DETERMINATION OF TOXIC ELEMENT CONTAMINATION IN THYME AND THYME-CONTAINING PRODUCTS CONSUMED IN LEBANON

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Abstract

Thyme herbs constitute a major part of the Mediterranean diet and are gaining worldwide popularity. However, their chemical contamination with toxic metals may put consumers at a health risk. Thus, the aim of this study was to assess the incidence of Arsenic (As), Cadmium (Cd), Lead (Pb) and Mercury (Hg) in thyme herbs and thyme-containing products. For this, 180 samples pooled into 36 composite samples of the most consumed types of thyme-containing products and thyme herbs were collected twice at three-month intervals. Samples were analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and the four heavy metals were detected in all the samples. Eleven percent, 22%, and 97% of samples had unacceptable levels of As, Hg and Pb respectively according to the international standards set by Codex Alimentarius and all the samples had acceptable limits of Cd. The dried thyme herbs were more contaminated with the four toxic elements than the thyme-containing products including cereal-based products, dairy products, herbal thyme tea and thyme salad. This study highlighted the importance of monitoring and enforcing regulatory actions related to the contamination of the food chain with chemical contaminants such as toxic elements to protect the health of consumers.

Chapter 1: Literature Review

1.1 Introduction:

The Mediterranean diet is rich in edible plants and herbs, especially in thyme also known as “Zaatar.” The consumption of thyme and thyme products in Lebanon has gained a wide popularity as thyme known as “Zaatar” is widely available in the country, tasty, nutritious, and affordable. Lebanese people use it in various recipes, as dried or fresh herb, for example thyme salads, thyme pizza (Mankoushe), thyme croissant, fatayer zaatar (Batal & Hunter, 2007).

Thymol and Carvacrol which are considered thyme’s main essential oils have many health benefits such as antioxidant actions, respiratory benefits, anti-inflammatory actions, neurological actions as well as gastrointestinal actions (Singletary *et al*., 2016). However, the nutritional benefits of thyme can be jeopardized by the presence of contaminants such as heavy metals in the plant.

Heavy metals such as lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg), are readily found in the environment and are considered a serious health concern because they are non-biodegradable, have long biological half-lives and hence can accumulate in the body and cause adverse effects (Al-Saleh & Abduljabbar, 2017). They are classified as environmental pollutants of chemical nature that can reach the human body through several routes. Ingestion of contaminated foods and beverages is considered one of the routes of exposure to these toxic metals and the contamination of these products is becoming inevitable those days (Żukowska & Biziuk, 2008). Therefore, efforts are made worldwide to estimate dietary exposure of individuals and populations to heavy metals from food and to assess the risk associated with such exposure.

Risk assessment involves the estimation of associated health risks of a hazard exposure on an organism. Four steps are involved: hazard identification, hazard characterization, exposure assessment and risk characterization. Toxicological reference values (example: Provisional tolerable weekly intake PTWI) or reference doses (example: benchmark dose lower confidence level BMDL) set by international organizations such as The European Food Safety Authority (EFSA) and Joint Expert Committee for Food Additives (JECFA) are used to evaluate risk. Margin of Exposure (MOE) approach is recommended by JECFA and EFSA to evaluate the risk of carcinogenic or genotoxic contaminants. A small MOE represents a higher risk than a large MOE. (EFSA 2005). The risk assessment results can be used by the risk management to establish preventative measures (Joint Expert Committee on Food Additives, 2006a)

Dietary exposure is a key step to assess the risk of heavy metals from thyme and thyme products. It is the quantification of the intake of chemicals (heavy metal) via food (Thyme and thyme products). Dietary exposure assessment is done by combining food consumption data with the concentration of chemicals in food data (Mbabazi, 2011a).

Many studies have been done to evaluate heavy metal contamination in herbs and spices as well as in other food products (Dghaim *et al*. 2015; Martín-Domingo *et al.*,2017; Dbaibo *et al*., 2020; Nasreddine *et al.*,2010; Lebbos *et al*.,2019); however, no studies have been made to evaluate the occurrence of heavy metals in thyme and thyme products nor to estimate the dietary exposure among the Lebanese population.

Hence, the objectives of this work are to assess the occurrence of heavy metals Pb, As, Cd and Hg in thyme and thyme products and to estimate the dietary exposure to these heavy metals among the Lebanese population.

1.2 Heavy metals: sources, routes of exposure and health effects

Heavy metals refer to metals with a high atomic weight and a density of more than 5 gr/cm^3. Their atomic weight can range from 5,63 to 3,200 (Hashemi *et al.*,2017; Tchounwou, Yedjou, Patlolla, & Sutton, 2012)

Some of these metals are relevant toxic elements such as Pb, Cd, As and Hg (Marín *et al*., 2018). They are non-essential elements and are not biodegradable (El-Kady & Abdel-Wahhab, 2018). They do occur naturally but their exposure can be increased by human activities and with increased advancements in industrialization, urbanization, and anthropogenic activities (Vahidinia *et al*., 2018).

Many factors influence the toxicity of heavy metals in humans including sociodemographic factors such as age, sex, genes, and nutritional status in addition to the route of exposure, dosage, and chemical species. For example, children and elderly are more likely to be affected by the accumulation of these metals in their body than younger adults (Rahimi 2013; Hashemi *et al.*,2017). Physiologic differences between males and females, including differences in pharmacokinetics and pharmacodynamics can influence the effect of heavy metals (Environmental health and toxicological information). Toxic responses can vary between species due to differences in metabolism by the body (Environmental health and toxicological information). Nutritional status such as diet and lifestyle can also influence the toxicity of substances especially if people consume considerable amounts of certain food items.

Biologically, it has been found that heavy metals have an impact on cellular organelles and elements including the membrane, mitochondria, lysosome, endoplasmic reticulum, nuclei, in addition to many enzymes that have a role in detoxifying, metabolizing, and repairing damage in the body (Tchounwou et al.,2012).

Lead, cadmium, arsenic, and mercury have an elevated level of toxicity and hence are considered a priority when it comes to public health importance. According to the substance priority list done by the Agency for Toxic Substances and Disease Registry (ATSDR) in 2015, As, Pb, and Cd were ranked 1st, 2nd, and 7th most potentially threatening substances to human health (Bassil *et al.*, 2018)

Due to their high toxicity, these toxins can lead to multiple organ damage even at low concentrations. In addition, they are categorized as known or possible human carcinogens according to the US (United States) Environmental Protection Agency and the International Agency for Research on Cancer (Tchounwou *et al*., 2012)

The WHO (2007) classified them among the top 10 chemicals of major public health concern (Bua *et al*., 2016). Some of the adverse health effects include mutations, cancer, or miscarriages. (Nordin & Selamat 2013 Cabrera *et al*., 2003)

Table 1 Sources, routes of exposure and health effects of heavy metals (cadmium, arsenic, lead, and mercury).

Heavy metal	**Sources**	**Routes of exposure**	**Health effects**	**References**
Cadmium	cadmium-containing phosphate fertilizers, inappropriate disposal of electronic wastes, industrial emission sources, nonferrous mining and metal refining industries, batteries, pigments	inhalation of fumes or dust in occupational settings, ingestion of contaminated crops	itai-itai disease (a disease of the kidneys and bones), kidney damage, lung cancer, liver disease, gastrointestinal cancer, cardiovascular disease	(Nordberg *et al.*, 2018) , (Liu *et al.*, 2018)
Arsenic	volcanic eruptions (volcanic rocks and their weathering products, geothermal fluids (water, gases) and volcanic exhalations), geological or via anthropogenic activities, mining operations, burning of coal and electric reefing industries, As-containing pesticides or veterinary or human medicinal drugs, preservatives	Ingestion, inhalation, and skin absorption	skin lesions, reproductive effects, cardiovascular effects, circulatory disorders, pulmonary disorders, neurological complications, diabetes, respiratory complications, hepatic and renal dysfunction, organ damage (acute toxicity), malignant tumors (chronic toxicity).	(Khan *et al*., 2020), (Abdul *et al*.,2015), CODEX 2018
Lead	mining, smelting, processing, use, recycling, or disposal, traditionally leaded gasoline, lead-based paints, and batteries industrial pollution ,agricultural practices, food packaging,	Ingestion, inhalation	behavioral abnormalities, hearing deficits, neuromuscular weakness, and impaired cognitive functions in humans, clinic	Assi *et al*.,2016 CODEX 2018

	glassware, ceramic products, and pesticides.		pathological changes through toxicity occurring in kidney and endocrine system,	
Mercury	chlor-alkali plants, coal-fired thermal power plants, gold and cement production, paper and pulp industry, municipal combustors, medical waste, incineration, combustion of fossil fuels ,forest fires	Ingestion, inhalation	damage to the nervous, respiratory, immune, and renal systems	Halder *et al.*, 2013 Renzoni, Zino, & Franchi, 1998

1.3 Chemical forms of heavy metals

i. Mercury

Mercury is one of the most studied toxic elements as it impacts human health negatively (Leopold *et al.,* 2010).

The toxicity of mercury is known to be correlated with its chemical form (Cabañero *et al*., 2005).

Mercury exists in three chemical forms: elemental or metallic mercury (Hg0), inorganic mercury (mercurous (Hg2 and mercuric (Hg2+) cations) and organic mercury (EFSA 2012). In general, organic mercury compounds are known to be more toxic than inorganic and elemental forms (Gao *et al.*, 2012). Methylated forms have a higher toxicity (by a factor of 10). (Silva *et al.*, 2005)

Methylmercury is regarded as the most toxic species regarding bioaccumulation and risk. (Cabañero *et al.*, 2005; Leopold *et al.*, 2010). It is the most prevalent form of organic mercury found in the food chain (EFSA 2012).

This form of organic mercury (methylmercury) is known to be found mostly in fish and seafood while inorganic mercury is common in other food groups like fruits and vegetables (Arnich *et al.*,2012; Ghasemidehkordi, *et al.*, 2018). In other words, Methylmercury's source from terrestrial food is limited. (Renzoni, Zino, & Franchi, 1998)

Indeed, speciation analysis is important in differentiating between the two forms of mercury (organic and inorganic) and providing more accurate information when assessing the relative health risk (Ghasemidehkordi *et al.*, 2018); however, the development of more sensitive, reagent-free, or reduced reagent consumption, and environmental friendly techniques is needed (Gao *et al*., 2012).

ii. Lead

According to the substance priority list done by the Agency for Toxic Substances and Disease Registry (ATSDR) in 2015 lead is ranked the 2nd most potentially threatening substance to human health (Bassil *et al.*,2018)

Diet and foodstuffs are known to be the primary source of non-occupational exposure. (Wang *et al*.,2019)

It can exist in two chemical forms: organic and inorganic chemical form (Kumar *et al.*, 2020). All the chemical forms are toxic; however, the organic form is more readily absorbed by the gastrointestinal tract than the inorganic form, but most lead that is found in the environment is in the inorganic form (Silva *et al*., 2005). The International Agency for Research on Cancer IARC classified inorganic lead as a probable carcinogen (Group 2A) (CODEX 2018).

iii. Arsenic

Arsenic is a ubiquitous element found in soil, water, and air (Jara & Winter, 2014). The consumption of contaminated foodstuffs and water is the main pathway of human exposure to arsenic (Devesa, Vélez, & Montoro, 2008). When a population is not exposed to contaminated water with arsenic, food would be the main contributor to the intake of inorganic As (D'Amato *et al.*, 2011).

Organic and inorganic arsenic which are two different chemical forms of arsenic, differ in their toxicities. (Devesa, Vélez, & Montoro, 2008). Inorganic species, arsenite (AsIII) and arsenate (AsV), are known to have a higher toxicity than organic arsenic compounds (Sadee, Foulkes, & Hill, 2015). According to sufficient epidemiological studies, the IARC classified inorganic As as a class 1 carcinogen as its exposure can increase the risk of skin, lung, and urinary bladder cancer (IARC 2019). Moreover, other long term health effects can arise from chronic exposure to dietary arsenic. These can include cardiovascular diseases, skin lesions, disrupted glucose metabolism and diabetes (EFSA 2009). Indeed, the available data related to arsenic speciation in different food commodities is limited (EFSA 2009).

According to the assumption made by the EFSA panel, the proportion of inorganic arsenic (the toxic form) may vary from 50 to 100% of the total arsenic reported in food commodities other than fish and seafood considering 70% as a good reflection of an overall average.

Processing of food like cooking or preservation methods may influence the chemical species of arsenic. These processes may either increase or decrease the inorganic arsenic present depending on the type of food, hence it is vital to evaluate the risk of arsenic on foods as consumed by people to have a more realistic assessment (Devesa, Vélez, & Montoro, 2008).

Data are insufficient to establish a heath-based guidance value for organic arsenic. (EFSA,2009)

iv. Cadmium

Cadmium and its compounds were classified by the IARC carcinogenic to humans (CODEX 2018). In nature and in the environment, cadmium is known to occur in its inorganic form. (EFSA,2009)

All the compounds related to cadmium are highly toxic to humans with dietary exposure being the most important pathway of exposure to the general population except for smokers (Silva *et al.*,2005).

1.4 Thyme and thyme products' consumption in Lebanon

Leaves and flowers of *O. syriacum* are used widely as a food, flavor, and seasoning ingredient in the traditional cuisine in Lebanon and throughout the Eastern Mediterranean. The consumption of thyme and thyme products in Lebanon has gained a wide popularity as thyme known as "Zaatar" is widely available in the country, tasty, nutritious, and affordable. Lebanese people use it in various recipes: Salads (Fattoush , thyme salad), Mankooshe (mixture: dried thyme, sesame, sumac, and salt), Fatayer Zaatar, herbal infusions ((Batal & Hunter, 2007; Global Environment Facility, implemented by the United Nations Development Program (UNDP) and Lebanese Agricultural Research Institute (LARI), Beirut (GEF-UNDP-LARI, 2013).

In 2008 the Lebanese export trade value for dried oregano herbs, zaatar mix, and oregano infusion herbs was approximately USD 1.9 million. In 2011 the size of this market was USD 2.4 million (20% increase in 3 years) (GEF-UNDP-LARI, 2013).

Origanum syriacum subsp. *Syriacum* is grown in many Middle Eastern countries such as Egypt, Jordan, and Syria; however, it is native to Lebanon. The plant is about 130 cm long with aromatic leaves and flowers (Benelli et al., 2019).

The genus *Origanum* (*Lamiaceae*) has 43 species and 18 hybrids organized in 3 groups and 10 sections. The section Majorana includes *Origanum syriacum L.* (Lukas *et al.*, 2009)

Thyme is commonly consumed herb in the Mediterranean region as it gives flavor, aroma, is cheap and is readily found in the market. It is known by the common name “za’atar” which is a mix of *Origanum syriacum*, roasted sesame seeds, sumac (Rhus coriaria) and salt (The herb society of America, 2005; Dbaibo, *et al*., 2020). Traditional uses of *Origanum* species include considering it as a remedy to treat several diseases such as those related to the respiratory tract as well as the gastrointestinal tract, and treating stomach pain as well as arthritis. Historically, it was considered an effective treatment of internal diseases, hemorrhoids, and sexually related conditions (Lukas *et al*., 2009).

1.5 Regulations and standards to evaluate the occurrence of heavy metals in food

Table 2 Acceptable limits of different heavy metals in some foodstuffs

Heavy metal	Limit (mg/kg)	Food matrix	Reference
Hg	0.1 (fresh wt.)	Food supplement	Commission regulation (EC (European Commission)) No 1881/2006
	0.5 (Methyl mercury)	Fishery products	Commission regulation (EC) No 1881/2006
	0.5	Instant tea	NL 610:2002 Lebanese Standards Institution LIBNOR
	0.1	Salt, food grade	Codex Alimentarius CXS193-1995
	0.001	Natural mineral waters	Codex Alimentarius CXS 108-1981
	0.001	Bottled drinking water	NL 162:1999 Lebanese Standards Institution LIBNOR
	1	spices	Food and Agriculture Organization/ World Health Organization 1984
Pb	0.2 (fresh wt.)	Legumes vegetables, cereals, and pulses	Commission regulation (EC) No 1881/2006
	0.02	Milk and secondary milk products	Codex Alimentarius 2018
	0.3	Zaatar	NL 677:2017 Lebanese Standards Institution LIBNOR
	0.2	Lebanese bread	NL 240:2010 Lebanese Standards Institution LIBNOR

	0.3	Leafy vegetables	Codex Alimentarius CXS 193-1995
	5	Instant tea	NL 610:2002 Lebanese Standards Institution LIBNOR
	0.2	Chanklish	Lebanese Standards Institution LIBNOR NL 507:2009
	0.01	Natural mineral waters	Codex Alimentarius CXS 108-1981
	0.05	Bottled drinking water	NL 162:1999 Lebanese Standards Institution LIBNOR
	0.2	Cereal grains	Codex Alimentarius CXS 193-1995
	1	Akkawi cheese	NL 495:2003
	1	Preserved tomato cans	NL 180:1990
	10	Spices	Food and Agriculture Organization/ World Health Organization 1984
Cd	0.2 (fresh wt.)	Bran, germ, wheat, and rice	Commission regulation (EC) No 1881/2006
	0.2	Lebanese bread	NL 240:2010 Lebanese Standards Institution LIBNOR
	0.2	Zaatar	NL 677:2017 Lebanese Standards Institution LIBNOR
	0.2	Leafy vegetables	Codex Alimentarius CXS 193-1995
	0.2	Wheat	Codex Alimentarius CXS 193-1995

	0.003	Natural mineral waters	Codex Alimentarius CXS 108-1981
	0.01	Bottled drinking water	NL 162:1999 Lebanese Standards Institution LIBNOR
	0.3	Spices	Food and Agriculture Organization/ World Health Organization 1984
As	0.35 (Inorganic arsenic)	Rice husked (brown)	Codex Alimentarius 1995
	0.2 (Inorganic arsenic)	Rice polished	Codex Alimentarius CXS 193-1995
	0.5	Zaatar	NL 677:2017 Lebanese Standards Institution LIBNOR
	1	Instant tea	NL 610:2002 Lebanese Standards Institution LIBNOR
	0.5	Salt, food grade	Codex Alimentarius 2019
	0.01	Natural mineral waters	Codex Alimentarius CXS 108-1981
	0.05	Bottled drinking water	NL 162:1999 Lebanese Standards Institution LIBNOR
	1	Spices	Food and Agriculture Organization/ World Health Organization 1984

1.6 Risk assessment of heavy metals in food products

Risk assessment is the process of evaluating the possibility of adverse health effects that may occur because of exposure to a hazard (chemical, biological or physical) (Żukowska & Biziuk, 2008). It is a four-step process that involves: hazard identification, hazard characterization, exposure assessment and risk characterization (EFSA, 2005; FAO/WHO, 2006).

1.6.1 Hazard identification

It involves identifying the toxic compounds in food to which the population is exposed. i.e.: Heavy metals.

1.6.2 Hazard characterization

For hazard characterization, adverse health effects that may arise from ingestion of the toxic agents should be specified. In the case of heavy metals, some of these effects can include carcinogenicity, multiple organ damage (kidneys, lungs, liver), reproductive, neurological, and respiratory disorders, lower IQ, and poor intellectual function in children (Nordin&selamat 2013; Cabrera *et al.,* 2003; Bassil *et al.,* 2018).

1.6.3 Exposure assessment

Exposure assessment is defined as the quantification of the intake of a chemical via food. It is done by combining data on the concentration of chemical in food with the food consumption data (WHO 2009).

To assess the concentration of heavy metal in food products several analytical methods can be used. These methods can include: Flame atomic absorption spectrometer (FAAS); atomic absorption spectrometry (AAS); inductively coupled plasma mass spectrometry (ICP-MS); inductively coupled plasma optical emission spectrometry (ICP-OES); inductively coupled plasma atomic emission spectrometry (ICP-AES); graphite furnace atomic absorption spectroscopy; (GFAAS) , direct mercury analyzer (DMA) and others (Helaluddin *et al.*, 2016).

1.6.3.1 Analytical methods used to determine heavy metals: advantages and disadvantages.

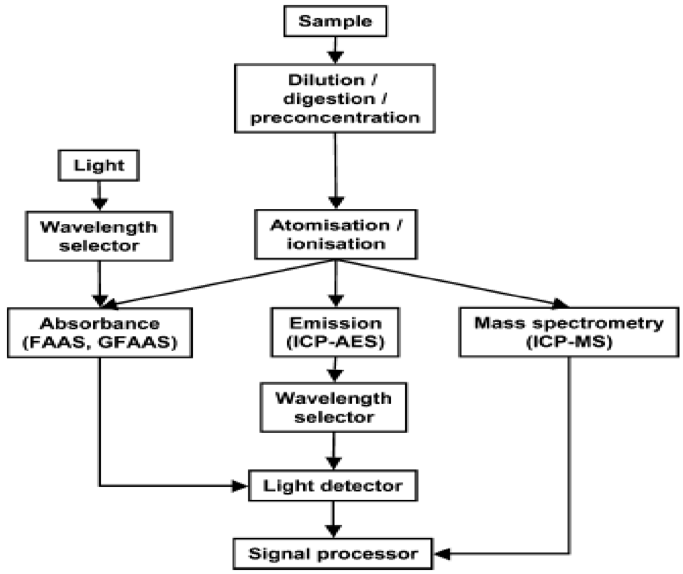

Figure 1: General diagram of spectrochemical techniques (Bolann et al., 2007)

Table 3: Methods for heavy metal analysis: advantages and disadvantages

Analytical Techniques	**Advantages**	**Disadvantages**
Flame atomic absorption spectrometer (FAAS)	-Relatively short measurement time Helaluddin *et al.*, 2016). -fast analysis (Helaluddin *et al.*, 2016(- suitable technique for determining metals at part per million (ppm) concentration (Helaluddin *et al.*, 2016). -good precision for many elements - repeatability (Helaluddin *et al.*, 2016) -moderate interferences (Helaluddin *et al.*, 2016) -relatively low cost (Helaluddin *et al.*, 2016)	-measuring multiple elements is time-consuming (Bolann *et al.*,2007) -the large sample volume required in the analysis (several mL) (Bolann *et al.*,2007) -inability of FAAS to analyze solid samples directly (Bolann *et al.*,2007) -high detection limits i.e.: analyte

		concentration is in the mg/L level, or below, precision is poor (Bolann *et al.*,2007)
Atomic absorption spectrometry (AAS)	-low detection limits (National research council 2004)	- Few elements, time-consuming, matrix effects (National research council 2004)
Inductively Coupled Plasma Mass spectrometry (ICP-MS)	-multielement capability (Bolann *et al.*,2007) - low detection limits (Bolann *et al.*,2007)	-high cost (Bolann *et al.*,2007) -difficult to use (need for trained technicians) Bolann *et al.*,2007 -matrix effects (National research council 2004)
Inductively coupled plasma/optical emission spectrometry (ICP-OES)	-limited spectral interferences, good stability, low matrix effects (National research council 2004)	-liquid samples only Bolann *et al*.,2007
Inductively coupled plasma atomic emission spectrometry (ICP-AES)	multi-element capability (Bolann *et al.*,2007) short time required (Bolann *et al.*,2007)	High detection limits Bolann *et al.*,2007
Graphite furnace atomic absorption spectroscopy (GFAAS)	-the ability to handle micro samples Bolann *et al.*,2007 -high sensitivity (Elemental 2001) -low detection limits; 10 to 100 times lower than the FAAS (Bolann *et al.*,2007) -few spectral interferences (Elemental 2001) -effective background corrections (Bolann *et al.*,2007) -moderate cost (Bolann *et al.*,2007) -important for speciation work (Bolann *et al.*,2007)	–much slower than FAAS (Bolann *et al.*,2007) limited working range, slow analysis (Helaluddin *et al*., 2016)

	-simpler and lower cost instrumentation (Bolann *et al.*,2007)	
Direct mercury analyzer DMA	-does not require any sample pretreatment -low levels of detection and quantification -capable of analyzing solid, liquid, and gaseous samples (Ferreira *et al.*,2015; Vieira *et al*., 2014)	-requires the user to take a hands-on role and remain present for lengthy periods of time with the instrument SHI *et al.*,2011

For quality control: Certified reference materials are used as a reference to guarantee method reliability and recovery percentages are calculated According to the AOAC method, percent recoveries of the certified reference materials should be between 75-125% of the given certified value (AOAC International 2013).

When reference materials are not available for any of the tested elements, spiking of the samples with a known concentration is usually done. (Lebbos *et al*.,2019)

Also, for quality control, spiking of samples with a known concentration of the tested metals can be done and recovery percentages calculated (Khan *et al*., 2014).

Sensitivity of the instrument can be estimated by determining the limits of detection (LOD) and the limits of quantification (LOQ). LOD and LOQ can be calculated with three and ten times the standard deviation of 10 replicate blank measurements respectively (Shim, Cho, Leem, Cho, & Lee, 2018).

1.6.3.2 Food consumption data

To obtain food consumption data, it is important to assess the dietary intake. Dietary intake can be assessed using one of two approaches: direct or indirect approach (Żukowska & Biziuk,2008)

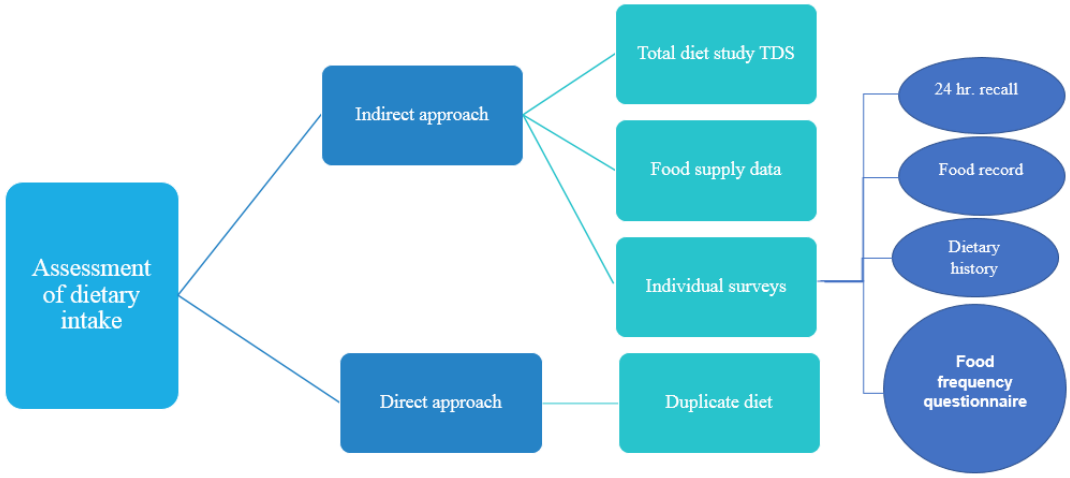

Table 4 Advantages and disadvantages of individual food consumption approaches (Żukowska & Biziuk, 2008)

Type of approach	**Advantages**	**Disadvantages**
Recall method	• detailed quantitative assessment of intake • estimates acute or chronic exposure	• difficulty in estimating portion size • 24 hr. recall: only 1 day
Food record	• no precoded food list • idea about various dietary practices	• possibility of incompliance • multiple contact with participants
Dietary history	• low burden for participants • estimate of habitual intake	• time consuming • recall bias
FFQ	• estimating intake for **lengthy periods of time** (weeks, months, years) • **easy** to complete • use of a **structured food list**	• time consuming • limitation of a precoded food list

According to FAO/WHO 2009, the general equation for dietary exposure is:

Dietary exposure= $\frac{concentration\ x\ consumption}{body\ weight}$ in mg/kg bw/d

1.6.4 Risk characterization

Risk characterization, is the final, integrative step of risk assessment process. It usually describes the nature and the magnitude of human risk (Fowle & Dearfield, 2000). Risk can be estimated using different methods.

1. <u>Comparison of dietary exposure with toxicologically **acceptable levels** such as Tolerable weekly intake TWI or oral reference dose Rfd or reference doses such as benchmark dose level BMDL</u>
    - Tolerable weekly intake (TWI) (set by European Food Safety Authority EFSA) µg/kg bw/week: It describes the amount of a substance that can be eaten every week throughout a person`s life with no risk of negative health effects (Lee *et al.*, 2006).
    - Benchmark dose (BMD) (set by set by European Food Safety Authority EFSA or by Codex Alimentarius): dose (based on dose-response data from key epidemiological studies) that corresponds to a specific change in an adverse response compared to the response in unexposed subjects, and the lower 95% confidence limit is termed the benchmark dose level (BMDL) (EFSA 2005) in µg/kg bw per day
    - Oral reference dose (Set by U.S (United States) Environmental Protection Agency US EPA): **(mg/kg bw/d):** The oral reference dose

Rfd approximates daily exposure to humans that is with no significant risk of harmful noncancer effects during one's life. (Jara & Winter, 2014)

Table 5 Risk characterization of certain heavy metals

Element	Chemical species	Toxicologically reference value	Toxicologically acceptable value	Reference
As	Total arsenic	No Health-based guidance value	-	-(Arnich *et al.*, 2012)
	Inorganic arsenic	BMDL $_{01}$	0.3-8 µg/kg bw/d (based on increased risk of lung, skin, and bladder tumors in humans)	EFSA 2009
	Inorganic arsenic	BMDL$_{0.5}$	3.0 µg/kg bw per day (2.0-7.0 µg/kg bw per day based on the range of estimated total dietary exposure	Codex Alimentarius 2018
Cd	Cadmium	TWI	2.5 µg/kg bw/week	EFSA 2009
Hg	Organic mercury (methylmercury)	TWI	1.3 µg/kg bw/week	EFSA 2012
	Inorganic mercury	TWI	4 µg/kg bw/week	

Pb	Lead	Reference doses	BMDL $_{01}$= 0.5 µg/kg bw/d (based on neurodevelopmental effects)	EFSA 2010
			BMDL $_{0.5}$= 1.5 µg/kg bw/d (based on cardiovascular effects)	
			BMDL 10= 0.63 µg/kg bw/d (based on nephrotoxic effects)	

* The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) concluded that the provisional tolerable weekly intake (PTWI) of 25 µg/kg b.w. set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and endorsed by the Scientific Committee of Food is **no longer appropriate.** The CONTAM Panel does consider it appropriate to calculate margins of exposure to support the risk characterization.

** The EFSA Panel on Contaminants in the Food Chain (The CONTAM Panel) concluded that the provisional tolerable weekly intake (PTWI) of 15 µg/kg b.w. established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is **no longer appropriate** as data had shown that inorganic arsenic causes cancer of the lung and urinary bladder in addition to skin, and that a range of adverse effects had been reported at exposures lower than those reviewed by the JECFA. The CONTAM Panel modelled the dose-response data from key epidemiological studies and selected a benchmark response of 1 % extra risk.

Table 6 Oral reference dose RFD for As, Cd, Pb and Hg

	Cd	**As**	**Pb**	**Hg**
Rfd (mg/kg bw/d)	**0.001** (Rahmani *et al.*, 2018)	**0.0003** (Jara & Winter, 2014) (Rahmani *et al.*, 2018)	**0.0004** (Rahmani *et al.*, 2018) (Ghasemidehkordi *et al.*, 2018)	**0.0001 (MeHg/organic) 0.0003 ($HgCl_2$/inorganic)** (Anyimah-Ackah, Ofosu, Lutterodt, & Darko, 2019)

2. <u>Non-carcinogenic risk can be evaluated using the target hazard quotient</u> **THQ.**

   The methodology for estimating THQ is described by the US EPA (US EPA 2000).

   The model is as follows (Liang *et al.*, 2019):

$$THQ = \frac{E_F E_D F_{IR} C}{R_{FD} W_{AB} T_A} \times 10^{-3}$$

Where **E_F is exposure frequency** (365 days/year);

E_D is the exposure duration (70 years), equivalent to

the average lifetime

F_{IR} is the food ingestion rate (g/person/day)

C is the metal concentration in foodstuffs (µg/g)

R_{FD} is the oral reference dose (mg/kg/day)

W_{AB} is the average body weight (60 kg for adults)

T_A **is the averaging exposure time for non-carcinogens** (365 days /year x number of exposure years, assuming 70 years)

If the value of THQ is greater than 1, then there is a risk that non-carcinogenic effects might occur. If the THQ is less than 1, then the risk of noncarcinogenic effects are negligible (Liang *et al.,* 2019)

Examples of non-carcinogenic risks can include impaired renal function, renal failure, liver damage, infertility, mental retardation, and coma (Sarkar *et al.,* 2016).

It has been reported that the exposure to two or more toxicants can lead to may in additive and/or interactive effects (Ullah, Maksud, Khan, Lutfa, & Quraishi, 2017). So, hazard index (also known as total target hazard quotient TTHQ) can also be calculated to evaluate aggregate noncarcinogenic risk of heavy metals. It is defined as the arithmetic sum of individual THQ of heavy metals. (Ghasemidehkordi *et al*., 2018)

Total THQ or HI = $THQ_1 + THQ_2 + THQ_{3\ldots}$ (Gupta, Ansari, Nasr, Chabukdhara, & Bux, 2018)

If the HI < 1, risk is not significant, however if HI$\geq$1 noncarcinogenic risk is possible. (Ghasemidehkordi *et al*., 2018)

Non-carcinogenic risk can also be expressed as **Hazard Quotient HQ**.

HQ for each toxic element can be estimated using the below models: (Tattibayeva *et al.*, 2015)

$$HQ = ADD/Rfd$$

$$ADD = C \times IR/ bw$$

Where **R_{FD} is the oral reference dose in mg/kg bw/d** (those reported by the Integrated Risk Information System (IRIS) of the US Environmental Protection Agency (EPA))

ADD is the average daily intake dose in mg/kg bw/d

C is the mean concentration of toxic elements obtained during analysis (mg/kg)

IR is the ingestion rate of product g/ (person/day),

BW is the average body weight (kg)

<u>3. Carcinogenic risk can be evaluated using:</u>

Margin of exposure approach: Margin of Exposure (MOE) approach is a methodology used to assess the risk of carcinogenic and genotoxic substances such as Pb and As (EFSA, 2005).

MOE is the ratio of no-observed-adverse-effect level (NOAEL) obtained from animal toxicology studies to the predicted, or estimated human exposure level or dose (EFSA, 2005; Wang *et al.*, 2019)

$$MOE = \frac{BMDL}{Dietary\ exposure\ estimate} \qquad \text{(EFSA, 2005)}$$

If the MOE is low, then it can be concluded that there exists a high carcinogenic risk for the exposed population. (EFSA, 2005; Brandon *et al.*, 2014)

If the MOE is high, then it can be concluded that there is an acceptable minimal risk for the exposed population (EFSA 2005; Brandon *et al*.,2014).

A MOE of less than 1 indicates that the population under study is at a high health risk for exposure (Juric *et al*., 2017).

1.7 Occurrence and risk assessment of heavy metals in food products

Dghaim *et al*. (2015), have done a study on 81 traditional herbs found in the UAE (United Arab Emirates) market. Out of the 81 samples they tested, 11 were Origanum Vulgare and 13 were Thymus Vulgaris. Among several metals tested, cadmium and lead were included in the analysis too. Results showed that the maximum level of cadmium in Oregano samples and in Thyme samples were 0.35 mg/kg and 0.63mg/kg, respectively. In terms of percentages, 9% and 27% of the total tested samples of Oregano and Thyme samples, respectively, exceeded the permissible limit of cadmium 0.3 mg/kg set by the FAO/WHO. Concerning lead, its concentration was 18.06 mg/kg for Oregano samples and 23.52 mg/kg for Thyme samples. In terms of percentages, 90% and 91% of the total tested samples of Oregano and Thyme samples, respectively, exceeded the permissible limit of 10mg/kg set by the FAO/WHO.

In Spain, Marín *et al.*, 2018 studied the occurrence of Pb, Cd, As and Hg in 81 different individual foodstuffs that were grouped into 12 food groups. Mercury was measured using cold vapor generation coupled with atomic fluorescence spectrometry (CV-AFS) and was only analyzed in fish and fish products. The other metals were measured using ICP-MS. Results showed that the second highest average Pb level was found in the cereal group 0.0438 mg/kg but was still below the normative limit set by the

EFSA. For Cd, the highest average level was found in fish (0.0816 mg/kg) while the average level found in the cereal group was 0.0271 mg/kg. All samples fell between the normative limits. As average level was highest in fish and fish products with a value of 2.1669 mg/kg. The average levels of Hg in fish products tested was 0.2515 mg/kg.

Barone *et al.*, 2018 measured the levels of toxic metals Hg, Cd and Pb in different cheeses found in Italy and assessed the risk of consuming such products by comparing the intake to the provisional weekly intake PTWI. The highest concentration of metals among hard and fresh cheeses were 0.13 µg/kg for Hg, 0.37 µg/kg for Pb and 0.01 µg/kg for Cd. Considering all concentrations of heavy metals in hard and fresh cheeses as well as highest concentration of metals found among all samples, none of the samples exceeded the safety weekly intake of Pb and Hg of 25 µg/kg bw and 5 µg/kg bw or the monthly intake for Cd of 25 µg/kg bw

Martín-Domingo *et al.*, 2017 assessed the levels of heavy metals in several herbal teas including thyme-containing tea. Their results showed that Cadmium in thyme (Thymus vulgaris) from some selling points have exceeded the Maximum Permissible limit of 0.3mg/kg set by the WHO and of 0.5mg/kg set by the European Pharmacopoeia for herbal medicines or herbal teas.

In Lebanon in 2012, Khozam *et al*. assessed the levels of toxic metals As, Cd, Hg and Pb in four of the most consumed types of white cheeses in the country regarding regional and seasonal variation. The forty samples of cheeses were collected in 10 sites geographically representative to the whole Lebanese territory. Results indicated that As and Pb concentrations (2.2 ±0.6 ng/g and 32.4±0.8 ng/g respectively) were below the permissible limit set by the Codex Alimentarius for dairy products As: 0.1–1.0 µg/g, Pb:

0.02 µg/g. Regarding Cd and Hg the mean ± SD was 0.14±0.04 ng/g and >LOD, respectively. However, according to the article there are no permissible limits for Cd and Hg in milk and dairy products in regulations related to food safety.

To study the concentrations of Pb and Cd in *O. syriacum* samples collected from several sale outlets in Lebanon, Dbaibo *et al*., 2020 collected four samples of dry ground leaves and 10 samples of dry ground leaves mixed with sesame and sumac (Zaatar). Out of the 4 leaf samples, none contained a toxic metal level higher than the MAL set by the WHO (2007). For cadmium, 2 out of the 10 samples contained a level higher than that set by the MAL at 0.3mg/kg. For Pb, the highest concentration in the 14 samples was found in one of the samples purchased from Beirut area at 26.33mg/kg. This value was more than double the limit of 10 mg/kg set by the WHO (2007). One other sample exceeded the permissible limit set for Pb by 0.43mg/kg. The rest of the samples were within the permissible and safe limits set by the WHO.

Nasreddine *et al.*,2010 found in the total diet study they conducted that the average concentrations of lead and cadmium in the tested food groups ranged between 0.54 and 16.43 µg/kg for lead and 3.07 to 30.23 µg/kg for cadmium. Vegetable-based products were the main contributors to lead and cadmium with 48.7 and 46.8% respectively. The second main contributors to lead and cadmium were bread and cereal products with 31.4 and 30.9 % respectively. The average cadmium and lead concentrations in bread and cereals group was 15 µg/kg fresh weight and 8.02 µg/kg fresh weight, respectively. To assess the intake and assess the relative risk, the average daily intake of lead was found to be 8.26µg/d which accounts for 3.2% of the PTWI of lead (25µg/kg BW/week) and the average daily intake of cadmium was found to be 15.82 µg/d which accounts of 21.7% of the PTWI OF cadmium (7µg/kg BW/week or 72.8 µg/d). Concerning dairy product group which contained strained yogurt or Labneh, the average concentrations of Pb and Cd were 0.54 µg/kg and 3.07 µg/kg, respectively.

In 2012, Khouzam, Pohl, Ayoubi, Jaber, & Lobinski, determined the levels of toxic metals As, Cd, Hg and Pb in three different varieties of Lebanese bread (white, brown and Saj) which were sampled at five geographical regions in Lebanon (Grand Beirut, South of Lebanon, North of Lebanon, Mount of Lebanon, and Beka'a) in wet and dry seasons. Mean concentrations of As, Cd, Pb and Hg in wet season were 9±2 µg/kg, 24 µg/kg, 18 µg/kg, and 0.5 µg/kg respectively and the mean concentrations of As, Cd, Pb and Hg in dry season were 33±4 µg/kg, 15 µg/kg, 28 µg/kg, and <LOD, respectively. They concluded that higher levels of As and Hg were found in Beirut region and higher levels of Pb was observed in the North region which they attributed this variation to higher levels of contamination possibly from production. For As, results showed that concentration significantly varied between the two seasons and they justified this difference by the possibility of seasonal variation of the composition of wheat grain and water.

The concentration of the trace elements As, Cd, Hg and Pb in Lebanese pita bread was studied by Lebbos *et al*., 2019 and the risk of exposure was evaluated by considering the concentration assessed relative to the amount consumed (based on a survey) and these were compared to tolerable daily intake (TDI), tolerable weekly intake (TWI) or toxicological reference points, such as benchmark dose limit (BMDL), to evaluate safety concerns. The results of the study showed that among the three most consumed brands in Lebanon, B3 brand was the highest source of trace element exposure. Cadmium consumption ranged on average from 0.13-0.2 µg/kg bw/week which was lower than the

European tolerable weekly intake TWI fixed by EFSA (2.5 µg/kg bw/week). Therefore, Cadmium was not considered a safety concern. For mercury, exposure ranged from 0.02-0.15 µg/kg bw/week which was lower than the European tolerable weekly intake TWI fixed by EFSA (1.3 µg/kg bw/week). Regarding Arsenic, exposure was more worrying and elevated levels were observed in all brands with a range of 235-400 µg/kg. In addition, the margin of exposures MOEs were very low suggesting that the situation in Lebanon is worrying. Considering a $BMDL_{0.1}$ of 0.5 µg/kg bw/d, the MOE of Pb ranged between 0.08 and 0.58 which is low and hence concluding that Pb intake via bread consumption in the Lebanese population is worrisome

This study provides, for the first time in Lebanon, an estimate of the concentrations of six toxic elements Pb, Cd, As and Hg in thyme and thyme products and characterizes the risk of heavy metals from the consumption of those products.

1.8 Objectives, research question and hypothesis

- The objectives of this study are:

To assess the occurrence of heavy metals Cd, Hg, Pb and As in thyme and thyme products marketed in Lebanon

To estimate the dietary exposure to these heavy metals among the Lebanese population

- The research question to be answered at the end of the study is:

“Is the Lebanese population exposed to toxic metals from thyme and thyme products consumption?”

- The hypothesis to be tested is as follows:

Due to poor environmental conditions as well as pollution, thyme and thyme products may be contaminated with heavy metals. This poses a health risk to the Lebanese population especially from highly consumed thyme and thyme products.

Chapter 2: Thesis

2.1 Introduction

Thyme has gained a wide popularity throughout history due to its active essential oil components (thymol and carvacrol) that have considerable positive impact on the health status of individuals in terms of their antioxidant, anti-inflammatory, neurological, respiratory, and gastrointestinal properties (Singletary, 2016).

Thyme, also known as "Zaatar", is one of the most consumed herbs in the Mediterranean region. In addition, Europe became one of the greatest importers of dried thyme herb worldwide. The increased interest in the consumption of thyme is its health benefit and the popular trend of following the Mediterranean diet. Statistics show a 2% increase in the annual growth rate of dried thyme imports in Europe from year 2015 to year 2019 (Cbi.eu, 2020). The frequent consumption of thyme and thyme products by the Lebanese people is related to the fact that Zaatar is readily grown and available, has a great taste, offers nutritional benefits and is affordable. The general population uses this plant in various forms and recipes; it can be consumed as dried or fresh herb, it is also used to prepare salads (thyme salads), thyme pie (Manooushe), thyme-flavored croissant, thyme-flavored "fatayer," among other food products (Batal & Hunter, 2007). That is why it is vital to ensure the safety of this frequently consumed food commodity.

Heavy metals are considered the main contributors to the chemical contamination of the food chain especially that they cannot be removed during routine cooking and processing methods. They gained thus a considerable attention worldwide and are recognized as public health concern (Lebbos et al., 2019; Marín et al., 2018).

These trace elements can occur naturally in the earth's crust because of volcanic eruptions, erosion, and weathering of rocks and/or can accumulate because of anthropogenic activities related to industries (mining, smelting, solid waste disposal) and agriculture (use of pesticides, fertilizers and irrigating with wastewater) (Arnich et al., 2012; Gupta et al., 2019). As a result, the contamination of the main environmental matrices which are air, soil and water, the transfer to the food chain and to humans became a major challenge (Lebbos et al., 2019).

Many studies have been done to estimate the dietary exposure to heavy metals from food commodities in different countries such as in Greece (Christophoridis et al., 2019), Spain (Marín et al., 2018a) , France (Arnich et al., 2012) , China (Wei et al., 2019), Saudi Arabia (Ali & Al-Qahtani, 2012), United Kingdom (Rose et al., 2010), and Argentina (Sigrist et al., 2016).

Few studies were done in Lebanon on heavy metals contamination of food products such as *Origanum syriacum* (dry ground leaves or dry ground leaves mixed with sesame, sumac, and salt) (Dbaibo et al., 2020); Lebanese cheese (Halloumi, double crème, Baladi, Labneh) (Khozam et al., 2012); Lebanese bread (Lebbos et al., 2019); Total diet study (Nasreddine et al., 2010).

Dbaibo et al., 2020 assessed the levels of only Cd and Pb in *Origanum Syriacum* herbs in Lebanon, but the authors didn't assess the levels of other heavy metals such as Hg and As in thyme and they didn't evaluate the contamination of thyme containing products.

Despite the frequent consumption of thyme and thyme products in Lebanon, an Eastern Mediterranean country, limited data is available on their contamination with chemical hazards as well as on the magnitude and severity of the dietary exposure of the general population. Food safety monitoring actions have lagged in the country, whereby evaluation on the national level, monitoring levels of heavy metals in food, the enforcement of regulatory actions and the available national data on food contamination levels are scarce (Nasreddine et al., 2016).

Even though, the Lebanese Standards Institution (LIBNOR) has put in place specific permissible limits related to heavy metals in some food commodities, including in Zaatar (NL 677:2017) , inspection, testing, and monitoring to ensure the conformity of the market food products to those limits are minimal.

It is essential to quantify the levels of heavy metals in a food product to evaluate their compliance to standards or regulatory limits, estimate the dietary exposure of the population and hence to assess the risk related to dietary intake (Marín *et al.,* 2018). Thus, the aim of this study was to assess the occurrence of heavy metals Pb, As, Cd and Hg in thyme and thyme products and their compliance to national and international standards.

2.2 Materials and methods

2.2.1 Collection of food samples and sampling methodology

After screening the Lebanese market, commonly commercialized thyme and thyme based- products were selected. Samples were divided into two categories: dried thyme herbs (Mixed thyme normal, Mixed thyme extra, Dried thyme herb, Tea thyme) and thyme based-products (thyme pie, cheese and thyme pie, thyme regular mix sandwich, thyme mix with nuts and seeds sandwich, pizza and pasta with thyme sauces, bread sticks with thyme (crunchy), sesame thick bread with thyme (soft), crackers with thyme, toast/bread with thyme, thyme croissant, traditional molded aged cheese with thyme/traditional strained yogurt balls with thyme, tea infusion, tea soaked, fresh thyme salad. For each of the above listed food items, a composite sampling approach was applied in this study. This consisted of purchasing the same food item from five different frequently consumed brands/varieties found in popular retail markets in Lebanon according to a food frequency questionnaire done in another study. The five samples were combined and blended to give a homogeneous sample representative of each food item. The contribution of each sub- sample to total weight was 20% w/w (Nasreddine et al., 2010).

For thyme-based products, 13 food items x 5 brands/varieties = 65 individual thyme-based products were collected and 13 composite samples were tested.

For dried thyme herbs, 3 food items x 5 brands/varieties = 15 individual thyme herbs items were collected and 3 composite samples were tested.

This complete set of samples was collected twice. The first sample collection was done in September 2019, and the second one after six months in February 2020 to consider variability between different production dates. Therefore, 180 individual samples (30 thyme herbs + 150 thyme-based products) were collected and 36 composite samples (6 thyme herbs + 30 thyme-based products) were tested.

2.2.2 Sample preparation

All food items were tested as collected and were not subject to any cooking or preparation except pasta, pizza, and tea (infusion and soaked). Pasta and pizza were prepared following the instructions of a traditional cookbook (Kamal and Othman 1992) using tomato thyme sauces as well as dried thyme as a seasoning herb. Raw ingredients were purchased from the market and step by step cooking instructions were followed to produce a typical dish cooked at a Lebanese home or restaurant. Tea was prepared as infusion (soaking the herbs in hot water) and soaked (boiling the herbs with water) using commercial bottled drinking to simulate consumer's preparation habits. To prepare the tea infusion, 0.75g of the composite tea sample was taken and infused in 100 ml boiling water (drinking bottled water) for 5 minutes in a beaker covered with watch glass as per the consumer instructions written on the tea bag. To prepare the soaked tea, 0.75g of the composite tea sample was taken and soaked in 100 ml drinking bottled water and boiled for 5 minutes in a beaker covered with a watch glass as per the traditional tea preparation in Lebanon (Korfali, Mroueh, Al-Zein, & Salem, 2013).

Samples were homogenized using a blender/mixer system. The blender was properly and thoroughly cleaned with soap and deionized water between each brand/variety of food item. After proper homogenization, 20% weight was taken from each brand/variety of food items to create for each item a representative composite sample. Using clean plastic spoons, further mixing was done to ensure that the composite sample is properly homogenized.

Samples were stored at -18˚C until analysis.

2.2.3 Chemicals

Reagents used in this study were of high purity: lead, cadmium, mercury, and arsenic standard solutions (Merck, Darmstadt, Germany), Hydrogen peroxide solution 30% (Sigma Aldrich, Germany), Nitric acid 69% (BDH Laboratory supplies, England), Hydrochloric acid 37% (AnalaR Normapur, France) or of high analytical grade. A certified groundwater reference material (BCR®-610) from the Institute for Reference Materials and Measurements (IRMM) was used.

All solutions were prepared with analytical reagent grade chemicals and ultrapure water (18 MV.cm) obtained by purifying distilled water using a Milli-Q water purification system (Millipore S.A., St Quentin-en-Yvelines, France).

2.2.4 Sample digestion

The microwave digestion includes wet digestion, which relies on the oxidizing power of chemical reagents such as oxidants and acids. A microwave oven (Multiwave ECO, Anton Paar) was used for sample digestions. All the studied samples followed the same digestion protocol except for soaked and infused tea. About 0.5 g (accurate to 0.001 g) of previously homogenized samples were weighed and placed inside Teflon vessels with 8 mL concentrated nitric acid (69%, BDH Laboratory supplies, England) and 2 mL hydrogen peroxide (37%, Sigma Aldrich, Germany). To prepare the tea infusion, 0.75g of the composite tea sample was taken and infused in 100 ml boiling water (drinking bottled water) for 5 minutes as per the consumer instructions written on the tea bag. Then, 10 g of the solution were taken and placed inside Teflon vessels with 8 mL concentrated nitric acid (69%, BDH Laboratory supplies, England) and 2 mL hydrogen peroxide (37%, Sigma Aldrich, Germany). To prepare the soaked tea, 0.75g of the composite tea sample was taken and soaked in 100 ml drinking bottled water for 5 minutes as per the traditional tea preparation in Lebanon. Then, 10 g of the solution were taken and placed inside Teflon vessels with 8 mL concentrated nitric acid (69%, BDH Laboratory supplies, England) and 2 mL hydrogen peroxide (37%, Sigma Aldrich, Germany).

The sample digestion procedure was performed according to *Table* 7 Operating conditions for *the microwave oven digestion*: (1) 850 W at 180˚C for 10 min and (2) 850 W at 22 ˚C for 1 min for cooling. After microwave digestion, 2 mL HCl (37%, AnalaR Normapur, France) were added to all sample solutions (expect tea infusion and soaked tea) and the digested samples were then quantitatively transferred into 50-mL flasks. The contents were diluted 5 times with 3% nitric acid (prepared with ultrapure deionized water), labeled accurately and stored at 4˚C until ICP-MS analysis.

2.2.5 ICP-MS analysis

Elemental analyses were carried out using Thermo iCAP Q / iCAP RQ ICP-MS instrument (Thermo Fisher Scientific Inc., Bremen, Germany) operating under argon gas of spectral purity (99.9995%, Air Products Middle East, Dubai, U.A.E.). The instrument parameters are shown in Table 2. Before each experiment, the instrument was tuned for daily performance, using iCAP Q / RQ TUNE solution (supplied by Thermo scientific, Bremen, Germany). It is an aqueous multi-element standard solution of Ba, Bi, Ce, Co, In, Li and U 1.0 µg/L (each) in 2% HNO_3 + 0.5% HCl. Each solution was measured 3 times and the quantification of cadmium, lead, arsenic, and mercury was carried out using external calibration curves. Standard solutions were prepared in 3% nitric acid, and for each element, the calibration curve was plotted using eight different concentrations (Figures 1, 2, 3, 4). A certified reference material (BCR-610, water sample) was used to determine the accuracy of the method. Limit of detection (LOD) values for the method, for each element, were determined by blank determination assays, as 3 times standard deviation of 20 blank replicates. Limit of quantification (LOQ) values were calculated as 2 times LOD for each element. The results of LOD and LOQ are displayed in *Table 8 LOD and LOQ of As, Cd,* *Hg and Pb*. The analytical quality control was confirmed by the recovery experiments for cadmium, lead, arsenic, and mercury, spiking at two selected concentrations, before and after microwave digestion. The torch position, ion lenses, gas output, resolution axis (10% of peak height) and background (< 20 shots) were optimized with the tuning solution (1 mg L^{-1}) to carry out a short-term stability test of the instrument, to maximize ion signals and to minimize interference effects due to high oxide levels.

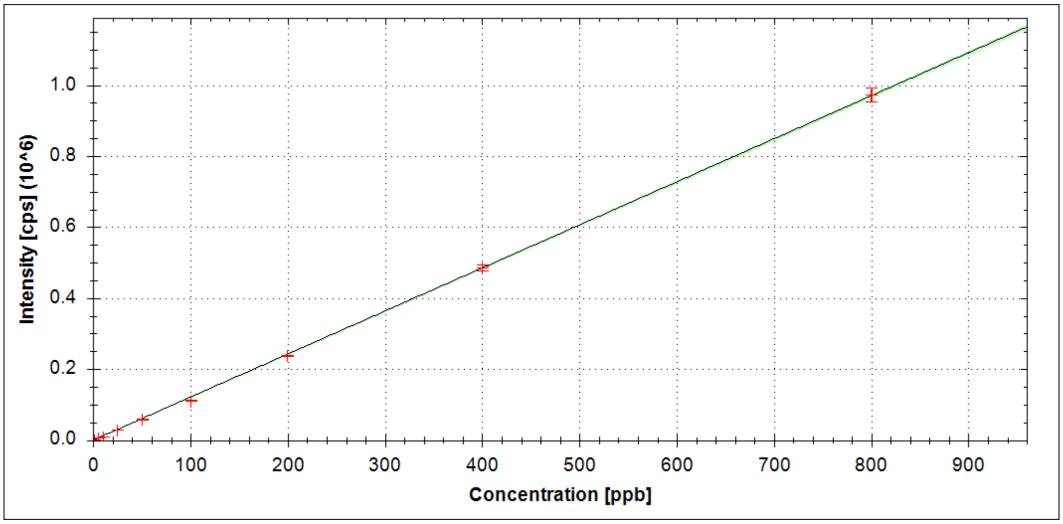

Figure 1 Calibration curve of Arsenic

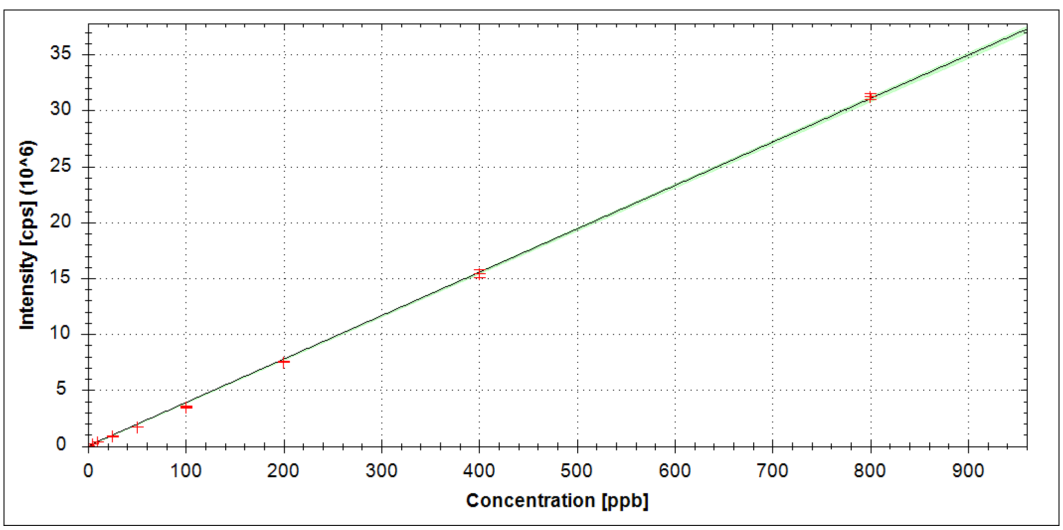

Figure 2 Calibration curve of Cadmium

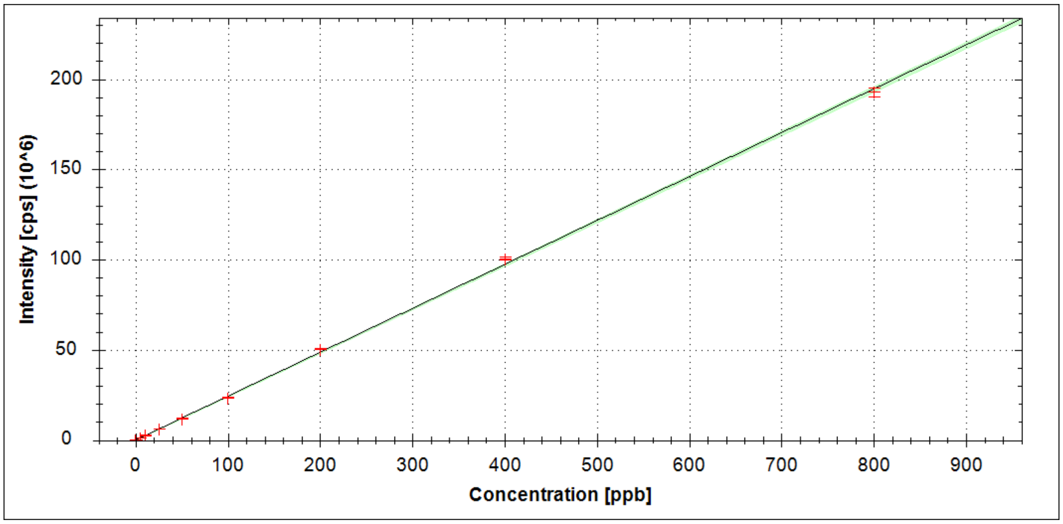

Figure 3 Calibration curve of Lead

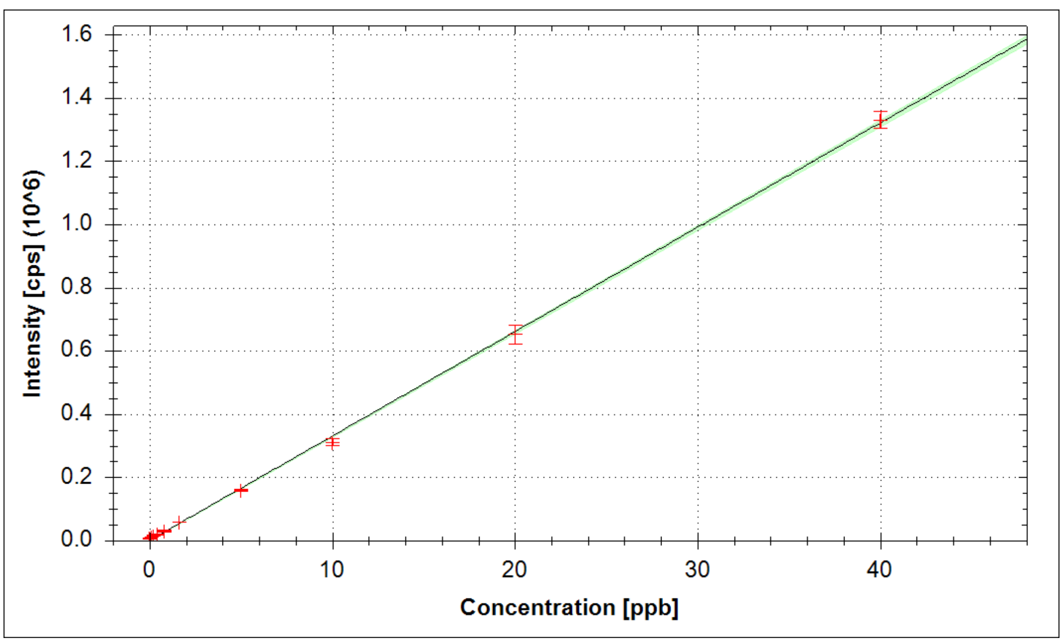

Figure 4 Calibration curve of Mercury

Table 7 Operating conditions for the microwave oven digestion

Step	Temperature (˚C)	Ramp time (Min)	Hold time (Min/sec)	Fan (level)	Power (W)
1	180	20	10 mins	1	850
2 (Cooling)	22	10	30 sec	3	850

Table 8 LOD and LOQ of As, Cd, Hg and Pb

Element	LOD (ppb)	LOQ (ppb)
Arsenic	0.054	0.108
Cadmium	0.031	0.062
Mercury	0.038	0.076
Lead	0.038	0.075

2.2.6 Quality control parameters

All analyses were performed according to protocols of quality assurance, including triplicate readings, reagent blanks, spiking of samples and certified reference materials as per the AOAC guidelines (AOAC International 2013).

For all the tested metals, the correlation coefficient of the reference curve R^2 was satisfactory (R^2 >0.995).

The **accuracy** of the method was checked by analysing a certified reference material groundwater CRM (BCR-610) and rice flour reference material (IRMM-804)

(N. Khan, Jeong, et al., 2014). The recovery percentages obtained for the reference material is presented in *Table 9 Heavy metals in water and in rice flour reference materials*. The percentage recoveries were within the acceptable range (75-125%) (AOAC International, 2013).

The **analytical quality control** and the precision for the four tested heavy metals were verified by applying the spiking methodology at two selected concentrations (one high and one low) (Table 6), and then calculating the recovery percentages for each metal (Shim, Cho, Leem, Cho, & Lee, 2018). Thyme pie, sandwich thyme mix with nuts and seeds, bread sticks with thyme, pizza & pasta, thyme mix extra with nuts and seeds, thyme croissant, traditional molded aged cheese with thyme, infused tea with thyme were chosen for the spiking experiments at low and high concentrations. Low spiking concentrations chosen were 10 times the LOQ and the high spiking concentrations were approximately equal to typical values or permissible limits for each matrix (AOAC International 2013). The same digestion protocol was followed for spiked samples. Approximately 0.5 grams of each of the composite samples chosen (except for infused tea) were weighed in a clean microwave vessel. Afterwards, each of the following chemical reagents were added to each of the microwave vessels: 8 mL of 69% nitric acid and 2 mL of 30% hydrogen peroxide in addition to Pb, Cd, As, and Hg standards prior to microwave digestion.

After the microwave digestion cycle, 2ml of 37% hydrochloric acid was added to each of the digestion vessels (except tea infusion) and the solutions were then quantitatively transferred to properly labeled polypropylene tubes.

To prepare the tea infusion, 0.75g of the composite tea sample was taken and infused in 100 ml boiling water for 5 minutes as per the consumer instructions written on the tea bag.

Then, 10 g of the solution were taken and the following chemical reagents were added to each of the microwave vessels: 8 mL of 69% nitric acid and 2 mL of 30% hydrogen peroxide in addition to Pb, Cd, As, and Hg standards prior to microwave digestion.

Unspiked samples were also analyzed following the same protocol to calculate the spike recovery.

Recovery percentages were calculated to express trueness (Llorente-Mirandes *et al*., 2014). The following formula was used: Recovery (%) = (a/ (b+c))*100 where a is the Pb, Cd, As, and Hg concentration measured in the extracts of samples which were spiked with standards solutions; b is the Pb, Cd, As, and Hg concentration measured in the unspiked sample and c was the known concentration added to the sample. Results of recovery percentages were between 72.1 %- 125.4% which is acceptable according to the AOAC International 2013 method (70-130%) and are presented in *Table 11 Recovery range of Elements from Spiked Samples before Microwave digestion*

Table 9 Heavy metals in water and in rice flour reference materials

Heavy metal / reference material	**Limit (mg/kg)**	**Recovery %**
Water reference material		
Arsenic (As)	0.01±0.4	105.1
Cadmium (Cd)	0.003±0.08	108.3
Lead (Pb)	0.08 ± 0.13	120.6
Rice flour reference material		
Arsenic (As)	0.049 ± 0.04	99.5

Cadmium (Cd)	1.61 ± 0.07	104.6
Lead (Pb)	0.42 ± 0.07	112.5

Table 10 Spiking concentrations (ppm) at two different levels (low and high) for eight chosen food products

Food matrix	Level 1 spiking (low concentration) (ppm)	Level 2 spiking (high concentration) (ppm)
Thyme pie (manooushe only zaatar)	Hg 0.7 Pb 0.7 As 0.1 Cd 0.6	Hg 0.1 Pb 1 As 1 Cd 0.2
Sandwich thyme mix with nuts and seeds (zaatar mix extra with nuts, seeds)	Hg 0.7 Pb 0.7 As 0.1 Cd 0.6	Hg 0.1 Pb 0.2 As 0.2 Cd 0.2
Bread sticks with thyme (kaak bi zaatar crunchy)	Hg 0.7 Pb 0.7 As 0.1 Cd 0.6	Hg 0.1 Pb 0.2 As 0.2 Cd 0.2
Pizza & pasta	Hg 0.7 Pb 0.7 As 0.1 Cd 0.6	Hg 0.1 Pb 1 As 0.2 Cd 0.2
Thyme mix extra with nuts and seeds (zaatar mix extra with nuts, seeds)	Hg 0.7 Pb 0.7 As 0.1 Cd 0.6	Hg 1 Pb 10 As 1 Cd 0.3
Thyme croissant (croissant zaatar)	Hg 0.7	Hg 0.1

	Pb 0.7 As 0.1 Cd 0.6	Pb 0.2 As 0.2 Cd 0.2
Cheese with thyme (chanklish with zaatar)	Hg 0.7 Pb 0.7 As 0.1 Cd 0.6	Hg 0.1 Pb 0.2 As 0.2 Cd 0.2
Infused tea with thyme (zaatar tea infusion)	Hg 0.7 Pb 0.7 As 0.1 Cd 0.6	Hg 0.5 Pb 5 As 1 Cd 0.2

Table 11 Recovery range of Elements from Spiked Samples before Microwave digestion

Element	Recovery range %
Arsenic	78.1-125.4
Cadmium	87.4-122.7
Mercury	76.5-123.9
Lead	72.1-123.6

2.2.7 Data analysis

For each food item, the heavy metal content was assessed in duplicate (two different collections) and each test was done by ICP-MS in three replicates. Average values were presented with related standard deviation (SD).

2.2.8 Results, discussion, and conclusion

Heavy metals content in thyme and thyme products was assessed according to the limits set Internationally by Codex Alimentarius and locally by the Lebanese Standards Institution (LIBNOR) *Table 12 Maximum allowed limits (mg/kg) of different heavy metals in tested foodstuffs according to LIBNOR and CODEX standards*. Percentages of acceptable and unacceptable concentrations are presented below.

Table 12 Maximum allowed limits (mg/kg) of different heavy metals in tested foodstuffs according to LIBNOR and CODEX standards

Tested matrix	As		Cd		Pb		Hg	
	LIBNOR	CODEX	LIBNOR	CODEX	LIBNOR	CODEX	LIBNOR	CODEX
Thyme based products								
Thyme pie	0.5[a]	0.2[d]	0.2[e]	0.2[g]	0.2[f]	0.2[r]	0.5[h]	0.1[i]
Cheese and thyme pie	0.5[a]	0.2[d]	0.2[e]	0.2[g]	0.2[f]	0.2[r]	0.5[h]	0.1[i]
Thyme regular mix sandwich	0.5[a]	0.2[d]	0.2[e]	0.2[g]	0.2[f]	0.2[r]	0.5[h]	0.1[i]
Thyme mix with nuts and seeds sandwich	0.5[a]	0.2[d]	0.2[e]	0.2[g]	0.2[f]	0.2[r]	0.5[h]	0.1[i]
Pizza and pasta with thyme sauces	0.5[a]	0.2[d]	0.2[e]	0.2[g]	0.2[f]	0.2[r]	0.5[h]	0.1[i]
Bread sticks with thyme (crunchy)	0.5[a]	0.2[d]	0.2[e]	0.2[g]	0.2[f]	0.2[r]	0.5[h]	0.1[i]
Sesame thick bread with thyme (soft)	0.5[a]	0.2[d]	0.2[e]	0.2[g]	0.2[f]	0.2[r]	0.5[h]	0.1[i]
Crackers with thyme	0.5[a]	0.2[d]	0.2[e]	0.2[g]	0.2[f]	0.2[r]	0.5[h]	0.1[i]
Toast/bread with thyme	0.5[a]	0.2[d]	0.2[e]	0.2[g]	0.2[f]	0.2[r]	0.5[h]	0.1[i]
Thyme croissant	0.5[a]	0.2[d]	0.2[e]	0.2[g]	0.2[f]	0.2[r]	0.5[h]	0.1[i]
Cheese with thyme	0.5[a]	0.2[d]	0.2[b]	0.2[l]	0.2[j]	0.02[k]	0.5[h]	0.1[i]
Tea infusion	0.05[s]	0.01[n]	0.01[s]	0.003[o]	0.05[s]	0.01[p]	0.001[s]	0.001[q]
Tea soaked	0.05[s]	0.01[n]	0.01[s]	0.003[o]	0.05[s]	0.01[p]	0.001[s]	0.001[q]
Fresh thyme salad	0.5[a]	0.2[d]	0.2[b]	0.2[l]	0.3[c]	0.3[m]	0.5[h]	0.1[i]

Dried thyme herbs								
Mixed thyme normal	0.5[a]	0.2[d]	0.2[b]	0.2[l]	0.3[c]	0.3[m]	0.5[h]	0.1[i]
Mixed thyme extra	0.5[a]	0.2[d]	0.2[b]	0.2[l]	0.3[c]	0.3[m]	0.5[h]	0.1[i]
Dried thyme herb	0.5[a]	0.2[d]	0.2[b]	0.2[l]	0.3[c]	0.3[m]	0.5[h]	0.1[i]
Tea thyme	0.5[a]	0.2[d]	0.2[b]	0.2[l]	0.3[c]	0.3[m]	0.5[h]	0.1[i]

[a] **Arsenic** Thyme.NL 677:2017 Lebanese Standards Institution LIBNOR
[b] **Cadmium** Thyme.NL 677:2017 Lebanese Standards Institution LIBNOR
[c] **Lead** Thyme.NL 677:2017 Lebanese Standards Institution LIBNOR
[d] **Arsenic** Rice polished Codex Alimentarius CXS 193-1995 (The ML is for inorganic arsenic (As-in)).
[e] **Cadmium** Lebanese bread. NL 240:2010 Lebanese Standards Institution LIBNOR
[f] **Lead** Lebanese bread. NL 240:2010 Lebanese Standards Institution LIBNOR
[g] **Cadmium** Wheat. Codex Alimentarius CXS 193-1995
[h] **Mercury** Instant tea.NL 610:2002. Lebanese Standards Institution LIBNOR
[i] **Mercury** Salt, food grade. Codex Alimentarius CXS 150-1985.
[j] **Lead** Chanklish.NL 507:2009 Lebanese Standards Institution LIBNOR
[k] **Lead** Milk and secondary milk products. Codex Alimentarius 2015
[l] **Cadmium** Leafy vegetables. Codex Alimentarius CXS 193-1995
[m] **Lead** Leafy vegetables. Codex Alimentarius CXS 193-1995
[n] **Arsenic** Natural mineral waters (Calculated as total As in mg/l) CXS 108-1981
[o] **Cadmium** Natural mineral waters CXS 108-1981
[p] **Lead** Natural mineral waters CXS 108-1981
[q] **Mercury** Natural mineral waters CXS 108-1981
[r] **Lead** Cereal grains. Codex Alimentarius CXS 193-1995
[s] **Arsenic, Cadmium, Lead, Mercury** Bottled drinking water. NL 162:1999. Lebanese Standards Institution LIBNOR

Arsenic

Arsenic was detected in all the 36 food composite samples tested. The average concentration was 0.087 mg/kg and ranged between 0.001 and 0.609 mg/kg. Results showed that 89% (32/36) of the composite samples tested were acceptable according to both national and international standards, while 11% (4/36) were above the acceptable limits (0.5 and 0.2 mg/kg for LIBNOR and Codex Alimentarius, respectively).

Acceptable levels of arsenic concentrations were found in all the products except tea thyme and dried thyme herbs that showed the highest concentrations of 0.609 and 0.557 mg/kg, respectively.

In contrast, the lowest mean concentration of As was found when water was added to prepare both soaked and infused tea (0.001 mg/kg).

Thyme tea was similarly contaminated with As in both Italy and Tunisia but at lower concentrations (0.33 and 0.155 mg/kg, respectively) (Potortì *et al*., 2020).

In another study done by Reinholds *et al.*, (2016) in Latvia, dried thyme and oregano showed the highest two levels of As (0.3 and 0.37 mg/kg respectively) among other five different condiments black pepper (0.06 mg/kg), paprika (0.1mg/kg), nutmeg (0.03 mg/kg) and basil (0.13 mg/kg).

In the dairy category, mean concentration of As in four cheese varieties (Halloumi, Double crème, Baladi and Labneh) found in the Lebanese market was 0.0022 mg/kg which is lower than the amount available in our tested cheese with thyme (0.007 mg/kg) (Khozam *et al.*, 2012).

However, higher concentrations of As were detected in Lebanese pita bread (0.4 mg/kg) by Lebbos *et al.*, 2019 as compared to the ones obtained in the current study for wheat-containing thyme products (i.e. thyme pie, thyme and cheese pie, thyme regular mix sandwich, thyme mix with nuts and seeds sandwich, bread sticks with thyme, sesame thick bread with thyme, crackers with thyme and toast/bread with thyme).

The main contributors to the level of arsenic in Lebanese bread samples were the contaminated water used in the process of bread preparation and the irrigation water used for wheat plants cultivation (Lebbos et al., 2019). The concentration of As was between 1.2 and 4.5 µg/L in drinking water in Lebanon and between 0.8±0.3 µg/L and 1.2±0.2 µg/L in the irrigation water for wheat in the Bekaa region (Lebbos et al., 2019).

Organic and inorganic arsenic, which are two different chemical forms of arsenic, differ in their toxicities. (Devesa, Vélez, & Montoro, 2008). Inorganic species, arsenite (AsIII) and arsenate (AsV), are known to have a higher toxicity than organic arsenic compounds (Sadee, Foulkes, & Hill, 2015). According to sufficient epidemiological studies, the IARC classified inorganic As as a class 1 carcinogen as its exposure can increase the risk of skin, lung, and urinary bladder cancer (Centre international de recherche sur le cancer, 2012). Moreover, other long-term health effects can arise from chronic exposure to dietary arsenic. These can include cardiovascular diseases, skin lesions, disrupted glucose metabolism and diabetes (EFSA 2009).

One of the main sources of exposure to arsenic are practices used by farmers to enhance their farming systems, foods grown on contaminated soil, food irrigated with contaminated water and foods washed and prepared with As-containing water (Cai et al., 2015; Kapaj, Peterson, Liber, & Bhattacharya, 2006).

Cadmium

Cadmium was detected in all the samples at levels within the acceptable limits set by both standards. The average concentration was 0.027 mg/kg and ranged between 0.001 and 0.087 mg/kg.

Among all the products tested, mixed thyme extra in the dried herb category had the highest concentration of Cadmium (0.0875 mg/kg) while soaked tea had the lowest level of contamination with Cd (0.01 mg/kg).

In the category of thyme-containing products, thyme croissant was mostly contaminated with Cd with an average value of 0.035 mg/kg.

The Cd values in dried thyme herbs (0.054 mg/kg) obtained in this study were lower than those reported in a study done in UAE by Dghaim *et al.*, 2015 in which results showed that 9% of Oregano samples (< 0.1-0.35 mg/kg) and 27% of thyme samples (<0.1-0.63 mg/kg) exceeded the permissible limit of 0.3 mg/kg set by the FAO/WHO for medicinal herbs and plants.

For milk and dairy products, a Total Diet Study (TDS) done in Valencia, Spain has found a mean concentration of Cadmium at 0.0085 mg/kg in milk, cheese, yogurt, custards and smoothie, butter, and soybean products (Marín et al., 2018). This level was close to the one found in our tested cheese with thyme samples (0.009 mg/kg).

In the bread and cereals category, a previous TDS done in Lebanon (Nasreddine *et al*., 2010), showed that the mean concentration of Cd was 0.015 mg/kg which is like the current average concentration of 0.019 mg/kg in the same category (Table 2).

In contrast to the acceptable Cd levels found in our tested herbal tea thyme, high contamination levels (0.29 mg/kg) were found in collected samples from several supermarkets, street herbal markets as well as herbal stores in Spain (Martín-Domingo et al., 2017).

The difference in the concentration of toxic metals in herbal teas and dried herbs could be explained by several factors including the difference in metal uptake, which is influenced by the soil characteristic like pH, climate factors and anthropogenic conditions like pollution and the presence of industries next to the plants (Martín-Domingo *et al.*, 2017).

In nature and in the environment, cadmium is known to occur in its inorganic form. (EFSA, 2009)

All the compounds related to cadmium are considered highly toxic to humans.

According to the European commission, the main source of Cd exposure is through the food chain for the non-smoking population and more specifically the food groups that contribute mostly to this exposure are cereals and cereal-based products, vegetables in addition to nuts and pulses (European commission, Cadmium in food, n.d.)

Cadmium and its compounds were classified by the IARC carcinogenic to humans (CODEX 2018). Adverse health issues have been reported following Cd toxicity. These include severe pain in joints and spine (Itai-itai disease), kidney damage, hypertension, and genetic mutations (P. Singh et al., 2020).

Cd could reach the food chain due to many human activities such as the use of chemical fertilizers, pesticides, herbicides, and animal manure in addition to the proximity of soil to industrial areas (Cai *et al.*, 2015).

Lead

The average Pb concentration was 0.55 mg/kg and ranged between 0.014 and 1.21 mg/kg. Lead was detected in all the samples but only 14% (5/36) and 3% (1/36) were acceptable according to Libnor and Codex Alimentarius standards, respectively.

Toast bread with thyme was acceptable according to both standards while infused and soaked tea were only acceptable according to the local standard. *Table 14 Percentage of acceptable and unacceptable toxic element content in thyme products and dried thyme herbs in first and second collection according to LIBNOR and Codex Alimentarius Standards*.

Interestingly, while the highest mean Pb concentration was detected in the dried tea thyme samples *Table 13 Average heavy metal content in thyme-containing products and dried thyme herbs.*, the lowest ones were found after preparation of the infused and soaked tea. The addition of non-contaminated drinking water can thus help to reduce or dilute the heavy metals levels present in the dried herb.

In a TDS done in Berlin, Germany (Kolbaum et al., 2019), the mean Pb concentrations in milk and dairy products (1.31-1.51 µg /kg), spices (32.29- 32.41 µg/kg), tea and infusions (5.58- 5.63 µg/kg) were below the levels found in similar food groups in the current study *Table 13 Average heavy metal content in thyme-containing products and dried thyme herbs.*

Lebanese pita bread (Lebbos et *al.*, 2019) showed lower levels of lead contamination (0.26 mg/kg) than our tested sandwiches (made with pita bread) that had the additional contribution of thyme mix (0.785 mg/kg) or thyme mix with nuts (0.401 mg/kg).

The average concentrations of Pb in dry ground thyme leaves (*Origanum syriacum)* (5.67 mg/kg) and in thyme mixture (*Origanum*, sumac, sesame, and salt) (8.55 mg/kg) previously collected from several stores in Beirut, Lebanon (Dbaibo et al., 2019), showed much higher contamination levels than the same products assessed in our study.

This variability can be related to several factors such as harvesting location sites, soil contamination, irrigation water, environmental pollutions, emerging food brands coming from non-reputable sources and lack of inspection efforts in Lebanon as the situation in the country is unstable politically and economically. Thus, there was a positive correlation between the presence of toxic metal Pb in soil and its level in the *Origanum syriacum* tissues, which in turn is reflected in the products derived from the plant (Dbaibo et al., 2020).

According to the substance priority list done by the Agency for Toxic Substances and Disease Registry (ATSDR) in 2015, lead is ranked the 2nd most potentially threatening substance to human health (Bassil *et al.,*2 018). The International Agency for Research on Cancer IARC classified inorganic lead as a probable carcinogen (Group 2A) (CODEX 2018). Elevated levels of Pb exposure can have detrimental effects on vulnerable populations such as pregnant women leading to miscarriages; it can also lead to a damage in the brain. (Singh et al., 2020). Diet and foodstuffs are known to be the primary source of non-occupational exposure (Wang *et al*.,2019).

Pb can exist in two chemical forms: organic and inorganic (Kumar *et al.*, 2020). All the chemical forms are toxic; however, the organic form is more readily absorbed by the gastrointestinal tract than the inorganic form, but most lead that is found in the environment is in the inorganic form (Silva *et al*., 2005).

In addition, the presence of Pb in the environment (air, soil, and water) could be associated with anthropogenic activities and products such as industrial activities agrochemicals, oil-processing activities, water from old pipes, plumbing materials, and natural events such as volcanic eruptions, water from mining areas and geochemical weathering (Kumar et al., 2020). Moreover, the source of Pb contamination in foodstuff can be due to the food contact material and/or the water used during food preparation (Lebbos et al., 2019).

Mercury

Mercury was detected in all samples while 92% (33/36), and 78% (28/36) of the products were accepted according to LIBNOR and Codex Alimentarius, respectively.

The average concentration was 0.076 mg/kg and ranged between 0.003 and 0.119 mg/kg.

Tea thyme and dried thyme herb were acceptable with reference to the national standards but were above the permissible international limits. However, tea infused and soaked were rejected according to both standards. Despite the high dilution effect of added water for tea preparation, the two samples remained unacceptable. This highlights the need to keep the contamination levels as low as possible in spices and herbs.

In Greece (Christophoridis et al., 2019), the mean concentration of Hg (0.002 mg/kg) and the range (0.0002-0.006 mg/kg) in fifteen cheese samples (cow cheese, blue cheese, Kaseri cheese of Macedonia, Kaseri cheese, Feta cheese of central Greece, Feta cheese, Feta cheese of W. Macedonia, Kefalograviera, Gruyere of Cyclades, Metsovone cheese, Cottage cheese, Mozzarella cheese, Parmesan cheese, Mizithra cheese and cream) were much lower than the values obtained in the present study for Hg in Cheese with thyme (0.047 mg/kg). Dairy products might have elevated levels of contamination due to a high chemical affinity of toxic metals such as Hg to certain products in dairy such as casein (Christophoridis et al., 2019a)

Kowalska (2021) have recently done a study to assess the safety of commonly consumed herbs, spices, tea, and coffee in Poland. Thyme samples showed a low level of Hg contamination (< LOQ of 0.005 mg/kg) in contrast to our study which showed a higher level of Hg in dried thyme herb (0.119 mg/kg).

All the cereals-containing products in the current study showed higher levels of mercury (range 0.073-0.092 mg/kg) as compared to similar ones in a TDS done in France (Guérin et al., 2018). For example, rice and wheat products, croissant like pastries and pasta had lower contamination ranges of 0.00025-0.0005, 0.00-0.0005, and 0.00- 0.005 mg/kg, respectively.

In Lebanon, the mean concentration of Hg in three varieties of Lebanese bread white, brown and Saj bread from five different geographical seasons and at two different seasons: wet and dry was found to be 0.0005 mg/kg (Bou Khouzam et al., 2012). This concentration was lower than our tested bread combined with thyme herbs mixes (0.087-0.073 mg/kg).

Mercury is one of the most studied toxic elements as it impacts human health negatively (Leopold *et al.*, 2010). The toxicity of mercury is known to be correlated with its chemical form (Cabañero *et al*., 2005). Mercury exists in three chemical forms: elemental or metallic mercury (Hg0), inorganic mercury (mercurous (Hg2 and mercuric (Hg2+) cations) and organic mercury (EFSA 2012). In general, organic mercury compounds are known to be more toxic than inorganic and elemental forms (Gao *et al.*, 2012). Methylated forms have a higher toxicity (by a factor of 10). (Silva *et al.*, 2005)

Methylmercury is regarded as the most toxic species regarding bioaccumulation and risk (Cabañero *et al.*, 2005; Leopold *et al.*, 2010). It is the most prevalent form of organic mercury found in the food chain (EFSA 2012). Effects of mercury toxicity lead to negative and harmful impact on several organs including the lungs, kidney, digestive system, and immunity (Singh et al., 2020).

The presence of Hg in food products could be related to several reasons like the animal consumption of contaminated feed and/or water, the contamination of pasturelands with Hg-containing pesticides and fungicides (Christophoridis et al., 2019a). According to Ghasemidehkordi et al. (2018a), vegetables and herbs could be contaminated with Hg as they can absorb and accumulate the toxic element in their tissues. This contamination can emerge from contaminated soils, contaminated irrigation water and deposits from polluted air. The source of contamination can be due to the combustion of fossil fuels, pulp and paper industries, medicinal waste products, production of cement and power plants. (Ghasemidehkordi et al., 2018a).

In summary, a higher heavy metal content of As, Pb, Cd and Hg was seen in the dried thyme herb category than in the thyme products category. This could be related to an increased level of contamination in herbs food matrix than in wheat, dairy, and fresh vegetables matrices.

Table 13 Average heavy metal content in thyme-containing products and dried thyme herbs.

Thyme products categories	**Average As value of both sample collections in mg/kg ± SD**	**Average Cd value of both sample collections in mg/kg ± SD**	**Average Pb value of both sample collections in mg/kg ± SD**	**Average Hg value of both sample collections in mg/kg ± SD**
Thyme products				
Cereal based products				
Thyme pie	0.03 ± 0.023	0.023 ± 0.006	**0.76 ± 0.384**	0.092 ± 0.005
Cheese and thyme pie	0.012 ± 0.008	0.012 ± 0.004	**0.409 ± 0.004**	0.087 ± 0.001
Thyme regular mix sandwich	0.02 ± 0.004	0.014 ± 0.001	**0.785 ± 0.437**	0.087 ± 0.003
Thyme mix with nuts and seeds sandwich	0.016 ± 0.009	0.022 ± 0.000	**0.401 ± 0.082**	0.073 ± 0.015
Pizza and pasta with thyme sauces	0.015 ± 0.006	0.015 ± 0.000	**0.447 ± 0.079**	0.079 ± 0.003
Bread sticks with thyme (crunchy)	0.021 ± 0.018	0.022 ± 0.004	**0.51 ± 0.062**	0.079 ± 0.008
Sesame thick bread with thyme (soft)	0.008 ± 0.006	0.015 ± 0.001	**0.364 ± 0.018**	0.084 ± 0.006
Crackers with thyme	0.019 ± 0.004	0.016 ± 0.003	**0.374 ± 0.049**	0.085 ± 0.003
Toast/bread with thyme	0.008 ± 0.000	0.015 ± 0.002	0.189 ± 0.216	0.086 ± 0.001
Thyme croissant	0.009 ± 0.004	0.034 ± 0.026	**0.406 ± 0.209**	0.079 ± 0.003
Average concentration of cereal based products subcategory	0.016 ± 0.007	0.019 ± 0.007	0.350 ± 0.210	0.083 ± 0.006
Dairy products				
Cheese with thyme	0.007 ± 0.000	0.009 ± 0.003	**0.422 ± 0.108**	0.047 ± 0.044
Herbal thyme tea				
Tea infusion	0.001 ± 0.000	0.002 ± 0.001	**0.014 ± 0.001**	**0.003 ± 0.001**
Tea soaked	0.001 ± 0.000	0.001 ± 0.000	**0.027 ± 0.016**	**0.004 ± 0.001**
Average concentration of herbal thyme tea subcategory	0.001 ± 0.000	0.002 ± 0.001	0.02 ± 0.009	0.004 ± 0.000
Salad				
Fresh thyme salad	0.082 ± 0.041	0.029 ± 0.006	**0.718 ± 0.165**	0.082 ± 0.001
Average concentration of thyme products category	0.018 ± 0.020	0.016 ± 0.090	0.0690±0.030	0.416 ±0.236
Dried thyme herbs				
Mixed thyme normal	0.091 ± 0.011	0.041 ± 0.005	**0.834 ± 0.060**	0.09 ± 0.001
Mixed thyme extra	0.063 ± 0.015	0.088 ± 0.015	**0.999 ± 0.750**	0.088 ± 0.013
Dried thyme herb	**0.557 ± 0.051**	0.055 ± 0.019	**1.031 ± 0.053**	**0.119 ± 0.005**
Tea thyme	**0.609 ± 0.106**	0.065 ± 0.031	**1.21 ± 0.222**	**0.107 ± 0.002**
Average concentration of dried thyme herbs category	0.33 ± 0.293	0.062 ± 0.020	1.019±0.154	0.101 ± 0.015

[1] Note: Average heavy element concentrations (mg/kg) ± SD with values that exceeded the national limit underlined, values that exceeded the international limit in **bold** and values that exceeded both limits **underlined and in bold**

Table 14 Percentage of acceptable and unacceptable toxic element content in thyme products and dried thyme herbs in first and second collection according to LIBNOR and Codex Alimentarius Standards

National/ international standard	heavy metals	As	Cd	Hg	Pb
LIBNOR standards	% of acceptable heavy metal concentration (n/N)[a]	89% (32/36)	100% (36/36)	92% (33/36)	14% (5/36)
	% of unacceptable heavy metal concentration (n/N) [a]	11% (4/36)	0%	8% (3/36)	86% (31/36)
Codex Alimentarius standard	% of acceptable heavy metal concentration (n/N)[a]	89% (32/36)	100% (36/36)	78% (28/36)	3% (1/36)
	% of unacceptable heavy metal concentration (n/N) [a]	11% (4/36)	-	22% (8/36)	97% (35/36)

[a]n/N: Number of acceptable samples/total number of samples in both categories at first and second collection

2.3 Conclusion

Our research investigated the occurrence of toxic metals in thyme herbs and thyme-containing products from the most common brands available in the Lebanese market. It is the first study done in Lebanon to assess the safety of popular and highly consumed food commodities that are thyme herbs and related based products. Results showed that some toxic metal concentrations were higher than permissible limits (national and international) as well as higher than similar food products in studies done in various countries.

Toxic metals accumulate in the food chain and are non-biodegradable and the chronic exposure to them leads to an alteration in the human body metabolism. Some of the preventive and mitigation processes can include careful monitoring and regulation of the use of fertilizers that contain heavy metals, the proper management of waste products from industries and mining areas, and an alternative to the use of contaminated irrigation water and wastewater.

This study was the first study done in Lebanon to assess the level of contamination of four heavy metals Pb, As, Cd and Hg in thyme and thyme containing products found in popular markets in Lebanon and frequently consumed by the Lebanese population.

Further studies are needed to assess the exposure and estimate the risk from consumption of these products including carcinogenic and non-carcinogenic risks.

In addition, as the biotoxicity and carcinogenicity of toxic elements depend on their chemical form (organic or inorganic) , further studies that include speciation analysis could provide more accurate information on the form of toxic elements present to characterize with higher precision the health risk of consumers from consumption of contaminated food products.

The findings of the current study highlight the need for planning and applying good agricultural practices, the need to implement a rigid food safety system, the importance of routinely testing various products to monitor levels of toxic metals in the Lebanese diet and identify the source of contamination to minimize their levels to a minimum value, and the importance of regular update of regulations and standards related to toxic metals in food.

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