LEBANESE EXPOSURE TO HEAVY METALS VIA CONSUMPTION OF PITTA BREAD

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ABSTRACT

Recently, high levels of heavy metals in several Lebanese foods were reported, which has raised the issue that today, many Lebanese products could be contaminated with heavy metals and toxins. Knowing that no regulations, effective control or corrective actions from the Lebanese government are present, there is a need to assess the exposure of the Lebanese to those contaminants through its consumption. The objective of this study was to assess the exposure of the Lebanese population to heavy metals through the consumption of white pitta bread and to evaluate the health risks of its consumption in comparison to the recommended reference limits.

This study compiled all the previous studies published between 2000 and May 2020 related to cereal product consumption in Lebanon and heavy metal levels. The exposure assessment was conducted following the Joint expert committee of FAO and WHO for Food additives and contaminants.

Nickel 1292 µg/kg, Arsenic 400 µg/kg and Aluminum 11580 µg/kg were the highest heavy metals found in bread above the recommended limits. The exposure of Lebanese population was higher than the safety regulations for Arsenic 3.03 µg/kg.bw and Nickel 9.733 µg/kg.bw. Children aged 6 to 9 years old and people living in South Lebanon and Nabatieh regions were the most exposed population to heavy metals. Numerous bakeries did not apply food safety standards, which will lead to threatening health risks. Recommendations are to revise LIBNOR standards to make it more appropriate to the Lebanese population consumption level of bread products and to conduct effective control on raw material safety and bakeries application of safety standards and use of preservatives.

Keywords: Food Safety, Heavy Metals, Exposure Assessment.

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LIST OF ABBREVIATIONS

PTWI: Provisional Tolerable Dietary Intake

PTMI: Provisional Tolerable Monthly Intake

BMDL: Benchmark Dose Lower Limit

TDI: Tolerable Daily Intake

TDS: Total Dietary Study

Cd: Cadmium

Pb: Lead

Hg: Mercury

Al: Aluminum

Cr: Chromium

Ni: Nickel

Mn: Manganese

Cu: Cupper

Zn: Zinc

Fe: Iron

Co: Cobalt

CHAPTER 1. INTRODUCTION

1.1. General Information

Despite its late resurgence, heavy metals have slowly made its way into food safety's everyday topic, but is yet a largely unexplored subject in the Lebanon. Moreover, the evaluation of heavy metals via consumption of pitta bread could cover several levels of food safety principles. Throughout this report, consumption of bread and its heavy metal content will be discussed.

1.2. Problem Statement

Several countries have introduced the exposure of heavy metals as part of the Food Processing Safety Manufacturing Departments. As a matter of fact, the base of the Mediterranean diet that consists of wheat products, present a valid reason for conducting several researches on the exposure to heavy metals.

1.3. Objective

The objective of this study was to assess the exposure of Lebanese to heavy metals via white pitta bread and its health risks.

1.4. Outline

A specific organization for the completion of the aims and objectives set was followed. It is important to ensure that all possible gaps are filled and as much information is provided in order to guarantee to the average reader a better understanding of the exposure of heavy metals via pitta bread. For that reason, the first chapter introduces the idea of bread and heavy metals. The second chapter presents an overview on the history, specification and market of bread. The third chapter highlights the key calculations for the exposure of heavy metals and assessment of its health risks.

Finally, the fourth chapter provides a conclusion on the whole project and introduces recommendations for future designs.

CHAPTER 2. LITERATURE REVIEW

2.1. History

It is said that the first bread ever made was in Egypt, while the best bakers came from Phoenicia (Mediterranean civilization that originated in the Levant, specifically Lebanon). The universality of bread was shared among the Roman, Greek, Egyptian civilizations, and many others (Pasqualone, 2018).

Lebanese pitta bread and bakery products are highly consumed in Lebanon. Pitta bread, the most famous between Lebanese bakeries is known to be a flat bread with a pocket between the two layers. (Quail *et al.*, 2016).

Pitta bread is the product that results from a mix of wheat flour, water, salt and yeast that is kneaded, fermented and baked to the correct temperature (LIBNOR NL 240, 2002). “Pitta” is a Greek term that literally translates to ‘flat’ (Hashem *et al.*, 2012). It is traditional Arabic bread that has been around for thousands of years. It is a Lebanese staple food and widely present in every Lebanese bakery, home, and on every Lebanese table and used in hospitals, nursing homes, fed to kids, elderly and pregnant women. Arabic bread is popular in many countries and is known by the following names: khobz, shami, mafrood, burr, bairuti, Lebanese, pitta, and pocket (Quail *et al.*, 2016).

A large pitta loaf that weigh 122 grams, contains approximately 170 calories, while a small pitta loaf has about 74 calories (depending on the ingredients and on the brand). Most of the calories come from the high carbohydrate content. Pitta bread is a rich source of carbohydrates (78.8%) with low dietary fiber content (5.1%). Pitta bread is characterized by having a low quantity of fat (1.6%) and a fair amount of proteins (12.98%) (Moussa *et al.*, 1992)

2.2. Process

Pitta bread is made most commonly with moderate ash white flour (76–80% extraction). It is also produced with high-extraction flours and meals. The bread can range from very thin (4 mm) to quite thick (20 mm). A typical formula would be flour 41.5%, yeast 1%, salt 1.5%, and water ~ 56%.

The dough is ideally mixed to full development and may be processed immediately or allowed a bulk fermentation period of approximately an hour. After scaling, the dough is rounded and rested before sheeting. On an automated line, the sheeting is usually completed with three sets of rollers. The first roller gently flattens the dough to allow the dough pieces to enter the second roller, which flattens the dough in the same direction but more aggressively on this occasion. The dough is then turned at right angles for the third sheeting. The final roller is set to produce the desired final thickness, and also by sheeting at right angles, this typically produces a round thin dough piece. The sheeted dough piece is then proofed for ~ 20 min. During the proof, a fine cell structure develops and a thin dry skin forms on the surface of the dough.

The dough is then baked at a high temperature, with thinner dough pieces requiring higher baking temperatures for shorter baking times. Oven temperatures can be over 600°C with baking times less than 20 seconds. During the baking period, the very hot temperature forms a crust of un-gelatinized starch on the surface of the dough piece, which provides a barrier to gas transfer. Gases within the cell structure expand and steam is generated. The gases spread through the now discontinuous cell structure, and once the gas pressure is adequate, it forces the upper and lower layers of the bread apart. The two layers are formed when the bread 'pockets' during baking at high temperatures of 350–600°C. Ideally, the layers are of even thickness, and this is largely achieved through a similar heating rate from the top and the bottom as this sets the crumb structure before the layers are separated (Quail *et al.*, 2016)

2.3. Specifications

According to LIBNOR standards NL 240, 2002, the Lebanese bread is classified under four different types including the white, whole wheat, brown and bran.

White Lebanese bread is the one, which has a semi-circular shape, and is made of two separate layers, with a gap between them. The thickness is not more than 3 mm.

Brown Lebanese bread, has a semi-circular shape, and is made of two separate layers, with a gap between them. It has a sickness of not more than 4 mm and is made of the whole wheat (with skin), which gives it the brown coloring.

Bran Lebanese bread has a semi-circular shape, and is made of two separate layers, with a gap between them. It has a sickness of not more than 5 mm and made of a mix of wheat flour with bran that increases 3% of the fiber content.

Whole-wheat pitta bread is characterized by the use of whole-wheat flour, which consists of the germ, starchy endosperm and bran, which are present in the same proportions as the original wheat grain (LIBNOR NL 240, 2002).

2.4. Market

2.4.1. *Local Brands*

The current leading brands whose products are available and distributed across more than one region in Lebanon are: Moulin D’Or, Wooden Bakery, Pain D’Or, Chamsine, and Al Sultan (listed in no particular order) (The Daily Star, 2017).

The Lebanese government maintains price ceilings for two food items, Arabic bread and chicken (Hunter, 2008). The price of a standard weight of Arabic bread paid by consumers is set at 1500 LBP (Daou and Mikhael, 2016). The consumer price of the pack of bread has been fixed, while the weight for the pack has gradually fallen over time with the explicit approval of the government: The pack measured 1.25 kg in 1996, 1.0 kg in 2010, and 900 g in 2012 (Quail *et al.*, 2016).

2.4.2. *Local wheat*

Lebanon plants, harvests and mills wheat locally. Today, Lebanon counts thirteen mills of which two in the Bekaa region, one in the north, one in the south, three in Beirut (Bakalian, Modern Mills and Crown Flour Mills) and 6 in Mount Lebanon. Crown Flour Mills is estimated to have around a 24% market share, Bakalian 18% and Modern Mills around 13%.

In 2013 and according to the Ministry of Agriculture, the planted area of wheat in Lebanon totaled 9,600 hectares with each hectare of irrigated land yielding 5 metric tons and each hectare of rain-fed land yielding 3.25 metric tons. In general, total wheat production can hover between 45,000 and 70,000 metric tons depending on the season, i.e. on the status of the rain and the heat.

Market researchers believe there are many hurdles to Lebanon becoming self-sufficient. Lebanon does not have enough agricultural space to grow the quantities of wheat needed to meet local demand. However, not only is local production not enough but also the characteristics of the wheat grown in Lebanon are not suitable for the production of Arabic bread (BLOMINVEST Bank, 2016).

2.4.3. *Imported Wheat*

The soft type of wheat in Lebanon has low protein and gluten content and the other type is the durum wheat which is only suitable for semolina. Therefore, to produce Arabic bread, domestically produced wheat, must inevitably be mixed with imported wheat.

Local production manages to cover only 10% of the 450,000 metric tons of consumption. Lebanon therefore turns to imports to supply the country with wheat. In 2015, Lebanon imported 625,661 tons of wheat with a value of $142.52M compared to 615,416 tons with a value of $176.68M back in 2014. The imported volume of wheat exceeded the 450,000 metric tons' mark probably due to the influx of Syria refugees into the country (BLOMINVEST Bank, 2016)

2.5. Recommended daily intake of cereals

The consumption of cereals, especially whole grains, has been associated with a decreased risk of cardiovascular diseases, overweight, abdominal obesity, hypercholesterolemia, and insulin resistance. Higher intakes of breads and cereals can help in achieving dietary targets of lower fat consumption, and as cereals are a major source of resistant starch, they are an important dietary component for colon health. The Food and Drug Administration (FDA) in the United States has approved the health claim that 'diets rich in whole-grain foods and other plant foods, and low in total fat, saturated fat, and cholesterol may reduce the risk of heart disease and certain cancers' (Hwalla *et al.,* 2013).

The cedar food guide (Fig1.) provides a graphic illustration of the food groups and the recommended intakes from each for Lebanese adults, to ensure a varied and balanced diet providing 2,000 calories (equivalents of a serving of each of the five food groups). It also provides a graphic illustration of the recommendations on safe water consumption and engagement in physical activity for improving general health. The recommended intakes from each of the five food groups and the serving equivalents for each group are adapted from recommendations of the United States Department of Agriculture (USDA MyPlate, 2011). Presented below is the recommended daily intake of cereals based on a 2000-calorie diet (Fig 2.).

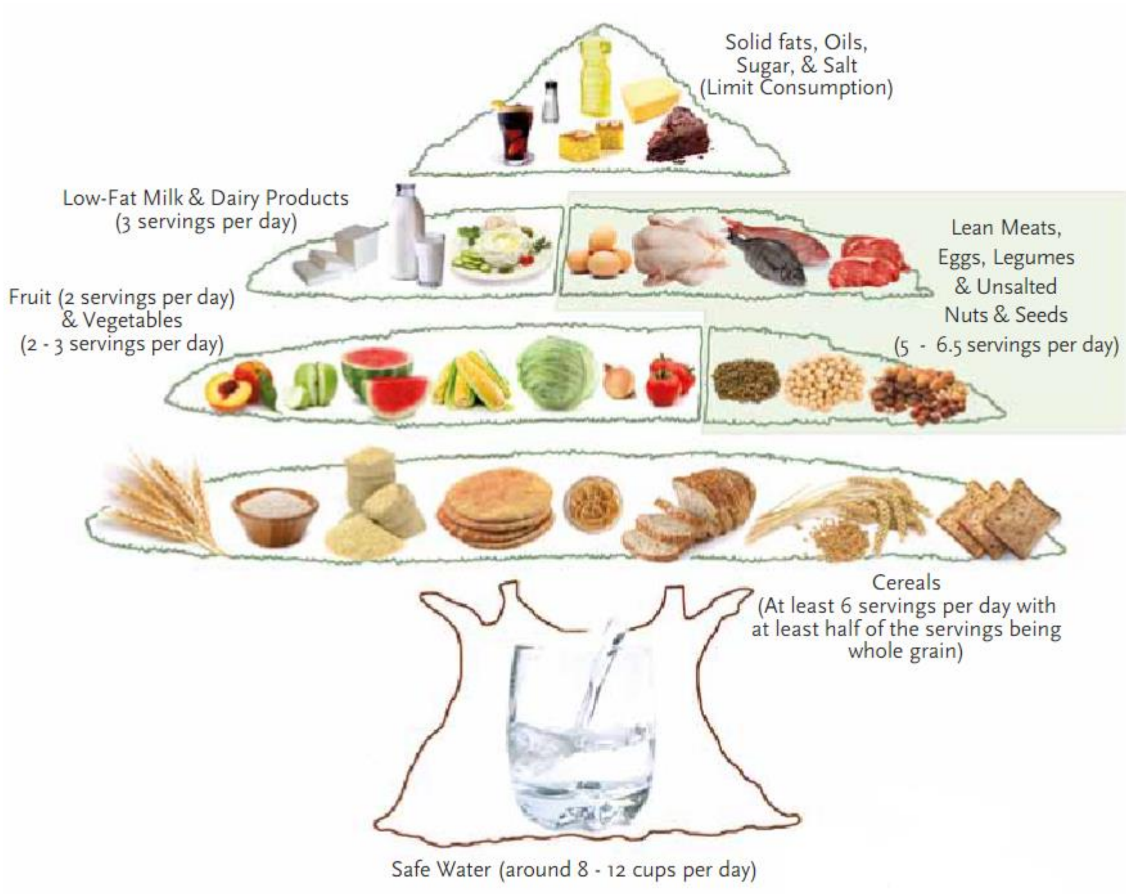

Figure 1- The Lebanese Cedar Food Guide (Hwalla *et al*., 2013)

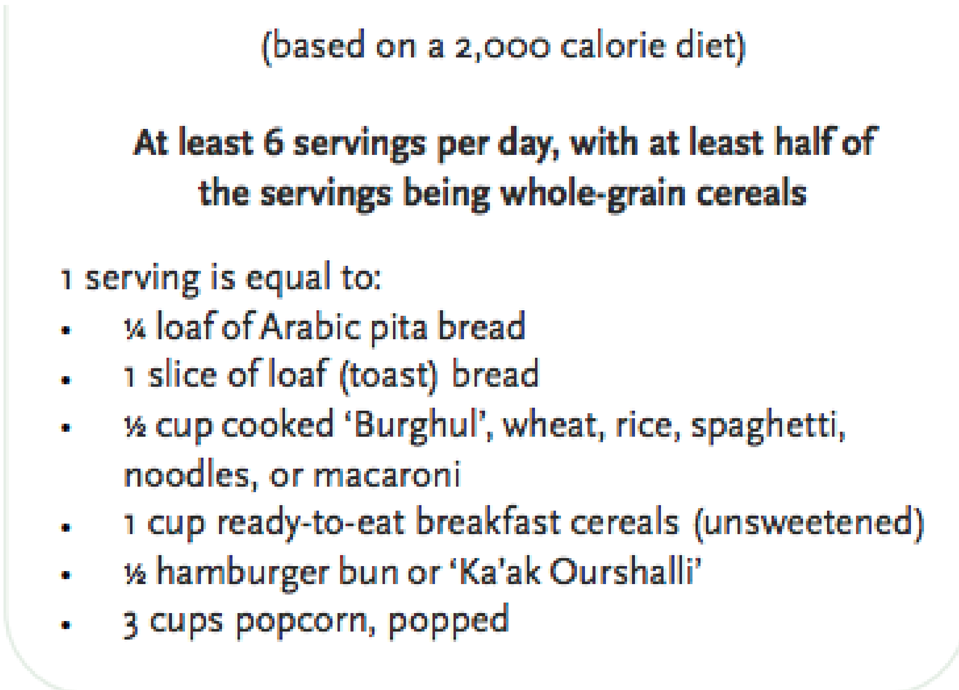

Figure 2- Recommended Daily Intake of Cereal (Hwalla *et al*., 2013)

2.6. Bread consumption

Several studies showed that the Lebanese highly consume bakery products and bread (Table 1). Nasreddine *et al.* (2006) conducted a study aiming to investigate, measure and assess the food consumption patterns of adults living in Beirut, and to identify inadequate or excessive intake of food groups particularly linked to non-communicable diseases. Results showed that cereals consumption was 324.5g/day providing 35.0% of daily energy intake, with bread being the most highly consumed (146.2 g/day) in this food group, kaak 6.2 g/d and toast 3.2 g/d (Nasreddine *et al.*, 2006).

Studies also showed that, although there has been a consistent decline in cereal consumption over the past three decades in Lebanon, cereals remain the principal staple food in the country (Hwalla *et al.*, 2013).

Cereals and cereal-based products contribute to almost ⅓ of daily energy intake among the Lebanese adult population, with wheat (mainly consumed as bread) being the major staple cereal, followed by rice. Nevertheless, a trend towards an increased consumption of refined cereals such as refined flour and pasta, as well as white rice, at the expense of whole-grain cereals has been witnessed (Hwalla *et al.*, 2013).

In 2015, Investment Development Authority of Lebanon (IDAL) created a fact book about Lebanese agro-food sector. Agro-food products account for 12.5% of total exports, and have grown in volume at an average rate of 31.7 % from 2011 until 2014. Agro-food exports have continued to grow steadily despite the general decline in industrial exports (Fig. 3).

The report showed that the highest share of agro-food establishments is involved in the production of baked goods, with nearly 30 % of total enterprises. Baked goods occupy a significant share in the diet of Lebanese households, and therefore form the vast majority of agro-food companies (IDAL, 2015). Nearly 24% of total household consumption goes to food products, and 65% of this share is allocated to agro-food products. Within this category, fresh meats occupy the highest share with 24% of total consumption, while grains and cereals rank second at 22%, and the share of dairy products stands at 16.93% (Fig. 4) (IDAL, 2015).

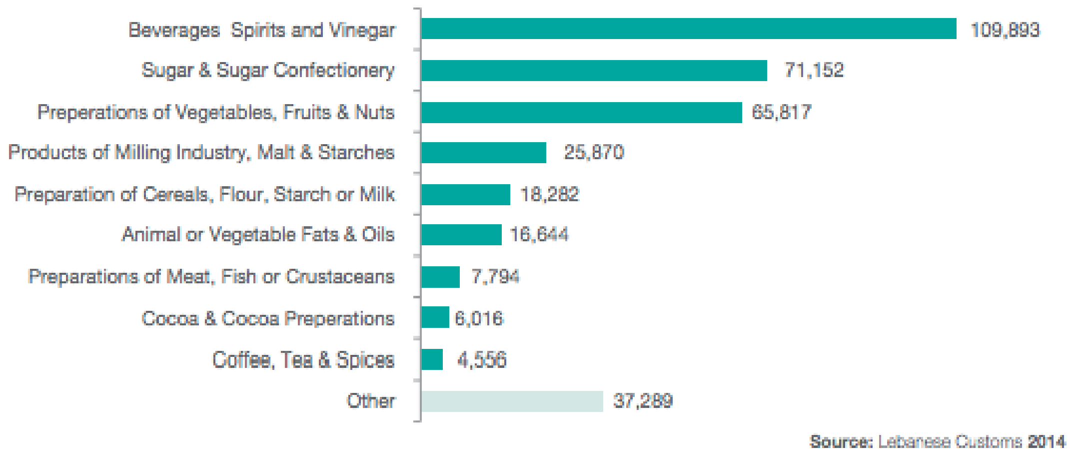

Figure 3- Agro-food Exports, Net Weight Tons (IDAL, 2015).

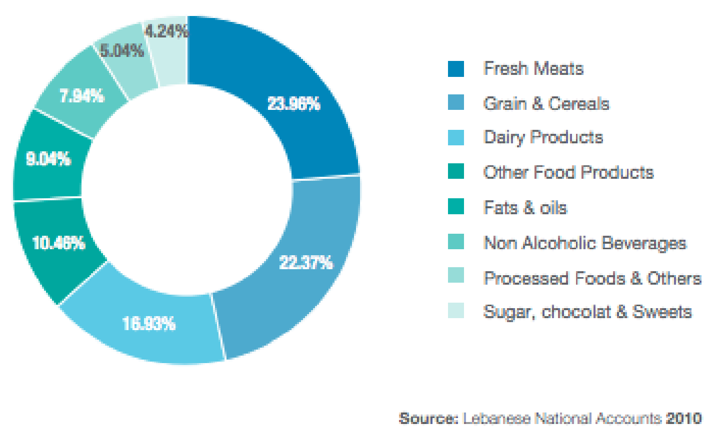

Figure 4- Household Consumption of Food in Lebanon by Type of Product (IDAL, 2015).

A study done in 2015 showed that daily bread consumption was higher among males than females among different types of bread. Consumption levels were as follows: white pitta 88.6 g/d (± 135.21), brown pitta 39.74 g/d (± 71.63), white baguette 14.06 grams per day (± 43.36), brown baguette 5.12 grams per day (± 13.83), markouk 11.83 grams per day (± 38.7) and tannour 3.77

grams per day (± 13.23). Consumption trends of bread were affected by several factors: gender, province, type of bread and the interaction of some variables together **(**Barakat, 2015).

According to the Lebanese owner's union, Lebanon consumes approximately 20,000 to 22,000 tons of flour per month, with every ton of flour making 1070 bread bags. In 2012, Lebanon produced 785,000 bags of bread a day, almost 23.5 million a month. In other words, Lebanese spend 23.5 million US $ each month (at 1 $ to a bag) on bread. Lebanese bread turned out to be the top selling good at 4 out of the five leading bakeries across Lebanon despite the diversification of products especially in modern bakeries (Chbeir *et al.,* 2017).

A survey of white pitta bread consumption was achieved among one thousand Lebanese individuals, grouped into adults (above 15 years old, men, and women) and young people (6–9 and 10–14 years old). The survey makes it possible to estimate the consumption of white pita for the different categories of the population. Adult men were the highest consumers followed by teenagers (10–14 years old) and women and children (6–9 years old): daily white pitta consumption medians were 282, 143, 126, and 71 g/day, respectively (Lebbos *et al*., 2018).

Bou-Mitri *et al.,* (2020) reported that the consumption of bread varied between 126.5 g/d and 146.2 g/d. Most of the Lebanese purchase (72.5%) and consume (69.2%) pitta bread. Moreover, 37% of them prefer white bread, 16.8% whole grain, 14.7% brown and 10.7% multi-cereal (Bou-Mitri *et al.*, 2020).

Recently, the Food and Agriculture Organization (FAO) (2019) reported that in Lebanon, the cereal import requirements, mainly wheat for human consumption and maize to feed livestock and poultry, are forecasted at an average level of 2 million tons, similarly to the previous year. The current import requirement is almost double the quantity that was needed in year 2011 (FAO, 2019).

2.7. Contaminants in Bread

Many contaminants can decrease the safety and quality of breads and bakery products including molds, heavy metals, toxins, pesticides, preservatives, packaging material, gas used in baking, environmental pollutants and contaminated raw material (Viegas *et al.,* 2018).

Moreover, studies have shown that bakery products could be subject to physical, chemical and microbiological spoilage. Many industrially produced baked goods emerge from the baking process with a surface that is essentially sterile but post bake handling can quickly lead to fungal, microbial surface contamination as a result of exposure to airborne contaminants as well as equipment contact (Saranj and Geetha, 2012). Further studies have also reported heavy metals contamination of baked goods and bread (Nasreddine *et al.,* 2007; Bou Khouzam *et al.,* 2012; Lebbos *et al.,* 2018).

2.8. Heavy metals definition

Heavy metals are naturally occurring elements that have a high atomic weight and a density at least 5 times greater than that of water. Their multiple industrial, domestic, agricultural, medical and technological applications have led to their wide distribution in the environment; raising concerns over their potential effects on human health and the environment. Their toxicity depends on several factors including the dose, route of exposure, and chemical species, as well as the age, gender, genetics, and nutritional status of exposed individuals. Because of their high degree of toxicity, arsenic, cadmium, chromium, lead, and mercury rank among the priority metals that are of public health significance. These metallic elements are considered systemic toxicants and are known to induce multiple organ damage, even at lower levels of exposure (Tchounwou *et al.*, 2012). They are also classified as human carcinogens (known or probable) according to the U.S. Environmental Protection Agency, and the International Agency for Research on Cancer (Tchounwou *et al.,* 2012).

2.9. Source of contaminants

Various sources of contaminations by heavy metals have been reported in the literature including water contamination, soil pollution, baking fuel contamination, handling and processing, baking utensils and environmental pollutants (Rather *et al.,* 2017).

The most important source of pollution is agricultural soil pollution with heavy metals where they are transferred to roots of agricultural plants such as wheat, rice, and other cereals. Another way of heavy metal contamination is preparation and processing operations in the consumed breads. During the processing and preparation of breads, some materials are added such as water, salt, yeast, and baking soda, which can contaminate the breads with heavy metals. In addition, bread baking in the metal trays, contaminated bakery ovens due to lack of surveillance, and fuels contaminated with heavy metals are other contamination parameters. Secondary pollution with heavy metals includes packaging and air pollution (Naghipour *et al.,* 2014).

Moreover, contamination of bread could derive from the production e.g., old metal containers used during bread processing. The regional variation on elemental composition of bread is due not only to the impact of manufacturing process (contamination by and/or loss of certain elements), but it is also reflected by the composition of the main ingredients of bread and notably the wheat grains (Bou Khouzam *et al.*, 2012).

2.9.1. *Water contamination*

Sipra *et al.* (2013) reported that the wheat irrigation with municipal wastewater in Pakistan resulted with high levels of heavy metals including zinc (Zn)> iron (Fe)> manganese (Mn)> cupper (Cu)> lead (Pb)> cadmium (Cd)>nickel (Ni)> chromium (Cr) in wheat plants with concentrations above recommended dietary limits (Sipra *et al.*, 2013).

In Pakistan, higher heavy metals levels (Cd, Cr and Ni) were also reported in wheat crop when contaminated water was used for irrigation as compared to crops irrigated with non-contaminated water (Khan *et al.*, 2015).

2.9.2. *Fuel contamination*

Ahmed *et al*. (2000) reported the influence of fuel type used to bake bread on the spectrum and concentrations of some polycyclic aromatic hydrocarbons (PAHs) and assessed heavy metals in baked bread. Samples collected from "mazot", solar and solid waste operated bakeries have had a wide spectrum of PAHs, in comparison to that detected in bread samples collected from electricity operated bakeries. Pb had the highest concentrations in the four groups of bread samples, followed by Ni, while the concentrations of Zn and Cd were the least (Ahmed *et al.*, 2000).

2.10. Health risks of heavy metals

The Codex in reference with JECFA amended in year 2018, issued that the maximum level of Cd in wheat and of Pb in cereal grains should be 0.2 mg/kg. However, maximum levels were not indicated for other heavy metals in bread (CX 193-1995).

The consumption of food contaminated with heavy metals above dietary limits could lead to various health risks. Studies have shown that excess Cd causes renal dysfunction, obstructive lung disease, lung cancer, damage to human respiratory system. Excess Pb leads to acute or chronic damage to the nervous system of humans and other behavioral disorders and Al at elevated levels can lead to loss of memory, severe trembling, and damage to central nervous system (Khan *et al.*, 2015). Excess Ni has notable human health toxicity effects identified from human and/or animal studies include respiratory cancer, non-cancer toxicity effects following inhalation, dermatitis, and reproductive effects (Buxton *et al*., 2019). In addition to skin cancer, long-term exposure to As may also cause cancers of the bladder and lungs, developmental effects, diabetes, pulmonary disease, and cardiovascular disease. The International Agency for Research on Cancer (IARC) has classified As and As compounds as carcinogenic to humans and has also stated that arsenic in drinking water is carcinogenic to humans (WHO, 2018).

A comprehensive analysis of published data indicates that heavy metals such as As, Cd, Cr, Pb, and Hg, occur naturally. However, anthropogenic activities contribute significantly to environmental contamination. These metals are systemic toxicants known to induce adverse health effects in humans, including cardiovascular diseases, developmental abnormalities, neurologic and neurobehavioral disorders, diabetes, hearing loss, hematologic and immunologic disorders, and various types of cancer. The main pathways of exposure include ingestion, inhalation, and dermal contact (Tchounwou *et al.*, 2012).

2.11. Background

The assessment of heavy metals in bread was reported in several studies worldwide:

Pb and Cd levels in 60 samples of bread and the exposure via bread consumption were assessed in Nigeria. The study findings showed that Pb content ranged between 0.01 and 0.071 mg/kg with 100% of bread samples violated the permissible limit. On the other hand, Cd content ranged between 0.01 and 0.03 mg/kg with all bread samples being below the permissible limits. Consumption of Pb 0.03-0.23 µg/kg.bw/day and Cd 0.033-0.36 µg/kg.bw/day were both under the tolerable daily intake (TDI) (Udowelle *et al.,* 2017)

The content in heavy metals of 40 samples in different types of breads in Iran showed that the mean content of Cd and Pb were 0.73 and 0.07 mg/kg in barbari bread and 0.09 and 0.9 mg/kg in baguette, respectively. Daily intake of the studied heavy metals was Pb 2.1-5.8 µg/kg, Cd 0.1-0.8 µg/kg (Naghipour *et al.,* 2014).

A study conducted in Nigeria in 2014, aimed to evaluate the levels of bromate (Br) and trace metals in the bread loaves sold within Ile-Ife and its surroundings. Br levels in the analyzed bread samples ranged from 2.051 ± 0.011 µg/g to 66.224 ± 0.014 µg/g while the trace metal levels were of the order: 0.03–0.10 µg/g Cobalt (Co)< 0.03–0.10 µg/g Pb <0.23–0.46 µg/g Cu <2.23–6.63 µg/g Zn < 25.83–75.53 µg/g Mn. The levels of Mn found in the bread samples far exceeded the daily intake of 2–3 mg/day recommended by WHO. The bread samples contained both essential and toxic trace metals to levels that could threaten the health of consumers (Oyekunle *et al.,* 2014).

A study done in Spain in 2013 aimed to determine the content of 12 metals in 50 samples of wheat flour coming from a wheat flour industry. Results showed that wheat flour type A (bread flour made of 3 types of wheat: 1 from Canada and 2 from France, had a slight higher content of Cd

(0.027 ± 0.002 mg/kg), in contrast to Pb, whose medium content is higher (0.056 ± 0.045 mg/kg) in wheat flour type B (medium strength wheat flour made of another mixing of wheat flour from Sweden, France, Canada and Germany). In both cases levels do not exceed the maximum permitted by Regulation (CE) 1881/2006 that fixes a limit of some contaminants in food, specifically in cereals like wheat. The maximum limit permitted for Cd and Pb is 0.20 mg/kg fresh weight (Tejera *et al.*, 2013).

2.12. Heavy metal contamination in Lebanese bakery products

Several studies assessed heavy metals contamination in Lebanese bread (Table 2). Lebbos *et al.*, (2018) assessed the level of the following elements As, Cd, Pb, Hg, Ni, Co in three Lebanese pitta bread brands. Results showed that the content of all assessed heavy metals in bread was below the Lebanese standard limit. There were no safety concerns for Hg, Cd, Cr or Co (except the 95th percentile of 6–9 years old). An excess of the Ni (TDI) was observed for the most exposed populations. Moreover, the population between 6-9 years old was exposed to heavy metals above toxicological reference values. The gap of this study is that only three brands of bread were studied which does not represent the wide collection of brands present in the country (Lebbos *et al*., 2018).

In a study done in five Lebanese governorates (Grand Beirut, South, North, Mount Lebanon, and Beka'a) in 2012, to determine the element composition of three types of the most popular Lebanese bread, including white Arabic bread, brown or whole wheat bread, and Saj. High concentrations of As and Hg were observed in Beirut. Also, bread from North Lebanon showed much higher concentrations of Al, Fe, Cu, Cr, Ni, Pb, Si, and Zn as compared to bread from other regions, possibly due to some kind of contamination (Bou Khouzam *et al.*, 2012).

Nasreddine *et al.* (2007) reported that among all the assessed elements and food products in Lebanon, the average concentrations of Pb in the analyzed food groups ranged between 2-35 mg/kg and was the highest in cereal-based products such as breads, pastries and pizzas. The average concentrations of Cd in the food samples varied between 1 and 17 mg/kg. The food products presenting the highest concentrations of Cd were cereal-based products. The analytical results showed that 86, 65 and 100% of the data obtained for Pb, Cd and Hg, respectively, were above the limits of quantification (LOQ). The gap of this study is that it dated more than ten years and does not comply with the most current criteria in Lebanon (Nasreddine *et al.,* 2007).

Considering the levels of heavy metals in Lebanese pitta bread compared to the safe limits set by CODEX and LIBNOR. It was noticed that heavy metals levels above the limits were Ni (1.292 mg/kg) and Pb (0.260 mg/kg). Other heavy metals could be higher however there was no standards regulations set for them yet.

The following graph represents the different levels of heavy metals according to the following studies previously done in Lebanon (Lebbos *et al*., 2019; Bou Khouzam *et al*., 2012; Nasreddine *et al*., 2007) (Fig. 5).

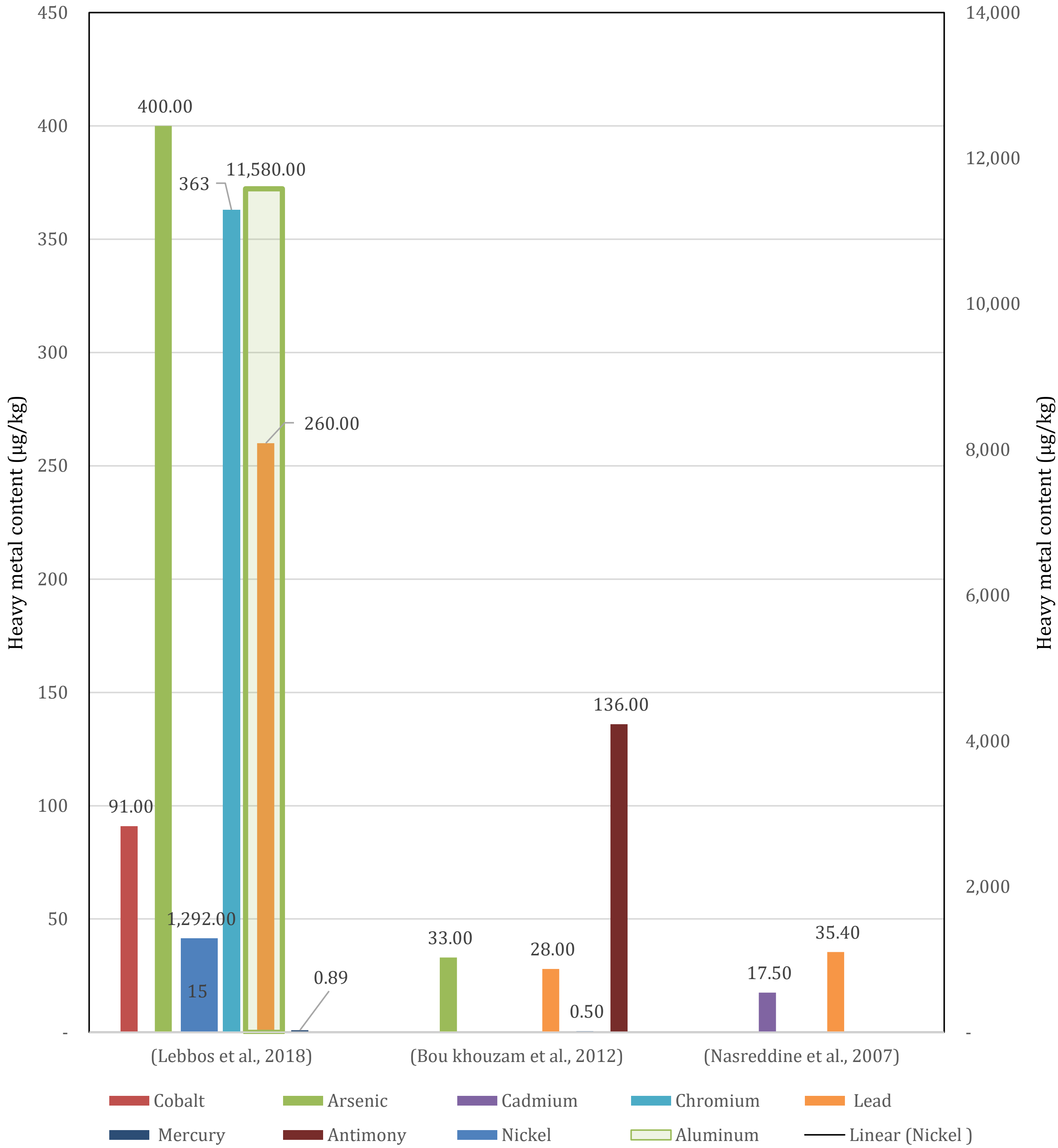

Figure 3- Heavy Metals Content in Lebanese White Pitta Bread

2.13. Daily intake of heavy metals from bread and bakery products in Lebanon

The daily intake of toxic elements in Lebanon was assessed by various studies. Bou Khouzam *et al.,* (2012) reported that the intake of As, Cd and Pb was 4.5 μg/day, 2.1 μg/day, and 3.8 μg/day, respectively. All of the toxic element values were below their corresponding appropriate provisional tolerable weekly intake (PTWI) (0.002 mg/day As, 260 μg/day for Pb, 72.8 μg/day for Cd) (Bou Kouzam *et al.,* 2012).

Nasreddine *et al*., (2007) assessed the average dietary intakes of Pb, Cd and Hg and they represented 7, 17 and 5.6%, respectively, of the PTWI. The estimated intakes of Pb are compared with those from other countries. It was noticed that in 2006, Lebanon had lower amounts of heavy metals compared to several countries like China, Egypt and Spain whereas it has higher levels compared to Sweden (Table 1).

These authors also reported that cereal products contributed to more than 45.3% of the exposure of the Lebanese urban population to Pb followed by drinking water 16.22 %, vegetables and potatoes 17.63%, fruits and fruit juices 9.9%, dairy products 6.4%, meat and poultry 4.6% (Fig 6) (Nasreddine *et al.,* 2007).

A recent study also assessed the consumption of metal contaminants via white pitta bread, in Lebanon. Based on 800 adults and 200 children responding from 5 different regions, the results showed that the content of heavy metals in bread was below the Lebanese standard limit, however, the population between 6 and 9 years old were exposed to heavy metals above toxicological reference values (Lebbos *et al.,* 2018).

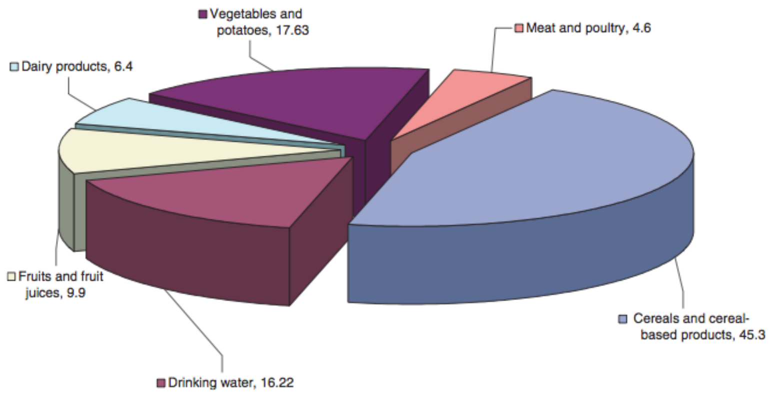

Figure 4- Contribution of the Different Food Groups to the Average Daily Exposure to Lead (Nasreddine *et al.*, 2006)

Table 1: Comparison of the Average Dietary Exposure to Lead, Cadmium and Mercury as Determined by Different Countries (Nasreddine et al., 2006)

Country	Type of study	Lead ($\mu g\ day^{-1}$)	Cadmium ($\mu g\ day^{-1}$)	Mercury ($\mu g\ day^{-1}$)	References
Australia	TDS[1], 1996; adult man (25–34 years)	32.1	23.6	10.7	Hardy (1998)
Canada	TDS, 1986–88; average individual; water and alcohol included	23	13	–	Dabeka and McKenzie (1995)
China	TDS, 1990; men (18–45 years); water and alcohol included	86.3	13.8	10.3	Chen and Gao (1993)
China	TDS, 2000; average individual; water and alcohol included	81.2	21.2	6.9	Gao (2004)
Cuba	–	514	–	–	Moy (1999)
Czech Republic	TDS, 1997; average individual	21.9	13.7	0.64	Ruprich (1998)
Denmark	1988–92	27	17	–	NFAD (1995)
Egypt	TDS; average individual; water and alcohol not included	242	19.7	76.4	Saleh et al. (1998)
Finland	1986	15.8	11.3	1.9	FAO/UNEP/WHO (1991)
France	TDS, 2000–03; adult (>15 years); water and alcohol included	18	2.7	9.7	Leblanc et al. (2004)
Guatemala	1986	254	29	10.8	FAO/UNEP/WHO (1991)
Italy	–	280	–	–	Kumpulainen (1996)
Japan	TDS, 1988; adults; water and alcohol included	84	–		FAO/UNEP/WHO (1991)
Japan	TDS, 1984–93; adults	–	32 (1991), 35 (1992)	4.3 (1991), 9.9 (1992)	Tsuda et al. (1995)
Spain	TDS, 1981; water not included, alcohol included	37–521	16–29	4–8	Cuadrado et al. (1995)
Spain	TDS, 1992–95; adults (25–60 years); water not included; alcohol included	28.2	10.7	18.5	Jalón et al. (1997)
Sweden	TDS, 1987; average individual; water not included; alcohol included	17	12	1.8	Becker and Kumpulainen (1991)
The Netherlands	TDS, 1984–86; men (18 years); water included; alcohol not included	32	21	0.7	Van Dokkum et al. (1989)
UK	TDS, 1994; average individual, water and alcohol included	24	14	4	Ysart et al. (1999)
USA	TDS, 1991–96; men (25–30 years); water and alcohol included	4.2–18.8	11.5–14.2	1.25	Egan et al. (2002)
New Zealand	TDS, 1997–98; men (19–24 years); water and alcohol included	12.0	24	7.3	Vannoort et al. (2000)
Lebanon (Beirut)	TDS, 2004; adults (25–54 years); water included, alcohol not included	18.5	12.3	3.0	Present study

1 Total Dietary Study

2.14. Rational

The data on heavy metal contamination in food products and Lebanese exposure are not conclusive due to various limitations. Accordingly, this study aimed to assess the exposure of all the Lebanese population to heavy metals via the consumption of white pitta bread using the studies published between 2000 till April 2020. Moreover, trends of the exposure versus time were assessed to identify possible causes of these contaminations.

This study estimated the exposure of the Lebanese to heavy metals using a systematic analysis, thus it assessed the risks of these contaminations. This study is of interest to all the Lebanese consumers, manufacturers and policy makers. It permitted to define the sources of exposure, populations at risk, and therefore recommendations could be drawn.

CHAPTER 3 MANUSCRIPT

3.1. Introduction

Bread is a Lebanese staple food that is found on each authentic Lebanese table. It is used in hospitals, nursing homes, fed to kids, elderly and pregnant women. A wide variety of bread is available including Lebanese pitta bread (or khobz), tanour, markouk, baguette among others (Quail *et al.*, 2016). It is most commonly made with flour, water, yeast, salt and sugar. It could be prepared with white flour, whole-wheat flour, brown flour, and bran depending on the bread type (LIBNOR NL 240, 2002).

Raw ingredients could be produced locally or imported from various origins. The consumption of these breads in Lebanon constitutes a major part of the Lebanese diet (BLOMINVEST Bank, 2016). According to Nasreddine et al. (2006), cereal consumption among Lebanese population was 324.5 g/day providing 35.0% of daily energy intake, with bread being the most highly consumed 146.2 g/day. Numerous studies showed that the Lebanese highly consume bakery products and mostly white pitta bread, Bou-Mitri *et al*. (2020); Lebbos *et al*. (2018); Barakat, (2015); Nasreddine *et al*. (2006).

Cereal-based products could be polluted with various contaminants including heavy metals, pesticides, toxins and microorganisms due to the use of contaminated raw ingredients, environmental pollutants and unsafe process and packaging (Kim *et al*., 2012). These hazardous substances contained in foods are known to not only have strong direct toxicity in the human body but also chronically disturb the endocrine system. Accordingly, studies assessing amounts of hazardous substance intake via food intake and related degrees of hazards have been conducted throughout the world, and individual countries have prepared food safety control institutions to regulate these hazards (Kim *et al*., 2012).

Mercury is used in fluorescent lamps, batteries, and measuring instruments. Organic mercury has been linked to central nervous system toxicity, immunotoxicity, genetic toxicity, reproductive toxicity, teratogenicity, and nephrotoxicity (US EPA, 2020). On another hand, Pb is present in water, air, paint, dust and toys. Humans poisoned by it show symptoms such as headaches, sleep disturbances, and central nervous system disorders (Wani *et al*., 2015). Cd exists in the air, water, soil, and foods, and if poisoned by it, humans can develop severe fractures (Kim *et al*., 2012). In 2010 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) proposed that indicating Cd monthly tolerable intake would be more appropriate than indicating it weekly considering Cd's long half-life. Thus, Cd intake is now specified according to tolerable monthly intake levels (25 μg/kg.bw/month) (JECFA, 2010b).

As per Al, it exists in soil, water, animal and plant, and high exposure can lead to severe conditions including hypercalcemia, anemia, and progressive encephalopathy (Krewski *et al*., 2007). JECFA judged that Al compounds could affect the nervous and reproductive systems at levels lower than the level that had been reported earlier (7 mg/kg.bw) and readjusted the PTWI of Al to 1 mg/kg.bw (FAO/WHO JECFA). Hazard assessments are important tools that can be utilized in estimating hazards to human health and safety and establishing related countermeasures to reinforce food safety (Kim *et al.,* 2012).

International scientific committees such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR), regional scientific committees such as those of the European Union, and national regulatory agencies generally use the safety factor approach for establishing acceptable or tolerable intakes of substances that exhibit thresholds of toxicity. The acceptable daily intake (ADI) is used widely to describe "safe" levels of intake; other terms that are used are the reference dose (RfD) and tolerable intakes that are expressed on either a daily (TDI or tolerable daily intake) or weekly basis. JECFA uses the term PTWI, or provisional tolerable daily intake, or BMDL (Benchmark Dose Lower Limit) for contaminants that may accumulate in the body. The weekly designation is used to stress the importance of limiting intake over a period of time for such substances. (Herman and Younes, 1999)

In Lebanon, assessment of heavy metal exposure was observed in three studies. The findings showed that bread contributed to the main exposure. Other studies on heavy metal levels in bread and exposure were assessed; however, they contain several gaps. Lebbos *et al.* (2019) assessed only three bread brands, which might not be a representative number considering the wide variety of brands available in Lebanon. Bou Khouzam *et al.* (2012) assessed 60 samples of three toxic elements (As, Cd, Pb), which do not prove the overall safety of the product since there might be other toxic heavy metals present. Nasreddine *et al.* (2005) assessed the exposure of urban Lebanese population (N=444) in spring and summer seasons only which could lead to under or overestimation of intake. Also, the survey included only adult people living in the district of Beirut, thus preventing the extrapolation of the results to the whole country. Therefore, there is a need to have a new horizon and set grounds for future studies based on the most toxic and health concerning issues highlighted in the present study.

The objective of this report was to assess the Lebanese exposure to heavy metals via the consumption of white pitta bread and to measure the health risks of its consumption according to the safe limits.

3.2. Material & Methods

In the present study the European Food Safety Authority guide (EFSA, 2010) and the PRISMA protocol (Moher *et al.,* 2009) for reporting, were adopted (Fig 7). Literature search of PubMed database was conducted from 2000 to April 2020 using the Boolean script. Furthermore, Google Scholar database were also reviewed to capture additional published papers. Different terms and combinations were included in the search: bread, pitta bread, heavy metals, Cd, Pb, Hg, As, Al exposure, bread consumption, Lebanese food consumption, total diet, basket analysis, risk assessment.

Data were compiled and information about Lebanese bread consumption level and heavy metals content in bread were extracted and used for the calculation of exposure. Several evaluation criteria were adopted to divide the data and interpret the results (gender, age, region, season and year).

Inclusion and exclusion criteria used were as follow: all published papers in peer-reviewed journals in English, French and Arabic were included. Papers with primary data on Pb, Hg, As, and Cd concentration in bread and pitta bread; data on Pb, Hg, As, and Cd human exposure or dietary intake in bread and pitta bread; data on risk characterization, including hazard quotient or hazard index of Pb, As, Hg, and Cd in bread and pitta bread; and relevant toxicological research output was included in this review.

Studies done in Lebanon from 2000 to 2020 were collected. Four studies previously assessed the consumption of Lebanese to white pitta bread (Bou-Mitri *et al*., 2020; Lebbos *et al*., 2018; Barakat, 2015; Nasreddine *et al*., 2006). Moreover, three studies done in Lebanon concerning heavy metals content in white pitta bread were Lebbos *et al*. (2018), Bou Khouzam *et al*. (2012) and Nasreddine *et al.* (2007).

The concentration, intake, and risk data were extracted, described, summarized, tabulated, and synthesized. To ensure uniformity of the analysis, units were converted, the assessment of intake technique were specified and the technique for analysis were also specified and tabulated. The research studies collected contain different evaluation criteria; technique used to assess heavy metal content, population age, region, type of bread, specific brand, date, seasons and sample size.

Following the JECFA guidelines, the exposure of Lebanese to heavy metals from the consumption of white pitta bread was calculated. Dietary exposure assessment combines food consumption data and the concentration of the food additive in food. The resulting dietary exposure estimate were then compared with the appropriate reference value for the heavy metal if available, as part of the risk characterization. Three elements were taken into account in assessing the dietary exposure to heavy metals: (1) the concentration of the heavy metal in food; (2) the amount of food consumed; and (3) the average body weight of the population (kg).

The general equation for dietary exposure is:

$$\text{Dietary exposure} = \sum \frac{\text{Concentration of heavy metal } (\mu g/kg) \times \text{Food consumption } (g/d)}{\text{Body weight } (kg)}$$

(CODEX, CAC/GL 3-1989, 2014)

The levels of heavy metals were reported in microgram per kilogram (µg/kg), the consumption level was reported in gram per day (g/d), and the exposure was calculated in microgram per kilogram body weight (µg/kg.bw). For the purposes of dietary exposure estimates, an average body weight of 60 kg for adults and 15 kg for children were assumed for most populations in the world

(CODEX CAC/GL 3-1989). The weight of teenagers was estimated to be 40 kg. A weight of 72.8 kg was used which was indicated in the study of Nasreddine *et al.* (2006) as the mean weight of the sample.

According to essential elements with nutritional value, risk assessment can be calculated by insufficient intake or intake excess. The present study focused only on excess intake using toxicological reference points or safety levels for each element case by case in order to determine safety concerns due to excess intake related to bread consumption in Lebanon.

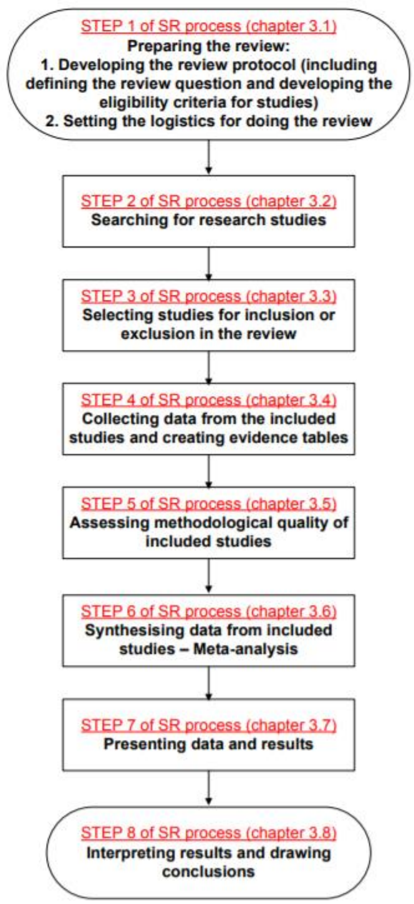

Figure 5- Core Steps for Performing a Systematic Review (Adapted from The Cochrane Handbook for Systematic Reviews of Interventions, Higgins and Green (editors), 2009).

3.3. Results

3.3.1 Consumption of white pitta bread

Bread consumption in Lebanon was assessed through four papers including Bou-Mitri *et al*. (2020), Lebbos *et al*. (2018), Barakat (2015) and Nasreddine *et al*. (2006). The overall studies showed that white pitta was the main type of bread consumed among Lebanese. The pitta consumption varied according to region, age, and gender (Table 2). All the studies showed that men's consumption was higher than women's, with values raging between 113.5 g/d (Nasreddine *et al*., 2006) - 135.9 g/d (Barakat, 2015) - 282 g/d (Lebbos *et al*., 2018) as compared to females 44.3 g/d (Barakat, 2015) - 167 g/d (Lebbos et al., 2018) - 182.7 g/d (Nasreddine *et al*., 2006) Furthermore, Lebbos *et al*. (2018) was the only study reporting values for children and teenagers. Teenagers consume on average 171 g/d, and children consume the least amount of bread between all age groups 113 g/d. However, teenagers and men can reach a maximum consumption up to 1000 g/d compared to women and children that can reach a maximum amount of 750 g/d. In addition, Lebanese living in South Lebanon and Nabatieh regions had the highest consumption of bread 355 g/d (Lebbos *et al*., 2018), compared to those living in the North of Lebanon 150 g/d (Lebbos et al., 2018) - 158.6 g/d (Barakat, 2015), Mount-Lebanon 150 g/d (Lebbos *et al*., 2018) - 72.8 g/d (Barakat, 2015), Bekaa 150 g/d (Lebbos *et al*., 2018). Data also showed that there were no significant differences between the consumption of bread in North, Mount Lebanon, and Bekaa regions according to Lebbos et al. (2018). The lowest consumption was observed in Beirut area 135 g/day (Lebbos *et al*., 2018) - 86.45 g/d (Barakat, 2015). In addition, data from 2006 to year 2020 showed an increase of bread consumption throughout the years from 112 g/d (Nasreddine *et al*., 2006) to 355 g/d (Lebbos *et al*., 2018).

Table 2: Lebanese Consumption of Bread According to Previous Studies Done

Source	Product	Population Characteristics		Sample Size: N	Consumption (g/d)		
					Min	Av. (mean)	Max
Bou-Mitri et al., 2020	Bread	Region	Mount-Lebanon	290	- [1]	126.5 [2]	-
		Gender	65% were females				
		Age	34				
Lebbos et al., 2018	White Pitta Bread	Age Group	Children	91	63	113	750
			Teenagers	113	31	171	1000
			Women	258	31	167	750
			Men	300	31	282	1000
			Young people and adults	762	31	206	1000
		Region	South Lebanon and Nabatieh	-	-	355	-
			North Lebanon, Mount Lebanon, Bekaa	-	-	150	-
			Beirut	-	-	135	-
Barakat, 2015	White Pitta Bread	Gender	Male	190	-	135.9	-
			Female	203	-	44.33	-
		Region	Beirut	-	-	86.45	-
			Mount-Lebanon	-	-	72.8	-
			North Lebanon	-	-	158.6	-
Nasreddine et al., 2006	Traditional Bread	Age	25-54 years	444	-	146.2	-
		Type	Urban Population				
		Gender	Female	210	-	182.7	-
			Male	234	-	113.5	-
		Male	25-34 years	210	-	192.6	-
			35-44 years		-	185.1	-
			45-54 years		-	166.2	-
		Female	25-34 years	234	-	112.8	-
			35-44 years		-	111.8	-
			45-54 years		-	116.6	-

[1] Data Not Available

[2] Personal calculations based on (Bou-Mitri *et al.*, 2020) taking into account ¼ loaf=30g, ½ loaf=60g, 1 loaf=120g, 2 loaves=240g; Mean Consumption =48 210 g/381=126.5g/day

3.3.2 Heavy metal content in bread

Pb values increased significantly between year 2007 and 2019 from 35.4 µg/kg (Nasreddine *et al*., 2007) to 260 µg/kg (Lebbos *et al*., 2018) respectively. Ni value according to Lebbos *et al. (*2019) was (1292 µg/kg) however; it was not assessed in the other two studies. Cd and Hg had almost similar values in the three present studies. As was significantly higher in Lebbos *et al.* (2019) (400 µg/kg) than in Bou Khouzam *et al.* (2012), (33 µg/kg). The level of Al was detected in Bou Khouzam study (11 580 µg/kg) while it was not analyzed in the other two studies. Furthermore, As and Pb in Bou Khouzam *et al.* (2012) had a higher concentration during the dry season compared to Cd, Al, and Sb, which had a higher concentration during the wet season (Table 3).

Table 3: Heavy Metal Content in Lebanese Pitta Bread from Previous Studies Done in Lebanon

Heavy metal	Level (µg/kg)			
	Lebbos et al., 2018	Bou Khouzam et al., 2012		Nasreddine et al., 2007
		Wet season	Dry season	
Nickel	1292	- [1]	-	-
Cobalt	91	-	-	-
Arsenic	400	9	33	-
Cadmium	<15	24	15	17.5
Chromium	363	-	-	-
Lead	260	18	28	35.4
Mercury	0.89	0.5	<LOD[2]	-
Aluminum	-	11580	5880	-
Antimony	-	136	63.64	-

[1] Data Not Available

[2] Limit of Detection

3.3.3 Exposure level of Lebanese to heavy metals through consumption of white pitta bread

Minimum, average and maximum exposure calculations were done using the minimum, average and maximum daily consumption of bread, the level of heavy metals and the individual weight were presented in (Table 4.). The calculations showed that maximum daily exposure level according to different gender and age groups for Ni were as follow (64.6 µg/kg.bw children>32.3 µg/kg.bw teenagers>21.533 µg/kg.bw men>16.150 µg/kg.bw women) and were higher than the reference value (TDI: 2.8 µg/kg.bw) and for As as follow (20 µg/kg.bw children >10 µg/kg.bw teenagers >6.667 µg/kg.bw men > 5 µg/kg.bw women) and were higher than the reference value (BMDL 0.5-3 µg/kg.bw/day). The average daily exposure levels for Ni by age and gender were as follow (9.733 µg/kg.bw children >5.523 µg/kg.bw teenagers >6.072 µg/kg.bw men >3.596 µg/kg.bw women) and by region (7.644 µg/kg.bw South Lebanon and Nabatieh >3.230 µg/kg.bw North Lebanon, Mount Lebanon and Bekaa >Beirut 2.907 µg/kg.bw) and were higher than the reference value (TDI 2.8 µg/kg.bw), and for As in children (3.013 µg/kg.bw) and was higher than the reference value (BMDL 0.5-3 µg/kg.bw/day). Other heavy metals exposure like Co, Cd, Cr, Pb, Hg and Al were not above the reference values for any of the calculations reported.

Table 4: Exposure level of Lebanese population to heavy metals through white pitta bread consumption

Source	Population	Daily consumption of bread (g/d)			Heavy metal	Seasons	Level of heavy metals in bread (μg/kg)	Body weight (kg)	Daily Exposure level (μg/kg.bw)			Weekly Exposure level (μg/kg.bw)		
		Min	Av.	Max					Min	Av.	Max	Min	Av.	Max
Lebbos et al., 2018	Children (6-9 yrs)	63	113	750	Nickel		1292	15	5.426	9.733	64.600	37.985	68.131	452.200
		63	113	750	Cobalt		91	15	0.382	0.686	4.550	2.675	4.799	31.850
		63	113	750	Arsenic		400	15	1.680	3.013	20.000	11.760	21.093	140.000
		63	113	750	Cadmium		15	15	0.063	0.113	0.750	0.441	0.791	5.250
		63	113	750	Chromium		363	15	1.525	2.735	18.150	10.672	19.142	127.050
		63	113	750	Lead		260	15	1.092	1.959	13.000	7.644	13.711	91.000
		63	113	750	Mercury		0.89	15	0.004	0.007	0.045	0.026	0.047	0.312
	Teenagers (10-14 yrs)	31	171	1000	Nickel		1292	40	1.001	5.523	32.300	7.009	38.663	226.100
		31	171	1000	Cobalt		91	40	0.071	0.389	2.275	0.494	2.723	15.925
		31	171	1000	Arsenic		400	40	0.310	1.710	10.000	2.170	11.970	70.000
		31	171	1000	Cadmium		15	40	0.012	0.064	0.375	0.081	0.449	2.625
		31	171	1000	Chromium		363	40	0.281	1.552	9.075	1.969	10.863	63.525
		31	171	1000	Lead		260	40	0.202	1.112	6.500	1.411	7.781	45.500
		31	171	1000	Mercury		0.89	40	0.001	0.004	0.022	0.005	0.027	0.156
	Women	31	167	750	Nickel		1292	60	0.668	3.596	16.150	4.673	25.172	113.050
		31	167	750	Cobalt		91	60	0.047	0.253	1.138	0.329	1.773	7.963
		31	167	750	Arsenic		400	60	0.207	1.113	5.000	1.447	7.793	35.000
		31	167	750	Cadmium		15	60	0.008	0.042	0.188	0.054	0.292	1.313
		31	167	750	Chromium		363	60	0.188	1.010	4.538	1.313	7.072	31.763
		31	167	750	Lead		260	60	0.134	0.724	3.250	0.940	5.066	22.750
		31	167	750	Mercury		0.89	60	0.000	0.002	0.011	0.003	0.017	0.078
	Men	31	282	1000	Nickel		1292	60	0.668	6.072	21.533	4.673	42.507	150.733
		31	282	1000	Cobalt		91	60	0.047	0.428	1.517	0.329	2.994	10.617
		31	282	1000	Arsenic		400	60	0.207	1.880	6.667	1.447	13.160	46.667
		31	282	1000	Cadmium		15	60	0.008	0.071	0.250	0.054	0.494	1.750
		31	282	1000	Chromium		363	60	0.188	1.706	6.050	1.313	11.943	42.350
		31	282	1000	Lead		260	60	0.134	1.222	4.333	0.940	8.554	30.333
		31	282	1000	Mercury		0.89	60	0.000	0.004	0.015	0.003	0.029	0.104
	Young people and adults	31	206	1000	Nickel		1292	60	0.668	4.436	21.533	4.673	31.051	150.733
		31	206	1000	Cobalt		91	60	0.047	0.312	1.517	0.329	2.187	10.617
		31	206	1000	Arsenic		400	60	0.207	1.373	6.667	1.447	9.613	46.667
		31	206	1000	Cadmium		15	60	0.008	0.052	0.250	0.054	0.361	1.750
		31	206	1000	Chromium		363	60	0.188	1.246	6.050	1.313	8.724	42.350
		31	206	1000	Lead		260	60	0.134	0.893	4.333	0.940	6.249	30.333
		31	206	1000	Mercury		0.89	60	0.000	0.003	0.015	0.003	0.021	0.104

Source	Population	Daily consumption of bread (g/d)	Heavy metal	Seasons	Level of heavy metals in bread (µg/kg)	Body weight (kg)	Daily Exposure level (µg/kg.bw)	Weekly Exposure level (µg/kg.bw)
Lebbos et al., 2018	South Lebanon and Nabatieh	355	Nickel		1292	60	7.644	53.510
		355	Cobalt		91	60	0.538	3.769
		355	Arsenic		400	60	2.367	16.567
		355	Cadmium		15	60	0.089	0.621
		355	Chromium		363	60	2.148	15.034
		355	Lead		260	60	1.538	10.768
		355	Mercury		0.89	60	0.005	0.037
	North Lebanon, Mount Lebanon, Bekaa	150	Nickel		1292	60	3.230	22.610
		150	Cobalt		91	60	0.228	1.593
		150	Arsenic		400	60	1.000	7.000
		150	Cadmium		15	60	0.038	0.263
		150	Chromium		363	60	0.908	6.353
		150	Lead		260	60	0.650	4.550
		150	Mercury		0.89	60	0.002	0.016
	Beirut	135	Nickel		1292	60	2.907	20.349
		135	Cobalt		91	60	0.205	1.433
		135	Arsenic		400	60	0.900	6.300
		135	Cadmium		15	60	0.034	0.236
		135	Chromium		363	60	0.817	5.717
		135	Lead		260	60	0.585	4.095
		135	Mercury		0.89	60	0.002	0.014

Source	Population	Daily consumption of bread (g/d)	Heavy metal	Seasons	Level of heavy metals in bread (µg/kg)	Body weight (kg)	Daily Exposure level (µg/kg.bw)	Weekly Exposure level (µg/kg.bw)
Bou Khouzam et al., 2012; Barakat. N, 2015	Male	135.9	Arsenic	wet	9	60	0.020	0.143
		135.9		dry	33	60	0.075	0.523
		135.9	Cadmium	wet	24	60	0.054	0.381
		135.9		dry	15	60	0.034	0.238
		135.9	Lead	wet	18	60	0.041	0.285
		135.9		dry	28	60	0.063	0.444
		135.9	Aluminum	wet	11580	60	26.229	183.601
		135.9		dry	5880	60	13.318	93.227
		135.9	Antimony	wet	136	60	0.308	2.156
		135.9		dry	63.64	60	0.144	1.009
		135.9	Mercury	wet	0.5	60	0.001	0.008
		135.9		dry	0	60	0.000	0.000
	Female	44.33	Arsenic	wet	9	60	0.007	0.047
		44.33		dry	33	60	0.024	0.171
		44.33	Cadmium	wet	24	60	0.018	0.124
		44.33		dry	15	60	0.011	0.078
		44.33	Lead	wet	18	60	0.013	0.093
		44.33		dry	28	60	0.021	0.145
		44.33	Aluminum	wet	11580	60	8.556	59.890
		44.33		dry	5880	60	4.344	30.410
		44.33	Antimony	wet	136	60	0.100	0.703
		44.33		dry	63.64	60	0.047	0.329
		44.33	Mercury	wet	0.5	60	0.000	0.003
		44.33		dry	0	60	0.000	0.000

Source	Population	Daily consumption of bread (g/d)	Heavy metal	Seasons	Level of heavy metals in bread (µg/kg)	Body weight (kg)	Daily Exposure level (µg/kg.bw)	Weekly Exposure level (µg/kg.bw)
Nasreddine et al., 2006	Men	182.7	Lead		35.4	72.8	0.089	0.622
		182.7	Cadmium		17.5	72.8	0.044	0.307
	Women	113.5	Lead		35.4	72.8	0.055	0.386
		113.5	Cadmium		17.5	72.8	0.027	0.191
	Men:							
	25-34 years	192.6	Lead		35.4	72.8	0.094	0.656
		192.6	Cadmium		17.5	72.8	0.046	0.324
	35-44 years	185.1	Lead		35.4	72.8	0.090	0.630
		185.1	Cadmium		17.5	72.8	0.044	0.311
	45-54 years	166.2	Lead		35.4	72.8	0.081	0.566
		166.2	Cadmium		17.5	72.8	0.040	0.280
	Women:							
	25-34 years	112.8	Lead		35.4	72.8	0.055	0.384
		112.8	Cadmium		17.5	72.8	0.027	0.190
	35-44 years	111.8	Lead		35.4	72.8	0.054	0.381
		111.8	Cadmium		17.5	72.8	0.027	0.188
	45-54 years	116.6	Lead		35.4	72.8	0.057	0.397
		116.6	Cadmium		17.5	72.8	0.028	0.196

Table 5: Heavy Metals Reference Values

Heavy metal	Reference name	Reference Value
Arsenic	BMDL	0.5-3 µg/kg bw/day
Aluminum	PTWI	1 mg/kg bw
Mercury	PTWI	4 µg/kg bw
Cadmium	PTMI	25 µg/kg bw
Nickel	TDI	2.8 µg Ni/kg.bw
Chromium	TDI	0.3 mg/kg.bw/day
Antimony	TDI	6 µg/kg.bw/week

Maximum exposure level was meticulously visualized through (Fig.6) as a worst-case scenario. Calculations were done for each heavy metal using the maximum level of consumption of bread 1000 g/d. The maximum exposure levels: As (26.6 µg/kg.bw/d), Ni (86 µg/kg.bw/d) and Sb (15.8 µg/kg.bw/week), were higher than their reference values respectively (BMDL: 0.5-3 µg/kg.bw/d, TDI: 2.8 µg/kg.bw/d, TDI: 6 µg/kg.bw/week).

The comparison of different age groups compared to each other and compared to reference values was represented in (Fig.7). Results showed that As was higher in children than other age groups but was not higher than the reference level BMDL (0.5-3 µg/kg.bw/day). Ni was the highest in children (6-9 years old) followed by men then teenagers then women and was higher than the reference values TDI (2.8 µg/kg.bw/d) in all age groups.

Comparison of exposure between men and women were visualized in (Fig.8). It was noticed than men are much more exposed to heavy metals than women; however, only Ni (6.072 µg/kg.bw/d) was higher than the TDI (2.8 µg/kg.bw).

The average exposure level differences between regions were pictured in (Fig.9). It was observed that the highest exposure to heavy metals were present in South Lebanon and Nabatieh regions, that are rural areas compared to the lowest levels present in Beirut area.

The average exposure levels differences throughout the years were reported in (Fig.10) based on all the studies previously done in Lebanon and new calculations from the present study. It was noticed that the exposure to heavy metals increased between years 2006 and 2020 especially for As (0.074 to 1.88 µg/kg.bw/d) and Cd (0.04 to 2.12 µg/kg.bw/month). Other heavy metals were not reported in all the studies so comparison was not possible.

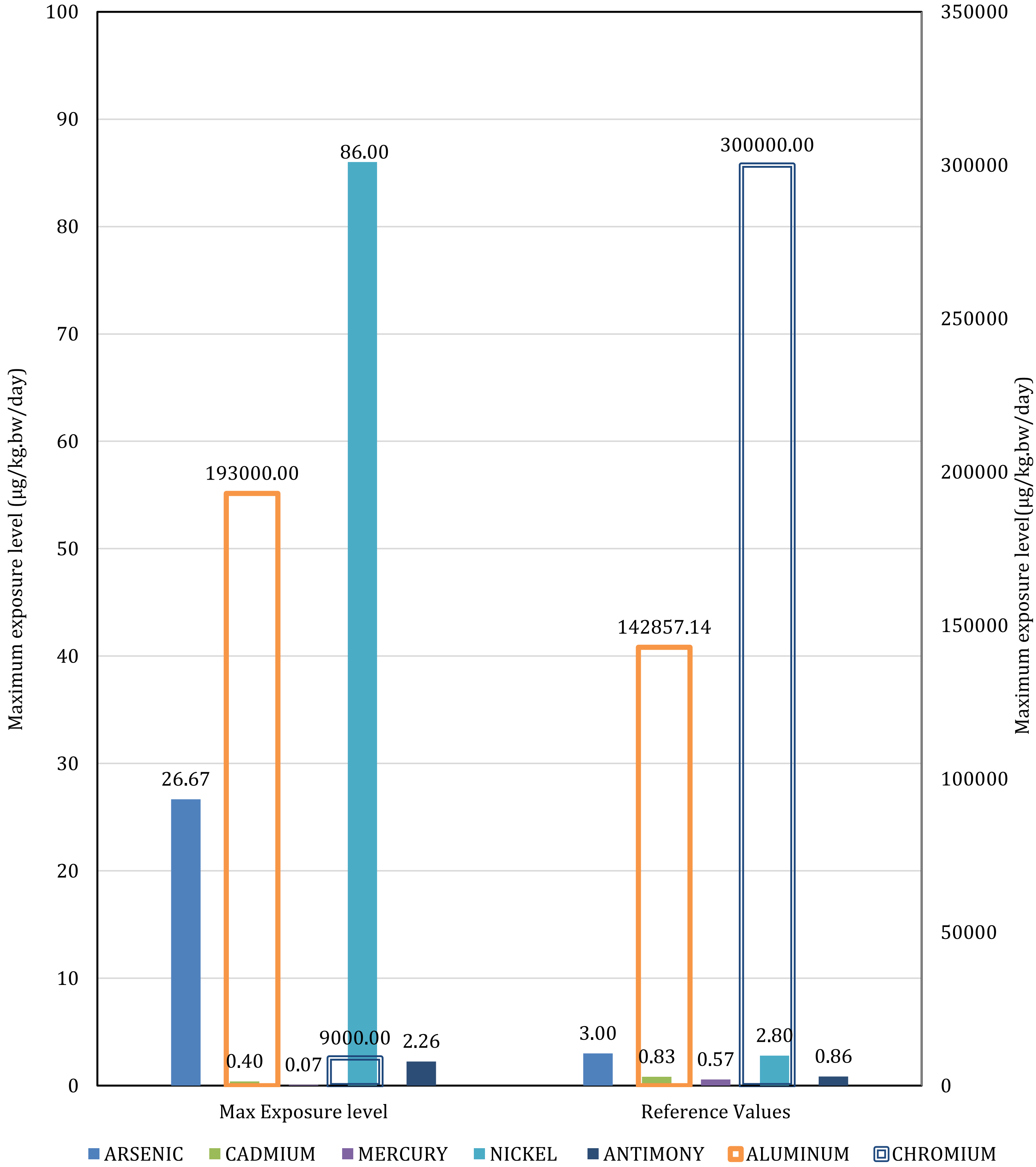

Figure 6-Maximum exposure level of Lebanese population to heavy metals through white pitta bread consumption compared to reference values

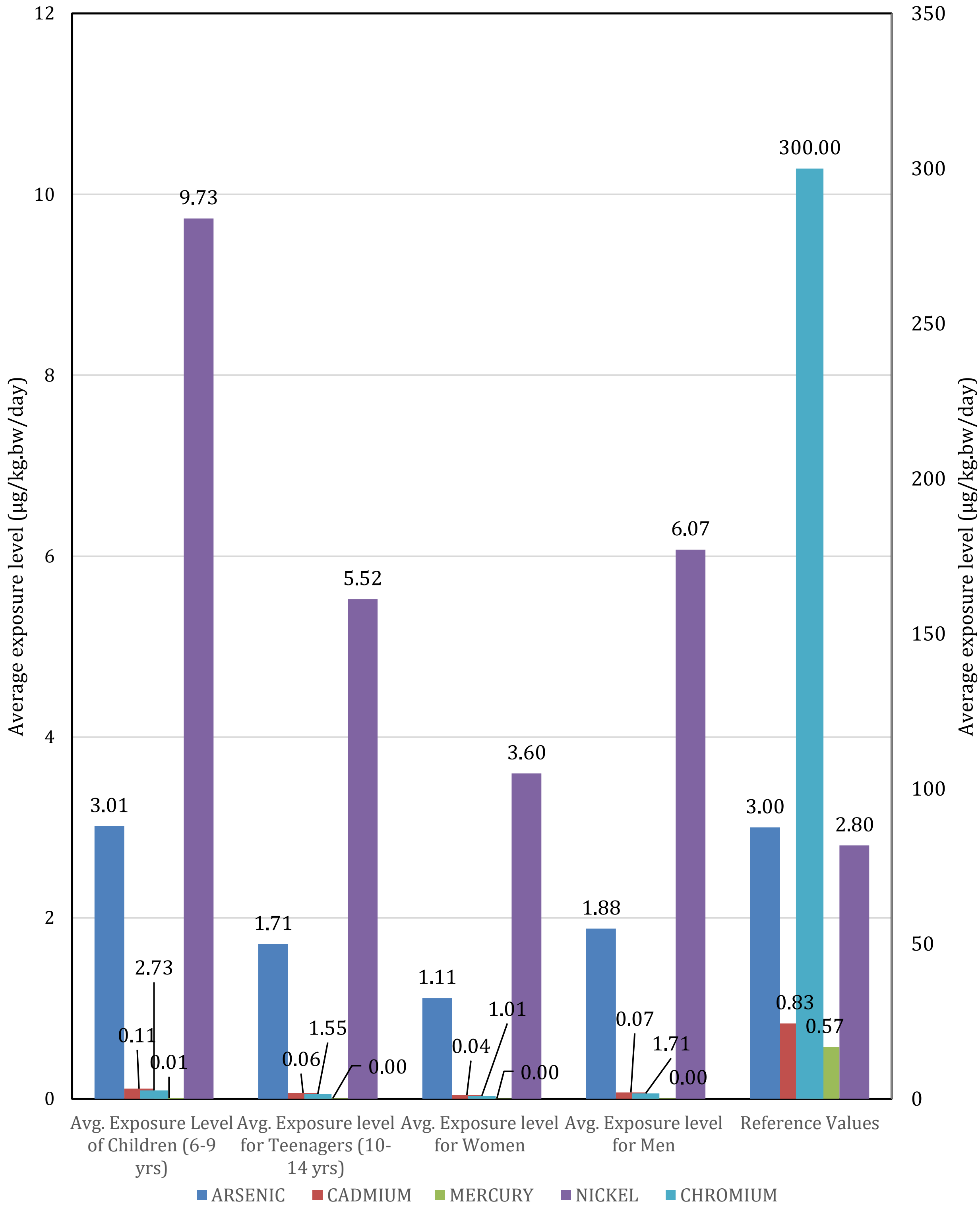

Figure 7 -Average exposure levels of different age groups compared to reference values

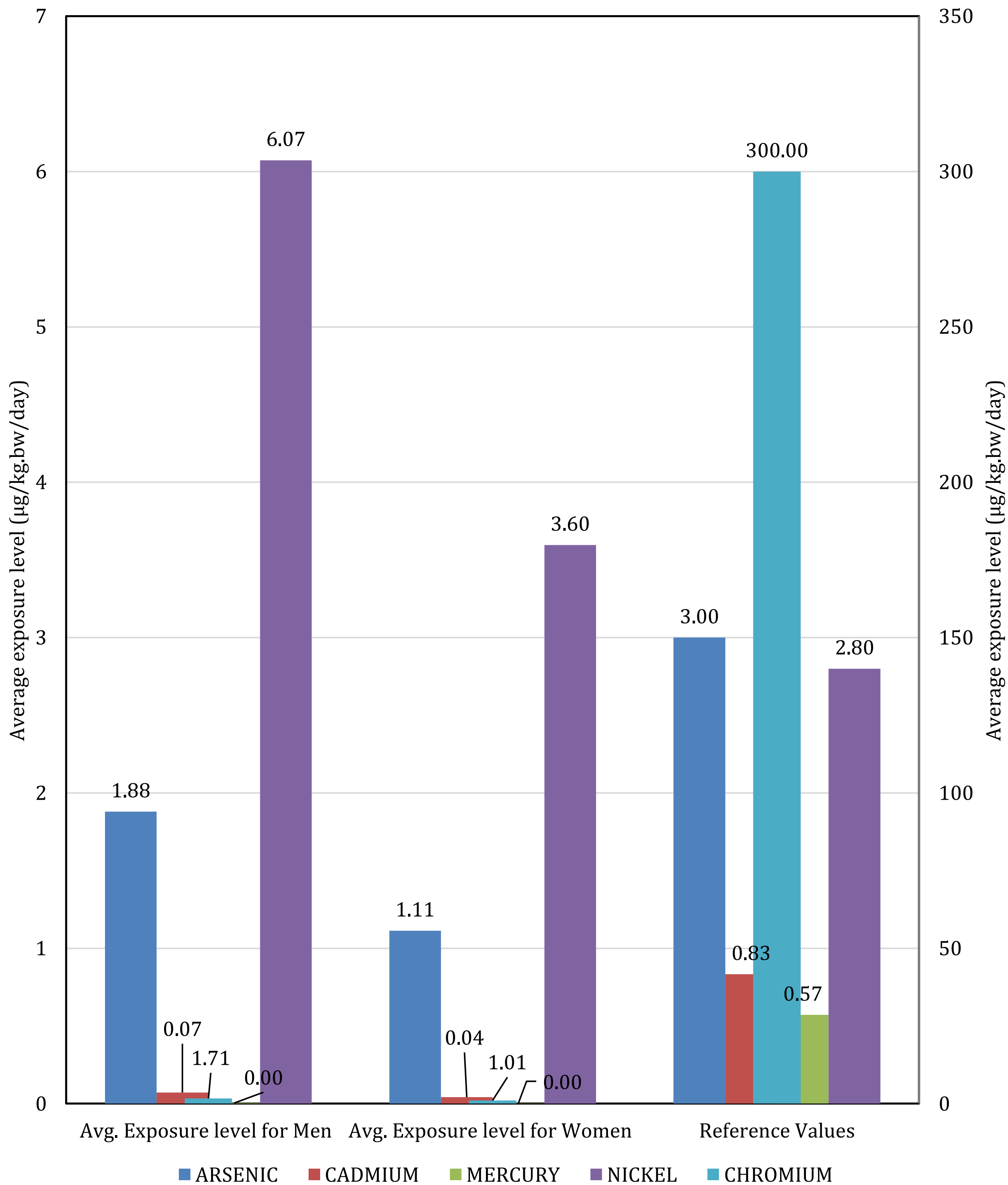

Figure 8-Average exposure level for men and women compared to reference values

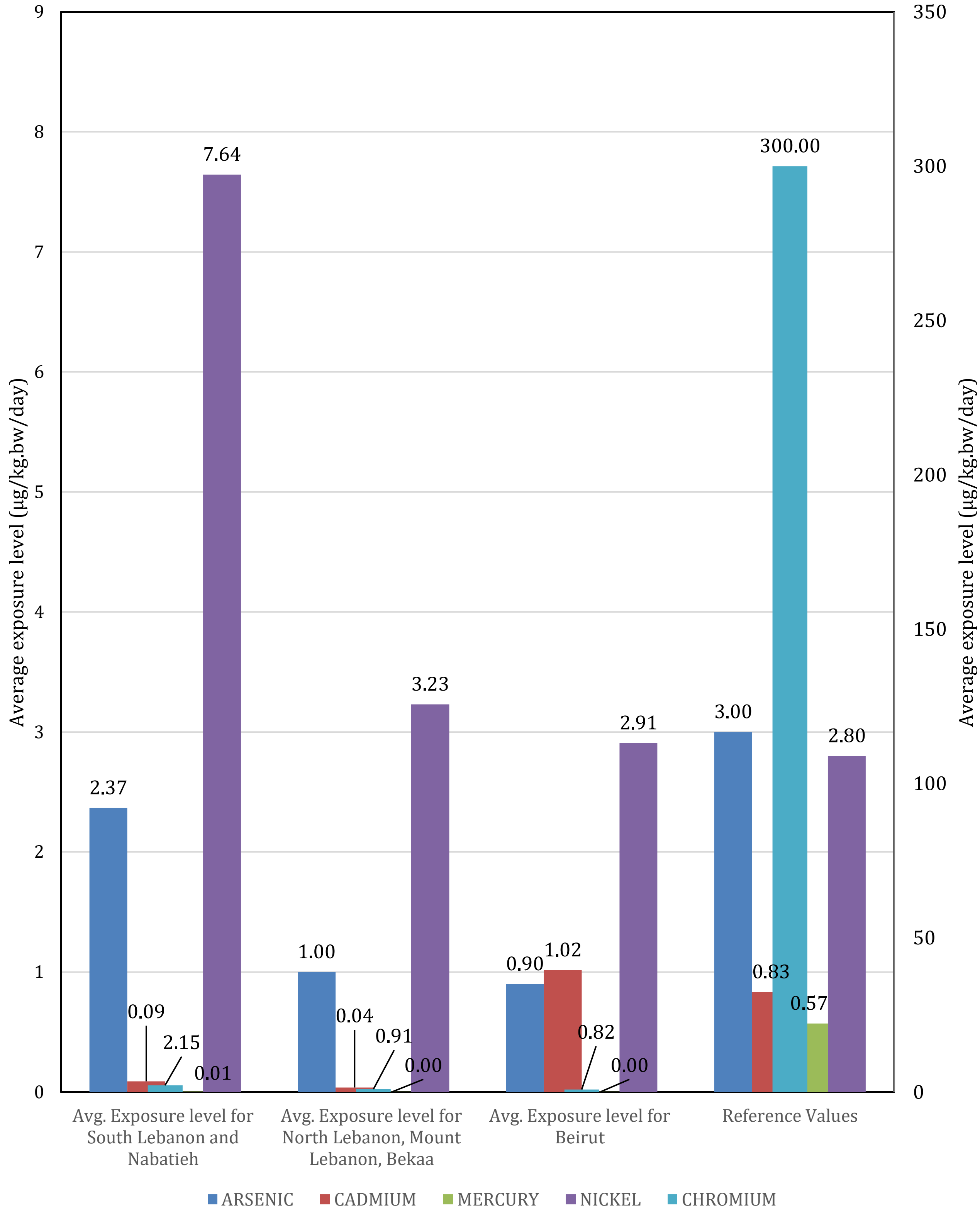

Figure 9-Average exposure level in different regions compared to reference values

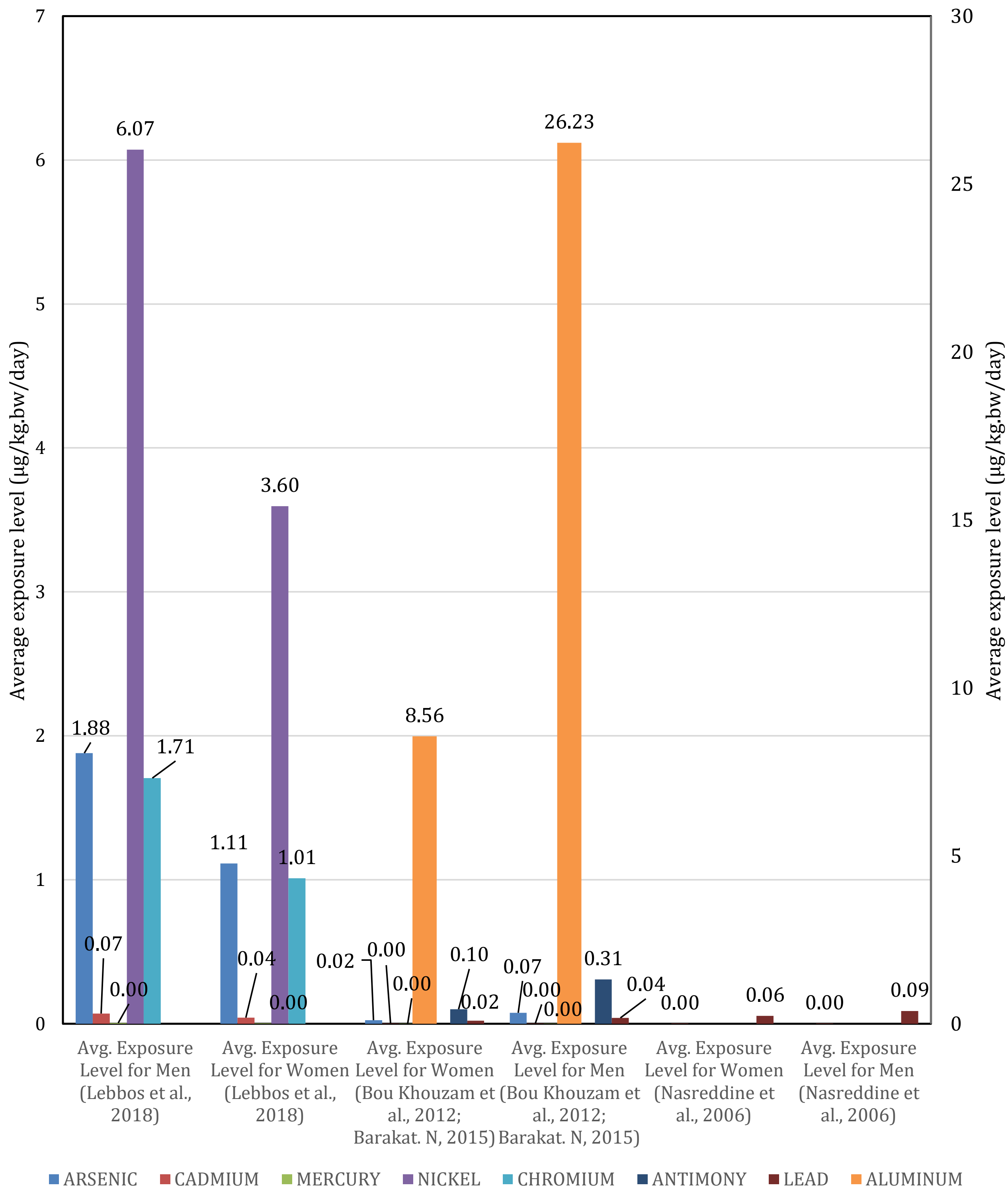

Figure 10- Average exposure level for men and women throughout the years

3.4. Discussion

3.4.1 Result analysis

3.4.1.1 Consumption of bread

Results showed that men consumed more bread as compared to women (Table.4). This might be explained by the higher energy level men usually consume especially that their maximum intake can reach 1000 g/d compared to women that are considered more health-conscious and followers of dietary recommendations. In Finland, women were more interested in and reported much more active seeking of health-related information, paid more attention to potential worldwide pandemics and were much more attentive as to how the goods they purchase in everyday life affect their health than men did (Ek, 2013). Differences of consumption by region might be correlated to the fact that rural areas have more agriculture and elderly populations that usually consume a higher amount of bread (Nasreddine *et al.*2006). Furthermore, Lebanese children consumed 113 g/d and teenagers 171 g/d which is much higher compared to a study done in Riyadh which evaluates the frequency and patterns of bread consumption among students, 60% of the surveyed respondents reporting that they consumed 1-2 servings (80g) of bread daily (Aljobair, 2017).

3.4.1.2. Increased consumption throughout the years

Additionally, the consumption increase throughout the years might be due to the increased refugee population, which increased the market demand. As of October 2017, the Government of Lebanon (GoL) estimates that the country hosts 1.5 million Syrians who have fled the conflict in Syria (including 997,905 million registered as refugees with UNHCR), along with 34,000 Palestine Refugees from Syria (PRS), 35,000 Lebanese returnees, and a pre-existing population of more than 277,985 Palestine Refugees in Lebanon (PRL). Over the past five years, the percentage of displaced Syrian households living below the poverty line ($3.84/ capita/day) has been increasing. In 2017, 76% live in poverty, compared to 71% in 2016, 69% in 2015 and 49% in 2014. A further 58% is deemed severely socio-economically vulnerable, that is, currently living below a survival minimum of $435/month for a household of five, compared to 53% in 2016, 52% in 2015, and 26% in 2014 (UNHCR, 2018) (Eglite, 2017).

3.4.1.3. Heavy metal content in bread

The toxic heavy metals present in white pitta bread in Lebanon were listed as follow from highest to lowest: Al>As>Pb>Sb>Cd>Hg (Table.5). Cr and Ni were considered essential elements, however they can have toxic effects at high concentrations or when present at particular oxidation states, e.g., Cr (VI). The highest level of heavy metal present in white pitta bread was Al 11.58 mg/kg; this might be due to additives used in bread making, higher than the permissible limit or contact with materials and containers at the production and distribution stage. Furthermore, Al-containing food additives have been used in food processing for over a century, as firming agent, raising agent, stabilizer, anticaking agent and coloring matter, and some are permitted to be used in food in many countries. Al is also present in food naturally (normally at low levels) (Yokel, 2012). Reported levels for Al in this study were similar to those reported in the urban areas of Pakistan (14.6 ± 3.9 mg/kg for no brand bread and 4.8 ± 2.0 mg/kg for brand bread) (Jalbani *et al.*, 2007) and in Egyptian bread 16.9 mg/kg in white wheat bran (Iskander *et al.*, 1990).

Pb increase throughout the years can be due to a higher contaminated soil, water or lead-contaminated paint inside the bakeries. This was previously discussed in Karrari et al. (2012). During the wet season, Pb level was 18 µg/kg, which was similar to Finnish bread values of 14 and 8 µg/kg (Tahvonen, 1994). Also, high As levels 400 µg/kg, might be due to contaminated water used in making bread or for irrigation (Sipra *et al*., 2013). This level was much higher than As content found in a study done in India, 43.64 ± 48.19 µg/kg in wheat, used for making the home-made bread (*chapatti*). The study presents a probabilistic assessment of increased cancer risk from wheat-based food where As exposure was endemic (Suman *et al*., 2020). Seasonal variations could be due to the composition of wheat grain and water, which are the main ingredients of bread in addition to the type and composition of soil (Bou Khouzam *et al*., 2018)

According to Bou Khouzam *et al*. (2018) the data of each element and each geographical region within one season showed no significant changes between mean concentrations of almost all the elements as a function of the region. The notable exception was the high concentrations of As and Hg observed in the Beirut region. Also, bread from North Lebanon showed much higher concentrations of Al, Fe, Cu, Cr, Ni, Pb, Si, and Zn as compared to bread from other regions. This might be due to the contamination of bread from production e.g., old metal containers used during bread processing. The regional variation on elemental composition of bread is due not only to the impact of manufacturing process (contamination by and/or loss of certain elements), but it is also reflected by the composition of the main ingredients of bread and notably the wheat grains. A previous study on the variation of elemental concentration (Na, K, Mn, Cu, Fe, Zn, and Se) from different cultivars in the Canary Islands showed a significant difference in the concentration of these elements, which might be associated with the genotype species and the environmental effects (type of soil and water composition) (Tejera *et al*., 2013)

3.4.2 Heavy metals Comparison between white bread and whole-grain bread:

Bou Khouzam *et al.* (2012) showed that nutrients, such as Cu, Zn, Mn, Mo, Fe, Co, and Se are present in brown and whole-wheat grain flour-based bread in much higher concentrations than in white flour-based bread. Indeed, brown and whole wheat flour are products of whole grains, which contain higher levels of trace elements in comparison with white flour owing to the presence of the outer kernel layers where minerals are concentrated. This was confirmed by a study on buckwheat, the bran of which has a higher concentration of Se, Zn, Co, Ni, and Ag than the flour part (Thielecke and Nugent, 2018). It is well known that wholegrain are nutrient dense and research demonstrates that the healthiest diets are characterized by a higher intake of wholegrain. It is true that wholegrain contain a higher level of contaminants than refined ones (white) but it also removes 50 to 80% of phytonutrients from whole grains, which also reduce the health benefits (Thielecke and Nugent, 2018). The observational and preclinical evidences presented by Thielecke and Neugent (2018) suggested that these phytonutrients, (vitamins, minerals and fibers) might exert a potentially protective effect against mycotoxins and toxic metals in particular. Further, the consumer also has a choice in mitigating any risk from contaminants, and to do so best by continuing to eat a healthy balanced diet, rich in nutrient dense foods, and including whole grain foods. Such a diet will ensure adequate trace mineral status and sufficient intakes of nutrients, antioxidant and phenolic compounds dietary fibers. Wholegrain contain more contaminants than refined grains (white); however, the nutritional benefit of wholegrain outweighs the risks posed by the contaminants (Thielecke and Nugent, 2018).

3.4.3 Exposure Assessment

The maximum exposure levels highlighted in (Fig.6), showed that the highest exposure to heavy metals were to As, Ni, and Sb that was above the reference values. The main sources of high As, Ni, and Sb levels could be due to a contaminated soil and the use of contaminated water during irrigation of the crops. These results represented the worst-case scenario, in case the individual consumption reached a level of 1000 g/d. Since it was found to be higher than the limits for the three heavy metals, further analyses were done.

Average exposure calculations that showed the greater Ni level in children (6-9 years old), followed by men, teenagers and women higher than the reference values (Fig.7) might be due to Ni occurring in air, water, sediments, and soil from various natural sources and anthropogenic processes. Ni is introduced into the environment and is circulated through the system by chemical and physical processes and through biological transport mechanisms of living organisms. Ni could be present in pots and pans used in the bakeries (Buxton *et al*., 2019). Children might be more exposed to Ni compared to other age groups because of their smaller body weight and highest absorption rate; additionally they are more exposed to Ni through direct consumption of soil through their hands, food and toys. There were no conclusive studies confirming why children are more at risk for Ni absorption. The current exposure results present in Lebanese children were much higher than the ones found in children living in urban areas in Germany (0.63-31 µg/kg.bw/d) (Wittsieppe *et al*., 2009).

Men were more exposed to heavy metals than women (Fig.8) due to the fact that men consume more energy per day and that women are usually more interested about reducing their carbohydrate intake and limiting their calorie intake. Furthermore, gender differences according to heavy metals were analyzed by a study done in 2007 where there was a markedly higher prevalence of Ni-induced allergy and hand eczema in women compared to men, mainly due to differences in exposure. Cd retention was generally higher in women than in men, and the severe cadmium-induced Itai-itai disease was mainly a woman's disease. Gender differences in susceptibility at lower exposure were uncertain, but recent data indicate that Cd has estrogenic effects and affect female offspring. Men generally had higher blood lead levels than women. Pb accumulated in bone and increased endogenous Pb exposure has been demonstrated during periods of increased bone turnover, particularly in women in pregnancy and menopause (Vahter *et al*., 2007). Recent data indicate that boys were more susceptible to neurotoxic effects of Pb and methyl Hg following exposure early in life, while experimental data suggest that females are more susceptible to immunotoxic effects of Pb (Vahter *et al*., 2007)

Elevated exposure to heavy metals in South Lebanon and Nabatieh regions that are rural areas (Fig.9), might be due to more agriculture available in this region and the fact that Lebanese traditions related to food are more applied among the elderly population living in rural areas compared to urban areas, which leads to a higher exposure to heavy metals from bread. These results can be explained by health risk assessment results that were done in Iran that showed that heavy metal contents in rural samples were higher than those in urban samples for wheat, flour and bread (Ghanati *et al*., 2019).

The increase in exposure to heavy metals throughout the years (Fig.10) could be due to a higher environmental pollution (air, soil and water), the economic recession in Lebanon, which obligates people to buy the cheapest products available in the market, and using a higher quantity of pesticides than the permissible limits.

3.4.4 Gaps of the present study:

This study was not without limitations. In fact, this study used previous published data. The heavy metal related data were obtained using two different techniques including the Inductive Coupled Plasma (ICP) spectrometry and Atomic Absorption Spectroscopy (AAS). We had to combine all the studies together and evaluate them since there were only three studies previously done in Lebanon about heavy metal content in bread. This might lead to non-conformity of the results. In addition, references values used to compare the exposure results calculated in the present study were not related to a specific method used, which might lead to limitations of the results.

CHAPTER 4. CONCLUSION & RECOMMENDATION

The Lebanese are exposed to certain heavy metals from consumption of white pitta bread above the recommended limits. These results confirm that numerous bakeries in Lebanon might have not been applying food safety standards and regulations. The exposure of Lebanese population is higher than the limits mainly for arsenic and nickel, which might cause severe health risks on the long run. Children aged 6-9 years old and people living in South Lebanon and Nabatieh regions were the most exposed population. Other heavy metals exposure like Co, Cd, Cr, Pb, Hg and Al were not of safety concern. Heavy metals exposure might be due to an increase of soil, water and air pollution and the rise in use of pesticides in wheat agriculture. Although the results indicated that it is unlikely for some heavy metals to cause adverse health effect like cadmium, mercury and lead for the general population, the adverse health effect cannot be ruled out since bread is not the only food highly consumed by the Lebanese. It is recommended that information on bread packaging label including specific food additives used to be accurate. It is also advised to develop alternative technique to reduce the use of heavy metal-containing food additives and utensils during food processing. It would be interesting to study the effect of food additives that are widely used in the production of bread and check if the bakeries in Lebanon are abiding by the permissible limits. In addition, a number of individual studies that addressed metals interactions reported that co-exposure to metal/metalloid mixtures of arsenic; lead and cadmium produced more severe effects at both relatively high dose and low dose levels in a biomarker-specific manner. Hence, research is needed to further elucidate the molecular mechanisms and public health impact associated with human exposure to mixtures of toxic metals.

The public is advised to maintain a balanced diet so as to avoid excessive exposure to heavy metals from a small range of food items and to make reference to the information in the ingredient list on the label to make informed food choices. Finally, governmental recommendations are to revise LIBNOR standards to make it more appropriate to the Lebanese population consumption level of bread products and to conduct effective control on raw material safety and bakeries application of safety standards and use of preservatives.

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