An-Najah National University

Faculty of Graduate Studies

Determination of Lead, Cadmium and Arsenic in Human Milk of Lactating Women in Jenin, Palestine

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This Thesis was defended successfully on12/1/2021,and approved by:

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Dedication

First of all, my greatest gratitude to Allah

To my family, especially my father and mother.

To my beloved husband: Jihad Jawabreh, thank you for encouraging me and for deep belief in me and my endeavors.

To my parent in-laws, especially in laws mother who took care for my children during my absence.

To my sisters, brothers and friend who supported me through my graduate studies

الى من زرعت احلامي وافكاري بين احضان قلبها الطاهر

الى من ستبقى لي قدوة امي الحنون

الى من قدم لنا في صغرنا ابي العزيز

الى نور قلبي وسندي وتؤام روحي زوجي:جهاد جوابرة

الى من احتضنتوا اولادي في غيابي عائلة زوجي

الى اخوتي واخواتي واصدقائي وكل من قدم لي الدعم خلال مسيرتي

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الإقرار

أنا الموقعة أدناه مقدمة الرسالة التي تحمل العنوان:

Determination of Lead, Cadmium and Arsenic in Human Milk of Lactating Women in Jenin, Palestine

أقر بأن ما اشتملت عليه هذه الرسالة إنما هي نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة كاملة، أو أي جزء منها لم يُقدَّم من قبل لنيل أية درجة أو لقب علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name:	**اسم الطالب:**
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Date:	**التاريخ:**

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List of Abbreviations

BMI	Body Mass Index
IQ	Intelligence Quotient
AAS	Atomic Absorption Spectrophotometry
ET.AAS	Electro Thermal Atomic Absorption Spectrophotometry
LOD	Limit Of Detection
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization
WHO	World Health Organization
IARC	International Agency for Research on Cancer
µg/L	Micro gram per Liter
Cadmium	Cd
Arsenic	As
Lead	Pb
CDC	Center for Disease Control
R^2	Correlation coefficients

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Abstract

Contamination of human milk with toxic metals is of special interest because of harmful effect especially on infants. The aim of this study was to determine the levels of cadmium, lead and arsenic in human milk of lactating mothers living in Jenin. The breast milk samples were obtained from a total 100 lactating women. Samples were analyzed using atomic absorption spectrophotometry. Also a questioner was used to study some factors that could affect presence of these metals in breast milk. Our study showed that Arsenic and lead contamination was detected in all human milk samples with mean concentration of (means 8.1±10.6 μg/L and 2.1±3.5 μg/L) respectively, whereas, Cadmium was detected in 95% of the human milk samples with average concentration 1.7±1.6 μg/L. However, estimated weekly infant intake of toxic metals from breast milk was reported to be less than the limit set by WHO. Also, no significant relationship was found between levels of heavy metals in breast milk and cereals consumption, cosmetic, drinking water, taking medicine, fish consumption, age, education, occupation of mothers and place of residency. The analysis indicated that arsenic contamination was related to residence near industrial activities, milk consumption. The study is the first study that estimated the level of toxic metals in human milk of breast milk in Jenin. The results showed an urgent need for developing policies to protect infants from exposure to these toxic heavy metals.

Chapter One
Introduction

Human milk is the best way to fed infants with the appropriate amount of nutrient such as carbohydrates, protein, fat, minerals and vitamins which are essential for their health growth and immunological development (Winiarska-Mieczan, 2014). Breast milk may be contaminated with toxic metals because it is considered as one of the body's routes of excretion of various toxins, which adversely affects health of infants (Kunter et al., 2016). Infants are particularly sensitive to toxic metals effects because they are growing rapidly and their physiological systems are still developing during the first year (Bassil et al., 2017).

Toxic substances, such as cadmium (**Cd**), lead (**Pb**) and arsenic (**As**) have a special interest because of their abundancy and adverse health effect (Nassir et al., 2016). Lead contaminate the environment through human activities such as burning fuels and manufacturing, and also present in several products including cosmetics, cigarette, batteries, and crops grown in contaminated soil (Wong and Lye, 2008). Lead in breast milk come from the mother bones due to past exposure. It is released into the blood with calcium and finally released into breast milk (Chao et al., 2017). A percentage of 36-80% of all Pb intake for infants comes from mother’s milk in the first three months (Mends, E., 2014). Small concentration of **Pb** in breast milk can seriously harm infant nervous system, damage kidneys, decrease in growth and weakens immune system (Wong and Lye, 2008).

The main source of cadmium exposure during pregnancy includes cigarette smoking, water and diet (Wong and Lye, 2008). Cadmium toxicity cause hypertension impairs the kidney function and cancer (Wong and Lye, 2008).

Arsenic is ubiquitous in the nature, it present in air, water and soil (Winiarska-Mieczan, 2014). It is a dangerous metal especially for newborns. It is classified as type1 human carcinogen (Bassil et al., 2017), and also damages immune function which increases newborns mortality (Carignan et al., 2015).

Quantification of metals in breast milk is commonly detected by atomic absorption spectrophotometry (**AAS**) or Inductively Coupled Plasma Mass Spectrometry (**ICP-MS**). Some studies from various regions such as Lebanon, Poland, Spain, Iran, and Saudi Arabia, have reported a relationship between the presence of heavy metals in human milk, the socio-demographic status and dietary habits of nursing mothers (Rebelo and Caldas, 2016). However, no such study has been conducted in Palestine.

The aim of this study is to estimate the concentration of lead, cadmium and arsenic by atomic absorption spectrometry in breast milk samples collected from nursing mothers in Jenin.

1.1 Research problem

The exposure of breastfeeding mothers to toxic metals like lead, cadmium and arsenic are dangerous since it can contaminate the breast milk leading to negative impact on infant health.

No studies about breast milk contamination with cadmium and arsenic were conducted in Palestine. Furthermore, no studies about lead in breast milk were conducted in Jenin area. This study investigated the levels of toxic heavy metals, specifically Arsenic, Lead, and cadmium in breast milk samples from Jenin and compared them with international standards.

1.2 Literature Review

1.2.1 Heavy metals

Heavy metals are those metals which have a density more than 5.0 g\cm^3 in their standard state. They are widely spread in the environment where they accumulate. They enter human bodies and lead to many health problems especially to infants who are most vulnerable to them. The presence of heavy metals such as **As, Pb, Cd** in breast milk affects newborns even at a low concentration.

1.2.2 Arsenic

Arsenic is a heavy metal that exists naturally in volcanic ashes, bed rock, organic matter, air and water (Lars, 2003). People are exposed to **As** by consuming seafood, water and crops produced in a contaminated soil

(Carignan et al.2015), also **As** can enter the environment through the use of wood preservatives and arsenic pesticides (Lars. 2003). **As** is classified as a carcinogen connected with lung, skin bladder, kidney and liver cancer (IARC, 2016). Long- term exposure to **As** brings many problems to human as fetus mortality, low weight neonate, birth defects (WHO 2001) and impairs several systems such as immune, cardiovascular, respiratory system. High level of exposure to **As** can cause neurological disturbance and finally death (Lars, 2003). As a result WHO recommended maximum contamination level of 10 μg\L **As** in drinking water (WHO, 2004).

1.2.2 Lead

Lead also is a heavy metal which is presented naturally in little amounts (Mends,2014) and produced by human activities such as coal, gasoline and rubbish burning (Yurdakök, 2015), also mining, battery cosmetic, pesticides and smelting production industry(WHO, 2010). Humans are exposed to lead through the consumption of water, food (Dorea, 2004), cosmatics and medicine polluted with lead (Counter rt al., 2014). Low amount of **Pb** can lead to hyperactivity, aggressive behavior (Patrick, 2006; Bischoff et al., 2014) and reduced Intelligence Quotient (IQ) (Jusko et al., 2007). High amount of **Pb** in body can damage kidney, nerve, stomach, brain, liver and at last may lead to death (Levin et al., 2008). Many studies reported that 90% of absorbed **Pb** stored in mother's bones (Keil etal.,2011) and 36-80% of **Pb** in blood of infants comes from breast milk of lactating mothers especially in the first months of their life (Mead, 2012; Abdollahi, 2014)

1.2.3 Cadmium

Cadmium is a heavy metal which enters our bodies through Smoking and food such as legumes, cereals which are considered the major source of cadmium exposure (EFSA, 2009). International Agency for Research on Cancer (IARC) categorized **Cd** as a carcinogen linked with lung and prostate cancer (IARC, 2016). **Cd** accumulate in body tissue specially kidney and liver with half-life of 6-38 years, and 4-19 years respectively causes damage to it (Kjellstrom & Nordberg,1985). Women and babies who suffer from iron deficiency are more vulnerable to **Cd** accumulation. Breast milk is considered the main food for breastfed baby and being contaminated it with **Cd** leads to health problems such as hypertension, neurological disorder, mineral density loss and brain damage (Rahimi et al., 2009).

1.3 Previous studies

Many studies around the world reported the presence of toxic metals in breast milk, and The World Health Organization (WHO) has indicated the reference range for these toxic metals of population that is not exposed to contamination to be: **Pb** (2-5 μg/l), **As** (1 μg/l) and **Cd** (1 μg/l) (WHO, 1989.).

There is a large number of studies that determined levels of lead in breast milk. In most studies lead was analyzed by ET AAS with wide range of LOD (0.04-3.4 μg/l). Other studies used ICP-MS with LOD (0.01-3 μg/l) (Rebelo and Caldas, 2016). The number of samples used to analyze levels of lead in breast milk varied from 15 (Needham et al., 2011) to over 310 samples (Al-Saleh et al., 2003). Some studies show very high Pb levels (up to 1515 μg/l) (Örün et al., 2012) or reported very high mean value (391 μg/l) (Gürbay et al., 2012). The percentage of Pb contaminated samples among the studied women in various studies ranged from 40% (Bentum et al., 2010) to 100% of samples (Turan et al., 2001).

In most studies, Cadmium levels in breast milk were below 2 μg/l (Rebelo and Caldas, 2016), with maximum level of 43 μg/l in one study (Gürbay et al. (2012). The number of samples used to analyze **Cd** varied from 15 to 320 samples (Rebelo and Caldas, 2016). In some studies, all of samples were contaminated with **Cd** (Turan et al., 2001) while in other studies, **Cd** was not detected (Felip et al., 2014). Cadmium commonly analyzed by ET.AAS with LOD (range 0.01-0.5 μg/l) or by ICP-MS with LOD (range 0.0027-0.3 μg/l) (Rebelo and Caldas, 2016).

The Highest content of **As** in breast milk found was 149 μg/l in a regions known of contamination with **As** (Samanta et al., 2007). However in an area with low level of arsenic contamination, **As** levels were less than 0.62 μg/l (Rebelo and Caldas, 2016). The number of samples used to analyze **As** varied from 9 (Carignan et al., 2015) to 236 samples (Samanta et al., 2007). Common method used to quantify level of Arsenic in breast milk was AAS and ICP-MS, which has the lowest LOD (0.007–0.3 μg/L) (Rebelo and Caldas, 2016). In some studies no **As** contamination was detected (Gürbay et al., 2012) while in others, recorded 60 % of samples were contaminated (Bentum et al., 2010).

In Palestine a study was conducted to measure lead level in breast milk sample taken from 89 breast feeding women living in Nablus, Ramallah, and Jerusalem. Concentration of lead in breast milk samples was measured using a graphite furnace atomic absorption spectrophotometer, 17% of the samples were higher than the reference range set by World Health Organization (Shawahna et al., 2016). No similar studies were performed about Cd and As in Palestine.

1.4 Objective

1.4.1 General objective

The objective of this study is to determine cadmium, arsenic and lead concentrations in the breast milk of lactating women in Jenin.

1.4.2 Specific objectives

1. To determine level of toxic heavy metals, specifically Arsenic, Lead, and cadmium in breast milk for breastfeeding mothers in Jenin using Atomic Absorption Spectrophotometry.

2. To compare the measured values of toxic metals with local and international standard.

3. To find if metal levels in breast milk is related to nutritional and living condition.

Chapter Two
Methodology

One hundred randomly lactating women participated in this study. Human milk samples were taken from nursing mothers living in Jenin (Jenin city, al-Yammon, bit Qade, Yabad, Om dar, alkhalgan and Anin) during June to July 2019. An Approval letter was obtained from the Palestinian ministry of health to facilitate the collection of breastmilk samples from lactating mothers who visited the governmental health care center to Vaccinate or take care of their infants. The research was approved by the ethical committee of An Najah National University. The samples were taken from lactating mothers whose babies ages ranged from one day to a year.

2.1 Samples and data collection

Breast milk samples (10-20ml) were collected from lactating women living in Jenin using manual breast pumps, each sample was stored in 50ml a polyethylene tube container and frozen at -20C until the time of analysis (Winiarska-Mieczan, 2014; Kunter et al., 2016; Bassil et al., 2017).The samples were analyzed using graphite furnace atomic absorption spectrometry and the concentration of **Cd**, **As** and **Pb** concentration were determined. Women were asked to provide breast milk samples, and they were informed about the aim and importance of the study.

A questionnaire was used in order to get more information about the participated mothers' data, including the following:

1- Socio- demographic characteristics of each volunteer such as age, height, weight, educational level, occupational status, and smoking habits.

2- Medical history

3- Intake of food supplements (vitamin and iron)

4- Dietary habits

2.2 Toxic metals quantification

2.2.1 Breast milk samples preparation

Breast milk samples were taken from the refrigerator and put in water path at room temperature to melt. Nearly, 5g of breast milk samples were weighed into a porcelain crucible with 5ml of 70% nitric acid (HNO_3) (Altar-pure). The crucibles were covered and stored overnight. After that, the samples were placed on a hotplate until dried then burnt in a muffle furnace at 600C for 12 hour. The obtained ashed sample was diluted with 5ml of 70% HNO_3 and 3ml of deionized water then stored in a labeled polyethylene vial. Levels of Arsenic, Cadmium and Lead in human milk were measured and analyzed in triplicates using graphite furnace atomic absorption spectrophotometer technique (IcE-3000 SERIES, Serial number c113500021 designed in UK AA Spectrometer). It contains hollow cathode lamps to study these metals. The optimal wavelengths for detection were 193.7 nm, 228.8 nm and 283.3 nm for As, Cd and Pb respectively. The levels of As, Cd and Pb were determined in microgram per liter.

2.2.2 Standard solution preparation:

The standard solutions of different concentrations were prepared from 1000 ppm of **As**, **Cd** and **Pb** stock solutions which were prepared as follow:

Arsenic Standard solutions having concentration of 1000ppm were prepared by placing 0.0500g of Sodium citrate dehydrate $NaAsO_2$ (0.05M) (Aldrich), with distilled water and 1.0ml of 70%HNO_3 to reach 50ml as the final volume. While **Cd** standard solution (1000ppm) were prepared by dissolving 0.1372g of Cadmium nitrate-tetra-hydrate $Cd(NO_3)_2.4H_2O$ (Riedel-deHaen No. 1714) with distilled water and 1.0ml of 70%HNO_3 and diluted until final volume of 50ml. **Pb** standard solutions were prepared by dissolving 0.0799g of Lead nitrate $Pb(NO_3)_2$(Sigma) with distilled water and1.0ml of 70%HNO_3 reached to 50ml as a final volume. After that, different concentrations (0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 ppb) for each of different metal were obtained by serial dilutions were made of stock solution with 0.5 ml 70% HNO_3 and distilled water.

2.3. Estimation of infant daily intake of toxic metals

Estimated metal weekly intake in (µg/day (EMWI)) can be calculated using the following formula:

EMWI =**a** ×**b**×7/ [**b.w**].

Where "**a**" is the mean milk concentration of heavy metals (µg /L), "**b**" is the average amount of milk in Liters consumed per infant per day which is considered 0.75L/day (Kent, 2006), and [**b.w**.] is the average body weight (kg) of the infants, which is (5.956 Kg) reported by lactating mothers. **(**Bassil et al., 2017**;** Carignan et al., 2015; IPCS, 2009**)**

2.4 Detection limit

Limits of detection (**LOD)** were estimated using the following relation;

$$\mathbf{LOD = 3s/m}$$

LOD: limit of detection

s: the computed standard error.

m: slope of the calibration curve.

Chapter Three

Results and Discussion

A total 100 human milk samples were used to report the concentration of **Cd**, **Pb** and **As** for nursing women in Jenin.

The Calibration standards were used to build calibration curve to be used in the quantification of metals. The calibration equations, correlation coefficients and LOD are displayed in table 4.1 below.

Table (3.1): The Calibration standards

Heavy metal element	**Equation**	**R^2**	**LOD**(µg /L)
As(1-50)	Y=0.0087X+0.0005	R^2=0.9845	0.84
As(51-90)	Y=0.0095X-0.0014	R^2=0.9585	0.43
As(91-100)	Y=0.0103X-0.0006	R^2=0.98999	0.49
Pb(1-14)	Y=0.0338X+0.0113	R^2=0.974	0.04
Pb(15-50)	Y=0.0503X+0.0184	R^2=0.9825	0.07
Pb(91-100)	Y=0.1658X+0.0109	R^2=0.9947	1.17
Cd(1-38)	Y=0.1431X+0.0599	R^2=0.9753	0.02
Cd(39-48)	Y=0.1635X+0.0937	R^2=0.9281	0.02
Cd(49-100)	Y=0.21X+0.0555	R^2=0.9729	0.01

3.1 Analysis of the questionnaire results

3.1.1 Socio-economic and demographic characteristic of lactating mothers' who participated in the study

A total of 100 samples were used in this study. The questionnaire included: nursing women and infant's age, infant's weight, family income, mother's occupation, educational level, address, smoking habits, cosmetics use and geographical location of residence, also dietary habits such as consumption of (cereals, seafood, water, milk, and fresh vegetables) and medical history.

The mean Body Mass Index (**BMI**) calculated as (mass/ $(tall)^2$) of the women who participated were 25.17Kg/m^2 (SD=3).A bout 62% of lactating mothers age ranged from (20-30) years while 26% of them ranged (30-40) years. Infant's age ranged from 3 days to one year with mean 4 months while their weight ranged (2.5-11Kg). On the other hand, 45% of lactating mothers have family income (2000-3000) NIS\month while 25% of them have family income (1000-2000) NIS\month. Only 7%of the participants live in Jenin city and 93% live in Jenin villages. In term of location of residence; 71% of nursing mothers live near a public road, 19% live near a waste disposal site, 18% live near cultivation activity and the rest live near industrial activity. Most of lactating mothers were unemployed which was 94%. Interestingly, 40% of lactating mothers have university degrees while 60% of them have secondary education or less. About half of lactating mothers reported that they received health care at governmental clinic while 27% received health care at private clinic and the rest received health care at both. Most of nursing mothers said that their children don't have any health problems.

3.1.2 Effect of food consumption on the results

Lactating mothers were asked about supplementary intake, 90% reported that they were continuously taking it through the pregnancy period. On the other hand, 39% of breastfeeding mothers were taking medicine through the pregnancy and lactating period as advised by doctors. According to dietary habits, 56% of participants drink water more than 1.0 L/day. Around 77% of lactating mothers consume milk less than 0.5 L\week. The Majority of nursing women consume grains more than twice a week and nearly half of them eat fish more than 0.6 ounce per two month. Additionally, 94% of nursing women reported that they don’t have smoking habit and 24% of them don’t used cosmetics.

3.2 Breast milk heavy metals quantification

The results of analyzed sample revealed the main descriptive statistics for arsenic, cadmium and lead in breast milk samples as shown in Table 3.2.

Table (3.2): Occurrence and concentration of heavy metals in the human milk

Heavy Metals	Number of Breast milk samples	% Detected Samples	% sample exceed the WHO limits	Mean ±St (μg/L)	Range	
					MIN	MAX
Cadmium	97	95%	50%	1.7±1.6	0.2	8.5
Arsenic	95	100%	100%	8.1±10.6	0.9	65.9
Lead	100	100%	9%	2.1±3.5	0.1	14.9

Cadmium was detected in (97)95% of the human milk samples and, with average concentration 1.7±1.6 μg/L compared with other studies such as in Iran (Goudarzi et al., 2012), Spain (Garcia-Esquinas et al.,2011) and Turkey (Turan et al., 2001) which were 1.9, 1.3 and 1.7 μg/L respectively. In other studies such as Turkey (Gurbay et al., 2012) and Saudi Arabia (Al-saleh et al., 2003) reported higher **Cd** level with mean concentration 4.6 and 2.2 µg/L respectively. In contrast studies conducted in Lebanon (Bassil et al., 2017) and Poland (Ols Zowski, et al., 2016) recorded lower **Cd** level with mean concentration 0.9 and 0.1 µg/L respectively. In this study, the cadmium concentration in 50% of the detected samples exceeded the limits set by WHO (1 µg/L) as shown in **Figure 3.1** below. Whereas, the average **Cd** weekly intake of infant was 1.5 µg/kg b.w./week which is less than the limit reported by the European Food Safety Authority EFSA 2.5 µg/kg b.w./week (EFSA et al., 2014). Estimated value is less than intake reported in Turkey (4.40 µg/kg b.w./week) (Gürbayet al., 2012), Saudi Arabia (1.80 µg/kg b.w./week) (Al-Saleh et al., 2003), Taiwan (12.6 µg/kg b.w./week) (Chien et al., 2006) but higher than Greece (0.18 µg/kg b.w./week) (Leotsinidis et al., 2005) and Slovakia (0.50 µg/kg b.w./week) (Ursinyova and Masanova, 2005).

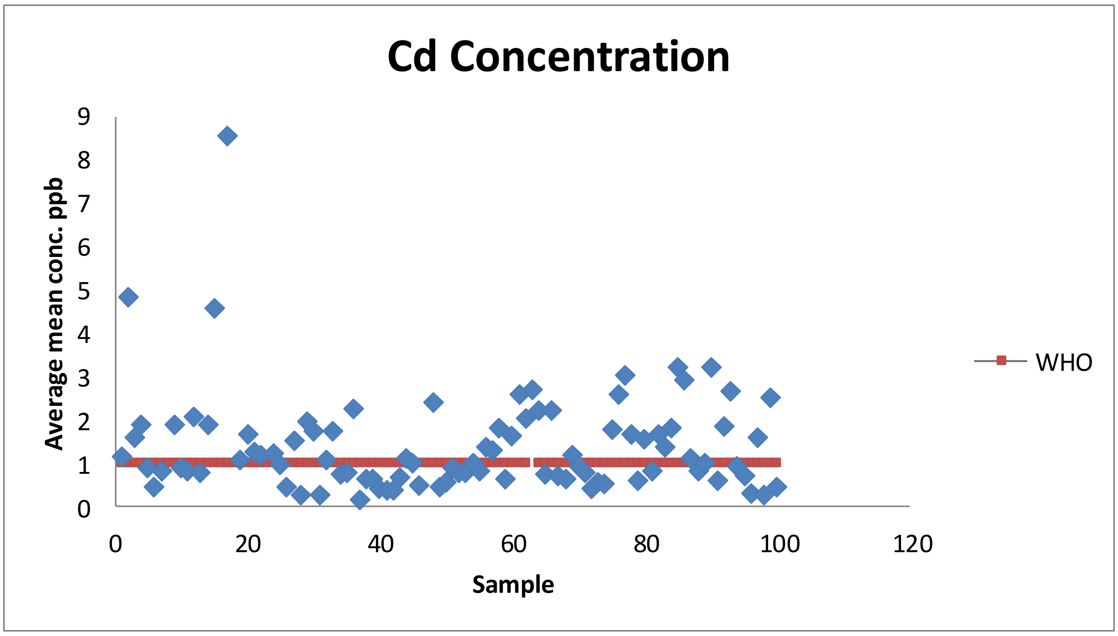

Figure (3.1): Cd concentration in breast milk

Lead contamination was detected in all breast milk samples with mean concentration 2.1±3.5 μg/L compared with Studies in Canada (Hanning et al.,2003) with a mean 2.1 μg/L and in Egypt (Moussa,2011) with a mean 1.7 μg/L. Studies from Lebanon, Turkey (Gurbay eh al.,2012), Spain (Garcia-Esquinasn et al.,2011) and Iran (Rahimi et al.,2009) reported high **Pb** mean concentration in human milk as18.17, 391, 15.56 and 10.4 μg/L, respectively. On the others hand others studies reported lower **Pb** levels, such as in United Arab Emirate (Abdulrazzaq et al., 2008) with a mean 0.019 μg/L, in Portugal (Almeida et al., 2008) with a mean 0.94 μg/L and in Brazil (Goncalves et al., 2010) with mean 0.3 μg/L. Results show that 9% of the detected samples in our study exceeded the limit reported by WHO (5 μg/L) as shown in **Figure 3.2** below. Also the results show that the average weekly intake of **Pb** of infant was 1.81 μg/kg b.w./week which is less than the limit used by the WHO 25 μg/kg b.w./week (Joint FAO/WHO Expert Committee on Food Additives, 1999). However, our estimation of **Pb** mean intake less than reported in many countries such as Poland (2.90 μg/kg b.w./week) (Winiarska-Mieczan, 2014), Taiwan (12.6 μg/kg b.w./week) (Chien et al., 2006), Greece (7.70 μg/kg b.w./week) (Leotsinidiset al., 2005) and Slovakia (5.40 μg/kg b.w./week) (Ursinyova and Masanova, 2005).

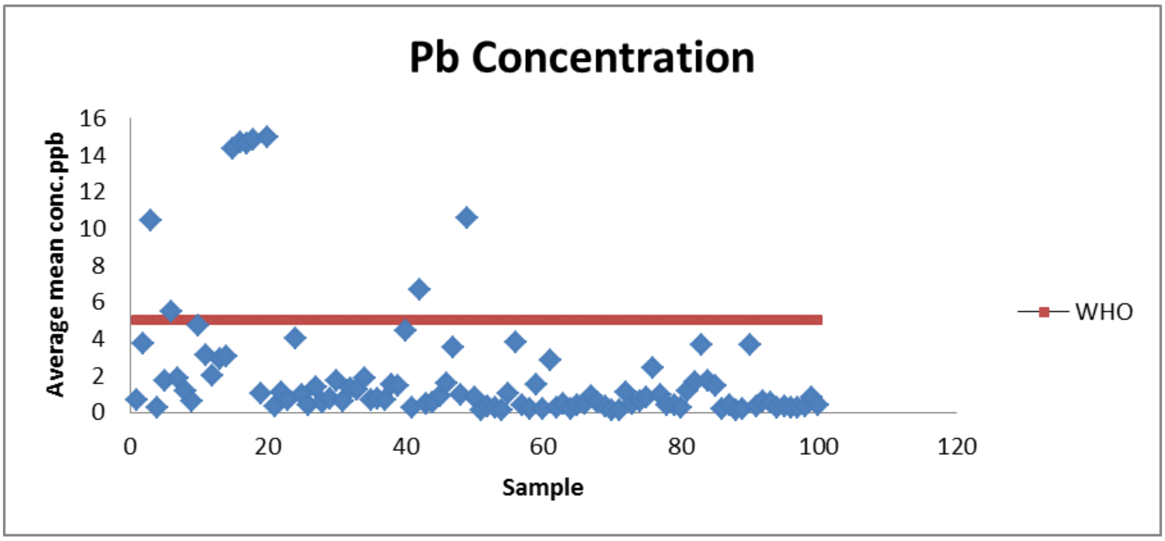

Figure (3.2): Pb concentration in breast milk

As shown in **Figure 3.3**, Arsenic contamination was detected in all of the breast milk samples with mean concentration of 8.1±10.6 µg/L. This result is comparable to studies in many locations in the world. Several studies such as Portugal (Almeida et al., 2008) with a mean 7.8 µg/L and Turkey (Gurbay et al., 2012) with a mean less than7.6 µg/L. However arsenic levels in Jenin was higher than that in Mexico (Gaxiola et al., 2014) with a mean 0.01 µg/L, United Arab Emirates (koscnovic et al., 2008) with a mean o.196 µg/L and United States (Sowers et al., 2002) with a mean 0.3 µg/L. In contrast, other studies reported higher **As** level up to 49 and 38 µg/L reported by Watanable et al., (2003) and Samanta et al., (2007) respectively. All samples in our study exceeded the limits set by WHO (1 µg/L). Also, results show that the estimated mean of infant weekly intake of **As** was 7.1 µg/kg b.w./week which is less than the limit reported by the WHO (14.7 µg/kg b.w./week) (Joint FAO/WHO Expert Committee on Food Additives, 1989). Other countries such as India reported higher infant **As** intake that was18.2 µg/kg b.w./week (Samanta et al., 2007) due to the high **As** content in water, while others such as in Bangladesh reported the intake was1.7 µg/kg b.w./week (Fangstrom et al., 2008) and was 5.5 µg/kg bw/week in Portuguese mothers (Almeida et al., 2008).

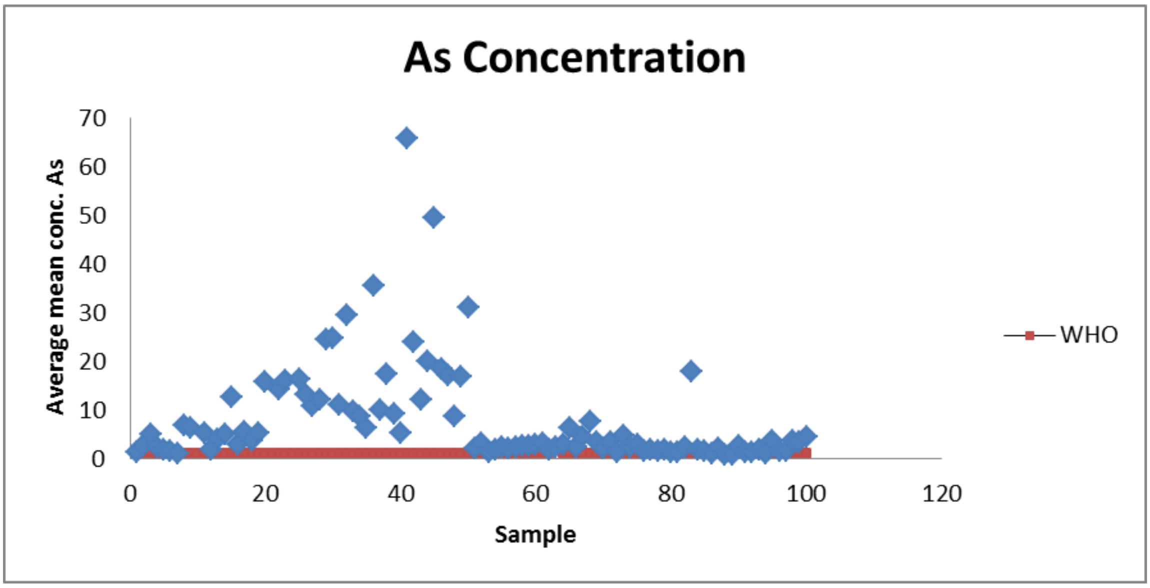

Figure (3.3): As concentration in breast milk

3.3 Possible factors that affect heavy metal levels in breast milk

Heavy metals in breast milk vary from one country to another. This difference could be due to many factors such as lactation period, methods which used to analyze samples, water used in analysis and nature of each country. Some of the factors are discussed in further paragraphs.

According to this study, lactating mothers who don't smoke had significantly higher **As** concentration in their breast milk (7.7±1.4 µg/L) compared with **As** concentration in smoking mothers (6% of participants) which was 2.76 ±3.73µg/L as shown in the **Figure 3.4**. The increase in **As** concentration in non-smoking women is not expected and may be due to insufficient aeration or another family member smoking in the house (Bahmani et al., 2018). However Bassil et al., (2017) reported high **As** concentration in human milk of lactating mothers who smoke was 3.7 μg/L compared with **As** concentration in non-smoking mothers which was 2.1μg/L. Also no significant difference was seen between **Pb** levels in breast milk of smoking mothers (0.9±1.0 μg/L) compared with non-smoking mothers (1.9 ± 0.5μg/L) as shown in the **Figure 3.4**. A study reported by Bassil et al., (2017) showed high **Pb** concentration of 22.6 μg/L in breast milk of lactating mothers who smoke compared with non-smoking mothers which was 17.6 μg/L. In regard to **Cd**, there was no significant difference between **Cd** levels in breast milk of smoking women (0.9±0.3 μg/L) compared with non-smoking mothers which was 1.37±0.15 μg/L as shown in the **Figure 3.4**. Similar **Cd** results were found in other studies (Goncalves et al., 2010; Turan et al., 2001). However, other studies reported high **Cd** level in human milk of nursing mothers who smoke compared with non-smoking mothers (Bassil et al; 2017, Orun et al., 2011 and Nassir et al., 2012).

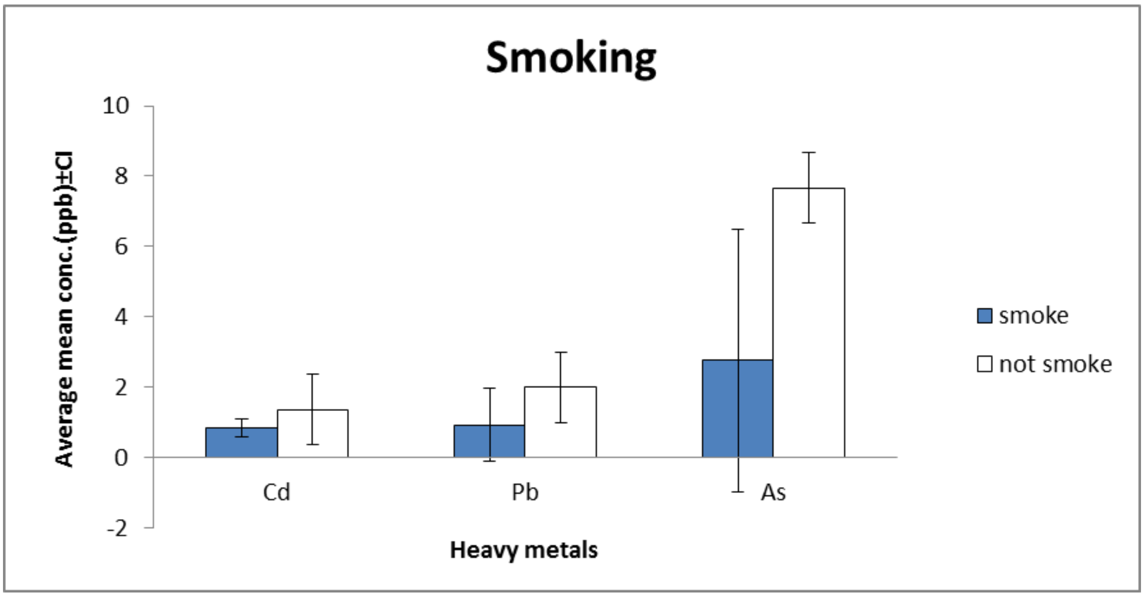

Figure (3.4): Heavy metals concentration in breast milk related to smoking. The error bars are at 80% Confidence Intervals.

Living near industrial activities could expose women to heavy metals. Results showed that women who live near industrial activity have large **As** mean level in their breast milk (24.2 μg/L) compared with women who live near cultivated areas (6.2 μg/L), waste disposal site (6.9 μg/L) and public road (7.7 μg/L) as shown in the **Figure 3.5**. Women can up take **As** or other heavy metals also if they live near from agricultural application or pesticides that contain **As** which are used in tobacco farming (Lazarevic et al., 2012) or near heavy traffic or live near industrial activities or from exposures to wood preservatives which produce **As** as by product (Lars, 2003). Study in Lebanon showed that lactating mothers who live near a waste disposal sites have higher **As** levels in their breast milk compared to women who live near agricultural or industrial or public road. On the other hand, in our study, all other residential areas whether close to agricultural or industrial or public road have the same **Pb** level pollution in breast milk as shown in the **Figure 3.5**. A study in Lebanon showed that lactating mothers who live near cultivation activities have higher mean **Pb** levels in their breast milk (25.7 µg/l) compared to those who don't live near cultivation areas (15.4 µg/l). Other studies, in Iraq and Iran reported that women who live near industrial areas had high mean level of **Pb** that is 38.6 and 23.7 µg/l respectively compared with those live in normal areas (Hasan et al., 2012; Soleimani et al; 2014). In regard to **Cd**, this study showed a significant low mean concentration of **Cd** 0.82 µg/L in the breast milk of mothers who live near industrial areas (7% of participants) compared with those who live near waste disposal site (1.7µg/L), agricultural (1.7 µg/L) and public road 1.3 µg/L as shown in the **Figure 3.5**. In contrast, other studies showed high mean level of **Cd** found in breast milk of women who live in industrial regions (Moussa, 2011; Hasan et al., 2012).

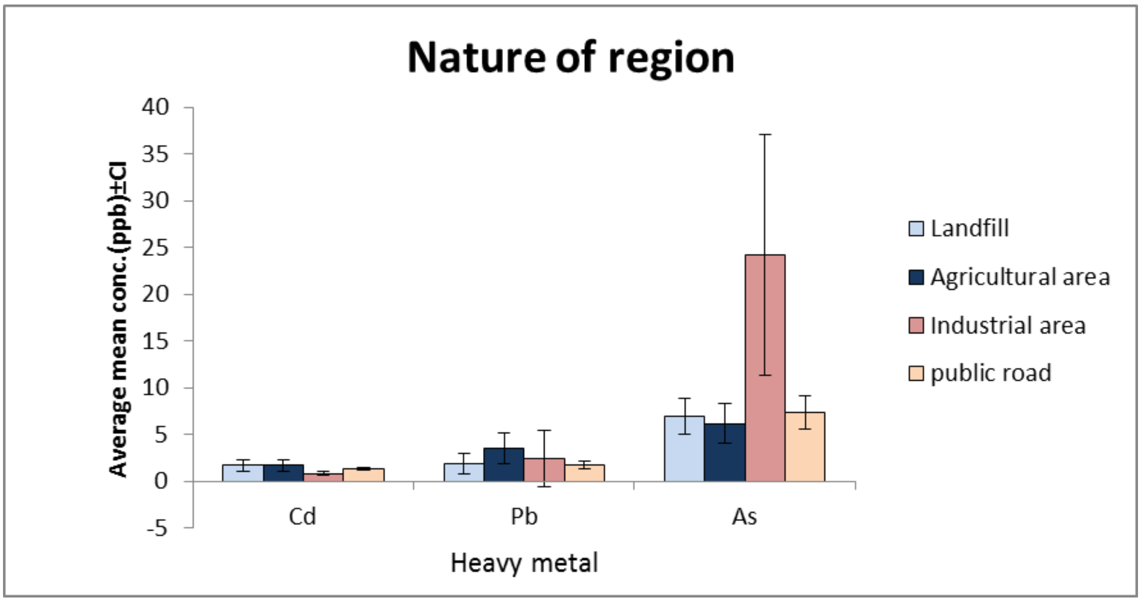

Figure (3.5): Heavy metals concentration in breast milk and the type of rejoin near the residence. The error bars are at 80% Confidence Intervals.

Interestingly, no significant differences in **Pb**, **Cd** and **As** levels in unemployed women compared with employed women (4% of participants) as shown in the **Figure 3.6** this could be due to similarities in the environment of the house and the place of employment. A Study in Iran reported the same result (Khanjani et al; 2018). However, a study conducted in Turkey showed that the median of **Cd** in breast milk was higher in unemployed mothers compared with employed mothers (E Orun et al; 2011).

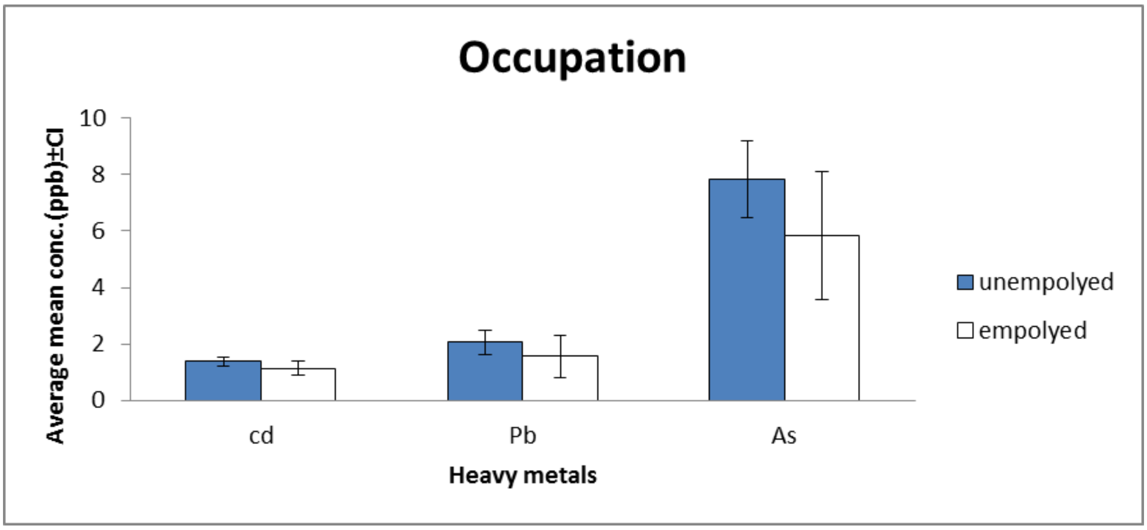

Figure (3.6): Heavy metals concentration in breast milk and employment. The error bars are at 80% Confidence Intervals.

In this study, mothers' age showed generally a non-significant relation with **Cd** or **Pb** and **As** levels in breast milk as shown in the **Figure 3.6.** The same result reported in others studies (García-Esquinas et al; 2011 and Orun et al; 2011). On the other hand, some studies reported high **Pb** level in human milk of older women (H Chao et al; 2014, R Sharma and S Pervez; 2005) while other studies showed an inverse relation between human milk **Pb** and lactating mothers age (Noronzi et al; 2010, Ursinyova et al.2005; Ettinger et al. 2004; Nassir et al; 2012).

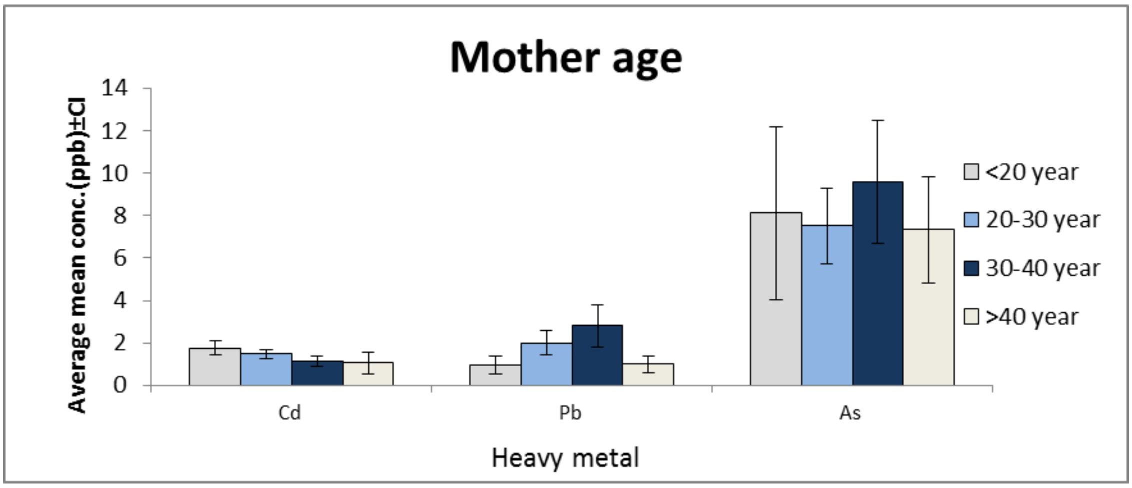

Figure (3.7): Heavy metals concentration in breast milk and mothers' age. The error bars are at 80% Confidence Intervals.

Also a non- significant correlation was found between **Cd**, **Pb** and **As** breast milk levels and level of education as shown in the **Figure 3.8**. Similar results were found for **Pb**, **Cd** levels and women's education (Soleimani et al; 2014, Orun et al; 2011, García-Esquinas et al; 2011). Some previous studies showed higher **Pb** levels for women with higher education (Chao et al; 2014), while others found the opposite; lower **Pb**, **As** and **Cd** Levels for women with lower educational levels (Bassil et al; 2017).

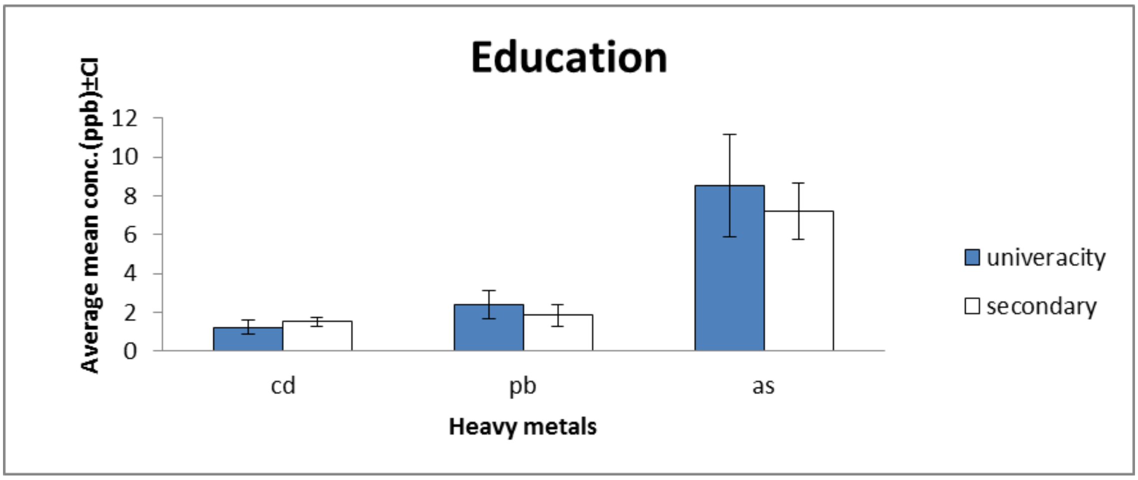

Figure (3.8): Heavy metals concentration in breast milk and education. The error bars are at 80% Confidence Intervals.

With respect to residence. There was no significant difference between the levels of **Cd**, **As** and **Pb** in breast milk and the place of residence if it is a city or a village as shown in the **Figure 3.9**. The reason for this could be that most of the studied lactating mothers live in villages (93%).

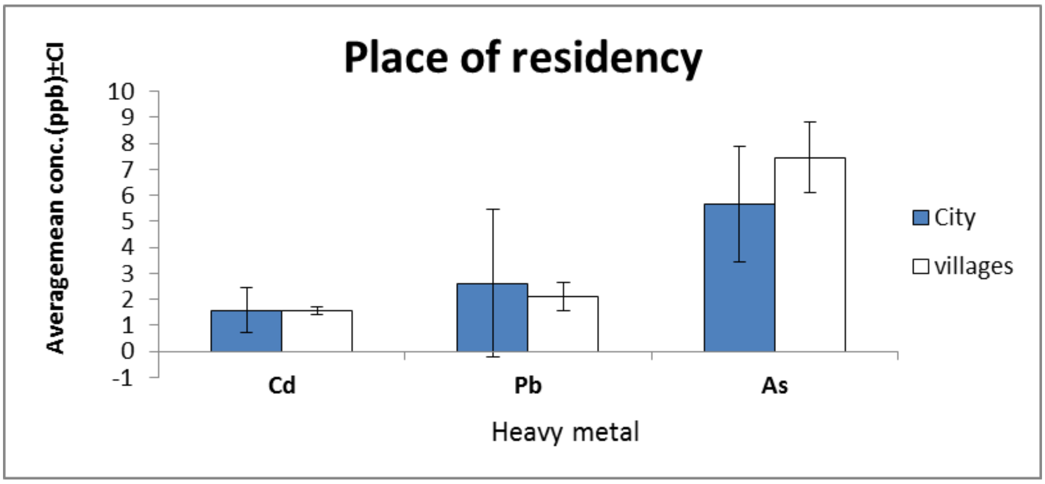

Figure (3.9): Heavy metals concentration in breast milk and place of residency. The error bars are at 80% Confidence Intervals.

Consumption of fish is expected to be a source of contamination sometimes if the fish came from polluted waters, however, in the current study fish consumption through lactating and pregnancy period had no effect on **Pb**, **Cd** and **As** level in breast milk of studied lactating mothers as shown in the **Figure 3.10**. Various studies reported results on the correlation between **As** and **Pb** and **Cd** in dietary food and human milk. In Spain, no type of food was associated with **Cd** levels in human milk (García-Esquinas et al; 2011). But a study in Saudi Arabia reported that higher **Pb** and **Cd** levels in human milk was related to not eating fish (Al-Saleh et al; 2003). In Italy and Croatia; **As** concentrations increased with higher fish consumption (Miklavcic et al., 2013, Bassil et al; 2017) as shown in Figure 3.10.

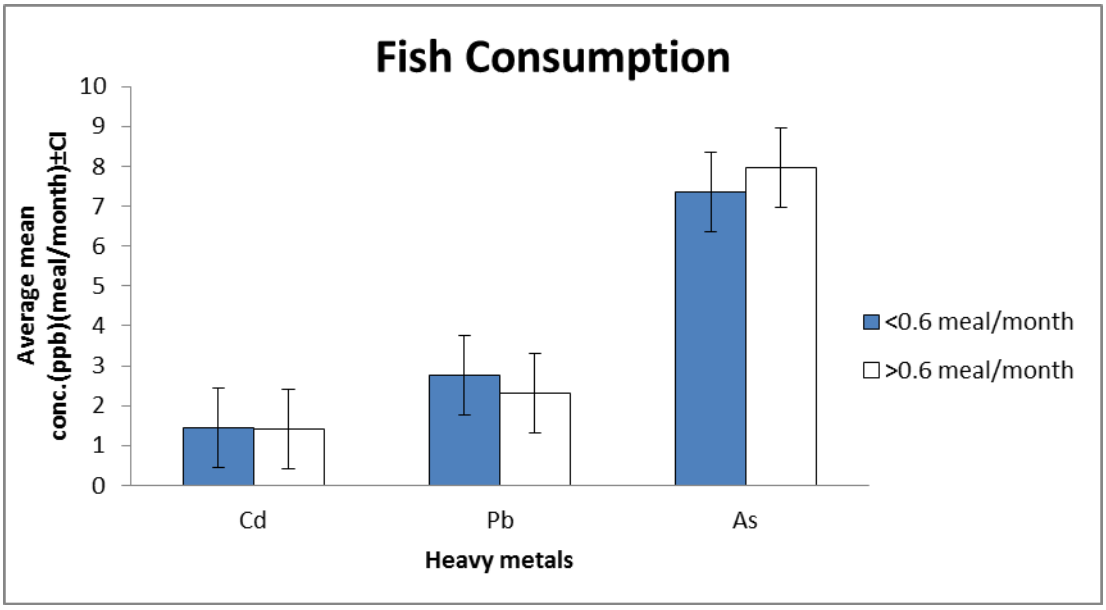

Figure (3.10): Heavy metals concentration in breast milk and consumption of fish. The error bars are at 80% Confidence Intervals.

Although rice (cereals) is considered as a source of **As** (shrain, 2017 & kahn et al, 2009), no research reported a correlation between rice consumption and **As** level in human milk (m.bassil et al; 2017). However, other studies reported that the consumption of cereal was related to higher **Cd** concentration in breast milk (Gundacker et al., 2007; Kharjani & Sim, 2006; 2007). In this study no significant relation between **Cd**, **As** and **Pb** levels in breast milk and cereal consumption as shown in **Figure 3.11**.

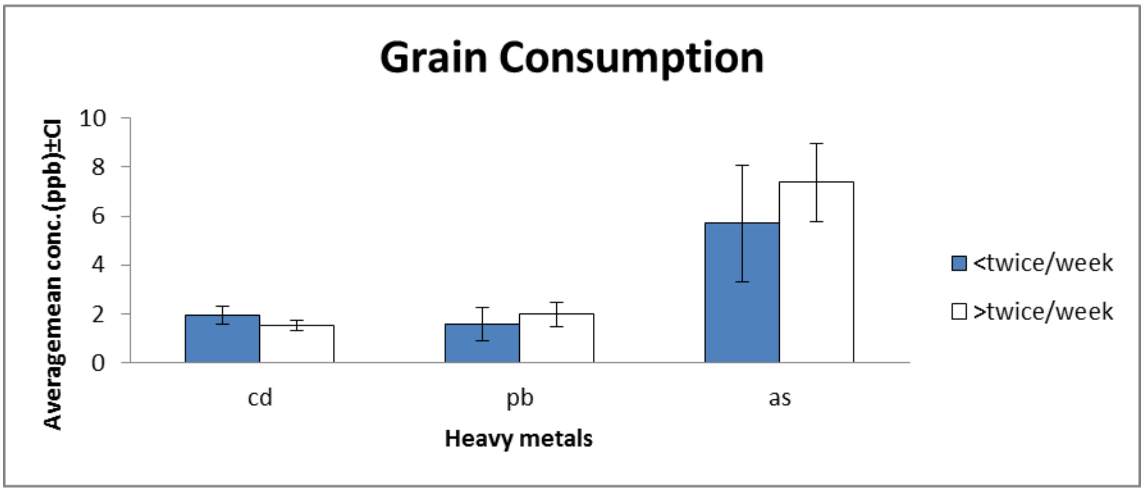

Figure (3.11): Heavy metals concentration in breast milk and seed consumption. The error bars are at 80% Confidence Intervals.

Milk could be polluted with heavy metals when animals eat polluted food or grass cultivated in area with industrial activities or heavy traffic. Also milk product could be also contaminated through industrial handling (Cabrera et al; 1995, Simsek et al; 2000). A Study in Iran found women who consumed dairy product were more polluted with **Pb** than women who consumed less (Khanjani, 2018). In this study, breast milk of the women who consumed milk of 0.6L /week were more significantly contaminated with **As** than mothers who consumed less. On the other hand, no significant correlation between **Cd** and **Pb** level and milk consumption as shown in the **Figure 3.12**.

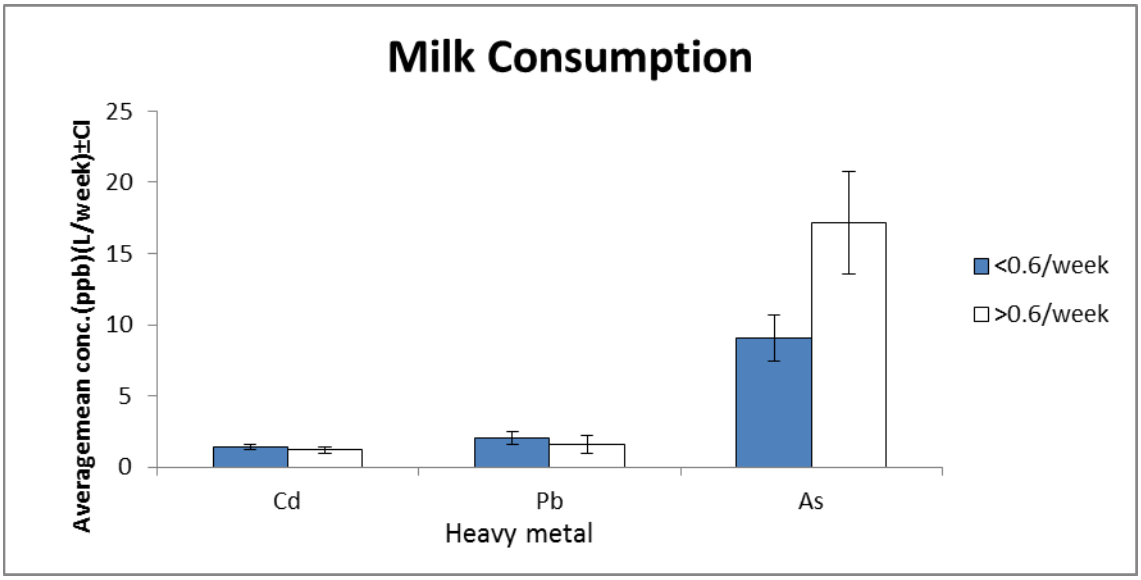

Figure (3.12): Heavy metals concentration in breast milk and grain consumption. The error bars are at 80% Confidence Intervals.

In this study frequent consumption of supplements was related to significantly high **As** and **Pb** levels in breast milk, but not significantly related to **Cd** level as shown in the **Figure 3.13**. Although some previous studies showed that frequent consumption of supplements were associated with decreased **Cd** level in human milk (Gundacker et al; 2007, Orun et al; 2011). The reason for increase **Cd** absorption with iron deficiency that both metals share the common transporters, so when iron decrease, the secretion of **Cd** to human milk increase(CDC, 2009; Sreedharan and Mehta, 2004; Kippler et al. (2009).

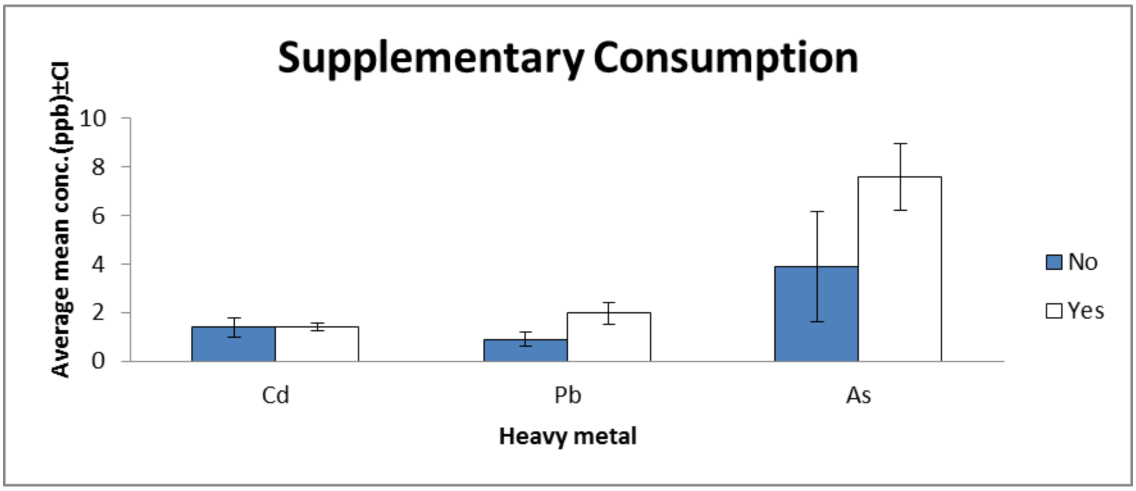

Figure (3.13): Heavy metals concentration in breast milk and supplementary consumption. The error bars are at 80% Confidence Intervals.

Pb and **Cd** contamination could reach women from cosmetics like eye shadows and lipsticks (Mousavi et al., 2013; Malakootian et al 2012; 2010). In this study, no-significant relation related to level of **As**, **Cd** and **Pb** in breast milk and cosmetics usage as shown in Figure 3.14.

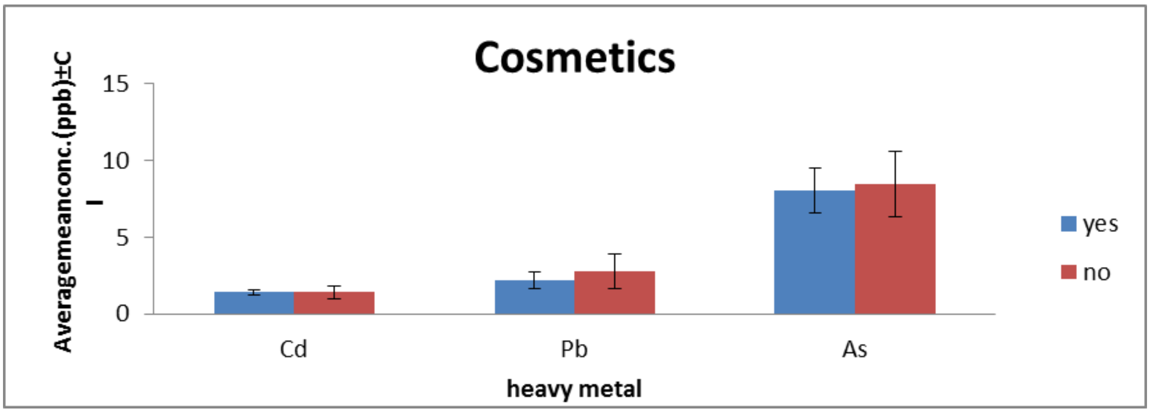

Figure (3.14): Heavy metals concentration in breast milk and cosmetics usage. The error bars are at 80% Confidence Intervals.

Water is considered as the main source of heavy metals exposure (Golpayegani et al., 2012). A study reported that the main source of **As** exposure of the nursing mothers is drinking water (Hughes et al., 2011). In this study non-significant association between levels **Cd**, **As** and **Pb** in breast milk related to consumption size of water as shown in the **Figure 3.15.**

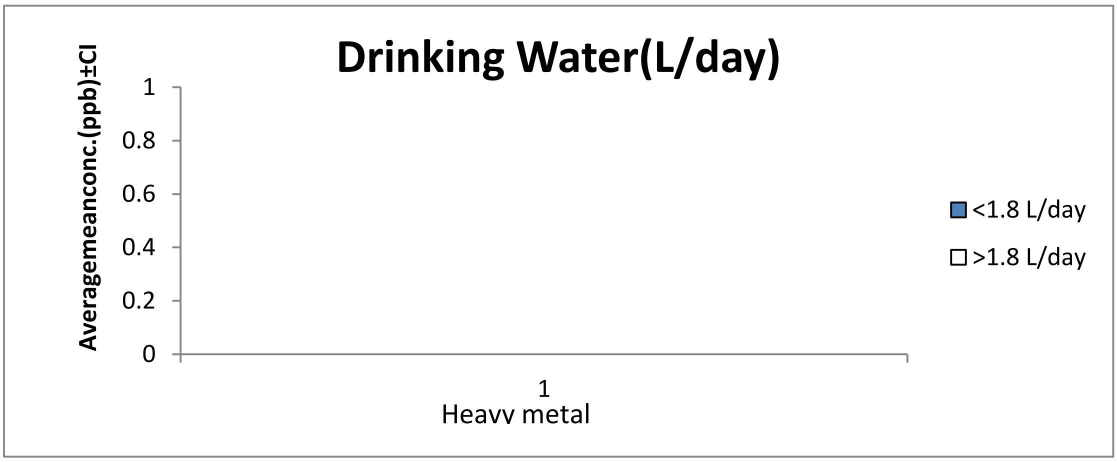

Figure (3.15): Heavy metals concentration in breast milk and Drinking water (L/day).The error bars are at 80% Confidence Intervals.

No statistically significant association was seen between **Pb**, **Cd** and **As** concentration in breast milk versus the consumption of vegetables in this study as shown in **Figure 3.16**. While In Madrid, Spain; Pb level increased with higher potato consumption. Others studies showed that main source among non-smokers is their diet of leafy vegetables. (Kharjani &Sim; 2006) (Kharjani & Sim; 2007).

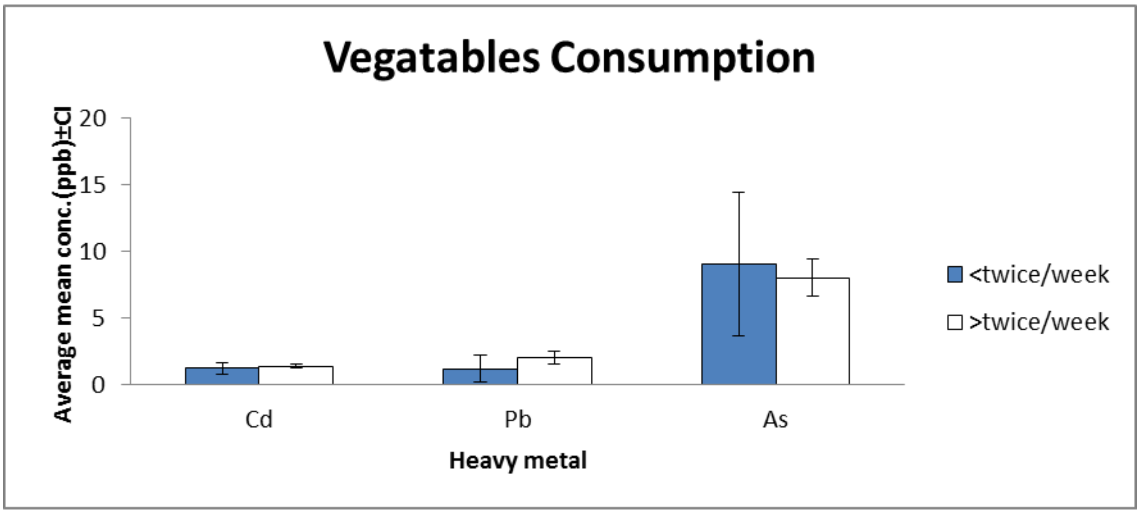

Figure (3.16): Heavy metals concentration in breast milk and vegetables consumption. The error bars are at 80% Confidence Intervals.

Also the present study outcomes revealed that there was no relationship between the income and taking medicine and the content of **Cd**, **As** and **Pb** in breast milk.

Conclusions

- This study is the first to give information on heavy metals in human milk of lactating women living in Jenin. It examined the occurrence of toxic metals in human milk and analyzed the correlation between toxic metals level, the socio-demographic status and dietary habits of mothers.

- The results of this study revealed that human milk were contaminated with lead, arsenic and Cadmium at varying degrees. Arsenic contamination was detected in all human milk samples with mean concentration of 8.1±10.6 μg/L**,** and all samples exceeded the limits by WHO (1 μg/L).Whereas, Cadmium was detected in 95% of the human milk samples with average concentration 1.7±1.6 μg/L while 50% of the detected samples with Cd exceeded the limits set by WHO (1 μg/L). Also Lead contamination was detected in all human milk samples with mean concentration 2.1±3.5 μg/L and 9% of the detected samples exceeded the limit set by WHO (5 μg/L).

- However, calculated weekly infant intake of heavy metals from human milk was found to be lower than the limit reported by WHO.

- The result of this study indicated that women live near industrial activities have more **As** in their breast milk compared to living near other places, and mothers who consume more than 0.5L/week of milk and milk product exposure have more **As** in breast milk compared to those who drink less milk. Whereas, women who live near waste disposal site, agricultural and public roads have higher **Cd** in breast milk compared to women who live near industrial activities.

- Also the study revealed that cereals consumption, cosmetic use, drinking water, taking medicine, fish consumption, age, education, occupation of mothers, place of residency and income don't affect levels of heavy metals in breast milk.

Recommendations

- More studies are needed to get information about the major source of these heavy metals in breast milk using larger number of samples and more sensitive method.
- As the lifestyle of nursing mothers has a direct effect on the presence of these heavy metals in human milk, it is necessary for enormous education to create awareness among mothers in this respect to prevent pollution of their milk.
- The study indicates a need for establishing and reinforcing national programs to avoid and reduce toxic metals environmental contamination.
- It is necessary to analyze the heavy metals levels in breast milk each period to ensure the safe limit for infants.

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Appendixes

Appendix (1) Protocol for human subject's research

AN-NAJAH UNIVERS

PROTOCOL FOR HUMAN SUBJECTS RESEARCH

NEW PROJECTS ONLY

Current Date of Submission: __January 31, 2019__________________

IRB office use only: Date received in IRB office (stamp)_______________

If this is a revision in response to an IRB Report of Action (ROA)-approval pending, indicate the date of the ROA: ___________________

Title of Research: Determination of Lead, Cadmium and Arsenic in human milk of lactating women/Case Study Jenin Governorate-Palestine.

Principal Investigator: Hanan Ahmed Ibrahim Saeed

Department/School_ graduated faculty / environmental science

Room # where mail can be sent ______________

Phone _0568524200 E-mail - hanansaed278@gmail.com

Other Investigator: Super visor

Department/School_ ______________________

Room # where mail can be sent ______________

Phone __ E-mail _

****Faculty Sponsor (for Student Research): Maather Feras Sawalha**

Department/School___ science faculty/ chemistry department

Room # where mail can be sent _14F1280_____________

Phone _0599675502 E-mail _ maather@najah.edu

Student Street Address ______________________________________

City ________________________State_________ Zip _____________

Type of Research (please check): Master's Thesis

** If the primary investigator is a student, check here to indicate that your faculty sponsor has read the entire application, including cover letters, informed consents, and data collection instruments, and asserts that this application is accurate and complete.

Dates Human Subjects Portion of Research Scheduled: from: _march______ to _June______.

Site(s) of Human Subject Data Collection: ___Jenin Governorate

(*NOTE: If sites are administratively separate from the University, please submit approval letters, or indicate when they will be forthcoming.)*

Funding Agency (if applicable):_______________________________

I. NATURE OF THE RESEARCH

In the judgment of the Principal Investigator, this research qualifies for which of the following types of review:

Review Type: expedited

II. PURPOSE OF RESEARCH

Briefly describe the objective(s) of the research (please keep description jargon free and use 100 words or less; the IRB will file this information in our descriptions of approved projects).

The main objective of this study is to know if infants of lactating women in Jenin are exposed to cadmium, lead and arsenic from the breast milk and the concentration of these metals in it.

- Specific objectives

1. To determine level of heavy metals, specifically Arsenic, Lead, and cadmium in breast milk for breastfeeding mothers in Jenin using Atomic Absorption Spectrophotometry.

2. To compare the measure value of metals with the reference range set by World Health Organization.

3-To find if metals level in breast milk is related to nutritional and living condition.

III. METHODS

Approximate number of subjects: _100 breast milk sample____________

Subjects will be (check only if applicable):

minors (under 18)

involuntarily institutionalized

mentally handicapped

Describe in detail how the subjects will be selected and recruited:

Breast milk samples (10-20ml) will be collected from 100 lactating women living in Jenin using manual breast pumps, each sample will be stored in a polyethylene container and frozen at -20C until time of analyze.

Describe <u>exactly what will be done</u> to subjects once they have agreed to participate in the project:

Women will receive breast pumps along with bottle, to collect 20 ml of milk.

Milk samples will be saved in freezer in 20 c0 and transfer in thermos bag to An Najah's lab and will be kept in freezer until analysis.

Mothers will be provided with the result of analysis if requested that.

The sample will be analysis in An najah,s university research lab. To estimate the level of heavy metals in each sample.

breast milk samples will be digested and analyzed by atomic absorption spectrophotometry Instruments.

What incentives will be offered, if any? _________________________

IV. RISKS/BENEFITS TO PARTICIPANTS

Identify possible risks to subjects:

(NOTE: These may be of a physical, psychological, social or legal nature. If subjects are vulnerable populations, or if risks are more than minimal, please describe what additional safeguards will be taken.)

There is will be no risk to subject.

What are the benefits and how will they be optimized?

The Result of research will show if any of targeted group have high level of heavey metals or not based on that we could recommended educational or nutritional change in targeted group.

Do benefits outweigh risks in your opinion? Yes

Are there potential legal risks to the Principal Investigator or University? No

V. INFORMED CONSENT

Describe how participants will be informed about the research before they give their consent. Be sure to submit with this protocol a copy of the informed consent/assent letter(s) you will use. Please prepare your informed consent letter at the 8th grade reading level or lower as dictated by the needs of the subjects. (See IRB website for required elements of an informed consent.)

Mothers will receive information sheet that explain the purpose and importance of the study, and a questionnaire which include background information

VI. PRIVACY/CONFIDENTIALITY

Please describe whether the research would involve observation or intrusion in situations where subjects have a reasonable expectation of privacy. If existing records are to be examined, has appropriate permission been sought; i.e. from institutions, subjects, physicians? What specific *provisions have been made to protect the confidentiality of sensitive information about individuals?*

Mother will collect the samples by them self and answer the questioner, then same number will be labeled on the sample and questioner, the name of mother will be hidden. Data and sample will exclusively use for research purposes.

Appendix (2) Questionnaire

السلام عليكم انا الطالبة حنان سعيد كلية الدراسات العليا جامعة النجاح اقوم باجراء بحث حول تقيم تراكيز بعض العناصر الثقيلة (الزرنيخ والرصاص والكادميوم) في حليب الام المرضع

ارجو من حضرتك مساعدتي بالاجابة على الاسئلة التالية: (علما بانه سيتم استخدام هذه البيانات لاغراض البحث ولن يتم الكشف عن الاسم او اي بيانات شخصية)

1- عمر الام:

أ. اقل من 20 ب. 20-30 ج. 30- 40 د- اكثر من 40.

2- **عمر المولود.......... وزن المولود............ طول الام .... وزن الام...........**

3- **مكان السكن:**

أ. قرية ب. مدينه ج. مخيم.

4- **العيادة التي اعتدت زيارتها للمراجعة:**

أ. حكومية ب. وكاله ج. خاصة.

5- **دخل الاسرة (بالشيكل):**

أ. 1000-2000 ب. 2000-3000 ج. 3000 -4000 د. اكثر من 4000

6- **عمل الام:**

أ - ربة منزل ب موظفه حكومية ج-موظفة في قطاع خاص

7- **التعليم:**

أ-ثانوي ب-مهني ج-جامعة دراسات عليا د-لاشي مما ذكر

8- اختاري كل ما ينطبق على مكان سكنك(اقل من 100م):

أ- قريب من مكب نفايات ب- قريب من انشطة زراعية

ج- قريب من طريق عام د- قريب من منطقة صناعية

9- معدل استهلاك الحليب في الاسبوع................. لتر

10- معدل تناول السمك في الاسبوع.............. مرة/وجبة

11- معدل استهلاك الماء................ لتر /يوم

12- معدل تناول الحبوب:

أ-مرتين فاكثر في الاسبوع ب- اقل من مرتين في الاسبوع

13- معدل تناول الخضروات الطازجة:

أ- مرتين فاكثر في الاسبوع ب- اقل من مرتين في الاسبوع

14- معدل تناول المعلبات في الاسبوع:

أ- مرتين فاكثر في الاسبوع ب- اقل من مرتين في الاسبوع

15- هل تدخنين؟

أ-نعم ب-لا

16- هل تستخدمين مساحيق التجميل؟

أ-نعم ب-لا

17- هل لديك فكرة عما يسببه وجود عناصر ثقيلة في الجسم؟

أ-نعم ب-لا

18- من اين حصلت على هذه المعلومات؟

أ- من طبيب ب – قراءة من مصادر مختلفة (النت, نشرات طبية)

19- هل يعاني احد اطفالك تاخر في التطور النفسي والحركي؟

أ-نعم ب-لا

20- هل لدى احد اطفالك تاخر في التحصيل الدراسي او خلل في تطوره العقلي؟

أ-نعم ب-لا ملاحظة:-

21- هل لدى احد اطفالك تاخر في التطور البدني؟

أ-نعم ب-لا

22- هل تقومي بتناول مكملات غذائية اثناء الحمل او الرضاعة؟

أ-نعم ب-لا

23- هل تتناولي اي ادوية ؟

أ-نعم ب-لا

24- من قام بنصحك باخذ هذه المكملات او الادوية؟

أ-طبيب ب-من نفسك ج-نصيحة من قريب او امهات سابقات

اتمنى لك ولطفلك دوام الصحة

جامعة النجاح الوطنية

كلية الدراسات العليا

تقييم مستويات الرصاص والكادميوم والزرنيخ في حليب الأمهات المرضعات في جنين: دراسة تجريبيه

اعداد

حنان احمد ابراهيم سعيد

اشراف

د. مآثر صوالحة

قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في العلوم البيئية بكلية الدراسات العليا في جامعة النجاح الوطنية، نابلس– فلسطين.

2021

تقييم مستويات الرصاص والكادميوم والزرنيخ في حليب الأمهات المرضعات في جنين: دراسة تجريبيه

اعداد

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اشراف

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الملخص

إن تلوث حليب الأم المرضع بالمعادن الثقيلة السامة له أهمية خاصة بسبب تأثيراته الضارة وخاصة على الاطفال الرضع.

الهدف من هذه الدراسة هو تقييم مستويات الكادميوم والرصاص والزرنيخ في حليب الامهات المرضعات وتوضيح علاقة الارتباط بين تراكيز هذه العناصر وعوامل مختلفة في جنين في فلسطين. حيث تم جمع 100 عينة من حليب الامهات المرضعات. تم تحليل عينات حليب المرضعات لتحديد مستوى الكادميوم والرصاص والزرنيخ باستخدام جهاز الامتصاص الذري. كما تم استخدام ورقة استبيان لتحديد العوامل المتعلق بوجود هذه المعادن في حليب الأمهات المرضعات.

أظهرت الدراسة تلوث جميع عينات الحليب بالزرنيخ والرصاص بمعدل(يعني 8.0532 ± 10.574 ميكروغرام / لتر و 2.05 ± 3.47 ميكروغرام / لتر) على التوالي, بينما 95٪ من عينات الحليب ملوثة بالكادميوم بمعدل 1.74 ± 1.56 ميكروغرام / لتر. كذلك بينت هذه الدراسة ان تناول الرضع الأسبوعي من المعادن السامة من حليب الأم أقل من الحد الذي وضعته منظمة الصحة العالمية. كما لا توجد علاقة بين مستويات المعادن الثقيلة في حليب الأم واستهلاك الحبوب ومستحضرات التجميل ومياه الشرب وتناول الأدوية واستهلاك الأسماك والعمر والتعليم ومهنة الأمهات ومكان الإقامة. بينما أظهرت دراستنا أن تلوث حليب الامهات بالزرنيخ مرتبط بالسكن بالقرب من الأنشطة الصناعية واستهلاك الحليب.

دراستنا هي أول دراسة تقيَم مستويات المعادن السامة في حليب الامهات المرضعات في جنين. حليب الامهات المرضعات ملوث بالعناصر السامة الثقيلة (الكادميوم والرصاص والزرنيخ) لذا نحن بحاجة الى إلى تطوير سياسات لحماية الرضع من التعرض لهذه المعادن الثقيلة السامة.