is 1.54 times higher than that of the healthy group. The average value of Ni concentrations in breast tissues of the cancer patients group is 1.5 times

higher than that of the healthy group. Whilst the mean value of Cd concentrations in kidney tissues of the cancer patients group is 1.45 times higher than that of the healthy group ($P < 0.05$). The results obtained show that toxic element concentrations in

tissue samples of the cancer patients group are higher than those of the healthy group. These results indicate an association between the accumulation of toxic elements and malignant tumours.

Table 4: Descriptive statistics of toxic element concentrations (mg/l) in breast tissues of the study groups.

Element	Statistical values	patients group n = 6	Healthy group n = 3
Pb	Mean $\pm$ Std. Error	1.65 $\pm$ 0.21	1.07 $\pm$ 0.26
	Std. Deviation	0.51	0.45
	P- Value*	P < 0.05	
Ni	Mean $\pm$ Std. Error	1.03 $\pm$ 0.11	0.69 $\pm$ 0.09
	Std. Deviation	0.26	0.16
	P- Value*	P < 0.05	
Cd	Mean $\pm$ Std. Error	0.68 $\pm$ 0.08	0.47 $\pm$ 0.07
	Std. Deviation	0.21	0.13
	P- Value*	P < 0.05	

Conclusions:

The levels of toxic elements in urine and tissue samples of the cancer patients group are significantly higher than those of the healthy group. These results show a correlation between the contamination of the toxic elements in the Iraqi environment and the cancerous diseases of these patients. Toxic metal concentrations are significantly higher in urine samples from males compared to females, which can be attributed to occupational exposure. The levels of toxic elements in urine samples of persons of ages between 31 and 50 years are high which could be due to their relatively higher exposure to the environment. The obtained mean values of toxic elements in urine samples of this study exceed the normal levels of toxic elements in urine sample (Hornig *et al.*, 2002). Concentrations of toxic elements in urine samples of smokers are significantly higher than those of non-smokers. This suggests that the smokers have the potential of getting toxic elements more than the non-smokers. Urine and tissue samples are good indicators for monitoring the internal exposure of toxic elements in human body. This study is useful in determining the toxic elements of the population within the study sites for toxicological protection and prevention from extreme exposure.

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