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Tracking trace elements from soil to Lebanese bread and Populations Exposure

Nada Lebbos

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Nada Lebbos Harfouche

on the 22nd of October 2021

Tracking Trace Elements from Soil to Lebanese Bread and Populations Exposure

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PREFACE

This work was carried out in the laboratories of LARI (Lebanese Agricultural Research Institute) Beirut - Lebanon, CEREGE Aix-Marseille University and Agrosup Dijon, University of Burgundy - Franche Comté under the supervision of Professor Pierre CURMI and the co-supervision of Professor Elias BOU-MAROUN in a project "Cèdre", in a collaboration with the Lebanese University - Faculty of Sciences II).

This work has led to the following:

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N. Lebbos, C. Daou, R. Ouaini, H. Chebib, M. Afram, P. Curmi, L. Dujourdy, E. Bou-Maroun, and Marie-Christine Chagnon. Lebanese Population Exposure to Trace Elements via White Bread Consumption, *Foods*, 2019. *Foods* 2019, 8, 574; <https://doi.org/10.3390/foods8110574>.

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N. Lebbos, E. Bou-Maroun, C. Daou, R. Ouaini, H. Chebib, C. Keller, M. Afram, P. Curmi, M.C. Chagnon. Chemical analysis of trace elements of toxicological concern in white Lebanese pita and risk characterization for the consumers. The 54th Congress of the European Societies of Toxicology (EUROTOX 2018) 2-5 September, 2018, Brussels.

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To my father's soul

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The harder the Goal, the greater the Victory

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TABLE OF CONTENTS

PREFACE	i
ACKNOWLEDGMENT	ii
TABLE OF CONTENTS	v
LIST OF TABLES	x
LIST OF FIGURES	xiii
LIST OF ABBREVIATIONS AND ACRONYMS	xvi
INTRODUCTION	1
PART I – BIBLIOGRAPHY	4
CHAPTER I-1 Trace Elements Contamination of Soils and Wheat Plants	5
I-1.1 Origin of trace elements contamination of soils	7
I-1.1.1 Natural origin	8
I-1.1.2 Anthropogenic origin	8
I-1.1.2.1 Agricultural practices	9
I-1.1.2.1.1 Organic Fertilizers	9
I-1.1.2.1.2 Inorganic Fertilizers	12
I-1.1.2.1.3 Pesticides	15
I-1.1.2.1.4 Irrigation water	16
I-1.1.2.2 Industrial stress	17
I-1.2 Mobility of trace elements in soils	18
I-1.2.1 Factors affecting the mobility of trace elements in soils	19
I-1.2.1.1 Soil parameters : soil pH, potential redox, and CEC	19
I-1.2.1.2 Soil constituents : Soil Organic Matter and Clay	20
I-1.2.2 Processes affecting trace elements mobility in soils	21
I-1.2.3 Trace elements uptake and translocation in plants	23
I-1.2.4 Effects of trace elements in wheat plants	24
I-1.3 The essential role of the element silicon in trace elements bioaccumulation in wheat plants	25
CHAPTER I-2 Bread and Trace Elements Exposure of the Lebanese Population via Lebanese White Bread	28
I-2.1 Challenges of trace elements food contamination	29
I-2.1.1 Challenges of trace elements food contamination worldwide	29
I-2.1.2 Challenges of food contamination in Lebanon and food safety	31
I-2.2 Lebanese bread Industry and trace elements contamination	32

I-2.2.1 Lebanese white bread composition	32
I-2.2.2 Lebanese white bread processing	34
I-2.2.2.1 Description and manufacturing process of Lebanese white bread	34
I-2.2.2.2 Packaging of Lebanese white bread	36
I-2.3 Nutritional benefits of Lebanese bread	36
I-2.4 Trace elements contamination of Lebanese bread	37
I-2.5 Potential Exposure and Risk Associated to TEs exposure of the Lebanese population via the daily consumption of the Lebanese white bread	39
I-2.5.1 Potential exposure to chemical compounds and associated risk	39
I-2.5.2 Potential exposure to trace elements in food and associated risk	41
I-2.5.3 Thresholds of trace elements in food and their monitoring	41
CHAPTER I-3 The international and national legislations of soil, wheat plant and bread	45
I-3.1 Standardization and regulatory aspect	46
I-3.1.1 Allowable limits of TEs in soil	46
I-3.1.2 Allowable limits of TEs in Inorganic Fertilizers	46
I-3.1.3 Allowable limits of TEs in Irrigation water	47
I-3.1.4 Allowable amounts of TEs in sewage sludge	47
I-3.1.5 Allowable limits of TEs in Cereal plants	48
I-3.1.6 Allowable limits of TEs in foodstuff and Bread	49
PART II – MATERIAL AND METHODS	50
CHAPTER II-1 Geological, hydrogeological, hydrological and climatic context of the Bekaa valley	51
II-1.1 Geology	52
II-1.2 Climate	53
II-1.3 The Litani River Basin	55
II-1.4 Site locations	58
II-1.5 Climate of the Central Bekaa valley	60
CHAPTER II-2 The studied matrices	61
II-2.1 Soil samples	62
II-2.1.1 Sampling	62
II-2.1.2 Physico chemical parameters of soil	62
II-2.1.2.1 Determination of soil pH	62
II-2.1.2.2 Determination of electrical conductivity EC	63
II-2.1.2.3 Soil texture	63
II-2.1.2.4 Determination of soil organic matter content (SOM)	63

II-2.1.2.5 Determination of total limestone	64
II-2.1.2.6 Determination of active limestone content	64
II-2.1.2.7 Determination of the available phosphorus content (Olsen method)	64
II-2.1.2.8 Determination of exchangeable bases : K^+ , Na^+ , Ca^{2+} and Mg^{2+}	65
II-2.1.3 Trace elements determination in soil samples	66
II-2.1.4 Silicon determination	67
II-2.2 Wheat samples	67
II-2.2.1 Wheat plant production	67
II-2.2.1 Sampling	68
II-2.2.2 Trace elements determination of the different wheat plant organs	68
II-2.3 Irrigation water samples	69
II-2.4 Bread samples	70
II-2.4.1 Survey of Lebanese bread consumption	70
II-2.4.2 Trace elements determination in bread samples	71
II-2.4.3 Exposure determination	72
PART III – RESULTS AND DISCUSSION	73
CHAPTER III-1 Validation of a new method for monitoring trace elements in Mediterranean cereal soils	75
III-1.1 Introduction	76
III-1.2 Material and methods	79
III-1.2.1 Chemicals	79
III-1.2.2 Sites and sampling	79
III-1.2.3 Samples pretreatment and digestion	80
III-1.2.4 Apparatus for trace elements analysis	81
III-1.2.5 Official or reference methods for trace element analysis	82
III-1.2.6. Statistical analysis	83
III-1.3 Results and Discussion	83
III-1.3.1 Choice of the best digestion method using the soil Certified Reference Material CMR (SQC001)	83
III-1.3.2 Validation of the microwave sulphuric digestion method followed by AAS using the soil Certified Reference Material CMR (SQC001)	85
III-1.3.2.1 Working range, linearity domain, limit of detection (LOD) and limit of quantification (LOQ)	85
III-1.3.2.2 Precision including repeatability and intermediate precision	87
III-1.3.2.3 Specificity	89
III-2.3.2.4 Comparison between the validated method (MSD + AAS) and the RTD method (HF-based digestion + ICP/ICPMS) of TE analysis. Accuracy or trueness	90
III-1.4 Conclusion	97

CHAPTER III-2 Geogenic and anthropogenic origin of trace elements in the Central Bekaa soils	98
III-2.1 Geogenic context	99
III-2.1.1 Soils in the landscape	99
III-2.1.2 Physicochemical characterization of the studied sites	105
III-2.2 Anthropogenic context	110
III-2.2.1 Trace elements provided by fertilization and amendments	110
III-2.2.2 Trace Elements contributions related to irrigation	111
III-2.2.2.1 Deep well water	111
III-2.2.2.2 Pathway of contaminants carried by surface water used for irrigation	115
III-2.2.2.3 Other contributions	117
III -2.3 Quantification and origin of trace elements available in the soil	119
III-2.4 Conclusion	122
CHAPTER III-3 Transfer of trace elements to wheat plant Analysis according to soil types, silicon status and origin of trace elements	124
III-3.1 Introduction	125
III-3.2 Trace element concentrations in soils	125
III-3.3 Trace elements and silicon availability in the 8 studied soils	128
III-3.4 The transfer of Pb, As and Cd from soil to wheat	131
III-3.5 Factors responsible for concentrations in grain	135
III-3.6 Conclusion	137
CHAPTER III-4 Lebanese Population Exposure to Trace Elements via White Bread consumption	138
III-4.1 Introduction	140
III-4.2 Materials and Methods	142
III-4.2.1 Survey	142
III-4.2.2 Chemicals	144
III-4.2.3 Sampling for Trace Elements Analysis	144
III-4.2.4 Sample Digestion for Trace Elements Analysis	144
III-4.2.5 Analysis of Trace Elements	145
III-4.2.6 Exposure Determination	145
III-4.2.7 Statistical Analysis	145
III-4.3 Results	146
III-4.3.1 Survey Results	146
III-4.3.2 Trace Element Contents in Pita	151
III-4.3.3 Trace Element Exposures	153
III-4.3.4 Risk Characterization (Intake Excess of TEs via Bread Consumption)	154

III-4.4 Discussion	159
III-4.4.1 Survey	159
III-4.4.2 Risk Characterization	159
III-4.5 Conclusions	167
GENERAL CONCLUSION	168
REFERENCES	172
ABSTRACT	194
RÉSUMÉ	196
ANNEX	a1
ANNEX 1 Survey and questionnaire related to Lebanese bread consumption	a2
ANNEX 2 Toxicological data sheet for As	a9
ANNEX 3 Toxicological data sheet for Cd	a19
ANNEX 4 Toxicological data sheet for Co	a27
ANNEX 5 Toxicological data sheet for Cr	a32
ANNEX 6 Toxicological data sheet for Pb	a39
PUBLISHED ARTICLES	a54
ARTICLE 1 Lebanese population exposure to trace elements via white bread consumption, <i>Foods</i> .	
ARTICLE 2 Validation of a new method for monitoring trace elements in Mediterranean cereal soils, <i>International Journal of Environmental Analytical Chemistry</i> .	

LIST OF TABLES

Table I-1. 1: World cereal production (2010)	6
Table I-1. 2: Concentration of As, Cd and Pb in P fertilizers marketed in China, European countries, Chile and Lebanon	13
Table I-1. 3: The average input of TEs from the application of P fertilizers to the cultivated soils in the Eastern Mediterranean countries and in the European countries	14
Table I-1. 4: The Cd contents in mg kg ⁻¹ of sedimentary and igneous phosphate rocks from different countries	4
Table I-2. 1: The main ingredients and manufacturing of different types of Lebanese bread	34
Table I-2. 2: The calorific values and nutrition facts of Lebanese breads	37
Table I-3. 1: Max allowable concentrations of trace elements (mg kg ⁻¹) in soil according to EU, WHO, NJDEP, CEQCs, MACs, and MEF.	46
Table I-3. 2: Maximum allowable concentrations of TEs in inorganic fertilizers TEs (mg kg ⁻¹)	47
Table I-3. 3: Maximum allowable concentrations of TEs (mg L ⁻¹) in irrigation water	47
Table I-3. 4: Limit values of TEs (mg kg ⁻¹ DM) in sewage sludge used in agricultural soil	48
Table I-3. 5: The limit values for TEs amounts which may be added annually to agricultural soil	48
Table I-3. 6: The concentrations of Cd, Pb and As (mg kg ⁻¹) in cereal plants as per EU, Germany regulations, FAO/WHO, China, and Australia/New Zealand	49
Table I-3. 7: The concentrations of TEs (mg kg ⁻¹) in foodstuffs and bread as per (NL 240, 2010) - Libnor and EC (No 1881, 2006)	49
Table II-1.1: The nine studied sites locations in the Bekaa Plain, their altitudes and annual rainfall	59
Table II-2. 1: The type and variety of wheat grown in the studied sites of the Bekaa region, and the different supplemental irrigation sources	68
Table III-1. 1: Comparison of the heating 3 acid digestion method (HAD) and the microwave sulphuric digestion method (MSD) using their relative recovery percentage of the soil Certified Reference Material CRM (SQC001)	84
Table III-1. 2: Calibration curves of the measured elements	86
Table III-1. 3: Working ranges, regression equations, limit of detection (LOD) and limit of quantification (LOQ) of the measured elements	87
Table III-1.3bis: LOQ/(Threshold value) ratio according to element and land use *	87
Table III-1. 4: Repeatability and intermediate precision results obtained after TE analysis of CRM soil using the microwave sulphuric digestion method (n = 9 for both operators)	89
Table III-1. 5: Specificity results expressed as mean recovery percentage and standard deviation. Three samples were analysed: CRM soil alone and CRM after the addition of two concentration levels for each element (n = 9)	90

Table III-1. 6: Comparison between the MSD method ($\text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2$ digestion) being validated and the RTD method ($\text{HF} + \text{HClO}_4$ digestion) for TEs in soil samples from the Bekaa governorate sites.	92
Table III-2. 1: Physicochemical characterization of the studied soils	106
Table III-2. 2: Total trace elements content of the studied sites	107
Table III-2. 3: The limits of TEs content (mg kg^{-1}) in phosphate fertilizers and their contribution to soils	111
Table III-2. 4: TEs contents ($\mu\text{g L}^{-1}$) in ultrafiltered irrigation water samples in the Bekaa studied sites	112
Table III-2. 5: The quantity of TEs carried by deep tube well irrigation water (338 mm yr^{-1}) to soil per year and per 100 years	115
Table III-2.6: The total and the available TEs (DTPA extraction) concentrations in the studied soil samples (mg kg^{-1})	119
Table III-3. 1: Phytoavailable concentrations of trace elements (assessed by DTPA extraction) and silicon (assessed by CaCl_2 extraction) in the soils of the 8 studied sites.	130
Table III-3. 2: As, Cd and Pb concentrations in stem, leaves and grain of wheat grown on the 8 soils	132
Table III-3. 3: Translocation ratios (factor) for the various plant parts and As, Cd and Pb	134
Table III-3. 4: plant parts-soil ratios. Bioaccumulation factors were calculated with total Pb, As and Cd concentrations in soil and with DTPA-extractable Pb, As and Cd concentrations for grain. S=stem; L=leaves; G=grain	135
Table III-4. 1: Sampling strategy according to the population distribution	143
Table III-4. 2: Pearson correlation matrix of the Principal Component Analysis	147
Table III-4. 3: Daily white pita consumption according to population categories and repartition by brands	148
Table III-4. 4: Trace element contents in white pita bread for the three major brands on the Lebanese market	151
Table III-4. 5: Pearson correlation matrix of the Principal Component Analysis	153
Table III-4. 6: Population categories exposure to Cd, Hg, Cr, Co, Ni, Pb & As trace elements via white pita consumption according the most consumed brands	154
Table III-4. 7: Population categories' exposure to Cd and Hg trace elements via B3 white pita consumption and comparison with the tolerable weekly intake (TWI) Exposure values are expressed in $\mu\text{g/kg}$ body weight/week	157
Table III-4. 8: Population categories exposure to Cr, Co, and Ni trace elements via white pita consumption and comparison with the tolerable daily intake (TDI) Exposure values are expressed in $\mu\text{g/kg}$ body weight/day	157

Table III-4. 9: Margin of exposure (MOE) to Pb and As trace elements via white pita consumption for the Lebanese population categories	158
Table III-2.1 Annex: Soil site classification according World Reference Base 2014	a48
Table III-2.2 Annex: The type and variety of wheat grown in the studied sites with the methods of fertilization and irrigation applied	a49
Table III-2.3 Annex: Wheat -Potato crop rotation in the studied sites, irrigation and fertilizers	a50
Table III-3.1: Correlation TEs in wheat plants and soil – Si (soil) – Physico-chemical parameters in soil	a51 a52
Table III-3.2 Annex: DTPA-extractable Pb, Cr, Co, Ni, Cu, Zn, As and Cd as a function of total	a53
Table III-4.1 Annex: The trace elements contents in studied soils in the Bekaa region and the three most consumed white Lebanese bread	

LIST OF FIGURES

Figure I-1. 1 : The different sources of soil TEs contamination	8
Figure I-1. 2 : The reduction and oxidation sequence in the soil. The redox status can be catalyzed either by heterotrophic bacteria (biotic) or produced by chemical elements/compounds (abiotic). The electron donor substrate is generally soil (natural organic matter) for the biotic reactions (Davranche et al., 2020). Humics in the figure means humic and fulvic organic matters	21
Figure I-2. 1 : The most popular Lebanese bread from the left : white pita bread (white flour, loaf of two layers), brown bread (whole wheat grain flour, loaf of two layers), and saj bread (brown and whole wheat flour, with one layer)	33
Figure I-2. 2 : The different steps of Lebanese white pita bread manufacturing	35
Figure I-2. 3 : The plastic bag containing Lebanese white pita bread	36
Figure I-2. 4 : The TEs in the environment (soil, water, air and plants), arriving to the food chain and then enter human body through the consumption of food and vegetables and cause potentially harmful effects for the health	40
Figure II-1. 1 : Bloc diagram of the geological fabric of Bekaa Valley (Lateef, 2007)	52
Figure II-1. 2 : Geological map of the Litani river basin (Shaban and Hamzé, 2018)	53
Figure II-1. 3 : Rainfall map of Lebanon 1950-1970 CNRS	54
Figure II-1. 4 : Space view showing Mount Lebanon Range facing Bekaa valley and Qaraaoun reservoir. Sources Tannouri 2010, in Shaban et al., 2018	54
Figure II-1. 5 : The geomorphology of the two sub-basins of the Litani River Basin : The Upper Litani River Basin from Baalbek to Qaraaoun (ULRB) and the Lower Litani River Basin from Qaraaoun reservoir to the Mediterranean Sea at Kasmieh (LLRB) (Nehme et al., 2014)	55
Figure II-1. 6 : Litani river sub-basins hydrological characteristics, ULRB : Upper Litani River Basin, LLRB : Lower Litani River Basin (Shaban and Hamzé, 2018)	56
Figure II-1. 7 : Drainage system of the Litani River Basin	57
Figure II-1. 8 : Upper Litani River Basin groundwater annual budget (Molle et al., 2017)	58
Figure II-1. 9 : The nine studied sites distributed in the Bekaa Plain along a transect crossing the Litani	59
Figure II-1. 10 : Average monthly precipitation and temperature in the Litani River Basin	60
Figure III-1. 1 : The location of the soil samples collected from Zahleh and western Bekaa administrative districts Lebanon : Mazraat Zahleh (B1), Tcheflik Qiqano (B2), Marj (B3), Haouch El Oumaraa (B4ab and B5), Maalaqah (B6), Dalhamiyeh (B7), Haouch El-Aamara (B8). Satellite Images © CNES/Airbus, Maxar Technologies, Map data © 2021	80
Figure III-1. 2 : Cartography by universal krieging of topsoil Pb distribution in Central Bekaa plain showing : (i) above a low geogenic background, an anthropic contamination along the roads with heavy traffic including the plume effect was controlled by the main northeast wind and (ii) a surface runoff gradient from the slopes near the town of Zahleh towards the Litani river	94

Figure III-2. 1 : Left Central Bekaa soil map (Gèze, 1956 in Nasser, 2018). Right Coverage of the Gèze soil reconnaissance map on the administrative map of untreated municipalities outlet and the position of the sites studied	100
Figure III-2. 2 : Light chestnut soils on western slope (B1, B2) and recent alluvium (B3)	101
Figure III-2. 3 : Light chestnut soils in recent alluvium (B4) and in an alluvial fan on Western slope (B5)	102
Figure III-2. 4 : Red soils developed on the eastern slope	103
Figure III-2. 5 : Dark chestnut soils in the Bekaa plain leaning against the anti-Lebanon range (B6 & B7)	104
Figure III-2. 6 : Site location on the satellite image of central Bekaa. Images© Landsat 2021, Google maps	104
Figure III-2. 7 : Variables correlation plots with principal components 1 and 2. The first two axes accounted for 71.32% of variance	107
Figure III-2. 8 : Sites repartition chart. Factor scores of the observations plotted on the first and third components. The first and the third axes accounted for 69.47% of variance	108
Figure III-2. 9 : The Litani river and its perennial tributaries in the upper Litani river catchment with location of the untreated municipality outlets	109
Figure III-2. 10 : The ARAYBY factory, installed on 2 ha in the industrial zone of Taanayel produces lead ingots and polypropylene chips since 1990 by recycling lead acid battery waste and is located 3 km from B4 and 5 Km from B3	113
Figure III-2. 11 : Variables correlation plots with principal components 1 and 2. The first two axes accounted for 67.69% of variance	114
Figure III-2. 12 : The Litani river and its perennial tributaries in the upper Litani river catchment with location of the untreated municipality outlets	116
Figure III-2. 13 : The ARAYBY factory, installed on 2 ha in the industrial zone of Taanayel produces lead ingots and polypropylene chips since 1990 by recycling lead acid battery waste and is located 3 km from B4 and 5 Km from B3	118
Figure III-2. 14 : Variables correlation plots with principal components 1 and 2. The first two axes accounted for 67.69% of variance	120
Figure III-2. 15 : Sites repartition chart. Factor scores of the observations plotted on the first and second components. The first and the third axes accounted for 67.69% of variance	120
Figure III-3. 1 : PCA performed for the 8 sites with soil physico-chemical properties, environmental characteristics and total trace elements (As, Pb and Cd) concentrations. For B9, the CEC (S), EC and pH missing values were replaced by the average calculated on the other 7 sites	127
Figure III-3. 2 : Same as (Figure III-3.1) but without total trace elements concentrations. For B9, the CEC (S), EC and pH missing values were replaced by the average calculated on the other 7 sites	127

Figure III-3. 3 : DTPA-extractable Pb, As and Cd as a function of total concentrations. Please note that total Cd concentrations were multiplied by 10 to fit the x axis	130
Figure III-3. 4 : PCA performed on the 8 sites with total and DTPA-extractable concentrations as variables	131
Figure III-3. 5 : PCA on the 8 sites with As, Cd and Pb concentrations in wheat parts	132
Figure III-3. 6 : ACP including all soils parameters and plant As, Cd and Pb concentrations	134
Figure III-3. 7 : Relationship between DTPA-extractable As concentration and As concentrations in the different parts of the wheat plant. Removing B3 improves greatly the relation between concentrations in the soil and grain	135
Figure III-3. 8 : Relationship between DTPA-extractable Cd concentration and Cd concentrations in the different parts of the wheat plant	136
Figure III-3. 9 : Relationship between DTPA-extractable Pb concentration and Pb concentrations in the different parts of the wheat plant	136
Figure III-4. 1 : Location of the fifty surveyed supermarkets in Lebanon within the five administrative regions according to population density	143
Figure III-4. 2 : Lebanese white pita consumption according to region. Letters represent homogeneous groups determined by a Kruskal–Wallis test and post-hoc Fisher’s test for least significant difference	148
Figure III-4. 3 : Results of crossings between regions, consumption, smoker or not, type of physical activity, socio-professional category, and area (urban or rural), using the most specific modalities as key views. Consumption is represented here by categories : less than 200 g/day (<200) ; from 200 to 400 g/day ; from 400 to 600 g/day ; and more than 600 g/day (600+). Sizes of circles are proportional to the number of observations by region	149
Figure III-4. 4 : Distribution of physical activity intensity by gender in adult population	150
Figure III-4. 5 : Principal component analysis showing trace element signatures of the B1, B2, and B3 pita brands	152

LIST OF ABBREVIATIONS AND ACRONYMS

ADI	ADI: Acceptable daily intake
ANSES	ANSES: Agence Nationale Sécurité Sanitaire Alimentaire
AUB	AUB: American University of Beirut
BMD	Benchmark dose
BMDL	The Lower confidence limit of the benchmark dose
CEC	Cation exchange capacity
CEQCs	Canadian Environmental Quality Guidelines
DOM	Dissolved organic matter
DTPA	Diethylenetriaminepentaacetic acid
EFSA	European Food Safety Authority
Eh	Redox Potential
EU	European Union
FAO	Food and Agriculture Organization
IARC	International Agency for Research on Cancer
INRA	National Institute of Agronomic Research
ITAB	Institute of Agriculture and Organic Food
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LARI	Lebanese Agricultural Research Institute
LIBNOR	Lebanese Standards Institution
MAC	Maximum Allowable Concentrations
MEF	Ministry of Environment – Finland
MOE	Margin of Exposure
MOPH	Ministry of Public Health
NJDEP	New Jersey Department of Environmental Protection
OM	Organic matter
PTDI	Provisional Tolerable Daily Intake
PTWI	Provisional Tolerable Weekly Intake
Si	Silicon
SOM	Soil organic matter
TDS	Total diet study
TEs	Trace elements
TWI	Tolerable Weekly Intake
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

INTRODUCTION: A WORLDWIDE PROBLEM CONCERNING TRACE ELEMENTS IN HUMAN FOOD

Trace elements (TEs) are now widely used all over the world, in many applications (industrial, medical, agricultural, etc.). Since the industrial revolution and the development of new agricultural tools and practices, the diversity of environmental contaminants has increased exponentially. TEs contaminations from natural processes and / or anthropogenic activities cause disorders in the different compartments of the environment: water, soil, air and plants (Baize et al., 2009; Chaza et al., 2018; Gupta et al., 2019). This leads to toxic effects on plants and then on humans by ingestion, inhalation or skin absorption (Singh et al., 2016).

These TEs can have a long residence time in the soil (Kabata-Pendias, 1995) and be absorbed by plants due to their concentrations and chemical forms. Where they accumulate in their edible and inedible parts (roots, stems, leaves and grains) (Singh et al., 2016). The bioaccumulation of TEs in edible parts entering the food chain can pose a risk to human health due to their possible toxicity (Gupta et al., 2019). These possible pathways of TEs in soil food crops, in addition to many other factors in food manufacturing such as handling, preparation, processing and packaging techniques, can influence the presence of TEs in foods (Kumar et al., 2019; Morgan, 1999).

Nowadays, TEs have become a high priority global concern, as they represent the main chemical contamination of food (Kumar et al., 2019). This has prompted certain international and European organizations such as FAO/WHO and the European Food Safety Authority (EFSA) to set the threshold concentrations of certain TEs (As, Pb, Cd, Al and Hg) per unit of daily consumption or unit of mass of the matrix (EFSA, 2009; WHO, 1996). Therefore, the exposure of the population to TEs in food has been extensively assessed by different countries such as France (Arnich et al., 2012), Spain (Marín et al., 2018), Italy (Barone et al., 2017), China (Tang and Huang, 2014), Saudi Arabia (Ali and Al-Qahtani, 2012), and Lebanon (Nasreddine et al., 2010).

Among these studied foods, the bread attracted attention of scientists in different countries: Egypt (Ahmed et al., 2000), Iran (Ghoreishy et al., 2018), Nigeria (Oyekunle et al., 2014) and Portugal (Soares et al., 2010), where the authors highlighted the tremendous efforts spared by their countries authorities to control any violation of the defined TEs contamination thresholds in bread. In Lebanon, studies on the TEs risk assessment in bread are scarce.

The Lebanese white bread is a daily diet of the Lebanese population, which enters their table anytime: at breakfast, at lunch, at dinner and even snacks. Although it is an essential food, and despite the national campaign for food safety, carried out by the Ministry of Health, the Ministry of Agriculture and FAO in 2014 to monitor food, it is one of the foods that does not receive much attention for its contamination with trace elements.

In the context of food safety, it is relevant to assess the contamination of the Lebanese white bread by TEs. To the best of our knowledge, until this date, no study in Lebanon has been undertaken about the routing of the TEs in bread production, from farm to fork

This thesis, carried out in a professional framework at LARI and supported by the Cèdre program, studies the contamination of soil, irrigation water, wheat plants and Lebanese white bread by TEs, as well as the exposure of the Lebanese population to these TEs. This work will be the first research project leading an interdisciplinary approach and exploring a study on soil, water, climate, plant, food and human health in Lebanon. It presents two main objectives:

The first objective consists in monitoring the behaviour of TEs in soils and their effects on wheat plants in such an environment. Studying TEs such as: As, Cd, Co, Cr, Cu, Hg, Ni and Pb in the following matrices: soil, wheat, and Lebanese white bread.

The second objective is of national interest and aims to assess the exposure of the Lebanese population to TEs in bread. In the same vein, the “Esteban” survey on the impregnation of the French population by As, Cd, Cr, Cu, Hg, Ni and Pb carried out in 2004-2006 (Esteban, 2021) showed levels of exposure greater than those encountered in Europe and North America, with the exception of Ni and Cu. Likewise, the health guideline values were exceeded for As, Hg and especially Cd. The Cd element which remains the most serious, showing cadmiuria greater than the value recommended by ANSES by nearly 50% of adults and that 100% of children have been contaminated by the consumption of breakfast cereals confirming the choice of these trace elements in bread for our study.

This manuscript is divided into three main parts:

- The first part is a bibliography part, which consists of a chapter studying trace elements contamination of soils and wheat plant, another chapter studying the bread and TEs exposure of the Lebanese population via Lebanese white bread, and a chapter

consisting of the legislative and regulatory aspects of TEs content in soil, wheat and bread.

- The second part details the methodological and experimental approach followed in the thesis to study the Bekaa valley, the trace elements in the studied matrices: soil, irrigation water, wheat plant and bread, and the silicon in soil samples. In addition to a statistical analysis and processing approach.

Chapter II-1: Geological, hydrogeological, hydrological and climatic context of the Bekaa valley.

Chapter II-2: The studied matrices.

- The third part presents and discuss the main results and is divided into four chapters:

Chapter III.1: Validation of a new method for monitoring trace elements in Mediterranean cereal soils.

Chapter III.2: Geogenic and anthropogenic origin of trace elements in the Central Bekaa soils.

Chapter III.3: Transfer of trace elements to wheat plant- Analysis according to soil types, silicon status and origin of trace elements.

Chapter III.4: Lebanese Population Exposure to Trace Elements via White Bread consumption.

The thesis ends with a summary of all the main results obtained and offers the perspectives that are drawn from the work.

PART I – BIBLIOGRAPHY

CHAPTER I-1

Trace Elements Contamination of Soils and Wheat Plants

In the earth's crust, there are two types of chemical elements depending on their abundance: major elements and trace elements. The "major elements" (O, Si, Al, Fe, Ca, Na, K, Mn, Mg, P, Ti and H) represent more than 99% by mass of the earth's crust, while the "trace elements" (Cd, Cr, Cu, Co, As, Zn, Hg, Ni, Pb, V, Sb, Zn and B) represent only 1%. The latter were called also in the literature "metallic trace elements" (MTEs) or "heavy metals" (HM) because of their metallic nature and their relatively high densities, atomic weights or atomic numbers. But some elements as As, Sb and B are classified as metalloids, this is why the term "trace elements" (TEs) will be used here to name all of these metals and metalloids. Several trace elements are physiologically essential for living organisms but when present in excessive concentrations, they can have harmful effects on microorganisms, animals, plants and humans (Antoniadis et al., 2019a). Other TEs are toxic regardless of their concentrations (As, Cd, Hg and Pb). Following previous studies based on the determination of TEs in the Bekaa soils, the choice of the following elements: As, Cd, Co, Cr, Cu, Hg, Ni, and Pb was made (Darwish, 2008, 2018; Kanbar et al., 2014; Roukoz and Issa, 2004).

The wheat plants are considered as staple food worldwide (Awika, 2011; Giraldo et al., 2019). It is the most domesticated food crops from ancient time, with great agronomic adaptability and ease of storage and conversion into different nutritional foods. It provides 20% of the daily protein and food calories in human nutrition (Giraldo et al., 2019). Wheat crops occupy the first place with 226 million ha cultivated in 2010 or 35% of the world surface in cereals, and only 27% of the grain production placing it behind corn and rice due to its lower yield per ha **Table I-1. 1** (Awika, 2011).

Table I-1. 1: World cereal production (2010)

Cereal	Cultivated area (Million ha)	Relative cultivated area (%)	Grain production (Million T)	Relative production (%)	Mean Yield (T ha ⁻¹)
Maize	160	25	825	35	5.2
Wheat	226	35	650	27	2.9
Rice	162	25	680	29	4.2
Barley	54	8	150	6	2.8
Sorghum	40	6	60	3	1.5
Total	642	100	2365	100	

Wheat comprises two species, durum wheat (*Triticum turgidum*) and soft wheat (*Triticum aestivum*) and a large number of cultivars adapted by humans to different environments and for different uses. Durum wheat is cultivated in various regions of the world and in the majority of the Middle East countries such as Egypt, Jordan, Syria and Lebanon (Almeselmani et al., 2011; Rawashdeh et al., 2007; Saab et al., 2019; Sall et al., 2019; Xynias et al., 2020). Soft wheat is mainly imported from countries such as Russia, Kazakhstan, Canada, Ukraine, Australia and USA, where it is used mostly for bread production (Mikhael, 2016).

The wheat grain accumulates more TEs such as Cd, Cr, As, Pb, Cu than other cereals like corn (Wang et al., 2018) Durum wheat has a great tendency to accumulate Cd in grains than common wheat and other cereals (Grant et al., 2008). In laboratory conditions, the accumulation of TEs depends on the cultivar and the organ of the plant (Vergine et al., 2017). Furthermore a same variety of wheat grown on different types of soil will accumulate more or less TEs in the grain depending on the soil characteristics (Baize et al., 2009).

The soil is composed of three phases (solid, liquid and gaseous) and can be divided in two functional compartments: the first known as the “TEs accumulator compartment” and formed of the solid phase, and the second known as “TEs vector compartment” and constituted by the liquid and gaseous phases (Azzi et al., 2017). The solid phase being composed of mineral constituents (primary minerals, oxides, hydroxides, sulfates, etc.) and organic constituents which can temporarily fix TEs. These trace elements coming from lithogenic or anthropogenic sources can be found in the different soil phases, thus making it possible to assess their mobility.

I-1.1 Origin of trace elements contamination of soils

One of the main challenges in the environmental pollution assessment is to understand how various TEs can reach the soil and whether they are of natural (lithogenic) and/or anthropogenic origin [Figure I-1. 1](#) (Serelis et al., 2017). In fact, special emphasis was paid for potentially toxic elements because their accumulation in soils and plants can easily threatened the human health through the food chain and drinking water.

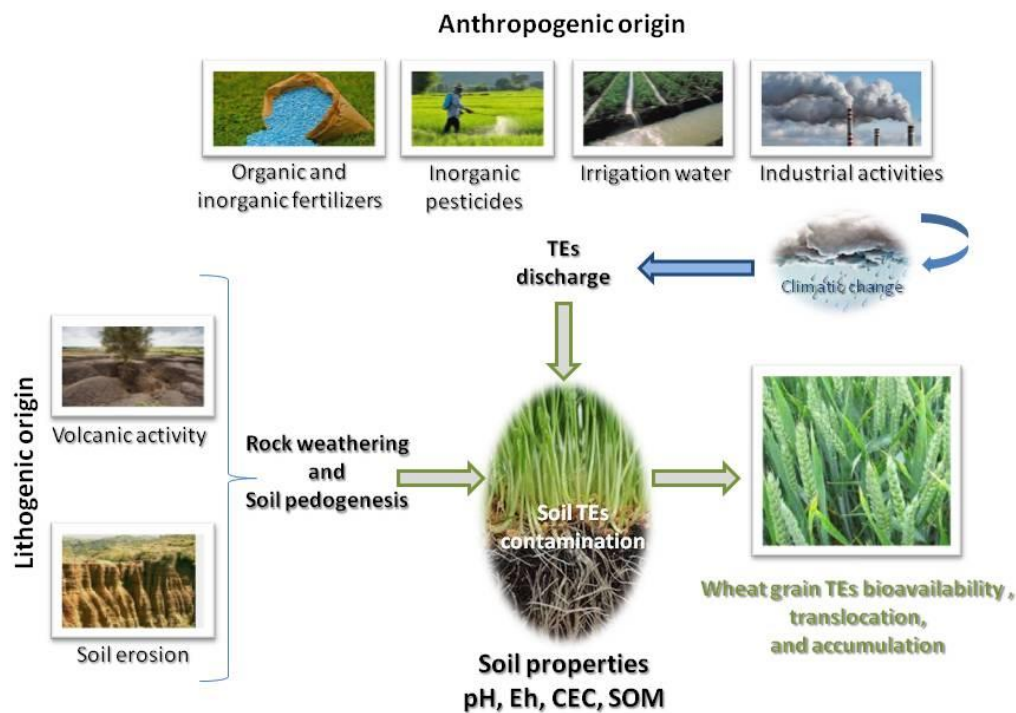


Figure I-1. 1: The different sources of soil TEs contamination

I-1.1.1 Natural origin

The TEs are naturally present in the earth's crust and concentrate in soils by mineral alteration and volcanic eruptions. The physical and chemical alteration of the rocks allows the release and precipitation of TEs as oxide. The TEs present in the topsoil are mainly coming from geogenic origin, where they are strongly linked within the crystal structures of primary minerals or secondary oxides and therefore are not available. Moreover, their amount in the original material, as showed by many studies, has a huge influence on the amount of TEs found in the topsoil (Baize, 2009; Serelis et al., 2017) with the exception of cadmium and mercury for which values of the natural pedogeochemical background are very low (Baize et al., 2006; Baize and Sterckeman, 2001).

I-1.1.2 Anthropogenic origin

Anthropogenic activities have led, following various studies, to an accumulation of TEs in soil and to high contents at the level of crops, such as As, Cd, Co, Cr, Cu, Pb, Ni and Hg. Agricultural practices and several industrial activities are responsible of topsoil contamination by trace elements. Different modes of contamination will be distinguished according its origin.

Agricultural practices intended to improve crop yields, such as organic and inorganic fertilizers, pesticides treatments and use of wastewaters for irrigation, lead to a “diffuse” contamination which is of moderate type. While the localized pollution within industrial areas causes a massive *punctual contamination* of a few hectares or tens of hectares. Finally, atmospheric contamination from point sources such industrial sites, heating or incineration plants and automobile traffic causes “proximity” contamination (Robert, 2001) whose intensity decreases with the distance from the source.

I-1.1.2.1 Agricultural practices

Agricultural practices, which are usually applied for farm production processes, help farmers to get better agricultural products. Sowing, weeding, manuring, harvesting, storing and other several methods usually focused on crop yields at a local scale. Many efforts are deployed nowadays to focus on a sustainable production at a global scale through a better soil management strategy. Despite all these efforts, most agricultural systems rely on the excessive and/or uncontrolled use of fertilizers, pesticides, and untreated wastewater, which are the main sources of contamination and accumulation of TEs in the soils and groundwaters (Atafar et al., 2010; Darwish and Jomaa, 2008; Schaeffer et al., 2016).

I-1.1.2.1.1 Organic Fertilizers

The organic fertilization is important for crop growth, allowing the development of plant roots in a healthy environment (Barłóg et al., 2020; Thomas et al., 2019). It consists of manure, slurry, methanization digestates and sewage sludges.

Manures and Slurry

The animal manure is well known as a crucial amendment to improve agricultural soil quality, and constitutes the main economically valuable source of plant nutrients (Barłóg et al., 2020; Thomas et al., 2019). The slurry defined as the excreta produced by livestock and mixed with rainwater and/or wash water, is a well-used fertilizer worldwide and one of the most profitable means of manage this waste (Font-Palma, 2019; Formentini et al., 2015). While addressing the land manure application as the best suitable solution for organic material recycling and soil enrichment, the risk of TEs soil contamination should be considered since

the animal manure might be rich in such pollutants. (Mantovi et al., 2003), demonstrated that the accumulation of Cu and Zn in agricultural soils and crop plants is resulting from the spreading of slurry in the soil. These TEs may originate from their excessive addition into the animal feed in order to prevent animal disease and gain weight (Baize et al., 2006; Burton and Turner, 2003). A four-years field monitoring study conducted by (Qian et al., 2018), on the land application of swine manure show a gradual increase of TEs (As, Hg, Cr, Cu, Zn and Mn) in land fields with the application duration, predicting an exceeding level of Cr, Cu and Zn according to the permissible thresholds for Chinese agricultural soils in the next 10-50 years.

Digestates

Svane and Karring, (2019) found that an intensive application of these fertilizers leads to a highly soil contamination with these TEs, while their levels in cereal crops still luckily remain below the regulatory standards. Unfortunately, these types of fertilizers might also contain potentially toxic elements such as Hg, Cd, Pb which may lead to their transfer to cereal crops (winter wheat and spring barley) as shown by (Barłóg et al., 2020) with a highly accumulation of Cd (below standards) and Pb (exceeded the European Union limits) in the grain of winter wheat.

Sewage Sludges

The sewage sludge resulting from domestic wastewater treatment plants are rich in nutrients and organic matter, where they are thoroughly used as low cost-effective fertilizers whilst responding to environmental regulatory pressure (Baize et al., 2006). Although its application, leads to high agricultural production, may cause a continuous introduction of potentially toxic TEs into the soil, mainly Cd (Baize et al., 2006; Kominko et al., 2017). Unluckily, the TEs present in the sewage sludge matrix, mainly due to wastewater, are more mobile hence available form than those coming from contamination of lithogenic origin (Kouchou et al., 2020; Merrington et al., 2003; Wuana and Okieimen, 2011). Sewage sludge provides soil with OM, fertilizing elements, as well as TEs in variable quantities depending on their origin, which may lead to soil contamination. And leads to norms for the products liable to be spread and on the soil liable to receive them.

Baize et al. (2006), focused in their study on the element cadmium in sludge, given its high toxicity, mobility and phyto-availability. A screening over 30 years showed a decrease in the Cd content of the sludge over time, due to different efforts in the treatment and purification of the sludge. Where the concentrations of Cd in the sludge of the studied station reported a value of 160 milligrams of dry matter in 1978, which decreases until reaching a value of 5.8 in 2002. The maximum limits of Cd concentrations in sludge are 85 mg kg⁻¹ according to the regulatory limits US EPA, 1993. The maximum Cd concentrations in sludge amended soils is 3mg kg⁻¹ following an average annual application over a period of 10 years of 0.15 kg ha⁻¹ year⁻¹ for soil with pH > 5 (DoE, 1996).

The concentrations of TEs were studied by (Merrington et al., 2003) in sewage sludge in 6 regions: Australia, Sweden, United Kingdom, New Zealand and United States. The authors reported values between 1.6 and 26 mg kg⁻¹, and between 20 and 60 mg kg⁻¹ of dry solids of Cd and Ni respectively in the United Kingdom and Australia. New Zealand and the United States have shown values between 311 and 700 mg kg⁻¹ of dry solids of Cu. And values between 99 and 480 mg kg⁻¹ and 559 and 2200 mg kg⁻¹ of dry solids of Pb and Zn respectively in the UK and USA. In soils amended by sewage sludge, the degradation of sludge OM can stimulate the diffusion of TEs in soils as demonstrated by several studies. The importance of dissolved organic matter (DOM) derived from anaerobically digested sewage sludge as a vehicle for TEs mobility.

The sludge organic matter has an influence on the bioavailability of TEs and varies according to its quantity and its quality (chemical structure). The chemical structure of sludge is changeable varying rapidly in a short period of time. Knowing that the organic matter of sludge is different from the organic matter of the soil. Many studies are being carried out to find out the relationships between the chemistry of organic matter in sludge. Other studies have been carried out to find the relationships between organic material chemistry in sludge, inputs and sludge treatment and inputs and treatment of sludge. Bioavailability measurements and C-NMR studies may allow us to determine the effect of organic matter quality in sludge on TEs availability.

I-1.1.2.1.2 Inorganic Fertilizers

The mineral fertilizers NPK, constitute: i) Nitrogenous fertilizers in liquid and solid forms, the most common of which are ammonia (NH₃), ammonium nitrate and urea. ii) Potash fertilizers from potash mines are generally potassium chloride, potassium sulfate, potassium carbonate or potassium nitrate, and iii) Phosphate fertilizers which are extracted from rock phosphates. These rocks, either of sedimentary or of igneous origin, contain TEs (Kratz et al., 2016) where those of sedimentary origin are the main origin of TEs in mineral fertilizers, which make up at least 85% of the raw material used for mineral P fertilizer production (Kratz et al., 2011), with mainly high Cd content as in Morocco, comparing to the igneous rocks.

However, these P fertilizers are essential for plant production used for food and feed, they are posing the main problems containing TEs such as As, Pb and especially Cd which can be transmitted in the soil, plants and thus in food (Jiao et al., 2012; Kratz et al., 2016; Molina-Roco et al., 2018; Spiegel et al., 2019). Few countries, especially Morocco and Western Sahara, China, Algeria, Syria and South Africa hold most of the P rock reserves (USGS, 2018). The majority of these phosphate reserves have already mined, and those that remain are of poor quality with low ore content and high concentrations of toxic TEs (Sverdrup and Ragnarsdóttir, 2014). According to FAO, 2017, Morocco is the largest exporter of rock phosphate in the world, constituting more than 50% of the world's phosphate reserves. The P fertilizers exported by Morocco to EU, New Zealand and Australia contain high levels of Cd, accounting for up to 60% of current cadmium emissions in EU soil and crops, according to the Policy Department of the European Parliament, 2017; WSRW, 2017 (Policy Dpt EU, 2017).

According to (Kratz et al., 2016), Cd was the only one that caused serious problems, with P fertilizers especially (superphosphate, triple superphosphate and rock phosphates) exceeding the current limit of 50 mg Cd per kg P₂O₅. The contributions of TEs to the soil through P-fertilizers have been well demonstrated in the literature (Molina-Roco et al., 2018; Spiegel et al., 2019; Thawornchaisit and Polprasert, 2009). A long-term fertilization with very high amounts of superphosphate (175 kg P ha⁻¹ year⁻¹) in a calcareous soil in Austria, led to significantly higher concentrations of Cd in topsoil (0-25cm) as per (Spiegel et al., 2019). Where the authors showed that Cd increased by + 25% in 0-25 cm soil depth from zero P, for a long-term fertilization of 175 kg P ha⁻¹ year⁻¹. In addition, the highest concentrations of Cd in the winter wheat grain were showed in the superphosphate fertilization. Basic slag

fertilization significantly enriched Cr concentrations in 0–25 cm with high fertilization. Cr increased by +32% in 0–25 cm soil depth with long-term basic slag fertilization of 175 kg P ha⁻¹ year⁻¹ compared to zero P. Even though, the literature shown that TEs accumulation in agricultural soils depends not only on the fertilization (type, amount and input frequency), but also on soil properties (pH, matrix) and climate conditions which vary between regions (Azzi et al., 2017; Barłóg et al., 2020; Spiegel et al., 2019).

In Lebanon, the majority of P-fertilizers are mainly imported from China, Turkey and Jordan, in the last decade The elements Cd, Pb and Cr had average values of 20.02 mg kg⁻¹, 42mg kg⁻¹ and 27.40 mg kg⁻¹ respectively (LARI, 2020). The Cd and Pb mean values exceeding the European standard limits (Cd: 6.26 mg kg⁻¹, Pb: 4.2 mg kg⁻¹, Cr: 70 mg kg⁻¹) (Verbeeck et al., 2020). The **Table I-1. 2** displayed the concentrations of As, Cd and Pb in P fertilizers marketed in different regions (China, European countries, Chile and Lebanon).

Table I-1. 2: Concentration of As, Cd and Pb in P fertilizers marketed in China, European countries, Chile and Lebanon

Region	Concentration (Mean mg kg ⁻¹)			Reference
	As	Cd	Pb	
China	13.5	2.6	30.0	(Yang et al., 2009)
Europe (12 countries)	7.6	7.4	2.9	(Nziguheba and Smolders, 2008)
Chile	15.1	12.3	10.4	(Molina et al., 2009)
Lebanon	-	20.02	42	(LARI, 2020)

The use of P fertilizers in cultivated wheat soils can lead to an increase of TEs such as Cd in crops (Barłóg et al., 2020). A ten-year field experiment conducted by (Chen et al., 2020), revealed that a long-term Calcium superphosphate, ~7% P, application may promote the bioaccumulation of As and Cd, and suppression of Zn, Cu, Pb and Ni in wheat grains. The authors found that an optimal P concentration of 50 kg P ha⁻¹ year⁻¹ to wheat crop didn't show any serious human health risk caused by the studied TEs through the ingestion of wheat grain.

A comparative table showing the average input of TEs from the application of P fertilizers in the cultivated soil in the Eastern Mediterranean countries compared to the European countries is presented below in **Table I-1. 3** (Azzi, 2017).

Table I-1. 3: The average input of TEs from the application of P fertilizers to the cultivated soils in the Eastern Mediterranean countries and in the European countries

Average input of TEs from P fertilizer	Country	
	Eastern Mediterranean countries	European countries
Cd (g ha ⁻¹ year ⁻¹)	6	1.6
Pb (g ha ⁻¹ year ⁻¹)	26	1
Zn (g ha ⁻¹ year ⁻¹)	922	43.1
Cu (g ha ⁻¹ year ⁻¹)	122	

In Lebanon, a continuous P fertilization application is carried out in the cultivated wheat soils in the Bekaa, where the amendment is ranged between 20-60 Kg ha⁻¹, still in the same range as (Chen et al., 2020), but with an application rate of 1860 Kg ha⁻¹ year⁻¹ higher than the average of the application on Asia 34 Kg ha⁻¹.

The Cd, presents in phosphate rocks from sedimentary and igneous deposits, has concentrations that can vary between countries and within deposits in the same country (Roberts, 2014). The author showed average values of 21 mg kg⁻¹ and 2 mg kg⁻¹ in sedimentary (from 35 deposits in 20 countries) and igneous (from 11 deposits in 9 countries) phosphate rocks respectively. Almost 85% of the world production of phosphate rock comes from sedimentary deposits. The **Table I-1. 4**, shows the Cd contents in mg kg⁻¹ of sedimentary and igneous phosphates rocks in Mediterranean countries (Roberts, 2014).

Table I-1. 4: The Cd contents in mg kg⁻¹ of sedimentary and igneous phosphate rocks from different countries

Country	Deposit	Average Cd mg kg ⁻¹	Range Cd mg kg ⁻¹
Jordan	El Hasa	5	3-12
	Shidyia	6	-
Syria	Khneifiss	3	-
Morocco	Undifferentiated	26	10-45
	Bou Craa	38	32-43
	Khouribga	15	3-27
	Youssoufia	23	4-51
Tunisia	-	40	30-56

In the European Union, nearly 85% of P fertilizers are imported. In 2017, Morocco was the 1st to supply the EU by 1.8 million tons, 1.6 million from Russia and 700,000 from Algeria. The European Commission has proposed a limit of 60 mg kg⁻¹ for 3 years after entry into force of the regulation, then 40 mg kg⁻¹ after 9 years and 20 mg kg⁻¹ after 12 years (EURACTIV, 2021). In EU, Finland is the only known source producing P fertilizers with low Cd content but its

production doesn't meet the needs of the European market, producing less than 1 million tons year⁻¹. The experts noted that only Russia meets the limit set by the EU (20 mg kg⁻¹ of Cd) in phosphate fertilizers and which could meet European fertilizer needs.

I-1.1.2.1.3 Pesticides

The application of pesticides with the aim of improving agriculture can cause damage in crops and shows an alteration of the food chain and the ecosystem (Hakeem et al., 2016). Several elements such as copper, iron, lead, sulphur, arsenic, mercury, zinc, tin, etc., are used immensely as pesticides in their organic or inorganic form (Jayaraj et al., 2016; Kovačič et al., 2013), i.e. methyl mercuric chloride, sodium arsenate, calcium arsenate, zinc phosphide. These TEs can be found in a significant quantity in agricultural soil due to the serious application of pesticides and high leaching potential, which may cause an important accumulation in the soil and be easily transferred and accumulated into the crops (Azzi, 2017; Chaza et al., 2018).

Since 1970s, the use of arsenic compound is known distinctively for wood preservatives purposes. Nowadays, it is used as weed-killers. In cotton field, the monosodium methanearsonate is used as a broadleaf weed herbicide as well as, substantial amounts of methylarsenic acid and dimethylarsenic acid are used as selective herbicides to control Johns grass (*Sorghum halepense*). Moreover, the arsenicals are used in viticulture in the form of sodium arsenite (Na_2AsO_3), calcium arsenite ($\text{Ca}(\text{AsO}_2)_2$), copper acetoarsenite-Paris Green ($\text{Cu}(\text{CH}_3\text{COO})_2 \cdot 3\text{Cu}(\text{AsO}_2)_2$), copper pyroarsenite ($\text{Cu}_2\text{As}_2\text{O}_5$), calcium arsenate ($\text{Ca}_3(\text{AsO}_4)_2$), and sodium arsenate ($\text{Na}_2\text{AsO}_4 \cdot 12\text{H}_2\text{O}$). The arsenate pesticides were banned in 1988 (USEPA, 1988). According to several studies, an increase in arsenic concentrations in agricultural soils has been explained as a result of different human activities and above all due to the excessive use of As-based pesticides where, the authors demonstrated that the As coming from this pesticide is more bioavailable than other anthropogenic sources (Juhasz et al., 2011; Punshon et al., 2017).

The element copper is a natural pesticide, known to be the most copper-based plant protection product used especially in organic fruit and grape production (Kovačič et al., 2013; Van Der Perk et al., 2004). In viticulture, it is applied to fight against downy mildew attacking vines. In Europe, and particularly in France, it is excessively used (3-15 times year⁻¹) in order

to avoid any mildew resistance development and as well, it is known to be an essential TE for the soil. This excessive use of copper fungicides may lead to an increase of Cu levels in soils (total content and bioavailable fractions of Cu) in layer 20-40 cm and even lower layers, and high Cu residues in grapes thus affecting the wine quality (Casali et al., 2008; Kovačič et al., 2013). According to (Adriano, 1986), the content of Cu in soils should not exceed the limits of 50 mg kg⁻¹. Due to this fact, around 12% of winegrowers have switched to organic farming recently in France. In 2018, the Institute of Agriculture and Organic Food (ITAB) and the National Institute of Agronomic Research (INRA) presented a scientific opinion on the use of copper, and showed that it is impossible to stop using Cu in organic farming but you have to go to half the dose usually used. Accordingly, nowadays the restrictions of use of Cu fungicides in agricultural soils showed a lot of importance, where the Commission Regulation EC No. 473/2002, requested the decrease of the Cu input levels from 8 to 6 kg ha⁻¹.

The element sulfur, since 1990s, was hardly used in crop protection in several countries such as United States, China, Europe and others (Gammon et al., 2010; Lamichhane et al., 2016). Nowadays, it continues to be used as fungicide and is commonly applied to grapes, tomatoes, and sugar beet, against fungus. As well as Cu, it can be used in “organic” farming (Gammon et al., 2010; Weitbrecht et al., 2021). A study conducted by (Kwasniewski et al., 2014), showed that following an SO spraying, the concentrations of SO residues are low in white wine making, while the residue concentrations in red fermentations are high (application during the 8 weeks following harvest), which even exceed those associated to an application of H₂S. The excessive application of all mentioned above pesticides may cause TEs accumulation in soil, and a high contamination of water and crops (Gammon et al., 2010; Kanda et al., 2012).

I-1.1.2.1.4 Irrigation waters

The irrigation water is a serious and crucial constituent for the agriculture (Darwish et al., 2011). Climate change and the subsequent change in agricultural conditions raise the susceptibility of agricultural water use. Due to the changing in the agricultural environment, the wastewater is considered as a substitute water resource around the world, which may be rich in TEs (Cu, As, Cd, Zn, Pb, Cd). Several researchers reported high concentrations of these elements in soil and in vegetables, where, they cause damage in leaves, roots, fruits and productivity, due to wastewater use (Gupta et al., 2019; Jeong et al., 2016).

Lente et al. (2014), showed in their study high concentrations of Pb on vegetables due to the irrigation by waste water coming from the effluents of the industries in an industrial studied area in Accra, Ghana. Another study conducted on tomatoes plants showed also a very high level of TEs accumulation in tomatoes due to the irrigation by sewage water (Manzoor et al., 2018). In wheat and barley crops, the shoots and roots showed an increase in Cu, Pb and Cd contents, although treated wastewater was used according to Werfelli et al. (2021). Where the authors demonstrated that such continuous irrigation may lead to TEs accumulation specifically in the roots of plants. In addition, a research conducted by kanwal et al. (2020), to study the bioaccumulation of Pb in wheat, using wastewater irrigation (7 treatments : 0-1000 mg kg⁻¹ of Pb), showed that Pb is highly accumulated in grains (> threshold in the cultivated part) with the highest Pb concentrations. Therefore, many research showed that the TEs accumulation in crops become harmful to human body (Gupta et al., 2019; Singh et al., 2011; Woldetsadik et al., 2017).

Since the use of wastewater for irrigation can be source of these TEs in crops, the WHO has controlled and set limits on use, where it does not recommend using wastewater containing an excessive amount of industrial wastewater, especially since it may contain TEs such as Cd, Pb, Mn, Cu, Zn, Cr, Hg, As, Fe and Ni (WHO, 2006). In Addition to (WHO, 2006), the (USEPA, 2012), and (Australia, 2006) have recommended guidelines for the safe reuse of wastewater as agricultural water, in other terms of water quality. Furthermore, Cyprus, France, Greece, Italy, Portugal, Spain and South Korea have revised and changed their quality standards with the aim of widely using treated wastewater for agriculture (Agrafioti and Diamadopoulos, 2012; Angelakis, 2001, 2001; Korea, 2011; Ortega and Iglesias, 2009; Paranychianakis et al., 2015).

In Lebanon, urban discharges, rich in TEs, can also be mixed with irrigation water. Where raw wastewater is mainly used for irrigation, and in particular in large agricultural areas such as Bekaa plain and Akkar plain (Darwish, 2008; Moustafa and Hamze, 2019).

I-1.1.2.2 Industrial stress

Industrial activities can be sources of TEs in the atmosphere. Thus, the atmospheric solid depositions and precipitation spread pollutants from industrial areas to rural areas. Consequently, the accumulation of these elements depends mainly on the intensity of

industrial activities and its duration. In the developed countries, the TEs accumulation has recently declined due to strict legislation and good law enforcement, unlike in developing regions (Cherfi et al., 2014).

A study carried out by Zhou and Wang (2019), showed that agricultural soils close to industries are contaminated with Hg and the level of this contamination largely depended on the number of industries present within the 5 km radius of the studied site. Other studies have shown that crops such as cereals and vegetables, grown in soils contaminated with industrial pollutants can contain a significant amount of TEs (Kabata-Pendias, 1995; Zwolak et al., 2019).

Thus, Safari et al. (2018), studying Cd contamination of winter wheat cultivated in an alkaline soil near a large metallurgical plant in Zing Town of Zanjan (ZTZ) in Iran, showed that 22% of the study area, around ZTZ, was covered by polluted soils with Cd exceeding the permissible concentration of 5 mg kg^{-1} . While 29% of the sampled wheat grain plants had a Cd concentration above the threshold value of 0.2 mg kg^{-1} , 42% of them were grown in non-polluted soils, hence the need to consider the sensitivity of the crop to TEs uptake from the soil.

Since different studies have shown significant loads of TEs in agricultural soil and crops, due to industrial sources, regular monitoring of TEs in soil and crops is necessary near industrial areas.

I-1.2 Mobility of trace elements in soils

The mobility of an element designates its ability to be transferred from a soil compartment where it is retained with a certain energy into another one where it is retained with less energy (Juste, 1988). The ultimate compartment will be the soil solution (liquid phase). We can thus define two large compartments in the soil: the solid matrix and the aqueous phase. The mineral and organic fraction constitute the solid matrix. While the aqueous phase or soil solution is composed by ions and organic and inorganic colloids.

In the solid matrix, the mineral fraction consists of primary minerals, secondary minerals (kaolinite, illite, montmorillonite) and oxides and hydroxides of Fe, Mn, and Al. The organic fraction is formed by complex and biodegradable substances: humin, humic acids and fulvic acids which differ in their molecular weight, CEC, pH, as well as their contents in C, N, and O.

The TEs in soil are non-degradable, stable and persistent. They are found in different chemical forms which can influence their activity, mobility, and bioavailability. For instance, they can be exchangeable on the clay-humic complex, precipitated in mineral form, adsorbed on or occluded in the Fe, Al, and Mn oxides-hydroxides or included in organic molecules, as well as in crystalline networks of primary and secondary minerals (Baize, 1997).

The mobility and bioavailability of trace elements depend strongly on their chemical speciation in soils. In order to define their behavior, it is essential to know their total concentration, their chemical speciation, the properties of the soil as well as the environmental factors (Violante et al., 2010).

I-1.2.1 Factors affecting the mobility of trace elements in soils

I-1.2.1.1 Soil parameters: soil pH, potential redox, and CEC

The soil pH is the main factor affecting the solubility of TEs in soil, and playing a key role in TEs bioavailability and toxicity in soil and plants (Antoniadis et al., 2017; Gupta et al., 2019; Likar et al., 2015; Teng et al., 2015).

The redox potential (Eh) is a measure of the tendency of an environment to oxidize or reduce substrates. The degree of soil aeration is determined by soil characteristics (texture, biological activity, etc.), cultural practices and climatic conditions (such as rainfall). These factors contribute to the lack of oxygen, thus modifying the mobility of certain TEs such as Mn and Fe, where their reduced forms are more mobile in the soil than the oxidized ones. In addition, it acts on the soil components, which have the ability to fix TEs. Thus, under satisfactory aeration conditions of the soil, the ferric and manganic compounds are very poorly soluble and therefore immobilize the TEs associated with them. Conversely, under limiting aeration conditions, the Fe and Mn compounds are reduced and dissolved; they therefore release the trace elements associated with them.

The pH and the potential redox (Eh) of the soil, influence directly and indirectly the chemical processes involving TEs, thus determining their behaviours in the soil. The “pH + Eh” effects on TEs mobility are complex and element specific. This is explained by several factors:

- a. Competition for sorption
- b. Decrease in negative charge depending on the pH of the sorption complex

c. Dissolution of soil components.

When the soil solution pH decreases, the activity of cations (H^+ , Fe^{3+} , Al^{3+}) as well as their positively charged hydroxide in the soil solution, will increase. Thus, these cations will compete with the TEs for a negative sorption of the sites.

As the pH decreases, the total amount of negative sorption sites decreases. The negative charges on the solid phase which depend on pH are neutralized by protonation. In addition, positive charges appear (covalent bond of H^+ on hydrated oxides of Fe and Mn, or organic functional groups). Therefore, the overall negative charges on the sorption complex decreases.

I-1.2.1.2 Soil constituents: Soil Organic Matter and Clay

The soil organic matter (SOM) plays a key role in the sorption and retention of TEs from the soil and therefore the transfer of their complexed forms into plants (Antoniadis et al., 2017; Partey et al., 2016; Pérez-Esteban et al., 2013). In fact, it can affect the mobility of TEs in the soil and thus their availability (Antoniadis et al., 2017; Azzi et al., 2017; Cambier et al., 2014). An increase in the soil pH allows the dissociation of the groups: carboxyl, phenolic, alcoholic and carbonyl, in the SOM of the soil, favouring an increase in the affinity of ligand ions for the positively charged element.

The clay fraction is mostly composed of aluminum silicates, more or less hydrated, with a leafy or fibrous structure. The interstices between the sheets may contain water molecules and/or ions.

Clay minerals have large specific surfaces and high cation exchange capacities (depending on the clay type). Consequently, they can retain a large amount of TEs by adsorption. Thus, all of the negative charges located on the internal and external surfaces and at the edge of the sheets represent as many exchange sites on which the cations metals will be able to be adsorbed.

I-1.2.2 Processes affecting trace elements mobility in soils

Davranche et al. (2020) in a unifying framework show that “the transfer of electrons drives metal cycling in the soil”. This means that the role of parameters such as pH, redox potential

and CEC, as well as that of clays and organic matter or that of microorganisms, must be understood from the point of view of electron transfer.

In a drained soil, the air easily penetrates the superficial horizon. While in deeper horizons and waterlogged soils, O_2 decreases due to the consumption of O_2 by the biomass for respiration. Fe and Mn containing minerals are found in high quantity in soils, where their Fe and Mn redox state can act as electron donors or acceptors (Melton et al., 2014).

Even a non-redox sensitive species like cadmium can be indirectly affected by redox changes in Mn and Fe components which can precipitate or dissolve as a result of redox reactions, thereby providing or removing reactive sorption surfaces, or even triggering processes of co-precipitation (or occlusion) immobilizing Cd.

On the other hand, the natural organic matter plays an important role, presenting functional fractions able to promote the transfer of electrons to mineral surfaces, thus catalysing redox transformations. Thus, there will be a development of the electron transfer gradients in soils which may influence the redox state and nutrients bioavailability (N, S, Fe or Mn). These redox changes shape various microbial groups, which will be different in their aptitude to use these nutrients under definite redox states *Figure I-1. 2*.

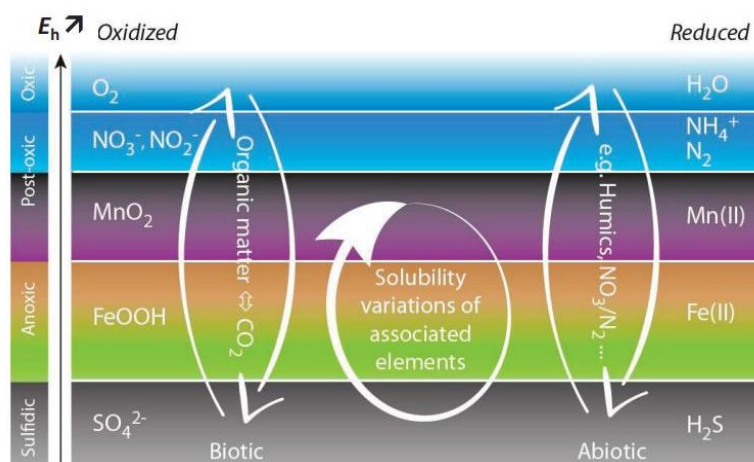


Figure I-1. 2: The reduction and oxidation sequence in the soil. The redox status can be catalyzed either by heterotrophic bacteria (biotic) or produced by chemical elements/compounds (abiotic). The electron donor substrate is generally soil (natural organic matter) for the biotic reactions (Davranche et al., 2020). Humics in the figure means humic and fulvic organic matters

In consequence, the microbes contribute to the biotic redox properties of the soils where the microorganisms form biofilms. The structure of these biofilms (gel-like structures) composed of cells allows a limited diffusion of chemical elements, thus avoiding their transport and loss out of cells surrounded by an exopolymer matrix. This causes specific physicochemical

properties (such as pH) of micro-environments, which influence the local redox properties of the biofilm and produce redox hot spots in the soil. In the mineral soil phases, the cycling of redox-sensitive elements is dependent on the redox-active minerals presence, particularly in environments exposed to varied redox conditions.

Among these minerals, the Mn (IV) oxides, one of the strongest oxidants in nature, play an important role, by controlling the redox state of Cr (hardly oxidizable) and releasing the toxic form Cr (VI) in nature (Tebo et al., 2004).

The Fe-bearing minerals can also be highly reactive. Mixed-valence Fe minerals carrying both Fe (II) and Fe (III) present strong redox properties. They are highly reactive, mainly in redox transition zones of the soil, where they are acting as durable reducing agents for: nitrites, chlorine compounds, nitroaromatics and Cr (VI) (Alowitz and Scherer, 2002).

Therefore, the Fe-oxyhydroxides and SOM are known as strong sorbents of metal(loid)s due to their high density of binding sites. Their nano-aggregate forms are the main phases that lead to TEs transport.

In SOM, the electron transfer activities of the dissolved and solid phase humic substances are commonly attributed to quinone-hydroquinone fractions.

In reduced environments, the humic substances, dissolved or adsorbed to solid phases, can act as redox buffers by receiving electrons from respiratory microbes and transferring these electrons to poorly accessible minerals, mediating electron transfer between microbes and electron acceptors (Macalady and Walton-Day, 2011).

According to a study by Zhou et al. (2015), the role played by electron transfer in biogeochemical cycles is better explained when acidic humic complexes – metal ions can be oxidized by oxidizing microbes Fe (II) and reduced by reducing microbes Fe (III).

I-1.2.3 Trace elements uptake and translocation in plants

In this part studying the plant, the elements As, Cd and Pb are presented since in our study only these elements were treated at the level of the wheat plant. Each plant has a specific physiology, morphology and anatomy. This diversity allows each plant to absorb and accumulate TEs in different ways (Yadav et al., 2018). In foliar uptake, small particles can diffuse through the stomatal and cuticular pathways to penetrate the interior of the plant leaf

(Xiong et al., 2014). Leaf area, size and density of stomata can affect leaf uptake of TEs (Shahid et al., 2016).

In root uptake, Chandran et al. (2012) showed that plants with many fine roots accumulate more TEs than those with few thick roots. In plant, the root absorbs the TEs available in the soil, where a quantity passes into the transpiration flow through the xylem vessels, arriving at the aerial part where they accumulate under the transpiration effect. The plants may accumulate more TEs when transpiration is flourishing (Hao et al., 2012). In this passive transport, the xylem sap is raised upwards by the transpiration stream and allows the distribution of TEs in the aerial tissues. At leaf level, these TEs can be redistributed or accumulate in photosynthetically active leaves. These TEs can pass from the leaves through the phloem to finally arrive in the growing vegetative part or the maturing fruits.

In wheat plant, the roots play an essential role in active uptake of metal ions. This root adsorption allows cations to bind to the negative cell wall of the roots due to the presence of cellulose, pectins and glycoproteins which in turn play a specific role of ion exchangers (Arif et al., 2016). Cations such as: Cd^{2+} , Fe^{2+} , Mn^{2+} , Ni^{2+} , Pb^{2+} , Zn^{2+} , are found available at the level of the root surface from the dissociation of their complex forms and strongly accumulated in the apoplast of the root (Krzyszowska, 2011).

When cations build up in the root apoplast, they can: (i) be retained in the root cells, (ii) move to the root stele, and then to the xylem and phloem tissues. Two ways are known for their passage through the xylem and phloem: passive uptake, and active uptake (Hossain, 2012).

In passive transport, metal ions from the soil solution are diffused into the root cell through the intercellular spaces, whereas active transport consists of the passage of metal ions using different carriers and transporters in the mediated plasma membrane (Barberon and Geldner, 2014).

1-1.2.4 Effects of trace elements in wheat plants

The presence of trace elements affects the availability of others whether in soil or at plant level (Chibuike and Obiora, 2014). In plant, the elements Ni and Cd compete for the same membrane supports (Clarkson and Lüttge, 1989).

Trace elements may have direct effects on the plant, such as inhibiting the cytoplasmic enzymes, or causing oxidative stress which leads to cell damage (Jadia and Fulekar, 2009).

Trace elements may also have indirect effects on plants, where they can substitute essential nutrients at cation exchange sites (Taiz and Zeiger, 2002). These TEs, easily absorbed by the roots of plants, are accumulated at any level of the plant and can be transferred to the edible part (Jolly et al., 2013).

In wheat plants, the P fertilizers application may increase the As contents in soil solution and in root and shoot of wheat plants (Tao et al., 2006). The As uptake in wheat plant varies from one variety to another as demonstrated by Zhang et al. (2009), where the authors showed a positive correlation between As content in soil and As content in wheat plant tissues, where the As concentrations in plant tissues were as follows: roots > stems > leaves and rachises > grains > glumes > awns. Another study conducted by Shi et al. (2019), showed a decrease in As concentrations in wheat plant roots in the period from tillering to maturity, as well as a decrease in its accumulation in plants. Showing that accumulation of As in wheat grain is influenced by the remobilization of the As from the leaves and stem to the ear.

The bioavailability of Pb is attributed to physico-chemical soil properties as per Wijayawardena et al. (2015). The use of waste water, fertilizers and pesticides resulted in high concentrations of Pb in studied wheat cultivars by Guo et al. (2018). The element Pb is considered as toxic element, affecting the growth and the development of crop plants and causing their death (Khan et al., 2015). In wheat plants, a Pb exposure can reduce the growth of wheat plant organs such as root and shoot length, as well as the fresh weight and the dry weight of the shoots. In addition, it can be accumulated in several parts of the plant and in particular in wheat grains (Khan et al., 2015). In wheat plants, a maximum reduction in stomatal conductance (82%) and transpiration rate (72%) was observed at high Pb concentration treatment (1000 mg Pb kg⁻¹) in a study conducted by Kanwal et al. (2020), concerning the bioaccumulation of Pb in the wheat plant following experimental trials where Pb treatments of (0 to 1000 mg Pb kg⁻¹) were applied.

The element Cd is mobile in acidic soil and will accumulate in wheat crops (Liu et al., 2015). Moreover, high exposure concentrations of Cd, even at short exposure times, may cause stress and complex phenomena in wheat plants (Stolt et al., 2003). During the period from tillering to maturity, the Cd levels in the roots of wheat plants decrease, in addition, the accumulation of Cd in roots decreased considerably at the lengthening stage, as well as in

stems because of the uptake and transport of Cd over time did not keep pace with biomass accumulation (Shi et al., 2019). Furthermore, wheat plants may accumulate above permissible levels of Cd in grains if grown on Cd contaminated soils ($0.11 - 1.37 \text{ mg kg}^{-1}$), and consequently wheat contaminated plants may transmit Cd to humans through ingestion (Liu et al., 2015).

I-1.3 The essential role of the element silicon in trace elements bioaccumulation in wheat plants

Silicon is a major constituent of the earth's crust (Tubaña et al., 2016). The silicon content of earth's rocks varies between 40 and 70%. In its soluble form, it is present as silicic acid Si(OH)_4 . Before its transfer to surface water, silicic acid dissolved in soil solutions will be trapped by plants roots which play an active role in the accumulation of silica in plant and in the storage of Si. The plant absorbs the silicon trapped by their roots under the form of Si(OH)_4 acid and transports it to the other parts of the plant. After the transfer of Si in all plant parts, the plant precipitates the Si in their aerial organs by forming the phytoliths ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$). After the plant death, the phytoliths are released by degradation of organic matter in the litter giving biogenic Si(Bsi) which again dissociates into Si(OH)_4 , allowing the storage in the soil to at concentrations varying from 0.09 to 23.4 mg L^{-1} depending on soil types (Khan et al., 2021; Meunier et al., 2018). Due to the intensification of agriculture, the soil solution becomes depleted of silicic acid. In the past 20 years, research has demonstrated that Si fertilization is well used given the benefits of silicon for crops (Tubaña et al., 2016). Accordingly, Si bioavailability increases with increasing silicon application in soil and varies with soil type. This will help the increase of Si absorption in stems, leaves and straws (Coskun et al., 2019). In crops, Si causes an increase in growth, development and reproduction of plants, under difficult conditions such as drought and salt stress, as per the Association of US Plant Food Control Officials (AAPFCO). Rice, sugarcane and wheat are known to be high-Si accumulating plants. Accordingly, the wheat biomass increased by 17% at vegetative stage, under drought and salt stress conditions, after Si fertilization (Tubaña et al., 2016). Wheat plants exposed to drought and treated with Si showed a maintenance of higher stomatal conductance, relative water content, and water potential than other non-treated plants (Guntzer et al., 2012).

The surface of the mineral-Si can carry a big quantity of exchangeable cations, while a small quantity of exchangeable H^+ , resulting in hydrolysis of exchangeable cations in the soil, then making a large amount of NaOH in the soil solution and increases the soil pH.

Several studies indicated that the increase in soil pH allows the decrease of the cation availability like: Cd, Pb, Zn and others. This was demonstrated by Khan et al. (2021), where the authors showed that the Si in the soil allows a decrease in the available Cd in the soil and thus its transport and its accumulation in rice grains.

In wheat plants, an increase of the application of Si available to plants in the soil, contributes to a decrease in the concentrations of Cd in shoots, while they showed an increase at the root level (Rizwan et al., 2012). Accordingly, The Si application found to decrease Cd translocation from root to shoots and then to grains (Naeem et al., 2015).

The total As, studied in rice plants showed a decrease of concentrations in different rice organs, between 24.1% and 51.9% after Si application, while the inorganic As concentrations showed a reduction between 20.1% and 52.3% (Li et al., 2018). In wheat plants, the Si may play an essential role in the detoxification of As (Hossain et al., 2018). According to authors, under an arsenic stress, the addition of Si to soil showed a significant increase in As in the roots but not in the shoots. The results of another study conducted in Norway by Kaur and Greger (2019), indicated that Si application in soil increases 10 times the available Si and lead to an increase of Si content in wheat grains by 12-18%, and decrease the As content in the same plant organ by 20-40%.

In China, the application of Si showed an attenuation of the toxicity of Cd and Pb in wheat plants and increased the grain yield by almost 44% as per Huang et al. (2019). This application increased the absorption of Si in roots and shoots, thereby reducing the build-up of Cd (13%) and Pb (63%) in wheat shoots and bran.

Wheat crops showed grain Cd contamination and low productivity in Cd-contaminated soil due to Cd stress (Yanardağ et al., 2016). The Si-soil application caused a decrease in plant available Cd fraction by coprecipitation and also by increasing soil pH. Furthermore, a decrease of Cd in wheat tissues was explained by the reduction in Cd-plant availability and also xylem Cd translocation to shoots (Naeem et al., 2015).

Zhou et al. (2021) studied a foliar Si and soil Si applications to wheat plants, in a neutral sandy loam soil, with a pH 7.15 and contaminated by Cd. They found a significant attenuation of Cd

toxicity through the regulation of Cd transport genes, which was expressed by an increase of grain yield and enzyme antioxidant activities in wheat tissues.

CHAPTER I-2

Bread and Trace Elements Exposure of the Lebanese Population *via* Lebanese White Bread

I-2.1 Challenges of trace elements food contamination

Trace elements show two faces, where they can be beneficial and/or toxic. Minerals are part of the human diet and are essential for normal health and function. The essentiality and toxicity of these elements show no relationship, while toxicity is a question of dose or exposure. In recent years, considerable researches have been carried out to better understand the physiological role and health consequences of TEs, below the challenges of TEs food contamination around the world and in Lebanon are well detailed.

I-2.1.1 Challenges of trace elements food contamination worldwide

Unfortunately, these elements enter the human food chain and may pose serious problems and cause risks for human health as showed by various studies in a number of developed countries such as Spain, Belgium, England and the United States. For instance, several studies have shown that element Pb may be excessive in some foods like tubers, legumes and cereals (Akinyele and Shokunbi, 2015), cereals (Brizio et al., 2016; Pirsheh et al., 2016), and rice (Abtahi et al., 2017).

The latter also presented the highest level of As among the cereal group analysed in a study carried out by Marín et al. (2018) on twelve selected foodstuff groups representing the most consumed food in the region of Valencia – Spain, with an average value of 0.147 mg kg⁻¹. These values were higher than those detected in France – (ANSES, 2011a) following a TDS study: 0.016 mg kg⁻¹ for white rice, and lower than those detected in Catalonia – Spain, where the average value of total As in rice was higher by 0.18 mg kg⁻¹ and also lower than the values found in Canada (average value of 1.240 mg kg⁻¹). This issue is underlined in the Middle Eastern countries and Saudi Arabia where populations consume rice on daily bases. Shraim (2017) showed the As levels content in rice product sold in Saudi Arabia markets and Brisbane – Australia, and rice produced in Bangladesh (in an As contaminated area). Noting that, this element values were above the threshold (0.2 mg kg⁻¹ day⁻¹) in rice grown in Bangladesh (WHO, 1996). The results of this investigation indicate that some rice varieties sold in the local markets of Almadinah Almunawarah, Saudi Arabia contain hazardous levels of one or more of the toxic elements (arsenic, chromium, and lead). Additionally, the values of As concentration measured in 70 rice samples on Saudi Arabia markets (average value of 0.136 ± 0.086 mg kg⁻¹), showed a variation from one country of origin to another: USA origin (0.257

mg kg⁻¹) > Thailand origin (0.200 mg kg⁻¹) > Pakistani rice (0.147 mg kg⁻¹) > Indian rice (0.103 mg kg⁻¹) > Egyptian rice (0.097 mg kg⁻¹). While rice samples from Suriname, Australia and France showed higher values respectively: 0.290, 0.188 and 0.183 mg kg⁻¹ respectively.

Canned foods such as canned vegetables, fruits, meat, drinks or fish are popular worldwide and are considered to be important sources of TEs. And since they are highly consumed, they make an important contribution to TEs. In Jordan, a first study of this type was carried out to measure the levels of Pb, Zn, Cr, Ni, Cu, As and Cd in different brands of canned fruits and vegetables present on the market, and compared them with other studies in other countries and the authorized limits. All the studied samples showed values above the authorized limits. In canned foods, the main role of the packaging material is to preserve the original characteristics of the food and to protect it. Accordingly, several studies have shown that the interactions between food and packaging are mainly due to the deterioration of these packaging (corrosion, dissolution of the coating layer, aging by oxidation of food components, etc.) and the nature intrinsic value of the preserved food (Bontos et al., 2016; Nouredine El Moussawi et al., 2019; Vaireanu et al., 2018). The TEs can be present in food following the use of stainless steel and Al materials during food preparation (Al, Ni), food packaging (Sn), unpainted steel containers (Fe, Cr, Cu and Zn). In addition, leaching of solder materials may lead to the appearance of Pb and Fe in canned foods (Kassouf et al., 2013; Nouredine El Moussawi et al., 2020). This release can lead to gastrointestinal disturbances at high concentrations (> 200 mg kg⁻¹) (Arnich et al., 2012; Korfali and Hamdan, 2013).

In fish and seafood, the TEs such as Pb, Cd, Hg and As are considered as part of food safety concern. Accordingly, the total Hg is known as a health concern for consumers, especially in the coastal regions of South-East Asia, the Western Pacific, the Baltic Sea and the Mediterranean area as per several studies in many countries, where the averages showed values close to the toxicological reference values established by JECFA. However, the global organizations have recommended limiting the consumption of certain shellfish (oysters and scallop fish) following the assessment of high levels of Cd intake among consumers in Canada (a consumption of 3 units per week shows values close to the threshold recommended by JECFA). The elements Pb and As pose no risk to humans due to fish ingestion (Chiocchetti et al., 2017). A study conducted by Sobolev et al. (2019) concerning the essential and non-essential TEs present in the most seven consumed fish by indigenous peoples of the European

Russian Arctic, showed similar concentrations of As as found in the literature (15-17 mg kg⁻¹) as reported by Sloth et al. (2005). The concentrations of Pb and Cd were lower than Hg in all fish species. The authors suggest that the Hg origin might be a resultant of atmospheric deposition due to human activities and/or an occurrence of natural processes.

In conclusion, the TEs in food can thus be the result of raw materials, food handling and manufacturing process such as direct contact between food and TEs, processing equipment, storage and packaging (Demirözü et al., 2003; Kassouf et al., 2013; Korfali and Hamdan, 2013).

1-2.1.2 Challenges of food contamination in Lebanon and food safety

In Lebanon, food industry constitutes about 18.2% of the factories, where the food safety plays an important and essential role in the national economy and public health. Despite the importance of the food sector for the Lebanese economy and industry, food safety remains a major problem (Kamleh et al., 2012).

Epidemics of food poisoning are still a problem among the Lebanese population (Vapnek, 2005). According to SOER (2010), Lebanon suffers from serious problems. Firstly, the energy availability, where the daily and prolonged power outages in the countries is the result of production shortages capacity. Secondly, the unsustainable water management practices, the state of wastewater management and the increasing water pollution showing that the majority of the population has no source of safe drinking water, in addition to the need of water filtration before use. All these problems may cause challenges to the Lebanese population and affect the food industry and then form barriers to food safety. Meeting the country's water demand over the medium and long-term poses a significant challenge to the Government of Lebanon. Accordingly, Harakeh et al. (2005) showed in their study, on Lebanese Shawarma sandwiches, that 47.5% of the samples contained high levels of *Salmonella*.

Other local studies conducted on the Lebanese food safety have indicated the presence of pathogenic microorganisms (levels exceeding the international standards) such as *E. coli*, *Salmonella*, *Staphylococcus aureus*, and *Listeria monocytogenes* (Borjac et al., 2019; Harakeh et al., 2009; Kassaify et al., 2010). A study conducted by the MOPH in Lebanon in 2012 (MOPH, 2012), indicates that in 2010 there were 1926 cases of food borne and waterborne diseases in Lebanon. Recently, food safety was of serious concern to the Lebanese people as a result,

of the November 2014 campaign, by the Minister of Public Health (MOPH, 2014). The latter published a list of restaurants, supermarkets and other food service providers that did not meet the requirements of the food regulation and standards with certain products having positive results for microorganisms such as: *Salmonella*, *E. coli* and obligatory aerobes. Despite the Lebanese people concerns, the food production control, and the food safety, the practices have not so much changed (MOPH, 2014).

Various studies have been conducted in Lebanon on food contaminants such as TEs in vegetables, breast milk, fish, meat and fruits, as well as on wheat (Tortoe et al., 2018). Regarding bread, few studies have been conducted on trace element contamination (Bou Khouzam et al., 2012; Nasreddine et al., 2010). Leafy vegetables, in Lebanon, showed high values of TEs (Pb, Cd, Cr and As) in comparison to Saudi Arabia and Turkey which also showed in industrial zones, high values of TEs (Fe, Mn, Zn, Cu, Pb, Cd and Hg), exceeding the limits of FAO, 1994 (Ali and Al-Qahtani, 2012; Demirezen and Aksoy, 2006; Obeid and Aouad, 2009). In addition, strawberries, cucumber and spinach presented the highest levels of Pb, Cd and Zn in Egypt according to Radwan and Salama (2006). The Cd shows levels above the EU norms ($0.1 \mu\text{g g}^{-1}$) in 30% of vegetables and legumes analysed samples and 45% of analysed fish samples in a study conducted by Korfali and Hamdan (2013).

I-2.2 Lebanese bread Industry and trace elements contamination

Bread is well known as the staple food, the most popular and the oldest in the world. Bread in one form or another, is the principal food for Man. Egypt was the first country producing bread, although the most excellent bakers came from Phoenicia. After that, bread became well known among the Roman, Greek, Egyptian civilizations and many others.

I-2.2.1 Lebanese white bread composition

Bread is a source of energy and minerals, some of them being essential for the body. It is a mixture of flour, yeast, water and salt. Certain additives are added to improve the nutritional profile of the bread, to increase its shelf life and/or to obtain a better quality. There are several types of bread that vary sensory and nutritionally depending on the type of flour used. In the Lebanese market, many types of bread [Fig I-2.1](#) may be found such as: “**White or plain**

Arabic bread”, “Brown bread or whole meal bread”, “Tannour bread”, and “Saj bread” (Bou Khouzam et al., 2012).



Figure I-2. 1: The most popular Lebanese bread from the left: white pita bread (white flour, loaf of two layers), brown bread (whole wheat grain flour, loaf of two layers), and saj bread (brown and whole wheat flour, with one layer)

The wheat flour is the main raw material of the bread which belongs to a group of staple foods and essentials for a balanced diet such as pastries and biscuits (Araujo et al., 2006). It is also known as essential on the manufacture of pizzas and other starch products (Doe, 2013). The quality of wheat flour is based on obtaining a final product with excellent sensorial properties like taste, color and smell, as well as an appropriate level of humidity, a correct content of ashes, and absence of other substances (Tejera et al., 2013).

Concentration of minerals in wheat products can be increased by using whole grain flour instead of white flour (Lopez et al., 2003). The flour used can come from wheat grown on farmland and watered with water that may contain TEs (Fakhri et al., 2018). The yeast is a leavening agent, used to help the dough rise due to the production of carbon dioxide at the fermentation stage. In pita bread making, the water gives the consistency of the dough (depending on the amount of water added). Its temperature may influence the temperature of the dough and the activity of the yeasts. The salt or sodium chloride “NaCl” plays an essential role in sensory properties, dough, texture, and shelf life of the bread. Accordingly, the salt content in the Lebanese white pita bread is presenting 1.3 % (± 0.66) comparing to brown pita 1.5% (± 0.77), saj bread 2.8% (± 1.16) and tannour 2.2% (± 0.96), following a study conducted by the American University of Beirut (AUB) in 2015 (Barakat, 2015). The sugar and other additives may be added for caramelization and to enhance flavor, color and texture of the bread (Dadzie-Mensah and Ayernor, 2004; Demiralp et al., 2000; Qarooni et al., 1989; Toufeili et al., 1999).

Table I-2. 1: The main ingredients and manufacturing of different types of Lebanese bread

Bread	Flour						Other ingredients and additives					Cooking time	Aspect	
	White wheat	Wholemeal wheat	Wheat	wheat bran	Whole corn	Corn	Yeast	Sugar	Salt	Water	Preservative	Vitamins	Minutes	nb of layers
Lebanese White	X						X	X	X	X	-	-	20 to 25	2
Lebanese Brown		X	X				X	X	X	X	E282	B1 / Folic acid	5 to 7	2
Tannour		X	X	X	X		X	X	X	X	-	-	5 to 7	1
Saj	X	X				X	X	-	X	X	-	-	3 to 5	1

(X) : contain, (-): doesn't contain, (nb of layers): number of layers, (B1): Thiamin.

I-2.2.2 Lebanese white bread processing

Pita bread is made with grain flour, water, salt and baker's yeast. The flatbreads are the most widely consumed bread category in the world. In Lebanon, as the majority countries of the Middle East region, some bread types are still produced at home, and others can be found in the markets.

I-2.2.2.1 Description and manufacturing process of Lebanese white bread

The majority of bread rolls go through the same basic steps when baking [Figure I-2. 2](#). The dry ingredients are weighed and transferred into the mess where water is added gradually. The dough goes through a fermentation stage. During this phase, the size and volume of the dough will increase due to the activity of the yeast. Once the lifting phase is over, the dough is divided into round pieces. The conveyor belts transfer the pieces of dough into the preheated oven. The cooking time and temperature vary depending on the type of bread. After cooling, the bread is packed in plastic bags.



Figure I-2. 2: The different steps of Lebanese white pita bread manufacturing

Flat breads are popular all over the world, having the same production stages as other types of bread: kneading the ingredients, then lifting and shaping, and finally cooking. They can be present under different types, depending on the consistency of the dough, the presence or absence of a rising phase, the cooking system used the thickness and the structure of the finished product. They are made either leavened or unleavened, and also divided into two major groups: one (single) layered and two (double) layered (Pahwa et al., 2016; Pasqualone, 2018), as showed in [Figure I-2. 2](#) and [Table I-2. 1](#). The two-ply flatbread known in Western countries, and called “Arabic bread” or “pita bread”, is made of a paste of a few millimetres and is not punched. In the oven, the dough swells and forms a “balloon”. This swelling is due to thermal expansion of carbon dioxide during fermentation, moisture in the dough and air trapped during kneading. After cooking, the bread cools and the balloon deflate, giving a soft loaf with an internal cavity. The two layers can be separated by cutting the bread along the edges. The word “pita” is not a classic Arabic word, but it is an illicit word meaning “food” and “bread”, or it is an Aramaic word “pita” which means flat bread or products bakery. The word

pita is used to indicate bread in the following regions: Lebanon, Syria, Jordan and Palestine (Braccini and Braccini, 2007; Pahwa et al., 2016; Pasqualone, 2018).

I-2.2.2.2 Packaging of Lebanese white bread

In our decades, food packaging is widely used, where it leaves food in a cost-effective manner that meets industry demands and consumer desires, preserves food safety, and minimizes environmental impact (Hahladakis et al., 2018).

The main role of food packaging is to: i) contain food, ii) protect food from external influences, and iii) provide information to consumers (Coles, 2003). Recently, the production of plastic packaging has become important for many reasons: inexpensive, multi-layered, durable and lightweight. This production consists of the use of different chemicals or “additives” resulting from the waste of plastic material (Al-Dayel et al., 2012; Hahladakis et al., 2018). These additives are found in plastic products to improve the properties of polymers and extend their lifespan. This huge use of these additives can lead to food contamination, which can lead to a human exposure as a result of packaging material in contact with food and migration. Traceability is one of the secondary functions that appear following the use of plastic packaging (Donatella et. Al., 2010). Transparent plastic containers are generally used to contain bread [Figure I-2. 3](#), it is question whether there is release of these TEs in the consumed product (Agunbiade et al., 2020; Marsh and Bugusu, 2007). It is mainly constituted from polyethylene knowing that migration of contaminants will take place in particular in the case of foods rich in fat and stored at high temperature (Silva et al., 2006).



Figure I-2. 3: The plastic bag containing Lebanese white pita bread

I-2.3 Nutritional benefits of Lebanese bread

Bread is one of the main constituents of human nutrition and well known as an ample source of grain-based nutrients. It is also considered as a good source of carbohydrates, magnesium,

iron, selenium, B vitamins and dietary fiber which are essential for humans. In fact, essential minerals found in Lebanese bread are Cu, Fe, Mn, Zn, and Se which account for 36%, 25%, 30%, 22%, and 32% of respective recommended dietary allowance (RDA) values given for these elements (Bou Khouzam et al., 2012).

The Arabic bread (Pita) is the most common type of bread consumed in Lebanon and the Arab region. In one hand, a large pita loaf of approximately 122 g may contain about 170 calories, and rich in carbohydrates with low dietary fiber content, noting that the majority of the calories come from the high carbohydrate content. In the other hand, the pita bread is characterized by having a low quantity of fat (1.6%), very low quantity of saturated fat and cholesterol, and a fair amount of 16 g of proteins, comparing to respectively 6 g and 3 g in a large and a small whole wheat pita loaf. In addition, the whole wheat pita bread may contain 10 mg of calcium and less than 2 mg of iron **Table I-2. 2**. Good bread quality is requiring good manufacturing process, as monitoring and corrective measures in manufacturing procedures and in the technological procedures of contamination and risk to the consumer (Mestek et al., 2015; Soares et al., 2010).

Table I-2. 2: The calorific values and nutrition facts of Lebanese breads

Bread	Mass (g)	Calorific value (cal)	Nutrition Facts						
			Carbohydrates (g)	Dietary fiber content (g)	Fat content (g)	Saturated fat & Cholesterol (g)	Proteins (g)	Ca (mg)	Fe (mg)
Lebanese White	122	170	50	14	5	Low quality*	16	10	< 2
Lebanese Brown	60	195	39	2	1.5	0.5	6	-	-
Tannour	38.5	96	22	2	-	0	5.4	-	-
Saj	80	270	58	0	1	0	8	-	-

*Saturated fat makes up less than 10 percent of the total calories of that meal

I-2.4 Trace elements contamination of Lebanese bread

As we mentioned previously, Tes represent the main source of chemical contamination in food and Tes are recognized worldwide as a danger to public health (Marín et al., 2018),

where they bioaccumulate and enter the food chain (Gupta et al., 2019; Kumar et al., 2019; Mitra et al., 2017).

Several few studies carried out in many countries have shown that the presence of TEs in bread are partly linked to cereals, but strongly linked to the process of bread preparation (kneading, lifting, shaping, and baking). In the cooking stage, the sources of trace element contamination are diverse, such as the trace element surfaces that come into contact with the material and the contamination of the air and the environment. In addition, all the fuel used during cooking can emit TEs residues during process (Ahmed et al., 2000; Khaniki et al., 2005). As a result, the flat bread samples collected from bakeries of Tehran, showed high levels in Pb, Cd and specifically in Ni comparing to those present in the Romanian bread (Khaniki et al., 2005; Storelli et al., 2017). Another study concerning the Iranian bread conducted in Isfahan region in 2018, showed a contamination of wheat bread by both elements Pb and Cd, this contamination was due to industrial and agricultural activities, where the results showed average values exceeding the limits allowed by international and national standards.

Consequently, many factors may influence the bread chemical contamination such as contamination of ingredients, processing and cooking. A study conducted by (Ertl and Goessler, 2018), studying concentrations of 27 elements, in cereals, flour, bread and pasta in Austria, showed that TEs concentrations (especially Ni, Cd, Pb, Al and Cr) may be affected during grinding and processing. The TEs in wheat flour showed successively values of $0.05 \pm 0.001 \text{ mg kg}^{-1}$, $0.036 \pm 0.016 \text{ mg kg}^{-1}$, $0.007 \pm 0.001 \text{ mg kg}^{-1}$, $0.06 \pm 0.0036 \text{ mg kg}^{-1}$, $0.005 \pm 0.001 \text{ mg kg}^{-1}$, $0.022 \pm 0.004 \text{ mg kg}^{-1}$, and less than 0.002 mg kg^{-1} for Pb, Cr, Co, Ni, As, Cd, and Hg respectively. The elements Al and Cr showed values 2 times higher in flour than in cereals due to grinding and processing. The cooking has been shown to be a source of lead contamination (Ghoreishy et al., 2018), while a study conducted by Yusà et al. (2018) did not take into consideration the effect of cooking and processing.

In Lebanon, a study conducted on the Lebanese population for total diet study "TDS" in 2010 (Nasreddine et al., 2010), and another in 2012 reported that the average consumption of bread is about 136.8 g per day (Bou Khouzam et al., 2012). Among the very rare studies in Lebanon concerning the trace element bread contamination, the latter determined the contents of 20 elements in different three varieties of Lebanese bread (i.e., white, brown pita, and Saj bread) throughout the country during the wet and dry seasons, where the

concentrations of the elements Ag, Co, Hg, and others in Lebanese bread were similar to those reported in other countries and where the toxic elements such as Cd and Pb were below maximum levels set by the Lebanese Standards Institution "LIBNOR" (Bou Khouzam et al., 2012). Due to the lack of sufficient data and information, our study was conducted in order to have a state of data of TEs contents in bread, as it is the case in other countries such as Egypt, Iran, South Africa, Nigeria, and Portugal and in Europe (Ahmed et al., 2000; ANSES, 2011; EFSA, 2012; Soares et al., 2010).

I-2.5 Potential Exposure and Risk Associated to TEs exposure of the Lebanese population via the daily consumption of the Lebanese white bread

I-2.5.1 Potential exposure to chemical compounds and associated risk

As shown above, the micronutrients are essential or necessary for human nutrition, some not essential or even toxic such as Pb, Hg, Cd and As (Counter and Buchanan, 2004). These contaminants can pass from farm to fork and enter the human body through the food chain, where it becomes toxic at sufficient high levels. The exposure to toxic elements can lead to risks and problems for human health such as disorders of the nervous and endocrine system, as well as reproductive and gastrointestinal tract problems, which are very well reported and documented (Babaahmadifooladi et al., 2019; Nordberg and Nordberg, 2016; WHO, 1996). For instance, the use of pesticides, fertilizers and other chemical compounds in different ways has shown numerous advantages at the society level expectation. However, sometimes these advantages are outweighed by certain disadvantages, in particular the toxic side properties of the chemical compounds used [Figure I-2. 4](#). The chemical exposure can lead to variable effects, such as death and chemical carcinogenesis (Oliveira et al., 2007). The chemical carcinogenesis is a multicausal process in which the normal cells become first initiated, then malignant and invasive. The first experimental work on chemical carcinogenesis was carried out in 1915, where at the end of the nineteenth century, the occupational chemicals exposure showed carcinogenic effects (Luch, 2005).

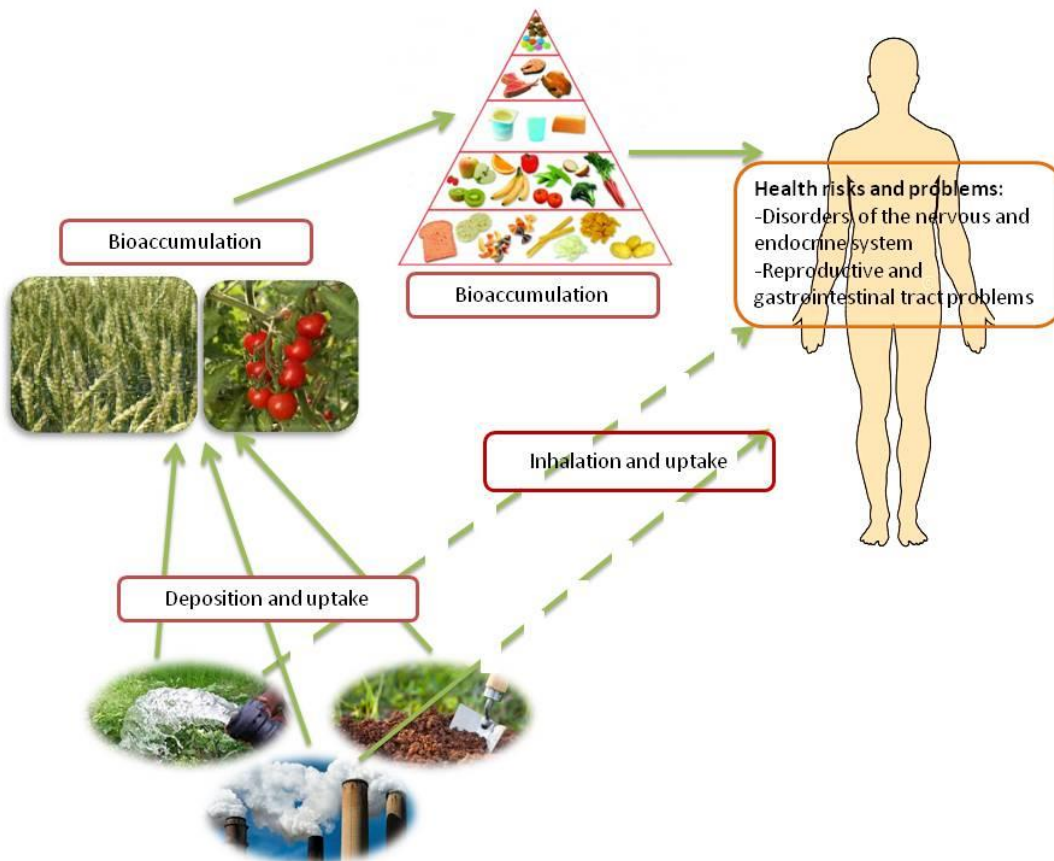


Figure I-2. 4: The Tes in the environment (soil, water, air and plants), arriving to the food chain and then enter human body through the consumption of food and vegetables and cause potentially harmful effects for the health.

As a result of endless human chemical exposure (different amounts) having mutagenic carcinogenic or reprotoxic properties, chemical carcinogens can appear as:

- i. exogenous: consumption of food and drinking water, and occupational exposure,
 - or ii. Endogenous: metabolism, hormones, pathophysiological conditions, etc.
- Several epidemiological studies have showed that lifestyle causes cancer such as diet, smoking, obesity, alcohol, etc (Valavanidis and Vlachogianni, 2010).

As a result, scientists have proven the importance of chemical carcinogenicity in human risk assessment.

Many agencies have estimated the carcinogenic risks of various chemicals agents such as the International Agency for Research on Cancer (IARC), the National Toxicology Program (NTP) and the European Commission through the regulation 1272/2008 (European Parliament and the Council of the European Union, 2008). Accordingly, after several studies, these organizations have set the limits of exposure and legislate to restrict the use of chemical

carcinogens in other applications and to protect the human population against carcinogenic risk from these substances (Cohen et al., 2019; Valavanidis and Vlachogianni, 2010).

I-2.5.2 Potential exposure to trace elements in food and associated risk

Following the existing literature, it is crucial to quantify the content of TEs in food in order to calculate roughly dietary exposure which is a requisite in the evaluation of risks associated to food consumption (Marín et al., 2018; van der Fels-Klerx et al., 2014). Recently, several studies have evaluated the exposure to TEs in foodstuffs, in different regions such as France (ANSES, 2011; Arnich et al., 2012), Spain (Marín et al., 2018), Italy (Storelli et al., 2017), China (Tang and Huang, 2014), Saudi Arabia (Ali and Al-Qahtani, 2012), and Lebanon (Nasreddine et al., 2010). It is essential to specify the food as consumed, in order to assess public health risks associated with substances such as TEs in food. The toxicity of micronutrients may depend on the chemical form of the element (Kelly et al., 2018). The risk arises on the fraction absorbed, therefore available or bioaccessible by the body (Babaahmadifooladi et al., 2019; Turdi and Yang, 2016). Therefore, it can be concluded that the origin of exposure and the estimation of TEs in food can lead to a complete assessment of their ingestion levels and potential risks to human health (Kim et al., 2015).

I-2.5.3 Thresholds of trace elements in food and their monitoring

In order to assess human dietary exposure to chemicals such as TEs, on one hand, the methodology of Total Diet Studies (TDS) should be monitored and performed. The TDS study is mainly based on daily food consumption, which represents the overall diet of the population. Such data are needed to identify risk groups and assess the risk to consumer health associated with chemical substances (ANSES, 2011).

On the other hand, in order to provide more realistic “background” exposure data, (ANSES, 2019) suggested the analysis of foods “as consumed”, that is to say washed, peeled and cooked if necessary. This type of study allows international comparisons in terms of consumer exposure. Accordingly, the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (ANSES, 2011; EFSA, 2011) have endorsed the Total Diet Study (TDS), where the FAO determines TDS by the amount of various chemical contaminants and nutrients that a person gets from their diet (type of food) in a day, a year or more.

Various studies in several countries, investigating the dietary exposure to TEs, have been carried out using the TDS approach (Ingenbleek et al., 2017; Papadopoulos et al., 2015; Turrini et al., 2018; EFSA, 2011). In France, a TDS allows to underline the necessity to reduce exposure to TEs such as Cd, Al, Ni, As, and Co by a diversified diet (food items and origins) (ANSES, 2019).

In Lebanon, a TDS approach study was carried out by Nasreddine et al. (2010) in order to assess, the dietary exposure of the population to six essential TEs (Co, Cu, Fe, Mn, Ni and Zn) and two toxic ones (Cd and Pb). This study, carried out in the (urban) capital of Beirut, provided us an idea on the evaluation of the adult population exposure to TEs, as well as a first estimation of TEs consumer exposure *via* food in Lebanon. In fact, the study of Nasreddine et al. (2010) showed that bread and cereals were the main dietary source of the following elements: Mn, Cu, Fe, and Ni respectively (0.97 mg day^{-1} , $486.95 \text{ } \mu\text{g day}^{-1}$, 4.87 mg day^{-1} and $54.73 \text{ } \mu\text{g day}^{-1}$). This is explained by the fact that Lebanese diet is highly dependent on grains, where the authors found that bread and grain products contained the highest levels of Mn, Ni and Fe respectively (3.0 mg kg^{-1} , $169.01 \text{ } \mu\text{g kg}^{-1}$ and 15.04 mg kg^{-1}). In this study, the main contributors to dietary Pb and Cd intake were vegetables ($4.02 \text{ } \mu\text{g day}^{-1}$ - 48.7% and $7.4 \text{ } \mu\text{g day}^{-1}$ - 46.8% respectively) followed by bread and cereal products ($2.6 \text{ } \mu\text{g day}^{-1}$ - 31.4% and $4.89 \text{ } \mu\text{g day}^{-1}$ - 30.9% respectively). In addition, bread and cereals contributed the most to the intake of Ni ($54.73 \text{ } \mu\text{g day}^{-1}$).

A long-term exposure to TEs and other chemical contaminants can lead to various health effects as well as chronic toxicity (Dorne et al., 2011). Trace elements can cause adverse health effects by affecting several organs and systems. The fundamental mechanisms of trace element toxicity include formation of free radicals thereby increasing oxidative stress, interaction with and damage of biological molecules, as well as damage to DNA, which is critical for carcinogenesis (Azeh Engwa et al., 2019). This is why an estimation of the daily intake of these toxic TEs *via* food consumption must be performed.

Accordingly, the International scientific committees such as FAO/WHO, JEFCA, European Union and others, mainly use the safety factor approach for establishing acceptable or tolerable intakes of substances that exhibit thresholds of toxicity.

The acceptable daily intake (**ADI**) for humans is widely used to describe “safe” levels of intake; and is defined as an estimate of the amount of a chemical that can be ingested daily over a

lifetime without appreciable risk to health (WHO, 2007). These levels of intake, ADIs are set due to several toxicological studies, including data from studies on various laboratory animals, where highest dose level may not produce observable toxic effect. The ADI is expressed in milligrams per kilogram of body weight per day (mg kg^{-1} per bw day^{-1}) and especially used for food additives.

In case of food contaminants such as trace elements, the tolerable intakes are expressed on either a daily basis (**TDI** or tolerable daily intake) or weekly basis, but the calculation is similar. It is an estimation of the ingested quantity of contaminants every day for all the life without having any corporeal effects on his or her health.

Furthermore, other terms can be used in order to assess the exposure to TEs levels in consumed food such as: **PTWI**, or provisional tolerable weekly intake, for contaminants that may accumulate in the body and mainly used by JECFA, and **PTDI** or Provisional Tolerable Daily Intake (**PTDI**) as showed by (Pipoyan et al., 2018) in their study of the exposure of the Armenian population to TEs present in consumed fruits and vegetables, that the PTDI values do not exceeded the FAO/WHO threshold values (500, 1000 and $20.71 \mu\text{g day}^{-1} \text{kg}^{-1}$ body weight for Cu, Zn, and Ni respectively).

If there are no Health-based Guidance Value available, the human health risk can be determined by the calculation of the Margin of Exposure (MOE), which was developed by (EFSA, 2005) in order to assess genotoxic and carcinogenic impurities in food. The MOE is calculated according to the following approach:

$$\text{MOE} = \frac{\text{BMDL}}{\text{Estimated Exposure Dose}}$$

Where the BMDL has the lower limit of the reference dose and which is presented by the unit of BMDL: mg kg^{-1} per bw day^{-1} or ppm. When the latter presents the reference, value derived from the reference dose (BMD) which extends the use of response data from animal studies.

Since the study of Nasreddine et al. (2010) showed that the bread was one of the major contributors of TEs to the Lebanese food basket, we found that it was more judicious to focus on this issue. There is an urgent need to specify the daily TEs intake by means of Lebanese bread all over Lebanon. Several factors were considered such as the bread type, the amount consumed which varies according to age, sex and socio-economic status of the consumer. All the data were collected by dint of a national survey of bread consumption, as well as bread

TEs analysis of the three most consumed brands in Lebanon. Using the survey data, population categories of exposures to TEs *via* white bread consumption were calculated and compared to the international health-based guidance values: tolerable daily intake (TDI), tolerable weekly intake (TWI) or toxicological reference points, such as bench mark dose limit (BMDL), to evaluate safety concerns.

CHAPTER I-3

The international and national legislations of soil, wheat plant and bread

I-3.1 Standardization and regulatory aspect

The human health is strongly correlated to the soil quality and especially to the degree of soil contamination. Consequently, soil contamination by TEs has attracted a great deal of attention in the world. The accumulated TEs in the soil may be transferred to plants and affect the human food chain and then human health.

I-3.1.1 Allowable limits of TEs in soil

Several countries have been developed and placed regulation limits for soil TEs concentrations such as European Union, WHO (World Health Organization), CEQCs (Canadian Environmental Quality Guidelines) and others **Table I-3. 1.**

Table I-3. 1: Max allowable concentrations of trace elements (mg kg^{-1}) in soil according to EU, WHO, NJDEP, CEQCs, MACs, and MEF

Regulation limits	Max allowable concentrations of trace elements (mg kg^{-1}) in soil								
	Cd	Pb	Cu	Zn	Ni	Cr	Hg	As	Co
EU limits (CEC, 1986)	3	300	140	300	75	-	-	-	-
WHO, 1996	0.8	85	36	50	35	100	-	-	-
(NJDEP, 1996)	100	600	-	-	-	100	-	-	-
CEQCs, 2007	1.4	70	63	200	50	64	6.6	-	-
Kabata -Pendias (MAC, 2011)	5	300	150	300	60	200	-	20	50
MEF (threshold value 2007)	1	60	100	200	50	100	0.5	5	20

EU: Limits according to the European Union Directive (CEC, 1986), WHO: World Health Organization (1996) NJDEP: New Jersey Department of Environmental Protection (1996), CEQCs: Canadian Environmental Quality Guidelines (2007), MAC: Maximum Allowable Concentrations as reported by Kabata-Pendias (2011; p. 24), MEF: Ministry of Environment, Finland, (2007)

I-3.1.2 Allowable limits of TEs in Inorganic Fertilizers

In recent years, agricultural production has increased remarkably in order to be able to feed the world (Hasler et al., 2017; Hazell and Wood, 2008). Farmers resort to advanced agricultural technologies, such as fertilization which is on the one hand necessary to meet global demand, but on the other hand, its excessive use, may cause environmental risks (serious environmental degradation), increase TEs concentrations in soil, where they accumulate and affect human health (Rahman and Zhang, 2018).

A large amount of chemicals, as fertilizers, is annually applied to agricultural soils. Europe was in the forefront of its use of these fertilizers until the 1990s, which subsequently showed a great reduction. Nowadays, Asia occupies the 1st place, while America has always remained in 2nd place since 1961 to 2018 (FAOSTAT, 2018). To limit the overuse of inorganic fertilizers,

and control the TEs of P-fertilizers, many countries and organizations settled limits of use of these fertilizers **Table I-3. 2**

Table I-3. 2: Maximum allowable concentrations of TEs in inorganic fertilizers TEs (mg kg^{-1})

Regulation limits	Max allowable concentrations of TEs (mg kg^{-1}) in P fertilizers								
	Cd	Pb	Cu	Zn	Ni	Cr	Hg	As	Co
FAO, 2014									
FGW, 2014	3	150	-	-	120	-	2	60	-
Germany, 2008	1.5	150	-	-	80	300	1	40	-
Finland, 2007	1.5	100	600	1500	100	300	1	25	-

I-3.1.3 Allowable limits of TEs in Irrigation water

The use of wastewater for irrigation has been controlled by WHO, which has set limits on use and does not recommend using wastewater containing an excessive amount of industrial wastewater (WHO, 2006). Where the latter may contain TES such as: Cd, Pb, Mn, Cu, Zn, Cr, Hg, As, Fe and Ni. Accordingly, (USEPA, 2012; WHO, 2006), have recommended guidelines for the safe reuse of wastewater as agricultural water, in other terms of water quality. For the aim of using treated wastewater in agriculture, many countries (Cyprus, France, Greece, Italy, Portugal, Spain and South Korea) have revised and changed their quality standards (Agrafioti and Diamadopoulou, 2012; do Monte, 2007; Paranychianakis et al., 2015). The **Table I-3. 3** showed the maximum allowable TEs concentrations in (mg L^{-1}) in irrigation water as per WHO/FAO and USEPA.

Table I-3. 3: Maximum allowable concentrations of TEs (mg L^{-1}) in irrigation water

Regulation limits	Max allowable concentrations of trace elements (mg L^{-1}) in irrigation water								
	Cd	Pb	Cu	Zn	Ni	Cr	Hg	As	Co
WHO/FAO, 2007	0.01	5	0.2	2	-	0.1	-	0.1	-
US EPA, 2010	0.005	0.015	1	2	-	-	-	-	-

I-3.1.4 Allowable amounts of TEs in sewage sludge

The use of sewage sludge improves agricultural production, containing high concentrations of Organic Matter, N, and P which are essential for plant growth (Zhang, 2019). However, the high concentrations of TEs present also in sewage sludge may allow deterioration of soil quality and ground water quality, which become a public health concern (Duan et al., 2017).

This ecological risk has been resolved by the establishment of limits of TEs in sewage sludge. This approach will prevent a further contribution of soil TEs contamination, which may influence the reduction of health risks associated with the consumption of contaminated plants. The **Table I-3. 4**, showed the TEs limits (mg kg^{-1} DM) in sewage sludge used in agricultural soil set by Mediterranean countries, DWAF, US EPA, and EU.

Table I-3. 4: The limit values of TEs (mg kg^{-1} DM) in sewage sludge used in agricultural soil

Regulation limits	Limit values of TEs (mg kg^{-1} DM) in sewage sludge used in agricultural soil								
	Cd	Pb	Cu	Zn	Ni	Cr	Hg	As	Co
France, 1997	20	800	1000	3000	200	1000	10	-	-
Italy, 2018	20	750	1000	2500	300	200	10	20	-
Cyprus, 2002	20-40	750-1200	1000-1750	2500-4000	300-400	-	16-25	-	-
DWAF, 2010	5	150	450	700	-	-	-	-	-
US EPA, 2012	39	300	1500	2800	420	-	-	-	-
EU (86/278/EEC)	20-40	750-1200	1000-1750	2500-4000	300-400	1000-1750	-	-	-

In order not to exceed these limits in the soil, several studies have been carried out over nearly ten years to define the appropriate amount of TEs which may be added to agricultural soil. In the **Table I-3. 5**, the limit values for amount TEs which may be added annually to agricultural soil as per S.I. No. 267/20001 and EU 86/278/EEC.

Table I-3. 5: The limit values for TEs amounts which may be added annually to agricultural soil

Regulation limits	Amounts of TEs which may be added annually to soil (kg ha^{-1} year $^{-1}$)								
	Cd	Pb	Cu	Zn	Ni	Cr	Hg	As	Co
S.I. No. 267/2001	0.05	4	7.5	7.5	3.0	3.5	0.10	-	-
EU (86/278/EEC)	0.15	15	12	30	3	-	0.1	-	-

I-3.1.5 Allowable limits of TEs in Cereal plants

In wheat plants, the TEs Concentrations varied according to genotypes, environmental factors such as soil, climate, and agricultural practices, and their availability in plants and soil. Therefore, many countries and organisations such as European Union (EU; CEC, 2006, 2008), Germany (MALB, 2010), China (Document GB2762-2012; USDA, 2014), Australia/New Zealand (FRLI, 2015), France, and FAO/WHO, 2017, had established and put in place legislations for the following TEs: As, Cd, and Pb in cereal and wheat plants **Table I-3. 6**.

Table I-3. 6: The concentrations of Cd, Pb and As (mg kg^{-1}) in cereal plants as per EU, Germany regulations, FAO/WHO, China, and Australia/New Zealand

Regulation limits	Max allowable concentrations of trace elements (mg kg^{-1}) in cereal plants			
	Cd	Pb	Hg	As
EU, 2008	0.1	0.2	-	-
Germany, 2010	0.1	0.2	-	-
China, 2014	-	0.5	-	0.2
Australia/New Zealand, 2015	-	0.2	-	1
INRA, 2004	0.2	0.2	0.03	-
FAO/WHO, 2017	0.1	0.2	-	-

EU – CEC (Commission of the European Communities), 2006.

EU – EC (Commission of the European Communities), 2008.

MALB (Materialien zur Altlasten – Bearbeitung im Land Brandenburg), 2010.

USDA (United States Department of Agriculture), 2014.

FRLI (Federal Register of Legislative Instruments), 2015.

FAO/WHO: Food and Agriculture Organization and World Health Organization

INRA, 2004: Courrier de l'environnement de l'INRA n°52, septembre 2004

I-3.1.6 Allowable limits of TEs in foodstuff and Bread

The foodstuffs consumption is the most possible path for human exposure to TEs. The evaluation of TEs contents (As, Pb, Cd, and others) in different foodstuffs (cereals, vegetables, fruits, fish, meat and bread) and the estimation of the potential health risks via this consumption is highly important. The **Table I-3. 7**, showed the TEs limits in food stuffs and bread in Lebanon and Europe

Table I-3. 7: The concentrations of TEs (mg kg^{-1}) in foodstuffs and bread as per (NL 240, 2010) – Libnor and EC (No 1881, 2006)

Regulation limits	Max allowable concentrations of trace elements (mg kg^{-1}) in foodstuffs and bread				
	Cd	Pb	Cu	Hg	As
(NL 240, 2010) (bread)	0.2	0.2	10	-	-
EC (No 1881, 2006) (foodstuffs)	0.1	-	-	0.1	0.1/0.3

NL 240, 2010): Libnor Lebanese Norms

EC (No 1881, 2006) (foodstuffs)

PART II – MATERIAL AND METHODS

CHAPTER II-1

Geological, hydrogeological, hydrological and climatic context of the Bekaa valley

II–1.1 Geology

Lebanon belongs to the unstable tectonic shelf of the Mediterranean region, which is affected by plate tectonic movements of the Dead Sea Rift System and has its extension forming the Bekaa Plain (Beydoun, 1988). Therefore, the folded mountain ranges with uplifted blocks created the three parallel physiographic units of Lebanon: Mount-Lebanon, Bekaa plain and Anti-Lebanon. The Bekaa depression, where de Litani river basin (LRB) is extended, is almost a graben structure located between the two uplifted mountain ranges *Figure II-1. 1* (Lateef, 2007).

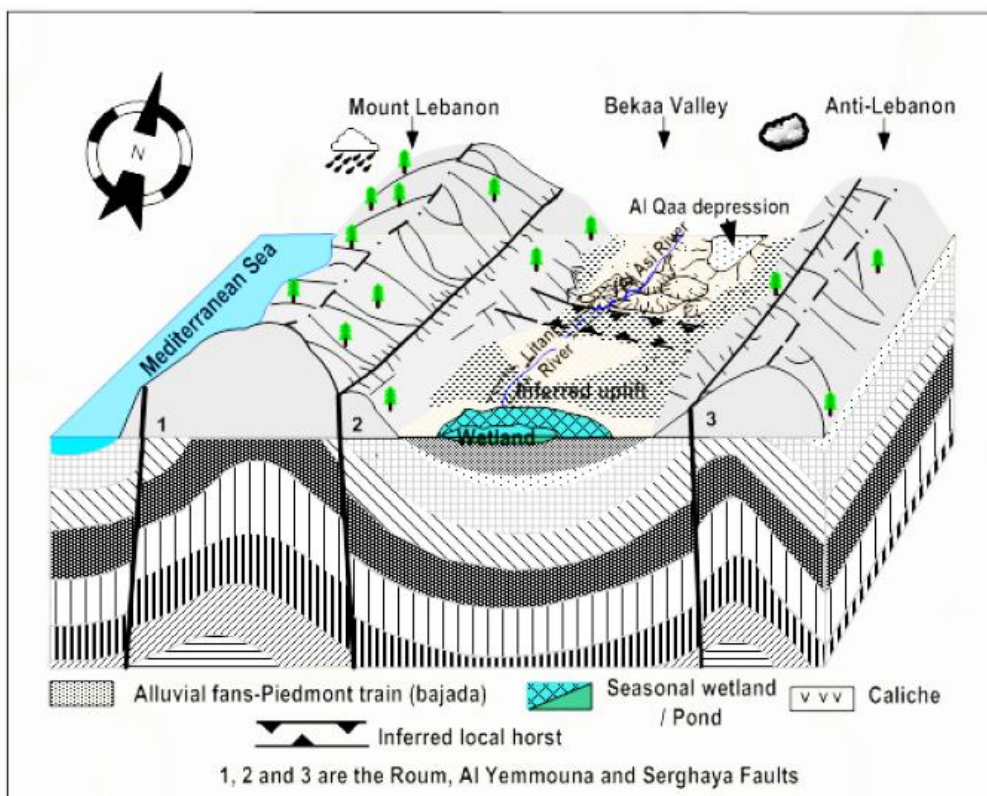


Figure II-1. 1: Bloc diagram of the geological fabric of Bekaa Valley (Lateef, 2007)

The exposed stratigraphic rock sequence among the Litani River Basin (LRB) is similar to the one of the entire Lebanon. It reveals rocks from the middle Jurassic up to the quaternary deposits *Figure II-1. 2*. Soils of the Central Bekaa mainly develop on Quaternary deposits with eventually a contribution of Miocene marly limestones from the slopes.

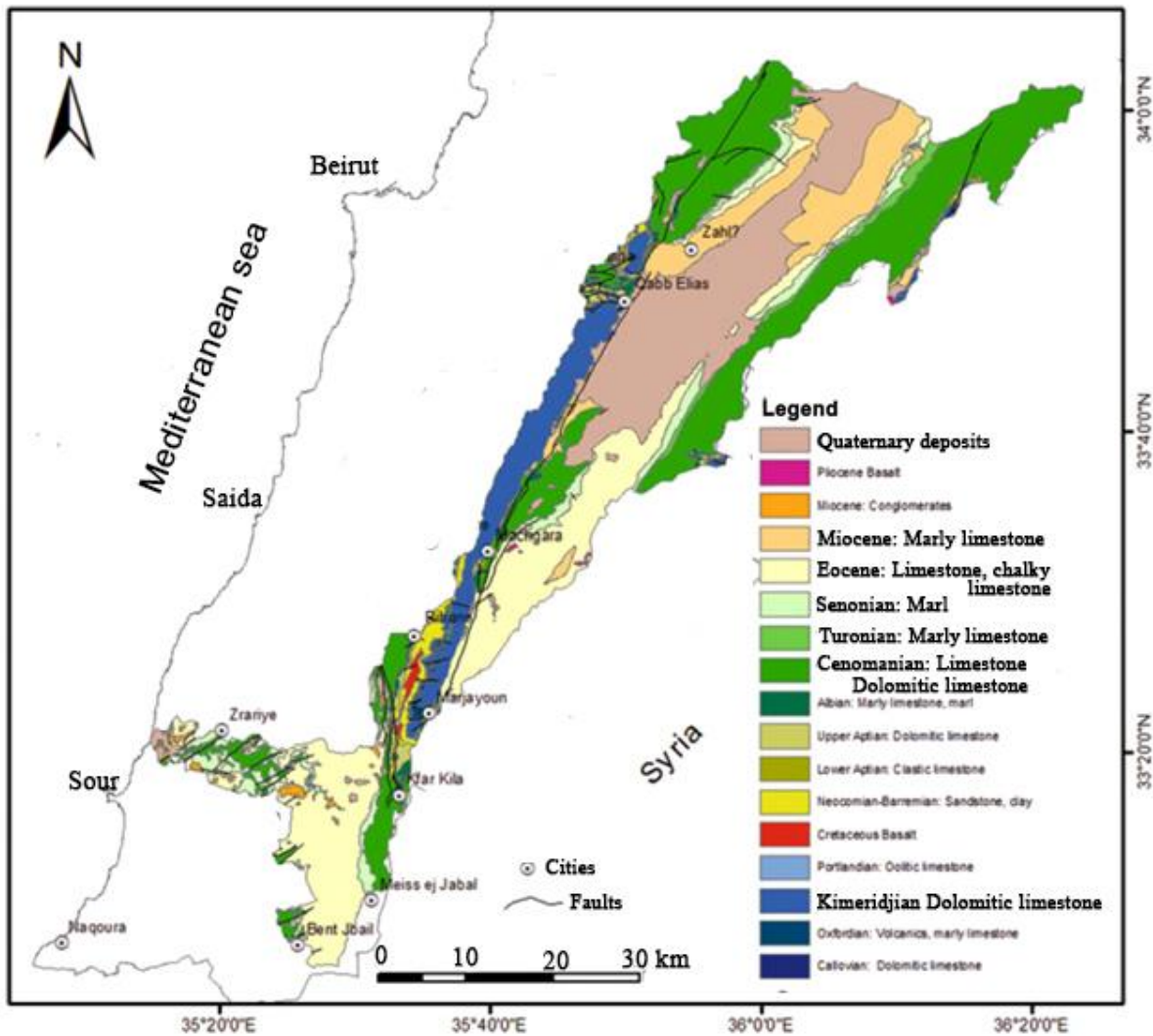


Figure II-1. 2: Geological map of the Litani river basin (Shaban and Hamzé, 2018)

II-1.2 Climate

The orientation of the Mount Lebanon and Anti-Lebanon mountains ranges perpendicular to the atmospheric flows coming from the Mediterranean leads to very different climatic regimes over short distances across Lebanon [Figure II-1. 3](#) While the coastal zone and the ranges of Mount Lebanon have a humid Mediterranean climate, the northern part of the Bekaa Plain has a semi-arid continental climate (Darwish et al., 2012).

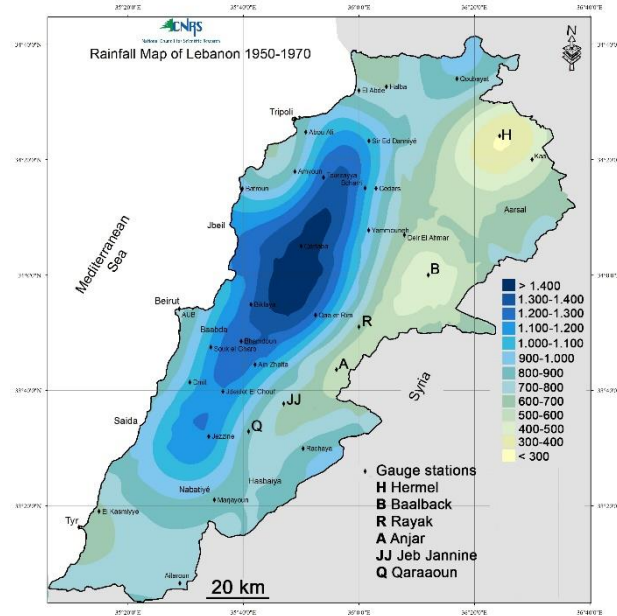


Figure II-1. 3: Rainfall map of Lebanon 1950-1970 CNRS

The accumulated solid precipitation surrounding the LRB above the 1500-m snow line [Figure II-1.4](#) is the integral feeding source of water, which is stored temporarily during 2 months in the form of snowpack. Therefore, a substantial portion of snowmelt recharges the terrain where the Litani River flows, whether directly from the running melted snow or from the issuing karstic springs, which are also fed mainly from snow. It was estimated that snow contributes to about 24% of the total hydrological budget in the LRB (Shaban and Darwich, 2013).



Figure II-1. 4: Space view showing Mount Lebanon Range facing Bekaa valley and Qaraaoun reservoir. Sources Tannouri 2010, in Shaban et al., 2018

II-1.3 The Litani River Basin

The relatively large areal extent of the Litani River catchment receives a considerable amount of water directly from precipitation as rainfall and snow of 875 mm year⁻¹. Therefore, the entire LRB, with an area of about 2140 km² [Fig. II-1.5](#) receives a precipitation annual budget of: $2140 \times 10^6 \times 875 / 1000 = 1870 \text{ Mm}^3 \text{ year}^{-1}$.

The Litani watershed presents an atypical morphology and hydrological behavior, linked to its karstic geology and active tectonics. The river takes its source north of the Bekaa plain, near Baalbek in Haouch el Barada (altitude 1000 m). The 170 km long river follows its course towards the South, parallel to the chain of Mount Lebanon, which is interrupted halfway by the Qaraaoun reservoir. It enters the reservoir at an altitude of 850 m and comes out at 800 m to flow into the Mediterranean Sea at Kasmieh by making a right-angled turn at the occasion of a transverse fault in the chain of Mount Lebanon [Figure II-1. 5](#).



Figure II-1. 5: The geomorphology of the two sub-basins of the Litani River Basin: The Upper Litani River Basin from Baalbek to Qaraaoun (ULRB) and the Lower Litani River Basin from Qaraaoun reservoir to the Mediterranean Sea at Kasmieh (LLRB) (Nehme et al., 2014).

Mapa 3D satellite images ©2021 Google

Therefore, two sub-basins were considered with very different geomorphology and hydrological characteristics:

- The Upper Litani River Basin (ULRB) from Baalbek to Qaraaoun reservoir, where the river flows in a straight channel in the vast Bekaa plain, showing a very gentle slope (2.5 m km^{-1})¹ resulting in slow stream-flow energy. This in turn promotes evaporation processes as well as sediments including pollutants to settle (Shaban and Hamzé, 2018). The mean annual flow of the Litani river in the Qaraaoun reservoir is $410 \text{ Mm}^3 \text{ year}^{-1}$ *Figure II-1. 6.*
- The Lower Litani River Basin (LLRB) from Qaraaoun reservoir to the Mediterranean Sea at Kasmieh, where the river acquires a turbulent regime and sinks into deep gorges to join the Mediterranean Sea where it discharges an annual flow of $320 \text{ Mm}^3 \text{ year}^{-1}$ *Figure II-1. 6.*

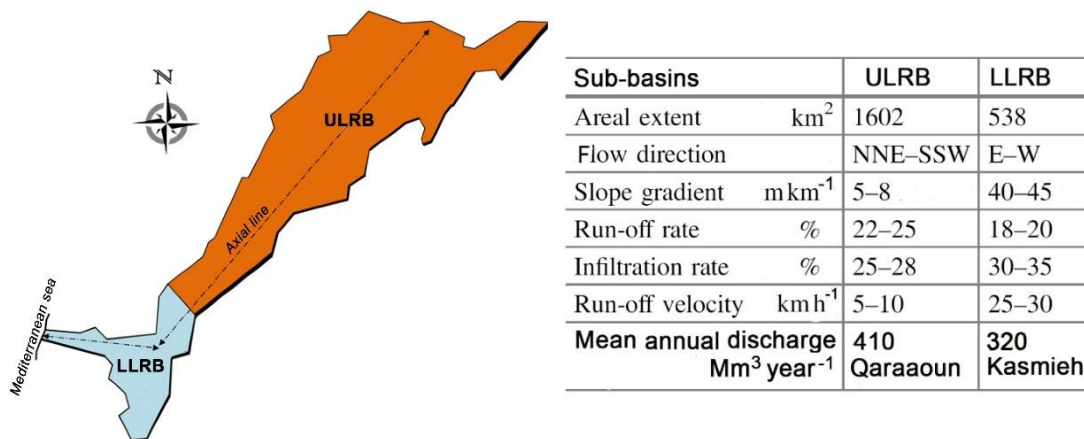


Figure II-1. 6: Litani river sub-basins hydrological characteristics, ULRB: Upper Litani River Basin, LLRB: Lower Litani River Basin (Shaban and Hamzé, 2018)

¹ If the identification of 2 different entities within the Litani river basin is recognized by all, the precise limit between these entities seems to vary between the authors. In this thesis, we have chosen to set the limit at the Qaraaoun reservoir as Nehme does, which allows us to more precisely define the slope gradient in the Central Bekaa, our main study site.

Drainage network and groundwaters

Several springs replenish the Litani River. Some directly flow into the primary tributaries, and others feed indirectly through percolation into the groundwater reservoirs. These springs are characterized by a diverse discharge magnitude and different hydrologic regimes and most of these springs are intermittent. In the ULRB, the density of the drainage network is higher on the Western side of the valley and more particularly in the central Bekaa [Figure II-1. 7](#).

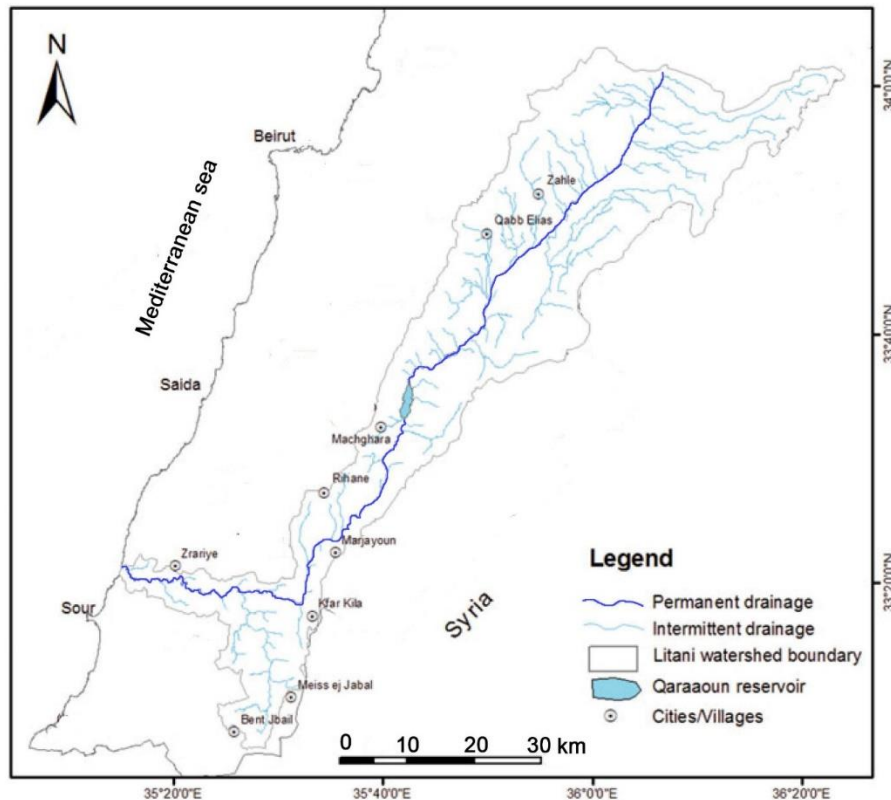


Figure II-1. 7: Drainage system of the Litani River Basin

The groundwater aquifers of the Bekaa comprise: 1) Quaternary and Neogene sediments that are layered and heterogeneous, with poor water bearing capacity and transmissivity and 2) 3 main layers of karstic aquifers (Eocene, Cretaceous and Jurassic) which outcrop on both sides of the valley [Figure II-1. 8](#). These represent water-bearing strata at depths exceeding several hundreds of meters but with considerable water volume and continuous discharge.

An extensive development of wells in the Eocene and Cretaceous aquifers, for crops irrigation, was observed in the ULRB and estimated between 6000-8000 boreholes (Shaban et al., 2018). In their “Policy White Paper on the Groundwater Governance in Lebanon: The Case of Central Beqaa”, (Molle et al., 2017) show that the assessment of these withdrawals from the

groundwater represents $120 \text{ Mm}^3 \text{ yr}^{-1}$ of which $82 \text{ Mm}^3 \text{ yr}^{-1}$ returned to the ground by infiltration. They also show *Figure II-1. 8* that the density of wells is twice as high on the eastern slope of the Bekaa, emphasizing the lower drainage density and a more arid microclimate.

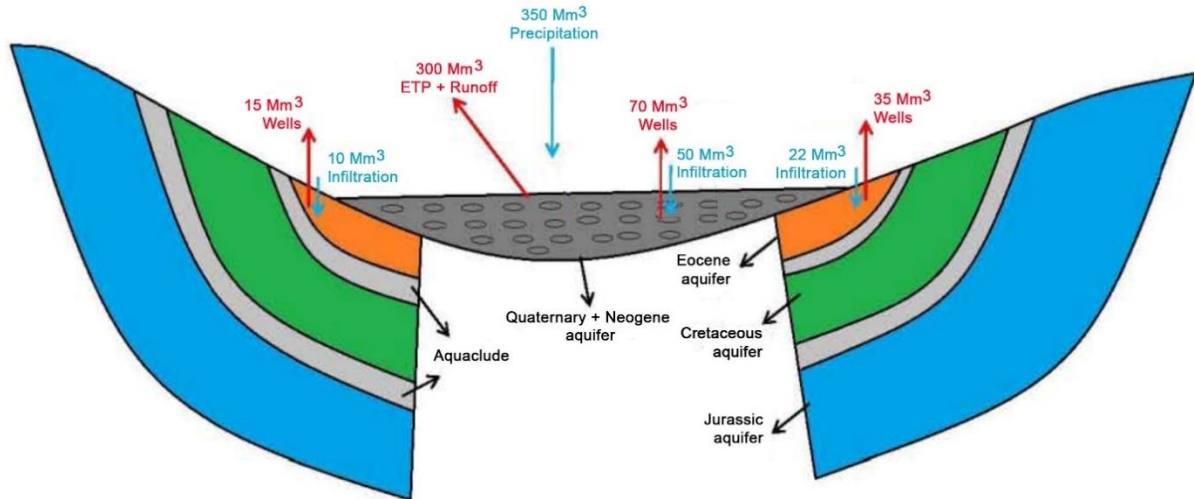


Figure II-1. 8: Upper Litani River Basin groundwater annual budget (Molle et al., 2017)

In conclusion of this presentation of the geological, hydrogeological, hydrological and climatic context of the Bekaa valley where our study sites are located, we will borrow a sentence from chapter 3 (Shaban et al., 2018) of the book “Le Litani river, Lebanon: Assessment and current challenges” edited by Shaban and Hamzé (2018) to which we have referred a lot:

“Snow has a significant contribution to feeding the river. The extension of the Litani River among the carbonate rocks and alluvial deposits makes it an open hydrogeological system that is fed from and feeds on the permeable and porous lithologies.”

II-1-4 Site locations

The choice of study sites was made considering the pedoclimatic diversity of the soils, relying on the field knowledge of LARI engineers and their network of experimental plots in the Central Bekaa (Zahleh district) and its borders (Western Bekaa and Baalbek districts).

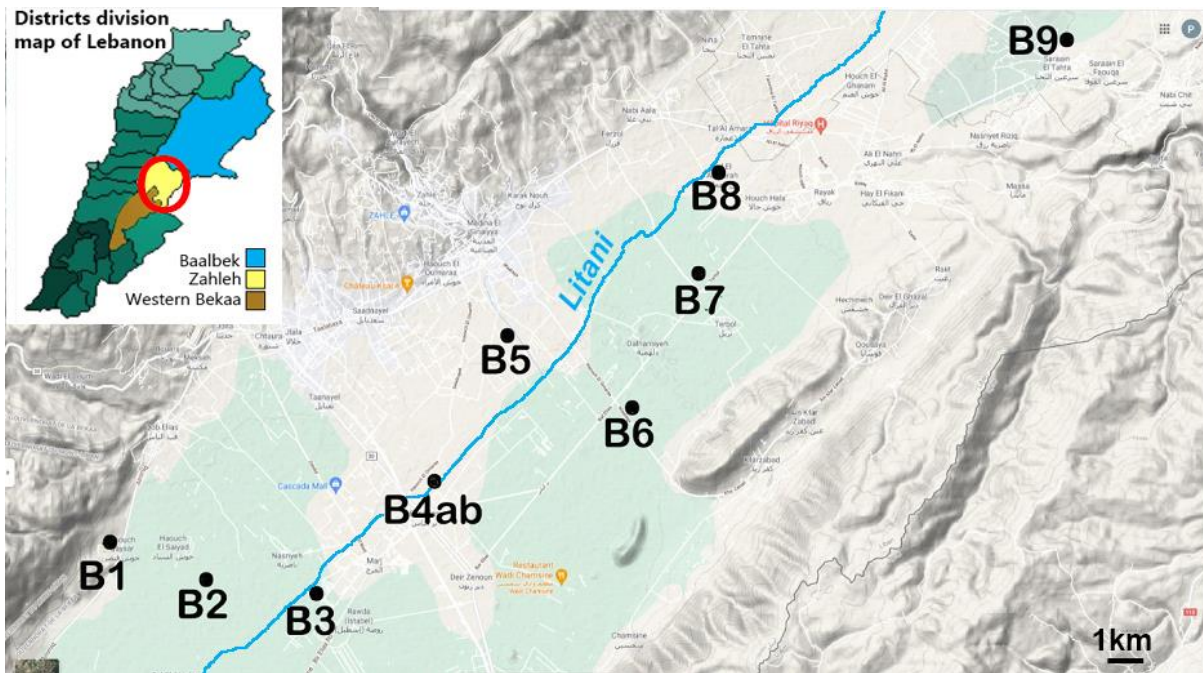


Figure II-1. 9: The nine studied sites distributed in the Bekaa Plain along a transect crossing the Litani

Table II-1.1: The nine studied sites locations in the Bekaa Plain, their altitudes and annual rainfall

Sites	GPS position		Altitude m	annual rainfall mm yr ⁻¹	Administrative affiliation		
	Nord	East			Municipality	District	Governorate
B1	33.765313	35.810416	880	750	Mzaraat Zahle	Zahle	Bekaa
B2	33.75663	35.834663	860	750	Tcheflik Qiqano	Zahle	Bekaa
B3	33.756292	35.866151	860	650	Marj BG	West Bekaa	Bekaa
B4a	33.779139	35.896194	860	650	Zahle Haouch El-Oumara	Zahle	Bekaa
B4b	33.780022	35.89817	860	650	Zahle Haouch El-Oumara	Zahle	Bekaa
B5	33.815278	35.920556	880	650	Zahle Haouch El-Oumara	Zahle	Bekaa
B6	33.796991	35.956955	860	550	Zahle Maallaqa	Zahle	Bekaa
B7	33.820752	35.969925	900	550	Dalhamiyet Zahle	Zahle	Bekaa
B8	33.854367	35.980539	905	550	Haouch El-Aamara	Zahle	Bekaa
B9	33.886595	36.081692	1000	550	Serraine Et-Tahta	Baalbek	Baalbek-Hermel

The nine sites are distributed along a transect crossing the Litani valley from southwest to northeast, starting from the piedmont of the eastern slope of Litani (B1) at 880 m altitude to the piedmont of the western slope (B9) at 1000 m altitude passing through the banks of the Litani (B3, B4 and B8) at 860 m above sea level. Three sites are located on the western slope (B1, B2 and B5) and three others on the eastern slope (B6, B7 and B9).

The average annual rainfall decreases from southwest to northeast by 750, 650 and 550 mm yr⁻¹ for B1 B2, B3 B4 B5 and B6 B7 B8 B9 respectively.

II-1-5 Climate of the Central Bekaa valley

Central Bekaa valley is characterized by a dry-Mediterranean climate with a mean annual precipitation around 600 mm (Darwish, 2008). The mean annual temperature is governed by altitude in the Litani River Basin (23-21 °C) at an altitude between 500 and 1000 m.

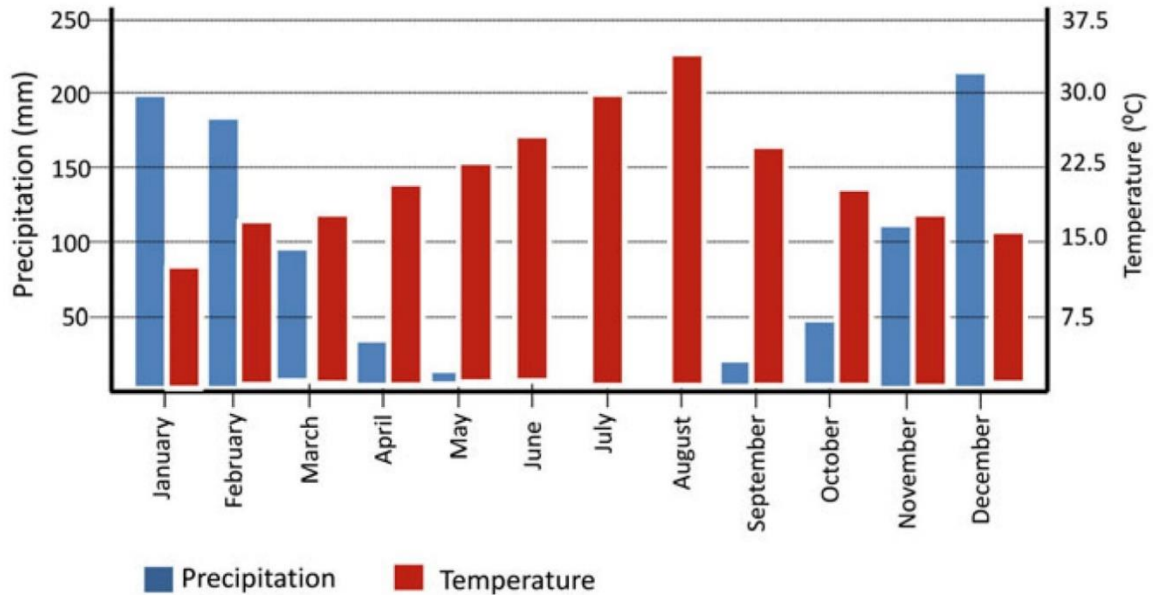


Figure II-1. 10: Average monthly precipitation and temperature in the Litani River Basin

The duration of the rainy season averages between 60 and 70 days/year, and it often extends from October to March reaching its climax in December [Figure II-1. 10](#). According to (CNRSL, 2015) there was an abrupt decrease in the rainfall rate by about 225 mm for the period between 1950 and 2015 in the middle Bekaa region (near Zahle area), showing the need of irrigation for cereal crops.

CHAPTER II-2

The studied matrices

II-2.1 Soil samples

II-2.1.1 Sampling

The French norm “AFNOR NF X 31-427 standard” was applied to the soil samples collection and respective wheat plants. In each plot, the topsoil layer (0-20 cm) was sampled, during harvesting period (from the last week of May and the first week of June), using a stainless-steel shovel at the corners, in the center and diagonally. The obtained 9 subsamples of each plot were mixed, homogenized, and carried in a clean coded polyethylene bag. The collected soil samples were brought the same day to LARI laboratories.

A quartering technique was applied to the overall homogenized sample to reduce the sample size and form a composite sample. A soil sample of almost 1 kg will undergo the pre-treatment.

The cleaned soil samples were dried according to the AFNOR NF X31-102 standard 24 in an oven (Model 700 Memmert) at 40°C, until constant weight. Then soil samples were sieved at 2 mm and stored in plastic bottles in the refrigerator protected from the direct sunlight and at a temperature below 4°C until analysis.

II-2.1.2 Physico chemical parameters of soil

The physico-chemical parameters of soil samples were measured as per the adopted norms. They were determined to characterize the studied soils and to evaluate their effect on the content and mobility of Tes in the soil. They included pH, conductivity (EC), soil texture, total organic carbon, total limestone, active limestone, Available phosphorus (Olsen method) and Exchangeable bases (EB).

II-2.1.2.1 Determination of soil pH

The method for measuring the pH (H₂O) of soil samples prepared for analysis adopted follows the AFNOR NF X 31-101 standard, it is an electrometric method. Where a mass of 40 g of prepared soil sample is mixed with 100 mL of mineralized water, then stirred at 35-45 rpm for one hour at room temperature of 20 ± 2°C. The mixture is allowed to stand for 30 minutes to settle. Then the same process is repeated.

The “electron corporation Orion 5 star” thermo model pH meter is used for pH measurement.

II-2.1.2.2 Determination of electrical conductivity EC

The electrometric method of measuring EC in soil samples is carried out according to ISO 11265: 1994. The soil sample is extracted in an extraction ratio of 1: 5 (m/V) with water at a temperature of $(20 \pm 2)^{\circ}\text{C}$ in order to dissolve the electrolytes. The specific electrical conductivity of the extract is measured at a temperature of 25°C .

The treated soil sample for pH measurement is used for measuring the electrical conductivity of the supernatant using a “Thermo” model connectimeter.

II-2.1.2.3 Soil texture

The soil texture is determined using the modified Bouyoucos method (Beretta, 2014). A volume of 50 mL of sodium hexametaphosphate solution (5%) was mixed to 50 g of soil sample in a stirring-cup. Then 5 mL of H_2O_2 was added and the mixture was stirred for 20 minutes at high speed. Distilled water is added to the mixture and a first reading of temperature and density is taken. After exactly 2 hours, a second reading is made. These readings allowed us to determine the % of clay and silt, while the % of sand was calculated by the difference of both %. The soil texture is determined by referring to the texture triangle.

II-2.1.2.4 Determination of soil organic matter content (SOM)

The method used is carried out according to the AFNOR NF X 31-109 standard. The WALKEY and BLACK method was applied, by adding a known quantity of an oxidant to the soil under well-defined conditions. In laboratory, SOM is oxidized by a mixture of potassium dichromate and H_2SO_4 . The excess potassium dichromate is titrated with a solution of ammoniacal ferrous sulphate (AFS) $\text{FeSO}_4, (\text{NH}_4)_2\text{SO}_4, 6\text{H}_2\text{O}$. The organic matter content is evaluated by the organic carbon thus determined.

The procedure consists of 2 steps, an oxidation step: in which 10 mL of potassium dichromate (0.2 M) is added to 0.5-1 g of soil and 20 mL of sulphuric acid H_2SO_4 , concentrated. For a complete oxidation, the mixture was left 30minutes at room temperature. And a titration step: a spatula of sodium fluoride NaF is then added to the mixture, and left until it cools. A volume of 1 to 2 mL of the indicator diphenylamine was added to obtain a dark blue colour. The titration was done by a standard solution of AFS.

II-2.1.2.5 Determination of total limestone

The method used follows the AFNOR NF X 31-105 standard. This method consists in a volumetric determination of carbon dioxide (CO_2) released under the action of a strong acid (hydrochloric acid HCl 6N) at room temperature. A 0.3 to 0.5 g soil sample was weighed using an analytical balance, to evaluate the carbonate content according to the BERNARD calcimeter method, where 10 mL of HCl 6N were used.

II-2.1.2.6 Determination of active limestone content

The method used follows the AFNOR NF X 31-106 standard. Its purpose is to assess the content of active limestone in soil samples prepared for analysis. Where the calcium fraction presents in carbonates is precipitated by an ammonium oxalate solution. The method adopted is the DROUINEAU method which covers an interval of 0 to 13% of active limestone. It consists on the determination of the amount of unreacted ammonium oxalate following a titration with a known concentration of potassium permanganate solution in the presence of sulfuric acid.

An extraction took place before analysis following this procedure: A volume of 100 mL of ammonium oxalate 0.2 N was added to 1 g of soil and agitated for 2 hours in a rocker stirrer, at temperature between 18 and 22°C. The obtained substrate was filtered by filtered paper and collected in a dry container. For analysis, 100 mL of demineralized water and 5 mL of concentrated H_2SO_4 were added to 10 mL of filtrate, which were carried out on a hotplate at 70°C. A titration with potassium permanganate KMnO_4 0.02N was performed. A blank test is measured under the same conditions.

II-2.1.2.7 Determination of the available phosphorus content (Olsen method)

The available phosphorus content was determined per Olsen method by extraction using the sodium bicarbonate (0.5 N; pH 8.5) for a fraction of 1/20 (mass/volume).

The phosphate extracted from 1.2 to 1.4 g of soil by using 25 mL of sodium bicarbonate solution NaHCO_3 through the phosphomolybdate method. After 30min shaking and filtration of the substrate, the blue molybdophosphate complex was formed in a H_2SO_4 matrix and reduced by ascorbic acid and catalyzed by antimony potassium tartrate. The absorbance was measured by spectrophotometer at a wavelength of 282 nm. The standard solutions for

phosphorus were prepared from a solution of 2 ppm and NaHCO_3 obtained from Sigma – Aldrich. A UV/Vis spectrophotometer type “Metertech SP 80001” was used for P determination.

II-2.1.2.8 Determination of exchangeable bases: K^+ , Na^+ , Ca^{2+} and Mg^{2+}

The method used follows the AFNOR NF X 31-108 standard. Its aim is to determine the content of exchangeable bases by ammonium acetate (1 mol L^{-1}) at pH 7 (free of carbonates) in soil samples after extraction by stirring with a mixing ratio. Extraction at 1/20 (m / V) followed by filtration.

The determination of the exchangeable cations K^+ and Na^+ flame is by emission spectrometry, while the exchangeable cations Ca^{2+} and Mg^{2+} by complexometric titration with EDTA.

The determination of the content of exchangeable K^+ and Na^+ is carried out by adding 50 mL of neutral ammonium acetate to 5 g of soil, the mixture is then stirred with a rocker stirrer for 45 minutes. A filtration by using filter paper was performed. The filtrate is used for the determination of K^+ and Na^+ by a flame photometer of the “BioTech Engineering Management Co. LTD” type at a wavelength of 766.5 nm. Calibration curves of 3 standards. Of concentrations 5, 10 and 15 ppm, were prepared from a solution of 100 ppm of KCl and NaCl. The demineralized water was used as a blank.

The determination of the Ca^{2+} and Mg^{2+} contents were carried out by a complexometric assay with EDTA after extraction of the cations exchangeable with ammonium acetate. This is the most common and reliable method

For the determination of Ca^{2+} alone, a 10 mL aliquot was diluted to approximately 100 mL with distilled water. Then a volume of 10 mL of NaOH, 5N was added in order to bring the pH to 12.0. A spatula of a colored indicator of pink color “murexide” was added. The mixture was then titrated with a solution of EDTA, 0.005N in order to have a persistent purple color.

For the determination of Ca^{2+} and Mg^{2+} , the same method was applied as that mentioned before, but 1mL of the colored indicator “metallochrome” of bright red color was used there. Titration with EDTA, 0.005N done until a persistent green color appears.

II-2.1.3 Trace elements determination in soil samples

The pre-treatment of soil samples consists of fine grinding with mortar and sieving to a diameter < 250 μm , according to standard NF ISO 11464. Two methods of digestion were used in order to mineralize a representative part of the composite soil samples after samples preparation. Where the first consists of using three-acid mixture (9 mL HNO_3 , 2 mL HClO_4 , and 2 mL H_2SO_4) and named by "HAD" or the heating 3 acids digestion. This mixture was added to 0.5 g of soil sample or CRM, and heated up to 350°C for 2h 30min in a heating block. After that, the mixture was stored an overnight at room temperature. The second method, known as "MSD" or the microwave sulphuric digestion, consists of the microwave usage, by adding 9 mL of H_2SO_4 and 3ml of H_2O_2 to 0.5 g of soil sample or CRM in a Teflon receptacle tightly closed. A microwave, type "Milestone – Ethos Plus", power of 1000 W was reached in 10 min and maintained at this level 10 min. Then, the power decreased gradually to 0 W, where the cooling step was maintained for 20 min. This method was suggested by Milestone and was subjected to a validation. After digestion, all samples were filtered and adjusted to 25 mL using HNO_3 1% before TEs analysis. The calibration standard solutions were prepared by successive dilution using HNO_3 , 1%; all plastic lab ware used for sampling and sample treatment was new or cleaned by soaking 24h first in 10% HNO_3 then in ultra-pure water.

The TEs analysis was conducted by Atomic Absorption Spectrometry (flame iCE 3000 series Thermo type, Thermo Fischer Scientific, Germany) for soil samples. Where liquid samples were introduced in the spectrometer with an auto sampler using blank and standards for the analysis of the following elements: As, Cd, Co, Cr, Cu, Ni and Pb. An acetylene/air flame was used. A direct mercury analyser, DMA-80 Milestone (Soriso, Italy) was used for a direct measurement in soil samples. For the official or reference method for TEs analysis in soil, a total digestion method was used by adding an acid mixture of 5mL of HF and 1.5 mL of HClO_4 to 0.25 g of soil following the standard ISO 14869-1. The elements: Co, Cr, Cu and Ni were then analyzed by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) following the standard ISO 22036, while As, Cd and Pb were analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) following the standard ISO 17294-2.

II-2.1.4 Silicon determination

For silicon analysis, the protocol of the “Silicate test – MERK” kit was applied for soil samples preparation. Where, a mass of 1-2 g of each soil sample was weighed exactly into polyethylene tubes in triplicates. Twenty ml of CaCl₂ SIGMA-ALDRICH, ≥ 97 % was added to each tube and stirred for 16 h by “LABINCO” type rotary agitator, then centrifuged for 10 min at 4000 rpm. This step is followed by filtration using syringe and Whatman 0.2 m cellulose acetate filter. Samples were stored in polyethylene tubes in refrigerator at 4°C before Si measurement. The analysis of Si was carried out by Molecular Absorption Spectrophotometry “Thermo” type at a wavelength of 820 nm.

II-2.2 Wheat samples

II-2.2.1 Wheat plant production

Wheat cultivated plots were chosen from farms monitored by engineers from the Lebanese Agronomic Research Institute (LARI). The choice of wheat varieties grown and the fertilization and amendment practices are those recommended by local agrobusiness companies or agricultural cooperatives. Irrigation practices combine withdrawals from the deep groundwater (deep wells) and traditional withdrawals from surface waters: rivers and irrigation canals.

The measurements and samples taken on the plant were carried out over two consecutive climatic years (June 2016 and June 2017).

The chosen wheat varieties, of durum and soft wheat, in our study were released and controlled by LARI. The durum wheat varieties: “Icarasha” and “Miki” were chosen, given their high seeding rate (250 kg ha⁻¹) and drought tolerance. These two cultivars were combined in all the studied sites. While, the soft wheat variety “Naama 12”, has specific characteristics for the Lebanese bread preparation according to the specifications requested by Lebanese flour mills. “Naama 12” is also cultivated in the sites B1, B5, B6, and B9 as shown in **Table II-2. 1**. A survey was carried out among the farmers concerning the supplementary irrigation (wells).

Table II-2. 1: The type and variety of wheat grown in the studied sites of the Bekaa region, and the different supplemental irrigation sources

Site	Wheat			P.T.W. ¹ Depth (m)	Irrigation Surface water
	Type and variety				
	Durum	Miki	Soft		
	Icarasha	Miki	Naame 12		
B1	X	X	X	80-90	-
B2	X	X	-	80-90	Canal from Kab Elias
B3	X	X	-	> 100	Litani River ²
B4a	X	X	-	100	Litani River
B4b	X	X	-	100	Litani River
B5	X	X	X	> 100	Canal from Berdawni River
B6	X	X	X	> 100	-
B7	X	X	X	80-90	-
B8	X	X	-	80-90	Litani River
B9	X	X	X	80-90	-

1 : Private Tubular Well

2 : Submersion

The studied sites were cultivated with potato-wheat crop rotation, and follow the following agricultural practices (irrigation and fertilization) presented in **Table III-2.3 Annex**.

II-2.2.1 Sampling

A manual sample of five wheat plants was taken from the same studied sites, with their roots using a pickaxe, at the corners, in the center and diagonally, to make a sum of 45 plants in each plot. The collected samples were carried in a coded paper bag, protected from sunlight, and brought the same day to LARI laboratories.

II-2.2.2 Trace elements determination of the different wheat plant organs

As soon as the wheat plants are in the laboratory, the length of each plant was measured. The measured plants were washed with tap water and ultra-pure water, and then dried in oven (Model 700 Memmert) at 104°C for 2h. The dried grains, leaves and stems were grinded separately in a zircon mortar. Finally, the obtained flour was kept in plastic containers at room temperature, away from sunlight.

A microwave type “Milestone – Ethos Plus” was used for mineralization of wheat plant organs. A mixture of 7 mL of HNO₃ and 1 mL of H₂O₂ was added to 0.5 g of wheat plant organ (stem,

leaf, and seed) directly in a Teflon receptacle and digested in triplicate. Same as soil samples, all wheat samples were filtered and adjusted to 25 mL using HNO₃ 1% after digestion and before TEs analysis. The Teflon bombs underwent, after each use, to an acid treatment by immersion in a 10% dilute HNO₃ solution.

The same elements as for soil samples were measured in wheat plant organs by using Atomic Absorption Spectrometer, Zeeman Graphite Furnace GF95Z, Thermo Electron Corporation M series. Each sample was analyzed in triplicate.

Mercury was analysed by direct mercury analyzer DMA 80 Milestone (Soriso, Italy) using 0.1 g of soil sample and wheat plant organ sample.

II-2.3 Irrigation water samples

A supplemental irrigation is applied during drought periods, by using private wells. These wells are deep tube wells, where water is lifted with the help of a pumping set operated by electricity.

The irrigation water sampling was carried out in November 2016 (rainfed period). The collected samples from the studied sites B1, B2, B3, B4a, B4b, B5, B8, and B9 were brought to the laboratory in 1.5 L plastic bottles, protected from the direct sunlight and at a temperature below 4°C (in the refrigerator) until TEs analysis.

The determination of the following elements: As, Cd, Co, Ni, Pb and Hg in irrigation water samples was performed by using the Atomic Absorption Spectrometer (AAS, Stafford House, UK) Zeeman Graphite Furnace GF95Z Thermo Electron corporation M series. The element mercury was analyzed by direct mercury analyzer DMA 80 Milestone (Soriso, Italy) using 0.1 g of irrigation water sample. Each sample was analyzed in triplicate. To guarantee the method reliability, a certified reference material (CRM; BCR-610) was analyzed at the same time as samples. The recovery percentages obtained for the reference material were 110.8% for As, 91.3% for Cd, and 102.7% for Pb.

II-2.4 Bread samples

II-2.4.1 Survey of Lebanese bread consumption

A daily consumption of Lebanese pita bread survey was conducted on a target sample of 1000 individuals. This survey was carried out in 5 regions covering the entire Lebanese territory. The present work used a cross-sectional study plan. Where participants were recruited using a four-stage stratified cluster sampling method.

The sampling was based on the distribution of the Lebanese population in governorates: 41% in Mount Lebanon, 20% in the north of Lebanon, including Akkar, 17% in the south of Lebanon, including Nabatieh, 13% in the Bekaa including Baalbeck and Hermel, and 9% in Beirut.

Individuals were equally distributed between men and women and grouped as follows: 800 adults (> 15 years old) and 200 young people (between 6 and 15 years old) (CAS, 2017). Indeed, Lebanese residents are 49.7% Women and 50.3% men; regarding age, 24% of them are less than or equal to 14 years old (CAS,2017).

A team of 8 data collectors trained and managed by a supervisor, collected the data in a period of about two-month from April 14th, 2017 – June 5th 2017. The questionnaire was filled in 50 supermarkets around the country, all over the day by 1000 individuals. It required approximately 7 minutes of time to be answered. The collectors interviewed face-to-face the participants in each supermarket, filling instantly the questionnaire online by using an electronic tablet.

The questionnaire composed of 4 pages, with a first introductive page, where the collectors can find out if the participants were eligible for the study according to the following criteria: if they were Lebanese, had lived at least fifteen years from the date of the survey, consumed Lebanese bread for the same predefined period and had no chronic or serious illness. Otherwise, children should meet the third criteria only; their answers were given by their parents. The 2nd and the 3rd containing 44 questions and figures such as the size of Lebanese pita bread, divided into 3 parts: *Part 1*. Consumption of Lebanese bread, *Part 2*. Sociodemographic data, and *Part 3*. State of health. And a final page explaining the intensity and measurement of physical activity which is characterized by the frequency, intensity, duration and type of practice that define the amount of physical activity in a space-time (day,

week, etc.). Finally, 992 Lebanese consumers who completed all measures were enrolled in this conducted study. The collected answers were transmitted in the “iSurvey” application for data harmonization. The questionnaire is presented in Annex 1. The results of the survey were used to determine the three most consumed brands of Lebanese white bread, among the 49 brands that are sold with different sales values in the Lebanese market and are distributed among the Lebanese governorates.

II-2.4.2 Trace elements determination in bread samples

Accordingly, the sampling of these brands was carried out in Lebanese supermarkets to assess the exposure of the Lebanese to seven TEs through the consumption of bread. The sampling plan has been drawn up in accordance with European Commission regulation (EC) No 333/2007 modified by Regulation (EU) No 836/2011 and on the establishment of methods of sampling and methods of analysis for the official control of lead, cadmium, mercury, tin contents inorganic, 3-MCPD and benzo (a) pyrene in food.

The collected samples have been labelled B1, B2, and B3. From each selected brand, three randomly sampled bags were collected and brought to the laboratory. Each bread bag had seven pitas where three were randomly picked. Each pita was cut into 12 circular sectors with a plastic knife on a polyethylene plate to prevent TEs contamination. Two pieces were randomly drawn for analysis, to finally have 18 circular pita pieces for each brand (3 bags x 3 pitas x 2 pieces). Then these pita pieces were ground into powder using an agate mortar.

Samples of 0.5 g each were directly weighed after grinding in 50 mL Teflon tube. Then, 7 mL of concentrated HNO_3 and 1 mL of H_2O_2 were added to the preparation. The Teflon bombs were closed and placed in the digester at 200°C for 15min (1000 W). Once the Teflon tubes had cooled for at least 45 min, 3 mL HNO_3 1% before the trace elements analysis. Each sample was digested in triplicate.

The TEs (As, Co, Cd, Cr, Pb, and Ni) analysis in bread samples was performed using an Atomic Absorption Spectrometer (AAS, Stafford House, UK) Zeeman Graphite Furnace GF95Z Thermo Electron Corporation M series. Each digested sample was analysed in triplicate.

The certified reference material (CRM; BCR-191) underwent the same digestion protocol and was analysed at the same time as the pita samples, to guarantee the method reliability. The recovery percentages obtained for CRM were between 86 and 110%, representing 93.2%, 110.8%, 86.3%, and 110.1% respectively for Hg, Pb, Ni, and Cd. While, a spiking by a known concentration (0.1 mg L^{-1}) was applied before digestion for the other elements: As, Co, and Cr which they don't have reference materials available. The spiked bread was treated and digested in the same way as the bread samples and analysed using an AAS. The recovery percentages were 103.08% for As, 101.29% for Co, and 96.37% for Cr.

For Hg analysis, an acid mineralization is not required for all samples. It was analysed by direct mercury analyzer DMA-80 Milestone (Soriso, Italy). A sample of 0.2 g was directly weighted in triplicate in a nickel boat or sample holder and then introduced in a quartz furnace (staying at drying temperature 60 sec at drying temperature: $200 \text{ }^{\circ}\text{C}$, 105 sec at ashing temperature: $650 \text{ }^{\circ}\text{C}$) for measurement.

II-2.4.3 Exposure determination

The exposure to TEs was calculated for each interviewee consuming Lebanese white pita bread. By taking into consideration the quantity of bread consumed per person, the body weight, and the metal content of the bread consumed according to the brand. The elements: As, Cr, Co, Ni, Pb, Hg and Cd were calculated and the results were expressed in terms of median and 95th percentile using Ms-Excel for different population categories. This exposure was expressed in $\mu\text{g kg}^{-1}$ of body weight per week for the elements Cd and Hg, while in $\mu\text{g kg}^{-1}$ of body weight per day for the elements: Cr, Co, Ni, Pb, and As.

PART III – RESULTS AND DISCUSSION

In the Mediterranean region, agricultural soils are seriously polluted with toxic trace elements (TEs) which could enter the food chain via the soil-plant trophic chain. For food safety reasons, the monitoring of TE concentrations in these agricultural soils is thus imperative. The most powerful monitoring method for TE measurements is based on perchloric acid (HClO₄) and hydrofluoric acid (HF) digestion, commonly used as reference total digestion (RTD) method, with consequent use of inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). Unfortunately, HF and HClO₄ manipulations are highly dangerous and ICP-OES and ICP-MS apparatus are very expensive, thus they are unaffordable, notably in developing countries. In this paper, an alternative, microwave sulphuric digestion (MSD) method, combined with atomic absorption spectroscopy (AAS) is proposed. First, the suggested method was validated on a soil certified reference material, for the determination of 7 TEs (As, Cd, Co, Cr, Cu, Ni and Pb). Second, the MSD method was applied on agricultural soil samples situated in the Bekaa valley, East Lebanon and results were compared to those of RTD method. The MSD method, coupled to AAS, offers a promising and feasible alternative to HF, as well as, aqua regia based methods.

CHAPTER III-1

Validation of a new method for monitoring trace elements in Mediterranean cereal soils

III-1.1 Introduction

Trace elements (TEs) are the most studied soil contaminants in the world, and come both from lithogenic sources (Rivera et al., 2015) and anthropogenic activities (Shang et al., 2019). They are persistent, non-degradable and even though some are essential for living organisms, they can adversely affect microorganisms, animals, plants and humans at high concentrations. As a matter of fact, TEs can accumulate in plants to harmful concentrations, becoming a threat for human health through the food chain (Antoniadis et al., 2019b). Accurate and routine determination of their concentration in soil is thus necessary to assess their risk to directly and indirectly enter the food chain.

Cereal production soils in the Mediterranean basin are regularly amended with organic matter and phosphates bearing TEs and repeatedly irrigated with untreated wastewater (Kassir et al., 2012; Korfali and Karaki, 2018). A regular monitoring of TE concentrations in these agricultural soils is of great interest. Arsenic, Cd, Co, Cr, Cu, Ni and Pb are the most monitored elements based on previous studies of TE determination in cereal production soils of the Bekaa valley, East Lebanon (Darwish, T. et al., 2008). Nevertheless, less than 6% of Lebanese arable lands have been checked so far for TE contamination (Darwish, 2018).

Although some countries introduced the nondestructive thick target particle induced X-ray emission technique for the determination of trace elements in as received soil samples, digestion methods are still widely practiced (Nsouli et al., 2004). However, the heterogeneous and complex soil matrix, associating organic compounds and mineral constituents with different physicochemical characteristics, does not allow a single solid phase digestion method for the determination of TEs. To be acceptable, precision and accuracy for digestion methods should be lower than 20% (Voica et al., 2012) for any set of targeted soil to be analysed on a routine basis.

The digestion of samples containing TEs can be done using a dry method: in this case, the sample is calcined in an oven at temperatures ranging between 450°C and 500°C. Dry ashing offers the advantage of requiring few reagents like acids to solubilize ashes. However, dry ashing can lead to a loss of analytes like As and Hg by volatilization.

To reduce the sample preparation time and losses by volatilization, wet digestion processes are an attractive alternative to dry ashing methods for several samples such as biological tissues, food and soils (Antoniadis et al., 2019b; Munoz et al., 2013). In this case, the sample is mineralized in a mixture of acid at their boiling point. Different acids are usually used to perform the wet mineralization: hydrochloric acid (HCl), nitric acid (HNO₃), sulfuric acid (H₂SO₄), perchloric acid (HClO₄), hydrofluoric acid (HF). Several combinations were used for the mineralization of soil samples (Funes Pinter et al., 2018; Kabata-Pendias and Mukherjee, 2007; Lin et al., 2019; Nguyen et al., 2019) but there is no consensus on the choice of the mineralization reagent.

It is admitted that HF combined with HClO₄ allows for a total dissolution of all elements present in the sample but induces Si volatilization. However, HF is dangerous to handle. High precautions and safe handling should be implemented in the laboratory to prevent accidents. Despite this, serious accidents can always occur.

To avoid handling HF, sample attacks using a mixture of other strong acids are usually adopted in routine analysis. HNO₃ has high oxidant properties, most often used in wet mineralization, especially when the analysis step is done by atomic absorption technics (Hoenig, 2001a). Its relatively low boiling point (120°C) limits its efficiency. Therefore, HNO₃ is commonly used in a mixture with H₂SO₄ or HClO₄ (boiling point at 340°C and 200°C respectively). HClO₄ should be handled carefully because when its concentration exceeds 85% above 150°C, it becomes unstable and presents a serious explosion hazard. The combination of HNO₃ with HCl is well-known as “aqua regia mixture”. It has a high dissolution power and is usually used to evaluate the trace element contamination level (Melaku et al., 2005). Aqua regia reagent gives a pseudo-total concentration compared with the HF total method, especially for the following elements (Cr, Ni, Zn and Pb) (Santoro et al., 2017).

Ammonium fluoride (NH₄F) and ammonium bifluoride (NH₄HF₂) are used as a substitute for HF. These compounds are safer than HF for the operator and have high boiling points (260°C and 239.5°C) allowing sample digestion at high temperature in open vessels (Zhang, 2019).

To optimize the mineralization time, microwave heating is often performed. Hydrogen peroxide (H₂O₂) is largely used in mixture with strong acids for the oxidation and destruction of organic matter (Funes Pinter et al., 2018; Lin et al., 2019a; Nguyen et al., 2019b). Its

oxidation power is greatly enhanced in the presence of H_2SO_4 due to the formation of H_2SO_5 (Hoenig, 2001). Recently, Magladi et al. (2019) used a mixture of NH_4HF_2 and HNO_3 in a microwave oven to dissolve completely a wide range of silicate rock samples for trace element determination. Certain analytes were not fully recovered using this method which limits its field of application.

Most often, TEs concentration in the mineralization solutions is determined using inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) following ISO 22036 ("ISO 22036:2008. Soil quality. Determination of trace elements in extracts of soil by inductively coupled plasma - atomic emission spectrometry (ICP - AES)," 2008, p. 22036) and ISO 17294-2 ("ISO 17294-2:2016. Water quality. Application of inductively coupled plasma mass spectrometry (ICP-MS). Part 2: Determination of selected elements including uranium isotopes," 2016, p. 17294) standards. These devices are very expensive and have high maintenance costs but combines low detection limits and a rapid multi-elemental determination in comparison with atomic absorption spectroscopy (AAS). Therefore, it is important to have available a method for TE analysis that is safe, green, inexpensive and accessible to a large number of laboratories. In agreement with green analytical chemistry, an eco-friendly infrared digestion method was recently developed for multi-element determination in soil sample by microwave induced plasma atomic emission spectrometry (MIP OES) (Cora Jofre et al., 2020). This method minimizes the reagents amounts, makes digestion safer and reduces waste.

The choice of a sample preparation method is crucial in the analysis of soil and sediment samples. Proper choice reduces matrix effects in complex samples and subsequently limits spectral and non-spectral interferences. The latter involves sample-introduction and plasma related-interferences. This last point becomes very important when using plasma-based atomic emission like ICP-OES and IC-PMS. Nitric acid is usually used to avoid spectral interferences caused by other acids (Bizzi et al., 2017).

In this study, two digestion methods were compared using a soil certified reference material: 1) heating 3 acid digestion (HAD) with a mixture of HNO_3 , $HClO_4$ and H_2SO_4 , and 2) microwave sulfuric digestion (MSD) using a mixture of H_2SO_4 and H_2O_2 . The latter is proposed as a green, simple and fast method for soil sample digestion. The use of H_2SO_4 in sample digestion in

combination with H₂O₂ is more advantageous than the use of NH₄F and NH₄F₂. The latter are safer than HF but remain more dangerous than H₂SO₄.

The measurement of As, Cd, Co, Cr, Cu, Ni and Pb concentrations was then conducted using a flame atomic absorption spectrometer (AAS). AAS is less efficient than the widely used plasma-based methods (ICP-OES and ICP-MS) because it is less sensitive and only allows the determination of single elements. However, AAS is cheaper than plasma-based methods and is available to a large number of laboratories. A validation of the selected digestion method was done using a certified reference material (CRM). Afterward, the validated method (MSD + AAS) was applied on agricultural soil samples collected from the Bekaa region, located in Lebanon, East Mediterranean. The results were compared to those obtained using the reference total digestion method (RTD) (HF-based digestion + ICP/ICPMS).

III-1.2 Material and methods

III-1.2.1 Chemicals

The standard solutions for the elements As, Cd, Co, Cr, Cu, Ni and Pb of concentrations 1000 ± 1 mg L⁻¹ were obtained from Sigma-Aldrich. Nitric acid HNO₃ 65%, extra pure, sulphuric acid H₂SO₄ 95% and, perchloric acid HClO₄ 70% and hydrogen peroxide H₂O₂ 30%, obtained from Sigma-Aldrich, were used for digestion. A 1% HNO₃ nitric acid was prepared from concentrated acid (65% HNO₃) and was used in the standard solution preparation. Ultrapure water (>18 MΩ cm⁻¹) was obtained from a Milli-Q apparatus (Boeco pure, Germany). The Certified Reference Material (CRM) (SQC001-LRAA8753) for trace elements (As, Cd, Co, Cr, Cu, Ni and Pb) in soil was purchased from Sigma Aldrich.

III-1.2.2 Sites and sampling

Lebanon is located in the eastern Mediterranean basin between 35° and 36°40' E and between 33° and 34°40' N. The Bekaa Valley, an agricultural region where more than 90% of Lebanese wheat production is produced (FAO, 2010), is mainly a plain area located between Mount Lebanon in the west and the Anti-Lebanon mountains to the East. Eight sites where soil samples were taken are selected along a transect crossing the central Bekaa valley from southwest to northeast [Figure III-1. 1](#). The soils of the sampling sites are Eutric and Vertic

Cambisols (B1, B2, B5, B6 and B7), and Eutric Fluvisols (B3, B4ab and B8) according to the World Reference Base (IUSS Working Group WRB, 2015). They are deep, predominantly non-calcareous clay, with neutral to weakly basic pH values (7.4 – 7.8) and low to medium organic matter (1.4% - 2.7%).

Most of these sites were irrigated by private deep wells taking water from the deep groundwater reservoir, and by surface waters when available nearby (Berdawni and Litani rivers). Farmers generally practice an annual wheat/potato succession.

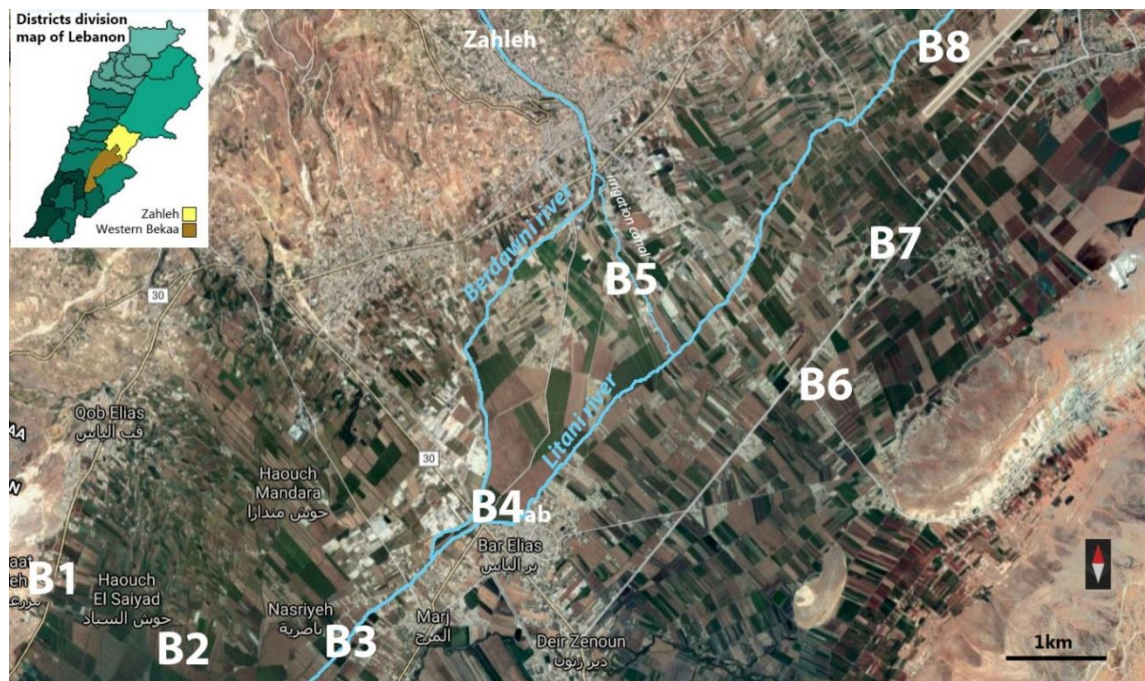


Figure III-1. 1: The location of the soil samples collected from Zahleh and western Bekaa administrative districts Lebanon: Mazraat Zahleh (B1), Tcheflik Qiqano (B2), Marj (B3), Haouch El Oumaraa (B4ab and B5), Maalaqah (B6), Dalhamiyeh (B7), Haouch El-Aamara (B8). Satellite Images © CNES/Airbus, Maxar Technologies, Map data © 2021

Topsoil layer (0 – 20 cm) was sampled using a stainless-steel shovel at the corners of the plot, in the centre and diagonally. The nine subsamples of the same plot were mixed, homogenized and carried in clean polyethylene bags. The samples were then brought to the laboratory where a quartering technique (Horwitz, 1990) was applied to the overall homogenized sample to reduce the sample size and form a composite sample. Only about 1 kg of soil sample underwent pretreatment.

III-1.2.3 Samples pretreatment and digestion

The composite soil samples were cleaned up from roots and gravels, dried according to the AFNOR NF X31-102 standard (“NF X31-102. Soils quality. Determination of residual moisture

content of soils analytical test samples,” 1981, pp. 31–102) in an oven (Model 700 Memmert) at 40°C, until constant weight and sieved at 2 mm. Then, soil samples were stored in plastic bottles protected from the direct sunlight and at a temperature below 4°C (in the refrigerator) until analysis.

Before mineralization, the soil samples were ground fine with a mortar and sieved (diameter < 250 µm) following the standard NF ISO 11464 (“ISO 11464:2006. Soil quality. Pretreatment of samples for physico-chemical analysis,” 2020, p. 2006). Two methods of digestion were used in order to mineralize a representative part of the composite soil samples:

i) For the heating 3 acid digestion (HAD), a three-acid mixture (9 mL HNO₃, 2 mL HClO₄, 2 mL H₂SO₄) was added to 0.5 g of soil sample or CRM, followed by heating up to 350°C for 2 h 30 min in a heating block. The mixture was then stored at room temperature for one night.

ii) For the microwave sulphuric digestion (MSD), a microwave type “Milestone – Ethos Easy” was used. Fifteen vessels can be processed simultaneously. This system controls the temperature in all vessels using a direct contactless temperature sensor. An acid mixture of 9 mL of H₂SO₄ and 3 mL of H₂O₂ was added to 0.5 g of soil sample or CRM in a Teflon receptacle tightly closed. A temperature of 200°C was reached in 10 min and maintained at this level 10 min. Then, the temperature decreased gradually where the cooling step was maintained for 20 min. This method was suggested by Milestone and was subjected to a validation.

All digested samples were filtered and adjusted to 25 mL using HNO₃ 1% before analysis.

III-1.2.4 Apparatus for trace elements analysis

The analyses of TEs were conducted by atomic absorption spectrometry (flame iCE 3000 series – Thermo type, Thermo Fischer Scientific, Germany). An acetylene/air flame was used. Liquid samples were introduced in the spectrometer with an auto sampler using blank and standards for the analysis of the following elements: As, Cd, Co, Cr, Cu, Ni and Pb.

Calibration standard solutions were prepared by successive dilution, all plastic labware used for sampling and sample treatment was new or cleaned by soaking 24 h first in 10% HNO₃ then in ultra-pure water.

III-1.2.5 Official or reference methods for trace element analysis

For the reference total digestion (RTD) method, soil sample mineralization was done by a mixture of HF (5 mL) and HClO₄ (1.5 mL) acids added to 0.25 g previously ground soil below 250 µm following the standard ISO 14869-1 ("ISO 14869-1:2001. Soil quality. Dissolution for the determination of total element content. Part 1: Dissolution with hydrofluoric and perchloric acids," 2001, p. 2001): 0.25 g of sample was transferred to a crucible and placed in a furnace. The temperature reached progressively 450 °C over 1 h. The temperature was maintained at 450 °C during 3h. After cooling, the ash was quantitatively transferred to a PTFE evaporating dish with a minimum amount of water. 5 mL of HF and 1.5 mL of HClO₄ were added to the PTFE dish. The mixture was heated on a hot plate until the dense fumes of HClO₄ and SiF₄ cease. Before complete dryness, the dish was removed from the hot plate and allowed to cool. 1 mL of HNO₃ 15 mol L⁻¹ and 5 mL of water were added to dissolve the residue. The latter was heated if needed to assist dissolution. The obtained solution was transferred quantitatively to a 50 mL volumetric flask.

HF decomposes silicates to form volatile SiF₄. The use of HClO₄ is necessary to avoid the precipitation of calcium in the form of fluoride (CaF₂). To avoid the risks of a brutal oxidation (acid ejection) of the organic matter (OM) by HClO₄, the OM was previously destroyed by calcining at 450°C. The hydrofluoric and perchloric acids were eliminated by evaporation at the end of the reaction. The residue was dissolved in diluted nitric acid 2%. Trace elements Co, Cr, Cu and Ni were then analysed by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES, Vista Pro, Varian, France) following the standard ISO 22036 ("ISO 22036:2008. Soil quality. Determination of trace elements in extracts of soil by inductively coupled plasma - atomic emission spectrometry (ICP - AES)," 2008, p. 2008), while As, Cd and Pb were analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Thermo series 2, France) following the standard ISO 17294-2 ("ISO 17294-2:2016. Water quality. Application of inductively coupled plasma mass spectrometry (ICP-MS). Part 2: Determination of selected elements including uranium isotopes," 2016, p. 17294).

III-1.2.6. Statistical analysis

All calculations related to the working range, linearity domain, limit of detection and limit of quantification were realised using R-4.0.0 (R Core team, 2020) and Rstudio 1.2.5042 (RStudio Team, 2016) with packages: “car” (Fox and Weisberg, 2018), “lmtest” (Zeileis and Hothorn, 2002) and “dplyr” (Wickham, 2020).

Analysis of variance was performed using XLSTAT 2018 to calculate repeatability and intermediate precision.

Information about various statistical analysis are detailed in following paragraphs, especially in section III-1.3.2.1 for working range, linearity domain, limit of detection and limit of quantification and III-1.3.2.2 for repeatability and intermediate precision.

III-1.3 Results and Discussion

III-1.3.1 Choice of the best digestion method using the soil Certified Reference Material CMR (SQC001)

To choose the best digestion method, the heating 3 acid digestion (HAD) and the microwave sulfuric digestion (MSD) methods were both applied on the soil CRM (SQC001).

HNO₃ is the most used acid in wet digestion. To overcome its limited efficiency due to its low boiling point, it was used in combination with H₂SO₄ and HClO₄ for the HAD digestion. Moreover, HClO₄ ameliorate the dissolution of sediment samples and insure a complete recovery of trace elements (Révillon and Hureau-Mazaudier, 2009). For the MSD digestion, H₂SO₄ was used because it has the highest boiling point. A mixture of H₂SO₄ and H₂O₂ was used because H₂SO₄ enhances the oxidation power of H₂O₂.

After digestion, the TEs As, Cd, Co, Cr, Cu, Ni and Pb were determined by AAS. The relative recovery, expressed in percentage was calculated using the following equation (Equation 1):

$$R(\%) = \frac{\bar{x}}{x_{ref}} \times 100 \quad (1)$$

where \bar{x} is the mean of CRM after digestion of the sample in triplicate, and x_{ref} is the assigned property value of CRM.

For the HAD method, the relative recovery for the elements analysed by AAS varied between 51.5% and 90.0% **Table III-1. 1** These values were very low and showed that HAD method using the mixture HNO₃, HClO₄, H₂SO₄ was not efficient enough to sufficiently solubilize the analysed trace elements.

Table III-1. 1: Comparison of the heating 3 acid digestion method (HAD) and the microwave sulphuric digestion method (MSD) using their relative recovery percentage of the soil Certified Reference Material CRM (SQC001)

Element	Soil CRM	HAD method		MSD method	
	Assigned value \pm SD	Mean Value \pm SD	Recovery	Mean Value \pm SD	Recovery
	mg kg ⁻¹	mg kg ⁻¹	%	mg kg ⁻¹	%
As	161.0 \pm 3.6	115.3 \pm 1.0	71.6	150.9 \pm 1.2	93.7
Cd	190.0 \pm 3.9	154.6 \pm 1.1	81.4	165.9 \pm 2.4	87.3
Co	177.0 \pm 3.6	91.2 \pm 1.0	51.5	199.3 \pm 4.7	112.6
Cr	87.9 \pm 2.3	67.8 \pm 0.7	77.1	98.7 \pm 1.0	112.3
Cu	258.0 \pm 5.3	232.3 \pm 0.3	90.0	230.1 \pm 2.3	89.2
Ni	127.0 \pm 3.2	111.2 \pm 1.1	63.5	125.0 \pm 1.1	98.4
Pb	138.0 \pm 4.0	80.6 \pm 1.2	80.6	155.4 \pm 1.0	112.6

SD = Standard deviation

For the MSD method, the relative recovery was in general higher and varied between 87.3% and 112.6%. These values were close to 100% and are within the accepted range for TE analysis (80 – 120%) (Hernández-Mendoza et al., 2013). Results show that sulphuric acid along with H₂O₂ represents an efficient mixture to solubilize Tes from different soil types, especially when microwave heating is used.

As the microwave sulphuric digestion of CRM gave acceptable recovery percentages, a validation procedure using this method was performed to check its applicability in our laboratory for trace element analysis in some Mediterranean soils.

III-1.3.2 Validation of the microwave sulphuric digestion method followed by AAS using the soil Certified Reference Material CMR (SQC001)

To validate the new digestion using a mixture of H₂SO₄ and H₂O₂, before applying it for trace element analysis in the soils, the following parameters were considered and determined: working range including linearity, limit of detection of the analytical system (LOD), limit of quantification (LOQ), precision including repeatability and intermediate precision, specificity and trueness.

III-1.3.2.1 Working range, linearity domain, limit of detection (LOD) and limit of quantification (LOQ)

The linearity of a method is often confused with the linearity of the instrument calibration function. In fact, the linearity of a method characterizes its accuracy, whereas instrumental linearity is concerned only with its own response. However, a response function does not have to be linear for correct quantification. Many methods make effective use of calibration models that are much more complex than just linearity (Gardiner, 1997; Hibbert and Gooding, 2005).

Calibration curves for each measured element were built using 4 or 5 points, depending on the element, each point was triplicated. For some elements, quadratic regression $y = \beta_0 + \beta_1x + \beta_2x^2 + \varepsilon$ gave better results and for some others, the best fit model was linear regression: $y = \beta_0 + \beta_1x + \varepsilon$. It can happen when wide dynamic ranges and/or not isotopically pure internal standards are considered (especially true in mass spectrometry) (Lavagnini and Magno, 2007). The independent variable x is assumed unaffected by error. β_0 , β_1 , and β_2 are the parameters of the model and ε represents a normally distributed random error, with mean zero and constant variance σ^2 [homoscedastic condition and $\varepsilon \sim \mathcal{N}(0, \sigma^2)$]. The β parameters are unknown and ordinary least-squares regression provides their estimates “b” by using a set of experimental data points (x_i, y_i) .

The adequacy of the model has been tested in several ways: (i) by the use of an analysis of variance test (ANOVA) called the lack of fit test (with replicate data); (ii) by inspection of the behaviour of the residuals and (iii) by the evaluation of the determination coefficient R^2 (square of correlation coefficient r). Models and main results of ANOVA tests are summarized

in **Table III-1. 2** and **Table III-1. 3**.

The estimated coefficients b_1 and b_2 were significantly different from zero and b_0 coefficient, depending on element, was not significantly different from zero. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the following equations (Lavín et al., 2018):

$$LOD = \frac{3}{b_1} \sqrt{\frac{s_{BL}^2}{n_{BL}} + s_{b_0}^2} \quad (2)$$

Where b_1 is the sensitivity and, s_{BL} is the standard deviation of the blank measurements, n_{BL} is the number of blank measurements and s_{b_0} is the standard deviation of the intersection with the vertical axis. In case of the quadratic model, when the instrument detector is working close to zero concentrations, the contribution of the 2nd degree term in the calibration curve is negligible.

$$LOQ \cong 3 \times LOD \quad (3)$$

Table III-1. 2: Calibration curves of the measured elements

Element	Model	Residual behaviour (normality, homoscedasticity, independence)
As	quadratic	Passed
Cd	quadratic	Passed excepted normality*
Co	quadratic	Passed excepted normality*
Cr	linear	Passed excepted autocorrelation *
Cu	linear	Passed
Ni	linear	Passed excepted normality & autocorrelation, presence of 1 outlier
Pb	quadratic	Passed

* Due to the presence of one or two extreme values (checked with Bonferroni Outlier Test) (Fox and Weisberg, 2018). The calculation of a reliable calibration model needed in general many values to be at least between 8 and 10 to verify the normality of the data by the Shapiro–Wilk test (Shapiro & Wilk, 1965) for instance, and to ascertain their scedasticity; and the number of concentration levels must range between 7 and 10 (Garden et al., 1980). In this study, the number of levels is between 4 and 5 with 3 replicates. So, the calibration models were evaluated with and without extreme values and coefficients were not drastically affected. It was decided to keep them.

Table III-1. 3: Working ranges, regression equations, limit of detection (LOD) and limit of quantification (LOQ) of the measured elements

Element	Working range mg kg ⁻¹	Regression equation $y = ax^2 + bx + c$ or $y = ax + b$	Standard errors on a, b and c	Regression quality R ²	LOD mg kg ⁻¹	LOQ mg kg ⁻¹
As	1.25 – 12	$y = -0.0032x^2 + 0.1599x - 0.0616$	0.0089; 0.0035; 0.0003	0.9997	0.166	0.498
Cd	5 – 15	$y = -0.0017x^2 + 0.0566x - 0.1077$	0.0119; 0.0027; 0.0002	0.9983	0.630	1.890
Co	3 – 12	$y = -0.0075x^2 + 0.1673x - 0.2153$	0.0034; 0.0011; 0.0001	0.9999	0.062	0.186
Cr	1 – 50	$y = 0.0637x + 0.0204$	0.0301; 0.0012	0.9956	1.736	5.041
Cu	5 – 15	$y = 0.1381x - 0.2347$	0.0030; 0.0003	1.000	1.764	1.915
Ni	1 – 15	$y = 0.1036x + 0.2119$	0.0610; 0.0065	0.9620	3.811	7.931
Pb	5 – 25	$y = -0.0158x^2 + 2.1287x - 3.2825$	0.2628; 0.0440; 0.0014	0.9999	0.370	1.110

High regression quality was obtained for all elements. Low detection limits, below 1 mg kg⁻¹ were obtained for all elements except Cr, Cu and Ni for which LOD was 1.736, 1.764 and 3.811 mg kg⁻¹ and LOQ was 5.041, 1.915 and 7.931 mg kg⁻¹ respectively **Table III-1. 3**. If we consider the threshold of Cr and Ni for multifunctional land use, where farmers can grow everything including the leaf succulent plants, are 50 and 40 mg kg⁻¹ respectively (OFEV, 2005), you can estimate the capacity of the method to quantify below the threshold value by the ratio LOD/(Threshold value) giving 0.10 and 0.20 for these two TEs respectively **Table III-1.3bis**. For field crops and fruit trees, these values are reduced by 4 and 2.5 times, giving 0.03 and 0.08 respectively, because of higher threshold values (200 mg kg⁻¹ for Cr and 100 mg kg⁻¹ for Ni) for the corresponding land use. This means high reliability of results to orient land use policy and protect public health from TEs transfer risks to food chain.

Table III-1.3bis: LOQ/(Threshold value) ratio according to element and land use *

Element	LOQ mg kg ⁻¹	Threshold value mg kg ⁻¹			
		Multifunctional land use		Field crops & Fruit trees	
		50 for Cr	40 for Ni	200 for Cr	100 for Ni
Cr	5.040	0.10		0.03	
Ni	7.931	0.20		0.08	

*capacity of the method to quantify below the threshold, the lower this ratio, the greater the capacity. Dimensionless ratio (values between 0 and 1)

III-1.3.2.2 Precision including repeatability and intermediate precision

Precision is a measure of how close results are to one another, usually expressed by the standard deviation (Barwick et al., 2014). Repeatability is the test results obtained using the same method, on the same sample in the same laboratory, with the same equipment, by the

same operator, in short intervals of time. Other terms are used to define repeatability, such as within-run, within-batch or intra-assay precision. Repeatability gives the smallest variation in results. Reproducibility is the test results obtained by different operators, different laboratories over a long period. It gives the largest variation in results. Between these two extremes, intermediate precision estimates result's variation in the same laboratory under routine conditions by different analysts and during an extended timescale. Other terms can be used to define intermediate precision such as between-run, between-batches, or inter-assay variation.

To determine repeatability and intermediate precision, two operators measured the metal concentrations in the soil CRM during each of the nine days. One-way ANOVA was applied on the obtained results. Repeatability was calculated as the within-group precision and expressed by the repeatability standard deviation S_r . It was calculated using the square root of the within-group mean square MS_w :

$$S_r = \sqrt{MS_w} \quad (4)$$

Intermediate precision was calculated as the within-group and between-group precision and expressed by the intermediate precision standard deviation S_i . It was calculated by combining the within- and between-group variance.

$$S_i = \sqrt{MS_w + \frac{MS_b - MS_w}{n}} \quad (5)$$

MS_b is the between-group mean square and MS_w the within-group mean square. Both values were calculated from the one-way ANOVA table. Relative standard deviation RSD varied from 0.3% to 1.5% for the first operator and from 0.1% to 1.2% for the second operator. The higher RSD was obtained for Co whatever the operator was. Repeatability standard deviation varied from 0.43 to 2.46 mg kg⁻¹. These values were relatively low and reflected good repeatability conditions **Table III-1. 4.**

Table III-1. 4: Repeatability and intermediate precision results obtained after TE analysis of CRM soil using the microwave sulphuric digestion method (n = 9 for both operators).

Parameters	Units	TE quantification						
		As	Cd	Co	Cr	Cu	Ni	Pb
Operator 1								
Mean	mg kg ⁻¹	151.5	165.5	186.3	98.5	233.8	123.7	126.4
SD	mg kg ⁻¹	0.5	2.4	2.7	0.6	2.1	0.6	1.0
RSD	%	0.3	1.4	1.5	0.6	0.9	0.5	0.8
Operator 2								
Mean	mg kg ⁻¹	151.0	167.3	182.4	98.1	235.0	124.0	126.9
SD	mg kg ⁻¹	0.9	0.7	2.2	0.4	0.2	0.1	0.5
RSD	%	0.6	0.4	1.2	0.4	0.1	0.1	0.4
S _r	mg.kg ⁻¹	0.70	1.76	2.46	0.49	1.52	0.43	0.76
S _i	mg kg ⁻¹	0.76	2.10	3.62	0.54	1.65	0.46	0.81
RS _i	%	0.5	1.3	2.0	0.5	0.7	0.4	0.6

SD = Standard Deviation; RSD = Relative Standard Deviation

S_r = repeatability standard deviation; S_i = intermediate precision standard deviation

RS_i = intermediate precision relative standard deviation

Intermediate precision varied between 0.46 and 3.62 mg kg⁻¹, which represented 0.4 to 2.0% as the relative standard deviation. These relatively low values reflect an acceptable variation of the results obtained by the two operators who applied the same method under routine conditions. The acceptable value for repeatability depends on concentration and was set at 8% for samples at the mg kg⁻¹ level (Horwitz, 2002).

III-1.3.2.3 Specificity

The lack of specificity of a method can be due to the widening of the absorption signal, but most of the time, it is due to the presence of interfering elements, which could increase or hinder the signal of a specific element. Interfering compounds are various constituents of the matrix and/or the reagents used to prepare the sample. It is what is called matrix effects. The International Conference on Harmonisation (International Conference on Harmonisation, 1996) defined specificity as “the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, this might include impurities, degradants, matrix, etc.” It was evaluated by analysing the CRM soil alone and after addition of known quantities of each element. Two levels of concentration for each element were

added to the CRM soil: 50 and 100 mg kg⁻¹. Samples were labelled CRM, CRM1 and CRM2. Samples were digested in triplicate and each sample was measured 3 times. The specificity was calculated using the recovery ratio expressed in percentage:

$$R(\%) = \frac{\text{experimental quantity after standard addition} - \text{experimental quantity before standard addition}}{\text{known added quantity}} \times 100 \quad (6)$$

The mean recovery of samples CRM, CRM1 and CRM2 and their respective standard deviations, for all the being studied elements are gathered in **Table III-1.5**.

Table III-1. 5: Specificity results expressed as mean recovery percentage and standard deviation. Three samples were analysed: CRM soil alone and CRM after the addition of two concentration levels for each element (n = 9).

Trace elements	Mean recovery percentage and standard deviation		
	%		
	Soil CRM	Soil CRM 1 + 50 mg kg ⁻¹ of each TE	Soil CRM 2 + 100 mg kg ⁻¹ of each TE
As	93.7 ± 0.9	96.1 ± 9.6	104.0 ± 4.9
Cd	87.3 ± 4.7	94.7 ± 9.2	92.0 ± 6.3
Co	112.6 ± 0.1	116.9 ± 0.3	117.8 ± 0.8
Cr	112.3 ± 3.5	111.5 ± 7.2	109.9 ± 5.7
Cu	89.2 ± 3.9	90.6 ± 4.0	99.1 ± 4.9
Ni	98.4 ± 0.9	98.1 ± 2.1	97.7 ± 1.7
Pb	112.6 ± 0.1	116.9 ± 0.2	117.8 ± 0.8

The specificity results show that the recovery percentages of all elements are within [80 – 120%] interval. Specificity values for TEs in soil have not been found in the literature. However, EU commission regulation No 2001/22/EC (Commission, 2001) mentions that recovery between 80 – 120% is acceptable for TEs such as lead, cadmium and mercury in foodstuffs. These results allow the application of the developed method in routine analysis without causing interference for As, Cd, Co, Cr, Cu, Ni and Pb.

III-2.3.2.4 Comparison between the validated method (MSD + AAS) and the RTD method (HF-based digestion + ICP/ICPMS) of TE analysis. Accuracy or trueness.

Accuracy is defined as “the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found” (International Conference on Harmonisation, 1996). Accuracy is sometimes defined as

trueness. Accuracy can be determined by analysing a CRM and comparing the experimental value to the assigned value. It is actually what we did in paragraph 3.1 where we obtained recovery percentages between 87 and 112%. Another way to assess accuracy is to work on real samples, to analyse them by the method being validated and to compare results with that obtained from a reference method. In this study, TEs in soil samples from the Bekaa governorate were determined by an MSD method using a mixture of H_2SO_4 and H_2O_2 followed by AAS measurements. Microwave digestion and element analysis were done in triplicate. Results from this method were compared to an external ISO reference which is the RTD method where the same samples were digested by HF and $HClO_4$ and analysed by ICP-AES or ICP-MS. The RTD method allows for total digestion of soil samples.

Results were compared in terms of recovery percentage calculated using equation 1. They are presented in **Table III-1. 6.**

1 Table III-1. 6: Comparison between the MSD method (H₂SO₄ + H₂O₂ digestion) being validated and the RTD method (HF + HClO₄ digestion) for Tes in soil samples from the Bekaa governorate sites.

Sites	Trace elements																	
	As			Co			Cr			Cu			Ni			Pb		
	RTD±SD	MSD±SD	Rec.	RTD±SD	MSD±SD	Rec.	RTD±SD	MSD±SD	Rec.	RTD±SD	MSD±SD	Rec.	RTD±SD	MSD±SD	Rec.	RTD±SD	MSD±SD	Rec.
mg kg ⁻¹			mg kg ⁻¹			mg kg ⁻¹			mg kg ⁻¹			mg kg ⁻¹			mg kg ⁻¹			
B1	6.8 ± 0.4	3.3 ± 0.0	48	18.3 ± 2.0	10.0 ± 0.0	55	94.1 ± 12.8	59.6 ± 0.1	63	22.0 ± 1.7	18.1 ± 0.1	82	56.1 ± 3.0	39.4 ± 0.2	70	13.8 ± 0.8	11.5 ± 0.0	83
B2	4.5 ± 0.2	3.1 ± 0.0	69	13.3 ± 1.7	9.7 ± 0.0	73	83.8 ± 11.6	60.7 ± 0.1	72	19.8 ± 1.6	16.3 ± 0.1	83	47.3 ± 2.7	25.6 ± 0.2	54	16.8 ± 1.0	12.3 ± 0.0	73
B3	4.4 ± 0.2	4.2 ± 0.0	95	11.4 ± 1.5	9.7 ± 0.1	85	75.1 ± 11.3	50.2 ± 0.1	67	17.2 ± 1.5	13.5 ± 0.1	78	43.6 ± 2.5	27.5 ± 0.2	63	24.5 ± 1.5	19.9 ± 0.0	81
B4a	7.3 ± 0.4	4.2 ± 0.0	57	11.4 ± 1.6	10.7 ± 0.0	94	55.8 ± 8.5	43.2 ± 0.1	77	16.6 ± 1.5	15.2 ± 0.0	91	39.4 ± 2.4	40.0 ± 0.2	102	9.1 ± 0.6	6.0 ± 0.0	66
B4b	7.5 ± 0.4	4.1 ± 0.0	55	11.7 ± 1.6	10.4 ± 0.1	89	57.2 ± 8.6	43.9 ± 0.0	77	14.5 ± 1.4	14.4 ± 0.2	100	39.7 ± 2.4	39.4 ± 0.6	99	8.8 ± 0.6	6.4 ± 0.0	73
B5	11.3 ± 0.6	9.9 ± 0.0	87	25.2 ± 2.3	17.3 ± 0.1	69	129.0 ± 16.7	68.2 ± 0.1	53	27.9 ± 1.8	15.6 ± 0.1	56	78.9 ± 3.9	46.8 ± 0.1	59	17.5 ± 1.1	15.5 ± 0.1	89
B6	6.4 ± 0.4	4.2 ± 0.0	66	14.9 ± 1.9	13.4 ± 0.1	90	71.7 ± 10.8	50.4 ± 0.0	70	23.0 ± 1.8	16.5 ± 0.0	72	52.0 ± 2.7	50.2 ± 0.1	96	15.0 ± 0.9	5.7 ± 0.0	38
B7	7.7 ± 0.4	4.3 ± 0.0	55	19.1 ± 2.5	17.0 ± 0.0	89	94.3 ± 10.7	90.4 ± 0.0	96	23.9 ± 1.9	16.8 ± 0.0	70	58.5 ± 3.0	50.4 ± 0.0	86	18.6 ± 1.1	5.8 ± 0.0	31
B8	9.7 ± 0.5	8.2 ± 0.0	85	23.0 ± 2.2	16.7 ± 0.1	72	114.0 ± 15.0	71.2 ± 0.1	62	25.9 ± 1.8	15.6 ± 0.0	60	64.2 ± 3.3	39.2 ± 0.1	61	19.5 ± 1.2	17.3 ± 0.1	89

2 RTD = Reference Total Digestion method mean value; MSD = Microwave Sulphuric Digestion method mean value; Rec. = mean Recovery; SD = Standard deviation

All recovery values were below 100%, which was predictable because the MSD with a mixture of H_2SO_4 and H_2O_2 does not induce a total mineralization of silicates compared with the RTD method using a mixture of HF and $HClO_4$. High recovery percentages were obtained for Co, Cr and Cu. Two-thirds of the calculated recoveries were higher than 70%, which is an acceptable value for trace analysis as defined by (AOAC 2002) (Horwitz, 2002). The other third was between 50 and 70% except for 3 values (As for B1 and Pb for B6 and B7). As, Co, Cr and Pb concentrations measured using the validated MSD method were correlated with values obtained using the RTD method. The relation was greatly improved for Pb ($R^2 = 0.9624^*$ with a slope = 1.0486) when B6 and B7 were removed. For Cr, the relationship was greatly improved when B7 was not considered ($R^2 = 0.9074^*$) and indicated a reduction of the recovery percentage with increased total concentrations. The most contaminated soil was B5, with the highest concentrations for As, Co, Cr, Cu and Ni. This plot is occasionally irrigated by pumping water from an irrigation canal from the Berdawni River, the catchment of which drains the town of Zahleh. Nevertheless, all soils remained in the lower range of concentrations found in soils worldwide for all elements (Kabata-Pendias and Mukherjee, 2007). Cobalt, Cr, Cu and Ni concentrations were correlated indicating a probable common geogenic origin. Arsenic was only correlated to Co and Ni, which may be related to a more scattered occurrence due to anthropogenic inputs (Darwish, T. et al., 2021). The lead concentration was not correlated to that of any of the other elements, probably because two soils (B6 and B7) had a very low recovery rate. These results confirm the reported low content in the soils of the Central Bekaa area and depicted from the spatial distribution of total Pb in the soil surface (Darwish, T. et al., 2004). The pattern of Pb concentration shows, above a weak geogenic background ($4 - 14 \text{ mg kg}^{-1}$), an anthropic contamination along heavy trafficked roads by atmospheric deposition (Gupta, 2020) and surface accumulation gradient of Pb from foot-slope, located downslope of Zahleh town, towards the Litani River ($14 - 34 \text{ mg kg}^{-1}$). This lead is carried by water runoff from Zahleh agglomeration [Figure III-1. 2](#). Where, topsoil samples (0-15 cm depth) were collected from Central Bekaa area (1200 ha) by grid system 1kmx2km, based on topography, lithology and land use. Sixty samples were collected in 1997 before the introduction of unleaded fuel in 2000. The digestion of samples was done by aqua regia mixture and Pb was analysed by means of ETA-AAS

device. The applied methodology of soil samples collection and preparation for analyses was quality-controlled, with reproducible results by means of reference samples from “The International Soil-Analytical Exchange Program” of the University of Wageningen, The Netherlands. A spatial analysis and Pb mapping were done using the universal kriging method with a matrix table of 5 m x 5 m resolution.

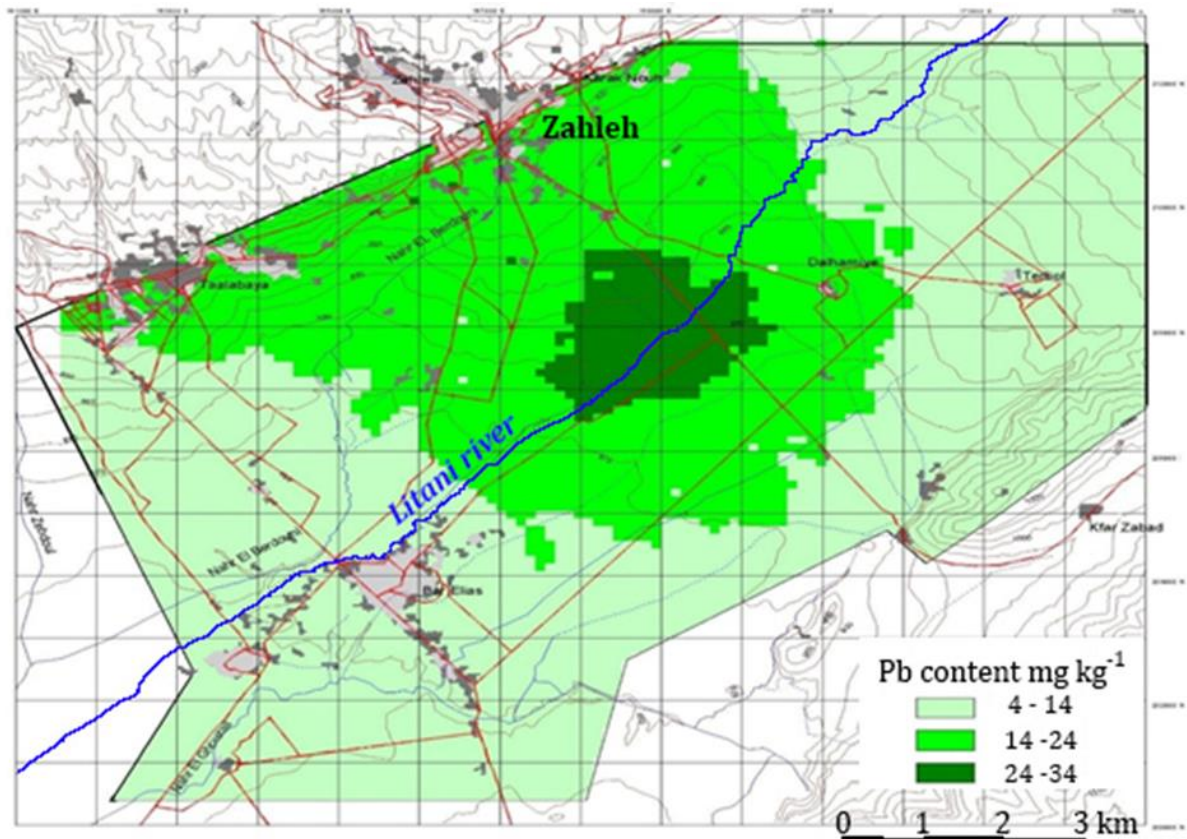


Figure III-1. 2: Cartography by universal kriging of topsoil Pb distribution in Central Bekaa plain showing: (i) above a low geogenic background, an anthropic contamination along the roads with heavy traffic including the plume effect was controlled by the main northeast wind and (ii) a surface runoff gradient from the slopes near the town of Zahleh towards the Litani river

Arsenic values obtained by the RTD method varied between 4.35 mg kg⁻¹ for B3 and 11.3 mg kg⁻¹ for B5. They were slightly above the commonly reported mean background As concentrations in surface soils (worldwide data) (Kabata-Pendias and Mukherjee, 2007), but still within the range for common, possible background values and multifunctional land use (OFEV, 2005).

Cadmium values were not shown in this study because they were below quantification limit (1.89 mg kg⁻¹) when using the MSD validated method. Cd could not be determined by this method. However, all values determined by the RTD method

varied between 0.23 mg kg⁻¹ for B4b and 0.48 mg kg⁻¹ for B8 and were defined within the global average soil Cd concentration (0.06 – 1.1 mg kg⁻¹) (Kabata-Pendias and Mukherjee, 2007). Our values are in agreement with those found by Kanbar et al. (0.28 – 2.8 mg kg⁻¹) in different Lebanese soils [52] and those found by Darwish in Bekaa soils (0.28 – 0.48 mg kg⁻¹) (Darwish, 2008).

Cobalt values obtained by the RTD method varied between 11.4 mg kg⁻¹ for B3 and 25.2 mg kg⁻¹ for B5. These values are close to the range of world values in surface soils (4.5 – 12 mg kg⁻¹) (Kabata-Pendias and Mukherjee, 2007). They are comparable to those obtained for arid and semiarid regions (16.5 – 26.8 mg kg⁻¹) (Jalali and Majeri, 2016). They are also in agreement with those found by Darwish in Bekaa soils (28.1-28.5 mg kg⁻¹) (Darwish, 2018).

Chromium values obtained by the RTD method varied between 55.8 mg kg⁻¹ for B4a and 129.0 mg kg⁻¹ for B5. These values are higher than the world median content of Cr in soils: 54 mg kg⁻¹. They are within the sandy and light loamy soils range, which contains Cr between 2 and 350 mg kg⁻¹ (Kabata-Pendias and Mukherjee, 2007). They are close to those found by Darwish in Bekaa soil samples (43.3 and 71.3 mg kg⁻¹) (Darwish, 2018) and do not exceed the limits for safe agricultural land use.

Copper values obtained by the RTD method varied between 14.5 mg kg⁻¹ for B4b and 27.9 mg kg⁻¹ for B5. These results are within the range for Cu concentration in soils (8 mg kg⁻¹ in acid sandy soils to 80 mg kg⁻¹ in heavy loamy soils) (Kabata-Pendias and Mukherjee, 2007). They are in general lower than those obtained in other Lebanese soils (25.3 – 54.2 mg kg⁻¹) (Kanbar et al., 2014), among others Taalabaya (Zahleh district) soils (35.7 mg kg⁻¹) (Darwish, 2018), but conform to the values obtained in Saadnaeal (Zahleh district) soils (17.0 mg kg⁻¹).

Nickel values obtained by the RTD method varied between 39.4 mg kg⁻¹ for B4a and 78.9 mg kg⁻¹ for B5. These values are within the range of Ni concentration in soils worldwide (0.2 – 450 mg kg⁻¹) (Kabata-Pendias and Mukherjee, 2007). They are in agreement with values obtained in Taalabaya soils (60.7 mg kg⁻¹) but higher than values obtained in Saadnaeal soils sampled at the same depth (33.7 mg kg⁻¹) (Darwish,

2018).

Lead values obtained by the RTD method varied between 8.84 mg kg⁻¹ for B4b and 24.48 mg kg⁻¹ for B3. They are lower than the overall mean value of Pb in different soils worldwide (25 mg kg⁻¹) (Kabata-Pendias and Mukherjee, 2007). They are in agreement with those obtained in other Lebanese soils (11.3 – 22.9 mg kg⁻¹) (Kanbar et al., 2014) and those obtained in Taalabaya soils (16.7 mg kg⁻¹) and Saadnaeal soils (10.0 mg kg⁻¹) (Darwish, 2018).

In summary, the results obtained in this study are representative of soils with limited trace elements contamination. The concentrations measured are within the same range than the values obtained by Darwish for surface soils and soil profiles of the Bekaa plain. He points to both a natural TE source with variation explained by the alluvial-colluvial origin of the parent material but also to a limited anthropogenic pollution due to a combination of inputs from cultivation and atmospheric deposition which is modulated by soil organic content.

The correlations between TEs values were higher following the RTD method than the validated one, suggesting that the RTD method can better trace the common origin of the elements, which was mostly of geogenic origin, as also indicated by the observed level of concentrations, which were close to the background level detected in North Lebanon (Nsouli et al., 2004).

Aqua regia, which is a reference method for pseudo-total metal concentrations in soils, gives extraction efficiency between 53 and 100%, depending on the element, as compared to total concentrations (calculated for the certified material METRANAL-33 in Shahbazi and Beheshti) (Shahbazi and Beheshti, 2019). Although it is widely used, resorption occurs (up to 470% for Cu) leading to underestimation of element concentrations (Guedes et al., 2020) and other mild extraction procedures may provide accurate results when trace element reactive fraction, not bound to silicates, is to be recovered (Guedes et al., 2020). Our results fall within the same range of efficiency as aqua regia and the validated method including the microwave digestion with H₂SO₄ + H₂O₂ may offer a more feasible and workable alternative.

III-1.4 Conclusion

The microwave digestion with $\text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2$ combined with flame-AAS measurement was validated with CRM and after comparison with HF-based digestion. It allows for routine but accurate characterization of calcareous and Ca-saturated soils with limited contamination in As, Cd, Cu, Co, Cr and Pb. The tested soils, used for wheat production, are irrigated with water of poor quality and need to be monitored for their trace element concentrations regularly to prevent transfer to the food chain.

Recent microwave digestion methods were implemented according to green chemistry, they have proved their efficiency for the determination of trace elements in soil samples (Cora Jofre et al., 2020; Funes Pinter et al., 2018; Lin et al., 2019b; Magaldi et al., 2019). In these studies, several reagents were used to dissolve soil or sediment samples such as: HNO_3 , HClO_4 , HF, HCl, NH_4HF_2 and H_2O_2 before trace element analysis using ICP-OES, ICP-MS or MIP-OES. These recent methods use problematic reagents difficult to handle safely and/or very expensive analytical devices.

The validated method offers an alternative to HF-based methods, as well as aqua regia. It is green, safe and cheap method for trace element analysis in soil samples. Other soil types will have to be tested to further extend its use to a larger range of soils to cover the soil diversity in the Mediterranean area.

CHAPTER III-2

Geogenic and anthropogenic origin of trace elements in the Central Bekaa soils

III-2.1 Geogenic context

Unable to access the sites that were in a conflict zone, we produced a photo-interpretation of the Bekaa valley using the 2021 satellite image from Google map, which is of exceptional quality. This allowed us to make a virtual geomorphological and pedological analysis and to classify our sites according to a decarbonation and reddening gradient increasing from the South-West to the North-East. This classification, considered as a conceptual model of the evolution of soils in the Bekaa valley, will allow us to approach the “geogenic” part of the contamination in trace elements.

The addition of spatial information on anthropogenic inputs: fertilizers, amendments, irrigation water (surface water, deep aquifers), untreated municipal liquid discharges, industrial pollution and considering the geometry of the systems (places of influx, direction of flow of surface water and aquifers, will allow discussion of the “anthropogenic part” of contamination.

III-2.1.1 Soils in the landscape

The Gèze soil reconnaissance map made in the 1950s is still relevant today (Gèze et al., 1956). Darwish **Table III-2.1 Annex** updated it mainly on the functional characteristics of the soils (structure, porosity, thickness, pebble load), giving the names according to the World Reference Base (WRB, 2014), while keeping the outlines of the cartographic units of Gèze. This old map allows us to distinguish in the Bekaa plain, the soils by their color in three categories: Light chestnut soils, Dark chestnut soils, and red soils *Figure III-2. 1*. These colors reflect a pedological evolution and different physicochemical compositions: strongly carbonated versus more or less decarbonated and reddened, going from light chestnut to red **Table III-2.1** p 104. Lamouroux (1972) shows that reddening was the result of the transformation of the carbonate residues into red amorphous ferruginous products during the weathering process of carbonate rocks under a Mediterranean climate. Therefore, this reddening gradient associated with decarbonation should be a good way to follow geogenic trace elements. Dark chestnut soils, for their part, result from an evolution in a

hydromorphic environment and lead to very clayey soils rich in smectites (Lamouroux et al., 1967).

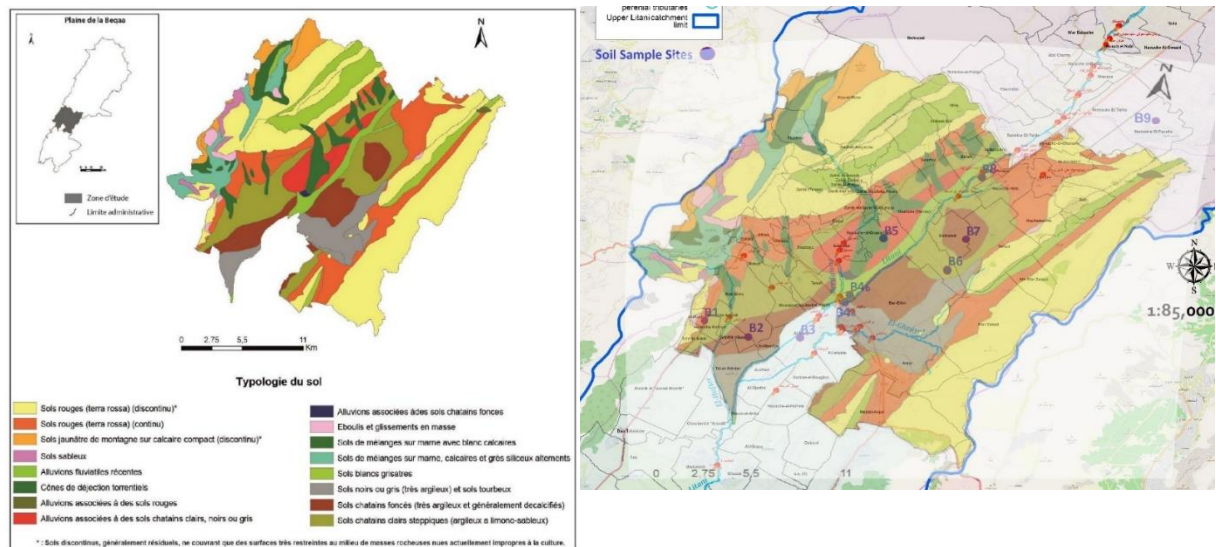


Figure III-2. 1: Left Central Bekaa soil map (Gèze, 1956 in Nasser, 2018). Right Coverage of the Gèze soil reconnaissance map on the administrative map of untreated municipalities outlet and the position of the sites studied

To carry out our geomorphological characterization of soils, we crossed different types of information:

- The GPS coordinates allowed us to position our sites on an administrative map of the Litani watershed on which we draped the Gèze soil reconnaissance map *Figure III-2. 1 on the right*.
- The Munsell color of the surface horizon of the soil, determined in the laboratory on dry samples sieved at 2 mm, which is compared to the color observed on the satellite map in the absence of plant cover;
- Finally, the geomorphological situation of the sites was apprehended using the topographic curves and thanks to the “3D vision” function of Google.

B1 – Mzaraat Zahle



B1 – Light chestnut soils developed on slope colluvium, located on the edge of an alluvial fan. Downstream a cardboard manufacturing industry (SICOMO) in the south and in joint ownership with a plastics manufacturing industry (PETCO) in the north (a). No surface water irrigation.

B2 – Tcheflik Qiqano and B3 – Marg



B2 – Light chestnut soils developed on slope colluvium, plot located in an agricultural environment, bordered on its north side by an irrigation canal fed by the El-Hafier river which drains Kab Elias city. **B3** site is bordering the Litani and flooded by the river (b). The light chestnut soil develops on the recent alluvium of the river.

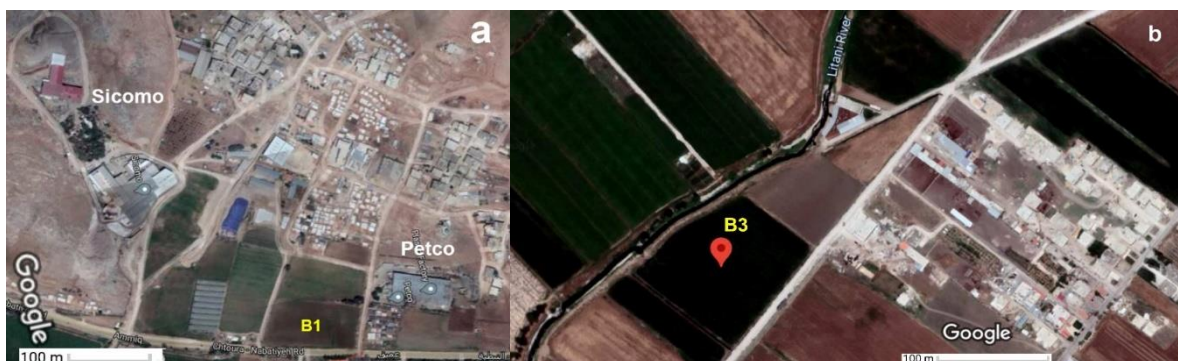


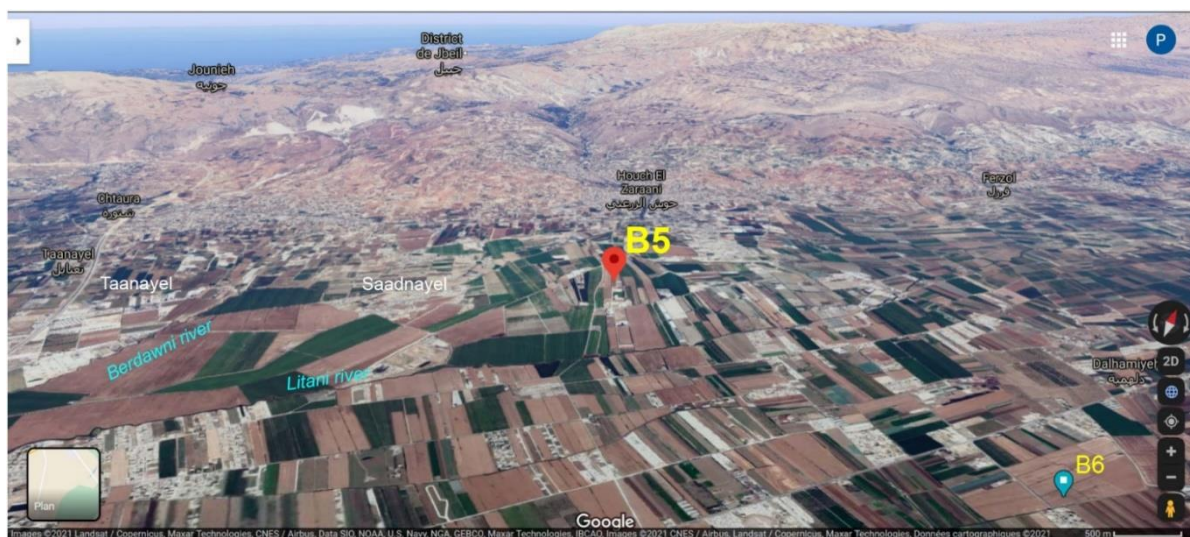
Figure III-2. 2: Light chestnut soils on western slope (B1, B2) and recent alluvium (B3)

B4ab- Zahleh Haouch El-Oumara Aradi



B4ab – Site bordering the Litani river, water is seen in the irrigation canals around the plots. The light chestnut soil develops on the recent alluvium of the river. This site is adjoining to the south with the municipality of Bar Elias and at one kilometer to the south of the industrial zone of Taanayel. Two plots were initially chosen to compare different practices.

B5 – Zahle Haouch El-Oumara Aradi



B5 – Light chestnut soil developed on the alluvial fan issued from Wadi El Aarayech. The relief is underlined by the orientation of the plots. The Berdawni river, whose course deviates towards the southwest at the exit of Zahleh, passes to the west of the agglomeration of Saadnayel then descends to the south, on the edge of the industrial zone of Taanayel.



This zoom on plot B5 shows the irrigation canals around the plots that are supplied, at the exit of Zahleh, by the Berdawni river.

Figure III-2. 3: Light chestnut soils in recent alluvium (B4) and in an alluvial fan on Western slope (B5)

B8 – Haouch El-Aamara



B8 site is located within the LARI. The red soil develops in old alluvium as evidenced by: (i) the terrace slope visible on the western edge of the plot (a) and (ii) the observation that the Litani leans and delimits the old red soils on the right bank while, in the recent alluvium deposited on the left bank, light brown soils are developing. For a long time, this plot benefited from additional irrigation by pumping water from the Litani before the drilling of a deep tube well. The pumping was done downstream a chicken slaughterhouse. Finally, it is located 600 West of the airstrip of Tal Aamara Airport.



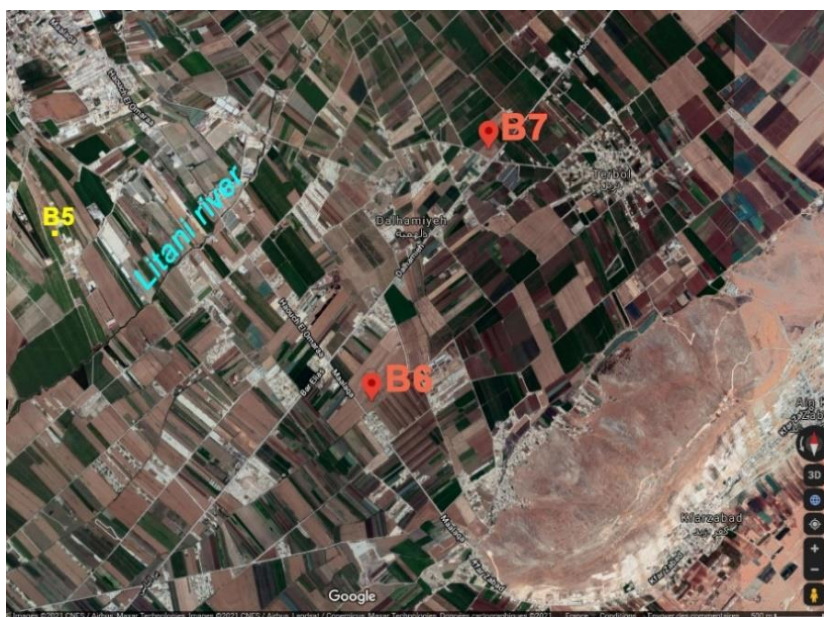
B9 – Serraine – Et-Tahta



B9 site is located at foot-slope of an eroded marly limestone slope. The red soil develops in the slope colluvium. It will also collect the sewers from the upslope villages built on the impermeable marly limestone.

Figure III-2. 4: Red soils developed on the eastern slope

B6 – Zahle Maallaqa Aradi and B7 – Dalhamiyet Zahle



B6 and B7 – Dark chestnut soils develop in the Bekaa plain, backed by the anti-Lebanon chain. The 2 plots are 2.2 km from the Litani river and irrigated only with deep tube wells. They are located in an agricultural environment. B7 adjoins Dalhamiyeh and remains 500 m from Terbol town.

Figure III-2. 5: Dark chestnut soils in the Bekaa plain leaning against the anti-Lebanon range (B6 & B7)

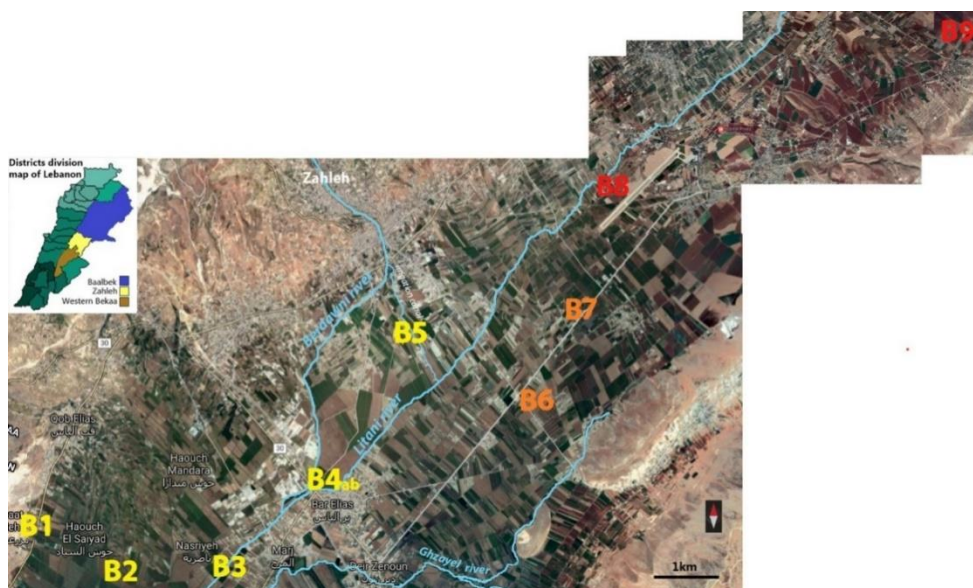


Figure III-2. 6: Site location on the satellite image of central Bekaa. Images© Landsat 2021, Google maps

The location of all the sites on the satellite image is a good illustration of the spatial organization of soils in the landscape. Light chestnut soils develop on the western slope of the Bekaa valley and in the recent alluvium while the red soils develop on the eastern slope and old alluvium. Dark chestnut soils develop in the Bekaa plain, backed by the anti-lebanon chain. It also underlines the relevance of the choice of sites

proposed by LARI engineers, which makes it possible to cover the diversity of the soils of the central Bekaa.

III-2.1.2 Physicochemical characterization of the studied sites

The classic agronomic analyzes of the plowed layer carried out at LARI make it possible to specify the main characteristics of the soil sequence Error! Reference source not found..

The soil texture is sandy clay, with no marked gradient along the sequence, site B5 is sandier and site B9 is more clayey. The reddening of the soils is confirmed, going from a Munsell hue of 10 YR for light brown soils to 2.5 YR for red soils. This reddening occurs considering a decreasing rainfall gradient ranging from 800 to 500 mm yr⁻¹. This reddening follows decarbonation: light brown soils have a CaCO₃ content greater than 50% while it is negligible in red soils which means a mass loss of 50% and a relative concentration of residual elements, including trace elements. Available phosphorus (P Olsen) is negatively correlated with CaCO₃ which correspond to well-known blocking of phosphorus ions by CaCO₃ (Morel et al.,1992).

Table III-2 1: Physicochemical characterization of the studied soils

Sites	Altitude m	Annual rainfall mm yr ⁻¹	Munsell soil color*	pH	EC μS cm ⁻¹	Clay	Silt	Sand	Total CaCO ₃ Active CaCO ₃ g kg ⁻¹	Total organic C	Total N	P Olsen mg kg ⁻¹	Exchangeable bases					
													Ca ⁺⁺	Mg ⁺⁺	K ⁺	Na ⁺	Sum	
													cmol ⁺ kg ⁻¹					
B1	880	700-800	10YR 4/2	7.5	504	363	156	481	406	104	27	2	17.4	56.3	8.8	0.7	2.0	67.7
B2	860	700-800	10YR 5/3	7.85	243	325	168	507	354	74	19	3	48.1	56.5	11.5	1.1	2.9	72.0
B3	860	600-700	5 YR 4/3	7.41	1276	341	187	472	559	189	21	2	60.4	56.3	11.5	1.0	2.6	71.4
B4a	860	600-700	10YR 4/3	7.53	270	266	166	568	597	200	21	2	16.4	42.6	22.9	0.3	2.1	67.9
B4b	860	600-700	10YR 4/2	7.25	2100	276	175	549	602	255	20	2	16.7	43.2	19.6	0.3	2.2	65.4
B5	880	600-700	2.5YR 4/4	6.93	367	206	167	627	397	72	12	1	108	29.7	11.5	0.5	2.7	44.4
B6	860	500-600	5YR -**			331	192	477	148	63	18	3	451	43.5	3.3	2.7	3.3	52.6
B7	900	500-600	5YR 4/2	7.54		328	196	476	147	64	17	3	448	43.6	3.2	2.6	3.7	53.1
B8	905	500-600	2.5YR 3/4	7.8	201	284	46	670	5	0	11	1	561	22.5	3.0	1.2	3.6	30.3
B9	1000	500-600	2.5YR-**			381	125	494	21		12		417					

A Principal Component Analysis was done using the physicochemical characteristics **Table III-2.1** and the total trace element content **Table III-2.2**. The results are presented in *Figure III-2. 7*.

Table III-2 2: Total trace elements content of the studied sites

Sites	Trace element total content (mg Kg ⁻¹)										
	As	Cd	Co	Cr	Cu	Hg	Mo	Ni	Pb	Sb	Zn
B1	6.84	0.281	18.3	94.1	22	0.0162	0.814	56.1	13.76	0.381	90.9
B2	4.52	0.286	13.3	83.8	19.8	0.0128	0.742	47.3	16.84	0.362	81.1
B3	4.35	0.255	11.4	75.1	17.2	0.0207	0.73	43.6	24.48	0.812	71.6
B4a	7.3	0.237	11.4	55.8	16.6	0.0126	0.711	39.4	9.09	0.322	56.1
B4b	7.51	0.233	11.7	57.2	14.5	0.0104	0.731	39.7	8.84	0.359	55.5
B5	11.3	0.426	25.2	129	27.9	0.0215	2.037	78.9	17.54	1.01	115
B6	6.37	0.345	14.9	71.7	23	0.0123	0.952	52	15	0.659	88.3
B7	7.74	0.329	19.1	94.3	23.9	0.0251	1.24	58.5	18.6	0.73	122
B8	9.65	0.475	23	114	25.9	0.0318	1.845	64.2	19.49	1	134
B9	7.043	0.415	15.25	81.34	20.1	0.02		49.08	18.6		82.23

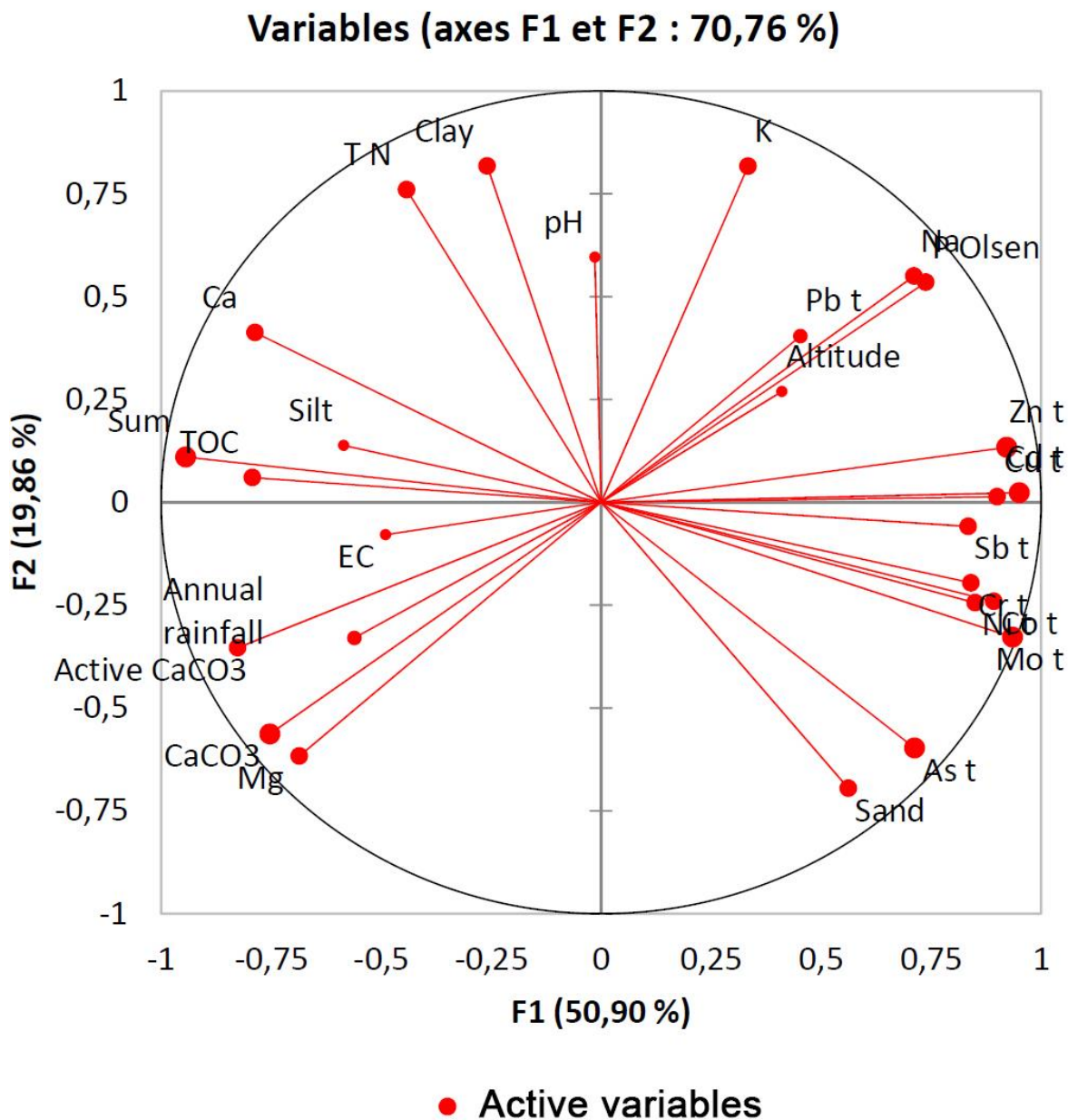
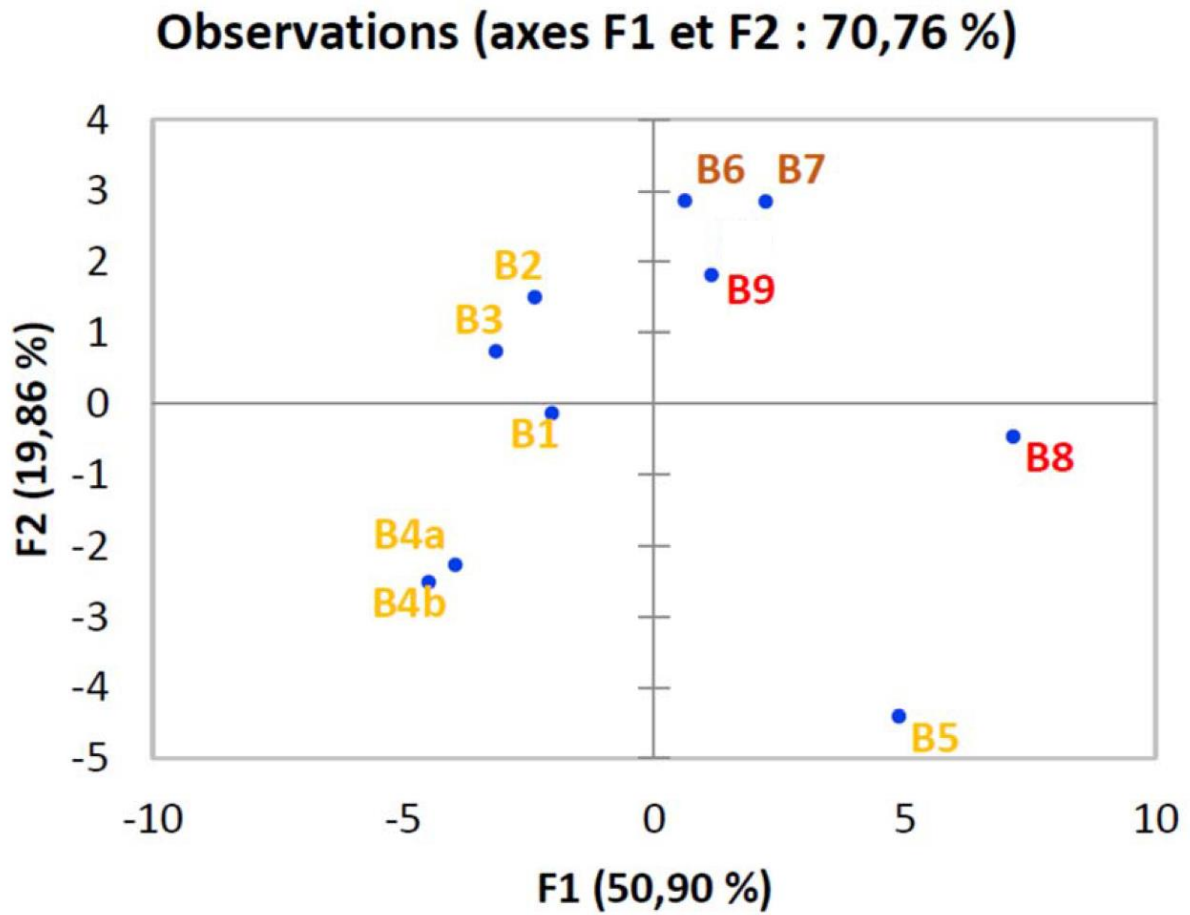


Figure III-2. 7: Variables correlation plots with principal components 1 and 2. The first two axes accounted for 71% of variance.

The majority of elements are correlated to each others. For example Sb is positively correlated to Cr, Cu, Ni, Zn, Co, Mo and Pb. These correlations show that Tes have probably the same geogenic origin.



- **Active observations**

Figure III-2. 8: Variables correlation plots with principal components 1 and 2. The first two axes accounted for 71% of variance.

The first axe represent Tes content, B4a and B4b are the sites having the lower Tes level and B5, B8 have the higher values of Tes.

From the individual chart we can observe 2 groups of sites and two individuals: first group gathering the Light chestnut soils (B1, B2, B3, B4a and B4b) with lower Tes contents; second group gathering dark chestnut soils B6, B7 and a red soil B9 with medium Tes contents and the light chestnut soil B5 and red light soil B8 with higher Tes content. This evolution is consistent with the formation of red Mediterranean soils from the decarbonation of light chestnut soils described by Lamouroux (1972). The

loss by dissolution of 50% of the mass of light chestnut soils leads to a relative increase in the Tes content which are concentrated in the residual clay fraction.

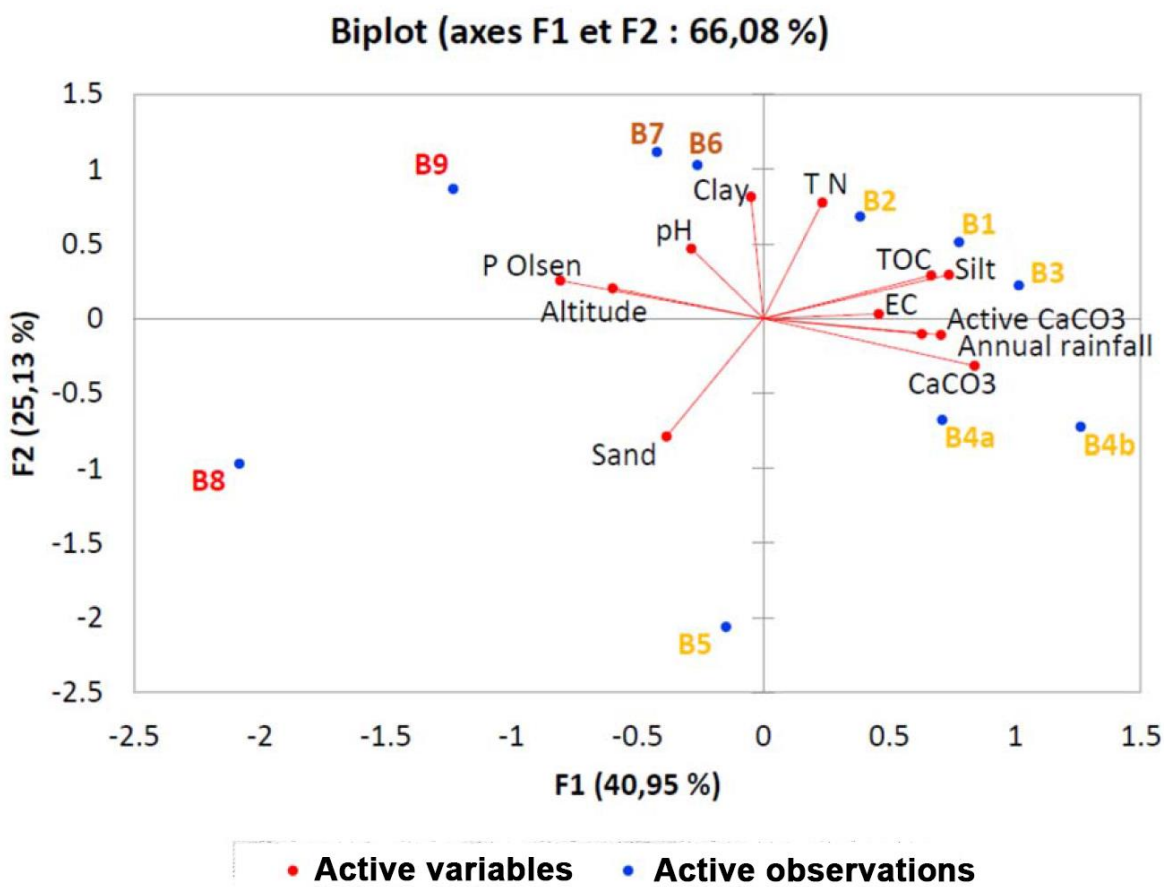


Figure III-2. 9: Biplot the site scores and variable loadings. Only physicochemical characteristics were used. The first two axes accounted for 66% of variance

Finally, this PCA was compared with the one using soil physicochemical characteristics as variables. The positioning of the sites is identical in both cases. This could suggest that the Tes concentrations have a geogenic origin and that Tes do not discriminate between sites.

III-2.2 Anthropogenic context

The types and varieties of wheat cultivated in the studied sites, the fertilization applied, as well as the type of irrigation used are presented in **Table III-2.2 Annex**. All the plots were cultivated by durum wheat (Icarasha and Miki mixed), while in addition to these two varieties of durum wheat, the sites B1, B5, B6, B7, and B9 were grown by soft wheat (Naama 12). Inorganic and organic fertilization is applied to all cultivated wheat plots, as well as organic manure (cows and poultry) is added in some plots. Deep well water irrigation was used in all studied sites, and surface water was used in B2, B3, B4a, B4b, B5, and B8.

III-2.2.1 Trace elements provided by fertilization and amendments

A Potato/Wheat crop rotation was applied in the studied sites **Table III-2.3 Annex**. The potato cultivation was taking place during March with full irrigation (dry period from sowing to harvesting). Before planting in March, an application of chicken manures was used (250 Kg N ha^{-1}), while during flowering and after flowering, a 2nd application of synthetic N fertilizer was used (100 Kg N ha^{-1}).

For wheat crop, the N fertilizers are applied twice, in the 1st application during the emergence stage and sowing stage (230 Kg N ha^{-1} and 60 Kg N ha^{-1} respectively), and the 2nd during the flowering stage (230 Kg N ha^{-1}).

In addition to the N fertilization, the P fertilizers were applied only during sowing stage of wheat plants (10 Kg P ha^{-1}). This application may cause a contribution of TEs to soil. The contribution of TEs to soils has been evaluated in relation to the use of phosphate fertilizers in several countries, where European countries give an average for: As, Cd, Cr, Ni, and Pb **Table III-2 3**.

Following analyzes carried out at LARI for the measurement of TEs in imported phosphate fertilizers, the elements Cd, Cr, and Pb showed average values of 20.02, 27.40, and 42 mg kg^{-1} respectively.

According to these values, an estimate of the contribution of these TEs could be done in the studied soils, following an application of 10 kg of phosphate fertilizers ha^{-1} per cultivation year.

Table III-2 3: The limits of TEs content (mg kg^{-1}) in phosphate fertilizers and their contribution to soils

Limits of TEs content (mg kg^{-1}) in phosphate fertilizers						
Country	As	Cd	Cr	Ni	Pb	Hg
Germany	40	1.5	2**	80	150	1
Finland	25	1.5	600	100	100	1
Turkey	-	-	270	120	-	-
Lebanon	-	50	400	-	150	-
US EPA, 1999	11.3	65	173.2	27.5	12.2	-

Contribution of TEs to soils ($\text{g}^{-1}\text{ha}^{-1}\text{yr}^{-1}$) phosphate fertilizers application						
Country	As	Cd	Cr	Ni	Pb	Hg
European countries (average)	2.3	1.6	20.7	3.6	1	-
France	1.3	2.5	28.6	5.7	2.3	-

*: US EAP, 1999; **: CrVI

Accordingly, a contribution of 0.2, 0.274, and 0.42 $\text{g ha}^{-1} \text{yr}^{-1}$ of Cd, Cr, and Pb respectively. This contribution shows us results that are still below the standards given by the European countries and France, for respectively, 1.6 et 2.5 $\text{g}^{-1} \text{ha}^{-1} \text{yr}^{-1}$ of Cd, 20.7 and 28.6 $\text{g}^{-1} \text{ha}^{-1} \text{yr}^{-1}$ of Cr, and 1 and 2.3 $\text{g}^{-1} \text{ha}^{-1} \text{yr}^{-1}$ of Pb.

III-2.2.2 Trace Elements contributions related to irrigation

As showed above, the irrigation used in the studied sites comes from deep tube wells with/without surface water **Table III-2.2 Annex**.

III-2.2.2.1 Deep well water

The determination of the following elements: Cd, Co, and Pb in irrigation water samples was performed by using the Atomic Absorption Spectrometer (AAS, Stafford House, UK) Zeeman Graphite Furnace GF95Z Thermo Electron corporation M series. The element mercury was analyzed by direct mercury analyzer DMA 80 Milestone (Soriso, Italy) using 0.1 g of irrigation water sample. Each sample was taken in triplicate, where each was analysed in triplicate too for all elements. To guarantee the method reliability, a certified reference material (CRM; BCR-610) was analyzed at the same time as samples. The recovery percentages obtained for the reference material were respectively 91.3% and 102.7% for Cd and Pb.

The levels of TEs in the ultrafiltered irrigation water samples of the studied sites B1, B2, B3, B4a, B4b, B5, B8, and B9 are shown in **Table III-2.4**.

Table III-2 4: TEs contents ($\mu\text{g L}^{-1}$) in ultrafiltered irrigation water samples in the Bekaa studied sites

Studied sites	Irrigation water samples			
	Tes (Mean concentration values \pm SD $\mu\text{g L}^{-1}$)			
	Cd	Co	Hg	Pb
B1	0.06 \pm 0.001	0.74 \pm 0.29	20.60 \pm 0.15	1.31 \pm 0.23
B2	0.09 \pm 0.06	0.84 \pm 0.17	4.80 \pm 0.10	1.70 \pm 0.30
B3	0.06 \pm 0.01	0.84 \pm 0.17	2.73 \pm 0.06	0.19 \pm 0.08
B4a	0.03 \pm 0.03	1.41 \pm 0.16	5.20 \pm 0.06	1.28 \pm 0.07
B4b	0.07 \pm 0.02	1.12 \pm 0.36	4.73 \pm 0.13	2.19 \pm 0.09
B5	0.06 \pm 0	0.84 \pm 0.17	21.6 \pm 0.1	1.12 \pm 0.15
B8	0.06 \pm 0	0.74 \pm 0.29	59.30 \pm 0.27	1.84 \pm 0.13
B9	0.07 \pm 0.03	0.65 \pm 0.33	7.53 \pm 0.15	1.64 \pm 0.27
SEQ	10	50	-	200

SEQ eauFrancais: for irrigation water according to Canadian recommendations

These samples showed values ranging between 0.03 and 0.09 $\mu\text{g L}^{-1}$ for Cd, between 0.65 and 1.41 $\mu\text{g L}^{-1}$ for Co, between 2.73 and 59.3 $\mu\text{g L}^{-1}$ for Hg, and between 0.19 and 2.19 $\mu\text{g L}^{-1}$ for Pb. These TEs contents in the irrigation water samples were lower than the standards as shown in **Table III-2 4**.

The following PCA showed the distribution of the studied sites according to the two first axes (explaining 71.32% of the variance) of a PCA using selected TEs concentrations of the irrigation water within the studied sites *Figure III-2. 10*. This showed that the elements Cd and Co are well represented on axis 1, and Hg is well represented on axis 2.

This indicates that the site **B8** is characterized by high values of Hg and Pb. The **B9** and **B2** are characterized by high values of Cd. While, **B4a** is characterized by high values of Co.

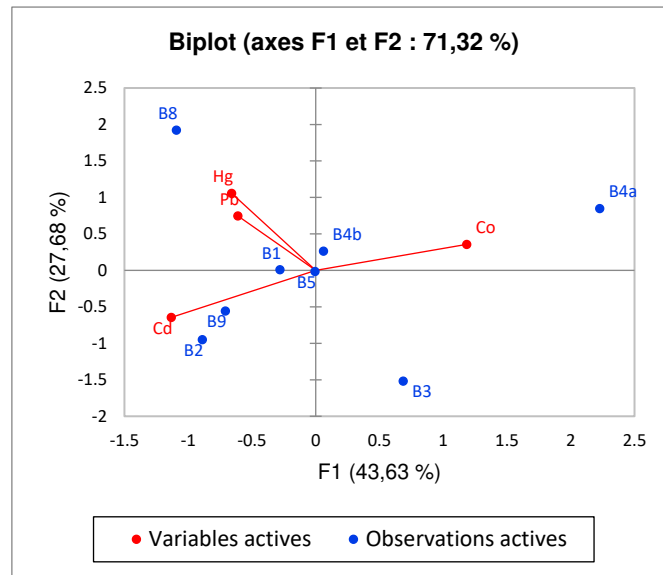


Figure III-2. 10: Variables correlation plots with principal components 1 and 2. The first two axes accounted for 71.32% of variance.

The PCA below showed the distribution of the studied sites according to the two axes 1 and 3 (explaining 69.47% of the variance) of a PCA using selected TEs concentrations of the irrigation water within the studied sites *Figure III-2. 11*. This showed that the element Pb is well represented on axis 3. This indicates that the sites **B2** and **B4b** are characterized by high values of Pb.

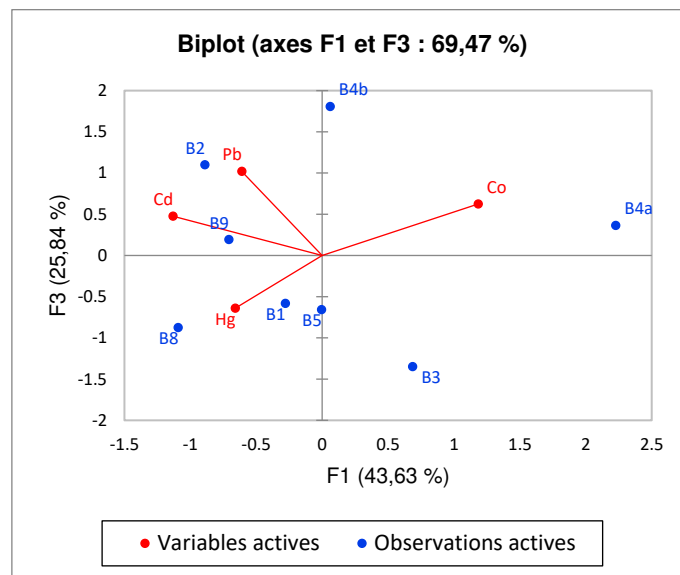


Figure III-2. 11: Sites repartition chart. Factor scores of the observations plotted on the first and third components. The first and the third axes accounted for 69.47% of variance

These PCA showed that there are no significant correlations between the 4 elements, which explains the fact that they do not come from the same source.

At the studied sites level, the irrigation water used in the site **B2** showed high values of Cd and Pb *Figure III-2. 10, Figure III-2. 11*. The element Cd showed values above the ones measured at 2 m depth (open water reservoir) and at 8 m depth for B2 and always above the levels at 8 m. This demonstrates that the Cd levels in deep well waters become closer to Cd values measured at surface water, which allows us to assume that after many years, the deep wells water become more contaminated with the element Cd. In addition, the concentrations of Pb in the same water are higher than waters at 2 m, 8 m and deep wells waters.

The site **B4a**, showed high values of Co in the irrigation water of $1.41 \mu\text{g L}^{-1}$, of the deep tube well, still lower than the values found by the study conducted by (Chen et al., 2021) in an agricultural area cultivated by wheat plants and irrigated by groundwater, sewage and industrial waste water. Where all the water samples showed high cobalt concentration than the maximum permissible value. However, all the soil and wheat plant samples were found within the maximum allowable range. The high cobalt concentration in irrigating water showed that the continuous use of this water may cause Co toxicity in living organisms.

In addition, the irrigation water of **B4b** showed the highest level of Pb and which is always higher than the water samples measured at 2 m (free water reservoir) and 8 m (Arab well) by (Darwish et al., 2008). The contamination of this water by Pb is comparable to the results of (Nehme et al., 2012).

B8, situated in Tal Al Amara, Zahle, and surrounded by other agricultural plots except from the north, where the "Ferzol street" passes all by side. The irrigation water used in this site, is characterized by high concentrations of Hg and Pb.

The Rayak Hospital presents at 3 km from B8 may lead to TEs contamination of water in the region such as Cd, Cr, Cu, Hg, Pb, and Zn. In addition, a study conducted by (Laffite, 2016) showed that the water of rivers presents next to hospitals showed high values of TEs such as Cd, Cr, Cu, Hg, Pb, and Zn, indicating the effect of hospital effluent waters on the contamination of rivers by toxic TEs.

The presence of Rayak Airport in this region may cause soil and water contamination by TEs as well as plants.

B9: The element Cd in the private well water has shown values above the ones measured at 2 m depth (open water reservoir) and at 8m depth for B9 (Darwish, 2008). This shows us that the Cd levels in deep wells waters become closer to Cd values measured at surface water,

which allows us to assume that after many years, the deep wells waters become more contaminated with the element Cd. This site collects all the sewers from the villages that precede it, carrying with them contaminants such as TEs.

Table III-2. 5: The quantity of TEs carried by deep tube well irrigation water (338 mm yr^{-1}) to soil per year and per 100 years

Studied sites	TEs IW ($\mu\text{g L}^{-1}$)				TEs soil carried by IW yr^{-1} ($\mu\text{g Kg}^{-1}$)				TEs soil per 100 yrs (mg Kg^{-1})			
	CdIW	CoIW	HgIW	PbIW	Cdsoil	Cosoil	Hgsoil	Pbsoil	Cd	Co	Hg	Pb
B1	0.06	0.74	20.6	1.31	0.10	1.28	35.71	2.27	0.010	0.13	3.57	0.23
B2	0.09	0.84	4.8	1.7	0.16	1.46	8.32	2.95	0.016	0.15	0.83	0.29
B3	0.06	0.84	2.73	0.19	0.10	1.46	4.73	0.33	0.010	0.15	0.47	0.03
B4a	0.03	1.41	5.2	1.28	0.05	2.44	9.01	2.22	0.005	0.24	0.90	0.22
B4b	0.07	1.12	4.73	2.19	0.12	1.94	8.20	3.80	0.012	0.19	0.82	0.38
B5	0.06	0.84	21.6	1.12	0.10	1.46	37.44	1.94	0.010	0.15	3.74	0.19
B8	0.06	0.74	59.3	1.84	0.10	1.28	102.79	3.19	0.010	0.13	10.28	0.32
B9	0.07	0.65	7.53	1.64	0.12	1.13	13.05	2.84	0.012	0.11	1.31	0.28

TEs: Trace elements, IW: Irrigation Water (Deep Tube Well)

The total quantity of irrigation water applied to the studied sites was 338 mm yr^{-1} , as shown in **Table III-2. 55**, this application may contribute the TEs: Cd, Co, Hg, and Pb into soil. The element Cd carried by the deep tube well irrigation water showed values in soil between 0.05 and $0.16 \mu\text{g kg}^{-1}\text{yr}^{-1}$ respectively in B4a and B2, between 1.13 and $2.44 \mu\text{g kg}^{-1}\text{yr}^{-1}$ for Co respectively in B9 and B4a, between 4.73 and $102.79 \mu\text{g kg}^{-1}\text{yr}^{-1}$ for Hg respectively in B3 and B8, and between 0.33 and $3.80 \mu\text{g kg}^{-1}\text{yr}^{-1}$ for Pb respectively in B3 and B4b.

III-2.2.2.2 Pathway of contaminants carried by surface water used for irrigation

Three rivers drains our study area: the Litani river and two of its tributaries: the Berdawni whose confluence with the Litani river is downstream of B4 site and the El-Hafier, of which a supply canal irrigates B2 site and which flows into the Litani at Chaoubark Ammick. The El-Ghazayel River does not irrigate any of our study sites [Figure III-2. 12](#).

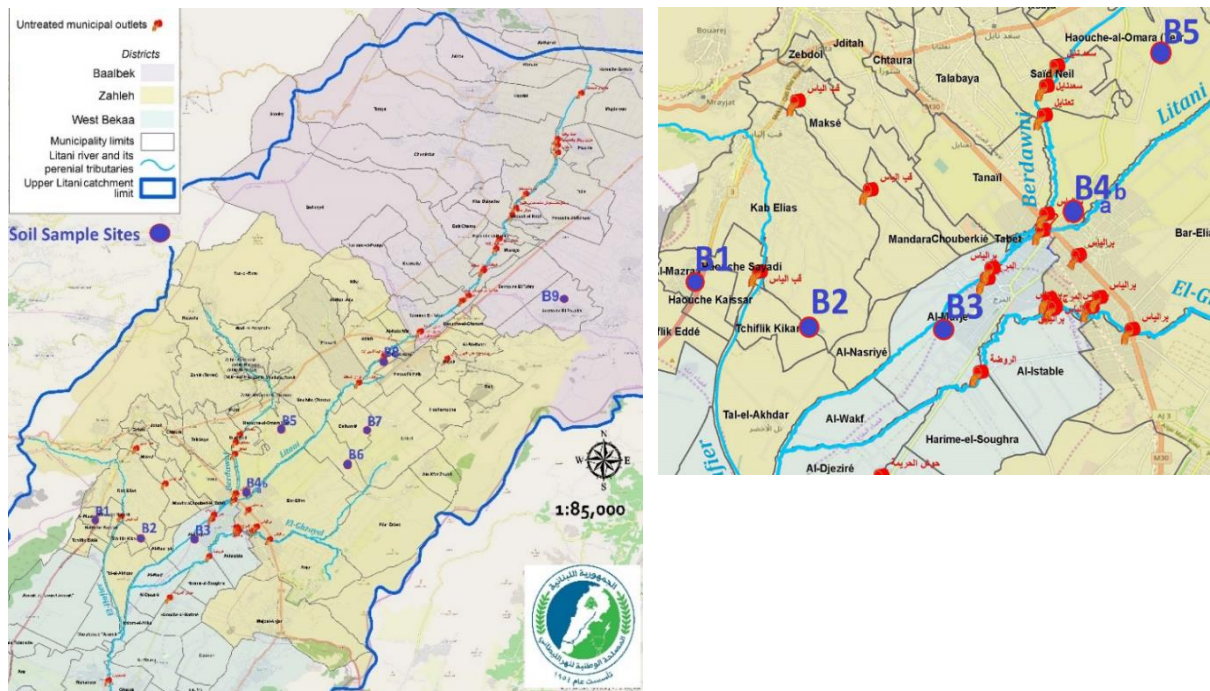


Figure III-2. 12: The Litani river and its perennial tributaries in the upper Litani river catchment with location of the untreated municipality outlets

This figure also shows the location of the 69 municipal untreated wastewater discharges in the hydrographic network, which represents an annual flow of 47 M m³ for an annual flow of the Litani at the entrance to the reservoir this Qaraaoun of 410 M m³. This represents 10% of the annual flow of the Litani and a relative proportion much higher in the dry season.

The breakdown of these untreated discharges in relation to our study sites is as follows:

- 44 municipalities upstream of B8 in the Litani
- 1 municipality between B8 and B4 in the Litani
- The Berdawni drains the wastewater from Zahle (a supply canal irrigates B5) and turns right, then runs alongside the industrial zones of Saadnayel and Taanayel from where it receives 4 municipal discharges including that of a recycling plant of batteries before flowing into the Litani downstream from B4 and before B3. The latter also receives wastewater from Bar Elias + 2 other municipalities.
- El- Hafier drains Kab Elias + 1 municipal Outlet and supplies B2 through an irrigation canal.

We do not know the area of influence of these outlets, but it may be low given the very low slope gradient of the valley (2.5 m km⁻¹) and thus promote the sedimentation of pollutants (Shaban. & Hamzé, 2018) rather than their transfer. The respective behavior of B4 and B3

sites which are very close but supplied by different waters and pollutants circuits which should allow us to shed light on the question of contributions of TEs by untreated wastewater.

In the Bekaa plain, few data on the wastewater quality used for irrigation is known. A study conducted by (Nehme et al., 2014), for the analysis of TEs such as Cd, Cr, and Pb, in the Litani river water and the bed sediments of the Lower Litani River Basin. The water samples showed values between 0.01 and 0.05 mg L⁻¹ for Cd, between 0.04 and 0.13 mg L⁻¹ for Cr, and between 0.07 and 0.12 mg L⁻¹ for Pb.

III-2.2.2.3 Other contributions

The atmospheric deposition can also be a source of contribution of TEs in soil and plants. As has been shown in a study carried out by Darwish et al. (2004). Where sixty soil samples taken in the central Bekaa area in 1997 before the introduction of unleaded gasoline in 2000 in Lebanon.

The authors showed a pattern of Pb concentrations from anthropogenic sources, found along high traffic roads because of atmospheric deposition with a surface accumulation gradient of Pb from the foot of the slope, located downstream from the slope of the town of Zahlé, towards the Litani river (14 – 34 mg kg⁻¹). This lead is transported by runoff from the agglomeration of Zahlé.

Among our studied sites, there are some which are near and even close to factories, and to the industrial zone of Zahlé, Saadnayel, and Taanayel. These can be sources of TEs contamination for soil, water and plants. As is the case of site B1, which is surrounded by two factories PETCO and SICOMO. In addition, the site B5 surrounded by the industrial zones of Zahle and Saadnayel.

The effluents of the industrial zones of Zahle, Taanayel and Saadnayel flowing into Berdawni cause the contamination of B3.

The battery factory “Arayby for industrial and trading company”, that collects batteries and melts lead, may cause a contamination by Ni and Pb. An atmospheric pollution in the B4a and B4b by Pb is well noted [Figure III-2. 13](#).



Figure III-2. 13: The ARAYBY factory, installed on 2 ha in the industrial zone of Taanayel produces lead ingots and polypropylene chips since 1990 by recycling lead acid battery waste and is located 3 km from B4 and 5 Km from B3

III -2.3 Quantification and origin of trace elements available in the soil

The total and available (DTPAextraction) TEs were measured in soil samples of the studied sites **Table III-2.6**. All the results are presented on the PCA [Figure III-2. 14](#), [Figure III-2. 15](#).

Table III-2.6: The total and the available TEs (DTPA extraction) concentrations in the studied soil samples (mg kg^{-1})

Sites	Concentration of trace elements in soil (mg kg^{-1})															
	Total TEs								Available TEs - DTPA extraction							
	As	Cd	Co	Cr	Cu	Ni	Pb	Zn	As	Cd	Co	Cr	Cu	Ni	Pb	Zn
B1	6.84	0.28	18.3	94.1	22	56.1	13.8	90.9	$13.4 \cdot 10^{-3}$	$27.4 \cdot 10^{-3}$	0.16	$3.6 \cdot 10^{-3}$	0.98	0.81	0.75	1.36
B2	4.52	0.29	13.3	83.8	19.8	47.3	16.8	81.1	$10 \cdot 10^{-3}$	$29.2 \cdot 10^{-3}$	0.11	$4.3 \cdot 10^{-3}$	1.62	0.89	1.4	9.56
B3	4.35	0.26	11.4	75.1	17.2	43.6	24.5	71.6	$23.9 \cdot 10^{-3}$	$61.5 \cdot 10^{-3}$	0.24	$3.6 \cdot 10^{-3}$	1.1	0.8	1.91	0.82
B4a	7.3	0.24	11.4	55.8	16.6	39.4	9.1	56.1	$7.3 \cdot 10^{-3}$	$30.9 \cdot 10^{-3}$	0.16	$3.6 \cdot 10^{-3}$	1.32	1.05	0.86	3.23
B4b	7.51	0.23	11.7	57.2	14.5	39.7	8.8	55.5	$10.5 \cdot 10^{-3}$	$34.1 \cdot 10^{-3}$	0.38	$3.4 \cdot 10^{-3}$	0.77	1.25	1.16	4.37
B5	11.3	0.43	25.2	129	27.9	78.9	17.5	115	$16.3 \cdot 10^{-3}$	$40.2 \cdot 10^{-3}$	0.16	$4.1 \cdot 10^{-3}$	1.73	0.98	1.08	9.39
B8	9.65	0.48	23	114	25.9	64.2	19.5	134	$22.6 \cdot 10^{-3}$	$46 \cdot 10^{-3}$	0.05	$4.3 \cdot 10^{-3}$	1.04	0.72	0.3	0.54
B9	7.04	0.42	15.3	81.3	20.1	49.1	18.6	82.2	$25.1 \cdot 10^{-3}$	$81.4 \cdot 10^{-3}$	0.69	$3.7 \cdot 10^{-3}$	1.66	1.14	0.24	1.9

The total TEs measured in the studied soils present values between 4.35 and 11.3 mg kg^{-1} of As respectively in B3 and B5. Between 0.23 and 0.48 mg kg^{-1} of Cd respectively in B4b and B8. The element Co showed values between 11.4 and 25.2 mg kg^{-1} respectively in (B3 and B4a) and B5. The values of Cr varied between 55.8 and 129 mg kg^{-1} in respectively B4a and B5. The Ni showed values between 39.4 and 78.9 mg kg^{-1} respectively in B4a and B5. The element Pb presented values varying between 8.8 and 24.5 mg kg^{-1} in respectively B4b and B3. And the values of Zn varied between 55.5 and 134 mg kg^{-1} in B4b and B8 respectively.

The available TEs by DTPA extraction **Table III-2.6** showed values between $7.3 \cdot 10^{-3}$ and $25.1 \cdot 10^{-3}$ mg kg^{-1} for As respectively in B4a and B9, between $27.4 \cdot 10^{-3}$ and $81.4 \cdot 10^{-3}$ mg kg^{-1} of Cd respectively in B1 and B9, between $3.4 \cdot 10^{-3}$ and $4.3 \cdot 10^{-3}$ mg kg^{-1} of Cr respectively in B4b and (B2, B8). And values between 0.05 and 0.69 mg kg^{-1} of Co respectively in B8 and B9, between 0.77 and 1.73 mg kg^{-1} of Cu respectively in B4b and B5, values ranging from 0.72 and 1.25 mg kg^{-1} of Ni respectively in B8 and B4b, between 0.24 and 1.91 mg kg^{-1} of Pb respectively in B9 and B3, and between 0.54 and 9.56 mg kg^{-1} of Zn respectively in B8 and B2.

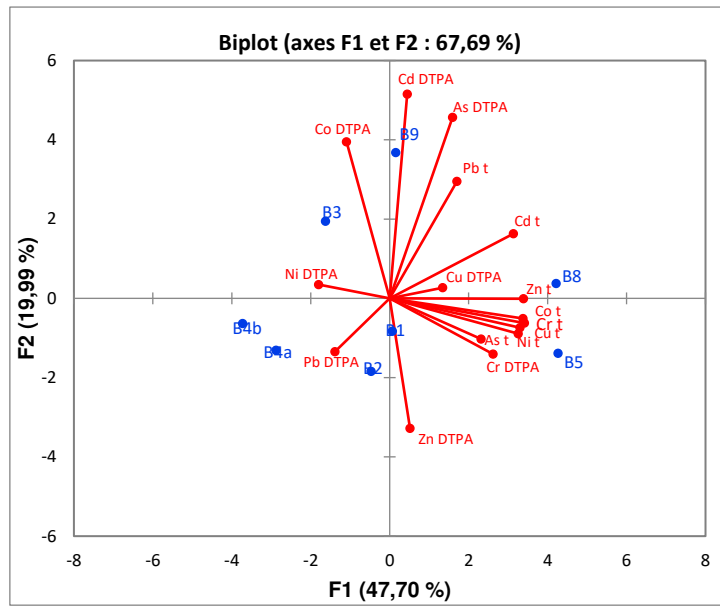


Figure III-2. 14: Variables correlation plots with principal components 1 and 2. The first two axes accounted for 67.69% of variance

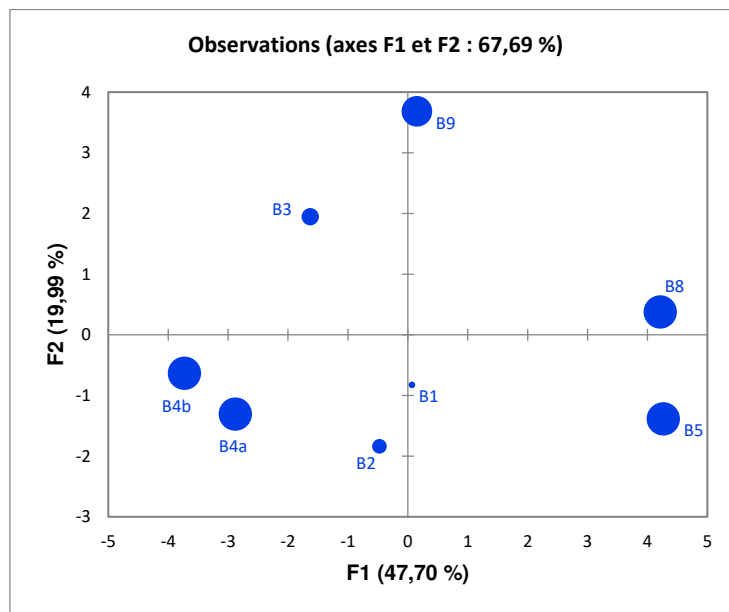


Figure III-2. 15: Sites repartition chart. Factor scores of the observations plotted on the first and second components. The first and the third axes accounted for 67.69% of variance

The distribution of the studied sites according to the two first axes (explaining 67.69% of the variance) of a PCA using total TEs and available TEs (DTPA extraction) concentrations in the soil. The first axe represents the total Tes content and the second axe represents the available Tes (DTPA extraction) content. The site B5 and the site B8 showed identical values of total soil Tes and higher than the other sites, while B8 showed higher values of available Tes (DTPA extraction) than B5.

The sites B4a and B4b have the lower total TEs levels, and B2, B4a and B5 showed the lower available TEs (DTPA extraction) concentrations. The sites B3 and B9 having the higher values of available TEs (DTPA).

This PCA shows that the total TEs are correlated to each other. While the available TEs (DTPA) are not correlated with each other nor with the total TEs except Cd DTPA / As DTPA, Cd DTPA / Co DTPA, Ni DTPA/ Pb DTPA, Zn DTPA/ Cr DTPA.

From the individual chart we can observe 4 groups of sites or clouds: first group gathering B3 and B9, second group gathering B4a and B4b, third group gathering B1 and B2, and and fourth group gathering B5 and B8.

In this graph, the element Cd is positively correlated to Cr, Cu, Ni, Pb, Co, Pb, and Zn. These correlations showed that these TEs have probably the same geogenic origin.

The sites B1 and B2 didn't show any significant values in total TEs and in available TEs (DTPA extraction).

The site B3 has a high concentration of Cd and the greatest concentration of total Pb. The source of these TEs in B3 may be due to the battery factory located at 5 km from the site B3, and to the use of the wastewater coming from Bar Elias.

The sites B4a and B4b are characterized by high values of available Pb (DTPA extraction). The presence of the battery factory "Aryby" located at 3 km from B4ab can be the main cause of the contribution of Pb. Where it's showed a significant contamination of Pb in soil.

The sites B5 and B8 are rich in total TEs. The TEs in B5 may be due to irrigation water coming from Zahle sewage. The effluents of the industries, Zahle and Saad Nayel industrial zone, may cause the TEs contamination of the site B5. But all these contaminations didn't show important values of TEs available (lowest in this PCA). While B8 is rich in the total TEs and have available TEs higher than B5 as shown above.

The presence of Rayak Airport, at 1.78 km east of the site B8, may be source of TEs contamination for this site. A study conducted in an agricultural soil (0-30cm), situated at 2 km around the International Hatay Airport in Hatay, Turkey. The studied soils showed concentrations between 0-0.220, 0-0.270, 0-0.780, 0.40-5.35, 0-3.95, and 0-1.45 mg kg⁻¹ respectively for Cd, Co, Cr, Cu, Ni, and Pb. Where the authors found that their results did not pose any TEs pollution problems (Özkan et al., 2017). The element Pb in the soil of the site B8

showed highest values (19.5 mg kg^{-1}) comparing to these results. This may explain to us that the presence of the airport does not present a risk for the contamination of neighboring soils, and therefore the presence of high levels of Pb in the soil of B8, may be due to other sources such as the used contaminated irrigation water from the Bekaa outlets.

In addition, the chicken slaughterhouses located near the site B8 may cause environmental problem and be a source of TEs contamination. Where the effluents discharged from the animal processing operations. For hygienic reasons, the chicken slaughterhouses use large amounts of water in animal processing operations. The effluents may contain high amounts of TEs which they are mainly coming from animal feed additives, food processing industry and chemical treatment (Marianna, M., 2012; ISBN 978-953-51-0204-5). However, long-term effluent discharge increases TEs levels in soil such as Cd, Co, Ni, Cu and Cr, which can lead to the pollution of surface and groundwater resources (Matheyarasu et al., 2014).

The B9 site is characterized by As DTPA, Cd DTPA, and Co DTPA. In addition, The DTPA extractable Cd was highest at site B9. Therefore, this allows us to expect high values of As, Cd, and Co in soil which may be due to the runoff from the higher villages [Figure III-2.14](#).

III -2.4 Conclusion

This interdisciplinary work aimed to follow the trace elements in the Lebanese white bread industry: From the soil to the consumed bread, from farm to fork, combining the agronomic and agrifood skills. But the field reality allowed us to study 2 segments of the chain: On the one hand, the transfer of trace elements from the soil to the wheat grain, taking into account the fertilization and the irrigation water. On the other hand, the exposure of the Lebanese population linked to the Lebanese bread consumption: wheat used for making bread from imports, as well as phosphate fertilizers.

Chapters III-1 and III-2 showed the soils studied in the Central Bekaa region, the geogenic and anthropogenic origin of the trace elements in these soils, as well as the new method used and validated for the analyses of the trace elements in these soils.

These chapters have shown us that between the source of the Litani and the site B8, there are 44 discharges, this site is rich in total and available trace elements and high Pb contents which may be due to the contaminated irrigation water by discharges of wastewater from the

district of Baalbeck. It has been observed that the site B3 can receive its contamination from the effluents of the industrial zones of Zahlé, Taanayel and Saadnayel flowing into Berdawni and from the wastewater coming from Bar Elias.

The presence of the battery recycling plant located 2 km from B4ab, which collects the batteries and melts the lead, can cause contamination by Ni and Pb. It may also be the main cause of Pb input, where it has shown significant contamination of Pb in the soil. In addition, atmospheric pollution in B4a and B4b by Pb is well noted.

Site B5, which is located downstream of the Zahlé industrial zone and is fed by irrigation canals, is rich in total trace elements (probably of geogenic origin: alluvial cone) and poor in available trace elements. This may be due to irrigation water coming from Zahlé wastewater.

The site B9 which has no Surface Water, showed high values of As, Cd and Co available in the soil, this could be due to runoff from villages located upstream.

The sites B8 and B4 as shown above, present various contaminations: B8 taking water from the Litani at the outlet of the Baalbeck district and which is very polluted, while B4 taking this water at the outlet of the Zahlé district and which is the least contaminated site. This difference in contamination is explained by the fact that the slope of the Litani in the Central Bekaa is very low, the water flow is slow which favors the sedimentation of trace elements. In addition, abundant ephemeral springs on the western slope of the central Bekaa, fed by the snowmelt, supply the Litani with unpolluted water and further reduce the concentrations of trace elements by dilution. Finally, the observation of a single untreated sewer discharge on this section crossing the mainly agricultural Zahlé district could explain the original situation.

In conclusion, we can summarize that the contamination of these sites is anthropogenic from industrial activities and runoff. In addition, we emphasize the need to consider the entire hydrological functioning of the system formed by the Bekaa Valley and the two mountain ranges that surround it to determine the origin of TEs contamination to manage it.

CHAPTER III-3

Transfer of trace elements to wheat plant

**Analysis according to soil
types, silicon status and
origin of trace elements**

III-3.1 Introduction

The set of soils used for the soil-plant transfer study is slightly different and smaller than that used for the soil-alone study and presented in the previous chapter. Arsenic, Cd and Pb were considered. They were selected because they belong to the most problematic elements for human health worldwide. Drinking water and foodstuffs are indeed the two main sources of inorganic As (ESFA, 2009; Schoof et al., 1999; Cubadda et al., 2010), and wheat has been identified as an emerging exposure route for arsenic in India (Suman et al., 2020). Cadmium is readily transferred to plants, especially wheat grain, while Pb was one of the four trace elements, together with Cd, mercury and tin that was given a special attention at the EU level with regulatory thresholds in foodstuffs: maximum levels for Pb and Cd in wheat grain were set up to 0.2 mg kg⁻¹ wet weight by the European Commission (COMMISSION REGULATION (EC) No 1881, 2006). As presented in Chapter III-1, wheat is the main cereal crop produced in Lebanon (GIEWS, 2020).

In the chapter, we intend to find out whether wheat might be a significant source of As, Cd and Pb, to decipher the link between the concentrations measured in wheat and those observed in soils. Because the set of sites is not similar to that presented and discussed in Chapter III-2, we will briefly present the 8 soils, their trace element concentrations and the relationship with the pedological, physico-chemical properties and environmental characteristics. We will then present and discuss the bioavailable concentrations as defined by DTPA extractions and, we will present and discuss As, Cd and Pb concentrations in wheat and their relationship with soil characteristics, including bioavailable Si.

III-3.2 Trace element concentrations in soils

All soils of the 8 sites **Table III-2.1** (cf Ch III-2) were in the lower range of concentrations found in soils worldwide for all trace elements, including As, Pb and Cd (Kabata-Pendias and Mukherjee, 2007). More in details, as for the main soil set (cf Ch III-2), As was only correlated to Co and Ni **Table III-3.1 Annex**, which may be related to a more scattered occurrence due to anthropogenic inputs (Darwish et al. 2021) or a common geogenic origin given the low measured As concentrations falling within the lower range of As concentrations in surface soils worldwide (Adriano, 2001). The B5 soil presented the highest As concentrations (and

other metals) probably due to its proximity with the town of Zahle and irrigation from the Berdawni River.

On the contrary Pb was not correlated to any of the other elements (cf Ch III-2 [Figure III-2.14](#)) highlighting a probable scattered contamination from traffic. Even in this eventuality, Pb concentrations were lower than the mean worldwide Pb value (25 mg kg^{-1}) Kabata-Pendias and Mukherjee (2007) and, within the ranges of values reported by Adriano (2001). The Pb concentrations of the studied sites are also in general agreement with the values of the Pb map provided by Darwish et al. (2021) and those obtained in other Lebanese soils ($11.3 - 22.9 \text{ mg kg}^{-1}$) (Darwish et al., 2008), including soils of Central Bekaa (Taalabaya and Saadnayel soils). The B3 site presented the highest Pb concentration, which can be explained by its location downstream of the Litani River, the Zahle and Bar Elias towns and, most likely, by a nearby recycling batteries smelter.

Cadmium concentrations were within the range of soil Cd average concentrations found worldwide (Adriano, 2001; Kabata-Pendias and Mukherjee, 2007). Our values were lower than those found by Kanbar et al. (2014) in some Lebanese soils, South of the Bekaa plain and, within the range of those found by Darwish. et al. (2008) in Central Bekaa ($0.28 - 0.48 \text{ mg kg}^{-1}$). However, unlike As and Pb, Cd concentrations were correlated to all other total trace element concentrations (except for As and Pb), strongly calling for a geogenic origin as also concluded by Nsouli et al. (2004) for North Lebanon soils.

We can conclude on both a natural TEs source with variation explained by the alluvial-colluvial origin of the parent material, and soil type of various ages and, a limited anthropogenic pollution due to inputs from irrigation and fertilizers and, atmospheric deposition from traffic emissions or smelter, especially for the B3 and B5 sites.

In a similar Mediterranean environment, (Austruy et al., 2016) calculated an average natural pedogeochemical background similar for Pb and Cd or slightly higher for As than the concentrations obtained for the 8 sites. They also found that background concentrations were higher for alluvial parent materials than for calcareous ones as observed at the studied sites. This is an additional support to a limited soil contamination. Unfortunately, it is not possible here to calculate similar average natural pedogeochemical background or enrichment factors because we had no data on deep soil horizons. However, again, calculating ratios between As, Pb and Cd and Fe or Al, we found that all sites fell within the same range except Pb for B3.

Another complementary approach is to relate total concentrations to the pedological and physico-chemical properties of the soils. In our case, As was positively and significantly correlated to the sand content, while Cd was positively correlated to P Olsen content and negatively to silt, calcium carbonate and organic matter contents. Phosphate fertilizers marketed in Lebanon are mainly imported from China, Turkey and Jordan. Their Cd and Pb mean concentrations have been found to reach $20.02 \pm 19.2 \text{ mg kg}^{-1}$ and $42 \pm 24.3 \text{ mg kg}^{-1}$ respectively (LARI, 2020), and these concentrations are in the upper range for phosphate fertilizers.

The significant correlation between Cd total concentrations and P Olsen content could be explained by agricultural practices and thus indicate further that, although Cd concentrations were low, they may already be higher than natural ones. The significant correlation between total Pb and total Si concentrations needs further investigation. The distribution of the studied sites according to the two first axes (explaining 70% of the variance) of an APC using selected environmental characteristics and soil physico-chemical properties together with all total trace element concentrations *Figure III-3. 1*, *Figure III-3. 2* is typical and similar to that observed with other sets of data, such as the use of the physico-chemical properties, rainfall and altitude only. This indicates that soil properties are the major parameters explaining the differences between sites and not TE concentrations: B5, B8 and B9 are scattered in the graph, B8 contributing most to the first axe. B4a and b are grouped.

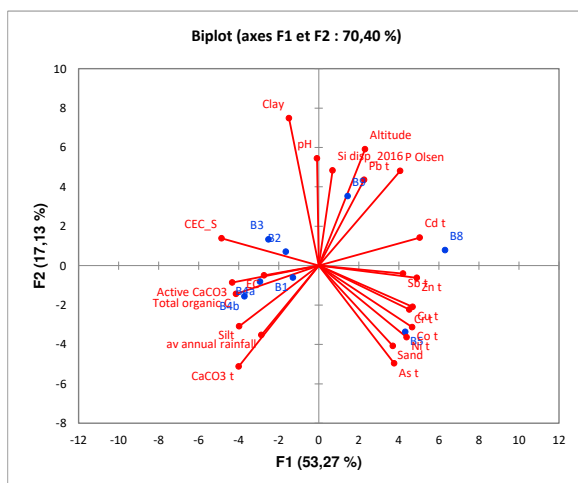


Figure III-3. 1: PCA performed for the 8 sites with soil physico-chemical properties, environmental characteristics and total trace elements (As, Pb and Cd) concentrations. For B9, the CEC (S), EC and pH missing values were replaced by the average calculated on the other 7 sites.

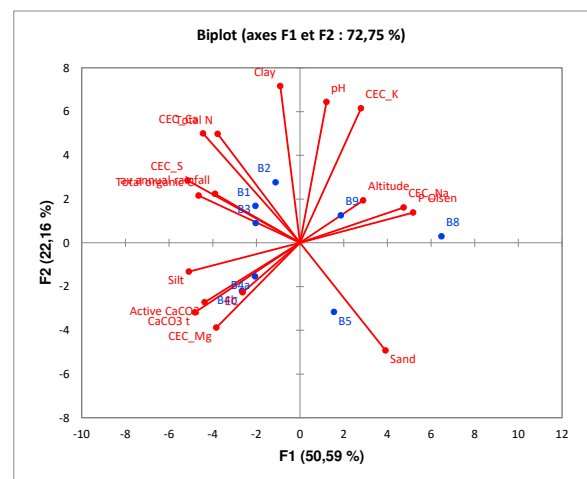


Figure III-3. 2: Same as (Figure III-3.1) but without total trace elements concentrations. For B9, the CEC (S), EC and pH missing values were replaced by the average calculated on the other 7 sites.

III-3.3 Trace elements and silicon availability in the 8 studied soils

Trace element phytoavailability was assessed by DTPA-extraction and bioavailable Si by CaCl_2 . It has been discussed extensively in the literature that total concentrations do not explain concentrations in plants. Therefore, several methods have been developed to predict phytoavailability. These methods -including DTPA extraction- have largely been applied for agricultural purposes (mostly to study the availability of micronutrients) and therefore for agricultural plants and soils. In this study we used DTPA because it is also most suitable for calcareous soils, as it is buffered at a pH 7.3 and therefore prevents CaCO_3 from dissolution and release of occluded metals (Lindsay and Norvell, 1978). The amount of DTPA-extracted TEs aims at assessing the size of a pool that might be depleted by a plant during a growth period, but the extracting power depends on the soil tested and the “equivalence” to plant uptake has been questioned (Miner *et al.*, 1997). However, DTPA extraction has been shown to correlate well with plant uptake, and recently was used to calculate DTPA-extractable Cd thresholds for paddy fields (Wu *et al.*, 2021).

Soluble silicon may mitigate TEs uptake thus interfere with TEs phytoavailability. Indeed, Si has been shown to be beneficial for crops exposed to biotic or abiotic stresses (Liang *et al.*, 2007), especially accumulating Si plants such as monocots, including wheat (Guntzer *et al.*, 2012). Soil Si bioavailability is therefore an emerging issue in agriculture (Yan *et al.*, 2018). Si is taken up from the soil solution by roots as $\text{Si}(\text{OH})_4$ (silicic acid). Si extracted with CaCl_2 ($\text{Si}_{\text{CaCl}_2}$) is used to assess this bioavailable pool (able to pass into solution – PAS) since the concentrations extracted are proportional to plant Si concentration or grain yield (Tubaña and Heckman, 2015). Caubet *et al.*, (2020) have investigated the impact of agriculture on this PAS pool and questioned the potential depletion in phytoavailable Si – and associated effects. Thus, we investigated the level of CaCl_2 -extractable Si in soil to further compare it to TE concentrations in wheat in the last part of this chapter. We assessed the level of CaCl_2 -extractable Si using the two thresholds proposed by (Caubet *et al.*, 2020): 20 mg kg^{-1} defined by Haysom and Chapman (1975) for sugarcane in Australia, and 40 mg kg^{-1} , an average value provided for rice based on data from silt loam in Louisiana soils (37 and 43 mg kg^{-1}) (Babu *et al.*, 2016).

DTPA-extractable As, Cd and Pb concentrations were low **Table III-3.3**. With ranges of respectively 0.24-1.9 mg kg^{-1} and 0.027-0.08 mg kg^{-1} , Pb and Cd concentrations agreed with

values measured by Ibrahim et al. (2017) in irrigated agricultural soils (resp. 0.3 to 2.1 mg Pb kg⁻¹ and 0.01 to 0.1 mg Cd kg⁻¹). They were also similar to concentrations found by Ragab et al. (2007) in Nile Delta fringe soils (alluvial calcareous and calcareous soils), in Egypt. The highest DTPA-extractable Cd concentration was found in the B9 soil, while B3 presented the second largest Cd concentration together with the largest Pb concentration. The reason is probably again the presence upstream of a recycling battery unit. On the contrary, the percentage of extractable Cd was surprisingly high, reaching 24% in B3 soil and not less than 9% in all other soils, while extractable Pb never reached more than 13%, including in B3 soil (nearly 8%). The proportion of extractable As was very low and always below 1%. However, DTPA might not be the best extracting agent for phytoavailable As because it might not desorb arsenate from soil, which is the phytoavailable form (Song et al., 2006). Indeed, using 0.05M ammonium phosphate, Zhao et al. (2010) obtained for uncontaminated neutral to alkaline wheat-cultivated soils 0.7 to 3.4 % of available As. These results speak for an enhanced Cd availability that needs to be checked against Cd concentrations in wheat.

As expected, none of the DTPA concentrations were correlated with their counterpart total concentrations (**Table III-3.2 Annex ; Figure III-3. 2**), probably because of the large variety of soils and parent materials of the studied sites. Only DTPA-extractable Cd was positively correlated with DTPA-extractable As and Co and, DTPA-extractable Pb with Sb. None of the selected elements correlated with phytoavailable Si. Correlation with the physico-chemical properties did not yield more explanation with only DTPA-extractable Pb being correlated positively with silt and CaCO₃ contents and negatively with P Olsen, DTPA-As concentrations positively correlated to P Olsen and DTPA-Cd concentrations correlated positively to altitude and negatively to rainfall. We did not find any correlation between DTPA concentrations and pH or organic matter content unlike observed in other situations (Wu, 2021) probably because of the narrow range of soil pH and organic carbon measured in these agricultural soils, conducted similarly in terms of fertilization, irrigation and crop rotations (cf Ch III-2). **Figure III-3.3** also indicates that the other measured trace elements, especially Co and Zn contribute to discriminate the sites.

CaCl₂-extractable Si concentrations were rather high compared to values obtained in India (Meunier et al., 2018) and in France (Caubet et al., 2020) in a large set of agricultural soils. More specifically, Caubet et al. (2020) found an average concentration of 21 mg Si kg⁻¹ (n=1986 soils) and a higher mean value for carbonated soils on sediments of 35 and a

maximum value of 134 mg Si kg⁻¹. Owing to their high available Si concentration, the B1 to B9 soils are all above the two thresholds proposed by Caubet et al. (2020) and plants requirements should be fulfilled and would contribute to alleviate abiotic stresses if/when any. Si concentrations in plants would have allow verifying this assumption and will have to be performed in the future. However, unlike these authors, we did not find any positive correlation between the available Si concentration and pH and the amount of clay fraction (< 2µm), probably because the set of samples was limited to 8 soils and, again, because the values were within a narrow range **Table III-3.1 Annex and Table III-3. 1**. Measurements replicated on samples collected in 2016 and 2017 gave a slightly difference in concentration but we obtained a significant positive correlation of 0,887 between 2016 and 2017 results, indicating that, although bioavailable Si concentration may vary from year to year, it was in a limited range and could be considered as a constant value on the short term.

Table III-3. 1: Phytoavailable concentrations of trace elements (assessed by DTPA extraction) and silicon (assessed by CaCl₂ extraction) in the soils of the 8 studied sites.

Sites	DTPA - extractable									DTPA/Total			CaCl ₂ -extractable
	Sb µgkg ⁻¹	Zn	Cr	Co	Ni	Cu	Pb	As	Cd	Pb	As	Cd	Si* mgkg ⁻¹
										%			
B1	2.8	1.36	3.6	0.16	0.81	0.98	0.75	13.4	27.4	5.4	0.2	9.7	48.5
B2	4.6	9.56	4.3	0.11	0.89	1.62	1.4	10	29.2	8.3	0.2	10.2	61.1
B3	4.6	0.82	3.6	0.24	0.8	1.1	1.91	23.9	61.5	7.8	0.6	24.1	79.7
B4 _a	3.2	3.23	3.6	0.16	1.05	1.32	0.86	7.3	30.9	9.5	0.1	13	62.3
B4 _b	3.1	4.37	3.4	0.38	1.25	0.77	1.16	10.5	34.1	13.1	0.1	14.6	46.2
B5	4.3	9.39	4.1	0.16	0.98	1.73	1.08	16.3	40.2	6.2	0.1	9.4	64
B8	2.3	0.54	4.3	0.05	0.71	1.04	0.3	22.6	46	1.6	0.2	97	59
B9	2.4	1.9	3.7	0.69	1.14	1.66	0.24	25.1	81.4	1.3	0.4	19.6	73.2

* : 2016 Values

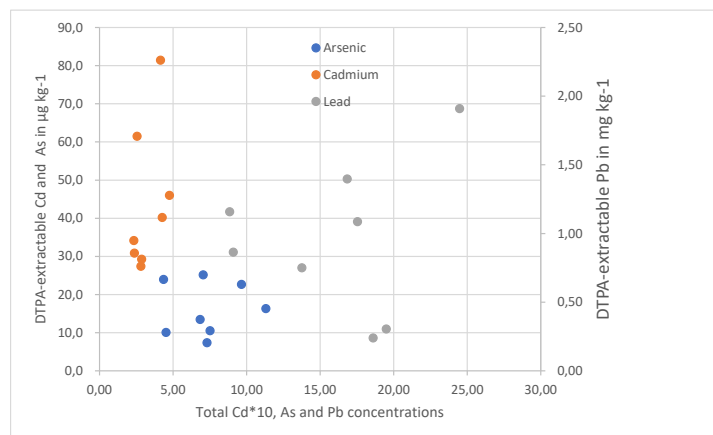


Figure III-3. 3: DTPA-extractable Pb, As and Cd as a function of total concentrations. Please note that total Cd concentrations were multiplied by 10 to fit the x axis

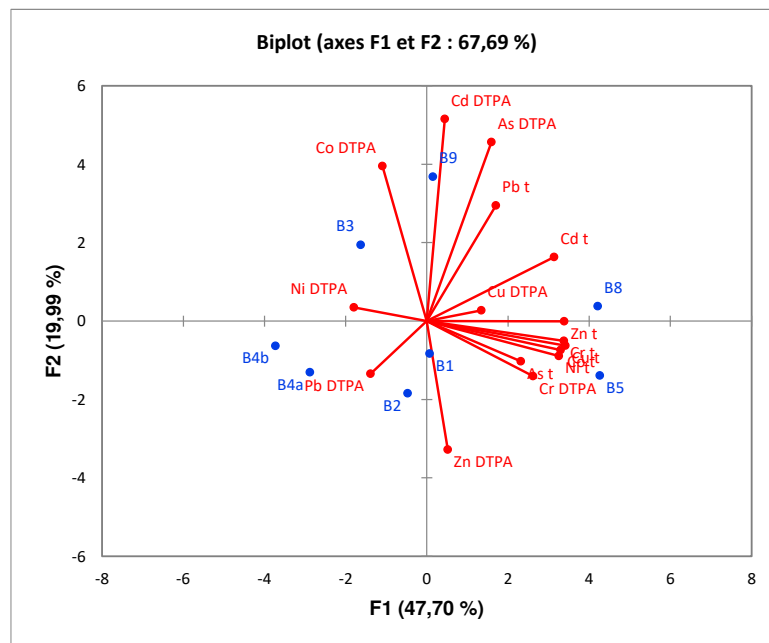


Figure III-3. 4: PCA performed on the 8 sites with total and DTPA-extractable concentrations as variables

III-3.4 The transfer of Pb, As and Cd from soil to wheat

Trace element concentrations in plants depend on various factors: the soil type and its physico-chemical properties, such as pH, EC, organic carbon content, texture etc..., the bioavailable concentrations and more generally speciation that itself depends on the origin of the trace elements (geogenic or anthropogenic) as observed above for B3. Environmental factors such as climate (rainfall and temperature) may impact indirectly plant concentrations through their effects on biomass production. The plant type and cultivars for crops may generate differences (Carlton-Smith and Davis, 1983) as well as agronomic practices, namely irrigation and fertilization. In our case, cultivars were mixed in the field: fields were grown either with a mixture of two durum wheat cultivars (*Icarasha* and *Miki*, at B2, B4a, B4b and B8) or durum (the two previous cv) and winter wheat (cv *Naama 12*) (B1, B9) were grown together, but no distinction was made when taking the plant samples. Consequently, the percentage of each cultivar in the final sample is uncertain and cv cannot be used as an explicative factor for Cd, As or Pb concentrations in plants. However, Cubadda et al. (2010) found no significant difference in As concentration in wheat grain neither between *T. aestivum* (n=80) and *T. durum* (n=60) nor between cultivars of each species, with average concentrations of resp. 8 ± 8 and 9 ± 8 $\mu\text{g As kg}^{-1}$ DW in a set of wheat grains sampled over the

entire Italian territory. They obtained an average As concentration in wheat grain of $9 \mu\text{g As kg}^{-1}$ DW, with values ranging from 2 to $55 \mu\text{g As kg}^{-1}$ DW. To our knowledge, no similar comparative study has been performed for Cd and Pb.

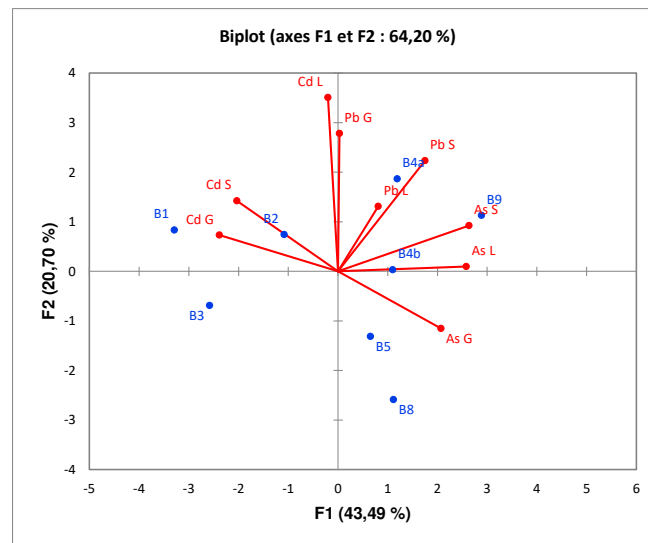


Figure III-3. 5: PCA on the 8 sites with As, Cd and Pb concentrations in wheat parts

Table III-3. 2: As, Cd and Pb concentrations in stem, leaves and grain of wheat grown on the 8 soils

	Pb			As			Cd			Root
	Stem	Leaf	Grain	Stem	Leaf	Grain	Stem	Leaf	Grain	
	mgkg^{-1}									
B1	0.15	0.06	0.02	0.55	0.48	0.4	0.68	0.31	0.24	0.075
B2	0.13	0.1	0.05	0.8	0.55	0.413	0.38	0.21	0.21	0.058
B3	0.1	0.07	0.02	0.5	0.34	0.159	0.17	0.25	0.18	0.242
B4a	0.81	0.16	0.02	0.87	0.7	0.35	0.15	0.33	0.17	0.067
B4b	0.8	0.11	0.02	0.8	0.64	0.57	0.13	0.19	0.18	0.05
B5	0.3	0.14	0.01	0.78	0.63	0.54	0.08	0.16	0.18	0.117
B8	0.07	0.05	0.01	0.8	0.8	0.66	0.14	0.13	0.15	0.167
B9	0.56	0.07	0.04	0.99	0.93	0.65	0.15	0.33	0.11	0.067
m	0.36	0.09	0.02	0.76	0.63	0.47	0.24	0.24	0.18	0.11
sd	0.31	0.04	0.01	0.16	0.18	0.17	0.2	0.08	0.04	0.07

Lead concentrations and, to a lesser extent As concentrations were the largest in stems, then in leaves and concentrations were the lowest in grain. Pb and As were thus not readily translocated to the edible part of the wheat plant **Table III-3. 2**. However, there was no significant correlation between the different plant parts for Pb while correlations were observed between stems and leaves and, leaves and grain As concentrations.

On the contrary, Cd concentrations in grain were always between 0.11 and 0.24 mg kg^{-1} w/w, that is a narrower range than stems and leaves and varying of a factor 2 while variation was larger for Pb and As. They were indeed not significantly lower than concentrations stems and

leaves but Cd concentrations in grain were correlated to those in stems indicating that Cd could readily reach the grain as also illustrated by the translocation ratios **Table III-3. 3**.

The European Commission (COMMISSION REGULATION (EC) No 1881, 2006) has proposed a threshold of 0,2 mg kg⁻¹ w/w for Cd and Pb in wheat grain. Considering a water content of maximum 15% (for conservation), it appears that none of the samples overcame the thresholds, although Cd was close to it, especially at B1 and to lesser extent at B2 sites. However, all concentrations were in the upper range of concentrations in wheat grain compiled by Denaix et al. (2010). Neither B1 nor B2 had been identified as problematic either in terms of total or phytoavailable Cd levels in soil.

Arsenic concentrations are still not enough documented to allow thresholds as for Cd and Pb according the opinion of the European Commission (COMMISSION REGULATION (EC) No 1881, 2006) amended 2015. But China (CMLCF, 2012) and the Codex Committee on Contaminants in Foods (2017) have both set a maximum level for As in polished rice of 0,2 mg As kg⁻¹ and 0,5 mg As kg⁻¹ for condiment and other grains. However some surveys have been performed in Europe, in Asia and in Iran, both in uncontaminated (Cubadda et al., 2010; Zhao et al., 2010) and polluted environments (Karimyan et al., 2020; Suman et al., 2020), to examine As concentration in wheat and assess the associated risks for human health. Most of the time, irrigation with groundwater contaminated with As was responsible for the high as content in grain (Suman et al., 2020). Concentrations measured in grain of half of the studied sites were above this threshold, ranging from 0,16 to 0,66, with a mean concentration of 0,47 mg As kg⁻¹. For comparison the Italian survey on As in wheat grain gave a range of 0,002-0,055 mg As kg⁻¹ for an average concentration of 0,09 among a set of 726 samples pooled into 141 composite samples (Cubadda et al., 2010), while Zhao et al. (2010) measured low As concentrations ($7,7 \pm 5,4 \mu\text{g kg}^{-1}$) in wheat grown on soils with low total concentrations (1,3 to 11 mg kg⁻¹) and $69 \pm 17 \mu\text{g kg}^{-1}$ in grain grown on soil with 29 mg As kg⁻¹. Suman et al. (2020) measured $43,6 \pm 48,2 \mu\text{g kg}^{-1}$ in 72 wheat grain samples harvested on contaminated Indian soils and Karimyan et al. (2020) calculated a mean concentration of 887 $\mu\text{g As kg}^{-1}$ in 27 wheat grain samples grown on soils with a mean As concentration of 311 mg As kg⁻¹. Consequently, the concentrations measured in the 8 composite wheat grain samples were far above concentrations measured in grain grown on uncontaminated soils and even Indian soils, but below concentrations measured by Karimyan et al. (2020), while total and DTPA-extractable

As concentrations in soils were low. These high concentrations deserve further in-depth investigation as well as the As speciation both in soil and grain to be able to better assess the risk for human health (Karimyan et al., 2020), while bioaccumulation factors (ratios) were low (**Table III-3. 4**).

Table III-3. 3 : Translocation ratios (factor) for the various plant parts and As, Cd and Pb.

Sites	As			Cd						Pb		
	L/S	G/L	G/S	S/R	L/R	G/R	L/S	G/L	G/S	L/S	G/L	G/S
B1	0.87	0.83	0.73	9.09	4.19	3.25	0.46	0.78	0.36	0.42	0.39	0.16
B2	0.69	0.75	0.52	6.43	3.52	3.55	0.55	1.01	0.55	0.73	0.52	0.38
B3	0.68	0.47	0.32	0.71	1.03	1.73	1.45	0.71	1.03	0.77	0.21	0.16
B4a	0.8	0.5	0.4	2.24	5	2.5	2.23	0.5	1.12	0.19	0.14	0.03
B4b	0.8	0.89	0.71	2.64	3.88	3.56	1.47	0.92	1.35	0.14	0.19	0.03
B5	0.81	0.86	0.69	0.71	1.41	1.52	1.98	1.08	2.14	0.47	0.04	0.02
B8	1	0.83	0.83	0.84	0.79	0.87	0.94	1.11	1.04	0.71	0.16	0.11
B9	0.94	0.7	0.66	2.24	4.92	1.71	2.19	0.35	0.76	0.12	0.56	0.07
m	0.82	0.73	0.61	3.11	3.09	2.21	1.41	0.81	1.04	0.44	0.28	0.12
sd	0.11	0.16	0.18	3.05	1.75	1.16	0.71	0.28	0.55	0.27	0.19	0.12

In *Figure III-3.6*, the sites appear to be grouped as previously

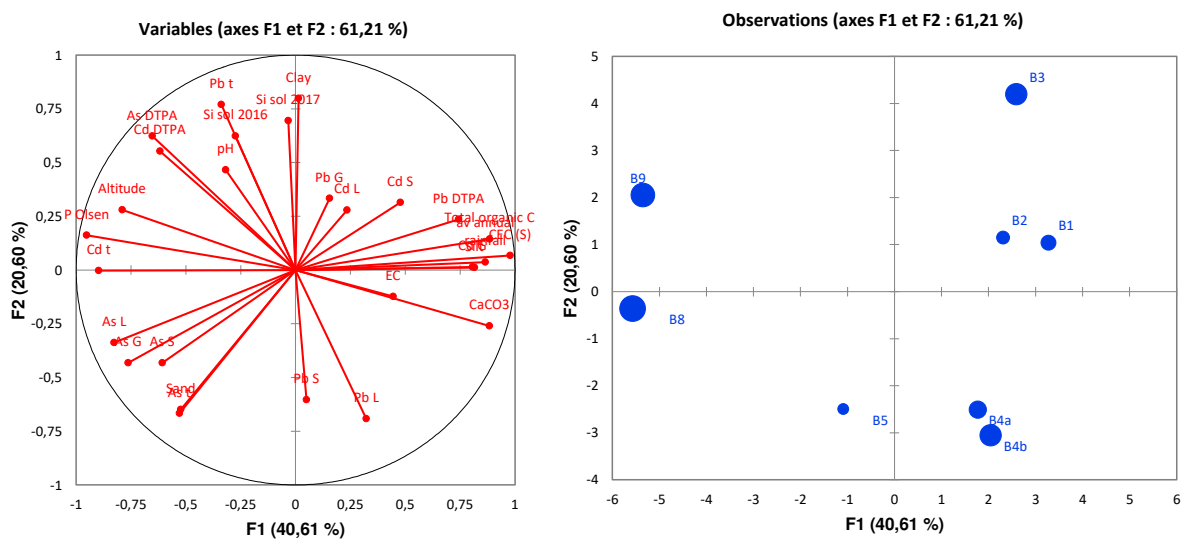


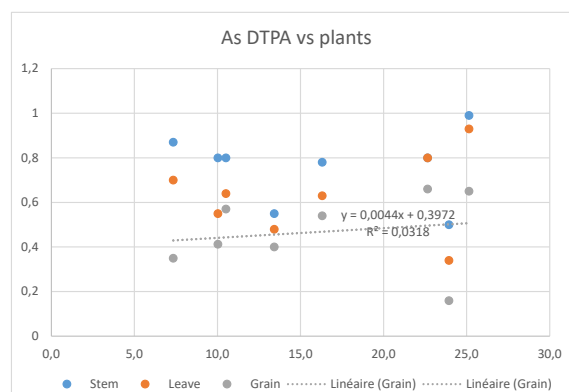
Figure III-3. 6: ACP including all soils parameters and plant As, Cd and Pb concentrations

Table III-3. 4: plant parts-soil ratios. Bioaccumulation factors were calculated with total Pb, As and Cd concentrations in soil and with DTPA-extractable Pb, As and Cd concentrations for grain. S = stem; L = leaves; G = grain.

Sites	Pb				As				Cd				
	S/Soil	L/Soil	G/Soil	G/Soil	S/Soil	L/Soil	G/Soil	G/Soil	R/Soil	S/Soil	L/Soil	G/Soil	G/Soil
	Tot	Tot	Tot	DTPA	Tot	Tot	Tot	DTPA	Tot	Tot	Tot	Tot	DTPA
B1	0.01	0.004	0.002	0.03	0.08	0.07	0.06	29.8	0.3	2.43	1.12	0.87	8.91
B2	0.01	0.006	0.003	0.04	0.18	0.12	0.09	41.2	0.2	1.31	0.72	0.72	7.08
B3	0	0.003	0.001	0.01	0.11	0.08	0.04	6.6	0.9	0.67	0.98	0.69	2.88
B4a	0.09	0.017	0.002	0.03	0.12	0.1	0.05	47.7	0.3	0.63	1.41	0.7	5.4
B4b	0.09	0.013	0.002	0.02	0.11	0.09	0.08	54.2	0.2	0.57	0.83	0.76	5.22
B5	0.02	0.008	0	0.01	0.07	0.06	0.05	33.1	0.3	0.19	0.39	0.42	4.41
B8	0	0.003	0	0.03	0.08	0.08	0.07	29.1	0.4	0.29	0.28	0.31	3.16
B9	0.03	0.004	0.002	0.16	0.14	0.13	0.09	25.9	0.2	0.36	0.79	0.28	1.4
m	0.03	0.007	0.002	0.04	0.11	0.09	0.06	33.46	0.34	0.81	0.81	0.59	4.81
sd	0.04	0.005	0.001	0.05	0.04	0.03	0.02	14.65	0.25	0.74	0.37	0.23	2.41

III-3.5 Factors responsible for concentrations in grain

In [Figure III-3. 6](#), it appears that there is no relation between As concentration in wheat and total or DTPA concentrations in soils unlike found by Karimyan et al. (2020) and Zhao et al. (2010) However, when B3 is removed, the correlation between As in grain and As-DTPA becomes significant ($R^2=0,706$), while the relation is also improved for As concentrations in leaves and As-DTPA [Figure III-3. 7](#). As-DTPA is also correlated to P Olsen content and bioavailable Si and As concentrations in leaves is correlated to Si, indicating possible common pathways. Lead concentrations in plant parts could not be related to DTPA-extractable Pb concentration nor any other soil parameter ([Figure III-3.8](#)) while Cd concentrations in roots were significantly and positively correlated to DTPA-extractable Cd concentrations [Figure III-3. 8](#).



[Figure III-3. 7](#): Relationship between DTPA-extractable As concentration and As concentrations in the different parts of the wheat plant. Removing B3 improves greatly the relation between concentrations in the soil and grain

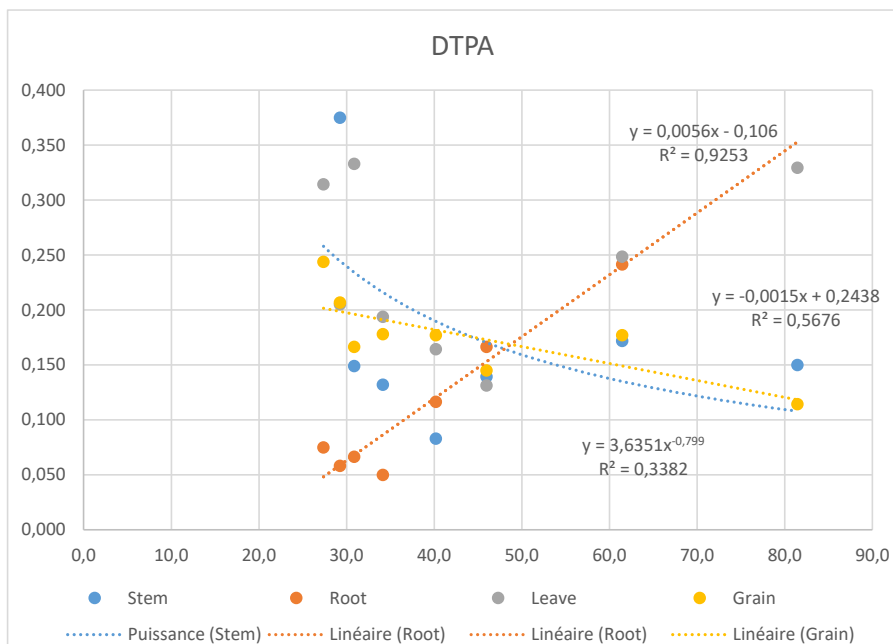


Figure III-3. 8: Relationship between DTPA-extractable Cd concentration and Cd concentrations in the different parts of the wheat plant

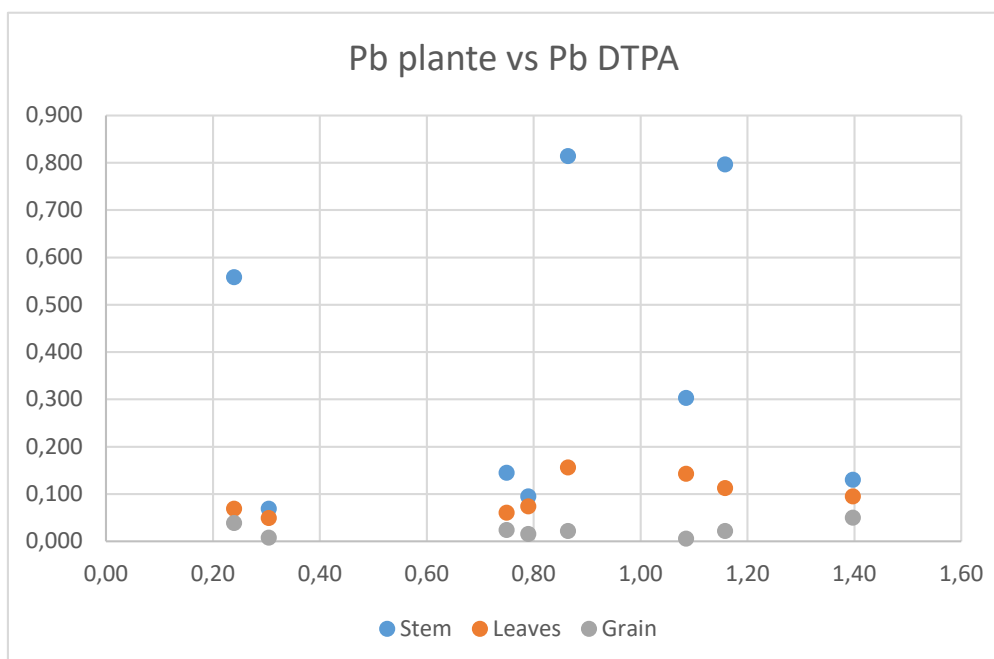


Figure III-3. 9: Relationship between DTPA-extractable Pb concentration and Pb concentrations in the different parts of the wheat plant

III-3.6 Conclusion

In 2007, Lebanon was self-sufficient for only 15% of its needs in wheat (FAO, 2008). Since then, areas cultivated with wheat have been highly variable (Caiserman et al., 2019), with 28% of the Central Bekaa area cultivated in wheat in 2011 (Hassan et al., 2013).

However, with the actual crisis, it might have to rely more on its domestic production. In that context, it was important to investigate the level of Cd, As and Pb in wheat, the relationships with soil and environmental factors to assess risks for human health and also identify ways to limit the potential exposure to metals and metalloids.

As far as the 8 studied sites are concerned, soil contamination appeared limited with only one site (B3) presenting a significant anthropic contamination associated to industrial activities. Irrigation, although it had been shown in Ch III-2 that some waters used for irrigation were contaminated, did not appear as a significant source of contamination for soils. However As was able to reach the grain since concentrations in grain were found high and correlated to DTPA-extractable As, while Pb concentrations in grain are very low and Cd concentrations were somewhat to be monitored. Indeed, Cd concentrations in grains were close to thresholds values, Cd concentration in roots were correlated to DTPA-extractable Cd and translocation factors were often above 1, indicating that Cd was readily translocated to the grain. Cd from fertilizers is proposed as a plausible source of contamination.

In the Chapter III-3, the site B3 showed significant anthropogenic contamination rather associated with industrial activities as shown before. The irrigation with well water did not appear to be a significant source of soil contamination. As concentrations in wheat grains showed high levels and correlated with As bioavailable. The Pb concentrations in wheat grains were very low. While the Cd concentrations presented values identical to the threshold values.

At the root level, the Cd concentrations were correlated with available Cd. The translocation factors were often greater than 1, indicating that Cd was easily translocated into grain, which is mainly due to the fertilizers.

CHAPTER III-4

Lebanese Population Exposure to Trace Elements via White Bread consumption

In the 2nd segment of this thesis, the trace elements in the consumed Lebanese white bread and the exposure of the Lebanese population to these trace elements were studied. To be able to know the consumption of Lebanese white bread and the exposure of the population to trace elements present in the bread, a survey was carried out as the 1st work.

The objective of characterizing the risk is to compare the exposure to the safety thresholds. To evaluate the population exposure, the contamination must be assessed, in other word to evaluate the amount of trace elements present in the consumed bread by measuring them. And on the other hand, the consumption of bread, in other words knowing the quantity of Lebanese bread consumed per day and per person and this was determined following the completion of an investigation.

This survey was carried out and which facilitated the assessment of the risk associated with a route of oral exposure to trace elements. Based on this survey carried out, among 49 brands consumed in Lebanon, 3 industrial bakeries B1, B2 and B3 were the most consumed. Where B1 has 28.9%, B2 30.1% and B3 17.1%. These 3 brands were chosen for analysis of: As, Cd, Co, Cr, Hg, Ni and Pb.

The population exposure was studied taking into account: the amount of bread consumed per person, the body weight and the trace elements content of the consumed bread according to the brand.

Exposures were calculated in B1 and B3, to be in the worst-case scenario, where results demonstrated that B3 showed the highest exposure to: As, Cd, Co, Cr, Hg and Pb. While for Ni, the brand B1 was the main source of exposure.

In conclusion, it was found that there are no risks for Hg, Cd, Cr, and Co. For the element Ni, the exposure presented an excess for the most exposed populations. While only the elements, As and Pb showed safety issues where the exposure margins are very low.

III-4.1 Introduction

Bread is a source of energy and minerals, some of them being essential for the body. Essential minerals found in Lebanese bread are Cu, Fe, Mn, Zn, and Se which account for 36%, 25%, 30%, 22%, and 32% of respective recommended dietary allowance (RDA) values given for these elements (Bou Khouzam et al., 2012).

Besides the quality of bread as nutriment, its safety must be assured and monitored in terms of putative contaminants like trace elements (TEs).

Indeed, trace elements represent the main food chemical contamination and are globally recognized as a public health hazard (Marín et al., 2018). They occur either from natural process (lithogenic and pedogenic routes) and/or anthropogenic activities. The latter include industrial, agricultural (pesticides, fertilizers), and untreated wastewater (Chaza et al., 2018). This leads to the contamination of the different environmental matrices, including water, soil, air, and plants (Baize et al., 2009; Gupta et al., 2019). In general, plants can uptake TEs mainly by absorbing them from polluted soils, and transporting them further to its different parts (roots, stem, leaves, and grains) (Mitra, 2017). The bioaccumulation of TEs in plants, especially those that enter the food chain, can be dangerous to human health due to the fact of their eventual toxicity (Gupta et al., 2019).

Trace elements such as lead, arsenic, cadmium, and mercury are relevant toxic elements (Counter and Buchanan, 2004). According to the existing literature, it is mandatory to quantify the contents of trace elements in foodstuffs in order to estimate dietary exposure, an essential step for risk assessment related to food consumption (Marín et al., 2018; van der Fels-Klerx et al., 2014).

Currently, many studies have been undertaken in different countries to assess exposure to TEs occurring in their relative foodstuffs such as France (Arnich et al., 2012), Spain (Marín et al., 2018), Italy (Storelli et al., 2017), China (Tang and Huang, 2014), Saudi Arabia (Ali and Al-Qahtani, 2012), and Lebanon (Nasreddine et al., 2010). In addition, the European Food Safety Authority (EFSA) panel on contaminants in the food chain has given its scientific opinion on the risks to public health related to the presence of As, Cd, Hg, and Ni in food (EFSA, 2012, 2009, 2015). Such studies should be representative of specific food as consumed by the population. In France, total diet studies (TDS) are national surveys routinely realized to assess public health risks associated with substances such as contaminants in food. They

underline the necessity to reduce exposure to elements such as Cd, Al, Ni, As, and Co by a diversified diet (food items and origins) (ANSES, 2019).

(Nasreddine et al., 2010) studied, using the TDS approach, the dietary exposure of the adult Lebanese urban population to six essential micro-nutriments (i.e., Co, Cu, Fe, Mn, Ni, and Zn) and two toxic heavy metals (i.e., Cd and Pb). This study constitutes a first estimate of the consumer exposure to trace elements through the diet in Lebanon. These authors determined that the average urban adult daily bread consumption was 136.8 g day⁻¹ and that mean exposure was found to be satisfactory for most trace elements. However, the survey included only adult people living in the district of Beirut, thus preventing the extrapolation of the results to the whole country; indeed, agricultural areas are sections of high bread consumption.

Previous studies in Egypt, Iran, South Africa, Nigeria, and Portugal determined TEs contents in bread (Ahmed et al., 2000; Soares et al., 2010). In Lebanon, Bou Khouzam and co-authors determined the contents of 20 elements in three varieties of Lebanese bread (i.e., white, brown pita, and Saj bread) throughout the country during the wet and dry seasons (Bou Khouzam et al., 2012). Toxic elements such as Cd and Pb were below maximum levels set by the Lebanese regulatory agency (Libnor) (NL 240, 2010).

Bread is an integral part of the daily diet, but the types of bread and amounts ingested vary according to the age, sex, and socio-economic status of the individual. As a result, and as consumer behavior evolves over time, there is a need for up-to-date information on daily TE intake via Lebanese bread consumption throughout Lebanon, including rural areas. The Lebanese standards (NL 240, 2010) provide only Pb, Cu, and Cd maximum levels in bread as food item contamination vectors. However, other TEs could be present (Bou Khouzam et al., 2012; Nasreddine et al., 2010). As exposition represents the combination of consumption of bread potentially contaminated, it is of first interest to compare exposure with safety levels in order to monitor any safety preoccupation concerns for the Lebanese population. For this reason, this study was directed by a public institute (Lebanese Agricultural Research Institute—LARI) to assess exposure to trace elements via Lebanese pita consumption.

As, Cd, Co, Cr, Hg, Ni, and Pb were selected in this study based on previous studies of trace element composition of Lebanese bread (Bou Khouzam et al., 2012; Nasreddine et al., 2010) and based on the Lebanese bread standard Libnor (NL 240, 2010). In contrast to

heavy metals known to be toxic, such as Pb, Cd or Hg, some elements, such as Cr and Co, are essential elements giving benefit to the body, the liver being a valuable source of elements (Kicińska, 2019). Co is a transition metal with two oxidation states Co (II) and Co (III) and is essential as part of vitamin B12 (cobalamin) involved in folate and fatty acid and the metabolism of proteins and nucleic acids (EFSA, 2017). In regards to Cr, the Cr (III) form has been postulated to be necessary for the efficacy of the insulin-regulating metabolism of carbohydrates, lipids, and proteins (WHO, 1996). However, EFSA (EFSA, 2014) in 2014 concluded there was no evidence of a beneficial effect associated with Cr intake in healthy, normoglycemic subjects.

Two risks may occur with trace elements: one is insufficient intake, and one is excess intake. In this study, we focused on risk characterization due to the fact of excess intake via pita consumption.

Therefore, the objective of this study was to assess exposure to the selected trace elements (TEs) of the different Lebanese population categories via pita consumption. First, a country survey of pita consumption was achieved among one thousand individuals, grouped into adults (above 15 years old, men and women) and young people (6–9 and 10–14 years old). Second, levels of the seven selected trace elements in pitas from the three most consumed brands were measured. Third, using the survey data, population categories of exposures to Tes via white pita consumption were calculated and compared to the international health-based guidance values: tolerable daily intake (TDI), tolerable weekly intake (TWI) or toxicological reference points, such as bench mark dose limit (BMDL), to evaluate safety concerns.

III-4.2 Materials and Methods

III-4.2.1 Survey

A questionnaire survey about the daily consumption of pita bread was conducted on a target sample of 1000 people. They were selected randomly in 50 supermarkets over the five administrative regions of Lebanon, taking into consideration the population distribution (CAS, 2019): 41% in Mount Lebanon, 20% in North Lebanon including Akkar, 17% in South Lebanon including Nabatieh, 13% in the Bekaa, and 9% in Beirut **Table III-4. 1**. The individuals

were represented by 50% men, 50% women, and grouped as follows: 800 adults (> 15 years old) and 200 young people (between 6 and 15 years old).

Table III-4. 1: Sampling strategy according to the population distribution.

Regions	Population Distribution		* Estimated Sample		Surveyed Supermarkets Realized Questionnaires		
	N	%	N	%	N	N	%
Beirut	361.366	9.6	90	9.0	5	82	8.3
Mount	1,484.474	39.5	410	41.0	20	410	41.3
North	763.712	20.3	200	20.0	10	200	20.2
South	659.718	17.6	170	17.0	8	170	17.1
Bekaa	489.865	13.0	130	13.0	7	130	13.1
Total	3,759,136	100	1000	100	50	992	100

* Lebanese Population, Central Administration for Statistics (CAS) Living Conditions Survey 2007 (CAS, 2019).

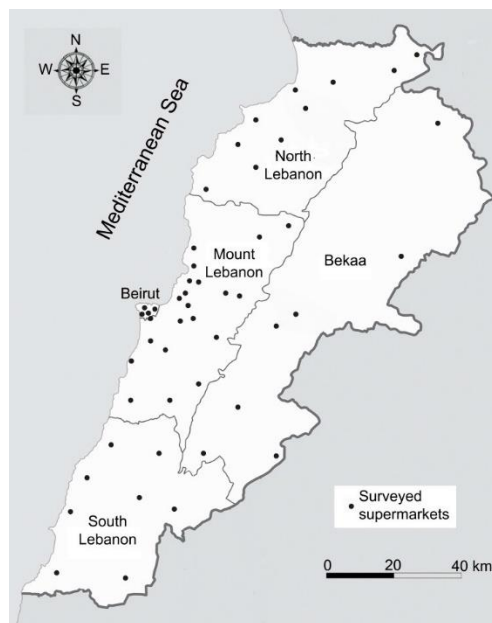


Figure III-4. 1: Location of the fifty surveyed supermarkets in Lebanon within the five administrative regions according to population density

Data were collected over a two-month period, from 14 April 2017 to 5 June 2017. The team of interviewers was composed of 8 data collectors trained prior to the fieldwork by a supervisor. Participants were eligible for the study if they were Lebanese, had been living in Lebanon for at least fifteen years before the date of the survey, and did not have a chronic or serious illness.

In each supermarket, eligible participants were interviewed face-to-face by a data collector who instantly filled an offline questionnaire on an electronic tablet, using multiple-choice questions and figures (e.g., size of the pita). In the case of children, parents aided us in collecting survey data.

In addition to information on the daily consumption of pita bread, age, gender, body weight, and socio-economic status of the interviewed individuals, data were also collected on the level of education, address (urban or rural), job description, and health status (e.g., smoking habit, alcohol consumption and physical activity of the individual). Each questionnaire consisted of 44 questions (Supplementary Materials, Survey) which required approximately 7 min to complete. The collected answers were transmitted in the “iSurvey” application for data harmonization.

III-4.2.2 Chemicals

Standard solutions (1 g L^{-1}) of Hg, As, Co, Cr, Cd, Ni, and Pb were purchased from Fluka. In addition, HNO_3 65%, H_2O_2 30%, and the BCR (Community Bureau of Reference) certified reference material for bread trace elements (BCR-191) were purchased from Sigma–Aldrich. Deionized water, obtained from a BOECO pure UV/UF water purification system, was used in all experiments.

III-4.2.3 Sampling for Trace Elements Analysis

Based on the survey data, three Lebanese white pita brands were determined as the most consumed by the Lebanese population. These brands were tagged as B1, B2, and B3. Three randomly sampled bags of each of the selected pita brands were collected. Each bread bag had seven pitas where three were randomly picked. Each pita was cut into 12 circular sectors with a plastic knife on a polyethylene plate to prevent trace element contamination. Two pieces were randomly drawn for analysis, creating 18 (3 bags \times 3 pitas \times 2 pieces) circular pita pieces for each brand. Before sample digestion, pita pieces were ground into powder using an agate mortar.

III-4.2.4 Sample Digestion for Trace Elements Analysis

Digestion of samples was done using an Anton Paar microwave (multiwave 3000—Rotor 8SXF 100, Graz, Austria). Samples of 0.5 g each were directly weighed after grinding in 50 mL Teflon tube. Then, 7 mL of concentrated HNO_3 and 1 mL of H_2O_2 were added to the preparation. The Teflon bombs were closed and placed in the digester at 200°C for 15 min

(1000 W). Once the Teflon tubes had cooled for at least 45 min, 3 mL of the digested samples were transferred to 25 mL polyethylene tubes and diluted with HNO₃ 1% before the trace elements analysis. Each sample was digested in triplicate.

III-4.2.5 Analysis of Trace Elements

The determination of trace elements (As, Co, Cd, Cr, Pb, and Ni) in pita samples was performed using an Atomic Absorption Spectrometer (AAS, Stafford House, UK) Zeeman Graphite Furnace GF95Z Thermo Electron corporation M series. Each pita sample was digested in triplicate and each digested sample was analyzed in triplicate. Mercury was analyzed by direct mercury analyzer DMA 80 Milestone (Soriso, Italy) using 0.2 g of pita sample.

To guarantee the method reliability, a certified reference material (CRM; BCR-191) underwent the same digestion protocol and was analyzed at the same time as the pita samples. The recovery percentages obtained for the reference material were 93.2% for Hg, 110.8% for Pb, 86.3% for Ni, and 110.1% for Cd. For the other elements, As, Co, and Cr, for which reference materials were not available, bread samples were spiked by a known concentration (0.1 mg L⁻¹) of each of the above listed elements before digestion. The spiked bread was treated and digested in the same way as the bread samples and analyzed using an AAS. The recovery percentages were 103.08% for As, 101.29% for Co, and 96.37% for Cr.

III-4.2.6 Exposure Determination

An exposure calculation was conducted for each respondent. They took into account the quantity of bread consumed by person, body weight, and the metal content of the consumed bread according to its brand. Results were expressed in terms of the median and 95th percentile using Ms-Excel for different population categories.

III-4.2.7 Statistical Analysis

In order to avoid a lack of representativeness of the selected samples of the surveyed population, a weighting adjustment technique was applied using auxiliary variables such as gender, age, and region by comparing the observed frequency distribution of each variable with its population distribution (Bethlehem, 2008).

Data processing and statistical adjustments were analyzed using Sphinx IQ2 (Le Sphinx®, Chavanod, France) for survey data and R Studio version 1.0.153 with R version 3.5.0 (R Core team, 2020; RStudio Team, 2016).

Kruskal–Wallis non-parametric tests (Sprent, 2001) completed by a multi-comparison Fisher’s test ($\alpha=0.05$) were conducted to test for significant differences in consumption by region. Analysis of variances (ANOVAs) completed by a multi-comparison Tukey’s test ($\alpha = 0.05$) were also executed to test differences in trace element contents among brands. They were performed with R using the “agricolae” package (De Mendiburu, 2020).

Median and percentile non-parametric tests ($\alpha = 0.05$) (Sprent, 2001) were realized to check significant differences in the level of trace element exposures between age and gender. They were performed using R and the “rcompanion” package (Mangiafico, 2019).

Principal component analysis (PCA) was applied to examine the relationships among the trace element contents of the breads using XLSTAT 2018. The results are presented by loading and score plots.

Multivariate correspondence analysis was done to explore relationships among consumption behaviors and socioeconomic data (Ganassali, 2014).

III-4.3 Results

III-4.3.1 Survey Results

Based on a target sample of 1000 people, the surveyed sample consisted of 992 individuals with the following demographic characteristics: a sex ratio of 0.992, a proportion of 19.4% young people (8.9% children aged from 6 to 9 years old and 10.5% teenagers aged from 10 to 14 years old) and 80.6% adults, and 70.6% living in an urban area. Compared to the overall Lebanese population (where the sex ratio is 0.962, 23% are young people, 77% adults, and 87% live in urban areas (CAS, 2019) there was a significant difference risk of 5% in terms of young people/adults and rural/urban ratios between the surveyed sample and the population distribution of Lebanon, which could lead to a bias. Consequently, a weighing adjustment in respect to these variables was performed to make a possible inference at the population level. The weighted numbers of respondents corresponded to 228 young people (i.e., 95 children and 133 teenagers) and 764 adults.

White pita was the type of bread most consumed according to the surveyed sample: 77%

reported white pita consumption and 23% brown or other. We limited our study to the 762 individuals consuming mainly white pita (W). This population also consumed brown pita (B) and other types of pita (O) in smaller proportions. The relative proportions of these different consumption modes were as follows: W only > W + B > W + O > W + B + O with 88.8%, 8.9%, 1.8%, and 0.4%, respectively **Table III-4. 2.**

Table III-4. 2: Pearson correlation matrix of the Principal Component Analysis.

Variables	As	Co	Cd	Cr	Hg	Ni	Pb
As	1						
Co	-0.41*	1					
Cd	0.43*	-0.10	1				
Cr	-0.03	-0.49*	-0.49*	1			
Hg	-0.04	-0.19	-0.53*	0.64*	1		
Ni	-0.59*	0.65*	0.09	-0.76*	-0.50*	1	
Pb	0.68*	-0.65*	-0.00	0.69*	0.39*	-0.98*	1

* Values significantly different from zero at a level of significance $\alpha = 0.95$

The survey makes it possible to estimate the consumption of white pita for the different categories of the population **Table III-4. 3.** Adult men were the highest consumers followed by teenagers (10–14 years old) and women and children (6–9 years old): daily white pita consumption medians were 282, 143, 126, and 71 g day⁻¹, respectively. The 95th percentiles were 750 g day⁻¹ for men and between 357 and 375 g day⁻¹ for other categories. Maximum consumption was 1000 g day⁻¹ for men and teenagers and 750 g day⁻¹ for women and children. White pita brand consumption was significantly dependent on the age of the Lebanese population. Children consumed mostly the B2 or B3 brands, teenagers consumed mostly the B1 brands, and adults consumed mostly the B1 or B2 brands. The whole sample consumption by brand, including young people and adults, was B2 > B1 > B3 representing, respectively, 30.1%, 28.9%, and 17.1% of the total sample and the 46 other brands representing the remaining 24.9%.

Table III-4. 3: Daily white pita consumption according to population categories and repartition by brands.

Population Categories	Individuals <i>n</i>	Daily White Pita Consumption						Brands Repartition				
		g/Day						%				
		min	M	95th P	Max	m	CI 95%	B1	B2	B3	Others *	Total
Children	91	63	71	357	750	113	94-131	24	31	30	14	99
Teenagers	113	31	143	375	1000	171	147-196	40	29	12	19	100
Women	258	31	126	375	750	167	153-181	29	31	16	24	100
Men	300	31	282	750	1000	282	260-304	29	30	13	27	99
Young people & adults	762	31	143	500	1000	206	195-218	28.9	30.1	17.1	24.9	101

M = median; 95th P = 95th percentile; min = minimum; max = maximum; m = mean; CI 95% = 95% confidence interval.

*" Others" correspond to an array of the 46 other pita brands.

White pita consumption varied according to region [Figure III-4.2](#), and the highest consumption corresponded to South Lebanon and Nabatieh (355 g day^{-1}). There were no significant differences between the North, Mount Lebanon, and Bekaa regions, which were two-fold lower than South Lebanon and Nabatieh. The lowest consumption was observed in Beirut (135 g day^{-1}). This last value is in agreement with the adult population of Beirut studied by (Nasreddine et al., 2010).

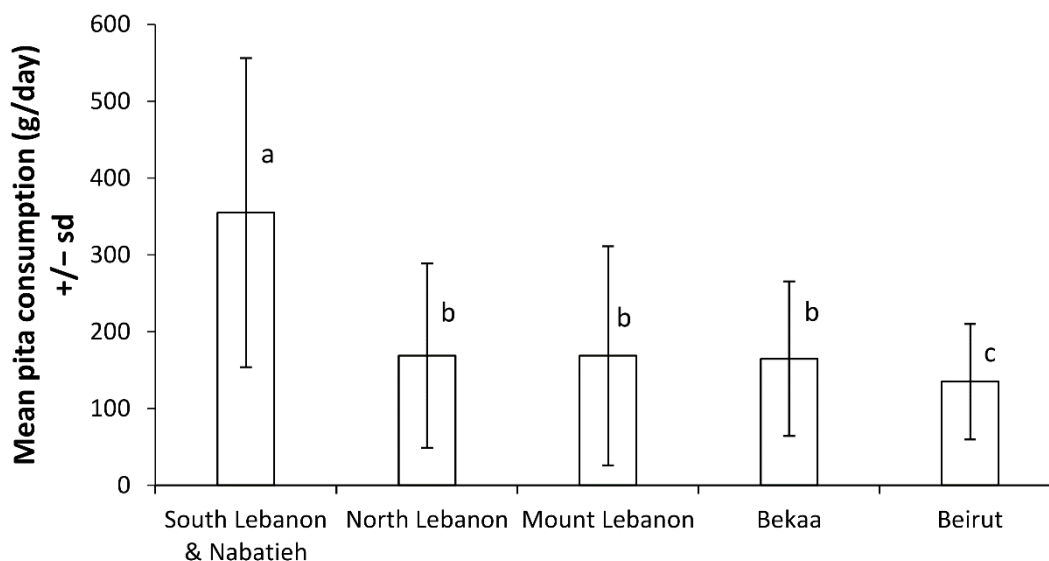


Figure III-4. 2: Lebanese white pita consumption according to region. The letters a, b, and c represent homogeneous groups determined by a Kruskal–Wallis test and post-hoc Fisher's test for least significant difference.

Regardless of the region, large standard deviations were observed in the distribution of bread consumption; the reason could be that, adults over 65 years old eat more bread, especially in rural areas (in South Lebanon, more than 600 g day^{-1} can be consumed, see [Figure III-4. 3](#)).

In order to better explore the relationships between adult consumption and regions, a data-mining tool was used, the purpose of which was to represent in only one table or figure the most significant results from several cross analyses (based on multivariate correspondence analysis (Ganassali, 2014). A pivot variable was first selected—the region—then other variables were crossed with it. *Figure III-4. 3* shows the results of crossing region and consumption, gender, socio-professional category, smoking habits, physical activity (4 categories: “No”, “Yes, strenuous”, “Yes, low”, and “Yes, medium”), and area (urban or rural), using the most specific modalities as key views.

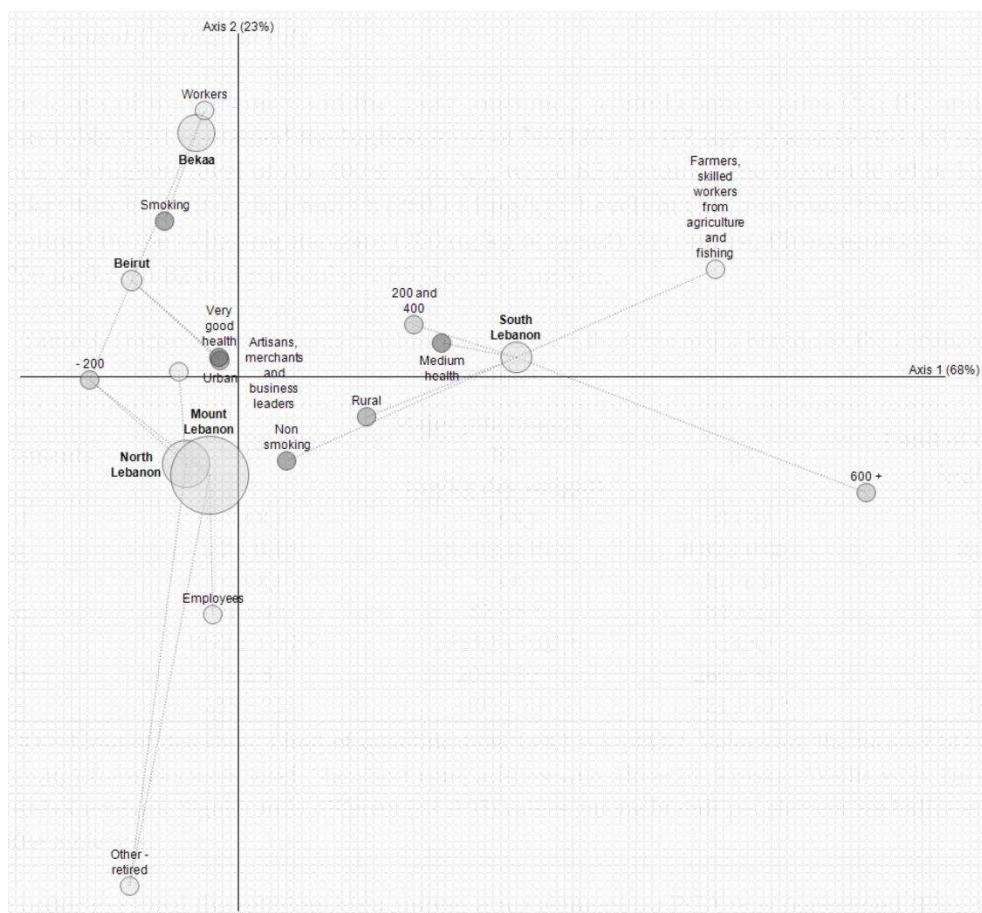


Figure III-4. 3: Results of crossings between regions, consumption, smoker or not, type of physical activity, socio-professional category, and area (urban or rural), using the most specific modalities as key views. Consumption is represented here by categories: less than 200 g/day (-200); from 200 to 400 g/day; from 400 to 600 g/day; and more than 600 g/day (600+). Sizes of circles are proportional to the number of observations by region.

In South Lebanon and Nabatieh, where consumption was the highest, it correlated to rural areas with more agriculture and elderly populations. The other parts were characterized by urban areas with high concentrations of workers and retired people.

In addition, 41% of total consumers of Lebanese white pita bread were smokers (at least

one cigarette per day, with a distribution of 68% men and 32% women) and 35% were alcohol drinkers (at least one glass per day, with a distribution of 67% men and 33% women, data not shown). Those behaviors may affect health and the detoxification process of alcohol and smoking are indeed well-known inducers of several xenobiotic metabolism enzymes.

Among the adult population, 85% reported engaging in physical activity which was qualified as low to medium by women and medium to high by men *Figure III-4.4*.

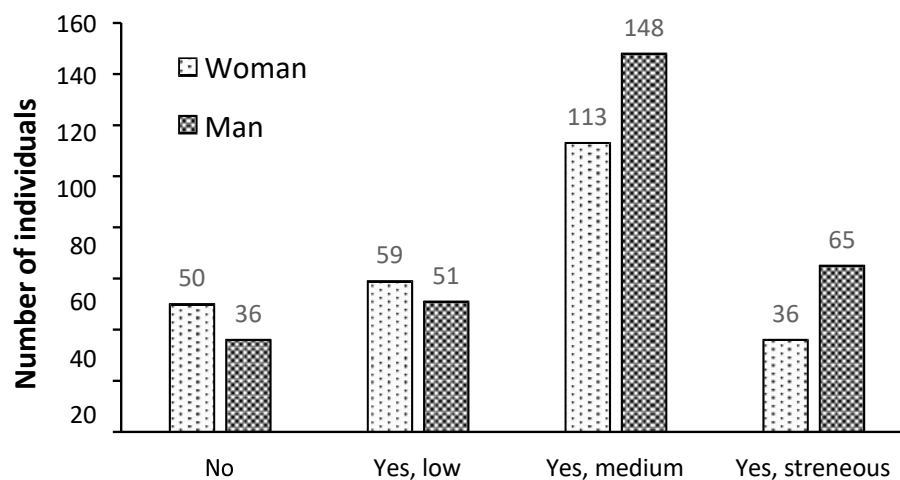


Figure III-4. 4: Distribution of physical activity intensity by gender in adult population

The correlation between consumption and health was also evaluated (data not shown). The pivot variable was still pita consumption per day and the other variables were gender, region, professional activity (student, retired, etc.), and health (smoking, physical activity). From this study, two typologies of adult consumers can be drawn: i) Women, quite young (under 20 years old), non-smokers, and who felt very healthy according to the survey. They were living in urban areas, Lebanon North–Mount Lebanon, with some level of pre-secondary education. The consumption of those aged 15–64 years old per day was approximately 163 g of pita (corresponding to 1 or 2 pitas). ii) Men between 40 and 59 years old who felt healthy. They were living in rural zones, with a technical secondary level education. Their consumption was approximately 267 g of pita per day, corresponding to 3 to 6 pitas for those 15–64 years old.

III-4.3.2 Trace Element Contents in Pita

The levels of trace elements in the most consumed white Lebanese pitas (B1, B2, and B3) are shown in **Table III-4. 4**. B1 contained the highest level of Ni ($1292.1 \pm 0.2 \mu\text{g kg}^{-1}$) and Co ($91 \pm 3 \mu\text{g kg}^{-1}$). B2 contained the highest level of As ($400 \pm 7 \mu\text{g kg}^{-1}$), while B3 contained the highest level of Hg ($0.89 \pm 0.06 \mu\text{g kg}^{-1}$), Cr ($363 \pm 10 \mu\text{g kg}^{-1}$), and Pb ($260 \pm 81 \mu\text{g kg}^{-1}$). Levels of Cd were lower than the maximum levels defined by the Lebanese standard ($200 \mu\text{g kg}^{-1}$ of bread). In contrast, Pb exceeded the maximum level ($200 \mu\text{g kg}^{-1}$) in B2 and particularly B3 ($260 \pm 81 \mu\text{g kg}^{-1}$).

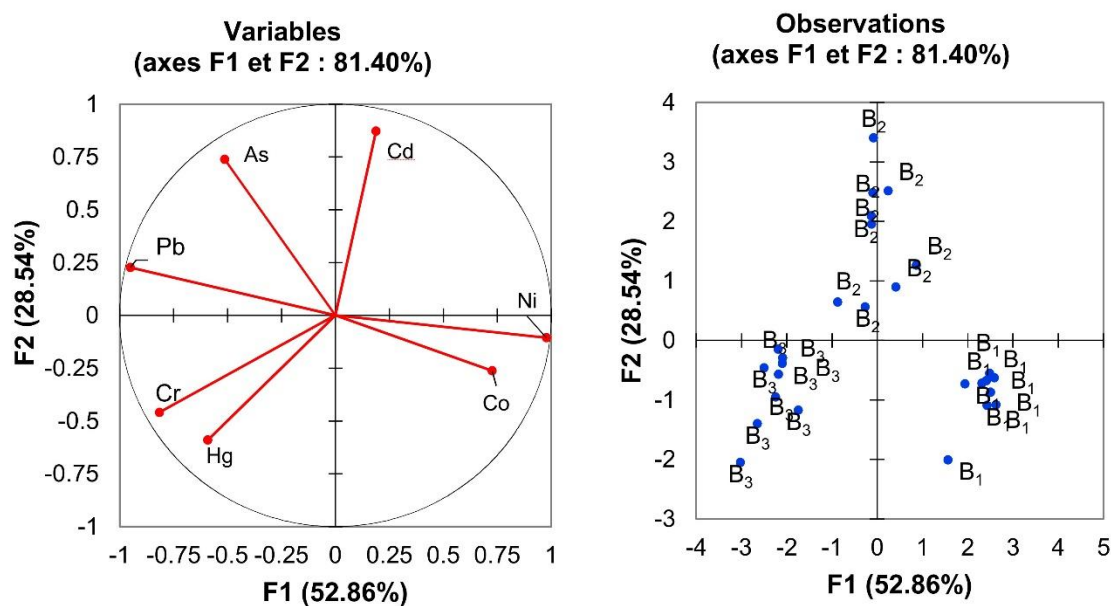
Table III-4. 4: Trace element contents in white pita bread for the three major brands on the Lebanese market.

Trace elements	Major pita brands			Limit of detection ($\mu\text{g/kg}$)
	B1	B2 ($\mu\text{g/kg dry weight}$)	B3	
Cd	6.5 ± 0.3^b	9 ± 2^a	6 ± 2^b	5
Hg	0.7 ± 0.1^b	0.70 ± 0.15^b	0.89 ± 0.06^a	0.04
Cr	40 ± 2^b	16 ± 2^c	363 ± 10^a	16
Co	91 ± 3^a	87 ± 5^a	84 ± 2^a	5
Ni	1292.1 ± 0.2^a	364.82 ± 0.02^b	<LOD	16
Pb	74 ± 5^c	203 ± 30^b	260 ± 81^a	21
As	235 ± 22^c	400 ± 7^a	321 ± 20^b	16

LOD = Limit of Detection. Limit of Quantification: LOQ = $3 \times \text{LOD}$.

Values are means \pm SD ($n = 9/\text{group}$) trace elements level. Statistical comparisons were done by Kruskal-Wallis with Tukey's post hoc analysis. Values significantly different ($P < 0.05$) between brands per element are indicated by different letters.

In order to reveal the relationships among trace elements, a PCA was performed *Figure III-4. 5*.



Figure

Figure III-4. 5: Principal component analysis showing trace element signatures of the B1, B2, and B3 pita brands.

Principal component analysis is a useful technique for exploratory data analysis, allowing to better visualize the variation present in a dataset with many interrelated variables. In our case, the dataset contained 27 observations (9 samples per brand) described by 7 variables (the trace elements content as mentioned in **Table III.4.4**). Principal component analysis allows to see the overall “shape” of the data, identifying which samples are similar to one another and which are very different. It makes possible the identification of potential groups of samples that are similar and to work out which variables make one group different from another. When many variables correlate with one another, they will all contribute strongly to the same principal component (Bereton, 2007).

Figure III-4. 5 shows typical plots from the PCA with a loading plot (on the left) and a score plot (on the right). The loading plot is used for interpreting relationships among variables and the score plot is used for interpreting relationships among observations.

All variables were well represented in the F1–F2 plot (sum of squared cosine above 0.75). The PCA shows that the following elements were correlated: Ni and Co, As and Pb as well as Cr and Hg. Lead and Co were anti-correlated, as well as Ni and Pb. There was no correlation between Cd and Co, Cd and Pb, Cr and As as well as Hg and As. The Pearson correlation matrix is provided in the table below.

Table III-4. 5: Pearson correlation matrix of the Principal Component Analysis.

Variables	As	Co	Cd	Cr	Hg	Ni	Pb
As	1						
Co	-0.41*	1					
Cd	0.43*	-0.10	1				
Cr	-0.03	-0.49*	-0.49*	1			
Hg	-0.04	-0.19	-0.53*	0.64*	1		
Ni	-0.59*	0.65*	0.09	-0.76*	-0.50*	1	
Pb	0.68*	-0.65*	-0.00	0.69*	0.39*	-0.98*	1

* Values significantly different from zero at a level of significance $\alpha = 0.95$

We observed three separate groups: B1, B2, and B3. The dispersion in B2 and B3 was larger than in B1; this dispersion can be explained by a more homogeneous sampling for B1.

III-4.3.3 Trace Element Exposures

The population categories' exposure to Cd, Hg, Cr, Co, Ni, Pb, and As via white pita consumption according the most consumed brands is presented in **Table III-4. 6**.

Using statistics, we observed that, for most of the cases, the B3 brand was the origin of the highest trace elements exposure. In regards to nickel, B1 was the main source of the highest exposure. We further decided to keep B3 Tes data to calculate exposures **Table III-4. 6**. Using a worst-case scenario for all element levels, except for nickel for which the B1 data were retained.

For young people, there was no significant difference among both populations in terms of exposure to any element. In contrast, we found significant differences between men and women for all elements except nickel. Men, at the 95th percentile, were always more exposed

Table III-4. 6: Population categories exposure to As, Cr, Co, Ni, and Pb trace elements via white pita consumption according the most consumed brands.

Trace element	Brand	Trace element exposure ($\mu\text{g}/\text{kg}$ body weight/day)							
		Children		Teenagers		Women		Men	
		M	95 th p	M	95 th p	M	95 th p	M	95 th p
As	B1	0.51 _a	1.86 _a	0.74 _a	2.23 _a	0.50 _a	1.60 _a	0.79 _a	1.61 _a
	B2	1.43 _b	7.65 _b	1.07 _{ab}	4.17 _a	0.77 _b	2.11 _a	1.05 _b	2.63 _a
	B3	1.54 _b	7.97 _b	1.58 _b	4.48 _a	1.07 _{c*}	2.19 _{a*}	1.53 _{c*}	3.77 _{b*}
Cr	B1	0.09 _a	0.32 _a	0.13 _a	0.38 _{ab}	0.09 _a	0.28 _a	0.14 _a	0.28 _a
	B2	0.06 _a	0.30 _a	0.04 _b	0.16 _a	0.03 _{b*}	0.08 _b	0.04 _{b*}	0.10 _{b*}
	B3	1.74 _b	9.00 _b	1.79 _c	5.06 _b	1.21 _{c*}	2.47 _{c*}	1.72 _{c*}	4.26 _{c*}
Co	B1	0.20 _a	0.72 _a	0.29 _{ab}	0.86 _a	0.20 _{ab}	0.31 _a	0.31 _a	0.62 _{ab}
	B2	0.31 _{ab}	1.66 _a	0.23 _a	0.91 _a	0.17 _{a*}	0.23 _{a*}	0.23 _{a*}	0.57 _a
	B3	0.41 _b	2.09 _a	0.42 _b	1.18 _a	0.28 _{b*}	0.40 _{b*}	0.40 _{b*}	0.99 _{b*}
Ni	B1	2.80 _a	10.26 _a	4.06 _a	12.26 _a	2.75 _a	8.83 _a	4.34 _a	8.86 _a
	B2	1.30 _a	6.99 _a	0.97 _b	3.81 _a	0.70 _{b*}	1.93 _b	0.96 _{b*}	2.40 _b
	B3	ND	ND	ND	ND	ND	ND	ND	ND
Pb	B1	0.16 _a	0.59 _a	0.23 _{ab}	0.70 _{ab}	0.16 _a	0.50 _a	0.25 _a	0.51 _a
	B2	0.72 _{ab}	3.88 _a	0.54 _a	2.12 _a	0.39 _{b*}	1.07 _b	0.53 _b	1.33 _b
	B3	1.25 _b	6.43 _a	1.28 _b	3.61 _a	0.86 _{c*}	1.77 _{c*}	1.23 _c	3.04 _{c*}

Trace element	Brand	Trace element exposure ($\mu\text{g}/\text{kg}$ body weight/day)							
		Children		Teenagers		Women		Men	
		M	95 th p	M	95 th p	M	95 th p	M	95 th p
Cd	B1	0.10 _a	0.36 _a	0.14 _a	0.43 _a	0.10 _a	0.31 _a	0.15 _a	0.31 _a
	B2	0.22 _a	1.16 _a	0.16 _a	0.64 _a	0.12 _a	0.32 _a	0.16 _a	0.40 _a
	B3	0.19 _a	1.00 _a	0.20 _a	0.56 _a	0.13 _{a*}	0.28 _{a*}	0.19 _{a*}	0.47 _{a*}
Hg	B1	0.01 _a	0.04 _a	0.02 _{ab}	0.06 _a	0.01 _{ab}	0.04 _{ab}	0.02 _a	0.04 _a
	B2	0.02 _{ab}	0.09 _a	0.02 _a	0.07 _a	0.01 _{a*}	0.03 _a	0.01 _{a*}	0.03 _a
	B3	0.03 _a	0.15 _a	0.03 _b	0.09 _a	0.02 _{b*}	0.04 _{b*}	0.03 _{b*}	0.07 _{b*}

III-4.3.4 Risk Characterization (Intake Excess of TEs via Bread Consumption)

In regards to essential elements with nutritional value, risk assessment can be calculated by insufficient intake or intake excess. This work focused only on intake excess using toxicological reference points or safety levels for each element case by case in order to determine if there is a safety concern due to the fact of an intake excess related to bread consumption in Lebanon. Of course, bread is not the only food consumed per day;

however, in this study we show that the most exposed population—men and teenagers—can consume up to 1000 g of bread per day.

Considering chromium levels in bread, represented mostly by Cr (III)—the major form in food (Novotnik et al., 2013), exposures to Cr ranged from 1.21 to 9 $\mu\text{g kg}^{-1} \text{bw day}^{-1}$ which represents only 3% of the European TDI (EFSA, 2014) for the most exposed population (children at the 95th percentile) **Table III-4. 7** in regards to pita consumption. Concerning cobalt, only the exposure related to children lead to an excess (131%) of the French TDI **Table III.4.9** (ANSES, 2019ANS).

Regarding cadmium, the most exposed population were children at the 95th percentile followed by teenagers and then men and women. The most exposed population represented 40% of the European tolerable weekly intake (TWI) fixed by the EFSA (EFSA, 2009) **Table III-4. 7**.

Exposures to mercury were also very low (ranging from 0.02 to 0.15 $\mu\text{g kg}^{-1} \text{bw week}^{-1}$) even for the most exposed population (children at the 95th percentile) leading to 11.5% of the TWI fixed by the (EFSA, 2012).

However, before concluding on safety in this survey in regards to Hg, Cd, Cr or Co, it is important to take into account exposure from other food items in the context of the Lebanese population's total diet.

Concerning nickel, the situation is already worrying when taking into account only pita consumption. Teenagers were the most exposed population followed by children and then adults, regardless of sex **Table III-4. 8**. The European TDI was exceeded for all exposures for teenagers and men and only at the 95th percentile (3 to 4 fold) for children and women.

Table III.4.9 shows that the margins of exposure (MOE) in regard to lead, determined with a $\text{BMDL}_{0.1}$ based on neurotoxic effect (EFSA, 2010), were very low (between 0.08 and 0.58) whatever the population. The most exposed populations in decreasing order were children, teenagers, men, and women. The calculated MOEs suggest a safety level of preoccupation regardless of the population in terms of lead exposure via bread consumption.

The same safety preoccupations can be observed with arsenic considering As is represented by its inorganic form. The EFSA has concluded that 0.3 to 8 $\mu\text{g kg}^{-1} \text{bw day}^{-1}$ should be used as a point of reference (EFSA, 2009a). Thus, these values were used for MOE calculation to assess risk characterization. **Table III.4.9** shows that the MOEs were very low regardless of the

population in both scenarios. The most exposed populations were children followed by teenagers and then men and women. The MOE data suggest a worrying risk in regard to As exposure linked to pita consumption regardless of the population in this survey.

Table III-4. 7: Population categories' exposure to Cd and Hg trace elements via B3 white pita consumption and comparison with the tolerable weekly intake (TWI). Exposure values are expressed in µg/kg body weight/week.

Trace Elements	Population Categories' Exposure to TEs via White Pita and Comparison with TWI															
	Children				Teenagers				Women				Men			
	M	M/TWI %	95 th P	95 th P/TWI %	M	M/TWI %	95 th P	95 th P/TWI %	M	M/TWI %	95 th P	95 th P/TWI %	M	M/TWI %	95 th P	95 th P/TWI %
Cd ^a	0.19	7.6	1.0	40.0	0.20	8.0	0.56	22.4	0.13	5.2	0.28	11.2	0.19	7.6	0.47	18.8
Hg ^b	0.03	2.3	0.15	11.5	0.03	2.4	0.09	6.7	0.02	1.5	0.04	3.1	0.03	2.3	0.07	5.6

The B3 brand, the origin of the highest trace elements exposure, was chosen for comparison with TWI values. M = median, 95thP = 95th percentile. ^A TWI for Cd = 2.5 µg/kg bw/week (EFSA, 2009).

^B TWI for methylmercury expressed as mercury, TWI for Hg = 1.3 µg/kg bw/week (EFSA, 2012b).

Table III-4. 8: Population categories exposure to Cr, Co, and Ni trace elements via white pita consumption and comparison with the tolerable daily intake (TDI). Exposure values are expressed in µg/kg body weight/day.

Trace Elements	Population Categories' Exposure to Tes via White Pita and Comparison with TDI															
	Children				Teenagers				Women				Men			
	M	M/TWI %	95 th P	95 th P/TWI %	M	M/TWI %	95 th P	95 th P/TWI %	M	M/TWI %	95 th P	95 th P/TWI %	M	M/TWI %	95 th P	95 th P/TWI %
Cr ^a	1.74	0.58	9.0	3.0	1.79	0.6	5.06	1.69	1.21	0.40	2.47	0.82	1.72	0.57	4.26	1.42
Co ^b	0.41	25.6	2.09	130.6	0.42	26.3	1.18	73.8	0.28	17.5	0.57	35.6	0.4	25.0	0.99	61.9
Ni ^c	2.80	100	10.26	367	4.06	145	12.26	438	2.75	98	8.83	315	4.34	155	8.86	317

The B3 brand, the origin of the highest trace elements exposure, was chosen for comparison with TDI values, except for nickel for which the B1 brand was retained. M = median, 95thP = 95th percentile. ^A TDI for Cr (III) = 300 µg/kg bw/day (EFSA, 2014b). ^b TDI for Co = 1.6 µg/kg bw/day (ANSES, 2017). ^C TDI for Ni = 2.8 µg/kg bw/day (EFSA, 2015).

Table III-4. 9: Margin of exposure (MOE) to Pb and As trace elements via white pita consumption for the Lebanese population categories.

Population Categories' Exposure to Tes via White Pita and Comparison with the Margins of Exposure																								
Tes	Children						Teenagers						Women						Men					
	Median			95 th Percentile			Median			95 th Percentile			Median			95 th Percentile			Median		95 th Percentile			
	Value	MOE	MOE	Value	MOE	MOE	Value	MOE	MOE	Value	MOE	MOE	Value	MOE	MOE	Value	MOE	MOE	Value	MOE	MOE	Value	MOE	MOE
	*	1	2	*	1	2	*	1	2	*	1	2	*	1	2	*	1	2	*	1	2	*	1	2
Pb ^a	1.25	0.40	—	6.43	0.08	—	1.28	0.39	—	3.61	0.14	—	0.86	0.58	—	1.77	0.28	—	1.23	0.41	—	3.04	0.16	—
As ^b	1.54	0.19	5.19	7.97	0.04	1.00	1.58	0.19	5.06	4.48	0.07	1.79	1.07	0.28	7.48	2.19	0.14	3.65	1.53	0.20	5.23	3.77	0.08	2.12

The B3 brand, the origin of the highest trace elements exposure, was chosen for the margin of exposure calculations. * Values of median and percentiles are expressed in µg/kg bw/day. ^A Pb margin of exposure calculated with BMDL_{0.1} = 0.5 µg/kg bw/day based on neurotoxic effect (E. EFSA, 2010). ^B As margin of exposure calculated with BMDL_{0.1} = 0.3 µg/kg bw/day for MOE 1 and BMDL_{0.1} = 8 µg/kg bw/day for MOE 2(EFSA, 2009).

III-4.4 Discussion

III-4.4.1 Survey

The direct input of the answers to the questions asked by the data collectors on electronic tablets made it possible to calculate exposure values to TEs at the level of the individual insofar as we had all the necessary data at this scale: age, gender, body weight, daily intake, and brand of pita. The most recent works on this topic were based on a survey conducted in 2001 and for which the calculation of exposure from the intake was based on the average body weight of the whole surveyed sample (Nasreddine et al., 2010, 2006). This survey was based on a nationally representative survey, a real sample covering 992 Lebanese consumers. To our knowledge, this was the first survey assessing the pita diet in Lebanon in different Lebanese regions covering young people and adults. Previous studies focused on adults in the Beirut area only. There was a large standard deviation in the distribution of bread consumption among adults in all regions. This could be explained by the fact that people aged 65 and over eat a lot of bread.

The use of the questionnaire responses provided a good overview of pita consumption by region, age, and gender. It allowed for the drawing of typologies of people living in these regions too. The combination of estimates on consumption from the survey and the analytical results from pita experiments made it possible to do a risk evaluation.

The exposure study was done on a population consuming mainly white pita (762 individuals out of 992). The remaining population who did not consume white bread would be, in principle, more exposed to trace elements based on the article by Bou Khouzam et al. (2012) which emphasizes that Lebanese brown bread is more contaminated than white.

III-4.4.2 Risk Characterization

In terms of risk characterization linked to the seven trace elements analyzed in the pita bread, a highly consumed foodstuff, two of the trace elements, Cd and Hg, appeared to not be a safety concern. The results for Cd are in accordance with Nasreddine et al. (2010) study conducted on the total diet in an adult urban population. Bread may not be the main food involved in the highest exposure to Cd, as these authors showed that the main contributors

to the dietary intake of Cd in a Beirut TDS were vegetables (46.8%) followed by breads and cereal-based products (30.9%). Same as for Cd, bread is not the main source of Hg, for which it is fish and seafood, which is a concern for high fish consumers (ANSES, 2019) and, particularly, pregnant women (EFSA, 2012b). We found between 0.70 and 0.89 $\mu\text{g kg}^{-1}$ of Hg in Lebanese pita which is by far less than in the study of Mestek and co-workers (Mestek et al., 2015) who found 13 $\mu\text{g kg}^{-1}$ of Hg in bread in the Czech Republic.

In our study, total chromium was analyzed. The B3 pita contained the highest level of Cr (0.36 mg kg^{-1} of dry weight). It is known that the major form of Cr in food is Cr (III), and the major contributor of Cr is bread (8%) followed by bakery products. For children, it is milk (9%) followed by pasta (6%) (Soares et al., 2010). In this study, the analytical data from the two brands (B1 and B2) were in accordance with Soares (Soares et al., 2010) team who quantified the total chromium contents ($47.3 \pm 20.0 \mu\text{g kg}^{-1}$) of dry weight white bread samples.

Compared to Cr (VI), Cr (III) is considered an essential element for humans and authorized in food supplements in Europe without any upper limit (Directive 2002 /46 EC, 2002). But, in 2010, the (EFSA, 2010a) recommended that total Cr does not exceed 250 $\mu\text{g/day}$, a value established by the WHO (WHO, 1996). Furthermore, Cr (III) can complex with lactate and picolinate, causing concern as it has been demonstrated to be genotoxic (EFSA, 2014). In contrast, Cr (VI) is known to be highly toxic and carcinogenic (Group 1, IARC, 2012). It also induces skin dermatitis, then the interconversion will be of relevance in terms of risk assessment.

Even if data on the presence of Cr (VI) in food are missing, it is considered that food is a large reducing medium and that oxidation of Cr (III) to Cr (VI) would not occur. Additionally, most of the Cr (VI) is considered to be reduced in the stomach to Cr (III) (EFSA, 2014b).

Humans are exposed to Cr mainly via food, but it can be present also in water. Concerning bottled water in Lebanese markets (Semerjian, 2014), a study of the physicochemical and heavy metal parameters in 32 samples of bottled waters, 25 of these samples showed values below 1 $\mu\text{g L}^{-1}$ for Cr, while the other samples showed values ranging between 1 and 4 $\mu\text{g L}^{-1}$ in the drinking water. Although naturally occurring Cr (VI) is rarely found in the environment, its presence in ground and surface waters, as a consequence of oxidation of Cr (III)-bearing minerals by Mn (IV) oxides, has been reported recently (Ščančar and Milačič,

2014). Furthermore, in terms of the total quantity found in bread, we do not know its respective part coming from the wheat flour or water used during the bread-making process. Wheat are among species that demonstrate the ability to bio-accumulate chromium the most, just after tomato (Gupta et al., 2019). An average value of Cr below 0.05 mg kg^{-1} of dry weight was measured in wheat seeds in Swedish long-term soil-fertility experiments between 1967 and 2003 (Kirchmann et al., 2009).

The recipient might also be a source of contamination if Cr is released. Indeed, migration of chromium from steel material occurred in the past mostly from Asia (China) and was above the upper legal limits for food contact material (RASFF, 2019).

The EFSA panel recommended the generation of data using sensitive analytical methodologies which specifically measure the content of Cr (III) and Cr (VI) in food and drinking water in different EU Member States (EFSA, 2014); the issue is the same for Lebanon. However, Cr speciation is difficult to conduct, especially in complex matrices. Chromium speciation depends on several factors such as pH, concentration, oxidation state, and complexation with natural components present in the matrix such as peptide (Ščančar and Milačič, 2014). Soares and co-workers (Soares et al., 2010) mentioned the presence of Cr (VI) in bread after extraction of Cr by alkaline solutions. However, (Novotnik et al., 2013) demonstrated that the only Cr species in bread is Cr (III) by spiking with enriched isotopes of ^{53}Cr (III) and ^{50}Cr (VI), a more accurate quantification, and using HPLC-ICP-MS. Thus, we made a characterization in regard to Cr (III) and, in this way, there were no safety concerns in this study for Cr (III) in Lebanese pita bread.

For cobalt, the exposure of young people under 15 years old at the 95th percentile exceeded the TDI by a percentage of 131%. Bread and cereals are significant source of Co exposure (18.1%), with a daily intake of cobalt estimated to be around $11.4 \text{ } \mu\text{g/day}$ according to the Nasreddine study (Nasreddine et al., 2010). Indeed, in their study they found $6.36 \text{ } \mu\text{g kg}^{-1}$ of cobalt in bread and cereals. In this study, the level of Co was $91 \text{ } \mu\text{g/kg}$ for the B1 pita brand, which is 7 fold more than in Australian bread and 13.5 fold higher than the data from the Nasreddine study (Nasreddine et al., 2010) but 1.8 fold less than in Ethiopian bread (Ergetie et al., 2012).

In Lebanon, imported flour samples used in Lebanese white pita presented values for cobalt between 2 ± 2 and $33 \pm 7 \mu\text{g kg}^{-1}$ which is far less than in the pita. Cobalt contamination can also occur during bread preparation via the water used. The mean level of Co in tap water is approximately $9 \mu\text{g L}^{-1}$ (IRAL, personal communication). In 2013, the Environmental Working Group's (EWG Tap Water, 2019) mission detected Co in only one sample (6 analyzed) and found $1.01 \mu\text{g L}^{-1}$ (EWG Tap Water, 2019). Furthermore, cobalt can also be released from food contact materials (FCMs) as it is used in ceramics (Saisine, 2010); thus, we can wonder if cobalt migration can occur from recipients used for making bread in Lebanon.

Several forms of cobalt (II) (sulphate, dichloride) are classified as presumed human carcinogens via the inhalatory route (European Parliament and the Council of the European Union, 2008). The IARC classified the soluble form of cobalt II in class 2B but as metal, Co was classified in 2A (IARC, 2012). Although Co (II) salts are able to induce genotoxicity in vitro and in vivo after oral or parenteral exposure; however, there is a lack of carcinogenesis studies in humans and animals following an oral route (EFSA, 2009). The EFSA concluded that it cannot be excluded that cobalt could have a non-threshold toxicological effect (EFSA, 2012). In France, ANSES made the recommendation to reduce food exposure to cobalt in infants under 3 years old (ANSES, 2019). Thus, for cobalt, the daily intake from all dietary sources may be quite pertinent to monitor in order to determine the total exposure of young people under 15 years old in Lebanon.

In regards to nickel, the B1 brand contained the highest value ($1292 \mu\text{g kg}^{-1}$), which was above values found in studies for Nigerian, Ethiopian, and Spanish bread (Cuadrado et al., 2000; Ergetie et al., 2012; Onianwa et al., 2001) but under those obtained in Iranian bread (Khaniki et al., 2005). However, for another brand the value ($365 \mu\text{g kg}^{-1}$) was in the same range.

The average daily intake of nickel was estimated at $126.27 \mu\text{g day}^{-1}$ bread and cereals contributing to a $54.73 \mu\text{g day}^{-1}$ Ni intake in the Lebanese adult urban population (Nasreddine et al., 2010).

In a French TDS study regarding the infant population, the value determined in bread was approximately $69.5 \mu\text{g kg}^{-1}$ (ANSES, 2019). The contribution of drinking water to total

exposure to nickel was very small across dietary surveys and age classes (EFSA, 2015). We do not have an explanation, but this high level could be due to the recipient used by bakers. Using the TDI $2.8 \mu\text{g kg}^{-1} \text{ bw day}^{-1}$ (EFSA, 2015) for oral chronic exposure, the data demonstrate that the percentage of Ni TDI was exceeded 3 to 4 fold for all populations with the 10–14 years old range being the most exposed. These data are in accordance with the TDS studies also showing a safety concern in France for very young people (<3 years old) (ANSES, 2019).

Nickel compounds and metallic nickel are classified as human carcinogens, respectively, group 1 and group 2B after inhalation (IARC, 2012). Available studies in animals suggest that exposure to nickel salts lead to renal effects and increases neonatal mortality with the kidney being the principal target. Reproductive and developmental toxicity were the critical effects after chronic oral exposure retained for risk characterization. However, oral exposure to nickel salts could be at the origin of contact dermatitis for sensitive populations. Indeed, oral nickel can also cause an oral and chronic effect in people dermally sensitized (EFSA, 2015).

In humans, absorption of Ni varies according to the method of exposure (in water it is 40 fold more absorbed compared to food; 0.7% and the bioavailability varies with the food type (ATSDR, 2005). There are no maximum levels in food for nickel; in contrast, a value of $20 \mu\text{g L}^{-1}$ is fixed in water intended for human consumption and in mineral waters in Europe (Commission Directive 2003/40/EC, 2003; Council Directive 98 83 EC, 1998).

In this study, arsenic exposure was more worrying, as we found a high level of arsenic for all brand analyzed ($235\text{--}400 \mu\text{g kg}^{-1}$) which is around 63 fold higher than those found in a bread study in the UK (Sadee et al., 2015) and 32 fold higher than those mentioned in a French infant TDS study (ANSES, 2019). (Bou Khouzam et al., 2012) mentioned seasonal variation in the arsenic concentration in bread ($33 \mu\text{g kg}^{-1}$ in dry season, which is 4 fold higher than in wet season). Arsenic was not studied by (Nasreddine et al., 2010) and calculated MOEs were very low for all bakeries and the population. These data are in accordance with the data and risk characterization in 19 countries in Europe (EFSA, 2009), for example, in France where there is a safety concern with MOEs lower than 36 (ANSES, 2019), suggesting that the situation in Lebanon is worrying. The inorganic form of As, which is known to be toxic, may cause human malignancies. It was classified as carcinogenic

(group 1) in Reference (IARC, 1987). The toxicity, due to the inorganic form, occurs by an epigenetic mechanism, and there is increasing evidence that early life exposure affecting fetal and infant growth (Vahter, 2008) may cause chronic disease later in life (Godfrey, 2000). This highlights the urgent need to obtain data on the effect of the toxicity of As on development (ANSES, 2019). Arsenic passes through the placental barrier in humans (Hall et al., 2007), resulting in similar levels (ATSDR, 2005) in both the fetus and mother. Both the inorganic form and its methylated metabolites are transferred (Concha et al., 1998). The effect of As on the endocrine system has also been investigated for many years showing a disruption on the gonadal, adrenal, and thyroid endocrine systems (Sun et al., 2016). Furthermore, it is having been shown a toxic effect due to an interaction between Cd and As (Lin et al., 2016; Su et al., 2017). Drinking water, crops irrigated with contaminated water, and food prepared with contaminated water are sources of exposure (Kapaj et al., 2006).

The EFSA's recommendation is to reduce inorganic arsenic exposure in food and develop robust validated methods to determine the inorganic form (EFSA, 2009). We do not distinguish between organic and inorganic form of As in this study. It is known that arsenic intake is higher from solid foods than from liquids.

Furthermore, speciation plays a major role in determining the amount of arsenic absorbed after consumption of As contaminated food as well as bioavailability with significant interspecies differences for organic arsenic. Regards to inorganic form, absorption can be high as demonstrated with contaminated rice (Juhasz et al., 2006; Raber et al., 2012).

Representative data on speciation are indeed scarce. The EFSA panel assumed a proportion of the contaminant to inorganic form (the toxic one) to vary between 50 (best case) to 100% (worst case) of the total arsenic reported in food commodities in Europe, other than fish and seafood (EFSA, 2009). Furthermore, in bread, 92% of the inorganic form has been found (D'Amato et al., 2011). Cereals and cereal-based products were identified as largely contributing to the daily exposure to inorganic arsenic, as it was quantified as 53 µg up to 72 µg of arsenic per kilo for all cereals and cereal product categories. In wheat samples, it was demonstrated that 91% to 95% of As was in the inorganic form (D'Amato et al., 2011; Raber et al., 2012). In wheat plants cultivated in the Bekaa region, the element As presented values

between 288.3 ± 14.7 and 743.7 ± 25.4 mg kg⁻¹. In the irrigation water used in the Bekaa sites cultivated for wheat plants, the As content varied between 0.8 ± 0.3 µg L⁻¹ and 1.2 ± 0.2 µg L⁻¹ (IRAL data). Indeed, the level of As in drinking water in Lebanon varied between 1.2 and 4.5 µg L⁻¹. Water used for making bread can be the main contributor to the level of arsenic in the pita with the main form recovered in drinking water as inorganic. It is important to note that change in the speciation of As can occur during food preparation (Devesa et al., 2008).

Concerning the level of lead in the different brands: B2 and B3 contents were more than 2.5 fold higher than B1 (74, 203, and 260 µg kg⁻¹, respectively). The order of magnitude of B1 is in accordance with Turkish, Iranian, Ethiopian, and Spanish studies on bread (Cuadrado et al., 2000; Ergetie et al., 2012) and 7 fold more higher than in data on the Czech Republic (Mestek et al., 2015). The B3 pita lead content was 32 fold higher than Lebanese data (Nasreddine et al., 2010) which focused on Beirut. The authors noticed that the lead exposure in Beirut was even two fold lower compared to a previous study (Nasreddine et al., 2006). Thus, our data were not in accordance, but it took into account all the Lebanese regions including rural ones. The lead origin may be from wheat. Several samples of Lebanese wheat cultivated in the Bekaa region were studied, showing values of lead varying between 4.5 and 23 µg kg⁻¹ but far less than in the bread, noting that the lead values in Lebanese wheat samples were lower than the values showing in wheat samples in Saudi Arabian markets (2.81 ± 0.08 mg kg⁻¹ dry weight) (Ali and Al-Qahtani, 2012). While, the lead levels, measured in five wheat flour samples in Lebanese markets varied between 0.0045 ± 0.002 and 0.023 ± 0.016 mg kg⁻¹ dry weight, they were lower than values presented in wheat flour samples in the Canary Islands and used for bread production (varying between 0.037 ± 0.013 and 0.056 ± 0.045 mg kg⁻¹ fresh weight) (Tejera et al., 2013).

Furthermore, lead can also be a contaminant issued from recipient food contact materials or water used for bread making.

Human data show that the developing nervous system is a critical target in children. Various water soluble and insoluble lead can induce tumors in rodents, especially renal tumors as a carcinogenic/promoter; brain gliomas were also observed. Classified lead compounds are probably carcinogenic to humans on the basis of limited evidence of

carcinogenicity in humans and sufficient evidence in animals (IARC, 2012).

It is important to note that due to the long lead half-life, chronic exposure is of most concern and children absorb lead after ingestion to a much greater extent (70% of the amount of lead ingested) than adults (20%) (ATSDR, 2010).

All MOEs calculated with exposure only via pita consumption in this study were very low, but in the same range than in France and Europe (ANSES, 2016, 2011; EFSA, 2010), suggesting an exposure safety concern and that it is necessary to decrease Pb exposure, for example, by varying the diet due to the difficulty in reducing Pb contamination.

For both lead and arsenic, this contamination occurred for all bread brands analyzed in this study and there is a safety concern Error! Reference source not found..

III-4.5 Conclusions

This was the first study conducted all over Lebanon showing the exposure of different categories of Lebanese population to trace elements via bread. A survey of daily consumption of pita bread was conducted on a sample of 992 people. Among the four categories (i.e., children, teenagers, women, and men), white bread was the most consumed. Men consumed the highest quantity (282 g/day). The survey revealed that three brands of white pita were the most consumed: B1, B2, and B3.

Seven trace elements (i.e., As, Cd, Co, Cr, Hg, Ni, and Pb) were quantified in pitas coming from B1, B2, and B3. B1 was characterized by high levels of Ni and Co. B2 was characterized by the presence of As and Cd, and B3 was characterized by the presence of Cr, Hg, and Pb. Lead concentrations exceeded the authorized limits of the Lebanese standard for bread in the two brands. Further analysis should be performed on soil, wheat, flour, and water in order to determine the source of the contamination and to reduce exposure.

The B3 brand was at the origin of the highest trace element exposure, except for nickel for which B1 was retained. In terms of risk characterization, there was no safety concern due to the fact of pita consumption for all studied categories with Hg, Cd, Cr, and for Co except the 95th percentile of the children category (6–9 years old). Nickel exposure showed an excess of the TDI up to 4 fold for the most exposed populations (95th percentile). There were safety concerns related to As and Pb, as the margins of exposure were very low. To our knowledge, this is the first time that exposure to As was studied in the Lebanese population. To confirm these observed data, it is urgent to analyze the inorganic arsenic form levels in bread. Experiments are currently being run to study any cocktail effects (synergistic) using pertinent bioassays due to the exposure to the sum of trace element identified in bread. The ultimate objectives are to protect the Lebanese population by reducing exposures to populations particularly exposed to those presenting safety concern and to make recommendations, as bread is only one part of the daily diet.

GENERAL CONCLUSION

&

PERSPECTIVES

In the context of food safety, it was relevant to study the Lebanese white bread since it constitutes an essential part of the daily diet of the Lebanese population. The study of trace elements in the Lebanese white bread and their traceability throughout the bread chain, allowed us to initiate from the wheat cultivated soils in the Bekaa plain, considering the cultivated wheat plants, and reaching the Lebanese white bread. This enabled us to study the exposure of the population to these elements.

The different themes addressed in this thesis are :

1. The approach of a new method of extraction of trace elements in the soil of the Bekaa Plain.
2. The analysis of geogenic and anthropogenic contamination of soils by TEs in the Bekaa Plain and their transfer to the cultivated wheat plants.
3. The exposure of the Lebanese population to trace elements present in Lebanese white bread consumed daily.

Our study allowed us to validate a new method for monitoring trace elements in the Mediterranean cereal soils. It consists of microwave digestion, with $\text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2$ combined with the measurement of flame-AAS, of wheat cultivated soil samples in the Bekaa region. This validated method is found efficient for routine TEs characterization of calcareous and Ca-saturated soils with limited contamination in As, Cd, Cu, Co, Cr and Pb, hence presenting a great substitute to advanced methods on Hydrofluoric acid, as well as aqua regia.

This cheap and safe method of analysis adapted to developing countries, should be verified on different soil types to further extend its use in the Mediterranean area.

We have chosen a field approach to take into account the bioclimatic and anthropogenic factors on the transfer of elements. The 9 sites chosen by LARI allow us to take into account the diversity of soils. The anthropogenic contamination mainly coming from industrial works of the industrial zones in Zahle, Taanayel, and Saadnayel present close to our sites. As well as sewage and runoff carried by surface water.

Among the sites studied in this work, the site B3 showed a contamination by trace elements in soils cultivated by wheat, where this contamination is of anthropogenic origin from intense industrial activities in the area. Due to the current crisis in Lebanon, it is essential to be able to rely more on the local production of wheat and thus the production of bread. In this

context, it was important to study the level of contamination by TEs of wheat produced locally, to be able to assess the risks to human health and to identify means of limiting potential exposure to metals and metalloids. In this study, the analysis of locally produced wheat grains showed high As values, correlated with As extractable by DTPA. The element Pb showed extremely low concentrations in wheat grains, while Cd values were close to limit values. The latter were found to correlate with Cd extractable by DTPA and translocation factors were often greater than 1, showing that Cd was easily translocated into the grain.

Although in this thesis, for the first time in Lebanon, the exposure of different categories of Lebanese population to trace elements via bread consumption was studied. A survey of a daily consumption of the Lebanese white bread was conducted. This survey was carried out at the level of 992 individuals distributed in 4 categories: children, teenagers, women, and men. This survey showed that white bread was the most consumed, especially by men (282 g per day). According to the questionnaire, three most famous bakery's brands of white bread designated by B1, B2, and B3 were collected and analyzed for the presence of seven trace elements (As, Cd, Co, Cr, Hg, Ni, and Pb).

In consequence, the brand B1 was characterized by high levels of Ni and Co, the brand B2 was characterized by the presence of As and Cd, while the brand B3 was characterized by the presence of Cr, Hg, and Pb. Where the Pb concentrations exceeded the authorized limits of the Lebanese standard for bread (NL 240:2010).

According to the Lebanese population's exposure, the B3 brand was at the origin of the highest TEs exposure, except for Ni for which B1 was retained. From a risk characterization point of view, there was no safety concern due to the fact of pita consumption for all studied categories with Hg, Cd, Cr, and for Co except the 95th percentile of the children category (6–9 years old). Nickel exposure showed an excess of the TDI up to 4 fold for the most exposed populations (95th percentile). There were safety concerns related to As and Pb, as the margins of exposure were very low.

Finally, this multidisciplinary thesis shed light on the Tracking Trace Elements from Soil to Lebanese Bread and Population's Exposure. Ranging from the characterization of the soil of the Bekaa plain, passing through the quality of wheat grown in the plain from a Tes point of view, the contamination of Lebanese white bread in the market, as well as the exposure of

the population to trace elements in the bread consumed. In addition, it encouraged a new method of extraction of trace elements from soil.

Following this study, other types of soil should be tested to further extend its use to a wider range of soils to cover the diversity of soils in the Mediterranean region.

According to the current crisis, it may have to rely more on its local production. In this context, it was important to study the level of Cd, As and Pb in wheat, the relationships with soil and environmental factors to assess the risks to human health and to identify ways to limit potential exposure to metals and metalloids.

To our knowledge, this is the first time that exposure to As was studied in the Lebanese population. To confirm these observed data, it is urgent to analyze the inorganic arsenic form levels in bread. The ultimate objectives are to protect the Lebanese population by reducing exposures to populations particularly exposed to TEs presenting safety concern and to make recommendations, as bread is the important part of the daily diet.

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ABSTRACT

In Lebanon, the agri-food industry represents 18% of the industrial sector and yet food safety remains a major problem. In this interdisciplinary thesis, we followed the Trace Elements (TEs) in the Lebanese white bread sector “from farm to fork” based on the reality on the ground, and on two links in the sector: (i) the geogenic or anthropogenic origin of TEs present in the soil and their transfer to the wheat grain, for which 10 plots cultivated in wheat, covering the diversity of soils, irrigation practices and anthropogenic environments of the central Bekaa were chosen; (ii) the exposure of populations to TEs present in bread, for which a survey of consumption habits across Lebanon was carried out on 992 individuals (children, adolescents, women and men) and the quantification of TEs present in bread from the 3 biggest bakeries. A new microwave digestion with $\text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2$ combined with flame – Atomic Absorption Spectrometry measurement was validated with Certified Reference Material and after comparison with Hydrofluoric-based digestion. It allows routine but accurate quantification of TEs As, Cd, Cu, Co, Cr and Pb, “green” and inexpensive, suitable for calcareous or calcium-saturated Mediterranean soils.

In soils, the total TE contents and the bioavailable quantity by DTPA extraction are not correlated. Thus, the B5 and B8 sites have the highest total contents, probably of geogenic origin, but B8 is richer in bioavailable forms linked to industrial activities. Likewise, the B3 and B9 sites, which have low to medium total TE contents, have on the contrary the highest bioavailable TE contents of anthropogenic origin.

In wheat, the level of Cd, As and Pb was studied in roots, stems, leaves and grains. The As contents in grains are high and correlate with the DTPA-extractable As in soil, while the Pb contents in the grain are very low. The Cd content in grains is close to the threshold values. Root Cd content correlates with extractable Cd and translocation factors are often greater than 1, showing that Cd is easily transferred to grain. The high Cd content (20 mg kg^{-1}) of phosphate fertilizers imported into Lebanon is the plausible source of this contamination, hence the importance of its monitoring.

The Lebanese bread consumption survey showed that white bread is consumed the most and men the greatest consumers (282 g d^{-1}). Arsenic, Cd, Co, Cr, Hg, Ni and Pb have been analyzed in white bread from the 3 most consumed brands. There is no health risk with respect to the elements Hg, Cd, Cr and Co, with the exception of the 95th percentile of 6-9 years old children.

Exposure to nickel, on the other hand, showed an excess of total food intake up to 4 times for the most exposed populations (95th percentile). There are food safety issues related to As and Pb because the exposure margins are very low.

Given the current crisis in Lebanon where it will be necessary to rely more on local production, it is important to monitor the levels of As, Cd and Pb in wheat, the relationships with the soil and environmental factors, to assess the risks to human health and identify ways to limit potential exposure to metals and metalloids. To our knowledge, this is the first time that exposure to Arsenic has been studied in the Lebanese population. These observations need to be confirmed, and there is an urgent need to determine the speciation of inorganic arsenic forms in bread.

RÉSUMÉ

Au Liban, l'industrie agro-alimentaire représente 18% du secteur industriel et cependant la sécurité sanitaire reste un problème majeur. Dans cette thèse interdisciplinaire, nous avons suivi les Éléments Traces (ÉT) dans la filière du pain blanc libanais « de la fourche à la fourchette » en se basant sur la réalité du terrain, et sur deux maillons de la filière : (i) l'origine géogénique ou anthropique des ÉTs présents dans le sol et leur transfert dans le grain de blé, pour lequel 10 parcelles cultivées en blé, couvrant la diversité des sols, des pratiques d'irrigation et des environnements anthropiques de la Békaa centrale ont été choisies ; (ii) l'exposition des populations aux ÉTs présents dans le pain, pour laquelle une enquête sur les habitudes de consommation sur tout le Liban a été réalisée sur 992 individus (enfants, adolescents, femmes et hommes) et la quantification des ÉTs présents dans le pain des 3 plus grandes boulangeries.

Une nouvelle méthode d'extraction et de quantification des ÉTs basée sur une digestion par micro-ondes avec l'acide sulfurique et le peroxyde d'hydrogène et combinée à la spectrométrie d'absorption atomique par flamme a été validée avec des matériaux de références certifiés et après comparaison avec la digestion « totale » basée sur une attaque à l'acide fluorhydrique. Elle permet une quantification de routine des ÉTs As, Cd, Cu, Co, Cr et Pb, « verte » et peu coûteuse, adaptée aux sols méditerranéens calcaires ou saturés en calcium.

Dans les sols, la teneur totale en ÉTs et la quantité biodisponible par extraction DTPA ne sont pas corrélées. Ainsi les sites B5 et B8 présentent les teneurs totales les plus élevées probablement d'origine géogénique, mais B8 est plus riche en formes biodisponibles liés aux activités industrielles. De même les sites B3 et B9 qui présentent des teneurs totales en ÉTs faible à moyenne, présentent au contraire les teneurs en ÉTs biodisponibles les plus fortes d'origine anthropique.

Dans le blé, le niveau de Cd, As et Pb a été étudié (racines, tiges, feuilles et grains). Les teneurs en As dans le grain sont élevées et corrélées à l'As du sol DTPA-extractible au, tandis que les teneurs en Pb dans le grain sont très faibles. La teneur en Cd des grains est proche des valeurs seuils. La teneur en Cd des racines est corrélée au Cd extractible et les facteurs de translocation sont souvent supérieurs à 1, montrant que le Cd est facilement transféré dans le grain. La teneur en Cd élevée (20 mg kg^{-1}) des engrais phosphatés importés au Liban est la source plausible de cette contamination, d'où l'importance de sa surveillance.

L'enquête sur la consommation du pain libanais a montré que le pain blanc est le plus consommé et les hommes les plus grands consommateurs (282 g j^{-1}). L'Arsenic, le Cd, le Co, le Cr, le Hg, le Ni et le Pb ont été analysés dans le pain blanc des 3 marques les plus consommées. Il n'y a pas de risque sanitaire vis-à-vis des éléments Hg, Cd, Cr et Co, à l'exception du 95^e centile des 6-9 ans. L'exposition au nickel par contre a montré un excès de l'apport alimentaire total jusqu'à 4 fois pour les populations les plus exposées (95^{ème} percentile). Il y a des problèmes de sécurité liés à l'As et au Pb car les marges d'exposition sont très faibles.

Compte tenu de la crise actuelle au Liban où il faudra s'appuyer davantage sur la production locale, il est important de suivre les teneurs en As, Cd et Pb dans le blé, les relations avec le sol et les facteurs environnementaux, pour évaluer les risques pour la santé humaine et identifier les moyens de limiter l'exposition potentielle aux métaux et métalloïdes. A notre connaissance, c'est la première fois que l'exposition à l'Arsenic est étudiée dans la population libanaise. Il faut confirmer ces observations, et il est urgent de déterminer la spéciation des formes d'arsenic inorganique dans le pain.

ANNEX

ANNEX 1: Survey and questionnaire related to Lebanese bread consumption

A study was conducted on the presence of metallic trace elements (ETM) throughout the Lebanese bread chain, from farm to fork. Some of these elements may cause health problems for the consumer, so the amount of Lebanese bread (likely to be contaminated) consumed per day and per person must be known, to assess the risk related to a route of oral exposure of trace elements.

Metallic trace elements are contaminants in the environment and foodstuffs, including bread. Once in excess of the environment, trace elements enter the soil, water or air and contaminate certain foods. They can cause a risk to human health if the consumer is too much exposed, for example, by ingesting cereals grown in contaminated soils. It is therefore important to monitor the presence of these contaminants in the bread and to know the exposure of the consumer by conducting a survey among the population in different regions of Lebanon.

Personal data collected will only be used for exposure calculations and will not be disclosed. Anonymity will be ensured throughout the study.

There is no intervention to undergo or therapeutic protocol to follow.

- **Do you have a chronic or serious illness?** Yes No
- **Do you eat Lebanese bread for more than 15 years?** Yes No

Do you live in Lebanon for more than 15 years? (Criteria for adults only)

Yes No

If you meet the above criteria, please complete this questionnaire, which will take you less than ten minutes

Date..... investigator.....

Part 1. Consumption of Lebanese bread

What is the kind of Lebanese bread eaten?

1 White bread 2 Brown bread 3 Other please specify:

What is the brand of bread most consumed?

1 Brand 1 2 Brand 2 3 Brand 3

4 Brand 4 5 Others please specify:

What is the size of pita consumed?

1 Small 2 Medium 3 Large

What is the number of pitas consumed per day?

1 2 3 4 Others please specify:

Part 2. Sociodemographic data

5. Age: | | |

Gender: 1 Man 2 Woman

Place of residence:

1 Beirut 2 Mount Lebanon 3 North Lebanon 4 South Lebanon and Nabatieh 5 Bekaa

Region: 1 Urban 2 Rural

Level of education:

1 Secondary 2 Technical secondary 3 Secondary
4 Technical University 5 Non-technical University

Economic activity:

1 Works 2 Seeks a job first
3 Unemployed 4 Studied
5 Study and work 6 Retired or annuitant
7 Other inactive

If you work (or have worked), the profession:

0 Armed Forces
 Members of the executive and legislative bodies, senior managers of public administration, executives and senior company executives
 Intellectual and scientific professions 3 Intermediate professions
4 Employees of administrative type 5 Service, store and market sellers
6 Farmers and skilled workers in agriculture and fisheries 7 Artisans and handcraft workers
8 Plant and machine operators and assembly workers 9 Workers and unskilled workers
10 Other specify:

Part 3. State of health**Are you a smoker?**1 Yes 2 No**If yes, please specify:**

Cigarette: a- Number of cigarettes / day: | | | b- Number of years of smoking: | | |
 Nargile: c- Number of nargile / week: | | | d- Number of years of tobacco: | | |

Are you an alcohol drinker?1 Yes 2 No**If yes, please specify:**a- 1 Occasional or 2 Regular b- Number of glasses / day: | | |

c- Number of years of consumption: | | |

How do you rate your health?1 Very good 2 Good 3 Fair 4 Bad 5 Very bad

a- Weight: |__| Kg b-Size: | | | |

Do you exercise?1 Yes, low intensity physical activity 2 Yes, moderate intensity physical activity 3 Yes, high intensity physical activity 4 No**If yes, how much time do you spend per week?** | | hours

Intensity and measurement of physical activity

Physical activity is a behavior that can be characterized by a frequency, intensity, duration and type of practice that define the amount of physical activity in a space-time (day, week, etc.).

MET: The intensity of a physical activity is most often expressed in MET (metabolic equivalent of task), defined as the ratio of energy expenditure related to physical activity on basic metabolism. 1 MET is the level of energy expenditure at rest, sitting on a chair (3.5mL O₂/mn/kg).

Physical activity of low intensity (<3 MET) It does not require effort (no breathlessness, no perspiration)

Examples:

Iron

Dust off

Clean the windows

To make the beds

Cook, do the dishes, do the shopping

Repair and wash the car

To vacuum

Sweep gently

Carry loads up to 6 kg when climbing the stairs

Clean

Physical activity of moderate intensity (about 3-6 MET)

It requires a moderate effort and significantly accelerates the heart rate. (Moderate shortness of breath, possible conversation, moderate sweating)

Examples:

walk briskly

dance

garden

engage in traditional hunting and gathering

actively participate in games and sports with children / take out your pet

do crafts (For ex: roof repairs, painting)

lift / move loads <11kg

High intensity physical activity (approximately > 6 MET)

It requires a great effort, the breath is shortened and the heart rate accelerates considerably.
(Marked breathlessness, difficult conversation, heavy transpiration)

Examples:

run

walk briskly / climb at fast pace

ride a bike at high speed

do aerobics

swim at high speed

play sports and competitive games (For ex: traditional games, football, volleyball, hockey, basketball)

doing hard work

lift / move heavy loads of 11 kg or more

ANNEX 2: Toxicological data sheet for As

Generalities

Arsenic is a chemical element with symbol As and atomic number 33.

It is a metalloid and a natural component of the earth's crust. It is widely distributed throughout the environment in the air, water and land. It is present in two forms, organic and inorganic, where the inorganic arsenic, is the more toxic form (EFSA, 2009).

Arsenic occurs naturally as trace component in rocks and sediments. It is released into groundwater due to these geologic sources and also as a result of human activities such as mining, various uses in industry, in animal feed, and as a pesticide. In drinking-water supplies, arsenic poses a problem because it is toxic at low levels and is a known carcinogen. The organic and inorganic arsenic compounds may enter the plant food chain from agricultural products or from soil irrigated with arsenic contaminated water (Ratnaike, 2003).

The primary use of arsenic is in alloys of lead (for example, in car batteries). Arsenic and its compounds, especially the trioxide, is used in the production of pesticides, treated wood products, herbicides, and insecticides (Naidu, R. et al. 2006). However, these applications are declining due to the toxicity of arsenic and its compounds:

- Contaminated water used for drinking, food preparation and irrigation of food crops poses the greatest threat to public health from arsenic.
- Long-term exposure to arsenic from drinking-water and food can cause cancer and skin lesions. It has also been associated with cardiovascular disease and diabetes.
- The early childhood exposure, has been linked to negative impacts on cognitive development and increased deaths in young adults.
- The most important action in affected communities is the prevention of further exposure to arsenic by provision of a safe water supply.
- Industrial activities: Agricultural pesticides and herbicides. Paints, fungicides, insecticides, wood preservatives, and cotton desiccants. Manufacture of semiconductors, light emitting diodes, and components of lasers and microwave circuits, in the processing of

glass, pigments, textiles, paper, metal adhesives, and ammunition Arsenic is present as a contaminant in many traditional remedies.

- Arsenic is also used in the hide tanning process and, to a limited extent, in pesticides, feed additives and pharmaceuticals. Arsenic trioxide is now used to treat acute promyelocytic leukaemia.
- Combustion of fossil products.

The International Agency for Research on Cancer (IARC) has listed As as a human carcinogen since 1980. Terminology Arsenicosis is a chronic illness resulting from drinking water with high levels of As over a long period of time. It is commonly known as As poisoning. Arseniasis means chronic arsenical poisoning, also called arsenicalism; the term arsenicism refers to a disease condition caused by slow poisoning with As. Arsenic exerts its toxicity by inactivating up to 200 enzymes, especially those involved in cellular energy pathways and DNA synthesis and repair.

In addition, (Ferrecio et al., 2000), showed in their study a positive correlation between ingestion of inorganic As and lung cancer in humans in Chile. It is already known that cigarette smoking is a main risk factor for lung cancer.

Arsenic exposure

Arsenic toxicity is a global health problem affecting many millions of people (Ratnaike, 2003). In Europe, the food is the main source of exposure to As for the population. EFSA opinion mainly focuses on inorganic arsenic which can be found in groundwater (EFSA, 2009).

Arsenic exposure occurs:

- from inhalation
- By absorption through the skin
- By ingestion of contaminated drinking water and food

Arsenic in food occurs as relatively nontoxic organic compounds (arsenobentaine and arsenocholine). Seafood, fish, and algae are the richest organic sources (Ratnaike, 2003).

People are exposed to elevated levels of inorganic arsenic (toxic) through drinking contaminated water, using contaminated water in food preparation and irrigation of food crops, industrial processes, eating contaminated food and smoking tobacco.

A long-term exposure to inorganic arsenic, mainly through drinking-water and food, can lead to chronic arsenic poisoning, neurotoxicity, skin lesions, cancers, diabetes, and glucose metabolism change, and cancer.

Human exposure:

1. Inhalation exposure

People who smoke tobacco can also be exposed to the natural inorganic arsenic content of tobacco because tobacco plants can take up arsenic naturally present in the soil. The exposure is greater when tobacco plants were treated by lead arsenate insecticide. According to the California Air Resources Board and the Department of Health Services in USA, 2020, smokers may breathe between 0.8 to 2.4 µg of inorganic arsenic per pack of 20 cigarettes (with approximately 40 percent of it being deposited in the respiratory tract). Ferreccio et al.(2000), found arsenic from tobacco and drinking water to have a synergistic effect.

2. Dermal exposure

Dermal contact when handling preserved wood products containing arsenic could result in arsenic exposure. However, very little is known regarding the chemical form, conditions for absorption, kinetics, or other information needed to make a statement regarding skin absorption in specific populations. Toxic effects have been reported in the occupational literature from splashes of arsenic trichloride or arsenic acid on worker's skin (Garb and Hine 1977). The dermal exposure may lead to illness, but to a lesser extent than ingestion or inhalation routes of exposure.

Children who play on wood structures treated with CCA have increased likelihood of dermal contact or ingestion of the arsenical through normal mouthing and play activities ("Guidelines for

Carcinogen Risk Assessment Risk Assessment Forum U.S. Environmental Protection Agency Washington, DC," 2005).

3. Ingestion exposure via drinking-water and food

The greatest threat to public health from arsenic originates from contaminated groundwater. The risk of As contamination is generally much higher in groundwater compared to surface water. Inorganic arsenic is naturally present at high levels exceeding $10\mu\text{g L}^{-1}$ in the groundwater of a number of countries, including Argentina, Bangladesh, Chile, China, India, Mexico, and the United States of America. Drinking-water, crops irrigated with contaminated water and food prepared with contaminated water are the sources of exposure (Kapaj et al., 2006).

Fish, shellfish, meat, poultry, dairy products and cereals can also be dietary sources of arsenic, although exposure from these foods is generally much lower compared to exposure through contaminated groundwater. In seafood, arsenic is mainly found in its less toxic organic form (EFSA, 2009). High consumers of rice in Europe, such as certain ethnic groups, are estimated to have a daily dietary exposure of inorganic arsenic of about $1\mu\text{g kg}^{-1}$ b.w. per day

In food, cereal grains and cereal based products, food for special dietary uses, bottled water, coffee and beer, rice grains and rice-based products, fish and vegetables were identified as largely contributing to the inorganic arsenic daily exposure in the European population.

The temporary WHO guideline for As in drinking water is $10\mu\text{g L}^{-1}$. On the basis of investigations initiated by the National Academy of Sciences, it was concluded that this standard did not eliminate the risks of skin, lung, and prostate cancer from long-term exposure to low As concentrations in drinking water.

Absorption

The exposure to arsenic occurs primarily through ingestion (mostly solid foods) and also through inhalation and absorption through the skin. It is found in foods mainly in relatively non-toxic organic form (arsenobentaine and arsenocholine).

Organic and inorganic arsenic compounds can enter the plant food chain from agricultural products produced or from soils irrigated with contaminated arsenic water.

Metabolism

The organic compounds may lead to high levels of arsenic in the blood but are rapidly excreted unchanged in the urine. The major site of absorption of As in the human body, is the small intestine by an electrogenic process involving a proton (H^+) gradient, where the pH is 5.0 (Ratnaïke, 2003).

The arsenic intestinal absorption is considered as a complex process having several underlying molecular pathways and mechanisms, and it all depends on the chemical form in which it is found (Calatayud et al., 2015).

The As in its both forms are absorbed, it undergoes hepatic characterization to form monomethylarsonic acid and dimethylarsinic acid that are less toxic but not really safe (DJ. Thompson, 1993, HV. Aposhian, 1997, (Ratnaïke, 2003).

Distribution

After acute poisoning, the studies show that the highest concentration of arsenic is in the kidneys and liver. In chronic arsenic ingestion, arsenic accumulates in the liver, kidneys, heart, and lungs and smaller amounts in the muscles, nervous system, gastrointestinal tract, and spleen. Though most arsenic is cleared from these sites, residual amounts remain in the keratin-rich tissues, nails, hair, and skin. After about two weeks of ingestion, arsenic is deposited in the hair and nails.

Elimination

Organic compounds cause raised arsenic levels in blood but are rapidly excreted unchanged in urine.

The elimination of the toxic is done by the vomitings and the faeces, the urines, the pharenas and the skin. For mineral compounds, it is quite slow for 15 to 40 days, vomiting and stool can eliminate most of the poison at the time of ingestion. elimination of organic compounds, introduced by the venous vein would be achieved in 48 hours.

Fifty percent of the ingested dose may be eliminated in the urine during three to five days. Dimethylarsinic acid is the dominant urinary metabolite (60%–70%) compared with monomethylarsonic acid. A small amount of inorganic arsenic is also excreted unchanged.

Toxicity in humans

Arsenic in drinking water can affect human health and is considered as one of the most significant environmental causes of cancer in the world (WHO, 2018). Inorganic arsenic known as carcinogenic found in water is highly toxic while organic arsenic found in seafood is less harmful to health Chronic arsenic toxicity results in multisystem disease.

Health effects

- ***Acute effects***

The acute arsenic poisoning is associated initially with nausea, vomiting, abdominal pain, and severe diarrhea. Encephalopathy and peripheral neuropathy are also reported. The immediate symptoms of acute arsenic poisoning include vomiting, abdominal pain and diarrhea. These are followed by lack of feeling and tingling of the extremities, muscle cramping and death, in extreme cases.

- ***Treatment:*** The elimination is done:

By gastric lavage as early as possible with pure or calcined magnesia.

By hydroelectrolytic re-equilibration by serum glucose infusion. of sodium and potassium.

By rehydration

By treatment of hepatic impairment by hepato-protectors.

- ***Long-term effects***

Alcoholics are the most predisposed, the general action of the As is that of a cellular poison which blocks the thiol groups of enzymes and proteins fixed on a radical R: it is a thioloprive body.

The first symptoms of long-term exposure to high levels of inorganic arsenic are usually observed in the skin, and include pigmentation changes, skin lesions and hard patches on the palms and soles of the feet (hyperkeratosis). These occur after a minimum exposure of approximately five years and may be a precursor to skin cancer.

A long-term ingestion of inorganic arsenic may cause developmental effects, diabetes, pulmonary disease, and cardiovascular disease. Arsenic-induced myocardial infarction, and may cause mortality. Arsenic is also associated with adverse pregnancy outcomes and infant mortality, with impacts on child health (Farzan). The exposure in early childhood has been linked to increases in mortality in young adults due to multiple cancers, lung disease, heart attacks, and kidney failure. In China, areas that have chronic arsenism also have increased levels of fluoride in the drinking water. There are suggestions that the combination of the two could increase the risk to human health due to potential synergism.

Numerous studies have demonstrated negative impacts of arsenic exposure on cognitive development, intelligence, and memory (Tolins et al. 2014).

Classification of Cancer

- **Skin Cancer**

A significant relationship between As exposure and skin cancer has been observed. The arsenite can play a role in the enhancement of UV-induced skin cancers. This element is associated with cancers of skin and internal organs, as well as with vascular disease.

- **Bladder Cancer**

In a U.S. study, there was an elevated risk of bladder cancer in smokers that were exposed to As in drinking water near $200 \mu\text{g L}^{-1}$, compared with smokers consuming lower As levels. These data suggest that As is synergistic with smoking at relatively high As levels ($200 \mu\text{g L}^{-1}$). Bates M. et al. (2004), highlighted that latency of As exposure causing bladder cancer can be very long (more than 40 years).

- **Lung Cancer**

The mortality from lung cancer was significantly increased with increasing As ingestion (Hopenhayn-Rich et al., 1998). In addition, As and cigarette smoke are synergistic, thus increasing the risk of lung cancer. In a recent Taiwanese study, residents in arseniasis-endemic areas were followed during an 8-year period.

An increased risk of lung cancer was associated with high levels of As exposure *via* drinking water. The authors suggested that reduction in As exposure should reduce the lung cancer risk in cigarette smokers.

The studies indicated that the mortality from lung cancer declined after the levels of As in the well water were reduced.

WHO response

Arsenic is one of WHO's 10 chemicals of major public health concern. WHO's work to reduce arsenic exposure includes setting guideline values, reviewing evidence, and providing risk management recommendations. WHO publishes a guideline value for arsenic in its Guidelines for drinking-water quality, these Guidelines are intended for use as the basis for regulation and standard setting worldwide. The current recommended limit of arsenic in drinking-water is $10 \mu\text{g L}^{-1}$, although this guideline value is designated as provisional because of practical difficulties in removing arsenic from drinking-water.

Risk characterization

Regarding the organic form of arsenic, there is no VTR available

The EFSA, 2009, concluded that the overall range of BMDL₀₁ values of 0.3 to 8 µg kg⁻¹ b.w. per day should be used instead of a single reference point in the risk characterization for inorganic arsenic.

Non-carcinogenic effects of chronic arsenic ingestion have been detected at arsenic doses of the order of 0.01 mg kg⁻¹ per day and above as per EPA, 2000.

ANNEX 3: Toxicological data sheet for Cd

Generalities

It is a metal found in the earth's crust (0.1mg kg^{-1}). It is discovered by Fredrich Stromeyer in 1817, has the symbol Cd and has an atomic number 48, an atomic mass 112.4 g mol^{-1} , a melting point 321°C , and a boiling point 767°C . The pure cadmium is a soft and silver-white metal. This metal is soluble in acids but not in alkalis, the cadmium chloride and cadmium sulfate are soluble in water.

In the atmosphere the cadmium (CdO) may be present in dust or fumes, this metal can be used in industries (metallurgy, electric batteries, paint pigments): oxide, chloride, sulphate, nitrate or sulphide. It is well known that we can find this element in cigarette smoke as fine particles of cadmium oxide, depositing mainly in the cells (ANSES, 2011). Cadmium oxide (CdO) may be present in the atmosphere in the form of dust or fumes. This last term applies to particles resulting mainly from the oxidation of Cd and which therefore have a finer granulometry than the dust resulting from the setting suspension of cadmium oxide powder. It can be present as effluents from many combustion processes such as waste incineration.

It is used in industrial and agricultural activities (phosphate fertilizer) as in Ni-Cd batteries, pigments, coatings and veneer. (Morrow 2001). Cadmium contained in agricultural soils, due to atmospheric deposition or direct application methods such as phosphate fertilizer application and municipal waste composting (Morrow 2001), and water can be taken by certain crops and aquatic organisms and accumulates in the food chain. In surface freshwater or groundwater, outside polluted areas, the cadmium concentration is generally less than $1\text{ }\mu\text{g L}^{-1}$. While, in France, the limit value for the concentration of cadmium in water intended for human consumption is $5\text{ }\mu\text{g L}^{-1}$.

Cadmium levels in the soil, principally derived from natural sources, phosphate fertilizers and sewage sludge will naturally impact upon this cadmium uptake of plant. However, this effect is secondary to the type of crop grown and the agricultural practices followed with respect to tillage, Aiming and crop rotation. Via the roots, the element cadmium entered the plants and thus cadmium easily enters the plants through their roots and passes to the food chain.

Exposure

The human may be exposed to cadmium through ingestion of food and drinking water or inhalation of particulates from ambient air or tobacco smoke. The majority of cadmium may enter the human body directly or indirectly from terrestrial foods (plants and meat from animals). This element may be transferred to plant via absorption from the soil or via deposition on the edible plant parts from the atmosphere.

In Lebanon, the exposure level may be even higher in light of the increased pollution due to the garbage crisis that started in 2015 (Azar et al., 2016)

As per (Nasreddine et al., 2006), and following a study on the dietary exposure of an adult urban population to three heavy metals (lead, cadmium and mercury) and to radionuclides, the average dietary intakes of cadmium is 17 percent, of the appropriate provisional tolerable weekly intakes (PTWI).

Routes of exposure

Oral exposure

1. Via food

For nonsmokers, food is the major source of cadmium exposure (NTP, 2005). The main source of cadmium exposure in human is diet especially the cadmium-rich diets such as fruits, vegetables, cereals, shellfish, crustaceans, oysters, scallops, mussels, offal and others. In addition, rice is considered as the most accumulating crop with high concentrations of cadmium if grown on cadmium-polluted soil.

2. Via drinking water

Cadmium exposure from drinking-water is relatively non important compared to diet exposure. However, impurities in the zinc of galvanized pipes and solders in fittings, water heaters, water coolers and taps can sometimes lead to increased cadmium levels in drinking water.

Absorption

A small amount of the cadmium in food and water (about 1–10%) will enter the human body through the digestive tract. If there is not enough iron or other nutrients in the diet, the cadmium is taken from the food more than usual.

The form in which cadmium is present in food, affects the cadmium absorption (Crews et al., 2000). A high cadmium level presents in food and drinking water will severely irritate the stomach, and lead to vomiting and diarrhea, and sometimes death. The estimated cadmium absorption ranged from 1.1 to 10.6% (Vanderpool and Reeves, 2001).

The unabsorbed cadmium is retained in the gastrointestinal tract where some may be trapped in the intestinal mucosa without passing into the blood or lymph. While the absorbed cadmium is first transferred to the liver through the blood where it is bond to proteins to form complexes that are transported to the kidneys. Once it is accumulated in kidneys, it damages filtering mechanisms and kidney.

A small quantity of cadmium absorbed for a long period, may lead to a build-up of cadmium in the kidneys which may cause damage of kidneys, or allows the bones to become fragile and break easily. The severity of bone effects increased after menopause.

The level of absorption depends on the type of absorption: 2% to 6% of the cadmium ingested is actually taken up into the body (ATSDR 1997), in contrast 30% to 64% of inhaled cadmium is absorbed by the body.

In conclusion, the kidney and liver are the two specific target tissues in which cadmium accumulates. It can be also accumulated in muscles.

The daily intake of cadmium is generally between 10 and 35 μg (generally less than 20 μg). Since January 2009, the European Food Safety Authority (EFSA) has set a lower tolerable weekly intake (DHT) of cadmium than in the past, equal to 2.5 $\mu\text{g kg}^{-1}$ of body weight per week, while set by JECFA, joint FAO/WHO committee is 7 $\mu\text{g kg}^{-1}$ body weight per week. Depending on the food the absorption rate is between 1 and 10%.

According to EFSA, the exposure rate of certain subgroups such as vegetarians, children, smokers and people living in heavily contaminated areas could reach more than twice the DHT.

Distribution

Most of the cadmium that enters the human body goes to kidney and liver where they can remain for long time.

In human body, the cadmium is mainly accumulated in the liver and kidneys and in other tissues particularly muscle, skin, and bone.

Systemic cadmium is widely distributed in the body, knowing that at birth, cadmium levels in the liver and kidneys are almost zero. In human life, the cadmium levels in the kidneys increase progressively up to the age of 50-60 years, with an average level of $0 - 50 \mu\text{g g}^{-1}$, then stabilize or decrease slightly. While, in the liver, the cadmium levels increase rapidly to $1 - 2 \mu\text{g g}^{-1}$ at 20-25 years of age, then increase much more slowly thereafter (ATSDR, 2008).

The most dangerous characteristic of cadmium is that it accumulates throughout lifetime. The cadmium coming from the lung or the gastrointestinal tract is transported in blood plasma. In the blood plasma, it binds to albumin. Cadmium bound to albumin is preferentially taken up by the liver. In the liver, cadmium induces the synthesis of metallothionein and a few days after exposure metallothionein-bound cadmium appears in the blood plasma. Because of its low molecular weight, cadmium-metallothionein is efficiently filtered through the glomeruli and thereafter taken up by the tubules

Metabolism

The preferred complexing agents of cadmium are metallothioneins (MT) which are low molecular weight proteins rich in cysteine and containing sulfhydryl groups. MTs are synthesized in the liver, kidneys and also other organs, including the intestines and lungs [OEHHA, 2006]. The cadmium can be transported in the blood by forming bound to MTs, albumin or erythrocytes. The small size of Cd-MT complex in the kidneys facilitates the passage of cadmium through the glomeruli to the renal tubules where it easily penetrates by pinocytosis into the tubular cells by binding in particular to apical megalin and cubilin. Pinocytosis vacuoles fuse with lysosomes whose enzymes degrade TM and release cadmium. Cadmium then recombines with MTs synthesized by tubular cells and can accumulate in the kidneys, in non-toxic complexed form, for several decades [OEHHA, 2006]. As it is absorbed, cadmium continues to accumulate in the kidneys until the renal MT is saturated. Free residual cadmium then causes lesions in the tubular cells (ANSES 2012).

Retention

The largest proportion of retained cadmium is that inhaled, compared to the ingested one. It is estimated that 42% of a cadmium is retained in the body 5 days after exposure (as per analysis of fecal excretion).

Women aged 53 to 64 are at the same time most sensitive to the retention of cadmium (which seems to decrease slightly) and osteoporosis (Hood, 2006). (Ernie Hood, « Putting a Load on Your Bones: Low-Level Cadmium Exposure and Osteoporosis » [archive] Environ Health Perspect. 2006;114(6): A369–A370).

Elimination and excretion

Most cadmium ingested is not absorbed and is excreted in the feces. Although oral absorption of cadmium is lowest, after ingestion more than 90% of the cadmium is contained in the feces. Intestinal elimination of ingested labeled cadmium takes several days, weeks or months, indicating retention. Cadmium in intestinal mucosal cells gradually evacuated by the fecal route [OEHHA, 2006]. Absorbed cadmium is excreted very slowly, with urinary and fecal excretion.

Toxicity

Cadmium, a well-known human carcinogen, can lead to renal disease, bone loss, and has a detrimental effect on cognitive ability (Cao et al., 2009; Jeong et al., 2015; Kippler et al., 2012; Sanders et al., 2015; Tian et al., 2009).

Human exposure to toxic chemicals is suspected of being responsible for a wide range of human health disorders. The kidney is the critical target organ of cadmium, due to excessive accumulation of cadmium in the kidneys it may cause also other health effects such as: stomach problems, bone demineralization, reproductive failure and even infertility, damage in the central nervous system, immune system and respiratory system also possibly of DNA damage or cancer development (OSHA, 2013).

They are characterized by degeneration of the proximal tubules and urinary leakage of molecular proteins that can be translated by various signs of proximal tubular involvement such as: enzymuria, aminoaciduria, glycosuria, hypercalciuria, hyperphosphaturia.

At a later stage, tubular involvement extends to the distal tubule, and glomerular involvement can be observed. In the case of severe intoxication, the lesions produced can lead to kidney failure.

Urinary phosphor-calcium leakage can lead to bone involvement that results in osteomalacia and predominant demineralization in the pelvis, which is the site of bone fissures and spontaneous fractures. More recent data on human exposure to cadmium in the general population have been associated with an increased risk of prostate cancer, kidney bladder, breast, uterus and testis. In the European Union, certain inorganic cadmium derivatives are known to be toxic to human fertility and fetal development and have a possible effect on human fertility and development fetal (Exposure of the French population to environmental chemical Institute for Public Health Surveillance). Several studies have found significantly higher cadmium body burdens in women with lower iron stores (Kipler et al. 2009).

Cadmium genotoxicity

Palus et al. (2003) noted from their study of workers occupationally exposed to cadmium that there are statistically significant increases in micronucleus levels and sister chromatid exchanges, as well as DNA fragmentation. And moreover, people living in polished areas showed an increase in lymphocyte micronuclei and severe chromosomal aberrations.

Carcinogenic effects in humans

In 1993, IARC has classified cadmium and its compound as cancerogenic for lung especially for workers exposed to cadmium. Although, as per Sorahan & Esmen (2004), a significant increase in cases of cancer of the pharynx was noticed with cumulative exposure to cadmium.

An increase in mortality rates from lung cancer was detected in case of individuals who worked in the Ni–Cd battery-producing industry.

A hospital-based case control study using cadmium measurements in toenails (Vinceti et al., 2007) showed a odds ratio significantly increased at the highest concentrations. A nested case-control study in a cohort did not find this association, using the same biological sample taken at baseline as the exposure measure (Platz et al.2002).

A cancer of the pancreas has been noticed in 2006 by Krieger et al.

In other hand, this metal has recently been shown to be an endocrine disruptor because of its estrogenic abilities at extremely low doses like other metals such as mercury and lead.

In animals, lead, cadmium, arsenic, mercury, zinc or nickel, can also induce endocrine disruption (Iavicoli et al., 2009; Bogdan et al., 2011). Cadmium in the blood produces in female rats the growth of the mammary glands, as well as an increase in the weight and size of the uterus. In rats' cadmium disrupts the hormonal system.

Risk characterization

The World Health Organization (WHO - ENHIS, 2009) has established a provisional tolerable weekly intake (PTWI) for cadmium at $7 \mu\text{g kg}^{-1}$ of body weight, this PTWI weekly value corresponds to a daily tolerable intake level of $70 \mu\text{g}$ of cadmium for the average 70 kg man and $60 \mu\text{g}$ of cadmium per day for the average 60 kg woman. In 2009, EFSA proposed a tolerable weekly intake of $2.5 \mu\text{g kg}^{-1}$ bw per week using a Benchmark Dose (BMD) approach modeling the dose-response relationship between urinary cadmium and excretion of beta-2 urinary microglobulin (EFSA, 2009). This notice relates to the construction of VTRs for cadmium and its compounds in relation to repeated exposure by inhalation.

According to the JECFA, a PTWI of cadmium is calculated on the assumption that 5% of all the dietary cadmium is absorbed and is proposed to be applied to long-term consumption. This PTWI is established to be $0.007 \mu\text{g g}^{-1}$ bw for cadmium per week (Obeid P. et al., 2014).

The U.S. Environmental Protection Agency (EPA) established a reference dose of food intake of cadmium of $0.001 \mu\text{g g}^{-1}$ bw per day, and the limit of intake of cadmium through drinking water of $0.0005 \mu\text{g g}^{-1}$ bw per day. Such difference of intake of reference doses between food and water is due to the difference in the levels of absorption of cadmium (EFSA, 2009).

ANNEX 4: Toxicological data sheet for Co

Generalities

Cobalt is a chemical element having a symbol "Co" and atomic number 27. It was discovered in 1730 (Moreno-Rojas *et al.*, 2016) and can be naturally present in the environment. The cobalt exists in different forms: Co^{2+} , Co^{3+} , the first form being the most stable. Cobalt is an essential biochemical element in the body, Co is a component of vitamin B12 (ATSDR 2004). It has been identified in high levels in liver, and also in muscles, lungs, lymph nodes, heart, skin, bones, hair, stomach, brain, kidneys, plasma, and bladder of unexposed subjects (INRS 2015).

It is also used in processing of ores such as nickel and copper, in manufacturing of rechargeable batteries, aircraft engines, and parts for gas turbine engines. In addition, it can be used in cemented carbides and diamond tools, in animal feed additives, drying agents for inks, paints and varnishes dyes and pigments, glass bleaches, floor coverings for enameling porcelains, humidity indicators, magnetic recording media, rubber adhesion promoters for radial steel belt tires and vitamin B12 (B. Shedd, 2013).

Human exposure

Exposure of the general population to cobalt is mainly dietary.

Via the food

Cobalt is present in high levels in chocolate, shellfish, dried fruits and oilseeds and pasta (EFSA, 2009). The element Co is known to be a component of vitamin B12, the major dietary sources of vitamin B12 being animal products such as meat (especially liver), fish, poultry, seafood, milk, cheese and eggs.

Naturally, food containing cobalt, according to Biego *et al.* (1998) is supplied by dairy products (32%), fish and seafood, crustaceans (20%), oils, sugars and condiments (16%) and plant products (9%) (AFFSA, 2009).

Via the drinking water

Concentrations in water vary with region, there are no regulations concerning cobalt levels in drinking water. In 1991 the International Agency for Research on Cancer (IARC) estimated from samples of uncontaminated water, that the cobalt concentration was in the order of 1 -10 $\mu\text{g L}^{-1}$. In the United States, cobalt concentrations measured in drinking water are generally less than 1-2 $\mu\text{g L}^{-1}$ (ATSDR, 2012).

Absorption

The gastrointestinal absorption of soluble cobalt compounds such as cobalt chloride is greater than that of insoluble compounds such as cobalt oxide (Lauwerys & Lison, 1994). The water-soluble cobalt salts are rapidly absorbed from the small intestine (*Leggett, 2008*). After absorption of Co^{2+} , the level of cobalt concentration in blood and serum is high in the beginning and then decreases rapidly and gradually, due to tissue uptake, mainly in liver and kidney, and excreted by urine or feces (Simonsen et al., 2012).

The Co is absorbed orally into the gastrointestinal tract at a rate varying between 5 and 45% (Lauwerys & Lison, 1994). The oral absorption varies and depends on the iron concentrations, the sex, the dose, the solubility of the compound and the nutritional status of the individuals. Thus, an iron deficiency will affect the absorption since the latter regulates the expression of carriers in the intestine and therefore a deficiency will induce an increase in the absorption of cobalt diet (INVS, 2016), ATSDR 2004). In addition, cobalt absorption is higher in women compared to men studies (Dominique Lison, 2015).

Cobalt is accumulated primarily in liver, kidney, pancreas, and heart. It does not appear to accumulate with age (AFSSA 2002).

Distribution

After it is breathed in or eaten, some of it leaves the body quickly in the feces.

Metabolism

In human body, the tissue partitioning and biokinetics of cobalt in cells and tissues are closely related to the uptake of calcium with cobalt partitioning primarily into tissues with a high calcium turn-over, and with cobalt accumulation and retention in tissues with a slow turn-over of the cells. In the human red cells, the membrane transport pathway for cobalt (Co^{2+}) uptake appears to be shared with calcium (Ca^{2+}) (Simonsen et al., 2011).

Elimination

In human body, most cobalt is eliminated by the urinary route and to a lesser extent by the fecal route (Lauwerys & Lison, 1994). Urinary excretion is independent of the dose and solubility of the compounds, whereas fecal excretion is proportional to the dose (INRS, 2016). The elimination is depending on the route of exposure, the dose and type of cobalt, and nutritional status (ANSES 2013).

After ingestion of cobalt dichloride, the elimination is mostly fecal. The fecal excretion is proportional to the dose, whereas urinary excretion is independent of the dose and solubility of the compounds (*Health Canada, 2011*).

The renal excretion is initially rapid but decreasing over the first days with retention in tissues for several years (Lauwerys and Lison, 1994; Mosconi et al., 1994). During the first 24h, 40% was eliminated, increasing to about 70% after a week. However, after 1 month about 20% was retained and about 10% after 1 year, with the possibility that this amount of cobalt is permanently retained (Simonsen et al., 2012).

Toxicity

Acute toxicity

The ingestion of cobalt may lead to cardiomyopathie, respiratory effects, gastrointestinal effects, vomiting and nausea. It can also cause death for important doses (IARC, 2005).

The toxic effects may occur after exposure to very high concentrations or following long-term chronic exposure to low concentrations of cobalt (Cámara-Martos & Moreno-Rojas, 2016).

Genotoxic toxicity

Cobalt particles can affect the integrity of DNA both by production of activated species of oxygen and by inhibiting the excision repair system (ANSES 2013). It can cause chromosomal aberrations in vivo and in vitro. A DNA breakage is induced by cobalt metal. The genotoxicity is attributed to oxidative stress and oxidative damage to DNA (Green et al., 2013). Similarly, cobalt can induce apoptosis by changing morphology and DNA fragmentation (De Boeck, Kirsch- Volders, & Lison, 2003). An oxidative DNA damage, leads to a pulmonary mutation that will contribute to an apparition of cancer (Behl et al., 2015).

Carcinogenic Toxicity

The element cobalt has been clearly identified as the cause of respiratory diseases especially when is in metallic form (Lison *et al.* 2001). The inhalation of cobalt in industries appears to increase the risk of lung cancer and especially during chronic exposure to tungsten carbide-related cobalt (ANSES, 2013); This complex is classified according to IARC as probably carcinogenic to humans (category 2A) whereas cobalt alone is classified as possibly carcinogenic to humans (category 2B) (Suh et al., 2016). Similarly, a higher risk of cervical cancer is observed in women working in a cobalt-containing environment, but these studies remain inconclusive (Cámara-Martos & Moreno-Rojas, 2016).

Risk characterization

The average exposure is estimated at $0.18 \mu\text{g g}^{-1} \text{bw}$ per day in adults (0.–7 - 0.21) and $0.31 \mu\text{g kg}^{-1} \text{bw}$ per day in children (0.29-0.36) in Europe.

The TDI limit ($1.6 \mu\text{g kg}^{-1} \text{bw}$ per day) in adults following an experimental study on rodents (ANSES 2009).

ANNEX 5: Toxicological data sheet for Cr

Generalities

The element Chromium (Cr) was discovered in 1798 by the French chemist Vaquelin in Siberian red lead ore (M. Costa et al. 1997). Chromium is a natural metallic element of the Earth's crust with symbol "Cr" and atomic number 24. It is the first element in group 6. The name of the element is derived from the Greek word *chrōma*, meaning color, because many chromium compounds are intensely colored. The term "total chromium" refers to trivalent and hexavalent compounds.

The Chrome III (trivalent) and Chrome VI (hexavalent) compounds are found in the environment. The industrial discharges are the main cause of the presence of the chromium VI (INERIS, 2005, ATSDR, 2012). Chromium metal is of high value for its high corrosion resistance and hardness.

In the United States, trivalent chromium Cr (III) ion is considered an essential nutrient in humans for glucides and lipid metabolism and to potentialize the insulin action. It is also designated an essential nutrient in Japan and for adults in France (ANSES, 2011). However, in 2014, the European Food Safety Authority, acting for the European Union, concluded that there was not sufficient evidence for chromium to be recognized as essential.

While chromium metal and Cr(III) ions are not considered toxic, hexavalent chromium (Cr(VI) is toxic and carcinogenic (group 1, IARC, 1990) .

The chromium VI in the environment comes mainly from the industries that use it. It can be rejected:

in the atmosphere (industries, or by re- suspension from soils) in waters (industries, soil erosion, atmospheric deposition, sewage treatment plants) in soils (spreading of sewage sludge, fertilizers, atmospheric deposition) (INERIS, 2005, 2010).

Although naturally occurring Cr (VI) is rarely found in the environment, its presence in ground and surface waters, as a consequence of oxidation of Cr (III)-bearing minerals by Mn (IV) oxides, has been reported recently (Scancar 2014).

Production and use of Chromium

- 90% of chromium are used in the production of stainless steels, special steels and alloys (INERIS, 2011).
- Chromium is also used in chemical industry, in metal or plastic surface treatments, in wood industry (conservation), in the production of pigments and dyes (dyes, paints, cosmetics, ceramics, etc.) (INERIS, 2011).
- Leather tanning (INERIS, 2011).
- In the manufacture of magnetic tapes, the manufacture of vitamins K (INERIS, 2011).

Human Exposure to Chromium VI

Exposure of the general population to total chromium is mainly oral following absorption of food and drinking water. Chromium VI is not intended to accumulate in fish and plants (INERIS, 2005), but according to (WHO, 2011), the main source of exposure to total chromium is food, of which drinking water. This tap water, however, represents less than 1% of the total daily dietary exposure to chromium, according to (ANSES, 2012). The French agency also states that among adults, the majority contributors to chromium intake are bread (8%), dry bakery products (5%) and alcoholic beverages (5%). In children, the majority contributors to chromium intake are milk (9%) and pasta (6%). The share represented by chromium VI could not be identified in these data. People living near plants emitting chromium VI into the atmosphere may be exposed to this compound by inhalation. Smokers are also exposed to chromium VI by this route as long as a cigarette contains it. Indoor air in smo'er's homes contains 10 to 400 times more chromium than in outdoor air (ATSDR, 2012).

Oral Exposure

1. Via the food

We lack data on the presence of CRVI in the food and it is considered that food is by and large reducing medium and that oxydation of CR III to CRVI would not be favored (EFSA, 2014).

2. Via the drinking water

In contrast, water intended for human consumption is usually treated with different oxidizing agents to make it potable, then it would promote the presence of CR (VI) over the trivalent form.

Occupational exposure

The respiratory route is the main route of occupational exposure to chromium VI. Indeed, people working in the aforementioned industries, especially metallurgies and tanneries, are likely to be exposed to this compound by inhalation: chromium VI can be found in fumes or dusts from industrial processes using it (ATSDR, 2012, INRS, 2009, INERIS, 2005).

Absorption

At human dietary exposure levels chromium absorption is relatively low (< 10 % of the ingested dose) and depends on its valence state and ligands. Most of the ingested Cr(VI) is considered to be reduced in the stomach to Cr(III), however, Cr(VI) is able to cross cellular membranes(efsa , 20014) . The interconversion of Cr(VI) to Cr(III) will be of relevance for risk assessment since, , Cr(VI) compounds are much more toxic than Cr (III) compounds.

Distribution

In humans, the hexavalent Cr exposure, after inhalation or ingestion, can have systemic effects that are distant from the site of exposure. the Cr VI is known as isostructural with sulfate and phosphate at physiological pH, it can be carried throughout the body and even into the brain. It is a competition between the extracellular reducing capacity and the uptake of chromate by cells. If the exposure is high, the levels of Cr VI may be elevated in many different organs. Depending on the genetic susceptibility of an individual, this could pose a significant risk of cancer induction in any organ.

In the United States, autopsy studies indicate that chromium concentrations in the body are the highest in kidney, liver, lung, aorta, heart, pancreas, and spleen at birth and tend to decrease with age. The levels in liver and kidney declined after the second decade of life. The aorta, heart,

and spleen levels declined rapidly between the first 45 days of life and 10 years, with low levels persisting throughout life. The level in the lung declined early, but increased again from mid-life to old age (Schroeder et al. 1962).

Elimination

Chromium may be transferred to infants *via* breast milk as indicated by breast milk levels of chromium in women exposed occupationally (Shmitova 1980) or *via* normal levels in the diet (Casey and Hambidge 1984). It has been demonstrated that in healthy women, the levels of chromium measured in breast milk are independent of serum chromium levels, urinary chromium excretion, or dietary intake of chromium (Anderson et al. 1993, Mohamedshah et al. 1998), but others (Engelhardt et al. 1990) have disputed this observation.

Chromium toxicities

Reproductive/Developmental Effects:

Chromium VI

Few studies have shown that there are reproductive effects in humans exposed by inhalation to chromium (VI), which can lead to complications during pregnancy and childbirth.

Oral studies have reported serious developmental effects in mice, such as reproductive abnormalities and effects, including reduction in litter size, reduction in sperm count and degeneration of the outer cell layer of the seminiferous tubules.

Chromium III

There is no information available on the effects of chromium (III) on reproduction or development in humans. In addition, a study conducted in mice fed high levels of chromium (III) in their drinking water didnt show a lear idea for reproductive effects, although various characteristics of the study preclude a definitive conclusion.

No developmental effects were reported in the offspring of rats fed chromium (III) during their period of development.

Carcinogenic effects in humans

Chromium VI was first recognized as a nasal carcinogen in Scotland in the late 1800s among chrome pigment workers. It is classified as a certain carcinogen for humans (IARC, 19–0 - US EPA, 1998) only during exposure by inhalation.

This classification was mainly based on studies carried out on worker populations (ANSES 2012, IARC 2012). In humans, the associated cancer is that of the lungs. Some data also imply an increase in nasal cancers and sinuses (IARC, 2012).

However, the relationship is no longer linear at low doses since chromium VI is transformed into chromium III in the stomach, then current scientific data do not allow to conclude on the link between exposure to chromium VI and these cancers in humans (IARC, 2012).

Carcinogenic effects in animals

In rodents, the exposure to high doses of oral chromium VI has been able to show an increase in stomach, intestines and oral cavity cancers (WHO, 2011, ASTDR, 2012, IARC, 2012); hepatic and gastrointestinal effects have been observed (US-EPA, 2010, ANSES, 2012, ATSDR, 2012). However, the relationship is no longer linear at low doses since chromium VI is transformed into chromium III in the stomach, current scientific data do not allow to conclude on the link between exposure to chromium VI and these cancers in humans (IARC, 2012). the ATSDR also reports effects on reproduction in animals.

Other toxic effects (for chronic exposure)

- Respiratory exposure to Cr VI: Irritation of the nasal mucosa, asthma, cough, shortness of breath, wheezing and to the development of chromium allergies.
- Oral exposure to Cr VI: In humans, chronic exposure to chromium VI *via* drinking water causes gastrointestinal effects (mouth ulcers, diarrhea, abdominal pain, dyspepsia and

vomiting) and hematological effects (anemia, leukocytosis and immature neutrophils) (ANSES, 2012).

The chronic exposure to chromium VI *via* drinking water causes gastrointestinal effects (mouth ulcers, diarrhea, abdominal pain, dyspepsia and vomiting) and hematological effects (anemia, leukocytosis and immature neutrophils) to human (ANSES, 2012).

Risk characterization: Chromium VI

Knowing that the toxicity of Cr VI comes mainly from food consumption, in France, ANSES did not create a VTR but, as part of a health risk assessment (ANSES, 2012), selected the following values for oral exposure:

- For non-carcinogenic effects (threshold effects): 1 $\mu\text{g kg}^{-1}$ bw per day, (as ATSDR in 2008, US-EPA in 2010 and WHO in 2011).
- For carcinogenic effects (no-threshold effects): 0.5 (mg kg^{-1} bw per day, (such as OEHHA * in 2011, US-EPA in 2010 and WHO in 2011).

Recent developments

In 2012, ANSES assessed the risk of exceeding the threshold values in drinking water ($50 \mu\text{g L}^{-1}$) and drew the following conclusions: In order to protect the health of the population, the maximum concentration of chromium VI in drinking water should be of the order of $6 \mu\text{g L}^{-1}$ (provisional objective). However, given the current analytical difficulties of such low chromium VI measurements, the agency proposes to measure total chromium in first intention and chromium VI if the limit is exceeded ($6 \mu\text{g L}^{-1}$). ANSES also asks to be able to determine exactly the share of exposure attributable to water in relation to food. This share is known (1%) for total chromium, but not for chromium VI. This would make it possible to define whether priority actions should focus on food or drinking water.

ANNEX 6: Toxicological data sheet for Pb

Generalities

Lead is a greyish blue metal, malleable and conductive. It is widespread throughout the environment. It can be present in two different forms: organic and inorganic.

The element lead is used in gasoline, aviation fuel, acid batteries for motor vehicles. However, also it can be in many other products, such as pigments, paints, solder, stained glass, lead crystal glassware, ammunition, ceramic glazes, jewelry, toys and in some cosmetics and traditional medicines. In addition, the drinking water delivered through lead pipes or pipes joined with lead solder may contain lead. Much of the lead in global commerce is now obtained from recycling.

Human exposure

Oral exposure

1. Via the food

The most important pathway whereby atmospheric lead enters the food chain is thought to be direct foliar contamination of plants. The air deposits raise the level of lead in soil, may result in an increased uptake of lead through the roots.

In addition, there is another source of lead in diet which is food containers, containing lead such as storage in lead-soldered cans which is banned actually in EU, ceramic vessels with lead glazes and leaded crystal glass which is strictly regulated under EU legislation related to food contact materials. Lead solder detected in can will eventually leach into the food product (Food safety, 2009).

Cereal products, potatoes, cereal grains (except rice), leafy vegetables and water are the most contributors of lead in Europe. Depending on the country but six categories (beverages; cereals and fine bakery wares; fruits and vegetables; salts, spices, soups and sauces; sweeteners, honeys and confectionery; meat and its products) contributed most to lead dietary exposure, contributing close to 83 % (Leblanc et al., 2005).

The most contaminated foods are crustaceans, mollusks, and offal. Noting that, bread and rusks, soups, vegetables, fruits, drinking water, non-alcoholic beverages, sugars and derivatives are considered as lead carriers (INVS, 2006). In Lebanon, the food groups that contributed most to the dietary intake of lead were cereals and cereal-based products (45.3%), vegetables and

potatoes (17.6%), drinking water (16.2%), and fruits and fruit juices (9.9%) (Nasserline et al., 2006).

In a published study of lead exposures in Lebanese adult urban populations (Nasreddine et al., 2006), the exposure assessment was performed using a TDS approach, as recommended by (WHO, 1996). Five “total diets” were collected during 2003–2004, it was noticed that the average concentrations of lead in different food groups (bread and toast, biscuit and toast, rice, pizza, pasta, cakes, fruits, vegetables, potatoes, cheese, milk, yogurt, meat chicken and others) was ranged between 2 and 35 $\mu\text{g kg}^{-1}$. The highest concentrations were observed in cereal-based products such as breads, pastries and pizzas, while the lowest concentrations were found in yoghurt-based products, potatoes and rice.

Via Drinking-water

Lead pipes used for household drinking water can contaminate water. The Drinking Water Directive, as recommended by WHO in 2013, sets a limit of 25 $\mu\text{g L}^{-1}$ for lead in drinking water, while it is 10 $\mu\text{g L}^{-1}$ for the EPA. In Lebanon, a study of Total Diet Study performed in 2006, it was shown that the lead concentration mean in drinking water samples was 3.0 $\mu\text{g kg}^{-1}$, far less than the range of lead concentration in food (2 to 35 $\mu\text{g kg}^{-1}$) (Nasreddine et al., 2006).

According to the infant total diet study published by ANSES in France in 2016, drinking water contributed at about 14% of the average lead exposure in children aged 13 to 36 months.

Absorption

Pb can be absorbed by the body through ingestion (Moore et al., 1980) and transmitted through the placenta (Angell et al., 1982). Indeed, the rate of absorption by the human body is often directly related to the chemical form and especially the solubility of the chemical species considered (Pichard, 2000).

The bioavailability of lead depends on its solubility in the gastrointestinal tract with an estimation of 15 to 20 % for adults (SKER and Bergdahl, 2007). There is an age dependency in lead absorption (rat pups absorb 40 to 50 times more than adult rats *via* the diet (EFSA, 2009). Pregnant woman

and children absorb lead after ingestion much greater (70% of the amount of lead ingested) than adults (20%) (ATDSR, 2000). Furthermore, nutritional iron status affects lead absorption leading to an increase when deficient, the same with a calcium deficiency (Ziegler et al., 1978 and EFSA, 2009).

Distribution

After lead absorption, the lead passes into the blood before reaching tissues. In the human blood, lead is found mainly in red blood cells (99%) and only 1% in plasma. Lead is rapidly mobilized by erythrocytes and then distributed in all soft organs. Lead enters a rapidly replenishing biological reserve and is distributed in the soft tissues (blood, liver, lungs, spleen, kidneys and bone marrow), or slowly renewed, and is then released into the body. Blood lead concentration is therefore a good indicator of exposure from any source. Blood lead represents approximately 2% of the total body pool. Lead is a cumulative toxicant distributes in the liver, kidneys, pancreas, ovaries, spleen, heart (Moore et al., 1977).

In the absence of a significant pathway of excretion, more than 90% of the absorbed lead is redistributed and fixed in the bones, where it accumulates, indeed in human adults 90 % of the total body burden of lead is found in the bones.

In contrast, concentrations in soft tissues are relatively constant (Barry, 1975). During breastfeeding, maternal lead can be transferred to infants and can contribute to lead infant blood between 40-80 % (Gulson et al, 1998). The most recent studies were performed in Mexico with a lead content in breast milk reaching 0.0011 mg L^{-1} (Ettinger et al., 2004). An Austrian study estimated a mean lead content of 0.0016 mg L^{-1} in breast milk (Gundacker et al., 2002).

Formula concentrates but also water can contribute to the lead content in ready-to-consume infant formula. Lower and upper bound lead content in ready-to-consume infant formula were calculated to be 0.0020 and $0.0047 \text{ mg kg}^{-1}$, respectively then infant formula might contain up more lead than breast milk (EFSA, 2009).

Metabolism

Once assimilated, lead is quickly present in the bloodstream (Bergeson, 2008). Then, it settles in the bones and teeth. Only a small amount of lead accumulated in the bones will be released and gradually eliminated in the urine.

The distribution of lead varies with the mode of absorption. In case of digestive absorption, hepatic and intestinal levels are three times higher than by pulmonary route (INSERM, 2006).

In the brain, lead easily passes the blood-brain barrier, it enters the brain very quickly, but this absorption is inhibited in the presence of albumin, cysteine or EDTA.

In bones, lead has been shown to inhibit osteoblastic activity by reducing osteocalcin synthesis (Long et al., 1990, Markowitz et al., 1988, Klein and Wirren, 1993), leading to morphometric or densitometric changes.

Teeth are known also to be a lead storage site, mainly at the dentine level, in areas adjacent to the pulp. In case of pregnant women, lead passes through the placenta in the first trimester and gives approximately the same maternal and umbilical blood lead levels (Bellinger et al., 1987, Goyer 1990, Wan et coll., 1996).

Elimination

The excretion of lead can be affected by several ways: urine (mostly 75% of the lead), feces, sweat, saliva, hair and nails (INSERM, 2006).

Urine elimination forms about 75% of the eliminated lead. According to Alessio et al. (1978a), lead that is in free form, is removed only by glomerol filtration when blood lead levels are within normal limits. While lead presents as not assimilated in the digestive system is eliminated by the feces (Thompson, 1971). In addition, lead elimination may be sweaty when the subject is exposed to heat (Asayama et al., 1975).

Toxicity

Numerous data from occupationally exposed groups in the population have been generated; neurotoxicity, mutagenicity, carcinogenicity, cardiovascular endocrine, and developmental

effects. Most of these endpoints were studied in animals. In 2016, lead was believed to have resulted in 540,000 deaths (Dapul H. et al., 2014).

More recently, it appears that exposure to lead during pregnancy may lead to epigenetic changes transmissible not only to children but also to the further generation (Sen A. et al., 2015).

Acute toxicity

In rodents, LD 50 values for salt lead are greater than 2,000 mg kg⁻¹ bw, depending on the form ranging from 300 up to 4000 after multiple oral short exposure (JECFA, 2000).

Chronic exposure

Lead effect is a poison at chronic exposure which represents a much higher risk. Muscle weakness, gastrointestinal symptoms, poor psychometric results, mood disturbances, and peripheral neuropathy symptoms can occur after lead exposure.

Saturnine gout has been identified as commonly occurring among individuals who were chronically exposed to lead.

It can seriously affect behavior, this effect at low exposure depending on the development state (IARC, 2006), the developing nervous system being the critical target. In human, attention deficit hyperactivity disorder (ADHD) demonstrated impulsivity leading to cognitive impairment (Cory-Slechta DA et al., 2003).

Lead genotoxicity

Lead chloride, acetate, oxide, and tetroxide were negative in several mutagenicity tests (*Salmonella typhimurium* and *Saccharomyces cerevisiae*). In contrast, lead chromate and bromide were positive in Ames test due to active moiety (chromate and bromide). A cytogenetic damage was noticed in a study using exfoliated buccal cells and where a measurement of Blood lead was performed, reporting abnormalities such as chromatid and chromosomal deficiencies and breaks fractures (Umair A., 2015, EFSA 2012), concluded that lead genotoxicity may be due to indirect genotoxin (ROS) and experiments suggest that lead is an indirect mutagen acting by inhibiting both nucleotide excision repair and base excision repair.

Reprotoxicity

Odland *et al.* in 1999 reported that exposure to organic lead affects the metabolism of steroid hormones in human and mice.

Several studies of men exposed occupationally to lead have shown: a reduction in fertility, semen volume and sperm counts, decreased sperm motility and an increase in abnormal sperm morphology. Current evidence suggests that chronic exposure to lead during pregnancy is associated with adverse outcomes including spontaneous abortion, premature birth, fetal growth restriction, neurodevelopmental delay and maternal hypertension (FAO/WHO 2011, UKTIS 2016).

Carcinogenic effects in humans

Various water soluble and insoluble lead can induce tumors in rodents (especially renal tumors) as carcinogenic/promoter agent, brain gliomas were also observed (IARC, 2006) but these effects were observed at high doses compared to human intake. The International Agency for Research on Cancer (IARC) classified lead compounds as probably carcinogenic to humans (Group 2A) on the basis of limited evidence of carcinogenicity in humans and sufficient evidence in animals (IARC, 2006).

But organic lead compounds were considered not to be classifiable for carcinogenicity (Group 3) because there was inadequate evidence for carcinogenicity in humans and animals (IARC, 2006).

Risk characterization

The Panel on Contaminants in the Food Chain of EFSA, 2009 identified developmental neurotoxicity in young children, but cardiovascular effects and nephrotoxicity in adults as the critical effects for the risk assessment. The respective BMDLs derived from blood lead levels in $\mu\text{g L}^{-1}$ (corresponding dietary intake values in $\mu\text{g kg}^{-1}$ b.w. per day) were: developmental neurotoxicity BMDL01, 12 (0.50); effects on systolic blood pressure BMDL01, 36 (1.50); effects on prevalence of chronic kidney disease BMDL10, 15 (0.63), and margin of exposure is applied. Most pharmaceutical companies have set a limit for maximum daily intake of lead as $1.0 \mu\text{g g}^{-1}$,

however prolonged intake at even this low level is hazardous to human body (Ab Latif Wan et al., 2015).

The daily intake of lead determined in the present study (18.5 mg day^{-1}) is in the same range of to those obtained in France, Sweden, Finland, the Czech Republic, Canada and the UK, but lower than intake determined in Cuba, Italy, Guatemala, Spain, Japan and China (Nassredine et al., 2006). Estimates of adult mean lead dietary exposure in European countries are in the range of 0.36 to $1.24 \text{ } \mu\text{g kg}^{-1} \text{ bw per day}$. The adult ⁹⁵th percentile lead dietary exposure is in the range 0.73 to $2.43 \text{ } \mu\text{g kg}^{-1} \text{ bw per day}$ and 0.36 to 1.24 on average and for infant (1-3 years age) 1.10 to 3.10 up to 1.71 to $5.51 \text{ } \mu\text{g kg}^{-1} \text{ bw per day}$ for high exposure (EFSA, 2010).

Assuming a body weight of 68 kg , total exposure from these categories would be $0.74 \text{ } \mu\text{g kg}^{-1} \text{ bw per day}$ in Egypt.

Regulation

The maximum levels for lead in foodstuffs are set up in the Regulation (EC) No 1881/2006. For Lebanon standard (n.d.), the maximum level of lead is 0.2 mg kg^{-1} of Lebanese white bread.

EPA has set the maximum contaminant level goal for lead in drinking water at zero because lead is a toxic metal that can be harmful to human health even at low exposure levels. Lead is persistent, and it can bioaccumulate in the body over time.

ANNEX 7: Tables Annex

Table III-2.1 Annex: Soil site classification according World Reference Base 2014 (cf Ch III-2)

Sites	Soil Class	Soil Group	Soil Unit
B1	Luvisols	Calcareous clay Luvisols	Calcic Luvisols
B2	Cambisols	Non calcareous clay Cambisols	Vertic Cambisols
B3	Vertisols	Calcareous Vertisols	Association of Calcic Vertisols, Eutric Vertisols, Luvic Chernozems and Gleyic Chernozems
B4ab	Fluvisols	Non Calcareous Fluvisols	Association of Eutric Fluvisols and Calcaric Fluvisols
B5	Cambisols	Non calcareous clay Cambisols	Association of Vertic Cambisols, Calcaric Fluvisols and Eutric Cambisols
B6	Cambisols	Non calcareous clay Cambisols	Association of Eutric Cambisols and Haplic Calcisols
B7	Fluvisols	Non Calcareous Fluvisols	Association of Eutric Fluvisols and Calcaric Fluvisols
B8	Fluvisols	Non Calcareous Fluvisols	Association of Eutric Fluvisols and Calcaric Fluvisols
B9	Regosols	Calcareous Regosols	Calcaric Regosols

Table III-2.2 Annex: The type and variety of wheat grown in the sites studied with the methods of fertilization and irrigation applied

Site	Wheat			Fertilization				Animal manure	Irrigation	
	Type and variety			Inorganic			cows and poultry	P.T.W ⁵	Surface water	
	Durum	Soft	CAN ¹	Ammonium sulfate ²	Potassium sulfate ³	Diammonium phosphate ⁴		Depth (m)		
Icarasha	Miki	Naame 12								
B1	X	X	X	X	X	X	X	X	80-90	-
B2	X	X	-	X	X	X	X	X	80-90	Canal from Kab Elias
B3	X	X	-	X	X	X	X	-	> 100	Litani River ⁶
B4a	X	X	-	X	X	X	X	X	100	Litani River
B4b	X	X	-	X	X	X	X	X	100	Litani River
B5	X	X	X	X	X	X	X	-	> 100	Canal from Berdawni
B6	X	X	X	X	X	X	X	-	> 100	-
B7	X	X	X	X	X	X	X	-	80-90	-
B8	X	X	-	X	X	X	X	-	80-90	Litani River
B9	X	X	X	X	X	X	X	X	80-90	-

(1) Calcium ammonium nitrate (N-NH₄) 13%, (N-NO₃) 13%, MgO 4%, Ca 6%; (2) Ammonium sulfate (NH₄)₂SO₄; (3) Potassium sulfate KNO₃; (4) Diammonium phosphate (NH₄)₂HPO₄: N 18%, P 46%, 10 kg (P) ha⁻¹ as per (Abi Saab et al., 2019); (5) Private Tubular Well; (6) Submersion.

Table III-2.3 Annex: Wheat-potato crop rotation in the studied sites, irrigation, and fertilizers

Crop rotation	Sown	Irrigation	Time of irrigation	1 st application of fertilization					2 nd application of fertilization						
				N fertilizer				P fertilizer	N fertilizer						
				Period	Manure	(NH ₄) ₂ SO ₄	Ammonium nitrate		Quantity (Kg N ha ⁻¹)	Quantity (Kg P ha ⁻¹)	Period	Synthetic N	(NH ₄) ₂ SO ₄	Ammonium nitrate	Quantity (Kg N ha ⁻¹)
Potato	Mar	Fully irrigated	Sowing to harvesting	Before planting	X	-	-	250	-	-	Flowering/ After flowering	X	-	-	100
Wheat	Oct/Nov	Rainfed/suppl.	During drought	Emergence	-	X	-	230	-	-	Flowering	-	X	-	230
				Sowing	-	-	X	60	X	10	Tillering	-	-	X	60

The use of poultry manures in agriculture has focused on its effects on tuber yield and quality, and on soil improvement properties with NO₃ (Rees et al., 2011). While their application, can be source of As in soil as reported by Darwish et al., 2021b (In press) and its accumulation with surface runoff downstream. Indeed, Arsanilic acid was reported to be used as growth promoter in broilers beside the possible risk of using organoarsenical herbicides which degrade into inorganic As (Chen et al., 2012).

Table III-3.1: Correlation TEs in wheat plants and soil – Si (soil) – Physico-chemical parameters in soil

Matrice de corrélation (Pearson):		As S	As L	As G	Pb S	Pb L	Pb G	Cd S	Cd L	Cd G	Pb dTPA	As dTPA	Cd dTPA	Si sol 2016	Si sol 2017	Altitude	Av annual rain	pH	EC	Clay	Silt	Sand	CaCO ₃	Active CaCO ₃	TOC	Total N	P Olsen	CEC (S)	As t	Cd t	Pb t
As S	1	0.904	0.701	0.577	0.303	0.317	-0.495	0.043	-0.682	-0.603	-0.071	0.241	0.007	-0.455	0.553	-0.516	0.281	-0.334	-0.177	-0.332	0.35	-0.436	-0.333	-0.625	-0.31	0.436	-0.551	0.332	0.378	-0.33	
As L	0.904	1	0.841	0.429	0.005	0.103	-0.422	0.035	-0.753	-0.857	0.198	0.377	-0.022	0.445	0.742	-0.719	0.34	-0.395	-0.061	-0.633	0.446	0.675	-0.52	-0.693	-0.596	0.709	-0.797	0.489	0.606	-0.201	
As G	0.701	0.841	1	0.204	-0.14	-0.027	-0.267	-0.337	-0.488	-0.787	0.178	0.179	-0.34	-0.51	0.6	-0.552	0.135	-0.191	-0.172	-0.658	0.551	-0.685	-0.549	-0.683	-0.596	0.671	-0.8	0.65	0.697	-0.186	
Pb S	0.577	0.429	0.204	1	0.625	0.091	-0.399	0.378	-0.337	-0.183	-0.434	-0.02	-0.218	-0.586	0.079	-0.212	-0.212	0.315	-0.237	0.274	0.018	0.353	0.506	0.037	-0.047	-0.231	0.056	0.126	-0.351	-0.752	
Pb L	0.303	0.005	-0.14	0.625	1	-0.124	-0.396	0.055	0.017	0.259	-0.648	-0.39	-0.075	-0.237	-0.409	0.182	-0.57	0.067	-0.717	0.538	0.238	0.607	0.488	0.022	0.123	-0.549	0.291	0.268	-0.316	-0.552	
Pb G	0.317	0.103	-0.027	0.091	-0.124	1	0.361	0.413	0.086	0.011	-0.223	0.064	0.039	-0.182	0.238	0.339	0.619	-0.174	0.604	0.221	-0.624	-0.117	-0.132	0.202	0.589	-0.139	0.25	-0.664	-0.288	-0.111	
Cd S	-0.495	-0.422	-0.267	-0.399	-0.396	0.361	1	0.353	0.799	0	-0.271	-0.425	-0.421	-0.227	-0.154	0.724	0.249	-0.16	0.535	0.133	-0.513	0.057	0.074	0.705	0.477	-0.331	0.428	-0.379	-0.303	-0.117	
Cd L	0.043	0.035	-0.337	0.378	0.055	0.413	0.353	1	0.036	-0.142	-0.104	0.201	0.18	-0.151	0.289	0.142	0.223	-0.168	0.573	0.332	-0.668	0.165	0.157	0.49	0.155	-0.266	0.249	-0.431	-0.421	-0.239	
Cd G	-0.682	-0.753	-0.488	-0.337	0.017	0.086	0.799	0.036	1	0.444	-0.559	-0.753	-0.512	-0.141	-0.67	0.937	-0.218	0.141	0.048	0.465	-0.33	0.518	0.321	0.785	0.642	-0.709	0.729	-0.272	-0.525	-0.232	
Pb dTPA	-0.603	-0.857	-0.787	-0.483	0.259	0.011	0	-0.142	0.444	1	-0.212	-0.216	0.235	0.533	-0.714	0.547	-0.383	0.507	-0.11	0.761	-0.389	0.738	0.621	0.44	0.618	-0.71	0.762	-0.522	-0.626	0.202	
As dTPA	-0.071	0.198	0.178	-0.434	-0.648	-0.223	-0.271	-0.104	-0.559	-0.212	1	0.863	0.637	0.635	0.641	-0.662	0.186	-0.121	0.369	-0.463	-0.006	-0.633	-0.538	-0.527	-0.632	0.707	-0.642	0.067	0.618	0.829	
Cd dTPA	0.241	0.377	0.179	-0.02	-0.39	0.064	-0.425	0.201	-0.753	-0.216	0.863	1	0.744	0.505	0.783	-0.71	0.224	-0.072	0.438	-0.232	-0.206	-0.515	-0.385	-0.517	-0.543	0.58	-0.575	-0.081	0.426	0.617	
Si sol 2016	0.007	-0.022	-0.34	-0.218	-0.075	0.039	-0.421	0.18	-0.512	0.235	0.637	0.744	1	0.836	0.334	-0.379	0.137	-0.257	0.22	0.064	-0.217	-0.187	-0.234	-0.355	-0.208	0.244	-0.194	-0.266	0.208	0.748	
Si sol 2017	-0.455	-0.445	-0.51	-0.598	-0.237	-0.182	-0.227	-0.151	-0.141	0.533	0.635	0.505	0.836	1	-0.017	-0.118	-0.062	-0.033	0.138	0.13	-0.193	-0.033	-0.128	-0.178	-0.072	0.089	0.005	-0.281	0.143	0.901	
Altitude	0.553	0.742	0.6	0.079	-0.409	0.238	-0.154	0.289	-0.67	-0.714	0.641	0.783	0.334	0.017	1	-0.65	-0.397	-0.392	0.454	-490	-0.057	-0.793	-0.683	-0.561	-0.627	0.72	-0.785	0.175	0.625	0.274	
Av annual rain	-0.516	-0.719	-0.552	-0.212	0.182	0.339	0.724	0.142	0.937	0.547	-0.662	-0.71	-0.379	-0.118	-0.65	1	-0.15	0.095	0.075	0.639	-0.462	0.581	0.357	0.767	0.802	-0.815	0.822	-0.431	-0.632	-0.266	
pH	0.281	0.34	0.135	-0.212	-0.57	0.619	0.249	0.223	-0.218	-0.383	0.186	0.224	0.137	-0.062	0.397	-0.15	1	-0.448	0.647	-0.511	-0.199	-0.575	-0.468	-0.064	0.216	0.489	-0.164	-0.467	0.128	0.175	
EC	-0.334	-0.395	-0.191	0.315	0.067	-0.174	-0.16	-0.168	0.141	0.507	-0.121	-0.072	-0.257	-0.033	-0.92	0.095	-0.448	1	-0.065	0.451	-0.231	0.595	0.772	0.346	0.225	-0.422	0.422	-0.215	-0.554	-0.22	
Clay	-0.177	-0.061	-0.172	-0.237	-0.717	0.604	0.535	0.573	0.048	-0.11	0.369	0.438	0.22	0.138	0.454	0.075	0.647	-0.065	1	-0.038	-0.779	-0.295	-0.215	0.331	0.225	0.139	0.075	-0.696	-0.155	0.298	
Silt	-0.332	-0.633	-0.658	0.274	0.538	0.221	0.133	0.332	0.465	0.761	-0.463	-0.232	0.064	0.13	-0.49	0.639	-0.511	0.451	-0.038	1	-0.597	0.84	0.698	0.588	0.569	-0.918	0.777	-0.441	-0.764	-0.223	
Sand	0.35	0.446	0.351	0.018	0.238	-0.624	-0.513	-0.668	-0.33	-0.389	-0.006	-0.206	-0.217	-0.193	-0.057	-0.462	-0.199	-0.231	-0.779	-0.597	1	-0.29	-0.265	-0.635	-0.538	0.464	-0.548	0.836	0.604	-0.099	
CaCO ₃	-0.436	-0.575	-0.688	0.353	0.607	-0.117	0.057	0.165	0.518	0.738	-0.633	-0.515	-0.187	-0.033	-0.793	0.581	-0.575	0.595	-0.295	0.84	1	0.932	0.887	0.554	-0.922	0.856	-0.292	-0.861	-0.435		
Active CaCO ₃	-0.333	-0.52	-0.549	0.506	0.488	-0.132	-0.074	0.157	0.321	0.621	-0.538	-0.385	-0.234	-0.128	-0.683	0.357	-0.468	0.772	-0.215	0.698	-0.265	0.932	1	0.632	0.483	-0.766	0.757	-0.312	-0.868	-0.494	
TOC	-0.625	-0.693	-0.683	0.037	0.422	0.202	0.705	0.49	0.785	0.44	-0.527	-0.517	-0.355	-0.178	-0.561	0.767	-0.064	0.346	0.331	0.588	-0.635	0.687	0.632	1	0.704	-0.769	0.867	-0.585	-0.858	-0.37	
Total N	-0.31	-0.596	-0.596	-0.047	0.123	0.589	0.477	0.155	0.642	0.618	-0.632	-0.543	-0.208	-0.072	-0.627	0.802	0.216	0.225	0.225	0.569	-0.538	0.564	0.483	0.704	1	-0.694	0.881	-0.764	-0.79	-0.252	
P Olsen	0.436	0.709	0.671	-0.231	-0.549	-0.139	-0.331	-0.266	-0.709	-0.71	0.707	0.58	0.244	0.089	0.72	-0.815	0.489	-0.422	0.139	-0.918	0.464	-0.922	-0.766	-0.769	-0.694	1	-0.904	0.378	0.849	0.43	
CEC (S)	-0.551	-0.797	-0.8	0.056	0.291	0.25	0.428	0.249	0.729	0.762	-0.642	-0.575	-0.194	0.005	-0.785	0.822	-0.164	0.422	0.075	0.777	-0.548	0.856	0.757	0.867	0.881	0.904	1	-0.65	-0.932	-0.305	
As t	0.332	0.489	0.65	0.126	0.268	-0.664	-0.379	-0.431	-0.772	-0.522	0.067	-0.081	-0.266	-0.281	0.175	-0.431	-0.467	-0.215	-0.696	-0.441	0.836	-0.292	-0.312	-0.585	-0.764	0.378	-0.65	1	0.663	-0.162	
Cd t	0.378	0.606	0.697	-0.351	-0.316	-0.288	-0.303	-0.421	-0.525	-0.626	0.618	0.426	0.208	0.143	0.625	-0.632	0.128	-0.554	-0.155	-0.764	0.604	-0.861	-0.868	-0.858	-0.79	0.849	-0.932	0.663	1	0.459	
Pb t	-0.330	-0.201	-0.186	-0.752	-0.552	-0.111	-0.117	-0.239	-0.232	0.202	0.829	0.617	0.748	0.901	0.274	-0.266	0.175	-0.22	0.298	-0.223	-0.099	-0.435	-0.494	0.37	-0.252	0.43	-0.305	-0.162	0.459	1	

Table III-3.2 Annex: DTPA-extractable Pb, Cr, Co, Ni, Cu, Zn, As and Cd as a function of total concentrations

Matrice de corrélation (Pearson (n)):

	Cr t	Cu t	Ni t	Zn t	Co t	As t	Cd t	Pb t	Pb DTPA	Cr DTPA	Co DTPA	Ni DTPA	Cu DTPA	Zn DTPA	As DTPA	Cd DTPA
Cr t	1	0.982	0.975	0.938	0.956	0.638	0.804	0.477	-0.256	0.692	-0.356	-0.521	0.360	0.241	0.387	0.031
Cu t	0.982	1	0.969	0.943	0.970	0.690	0.836	0.391	-0.385	0.710	-0.360	-0.501	0.405	0.215	0.336	0.009
Ni t	0.975	0.969	1	0.879	0.975	0.764	0.772	0.329	-0.277	0.592	-0.325	-0.374	0.366	0.297	0.286	-0.026
Zn t	0.938	0.943	0.879	1	0.926	0.619	0.876	0.482	-0.425	0.762	-0.382	-0.607	0.203	0.021	0.468	0.077
Co t	0.956	0.700	0.975	0.926	1	0.814	0.825	0.26	-0.443	0.599	-0.324	-0.387	0.250	0.161	0.300	-0.035
As t	0.638	0.690	0.764	0.619	0.814	1	0.663	-0.162	-0.522	0.282	-0.145	0.092	0.129	0.153	0.067	-0.081
Cd t	0.804	0.836	0.772	0.876	0.825	0.663	1	0.459	-0.626	0.644	0.042	-0.261	0.411	-0.004	0.618	0.426
Pb t	0.477	0.391	0.329	0.482	0.26	-0.162	0.459	1	0.202	0.394	-0.001	-0.610	0.276	-0.168	0.829	0.617
Pb DTPA	-0.256	-0.385	-0.277	-0.425	-0.443	-0.522	-0.626	0.202	1	-0.177	-0.27	-0.098	-0.089	0.322	-0.212	-0.216
Cr DTPA	0.692	0.710	0.592	0.762	0.599	0.282	0.644	0.394	-0.177	1	-0.529	-0.551	0.443	0.401	0.136	-0.12
Co DTPA	-0.356	-0.360	-0.325	-0.382	-0.324	-0.145	0.042	-0.001	-0.27	-0.529	1	0.670	0.167	-0.216	0.371	0.727
Ni DTPA	-0.521	-0.501	-0.374	-0.607	-0.387	0.092	-0.261	0.61	-0.098	-0.551	0.670	1	0.083	0.240	-0.321	0.108
Cu DTPA	0.360	0.405	0.366	0.203	0.25	0.129	0.411	0.276	-0.089	0.443	0.167	0.083	1	0.592	0.105	0.284
Zn DTPA	0.241	0.215	0.297	0.021	0.161	0.153	-0.004	-0.168	0.322	0.401	-0.216	0.240	0.592	1	-0.491	-0.400
As DTPA	0.387	0.336	0.286	0.468	0.300	0.067	0.618	0.829	-0.212	0.136	0.371	-0.321	0.105	-0.491	1	0.863
Cd DTPA	0.031	0.009	-0.026	0.077	-0.035	-0.081	0.426	0.617	-0.216	-0.120	0.727	0.108	0.284	-0.400	0.863	1

Table III-4.1 Annex: The trace elements contents in studied soils in the Bekaa region and the three most consumed white Lebanese bread



Soil samples	Trace element total content (mg Kg ⁻¹)						
	As	Cd	Co	Cr	Hg	Ni	Pb
B1	6.84	0.281	18.3	94.1	0.02	56.1	13.76
B2	4.52	0.286	13.3	83.8	0.01	47.3	16.84
B3	4.35	0.255	11.4	75.1	0.02	43.6	24.48
B4a	7.3	0.237	11.4	55.8	0.01	39.4	9.09
B4b	7.51	0.233	11.7	57.2	0.01	39.7	8.84
B5	11.3	0.426	25.2	129	0.02	78.9	17.54
B6	6.37	0.345	14.9	71.7	0.01	52	15
B7	7.74	0.329	19.1	94.3	0.03	58.5	18.6
B8	9.65	0.475	23	114	0.03	64.2	19.49
B9	7.043	0.415	15.25	81.34	0.02	49.08	18.6

Bread samples	Trace element total content (µg Kg ⁻¹)						
	As	Cd	Co	Cr	Hg	Ni	Pb
B1	235	<LOQ	91	<LOQ	0.7	1292	74
B2	400	<LOQ	87	<LOQ	0.7	364.82	203
B3	321	<LOQ	84	363	0.89	<LOQ	260

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Lebanese Population Exposure to Trace Elements via White Bread Consumption

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Abstract: The objective of this study was to assess Lebanese population exposure to trace elements (TEs) via white pita consumption. A survey of white pita 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 most consumed pita brands, labeled B1, B2, and B3, were selected. Levels of TEs (i.e., As, Cd, Co, Cr, Hg, Ni, and Pb) in B1, B2, B3 pitas were measured. The highest contents of TEs in pitas were: Ni (1292 µg/kg) and Co (91 µg/kg) in B1; As (400 µg/kg) and Cd (< 15 µg/kg) in B2; Cr (363 µg/kg), Pb (260 µg/kg), and Hg (0.89 µg/kg) in B3. The pita brand B3 was the source of the highest TEs exposure, except for Ni for which it was B1. Daily exposures to TEs due to the fact of pita consumption were compared to safety levels. 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 tolerable daily intake was observed for the most exposed populations. The very low margins of exposure for As and Pb suggest a worrying risk for the Lebanese population.

Keywords: survey; trace elements analysis; Lebanese pita; human exposure; risk characterization

1. Introduction

Bread is a source of energy and minerals, some of them being essential for the body. Essential minerals found in Lebanese bread are Cu, Fe, Mn, Zn, and Se which account for 36%, 25%, 30%, 22%, and 32% of respective recommended dietary allowance (RDA) values given for these elements [1].

Besides the quality of bread as nutriment, its safety must be assured and monitored in terms of putative contaminants like trace elements (TEs).

Indeed, trace elements represent the main food chemical contamination and are globally recognized as a public health hazard [2]. They occur either from natural process (lithogenic and pedogenic routes) and/or anthropogenic activities. The latter include industrial, agricultural (pesticides, fertilizers), and untreated wastewater [3]. This leads to the contamination of the different environmental matrices, including water, soil, air, and plants [4,5]. In general, plants can uptake TEs mainly by absorbing

them from polluted soils, and transporting them further to its different parts (roots, stem, leaves, and grains) [6]. The bioaccumulation of TEs in plants, especially those that enter the food chain, can be dangerous to human health due to the fact of their eventual toxicity [5].

Trace elements such as lead, arsenic, cadmium, and mercury are relevant toxic elements [7]. According to the existing literature, it is mandatory to quantify the contents of trace elements in foodstuffs in order to estimate dietary exposure, an essential step for risk assessment related to food consumption [2,8].

Currently, many studies have been undertaken in different countries to assess exposure to TEs occurring in their relative foodstuffs such as France [9], Spain [2], Italy [10], China [11], Saudi Arabia [12], and Lebanon [13]. In addition, the European Food Safety Authority (EFSA) panel on contaminants in the food chain has given its scientific opinion on the risks to public health related to the presence of Ni, Hg, and As in food [14–16]. Such studies should be representative of specific food as consumed by the population. In France, total diet studies (TDS) are national surveys routinely realized to assess public health risks associated with substances such as contaminants in food. They underline the necessity to reduce exposure to elements such as Cd, Al, Ni, As, and Co by a diversified diet (food items and origins) [17].

Nasreddine et al. [13] studied, using the TDS approach, the dietary exposure of the adult Lebanese urban population to six essential micro-nutriments (i.e., Co, Cu, Fe, Mn, Ni, and Zn) and two toxic heavy metals (i.e., Cd and Pb). This study constitutes a first estimate of the consumer exposure to trace elements through the diet in Lebanon. These authors determined that the average urban adult daily bread consumption was 136.8 g/day and that mean exposure was found to be satisfactory for most trace elements. However, the survey included only adult people living in the district of Beirut, thus preventing the extrapolation of the results to the whole country; indeed, agricultural areas are sections of high bread consumption.

Previous studies in Egypt, Iran, South Africa, Nigeria, and Portugal determined TEs contents in bread [18–22]. In Lebanon, Bou Khouzam and co-authors [1] determined the contents of 20 elements in three varieties of Lebanese bread (i.e., white, brown pita, and Saj bread) throughout the country during the wet and dry seasons. Toxic elements such as Cd and Pb were below maximum levels set by the Lebanese regulatory agency, (The Lebanese Standards Institution—Libnor) [23].

Bread is an integral part of the daily diet, but the types of bread and amounts ingested vary according to the age, sex, and socio-economic status of the individual. As a result, and as consumer behavior evolves over time, there is a need for up-to-date information on daily ETs intake via Lebanese bread consumption throughout Lebanon, including rural areas.

The Lebanese standards [23] provide only Pb, Cu, and Cd maximum levels in bread as food item contamination vectors. However, other TEs could be present [1,13]. As exposition represents the combination of consumption of bread potentially contaminated, it is of first interest to compare exposure with safety levels in order to monitor any safety preoccupation concerns for the Lebanese population. For this reason, this study was directed by a public institute (Lebanese Agricultural Research Institute—LARI) to assess exposure to trace elements via Lebanese pita consumption.

As, Cd, Co, Cr, Hg, Ni, and Pb were selected in this study based on previous studies of trace element composition of Lebanese bread [1,13] and based on the Lebanese bread standard Libnor, NL 240 [23]. In contrast to heavy metals known to be toxic, such as Pb, Cd or Hg, some elements, such as Cr and Co, are essential elements giving benefit to the body, the liver being a valuable source of elements [24]. Co is a transition metal with two oxidation states Co (II) and Co (III) and is essential as part of vitamin B12 (cobalamin) involved in folate and fatty acid and the metabolism of proteins and nucleic acids [25]. In regards to Cr, the Cr (III) form has been postulated to be necessary for the efficacy of the insulin-regulating metabolism of carbohydrates, lipids, and proteins [26]. However, EFSA [27] in 2014 concluded there was no evidence of a beneficial effect associated with Cr intake in healthy, normoglycemic subjects.

Two risks may occur with trace elements: one is insufficient intake and one is excess intake. In this study, we focused on risk characterization due to the fact of excess intake via pita consumption.

Therefore, the objective of this study was to assess exposure to the selected trace elements (TEs) of the different Lebanese population categories via pita consumption. First, a country survey of pita consumption was achieved among one thousand individuals, grouped into adults (above 15 years old, men and women) and young people (6–9 and 10–14 years old). Second, levels of the seven selected trace elements in pitas from the three most consumed brands were measured. Third, using the survey data, population categories of exposures to TEs via white pita consumption were calculated and compared to the international health-based guidance values: tolerable daily intake (TDI), tolerable weekly intake (TWI) or toxicological reference points, such as bench mark dose limit (BMDL), to evaluate safety concerns.

2. Materials and Methods

2.1. Survey

A questionnaire survey about the daily consumption of pita bread was conducted on a target sample of 1000 people. They were selected randomly in 50 supermarkets over the five administrative regions of Lebanon, taking into consideration the population distribution [28]: 41% in Mount Lebanon, 20% in North Lebanon including Akkar, 17% in South Lebanon including Nabatieh, 13% in the Bekaa, and 9% in Beirut (Table 1 and Figure 1). The individuals were represented by 50% men, 50% women, and grouped as follows: 800 adults (> 15 years old) and 200 young people (between 6 and 15 years old).

Table 1. Sampling strategy according to the population distribution.

Regions	Population Distribution *		Estimated Sample		Surveyed Supermarkets	Realized Questionnaires	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	<i>n</i>	%
Beirut	361,366	9.6	90	9.0	5	82	8.3
Mount Lebanon	1,484,474	39.5	410	41.0	20	410	41.3
North Lebanon	763,712	20.3	200	20.0	10	200	20.2
South Lebanon	659,718	17.6	170	17.0	8	170	17.1
Bekaa	489,865	13.0	130	13.0	7	130	13.1
Total	3,759,136	100	1000	100	50	992	100

* Lebanese Population, Central Administration for Statistics (CAS) Living Conditions Survey 2007 [28].

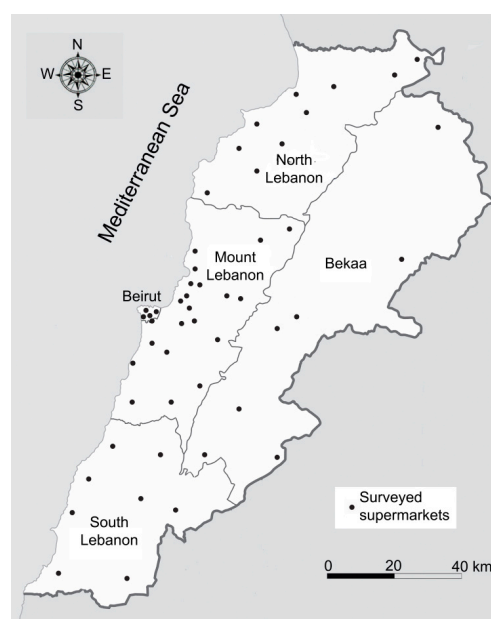


Figure 1. Location of the fifty surveyed supermarkets in Lebanon within the five administrative regions according to population density.

Data were collected over a two-month period, from 14 April 2017 to 5 June 2017. The team of interviewers was composed of 8 data collectors trained prior to the fieldwork by a supervisor. Participants were eligible for the study if they were Lebanese, had been living in Lebanon for at least fifteen years before the date of the survey, and did not have a chronic or serious illness.

In each supermarket, eligible participants were interviewed face-to-face by a data collector who instantly filled an offline questionnaire on an electronic tablet, using multiple-choice questions and figures (e.g., size of the pita). In the case of children, parents aided us in collecting survey data.

In addition to information on the daily consumption of pita bread, age, gender, body weight, and socio-economic status of the interviewed individuals, data were also collected on the level of education, address (urban or rural), job description, and health status (e.g., smoking habit, alcohol consumption and physical activity of the individual). Each questionnaire consisted of 44 questions (Supplementary Materials, Survey) which required approximately 7 min to complete. The collected answers were transmitted in the “iSurvey” application for data harmonization.

2.2. Chemicals

Standard solutions (1 g/L) of Hg, As, Co, Cr, Cd, Ni, and Pb were purchased from Fluka. In addition, HNO₃ 65%, H₂O₂ 30%, and the BCR (Community Bureau of Reference) certified reference material for bread trace elements (BCR-191) were purchased from Sigma–Aldrich. Deionized water, obtained from a BOECOpure UV/UF water purification system, was used in all experiments.

2.3. Sampling for Trace Elements Analysis

Based on the survey data, three Lebanese white pita brands were determined as the most consumed by the Lebanese population. These brands were tagged as B1, B2, and B3.

Three randomly sampled bags of each of the selected pita brands were collected. Each bread bag had seven pitas where three were randomly picked. Each pita was cut into 12 circular sectors with a plastic knife on a polyethylene plate to prevent trace element contamination. Two pieces were randomly drawn for analysis, creating 18 (3 bags × 3 pitas × 2 pieces) circular pita pieces for each brand. Before sample digestion, pita pieces were ground into powder using an agate mortar.

2.4. Sample Digestion for Trace Elements Analysis

Digestion of samples was done using an Anton Paar microwave (multiwave 3000—Rotor 8SXF 100, Graz, Austria). Samples of 0.5 g each were directly weighed after grinding in 50 mL Teflon tube. Then, 7 mL of concentrated HNO₃ and 1 mL of H₂O₂ were added to the preparation. The Teflon bombs were closed and placed in the digester at 200 °C for 15 min (1000 W). Once the Teflon tubes had cooled for at least 45 min, 3 mL of the digested samples were transferred to 25 mL polyethylene tubes and diluted with HNO₃ 1% before the trace elements analysis. Each sample was digested in triplicate.

2.5. Analysis of Trace Elements

The determination of trace elements (As, Co, Cd, Cr, Pb, and Ni) in pita samples was performed using an Atomic Absorption Spectrometer (AAS, Stafford House, UK) Zeeman Graphite Furnace GF95Z Thermo Electron corporation M series. Each pita sample was digested in triplicate and each digested sample was analyzed in triplicate. Mercury was analyzed by direct mercury analyzer DMA 80 Milestone (Soriso, Italy) using 0.2 g of pita sample.

To guarantee the method reliability, a certified reference material (CRM; BCR-191) underwent the same digestion protocol and was analyzed at the same time as the pita samples. The recovery percentages obtained for the reference material were 93.2% for Hg, 110.8% for Pb, 86.3% for Ni, and 110.1% for Cd. For the other elements, As, Co, and Cr, for which reference materials were not available, bread samples were spiked by a known concentration (0.1 mg/L) of each of the above listed elements before digestion. The spiked bread was treated and digested in the same way as the bread

samples and analyzed using an AAS. The recovery percentages were 103.08% for As, 101.29% for Co, and 96.37% for Cr.

2.6. Exposure Determination

An exposure calculation was conducted for each respondent. They took into account the quantity of bread consumed by person, body weight, and the metal content of the consumed bread according to its brand. Results were expressed in terms of the median and 95th percentile using Ms-Excel for different population categories.

2.7. Statistical Analysis

In order to avoid a lack of representativeness of the selected samples of the surveyed population, a weighting adjustment technique was applied using auxiliary variables such as gender, age, and region by comparing the observed frequency distribution of each variable with its population distribution [29].

Data processing and statistical adjustments were analyzed using Sphinx IQ2 (Le Sphinx®, Chavanod, France) for survey data and R Studio version 1.0.153 with R version 3.5.0 [30,31].

Kruskal–Wallis non-parametric tests [32] completed by a multi-comparison Fisher's test ($\alpha = 0.05$) were conducted to test for significant differences in consumption by region. Analysis of variances (ANOVAs) completed by a multi-comparison Tukey's test ($\alpha = 0.05$) were also executed to test differences in trace element contents among brands. They were performed with R using the "agricolae" package [33].

Median and percentile non-parametric tests ($\alpha = 0.05$) [32] were realized to check significant differences in the level of trace element exposures between age and gender. They were performed using R and the "rcompanion" package [34].

Principal component analysis (PCA) was applied to examine the relationships among the trace element contents of the breads using XLSTAT 2018. The results are presented by loading and score plots.

Multivariate correspondence analysis was done to explore relationships among consumption behaviors and socioeconomic data [35].

3. Results

3.1. Survey Results

Based on a target sample of 1000 people, the surveyed sample consisted of 992 individuals with the following demographic characteristics: a sex ratio of 0.992, a proportion of 19.4% young people (8.9% children aged from 6 to 9 years old and 10.5% teenagers aged from 10 to 14 years old) and 80.6% adults, and 70.6% living in an urban area. Compared to the overall Lebanese population (where the sex ratio is 0.962, 23% are young people, 77% adults, and 87% live in urban areas [36]) there was a significant difference risk of 5% in terms of young people/adults and rural/urban ratios between the surveyed sample and the population distribution of Lebanon, which could lead to a bias. Consequently, a weighing adjustment in respect to these variables was performed to make a possible inference at the population level. The weighted numbers of respondents corresponded to 228 young people (i.e., 95 children and 133 teenagers) and 764 adults.

White pita was the type of bread most consumed according to the surveyed sample: 77% reported white pita consumption and 23% brown or other. We limited our study to the 762 individuals consuming mainly white pita (W). This population also consumed brown pita (B) and other types of pita (O) in smaller proportions. The relative proportions of these different consumption modes were as follows: W only > W + B > W + O > W + B + O with 88.8%, 8.9%, 1.8%, and 0.4%, respectively (Supplementary Materials, Table S1).

The survey makes it possible to estimate the consumption of white pita for the different categories of the population (Table 2). Adult men were the highest consumers followed by teenagers (10–14 years old) and women and children (6–9 years old): daily white pita consumption medians were 282, 143, 126,

and 71 g/day, respectively. The 95th percentiles were 750 g/day for men and between 357 and 375 g/day for other categories. Maximum consumption was 1000 g/day for men and teenagers and 750 g/day for women and children. White pita brand consumption was significantly dependent on the age of the Lebanese population. Children consumed mostly the B2 or B3 brands, teenagers consumed mostly the B1 brands, and adults consumed mostly the B1 or B2 brands. The whole sample consumption by brand, including young people and adults, was B2 > B1 > B3 representing, respectively, 30.1%, 28.9%, and 17.1% of the total sample and the 46 other brands representing the remaining 24.9%.

Table 2. Daily white pita consumption according to population categories and repartition by brands.

Population Categories	Individuals		Daily White Pita Consumption g/Day					Brands Repartition %				
	n		min	M	95th P	max	m	CI 95%	B1	B2	B3	Others *
Children	91	63	71	357	750	113	94-131	24	31	30	14	99
Teenagers	113	31	143	375	1000	171	147-196	40	29	12	19	100
Women	258	31	126	375	750	167	153-181	29	31	16	24	100
Men	300	31	282	750	1000	282	260-304	29	30	13	27	99
Young people & adults	762	31	143	500	1000	206	195-218	28.9	30.1	17.1	24.9	101

M = median; 95th P = 95th percentile; min = minimum; max = maximum; m = mean; CI 95% = 95% confidence interval. * "Others" correspond to an array of the 46 other pita brands.

White pita consumption varied according to region (Figure 2), and the highest consumption corresponded to South Lebanon and Nabatieh (355 g/day). There were no significant differences between the North, Mount Lebanon, and Bekaa regions, which were two-fold lower than South Lebanon and Nabatieh. The lowest consumption was observed in Beirut (135 g/day). This last value is in agreement with the adult population of Beirut studied by Nasreddine et al. [13].

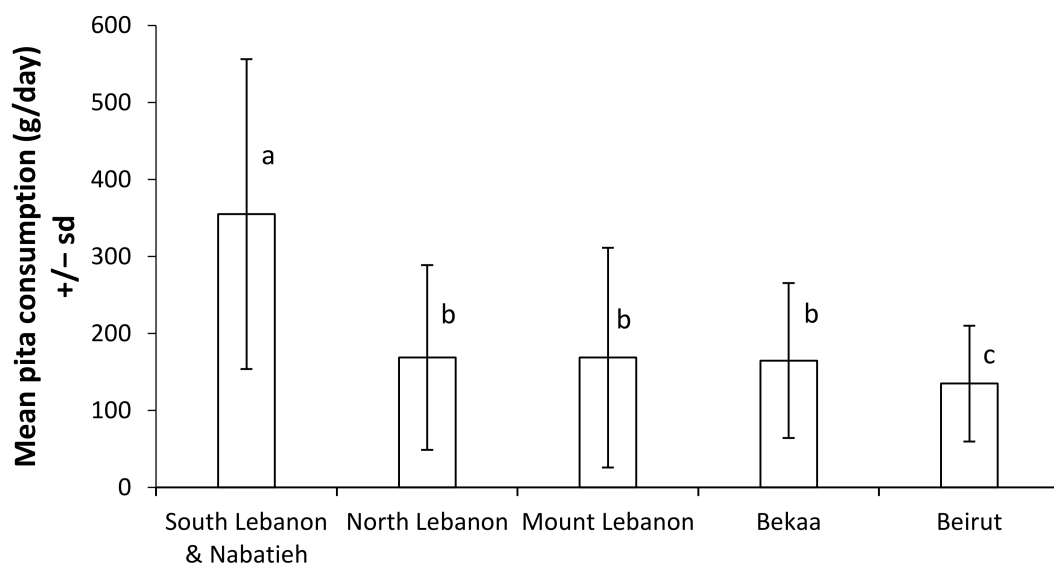


Figure 2. Lebanese white pita consumption according to region. Letters represent homogeneous groups determined by a Kruskal–Wallis test and post-hoc Fisher's test for least significant difference.

Regardless of the region, large standard deviations were observed in the distribution of bread consumption; the reason could be that adults over 65 years old eat more bread, especially in rural areas (in South Lebanon, more than 600 g per day can be consumed, see Figure 3).

In order to better explore the relationships between adult consumption and regions, a data-mining tool was used, the purpose of which was to represent in only one table or figure the most significant results from several cross analyses (based on multivariate correspondence analysis [35]). A pivot variable was first selected—the region—then other variables were crossed with it. Figure 3 shows the results of crossing region and consumption, gender, socio-professional category, smoking habits,

physical activity (4 categories: “No”, “Yes, strenuous”, “Yes, low”, and “Yes, medium”), and area (urban or rural), using the most specific modalities as key views.

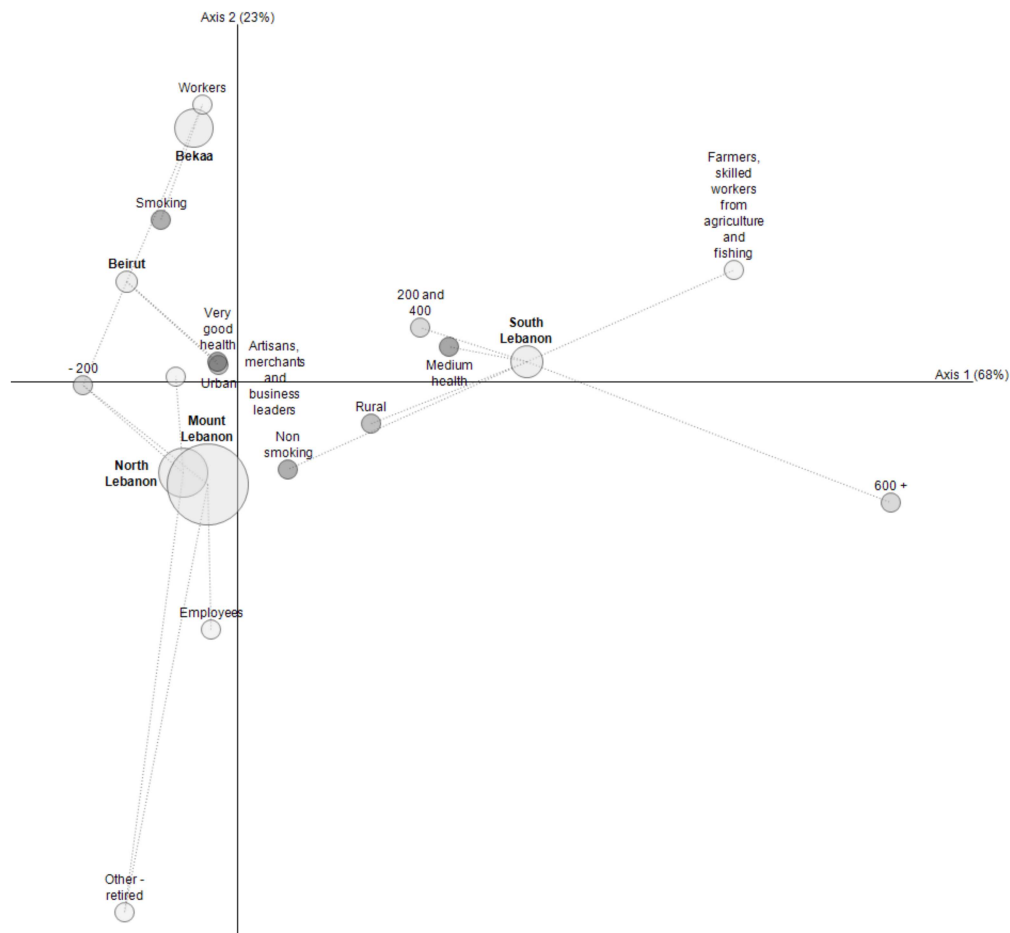


Figure 3. Results of crossings between regions, consumption, smoker or not, type of physical activity, socio-professional category, and area (urban or rural), using the most specific modalities as key views. Consumption is represented here by categories: less than 200 g/day (−200); from 200 to 400 g/day; from 400 to 600 g/day; and more than 600 g/day (600+). Sizes of circles are proportional to the number of observations by region.

In South Lebanon and Nabatieh, where consumption was the highest, it correlated to rural areas with more agriculture and elderly populations. The other parts were characterized by urban areas with high concentrations of workers and retired people.

In addition, 41% of total consumers of Lebanese white pita bread were smokers (at least one cigarette per day, with a distribution of 68% men and 32% women) and 35% were alcohol drinkers (at least one glass per day, with a distribution of 67% men and 33% women, data not shown). Those behaviors may affect health and the detoxification process of alcohol and smoking are indeed well-known inducers of several xenobiotic metabolism enzymes.

Among the adult population, 85% reported engaging in physical activity which was qualified as low to medium by women and medium to high by men (Supplementary Materials Figure S1).

The correlation between consumption and health was also evaluated (data not shown). The pivot variable was still pita consumption per day and the other variables were gender, region, professional activity (student, retired, etc.), and health (smoking, physical activity).

From this study, two typologies of adult consumers can be drawn: i) Women, quite young (under 20 years old), non-smokers, and who felt very healthy according to the survey. They were

living in urban areas, Lebanon North–Mount Lebanon, with some level of pre-secondary education. The consumption of those aged 15–64 years old per day was approximately 163 g of pita (corresponding to 1 or 2 pitas). ii) Men between 40 and 59 years old who felt healthy. They were living in rural zones, with a technical secondary level education. Their consumption was approximately 267 g of pita per day, corresponding to 3 to 6 pitas for those 15–64 years old.

3.2. Trace Element Contents in Pita

The levels of trace elements in the most consumed white Lebanese pitas (B1, B2, and B3) are shown in Table 3. B1 contained the highest level of Ni ($1292.1 \pm 0.2 \mu\text{g/kg}$) and Co ($91 \pm 3 \mu\text{g/kg}$). B2 contained the highest level of As ($400 \pm 7 \mu\text{g/kg}$), while B3 contained the highest level of Hg ($0.89 \pm 0.06 \mu\text{g/kg}$), Cr ($363 \pm 10 \mu\text{g/kg}$), and Pb ($260 \pm 81 \mu\text{g/kg}$). Levels of Cd were lower than the maximum levels defined by the Lebanese standard ($200 \mu\text{g/kg}$ of bread). In contrast, Pb exceeded the maximum level ($200 \mu\text{g/kg}$) in B2 and particularly B3 ($260 \pm 81 \mu\text{g/kg}$).

Table 3. Trace element contents in white pita bread for the three major brands on the Lebanese market.

Trace Elements	Major Pita Brands			Limit of Detection ($\mu\text{g/kg}$)
	B1	B2 ($\mu\text{g/kg}$ Dry Weight)	B3	
Cd	<LOQ	<LOQ	<LOQ	5
Hg	0.7 ± 0.1^b	0.70 ± 0.15^b	0.89 ± 0.06^a	0.04
Cr	<LOQ	<LOQ	363 ± 10	16
Co	91 ± 3^a	87 ± 5^a	84 ± 2^a	5
Ni	1292.1 ± 0.2^a	364.82 ± 0.02^b	<LOQ	16
Pb	74 ± 5^c	203 ± 30^b	260 ± 81^a	21
As	235 ± 22^c	400 ± 7^a	321 ± 20^b	16

LOD = Limit of detection. Limit of quantification: $\text{LOQ} = 3 \times \text{LOD}$. Values are the mean \pm SD ($n = 9/\text{group}$) trace elements level. Statistical comparisons were done by Kruskal–Wallis with Tukey’s post-hoc analysis. Significantly different ($p < 0.05$) values among brands per element are indicated by different letters.

In order to reveal the relationships among trace elements, a PCA was performed (Figure 4).

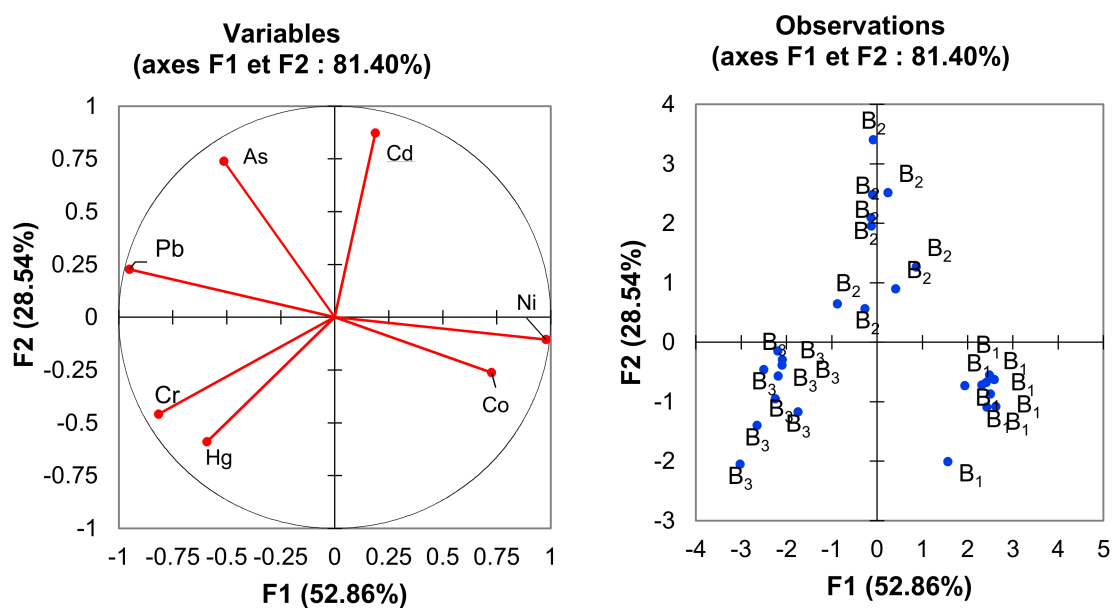


Figure 4. Principal component analysis showing trace element signatures of the B1, B2, and B3 pita brands.

Principal component analysis is a useful technique for exploratory data analysis, allowing to better visualize the variation present in a dataset with many interrelated variables. In our case, the dataset

contained 27 observations (9 samples per brand) described by 7 variables (the trace elements content as mentioned in Table 3). Principal component analysis allows to see the overall “shape” of the data, identifying which samples are similar to one another and which are very different. It makes possible the identification of potential groups of samples that are similar and to work out which variables make one group different from another. When many variables correlate with one another, they will all contribute strongly to the same principal component [37].

Figure 4 shows typical plots from the PCA with a loading plot (on the left) and a score plot (on the right). The loading plot is used for interpreting relationships among variables and the score plot is used for interpreting relationships among observations.

All variables were well represented in the F1–F2 plot (sum of squared cosine above 0.75). The PCA shows that the following elements were correlated: Ni and Co, As and Pb as well as Cr and Hg. Lead and Co were anti-correlated, as well as Ni and Pb. There was no correlation between Cd and Co, Cd and Pb, Cr and As as well as Hg and As. The Pearson correlation matrix is provided in Supplementary Materials Table S2. We observed three separate groups: B1, B2, and B3. The dispersion in B2 and B3 was larger than in B1; this dispersion can be explained by a more homogeneous sampling for B1.

3.3. Trace Element Exposures

The population categories’ exposure to Cd, Hg, Cr, Co, Ni, Pb, and As via white pita consumption according the most consumed brands is presented in Supplementary Materials Table S3. Using statistics, we observed that, for most of the cases, the B3 brand was the origin of the highest trace elements exposure. In regards to nickel, B1 was the main source of the highest exposure. We further decided to keep B3 TE data to calculate exposures (Table 4) using a worst-case scenario for all element levels, except for nickel for which the B1 data were retained.

Table 4. Population categories’ exposure to trace elements via white pita consumption: Cd and Hg are expressed in $\mu\text{g}/\text{kg}$ body weight/week while Cr, Co, Ni, Pb, and As are expressed in $\mu\text{g}/\text{kg}$ body weight/day

TEs	Population Categories’ Exposure to TEs via White Pita Consumption							
	Children		Teenagers		Women		Men	
	M ¹	95th P ¹	M ¹	95th P ¹	M ¹	95th P ¹	M ¹	95th P ¹
Cd	0.19	1.00	0.20	0.56	0.13 ^a	0.28 [*]	0.19 ^b	0.47 ^{**}
Hg	0.03	0.15	0.03	0.09	0.02 ^a	0.04 [*]	0.03 ^b	0.07 ^{**}
Cr	1.74	9.00	1.79	5.06	2.47 ^a	2.47 [*]	1.72 ^b	4.26 ^{**}
Co	0.41	2.09	0.42	1.18	0.28 ^a	0.57 [*]	0.40 ^b	0.99 ^{**}
Ni	2.80	10.26	4.06	12.26	2.77 ^a	10.26 [*]	4.34 ^a	12.26 [*]
Pb	0.69	3.59	0.71	2.01	0.48 ^a	1.77 [*]	1.23 ^b	3.04 ^{**}
As	1.54	7.97	1.58	4.49	1.07 ^a	2.19 [*]	1.53 ^b	3.77 ^{**}

The B3 brand, the origin of the highest trace elements exposure, was chosen for comparison with toxicological reference values except for nickel for which the B1 brand was retained. ¹ M = median, 95th P = 95th percentile. Statistical tests were performed separately for young people and adults for the median and the 95th percentile. Values with different letters (a,b) in each row indicate a significant difference among medians at $p < 0.05$. Values with different stars (*,**) in each row show significant difference among 95th percentiles at $p < 0.05$.

For young people, there was no significant difference among both populations in terms of exposure to any element. In contrast, we found significant differences between men and women for all elements except nickel. Men, at the 95th percentile, were always more exposed.

3.4. Risk Characterization (Intake Excess of TEs via Bread Consumption)

In regards to essential elements with nutritional value, risk assessment can be calculated by insufficient intake or intake excess. This work focused only on intake excess using toxicological reference points or safety levels for each element case by case in order to determine if there is a safety

concern due to the fact of an intake excess related to bread consumption in Lebanon. Of course, bread is not the only food consumed per day; however, in this study we show that the most exposed population—men and teenagers—can consume up to 1000 g of bread per day.

Considering chromium levels in bread, represented mostly by Cr (III)—the major form in food [38], exposures to Cr ranged from 1.21 to 9 $\mu\text{g}/\text{kg}$ bw/day which represents only 3% of the European TDI [27] for the most exposed population (children at the 95th percentile) (Table 5) in regards to pita consumption. Concerning cobalt, only the exposure related to children lead to an excess (131%) of the French TDI (Table 6) [39].

Regarding cadmium, the most exposed population were children at the 95th percentile followed by teenagers and then men and women. The most exposed population represented 40% of the European tolerable weekly intake (TWI) fixed by the EFSA [40] (Table 5).

Exposures to mercury were also very low (ranging from 0.02 to 0.15 $\mu\text{g}/\text{kg}$ bw/week) even for the most exposed population (children at the 95th percentile) leading to 11.5% of the TWI fixed by the EFSA [15].

However, before concluding on safety in this survey in regards to Hg, Cd, Cr or Co, it is important to take into account exposure from other food items in the context of the Lebanese population's total diet.

Concerning nickel, the situation is already worrying when taking into account only pita consumption. Teenagers were the most exposed population followed by children and then adults, regardless of sex (Table 6). The European TDI was exceeded for all exposures for teenagers and men and only at the 95th percentile (3 to 4 fold) for children and women.

Table 7 shows that the margins of exposure (MOE) in regard to lead, determined with a $\text{BMDL}_{0.1}$ based on neurotoxic effect [41], were very low (between 0.08 and 0.58) whatever the population. The most exposed populations in decreasing order were children, teenagers, men, and women. The calculated MOEs suggest a safety level of preoccupation regardless of the population in terms of lead exposure via bread consumption.

The same safety preoccupations can be observed with arsenic considering As is represented by its inorganic form. The EFSA has concluded that 0.3 to 8 $\mu\text{g}/\text{kg}$ bw/day should be used as a point of reference [16]. Thus, these values were used for MOE calculation to assess risk characterization. Table 7 shows that the MOEs were very low regardless of the population in both scenarios. The most exposed populations were children followed by teenagers and then men and women. The MOE data suggest a worrying risk in regard to As exposure linked to pita consumption regardless of the population in this survey.

Table 5. Population categories’ exposure to Cd and Hg trace elements via B3 white pita consumption and comparison with the tolerable weekly intake (TWI). Exposure values are expressed in µg/kg body weight/week.

Trace Elements	Population Categories’ Exposure to TEs via White Pita and Comparison with TWI															
	Children				Teenagers				Women				Men			
	M	M/TWI %	95thP	95thP/TWI %	M	M/TWI %	95thP	95thP/TWI %	M	M/TWI %	95thP	95thP/TWI %	M	M/TWI %	95thP	95thP/TWI %
Cd ^a	0.19	7.6	1.0	40.0	0.20	8.0	0.56	22.4	0.13	5.2	0.28	11.2	0.19	7.6	0.47	18.8
Hg ^b	0.03	2.3	0.15	11.5	0.03	2.4	0.09	6.7	0.02	1.5	0.04	3.1	0.03	2.3	0.07	5.6

The B3 brand, the origin of the highest trace elements exposure, was chosen for comparison with TWI values. M = median, 95thP = 95th percentile. ^a TWI for Cd = 2.5 µg/kg bw/week [40]. ^b TWI for methylmercury expressed as mercury, TWI for Hg = 1.3 µg/kg bw/week [15].

Table 6. Population categories exposure to Cr, Co, and Ni trace elements via white pita consumption and comparison with the tolerable daily intake (TDI). Exposure values are expressed in µg/kg body weight/day.

Trace Elements	Population Categories’ Exposure to TEs via White Pita and Comparison with TDI															
	Children				Teenagers				Women				Men			
	M	M/TWI %	95thP	95thP/TWI %	M	M/TWI %	95thP	95thP/TWI %	M	M/TWI %	95thP	95thP/TWI %	M	M/TWI %	95thP	95thP/TWI %
Cr ^a	1.74	0.58	9.0	3.0	1.79	0.6	5.06	1.69	1.21	0.40	2.47	0.82	1.72	0.57	4.26	1.42
Co ^b	0.41	25.6	2.09	130.6	0.42	26.3	1.18	73.8	0.28	17.5	0.57	35.6	0.4	25.0	0.99	61.9
Ni ^c	2.80	100	10.26	367	4.06	145	12.26	438	2.75	98	8.83	315	4.34	155	8.86	317

The B3 brand, the origin of the highest trace elements exposure, was chosen for comparison with TDI values, except for nickel for which the B1 brand was retained. M = median, 95thP = 95th percentile. ^a TDI for Cr (III) = 300 µg/kg bw/day [27]. ^b TDI for Co = 1.6 µg/kg bw/day [42]. ^c TDI for Ni = 2.8 µg/kg bw/day [14].

Table 7. Margin of exposure (MOE) to Pb and As trace elements via white pita consumption for the Lebanese population categories.

TEs	Population Categories’ Exposure to TEs via White Pita and Comparison with the Margins of Exposure																							
	Children						Teenagers						Women						Men					
	Median			95th Percentile			Median			95th Percentile			Median			95th Percentile			Median		95th Percentile			
	Value	M0E	M0E	Value	M0E	M0E	Value	M0E	M0E	Value	M0E	M0E	Value	M0E	M0E	Value	M0E	M0E	Value	M0E	M0E	Value	M0E	
Pb ^a	1.25	0.40	–	6.43	0.08	–	1.28	0.39	–	3.61	0.14	–	0.86	0.58	–	1.77	0.28	–	1.23	0.41	–	3.04	0.16	–
As ^b	1.54	0.19	5.19	7.97	0.04	1.00	1.58	0.19	5.06	4.48	0.07	1.79	1.07	0.28	7.48	2.19	0.14	3.65	1.53	0.20	5.23	3.77	0.08	2.12

The B3 brand, the origin of the highest trace elements exposure, was chosen for the margin of exposure calculations. * Values of median and percentiles are expressed in µg/kg bw/day. ^a Pb margin of exposure calculated with BMDL_{0.1} = 0.5 µg/kg bw/day based on neurotoxic effect [41]. ^b As margin of exposure calculated with BMDL_{0.1} = 0.3 µg/kg bw/day for MOE 1 and BMDL_{0.1} = 8 µg/kg bw/day for MOE 2 [16].

4. Discussion

4.1. Survey

The direct input of the answers to the questions asked by the data collectors on electronic tablets made it possible to calculate exposure values to TEs at the level of the individual insofar as we had all the necessary data at this scale: age, gender, body weight, daily intake, and brand of pita. The most recent works on this topic were based on a survey conducted in 2001 and for which the calculation of exposure from the intake was based on the average body weight of the whole surveyed sample [13,43].

This survey was based on a nationally representative survey, a real sample covering 992 Lebanese consumers. To our knowledge, this was the first survey assessing the pita diet in Lebanon in different Lebanese regions covering young people and adults. Previous studies focused on adults in the Beirut area only. There was a large standard deviation in the distribution of bread consumption among adults in all regions. This could be explained by the fact that people aged 65 and over eat a lot of bread.

The use of the questionnaire responses provided a good overview of pita consumption by region, age, and gender. It allowed for the drawing of typologies of people living in these regions too. The combination of estimates on consumption from the survey and the analytical results from pita experiments made it possible to do a risk evaluation.

The exposure study was done on a population consuming mainly white pita (762 individuals out of 992). The remaining population who did not consume white bread would be, in principle, more exposed to trace elements based on the article by Bou Khouzam et al. [1] which emphasizes that Lebanese brown bread is more contaminated than white.

4.2. Risk Characterization

In terms of risk characterization linked to the seven trace elements analyzed in the pita bread, a highly consumed foodstuff, two of the trace elements, Cd and Hg, appeared to not be a safety concern. The results for Cd are in accordance with the Nasreddine [13] study conducted on the total diet in an adult urban population. Bread may not be the main food involved in the highest exposure to Cd, as these authors showed that the main contributors to the dietary intake of Cd in a Beirut TDS were vegetables (46.8%) followed by breads and cereal-based products (30.9%). Same as for Cd, bread is not the main source of Hg, for which it is fish and seafood, which is a concern for high fish consumers [17] and, particularly, pregnant women [15]. We found between 0.70 and 0.89 $\mu\text{g}/\text{kg}$ of Hg in Lebanese pita which is by far less than in the study of Mestek and co-workers [44] who found 13 $\mu\text{g}/\text{kg}$ of Hg in bread in the Czech Republic.

In our study, total chromium was analyzed. The B3 pita contained the highest level of Cr (0.36 mg/kg of dry weight). It is known that the major form of Cr in food is Cr (III), and the major contributor of Cr is bread (8%) followed by bakery products. For children, it is milk (9%) followed by pasta (6%) [22]. In this study, the analytical data from the two brands (B1 and B2) were in accordance with Soares' [22] team who quantified the total chromium contents ($47.3 \pm 20.0 \mu\text{g}/\text{kg}$) of dry weight white bread samples.

Compared to Cr (VI), Cr (III) is considered an essential element for humans and authorized in food supplements in Europe without any upper limit [45]. But, in 2010, the EFSA [46] recommended that total Cr does not exceed 250 $\mu\text{g}/\text{day}$, a value established by the WHO [26]. Furthermore, Cr (III) can complex with lactate and picolinate, causing concern as it has been demonstrated to be genotoxic [27]. In contrast, Cr (VI) is known to be highly toxic and carcinogenic (Group 1, [47]). It also induces skin dermatitis, then the interconversion will be of relevance in terms of risk assessment.

Even if data on the presence of Cr (VI) in food are missing, it is considered that food is a large reducing medium and that oxidation of Cr (III) to Cr (VI) would not occur. Additionally, most of the Cr (VI) is considered to be reduced in the stomach to Cr (III) [27].

Humans are exposed to Cr mainly via food, but it can be present also in water. Concerning bottled water in Lebanese markets [48], a study of the physicochemical and heavy metal parameters

in 32 samples of bottled waters, 25 of these samples showed values below 1 µg/L for Cr, while the other samples showed values ranging between 1 and 4 µg/L in the drinking water. Although naturally occurring Cr (VI) is rarely found in the environment, its presence in ground and surface waters, as a consequence of oxidation of Cr (III)-bearing minerals by Mn (IV) oxides, has been reported recently [49]. Furthermore, in terms of the total quantity found in bread, we do not know its respective part coming from the wheat flour or water used during the bread-making process. Wheat are among species that demonstrate the ability to bio-accumulate chromium the most, just after tomato [5]. An average value of Cr below 0.05 mg/kg of dry weight was measured in wheat seeds in Swedish long-term soil-fertility experiments between 1967 and 2003 [50].

The recipient might also be a source of contamination if Cr is released. Indeed, migration of chromium from steel material occurred in the past mostly from Asia (China) and was above the upper legal limits for food contact material [51].

The EFSA panel recommended the generation of data using sensitive analytical methodologies which specifically measure the content of Cr (III) and Cr (VI) in food and drinking water in different EU Member States [27]; the issue is the same for Lebanon. However, Cr speciation is difficult to conduct, especially in complex matrices. Chromium speciation depends on several factors such as pH, concentration, oxidation state, and complexation with natural components present in the matrix such as peptide [49]. Soares and co-workers [22] mentioned the presence of Cr (VI) in bread after extraction of Cr by alkaline solutions. However, Novotnik et al. [38] demonstrated that the only Cr species in bread is Cr (III) by spiking with enriched isotopes of ⁵³Cr (III) and ⁵⁰Cr (VI), a more accurate quantification, and using HPLC-ICP-MS. Thus, we made a characterization in regards to Cr (III) and, in this way, there were no safety concerns in this study for Cr (III) in Lebanese pita bread.

For cobalt, the exposure of young people under 15 years old at the 95th percentile exceeded the TDI by a percentage of 131%. Bread and cereals are significant source of Co exposure (18.1%), with a daily intake of cobalt estimated to be around 11.4 µg/day according to the Nasreddine study [13]. Indeed, in their study they found 6.36 µg/kg of cobalt in bread and cereals. In this study, the level of Co was 91 µg/kg for the B1 pita brand, which is 7 fold more than in Australian bread and 13.5 fold higher than the data from the Nasreddine study [13] but 1.8 fold less than in Ethiopian bread [52].

In Lebanon, imported flour samples used in Lebanese white pita presented values for cobalt between 2 ± 2 and 33 ± 7 µg/kg which is far less than in the pita. Cobalt contamination can also occur during bread preparation via the water used. The mean level of Co in tap water is approximately 9 µg/L (IRAL, personal communication). In 2013, the Environmental Working Group's (EWG) mission detected Co in only one sample (6 analyzed) and found 1.01 µg/L [53]. Furthermore, cobalt can also be released from food contact materials (FCMs) as it is used in ceramics [54]; thus, we can wonder if cobalt migration can occur from recipients used for making bread in Lebanon.

Several forms of cobalt (II) (sulphate, dichloride) are classified as presumed human carcinogens via the inhalatory route [55]. The IARC classified the soluble form of cobalt II in class 2B but as metal, Co was classified in 2A [56]. Although Co (II) salts are able to induce genotoxicity in vitro and in vivo after oral or parenteral exposure; however, there is a lack of carcinogenesis studies in humans and animals following an oral route [57]. The EFSA concluded that it cannot be excluded that cobalt could have a non-threshold toxicological effect [58]. In France, ANSES made the recommendation to reduce food exposure to cobalt in infants under 3 years old [39]. Thus, for cobalt, the daily intake from all dietary sources may be quite pertinent to monitor in order to determine the total exposure of young people under 15 years old in Lebanon.

In regards to nickel, the B1 brand contained the highest value (1292 µg/kg), which was above values found in studies for Nigerian, Ethiopian, and Spanish bread [52,59,60] but under those obtained in Iranian bread [61]. However, for another brand the value (365 µg/kg) was in the same range.

The average daily intake of nickel was estimated at 126.27 µg/day bread and cereals contributing to a 54.73 µg/day Ni intake in the Lebanese adult urban population [13].

In a French TDS study regarding the infant population, the value determined in bread was approximately 69.5 µg/kg [39]. The contribution of drinking water to total exposure to nickel was very small across dietary surveys and age classes [14]. We do not have an explanation, but this high level could be due to the recipient used by bakers. Using the TDI 2.8 µg/kg bw/day [14] for oral chronic exposure, the data demonstrate that the percentage of Ni TDI was exceeded 3 to 4 fold for all populations with the 10–14 years old range being the most exposed. These data are in accordance with the TDS studies also showing a safety concern in France for very young people (<3 years old) [39].

Nickel compounds and metallic nickel are classified as human carcinogens, respectively, group 1 and group 2B after inhalation [62]. Available studies in animals suggest that exposure to nickel salts lead to renal effects and increases neonatal mortality with the kidney being the principal target. Reproductive and developmental toxicity were the critical effects after chronic oral exposure retained for risk characterization. However, oral exposure to nickel salts could be at the origin of contact dermatitis for sensitive populations. Indeed, oral nickel can also cause an oral and chronic effect in people dermally sensitized [14].

In humans, absorption of Ni varies according to the method of exposure (in water it is 40 fold more absorbed compared to food; 0.7% and the bioavailability varies with the food type [63]). There are no maximum levels in food for nickel; in contrast, a value of 20 µg/L is fixed in water intended for human consumption and in mineral waters in Europe [64,65].

In this study, arsenic exposure was more worrying, as we found a high level of arsenic for all brand analyzed (235–400 µg/kg) which is around 63 fold higher than those found in a bread study in the UK [66] and 32 fold higher than those mentioned in a French infant TDS study [39]. Bou Khouzam et al. [1] mentioned seasonal variation in the arsenic concentration in bread (33 µg/kg in dry season, which is 4 fold higher than in wet season). Arsenic was not studied by Nasreddine et al. [13] and calculated MOEs were very low for all bakeries and the population. These data are in accordance with the data and risk characterization in 19 countries in Europe [16], for example, in France where there is a safety concern with MOEs lower than 36 [39], suggesting that the situation in Lebanon is worrying.

The inorganic form of As, which is known to be toxic, may cause human malignancies. It was classified as carcinogenic (group 1) in Reference [67]. The toxicity, due to the inorganic form, occurs by an epigenetic mechanism, and there is increasing evidence that early life exposure affecting fetal and infant growth [68] may cause chronic disease later in life [69]. This highlights the urgent need to obtain data on the effect of the toxicity of As on development [39]. Arsenic passes through the placental barrier in humans [70], resulting in similar levels in both the fetus and mother. Both the inorganic form and its methylated metabolites are transferred [71]. The effect of As on the endocrine system has also been investigated for many years showing a disruption on the gonadal, adrenal, and thyroid endocrine systems [72]. Furthermore, it has been shown a toxic effect due to an interaction between Cd and As [73,74]. Drinking water, crops irrigated with contaminated water, and food prepared with contaminated water are sources of exposure [75].

The EFSA's recommendation is to reduce inorganic arsenic exposure in food and develop robust validated methods to determine the inorganic form [16]. We do not distinguish between organic and inorganic form of As in this study. It is known that arsenic intake is higher from solid foods than from liquids.

Furthermore, speciation plays a major role in determining the amount of arsenic absorbed after consumption of As contaminated food as well as bioavailability with significant interspecies differences for organic arsenic. Regards to inorganic form, absorption can be high as demonstrated with contaminated rice [76,77].

Representative data on speciation are indeed scarce. The EFSA panel assumed a proportion of the contaminant to inorganic form (the toxic one) to vary between 50 (best case) to 100% (worst case) of the total arsenic reported in food commodities in Europe, other than fish and seafood [16]. Furthermore, in bread, 92% of the inorganic form has been found [78]. Cereals and cereal-based products were identified as largely contributing to the daily exposure to inorganic arsenic, as it was quantified as 53 µg

up to 72 µg of arsenic per kilo for all cereals and cereal product categories. In wheat samples, it was demonstrated that 91% to 95% of As was in the inorganic form [77,78]. In wheat plants cultivated in the Bekaa region, the element As presented values between 288.3 ± 14.7 and 743.7 ± 25.4 mg/kg. In the irrigation water used in the Bekaa sites cultivated for wheat plants, the As content varied between 0.8 ± 0.3 µg/L and 1.2 ± 0.2 µg/L (IRAL data). Indeed, the level of As in drinking water in Lebanon varied between 1.2 and 4.5 µg/L [79]. Water used for making bread can be the main contributor to the level of arsenic in the pita with the main form recovered in drinking water as inorganic. It is important to note that change in the speciation of As can occur during food preparation [80].

Concerning the level of lead in the different brands: B2 and B3 contents were more than 2.5 fold higher than B1 (74, 203, and 260 µg/kg, respectively). The order of magnitude of B1 is in accordance with Turkish, Iranian, Ethiopian, and Spanish studies on bread [52,59] and 7 fold more higher than in data on the Czech Republic [44]. The B3 pita lead content was 32 fold higher than Lebanese data [13] which focused on Beirut. The authors noticed that the lead exposure in Beirut was even two fold lower compared to a previous study [43]. Thus, our data were not in accordance, but it took into account all the Lebanese regions including rural ones. The lead origin may be from wheat. Several samples of Lebanese wheat cultivated in the Bekaa region were studied, showing values of lead varying between 4.5 and 23 µg/kg but far less than in the bread, noting that the lead values in Lebanese wheat samples were lower than the values showing in wheat samples in Saudi Arabian markets (2.81 ± 0.08 mg/kg dry weight) [12]. While, the lead levels, measured in five wheat flour samples in Lebanese markets varied between 0.0045 ± 0.002 and 0.023 ± 0.016 mg/kg dry weight, they were lower than values presented in wheat flour samples in the Canary Islands and used for bread production (varying between 0.037 ± 0.013 and 0.056 ± 0.045 mg/kg fresh weight) [81].

Furthermore, lead can also be a contaminant issued from recipient food contact materials or water used for bread making.

Human data show that the developing nervous system is a critical target in children. Various water soluble and insoluble lead can induce tumors in rodents, especially renal tumors as a carcinogenic/promoter; brain gliomas were also observed. Classified lead compounds are probably carcinogenic to humans on the basis of limited evidence of carcinogenicity in humans and sufficient evidence in animals [56].

It is important to note that due to the long lead half-life, chronic exposure is of most concern and children absorb lead after ingestion to a much greater extent (70% of the amount of lead ingested) than adults (20%) [82].

All MOEs calculated with exposure only via pita consumption in this study were very low, but in the same range than in France and Europe [17,39,41], suggesting an exposure safety concern and that it is necessary to decrease Pb exposure, for example, by varying the diet due to the difficulty in reducing Pb contamination.

For both lead and arsenic, this contamination occurred for all bread brands analyzed in this study and there is a safety concern (Supplementary Materials Table S3).

5. Conclusions

This was the first study conducted all over Lebanon showing the exposure of different categories of Lebanese population to trace elements via bread. A survey of daily consumption of pita bread was conducted on a sample of 992 people. Among the four categories (i.e., children, teenagers, women, and men), white bread was the most consumed. Men consumed the highest quantity (282 g/day). The survey revealed that three brands of white pita were the most consumed: B1, B2, and B3.

Seven trace elements (i.e., As, Cd, Co, Cr, Hg, Ni, and Pb) were quantified in pitas coming from B1, B2, and B3. B1 was characterized by high levels of Ni and Co. B2 was characterized by the presence of As and Cd, and B3 was characterized by the presence of Cr, Hg, and Pb. Lead concentrations exceeded the authorized limits of the Lebanese standard for bread in the two brands. Further analysis should be

performed on soil, wheat, flour, and water in order to determine the source of the contamination and to reduce exposure.

The B3 brand was at the origin of the highest trace element exposure, except for nickel for which B1 was retained. In terms of risk characterization, there was no safety concern due to the fact of pita consumption for all studied categories with Hg, Cd, Cr, and for Co except the 95th percentile of the children category (6–9 years old). Nickel exposure showed an excess of the TDI up to 4 fold for the most exposed populations (95th percentile). There were safety concerns related to As and Pb, as the margins of exposure were very low. To our knowledge, this is the first time that exposure to As was studied in the Lebanese population. To confirm these observed data, it is urgent to analyze the inorganic arsenic form levels in bread. Experiments are currently being run to study any cocktail effects (synergistic) using pertinent bioassays due to the exposure to the sum of trace element identified in bread. The ultimate objectives are to protect the Lebanese population by reducing exposures to populations particularly exposed to TE presenting safety concern and to make recommendations, as bread is only one part of the daily diet.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2304-8158/8/11/574/s1>, Survey and questionnaire related to Lebanese bread consumption. Figure S1: Distribution of physical activity intensity by gender in adult population. Table S1-a: Cross-tabulation of consumed pita type occurrences for the total 992 respondents in the number of individuals. Table S1-b: Types of consumed bread for the subpopulation consuming mainly white pita (762 individuals). Table S2: Pearson correlation matrix of the principal component analysis. Table S3: Population categories' exposure to Cd, Hg, Cr, Co, Ni, Pb, and As trace elements via white pita consumption according to the most consumed brands.

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Supplementary data

Manuscript Title: Lebanese population exposure to trace elements *via* white bread consumption
Supplementary data

Survey and questionnaire related to Lebanese bread consumption.

A study was conducted on the presence of metallic trace elements (ETM) throughout the Lebanese bread chain, from farm to fork. Some of these elements may cause health problems for the consumer, so the amount of Lebanese bread (likely to be contaminated) consumed per day and per person must be known, to assess the risk related to a route of oral exposure of trace elements.

Metallic trace elements are contaminants in the environment and foodstuffs, including bread. Once in excess of the environment, trace elements enter the soil, water or air and contaminate certain foods. They can cause a risk to human health if the consumer is too much exposed, for example, by ingesting cereals grown in contaminated soils. It is therefore important to monitor the presence of these contaminants in the bread and to know the exposure of the consumer by conducting a survey among the population in different regions of Lebanon.

Personal data collected will only be used for exposure calculations and will not be disclosed. Anonymity will be ensured throughout the study.

There is no intervention to undergo or therapeutic protocol to follow.

- **Do you have a chronic or serious illness?** Yes No
- **Do you eat Lebanese bread for more than 15 years?** Yes No
- **Do you live in Lebanon for more than 15 years? (Criteria for adults only)**
 Yes No

If you meet the above criteria, please complete this questionnaire, which will take you less than ten minutes

Date.....

investigator.....

Part 1. Consumption of Lebanese bread

1. What is the kind of Lebanese bread eaten?

- 1 White bread 2 Brown bread 3 Other please specify:

2. What is the brand of bread most consumed?

- 1 Brand 1 2 Brand 2 3 Brand 3
 4 Brand 4 5 Others please specify:

3. What is the size of pita consumed?

- 1 Small 2 Medium 3 Large

4. What is the number of pitas consumed per day?

- 1 2 3 4 Others please specify:

Part 2. Sociodemographic data

5. Age: | __ | __ |

6. Gender: 1 Man 2 Woman

7. Place of residence:1 Beirut 2 Mount Lebanon 3 North Lebanon4 South Lebanon and Nabatieh 5 Bekaa**8. Region:** 1 Urban 2 Rural**9. Level of education:**1 Secondary 2 Technical secondary 3 Secondary4 Technical University 5 Non-technical University**10. Economic activity:**1 Works 2 Seeks a job first3 Unemployed 4 Studied5 Study and work 6 Retired or annuitant7 Other inactive**11. If you work (or have worked), the profession:**0 Armed Forces1 Members of the executive and legislative bodies, senior managers of public administration, executives and senior company executives2 Intellectual and scientific professions3 Intermediate professions4 Employees of administrative type5 Service, store and market sellers6 Farmers and skilled workers in agriculture and fisheries7 Artisans and handcraft workers8 Plant and machine operators and assembly workers9 Workers and unskilled workers10 Other specify:**Part 3. State of health****12. Are you a smoker?**1 Yes 2 No**13. If yes, please specify:**

Cigarette: a- Number of cigarettes / day: | __ | __ | b- Number of years of smoking: | __ | __ | Nargile: c- Number of nargile / week: | __ | __ | d- Number of years of tobacco: | __ | __ |

14. Are you an alcohol drinker?1 Yes 2 No**15. If yes, please specify:**a- 1 Occasional or 2 Regular

b- Number of glasses / day: | __ | __ |

c- Number of years of consumption: | __ | __ |

16. How do you rate your health?

1 Very good 2 Good 3 Fair 4 Bad 5 Very bad

17. a- Weight: | _____ | Kg b-Size: | __ | __ | __ |

18. Do you exercise?

1 Yes, low intensity physical activity 2 Yes, moderate intensity physical activity
3 Yes, high intensity physical activity 4 No

19. If yes, how much time do you spend per week? | ____ | hours

Intensity and measurement of physical activity

Physical activity is a behavior that can be characterized by a frequency, intensity, duration and type of practice that define the amount of physical activity in a space-time (day, week, etc.).

MET: The intensity of a physical activity is most often expressed in MET (metabolic equivalent of task), defined as the ratio of energy expenditure related to physical activity on basic metabolism. 1 MET is the level of energy expenditure at rest, sitting on a chair (3.5mL O₂/mn/kg).

Physical activity of low intensity (<3 MET) It does not require effort (no breathlessness, no perspiration)

Examples:

- Iron
- Dust off
- Clean the windows
- To make the beds
- Cook, do the dishes, do the shopping
- Repair and wash the car
- To vacuum
- Sweep gently
- Carry loads up to 6 kg when climbing the stairs
- Clean

Physical activity of moderate intensity (about 3-6 MET)

It requires a moderate effort and significantly accelerates the heart rate. (Moderate shortness of breath, possible conversation, moderate sweating)

Examples:

- walk briskly
- dance
- garden
- engage in traditional hunting and gathering
- actively participate in games and sports with children / take out your pet
- do crafts (For ex: roof repairs, painting)
- lift / move loads <11kg

High intensity physical activity (approximately > 6 MET)

It requires a great effort, the breath is shortened and the heart rate accelerates considerably. (Marked breathlessness, difficult conversation, heavy transpiration)

Examples:

- run
- walk briskly / climb at fast pace
- ride a bike at high speed
- do aerobics
- swim at high speed
- play sports and competitive games (For ex: traditional games, football, volleyball, hockey, basketball)
- doing hard work
- lift / move heavy loads of 11 kg or more

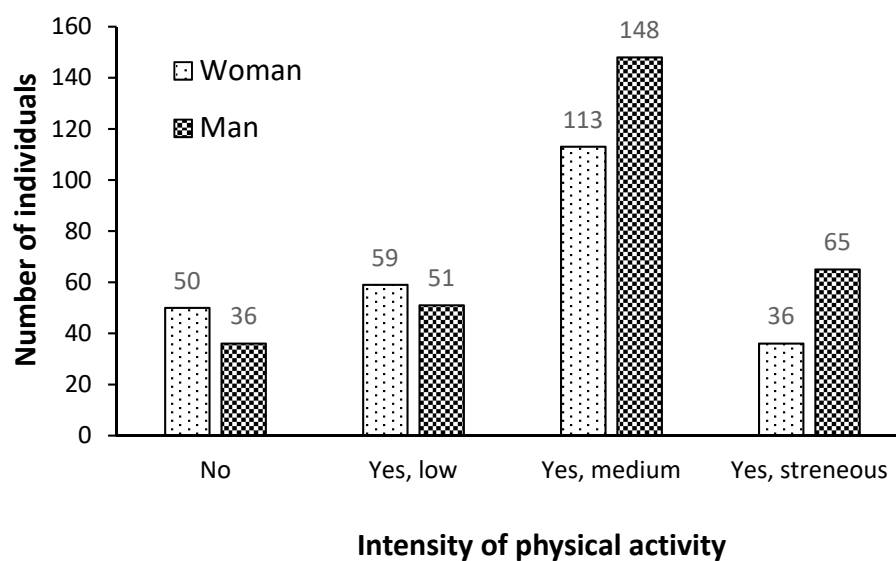


Figure S1: Distribution of physical activity intensity by gender in adult population.

Table S1-a. Cross-tabulation of consumed pita types occurrences for the total 992 respondent in number of individuals.

	White	Brown	Others ¹	Total
White	677			677
Brown	68	162		230
Others ¹	14	16	52	82
White+Brown+Others ¹	3			3
Total	762	178	52	992

¹Others: rye, 35; Saj, 22; Tannour, 9; whole wheat, 8; oat, 4; bran, 2; baguette, 1; gluten free, 1

Table S1-b. Types of consumed bread for the subpopulation consuming mainly white pita (762 individuals).

Types of consumed bread	Individuals	
	Number	%
White only	677	88.8
White + Brown	68	8.9
White + Others	14	1.8
White + Brown + Others	3	0.4
Total	762	100.0

Table S2: Pearson correlation matrix of the Principal Component Analysis.

Variables	As	Co	Cd	Cr	Hg	Ni	Pb
As	1						
Co	-0.41*	1					
Cd	0.43*	-0.10	1				
Cr	-0.03	-0.49*	-0.49*	1			
Hg	-0.04	-0.19	-0.53*	0.64*	1		
Ni	-0.59*	0.65*	0.09	-0.76*	-0.50*	1	
Pb	0.68*	-0.65*	-0.00	0.69*	0.39*	-0.98*	1

* Values significantly different from zero at a level of significance $\alpha = 0.05$

Table S3: Population categories exposure to Cd, Hg, Cr, Co, Ni, Pb & As trace elements via white pita consumption according the most consumed brands.

Trace element	Brand	Trace element exposure ($\mu\text{g}/\text{kg}$ body weight/day)							
		Children		Teenagers		Women		Men	
		M	95 th P	M	95 th P	M	95 th P	M	95 th P
As	B1	0.51 ^a	1.86 ^a	0.74 ^a	2.23 ^a	0.50 ^a	1.60 ^a	0.79 ^a	1.61 ^a
	B2	1.43 ^b	7.65 ^b	1.07 ^{ab}	4.17 ^a	0.77 ^b	2.11 ^a	1.05 ^b	2.63 ^a
	B3	1.54 ^b	7.97 ^b	1.58 ^b	4.48 ^a	1.07 ^{c*}	2.19 ^{a*}	1.53 ^{c*}	3.77 ^{b*}
Cr	B1	0.09 ^a	0.32 ^a	0.13 ^a	0.38 ^{ab}	0.09 ^a	0.28 ^a	0.14 ^a	0.28 ^a
	B2	0.06 ^a	0.30 ^a	0.04 ^b	0.16 ^a	0.03 ^{b*}	0.08 ^b	0.04 ^{b*}	0.10 ^{b*}
	B3	1.74 ^b	9.00 ^b	1.79 ^c	5.06 ^b	1.21 ^{c*}	2.47 ^{c*}	1.72 ^{c*}	4.26 ^{c*}
Co	B1	0.20 ^a	0.72 ^a	0.29 ^{ab}	0.86 ^a	0.20 ^{ab}	0.31 ^a	0.31 ^a	0.62 ^{ab}
	B2	0.31 ^{ab}	1.66 ^a	0.23 ^a	0.91 ^a	0.17 ^{a*}	0.23 ^{a*}	0.23 ^{a*}	0.57 ^a
	B3	0.41 ^b	2.09 ^a	0.42 ^b	1.18 ^a	0.28 ^{b*}	0.40 ^{b*}	0.40 ^{b*}	0.99 ^{b*}
Ni	B1	2.80 ^a	10.26 ^a	4.06 ^a	12.26 ^a	2.75 ^a	8.83 ^a	4.34 ^a	8.86 ^a
	B2	1.30 ^a	6.99 ^a	0.97 ^b	3.81 ^a	0.70 ^{b*}	1.93 ^b	0.96 ^{b*}	2.40 ^b
	B3	ND	ND	ND	ND	ND	ND	ND	ND
Pb	B1	0.16 ^a	0.59 ^a	0.23 ^{ab}	0.70 ^{ab}	0.16 ^a	0.50 ^a	0.25 ^a	0.51 ^a
	B2	0.72 ^{ab}	3.88 ^a	0.54 ^a	2.12 ^a	0.39 ^{b*}	1.07 ^b	0.53 ^b	1.33 ^b
	B3	1.25 ^b	6.43 ^a	1.28 ^b	3.61 ^a	0.86 ^{c*}	1.77 ^{c*}	1.23 ^c	3.04 ^{c*}

Trace element	Brand	Trace element exposure ($\mu\text{g}/\text{kg}$ body weight/week)							
		Children		Teenagers		Women		Men	
		M	95 th P	M	95 th P	M	95 th P	M	95 th P
Cd	B1	0.10 ^a	0.36 ^a	0.14 ^a	0.43 ^a	0.10 ^a	0.31 ^a	0.15 ^a	0.31 ^a
	B2	0.22 ^a	1.16 ^a	0.16 ^a	0.64 ^a	0.12 ^a	0.32 ^a	0.16 ^a	0.40 ^a
	B3	0.19 ^a	1.00 ^a	0.20 ^a	0.56 ^a	0.13 ^{a*}	0.28 ^{a*}	0.19 ^{a*}	0.47 ^{a*}
Hg	B1	0.01 ^a	0.04 ^a	0.02 ^{ab}	0.06 ^a	0.01 ^{ab}	0.04 ^{ab}	0.02 ^a	0.04 ^a
	B2	0.02 ^{ab}	0.09 ^a	0.02 ^a	0.07 ^a	0.01 ^{a*}	0.03 ^a	0.01 ^{a*}	0.03 ^a
	B3	0.03 ^a	0.15 ^a	0.03 ^b	0.09 ^a	0.02 ^{b*}	0.04 ^{b*}	0.03 ^{b*}	0.07 ^{b*}







M = Median, 95th P = 95th percentile. Different letters in each column are significantly different at $P < 0.05$.

*corresponds to a significant difference, respectively, medians and percentiles at $P < 0.05$ for a given brand.

N.D. = Not Determined because the Nickel content was below the detection limit.



Validation of a new method for monitoring trace elements in Mediterranean cereal soils

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ABSTRACT

In the Mediterranean region, agricultural soils are seriously polluted with toxic trace elements (TEs) which could enter the food chain via the soil-plant trophic chain. For food safety reasons, the monitoring of TE concentrations in these agricultural soils is thus imperative. The most powerful monitoring method for TE measurements is based on perchloric acid (HClO₄) and hydrofluoric acid (HF) digestion, commonly used as reference total digestion (RTD) method, with consequent use of inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). Unfortunately, HF and HClO₄ manipulations are highly dangerous and ICP-OES and ICP-MS apparatus are very expensive, thus they are unaffordable, notably in developing countries. In this paper, an alternative, microwave sulphuric digestion (MSD) method, combined with atomic absorption spectroscopy (AAS) is proposed. First, the suggested method was validated on a soil certified reference material, for the determination of 7 TEs (As, Cd, Co, Cr, Cu, Ni and Pb). Second, the MSD method was applied on agricultural soil samples situated in the Bekaa valley, East Lebanon and results were compared to those of RTD method. The MSD method, coupled to AAS, offers a promising and feasible alternative to HF, as well as, *aqua regia* based methods.

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1. Introduction

Trace elements (TEs) are the most studied soil contaminants in the world, and come both from lithogenic sources [1] and anthropogenic activities [2]. They are persistent, non-degradable and even though some are essential for living organisms, they can adversely affect microorganisms, animals, plants and humans at high concentrations. As a matter of fact, TEs can accumulate in plants to harmful concentrations, becoming a threat for

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human health through the food chain [3]. Accurate and routine determination of their concentration in soil is thus necessary to assess their risk to directly and indirectly enter the food chain.

Cereal production soils in the Mediterranean basin are regularly amended with organic matter and phosphates bearing TEs and repeatedly irrigated with untreated wastewater [4,5]. Regular monitoring of TE concentrations in these agricultural soils is of great interest. Arsenic, Cd, Co, Cr, Cu, Ni and Pb are the most monitored elements based on previous studies of TE determination in cereal production soils of the Bekaa valley, East Lebanon [6]. Nevertheless, less than 6% of Lebanese arable lands have been checked so far for TE contamination [7].

Although some countries introduced the nondestructive thick target particle induced X-ray emission technique for the determination of trace elements in as received soil samples, digestion methods are still widely practiced [8]. However, the heterogeneous and complex soil matrix, associating organic compounds and mineral constituents with different physicochemical characteristics, does not allow a single solid phase digestion method for the determination of TEs. To be acceptable, precision and accuracy of digestion methods should be lower than 20% [9] for any set of targeted soil to be analysed on a routine basis.

The digestion of samples containing TEs can be done using a dry method: in this case, the sample is calcined in an oven at temperatures ranging between 450°C and 500°C. Dry ashing offers the advantage of requiring fewer reagents like acids to solubilise ashes. However, dry ashing can lead to a loss of analytes like As and Hg by volatilisation.

To reduce the sample preparation time and losses by volatilisation, wet digestion processes are an attractive alternative to dry ashing methods for several samples such as biological tissues, food and soils [3,10]. In this case, the sample is mineralised in a mixture of acids at their boiling point. Different acids are usually used to perform the wet mineralisation: hydrochloric acid (HCl), nitric acid (HNO₃), sulphuric acid (H₂SO₄), perchloric acid (HClO₄), hydrofluoric acid (HF). Several combinations were used for the mineralisation of soil samples [11–14] but there is no consensus on the choice of the mineralisation reagent.

It is shown that HF combined with HClO₄ allows for a total dissolution of all elements present in the sample but induces Si volatilisation. However, HF is dangerous to handle. High precautions and safe handling should be implemented in the laboratory to prevent accidents. Despite this, serious accidents can always occur.

To avoid handling HF, sample attacks using a mixture of other strong acids are usually adopted in routine analysis. HNO₃ has high oxidant properties, most often used in wet mineralisation, especially when the analysis step is done by atomic absorption technics [15]. Its relatively low boiling point (120°C) limits its efficiency. Therefore, HNO₃ is commonly used in a mixture with H₂SO₄ or HClO₄ (boiling point at 340°C and 200°C, respectively). HClO₄ should be handled carefully because when its concentration exceeds 85% above 150°C, it becomes unstable and presents a serious explosion hazard. The combination of HNO₃ with HCl is well-known as '*aqua regia* mixture'. It has a high dissolution power and is usually used to evaluate the trace element contamination level [16]. *Aqua regia* reagent gives a pseudo-total concentration compared with the HF total method, especially for the following elements (Cr, Ni, Zn and Pb) [17].

Ammonium fluoride (NH_4F) and ammonium bifluoride (NH_4HF_2) are used as a substitute for HF. These compounds are safer than HF for the operator and have high boiling points (260°C and 239.5°C) allowing sample digestion at high temperature in open vessels [18].

To optimise the mineralisation time, microwave heating is often performed. Hydrogen peroxide (H_2O_2) is largely used in mixture with strong acids for the oxidation and destruction of organic matter [11–13]. Its oxidation power is greatly enhanced in the presence of H_2SO_4 due to the formation of H_2SO_5 [15]. Recently, Magdali et al. [19] used a mixture of NH_4HF_2 and HNO_3 in a microwave oven to dissolve completely a wide range of silicate rock samples for trace element determination. Certain analytes were not fully recovered using this method which limits its field of application.

Most often, TEs concentration in the mineralisation solutions is determined using inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) following ISO 22036 [20] and ISO 17294–2 [21] standards. These devices are very expensive and have high maintenance costs but combines low detection limits and a rapid multi-elemental determination in comparison with atomic absorption spectroscopy (AAS). Therefore, it is important to have available a method for TE analysis that is safe, green, inexpensive and accessible to a large number of laboratories. In agreement with green analytical chemistry, an eco-friendly infrared digestion method was recently developed for multi-element determination in soil sample by microwave induced plasma atomic emission spectrometry (MIP-OES) [22]. This method minimises the reagents amounts, makes digestion safer and reduces waste.

The choice of a sample preparation method is crucial in the analysis of soil and sediment samples. Proper choice reduces matrix effects in complex samples and subsequently limits spectral and non-spectral interferences. The latter involves sample-introduction and plasma related-interferences. This last point becomes very important when using plasma-based atomic emission like ICP-OES and ICP-MS. Nitric acid is usually used to avoid spectral interferences caused by other acids [23].

In this study, two digestion methods were compared using a soil certified reference material: 1) heating 3 acid digestion (HAD) with a mixture of HNO_3 , HClO_4 and H_2SO_4 , and 2) microwave sulphuric digestion (MSD) using a mixture of H_2SO_4 and H_2O_2 . The latter is proposed as a green, simple and fast method for soil sample digestion. The use of H_2SO_4 in sample digestion in combination with H_2O_2 is more advantageous than the use of NH_4F and NH_4F_2 . The latter are safer than HF but remain more dangerous than H_2SO_4 .

The measurement of As, Cd, Co, Cr, Cu, Ni and Pb concentrations were then conducted using a flame atomic absorption spectrometer (AAS). AAS is less efficient than the widely used plasma-based methods (ICP-OES and ICP-MS) because it is less sensitive and only allows the determination of single elements. However, AAS is cheaper than plasma-based methods and is available to a large number of laboratories. A validation of the selected digestion method was done using a certified reference material (CRM). Afterwards, the validated method (MSD + AAS) was applied on agricultural soil samples collected from the Bekaa region, located in Lebanon, East

Mediterranean. The results were compared to those obtained using the reference total digestion method (RTD) (HF-based digestion + ICP/ICPMS).

2. Material and methods

2.1. Chemicals

The standard solutions for the elements As, Cd, Co, Cr, Cu, Ni, and Pb of concentrations $1000 \pm 1 \text{ mg L}^{-1}$ were obtained from Sigma-Aldrich. Nitric acid HNO_3 65%, extra pure, sulphuric acid H_2SO_4 95% and, perchloric acid HClO_4 70% and hydrogen peroxide H_2O_2 30%, obtained from Sigma-Aldrich, were used for digestion. A 1% HNO_3 nitric acid was prepared from concentrated acid (65% HNO_3) and was used in the standard solution preparation. Ultrapure water ($<18 \text{ M}\Omega \text{ cm}^{-1}$) was obtained from a Milli-Q apparatus (Boeco pure, Germany). The Certified Reference Material (CRM) (SQC001-LRAA8753) for trace elements (As, Cd, Co, Cr, Cu, Ni and Pb) in soil was purchased from Sigma Aldrich.

2.2. Sites and sampling

Lebanon is located in the eastern Mediterranean basin between 35° and $36^\circ 40'$ E and between 33° and $34^\circ 40'$ N. The Bekaa Valley, an agricultural region where more than 90% of Lebanese wheat production is produced [24], is mainly a plain area located between Mount Lebanon in the west and the Anti-Lebanon mountains to the East. Eight sites where soil samples were taken are selected along a transect crossing the central Bekaa



Figure 1. The location of the soil samples collected from Zahleh and western Bekaa administrative districts Lebanon: Mazraat Zahleh (B1), Tcheflik Qiqano (B2), Marj (B3), Haouch El Oumaraa (B4ab and B5), Maalaqah (B6), Dalhamiyeh (B7), Haouch El-Aamara (B8). *Satellite Images © CNES/Airbus, Maxar Technologies, Map data © 2021.*

valley from southwest to northeast (Figure 1). The soils of the sampling sites are Eutric and Vertic Cambisols (B1, B2, B5, B6 and B7), and Eutric Fluvisols (B3, B4ab and B8) according to the World Reference Base [25]. They are deep, predominantly non-calcareous clay, with neutral to weakly basic pH values (7.4–7.8) and low to medium organic matter (1.4–2.7%).

Most of these sites were irrigated by private tube wells taking water from the deep groundwater reservoir, and by surface waters when available nearby (Berdawni and Litani rivers). Farmers generally practice an annual wheat/potato succession.

Topsoil layer (0–20 cm) was sampled using a stainless-steel shovel at the corners of the plot, in the centre and diagonally. The nine subsamples of the same plot were mixed, homogenised and carried in clean polyethylene bags. The samples were then brought to the laboratory where a quartering technique [26] was applied to the overall homogenised sample to reduce the sample size and form a composite sample. Only about 1 kg of soil sample underwent pretreatment.

2.3. Samples pretreatment and digestion

The composite soil samples were cleaned up from roots and gravels, dried according to the AFNOR NF X31-102 standard [27] in an oven (Model 700 Memmert) at 40°C, until constant weight and sieved at 2 mm. Then, soil samples were then stored in plastic bottles protected from the direct sunlight and at a temperature below 4°C (in the refrigerator) until analysis.

Before mineralisation, the soil samples were ground fine with a mortar and sieved (diameter <250 µm) following the standard NF ISO 11464 [28]. Two methods of digestion were used in order to mineralise a representative part of the composite soil samples:

i) For the heating three acid digestion (HAD), a three-acid mixture (9 mL HNO₃, 2 mL HClO₄, 2 mL H₂SO₄) was added to 0.5 g of soil sample or CRM, followed by heating up to 350°C for 2h30 min in a heating block. The mixture was then stored at room temperature for one night. ii) For the microwave sulphuric digestion (MSD), a microwave type 'Milestone – Ethos Easy' was used. Fifteen vessels can be processed simultaneously. This system controls the temperature in all vessels using a direct contactless temperature sensor. An acid mixture of 9 mL of H₂SO₄ and 3 mL of H₂O₂ were added to 0.5 g of soil sample or CRM in a Teflon receptacle tightly closed. A temperature of 200°C was reached in 10 min and maintained at this level 10 min. Then, the temperature decreased gradually where the cooling step was maintained for 20 min. This method was suggested by Milestone and was subjected to a validation.

All digested samples were filtered and adjusted to 25 mL using HNO₃ 1% before analysis.

2.4. Apparatus for TE analysis

The analyses of TEs were conducted by atomic absorption spectrometry (flame iCE 3000 series – Thermo type, Thermo Fischer Scientific, Germany). An acetylene/air flame was used. Liquid samples were introduced in the spectrometer with an auto sampler using blank and standards for the analysis of the following elements: As, Cd, Co, Cr, Cu, Ni and Pb.

Calibration standard solutions were prepared by successive dilution, all plastic labware used for sampling and sample treatment was new or cleaned by soaking 24 h first in 10% HNO₃ then in ultra-pure water.

2.5. Official or reference methods for trace element analysis

For the reference total digestion (RTD) method, soil sample mineralisation was done by a mixture of HF (5 mL) and HClO₄ (1.5 mL) acids added to 0.25 g previously ground soil below 250 µm following the standard ISO 14869–1 [29] (details are given in Supplementary Materials SM1). The HF decomposes silicates to form volatile SiF₄. The use of HClO₄ is necessary to avoid the precipitation of calcium in the form of fluoride (CaF₂). To avoid the risks of a brutal oxidation (acid ejection) of the organic matter (OM) by HClO₄, the OM was previously destroyed by calcining at 450°C. The hydrofluoric and perchloric acids were eliminated by evaporation at the end of the reaction. The residue was dissolved in diluted nitric acid 2%. Trace elements Co, Cr, Cu and Ni were then analysed by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES, Vista Pro, Varian, France) following the standard ISO 22036 [20], while As, Cd and Pb were analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Thermo series 2, France) following the standard ISO 17294–2 [21].

2.6. Statistical analysis

All calculations related to the working range, linearity domain, limit of detection and limit of quantification were realised using R-4.0.0 [30] and RStudio 1.2.5042 [31] with packages: 'car' [32], 'lmtest' [33] and 'dplyr' [34].

Analysis of variance was performed using XLSTAT 2018 to calculate repeatability and intermediate precision.

Information about various statistical analyses are detailed in the following paragraphs, especially in section 3.2.1 for working range, linearity domain, limit of detection and limit of quantification and 3.2.2. for repeatability and intermediate precision.

3. Results and discussion

3.1. Choice of the best digestion method using the soil certified reference material CMR (SQC001)

To choose the best digestion method, the heating three acid digestion (HAD) and the microwave sulphuric digestion (MSD) methods were both applied on the soil CRM (SQC001).

HNO₃ is the most used acid in wet digestion. To overcome its limited efficiency due to its low boiling point, it was used in combination with H₂SO₄ and HClO₄ for the HAD digestion. Moreover, HClO₄ ameliorate the dissolution of sediment samples and insures a complete recovery of trace elements [35]. For the MSD digestion, H₂SO₄ was used because it has the highest boiling point. A mixture of H₂SO₄ and H₂O₂ was used because H₂SO₄ enhances the oxidation power of H₂O₂.

After digestion, the TEs As, Cd, Co, Cr, Cu, Ni and Pb were determined by AAS. The relative recovery, expressed in percentage was calculated using the following equation (Equation 1):

$$R(\%) = \frac{\bar{x}}{x_{ref}} \times 100 \quad (1)$$

Table 1. Comparison of the heating three acid digestion method (HAD) and the microwave sulphuric digestion method (MSD) using their relative recovery percentage of the soil certified reference material CMR (SQC001).

Element	Soil CRM Assigned value \pm SD mg kg ⁻¹	HAD method		MSD method	
		Mean Value \pm SD mg kg ⁻¹	Recovery %	Mean Value \pm SD mg kg ⁻¹	Recovery %
As	161.0 \pm 3.6	115.3 \pm 1.0	71.6	150.9 \pm 1.2	93.7
Cd	190.0 \pm 3.9	154.6 \pm 1.1	81.4	165.9 \pm 2.4	87.3
Co	177.0 \pm 3.6	91.2 \pm 1.0	51.5	199.3 \pm 4.7	112.6
Cr	87.9 \pm 2.3	67.8 \pm 0.7	77.1	98.7 \pm 1.0	112.3
Cu	258.0 \pm 5.3	232.3 \pm 0.3	90.0	230.1 \pm 2.3	89.2
Ni	127.0 \pm 3.2	111.2 \pm 1.1	63.5	125.0 \pm 1.1	98.4
Pb	138.0 \pm 4.0	80.6 \pm 1.2	80.6	155.4 \pm 1.0	112.6

SD = Standard deviation

where \bar{x} is the mean of CRM after digestion of the sample in triplicate, and X_{ref} is the assigned property value of CRM.

For the HAD method, the relative recovery for the elements analysed by AAS varied between 51.5% and 90.0% (Table 1). These values were very low and showed that the HAD method using the mixture HNO₃, HClO₄, H₂SO₄ was not efficient enough to sufficiently solubilise the analysed trace elements.

For the MSD method, the relative recovery was in general higher and varied between 87.3% and 112.6%. These values were close to 100% and are within the accepted range for TE analysis (80–120%) [36]. Results show that sulphuric acid along with H₂O₂ represents an efficient mixture to solubilise TEs from different soil types, especially when microwave heating is used.

As the microwave sulphuric digestion of CRM gave acceptable recovery percentages, a validation procedure using this method was performed to check its applicability in our laboratory for trace element analysis in some Mediterranean soils.

3.2. Validation of the microwave sulphuric digestion method followed by AAS using the soil certified reference material CMR (SQC001)

To validate the new digestion using a mixture of H₂SO₄ and H₂O₂, before applying it for trace element analysis in the soils, the following parameters were considered and determined: working range including linearity, limit of detection of the analytical system (LOD), limit of quantification (LOQ), precision including repeatability and intermediate precision, specificity and trueness.

3.2.1. Working range, linearity domain, limit of detection (LOD) and limit of quantification (LOQ)

The linearity of a method is often confused with the linearity of the instrument calibration function. In fact, the linearity of a method characterises its accuracy, whereas instrumental linearity is concerned only with its own response. However, a response function does not have to be linear for correct quantification. Many methods make effective use of calibration models that are much more complex than just linearity [37,38].

Calibration curves for each measured element were built using 4 or 5 points, depending on the element, each point was triplicated. For some elements, quadratic regression $y = \beta_0 + \beta_1x + \beta_2x^2 + \varepsilon$ gave better results and for some others, the best fit

Table 2. Calibration curves of the measured elements.

Element	Model	Residual behaviour (normality, homoscedasticity, independence)
As	Quadratic	Passed
Cd	Quadratic	Passed excepted normality*
Co	Quadratic	Passed excepted normality*
Cr	Linear	Passed excepted autocorrelation *
Cu	Linear	Passed
Ni	Linear	Passed excepted normality & autocorrelation, presence of 1 outlier
Pb	Quadratic	Passed

*Due to the presence of one or two extreme values (checked with Bonferroni Outlier Test) [32]. The calculation of a reliable calibration model needed in general many values to be at least between 8 and 10 to verify the normality of the data by the Shapiro–Wilk test (Shapiro & Wilk, 1965) for instance, and to ascertain their scedasticity; and the number of concentration levels must range between 7 and 10 [43]. In this study, the number of levels is between 4 and 5 with 3 replicates. So, the calibration models were evaluated with and without extreme values and coefficients were not drastically affected. It was decided to keep them.

Table 3. Working ranges, regression equations, limit of detection (LOD) and limit of quantification (LOQ) of the measured elements.

Element	Working range mg kg ⁻¹	Regression equation $y = ax^2 + bx + c$ or $y = ax + b$	Standard errors on a, b and c	Regression quality R ²	LOD mg kg ⁻¹	LOQ mg kg ⁻¹
As	1.25–12	$y = -0.0032x^2 + 0.1599x - 0.0616$	0.0089; 0.0035; 0.0003	0.9997	0.166	0.498
Cd	5–15	$y = -0.0017x^2 + 0.0566x - 0.1077$	0.0119; 0.0027; 0.0002	0.9983	0.630	1.890
Co	3–12	$y = -0.0075x^2 + 0.1673x - 0.2153$	0.0034; 0.0011; 0.0001	0.9999	0.062	0.186
Cr	1–50	$y = 0.0637x + 0.0204$	0.0301; 0.0012	0.9956	1.736	5.041
Cu	5–15	$y = 0.1381x - 0.2347$	0.0030; 0.0003	1.000	1.764	1.915
Ni	1–15	$y = 0.1036x + 0.2119$	0.0610; 0.0065	0.9620	3.811	7.931
Pb	5–25	$y = -0.0158x^2 + 2.1287x - 3.2825$	0.2628; 0.0440; 0.0014	0.9999	0.370	1.110

model was linear regression: $y = \beta_0 + \beta_1x + \varepsilon$. It can happen when wide dynamic ranges and/or not isotopically pure internal standards are considered (especially true in mass spectrometry) [39]. The independent variable x is assumed unaffected by error. β_0 , β_1 and β_2 are the parameters of the model and ε represents a normally distributed random error with mean zero and constant variance σ^2 [homoscedastic condition and $\varepsilon \sim \mathcal{N}(0, \sigma^2)$]. The β parameters are unknown and ordinary least-squares regression provides their estimates 'b' by using a set of experimental data points (x_i, y_i) [40].

The adequacy of the model has been tested in several ways: (i) by the use of an analysis of variance test (ANOVA) called the lack of fit test (with replicate data) [41]; (ii) by inspection of the behaviour of the residuals and (iii) by the evaluation of the determination coefficient R^2 (square of correlation coefficient r). The models and main results of ANOVA tests are summarised in Tables 2 and 3. Further results are given in Supplementary Materials SM2 and SM3.

The estimated coefficients b_1 and b_2 were significantly different from zero and b_0 coefficient, depending on element, was not significantly different from zero. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the following equations [42]:

$$LOD = \frac{3}{b_1} \sqrt{\frac{s_{Bj}^2}{n_{Bj}} + s_{b_0}^2} \quad (2)$$

Where b_1 is the sensitivity and, s_{Bj} is the standard deviation of the blank measurements, n_{Bj} is the number of blank measurements and s_{b_0} is the standard deviation of the intersection with the vertical axis. In case of the quadratic model, when the instrument detector is working close to zero concentrations, the contribution of the 2nd degree term in the calibration curve is negligible.

$$LOQ \cong 3 \times LOD \quad (3)$$

High regression quality was obtained for all elements. Low detection limits, below 1 mg kg^{-1} were obtained for all elements except Cr, Cu and Ni for which LOD was 1.736, 1.764 and 3.811 mg kg^{-1} respectively (table 3). If we consider the threshold of Cr and Ni for multifunctional land use, where farmers can grow everything including the leaf succulent plants, are 50 and 40 mg kg^{-1} respectively [44], this means an error less than 3.5% and 9.5% for these two TEs, respectively. For field crops and fruit trees, these values are reduced by 4 and 2.5 times, which means high reliability of results to orient land use policy and protect public health from TE transfer risks to the food chain.

3.2.2. Precision including repeatability and intermediate precision

Precision is a measure of how close the results are to one another usually expressed by the standard deviation [45]. Repeatability is the test results obtained using the same method, on the same sample in the same laboratory, with the same equipment, by the same operator, in short intervals of time. Other terms are used to define repeatability, such as within-run, within-batch or intra-assay precision. Repeatability gives the smallest variation in results. Reproducibility is the test results obtained by different operators and different laboratories over a long period. This gives the largest variation in results. Between these two extremes, intermediate precision estimates result in variations in the same laboratory under routine conditions by different analysts and during an extended timescale. Other terms can be used to define intermediate precision such as between-run, between-batches, or inter-assay variation.

To determine repeatability and intermediate precision, two operators measured the metal concentrations in the soil CRM during each of the 9 days. One-way ANOVA was applied on the obtained results. Repeatability was calculated as the within-group precision and expressed by the repeatability standard deviation S_r . It was calculated using the square root of the within-group mean square MS_w :

$$S_r = \sqrt{MS_w} \quad (4)$$

Intermediate precision was calculated as the within-group and between-group precision and expressed by the intermediate precision standard deviation S_i . It was calculated by combining the within- and between-group variance.

$$S_i = \sqrt{MS_w + \frac{MS_b - MS_w}{n}} \quad (5)$$

Table 4. Repeatability and intermediate precision results were obtained after TE analysis of CRM soil using the microwave sulphuric digestion method ($n = 9$ for both operators).

Parameters	Units	TE quantification						
		As	Cd	Co	Cr	Cu	Ni	Pb
Operator 1								
Mean	mg kg ⁻¹	151.5	165.5	186.3	98.5	233.8	123.7	126.4
SD	mg kg ⁻¹	0.5	2.4	2.7	0.6	2.1	0.6	1.0
RSD	%	0.3	1.4	1.5	0.6	0.9	0.5	0.8
Operator 2								
Mean	mg kg ⁻¹	151.0	167.3	182.4	98.1	235.0	124.0	126.9
SD	mg kg ⁻¹	0.9	0.7	2.2	0.4	0.2	0.1	0.5
RSD	%	0.6	0.4	1.2	0.4	0.1	0.1	0.4
S_r	mg.kg ⁻¹	0.70	1.76	2.46	0.49	1.52	0.43	0.76
S_i	mg kg ⁻¹	0.76	2.10	3.62	0.54	1.65	0.46	0.81
RS_i	%	0.5	1.3	2.0	0.5	0.7	0.4	0.6

SD = Standard Deviation; RSD = Relative Standard Deviation.

S_r = repeatability standard deviation; S_i = intermediate precision standard deviation.

RS_i = intermediate precision relative standard deviation.

MS_b is the between-group mean square and MS_w the within-group mean square. Both values were calculated from the one-way ANOVA table. The relative standard deviation RSD varied from 0.3% to 1.5% for the first operator and from 0.1% to 1.2% for the second operator. The higher RSD was obtained for Co whatever the operator was. Repeatability standard deviation varied from 0.43 to 2.46 mg kg⁻¹. These values were relatively low and reflected good repeatability conditions (Table 4).

Intermediate precision varied between 0.46 and 3.62 mg kg⁻¹, which represented 0.4% to 2.0% as the relative standard deviation. These relatively low values reflect an acceptable variation of the results obtained by the two operators who applied the same method under routine conditions. The acceptable value for repeatability depends on concentration and was set at 8% for samples at the mg kg⁻¹ level [46].

3.2.3. Specificity

The lack of specificity of a method can be due to the widening of the absorption signal, but most of the time, it is due to the presence of interfering elements, which could increase or hinder the signal of a specific element. Interfering compounds are various constituents of the matrix and/or the reagents used to prepare the sample. It is what is called matrix effects. The International Conference on Harmonisation [47] defined specificity as 'the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically, this might include impurities, degradants, matrix, etc.' It was evaluated by analysing the CRM soil alone and after addition of known quantities of each element. Two levels of concentration for each element were added to the CRM soil: 50 and 100 mg kg⁻¹. Samples were labelled CRM, CRM1 and CRM2. Samples were digested in triplicate and each sample was measured three times. The specificity was calculated using the recovery ratio expressed in percentage:

$$R(\%) = \frac{\text{experimental quantity after standard addition} - \text{experimental quantity before standard addition}}{\text{known added quantity}} \times 100 \quad (6)$$

Table 5. Specificity results expressed as mean recovery percentage and standard deviation. Three samples were analysed: CRM soil alone and CRM after the addition of two concentration levels for each element ($n = 9$).

Trace elements	Mean recovery percentage and standard deviation %		
	Soil CRM	Soil CRM 1 + 50 mg kg ⁻¹ of each TE	Soil CRM 2 + 100 mg kg ⁻¹ of each TE
As	93.7 ± 0.9	96.1 ± 9.6	104.0 ± 4.9
Cd	87.3 ± 4.7	94.7 ± 9.2	92.0 ± 6.3
Co	112.6 ± 0.1	116.9 ± 0.3	117.8 ± 0.8
Cr	112.3 ± 3.5	111.5 ± 7.2	109.9 ± 5.7
Cu	89.2 ± 3.9	90.6 ± 4.0	99.1 ± 4.9
Ni	98.4 ± 0.9	98.1 ± 2.1	97.7 ± 1.7
Pb	112.6 ± 0.1	116.9 ± 0.2	117.8 ± 0.8

The mean recovery of samples CRM, CRM1 and CRM2 and their respective standard deviations, for all the being studied elements are gathered in Table 5.

The specificity results show that the recovery percentages of all elements are within [80–120%] interval. Specificity values for TEs in soil have not been found in the literature. However, EU commission regulation No 2001/22/EC [48] mentions that recovery between 80% and 120% is acceptable for TEs such as lead, cadmium and mercury in foodstuffs. These results allow the application of the developed method in routine analysis without causing interference for As, Cd, Co, Cr, Cu, Ni and Pb.

3.2.4. Comparison between the validated method (MSD + AAS) and the RTD method (HF-based digestion + ICP/ICPMS) of TE analysis. Accuracy or trueness

Accuracy is defined as 'the closeness of agreement between the value that is accepted either as a conventional true value or an accepted reference value and the value found' [47]. Accuracy is sometimes defined as trueness. Accuracy can be determined by analysing a CRM and comparing the experimental value to the assigned value. It is actually what we did in paragraph 3.1 where we obtained recovery percentages between 87% and 112%. Another way to assess accuracy is to work on real samples, to analyse them by the method being validated and to compare results with that obtained from a reference method. In this study, TEs in soil samples from the Bekaa governorate were determined by an MSD method using a mixture of H₂SO₄ and H₂O₂ followed by AAS measurements. Microwave digestion and element analysis were done in triplicate. Results from this method were compared to an external ISO reference, which is the RTD method where the same samples were digested by HF and HClO₄ and analysed by ICP-AES or ICP-MS. The RTD method allows for total digestion of soil samples.

Results were compared in terms of recovery percentage calculated using Equation 1. These results are presented in Table 6.

All recovery values were below 100%, which was predictable because the MSD with a mixture of H₂SO₄ and H₂O₂ does not induce a total mineralisation of silicates compared with the RTD method using a mixture of HF and HClO₄. High recovery percentages were obtained for Co, Cr and Cu. Two-thirds of the calculated recoveries were higher than 70%, which is an acceptable value for trace analysis as defined by (AOAC 2002) [46]. The other third was between 50% and 70% except for 3 values (As for B1 and Pb for B6 and B7). As, Co, Cr and Pb concentrations measured using the validated MSD method were correlated

Table 6. Comparison between the MSD method (H₂SO₄ + H₂O₂ digestion) are being validated and the RTD method (HF + HClO₄ digestion) for TEs in soil samples from the Bekaa governorate sites.

Sites	Trace elements																	
	As			Co			Cr			Cu			Ni			Pb		
	RTD±SD mg kg ⁻¹	MSD±SD	Rec. %	RTD±SD mg kg ⁻¹	MSD±SD	Rec. %	RTD±SD mg kg ⁻¹	MSD±SD	Rec. %	RTD±SD mg kg ⁻¹	MSD±SD	Rec. %	RTD±SD mg kg ⁻¹	MSD±SD	Rec. %	RTD±SD mg kg ⁻¹	MSD±SD	Rec. %
B1	6.8 ± 0.4	3.3 ± 0.0	48	18.3 ± 2.0	10.0 ± 0.0	55	94.1 ± 12.8	59.6 ± 0.1	63	22.0 ± 1.7	18.1 ± 0.1	82	56.1 ± 3.0	39.4 ± 0.2	70	13.8 ± 0.8	11.5 ± 0.0	83
B2	4.5 ± 0.2	3.1 ± 0.0	69	13.3 ± 1.7	9.7 ± 0.0	73	83.8 ± 11.6	60.7 ± 0.1	72	19.8 ± 1.6	16.3 ± 0.1	83	47.3 ± 2.7	25.6 ± 0.2	54	16.8 ± 1.0	12.3 ± 0.0	73
B3	4.4 ± 0.2	4.2 ± 0.0	95	11.4 ± 1.5	9.7 ± 0.1	85	75.1 ± 11.3	50.2 ± 0.1	67	17.2 ± 1.5	13.5 ± 0.1	78	43.6 ± 2.5	27.5 ± 0.2	63	24.5 ± 1.5	19.9 ± 0.0	81
B4a	7.3 ± 0.4	4.2 ± 0.0	57	11.4 ± 1.6	10.7 ± 0.0	94	55.8 ± 8.5	43.2 ± 0.1	77	16.6 ± 1.5	15.2 ± 0.0	91	39.4 ± 2.4	40.0 ± 0.2	102	9.1 ± 0.6	6.0 ± 0.0	66
B4b	7.5 ± 0.4	4.1 ± 0.0	55	11.7 ± 1.6	10.4 ± 0.1	89	57.2 ± 8.6	43.9 ± 0.0	77	14.5 ± 1.4	14.4 ± 0.2	100	39.7 ± 2.4	39.4 ± 0.6	99	8.8 ± 0.6	6.4 ± 0.0	73
B5	11.3 ± 0.6	9.9 ± 0.0	87	25.2 ± 2.3	17.3 ± 0.1	69	129.0 ± 16.7	68.2 ± 0.1	53	27.9 ± 1.8	15.6 ± 0.1	56	78.9 ± 3.9	46.8 ± 0.1	59	17.5 ± 1.1	15.5 ± 0.1	89
B6	6.4 ± 0.4	4.2 ± 0.0	66	14.9 ± 1.9	13.4 ± 0.1	90	71.7 ± 10.8	50.4 ± 0.0	70	23.0 ± 1.8	16.5 ± 0.0	72	52.0 ± 2.7	50.2 ± 0.1	96	15.0 ± 0.9	5.7 ± 0.0	38
B7	7.7 ± 0.4	4.3 ± 0.0	55	19.1 ± 2.5	17.0 ± 0.0	89	94.3 ± 10.7	90.4 ± 0.0	96	23.9 ± 1.9	16.8 ± 0.0	70	58.5 ± 3.0	50.4 ± 0.0	86	18.6 ± 1.1	5.8 ± 0.0	31
B8	9.7 ± 0.5	8.2 ± 0.0	85	23.0 ± 2.2	16.7 ± 0.1	72	114.0 ± 15.0	71.2 ± 0.1	62	25.9 ± 1.8	15.6 ± 0.0	60	64.2 ± 3.3	39.2 ± 0.1	61	19.5 ± 1.2	17.3 ± 0.1	89

RTD = Reference Total Digestion method mean value; MSD = Microwave Sulphuric Digestion method mean value; Rec. = mean Recovery; SD = Standard deviation

with values obtained using the RTD method. The relation was greatly improved for Pb ($R^2 = 0.9624^*$ with a slope = 1.0486) when B6 and B7 were removed. For Cr, the relationship was greatly improved when B7 was not considered ($R^2 = 0.9074^*$) and indicated a reduction of the recovery percentage with increased total concentrations. The most contaminated soil was B5, with the highest concentrations for As, Co, Cr, Cu and Ni. This plot is occasionally irrigated by pumping water from an irrigation canal from the Berdawni River, the catchment of which drains the town of Zahleh. Nevertheless, all soils remained in the lower range of concentrations found in soils worldwide for all elements [14]. Cobalt, Cr, Cu and Ni concentrations were correlated indicating a probable common geogenic origin. Arsenic was only correlated with Co and Ni, which may be related to a more scattered occurrence due to anthropogenic inputs [49]. The lead concentration was not correlated to that of the other elements, probably because two soils (B6 and B7) had a very low recovery rate. These results confirm the reported low content in the soils of the Central Bekaa area and depicted from the spatial distribution of total Pb in the soil surface [50]. The pattern of Pb concentration shows, above a weak geogenic background (4–14 mg kg⁻¹), an anthropic contamination along heavily trafficked roads by atmospheric deposition [51] and surface accumulation gradient of Pb from foot-slope, located down-slope of Zahleh town, towards the Litani River (14–34 mg kg⁻¹). This lead is carried by water runoff from Zahleh agglomeration (see Figure S1, Supplementary Materials SM4).

Arsenic values obtained by the RTD method varied between 4.35 mg kg⁻¹ for B3 and 11.3 mg kg⁻¹ for B5. They were slightly above the commonly reported mean background As concentrations in surface soils (worldwide data) [14], but still within the range for common, possible background values and multifunctional land use [44].

Cadmium values were not shown in this study because they were below the quantification limit (1.89 mg kg⁻¹) when using the MSD validated method. Cd could not be determined by this method. However, all the values determined by the RTD method varied between 0.23 mg kg⁻¹ for B4b and 0.48 mg kg⁻¹ for B8 and were defined within the global average soil Cd concentration (0.06–1.1 mg kg⁻¹) [14]. Our values are in agreement with those found by Kanbar et al. (0.28–2.8 mg kg⁻¹) in different Lebanese soils [52] and those found by Darwish in Bekaa soils (0.28–0.48 mg kg⁻¹) [7].

Cobalt values obtained by the RTD method varied between 11.4 mg kg⁻¹ for B3 and 25.2 mg kg⁻¹ for B5. These values are close to the range of world values in surface soils (4.5–12 mg kg⁻¹) [14]. They are comparable to those obtained for the arid and semiarid regions (16.5–26.8 mg kg⁻¹) [53]. They are also in agreement with those found by Darwish in Bekaa soils (28.1–28.5 mg kg⁻¹) [7].

Chromium values obtained by the RTD method varied between 55.8 mg kg⁻¹ for B4a and 129.0 mg kg⁻¹ for B5. These values are higher than the world median content of Cr in soils: 54 mg kg⁻¹. They are within the sandy and light loamy soils range, which contains Cr between 2 and 350 mg kg⁻¹ [14]. They are close to those found by Darwish in Bekaa soil samples (43.3 and 71.3 mg kg⁻¹) [7] and do not exceed the limits for safe agricultural land use.

Copper values obtained by the RTD method varied between 14.5 mg kg⁻¹ for B4b and 27.9 mg kg⁻¹ for B5. These results are within the range for Cu concentration in soils (8 mg kg⁻¹ in acid sandy soils to 80 mg kg⁻¹ in heavy loamy soils) [14]. They are in general lower than those obtained in other Lebanese soils (25.3–54.2 mg kg⁻¹) [52], among others

Taalabaya (Zahleh district) soils (35.7 mg kg^{-1}) [7], but conform to the values obtained in Saadnaeal (Zahleh district) soils (17.0 mg kg^{-1}).

Nickel values obtained by the RTD method varied between 39.4 mg kg^{-1} for B4a and 78.9 mg kg^{-1} for B5. These values are within the range of Ni concentration in soils worldwide ($0.2\text{--}450 \text{ mg kg}^{-1}$) [14]. These results are in agreement with values obtained in Taalabaya soils (60.7 mg kg^{-1}) but higher than values obtained in Saadnaeal soils sampled at the same depth (33.7 mg kg^{-1}) [7].

Lead values obtained by the RTD method varied between 8.84 mg kg^{-1} for B4b and 24.48 mg kg^{-1} for B3. These values are lower than the overall mean value of Pb in different soils worldwide (25 mg kg^{-1}) [14]. These results are in agreement with those obtained in other Lebanese soils ($11.3\text{--}22.9 \text{ mg kg}^{-1}$) [52] and those obtained in Taalabaya soils (16.7 mg kg^{-1}) and Saadnaeal soils (10.0 mg kg^{-1}) [7].

In summary, the results obtained in this study are representative of soils with limited trace elements contamination. The concentrations measured are within the same range than the values obtained by Darwish for surface soils and soil profiles of the Bekaa plain. He points to both a natural TE source with variation explained by the alluvial-colluvial origin of the parent material but also to a limited anthropogenic pollution due to a combination of inputs from cultivation and atmospheric deposition which is modulated by soil organic content.

The correlations between TE values were higher following the RTD method than the validated one, suggesting that the RTD method can better trace the common origin of the elements, which were mostly of geogenic origin as also indicated by the observed level of concentrations, which were close to the background level detected in North Lebanon [8].

Aqua regia, which is a reference method for pseudo-total metal concentrations in soils, gives an extraction efficiency between 53% and 100%, depending on the element as compared to the total concentrations (calculated for the certified material METRANAL-33 in Shahbazi and Beheshti) [54]. Although it is widely used, resorption occurs (up to 470% for Cu) leading to underestimation of element concentrations [55] and other mild extraction procedures may provide accurate results when the trace element reactive fraction, not bound to silicates, is to be recovered [55]. Our results fall within the same range of efficiency as *aqua regia* and the validated method including the microwave digestion with $\text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2$ may offer a more feasible and workable alternative.

4. Conclusion

The microwave digestion with $\text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2$ combined with flame-AAS measurement was validated with CRM and after comparison with HF-based digestion. This allows for routine but accurate characterisation of calcareous and Ca-saturated soils with limited contamination in As, Cd, Cu, Co, Cr and Pb. The tested soils, used for wheat production, are irrigated with water of poor quality and need to be monitored for their trace element concentrations regularly to prevent transfer to the food chain.

Recent microwave digestion methods were implemented according to green chemistry, they have proved their efficiency for the determination of trace elements in soil samples [11,12,19,22]. In these studies, several reagents were used to dissolve soil or

sediment samples such as: HNO_3 , HClO_4 , HF, HCl, NH_4HF_2 and H_2O_2 before trace element analysis using ICP-OES, ICP-MS or MIP-OES. These recent methods uses problematic reagents difficult to handle safely and/or expensive analytical devices.

The validated method offers an alternative to HF-based methods, as well as *aqua regia*. It is a green, safe and cheap method for trace element analysis in soil samples. Other soil types will have to be tested in order to further extend its use to a larger range of soils to cover the soil diversity in the Mediterranean area.

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Supplementary data

Supplementary Materials

Validation of a new method for monitoring trace elements in Mediterranean cereal soils

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SM 1 - Dissolution of soil samples for the determination of total element content according to the standard (NF ISO 14869-1)

0.25 g of sample was transferred to a crucible and placed in a furnace. The temperature reached progressively 450 °C over 1 h. The temperature was maintained at 450 °C during 3h. After cooling, the ash was quantitatively transferred to a PTFE evaporating dish with a minimum amount of water.

5 mL of HF and 1.5 mL of HClO₄ were added to the PTFE dish. The mixture was heated on a hot plate until the dense fumes of HClO₄ and SiF₄ cease. Before complete dryness, the dish was removed from the hot plate and allowed to cool. 1 mL of HNO₃ 15 mol L⁻¹ and 5 mL of water were added to dissolve the residue. The latter was heated if needed to assist dissolution. The obtained solution was transferred quantitatively to a 50 mL volumetric flask.

SM 2 - Statistical analysis for the validation of the regression model

The validation of the linear regression model is done using an ANOVA table and several tests performed on the different parameters such as:

- Student's test in order to validate the significance of the slope and the intercept (=> we check that they are different from 0),
- Fisher's tests on the significance of the regression model and on the linearity of the model,
- Durbin Watson's test to check the independence of the residuals (absence of autocorrelation),
- Shapiro's test to verify the normality of the residuals,
- Breush-Pagan's test to verify the hypothesis of homogeneity of the residuals.

In case of non-normality, it is possible to transform the data to make them normal. For autocorrelation, in the case of calibration, the phenomenon may appear and may be due to the

"trumpet effect" (i.e., small variability near concentration equal to 0 and large when the concentration increases).

In case of model failure, it is then necessary to compare the linear regression model with other models that may fit better such as weighted linear regression, quadratic regression, etc.

Once the whole model is validated, it is then possible to calculate LOD, LOQ and the inverse concentration in order. It is also possible to calculate recovery rates.

Table S1. Regression model assumptions

Element	Regression model $y = \beta_0 + \beta_1x + \varepsilon$ $y = \beta_0 + \beta_1x + \beta_2x^2 + \varepsilon$	Assumptions		
		Normality Shapiro's test	Autocorrelation Durbin Watson's test	Homoscedasticity Breush Pagan's test
		p-value*		
As	Linear	<i>0.0082</i>	<i>0.046</i>	0.6563
	Quadratic	0.5423	0.502	0.1207
Cd	Linear	<i>0.0028</i>	0.094	1
	Quadratic	<i>0.0113</i>	0.136	0.0799
Co	Linear	<i>0.0052</i>	<i>0.018</i>	0.1064
	Quadratic	<i>0.0404</i>	0.076	0.2279
Cr	Linear	0.0872	<i>0.016</i>	0.6621
	Quadratic	<i>0.0280</i>	<i>0.006</i>	<i>0.0180</i>
Cu	Linear	0.4309	0.834	0.9643
	Quadratic	0.1509	0.766	0.0799
Ni	Linear	<i>0.0098</i>	<i>0.032</i>	0.2322
	Quadratic	0.0662	<i>0.010</i>	<i>0.0034</i>
Pb	Linear	<i>0.0299</i>	<i>0.044</i>	0.1384
	Quadratic	0.1881	0.118	0.0908

* In italics, it means that the assumption of model is not met.

SM 3 - Detection and quantitation limits

Linear regression model

$$x_{LOD} = \frac{b_0 + 3s_{b_0}}{b_1} \text{ and } x_{LOQ} = \frac{b_0 + 10s_{b_0}}{b_1} \quad \text{Eq. S1}$$

Where b_1 is the sensitivity and, s_{Bl} is the standard deviation of the blank measurements, n_{Bl} is the number of blank measurements and s_{b_0} is the standard deviation of the intersection with the vertical axis.

Quadratic regression model

$$x_{LOD} = \frac{3}{b_1} \sqrt{\frac{s_{Bl}^2}{n_{Bl}} + \frac{Res^2}{12} + s_{b_0}^2} \text{ and } x_{LOQ} \cong 3 \times x_{LOD} \quad \text{Eq. S2 (from Lavin et al., "On the$$

Determination of Uncertainty and Limit of Detection in Label-Free Biosensors", Sensors(18), 2018. <https://doi.org/10.3390/s18072038>)

In case of the quadratic model, when the instrument detector is working close to zero concentrations, the contribution of the 2nd degree term in the calibration curve is negligible.

Table S2. Limits of detection and quantification obtained with each model

Element	Regression model	Limit of	Limit of
	$y = ax + b$ $y = ax^2 + bx + c$	Detection LOD	Quantification LOQ
		mg kg ⁻¹	
As	Linear	0.944	2.711
	<i>Quadratic*</i>	<i>0.166</i>	<i>0.498</i>
Cd	Linear	4.037	10.176
	<i>Quadratic</i>	<i>0.630</i>	<i>1.890</i>
Co	Linear	5.285	12.089
	<i>Quadratic</i>	<i>0.062</i>	<i>0.186</i>
Cr	<i>Linear</i>	<i>1.736</i>	<i>5.041</i>
	Quadratic	1.750	5.250
Cu	<i>Linear</i>	<i>1.764</i>	<i>1.915</i>
	Quadratic	0.187	0.560
Ni	<i>Linear</i>	<i>3.811</i>	<i>7.931</i>
	Quadratic	3.403	10.208
Pb	Linear	0.538	2.699
	<i>Quadratic</i>	<i>0.370</i>	<i>1.110</i>

* In italics the selected model.

SM 4 - Cartography by universal kriging of topsoil Pb distribution in Central Bekaa plain

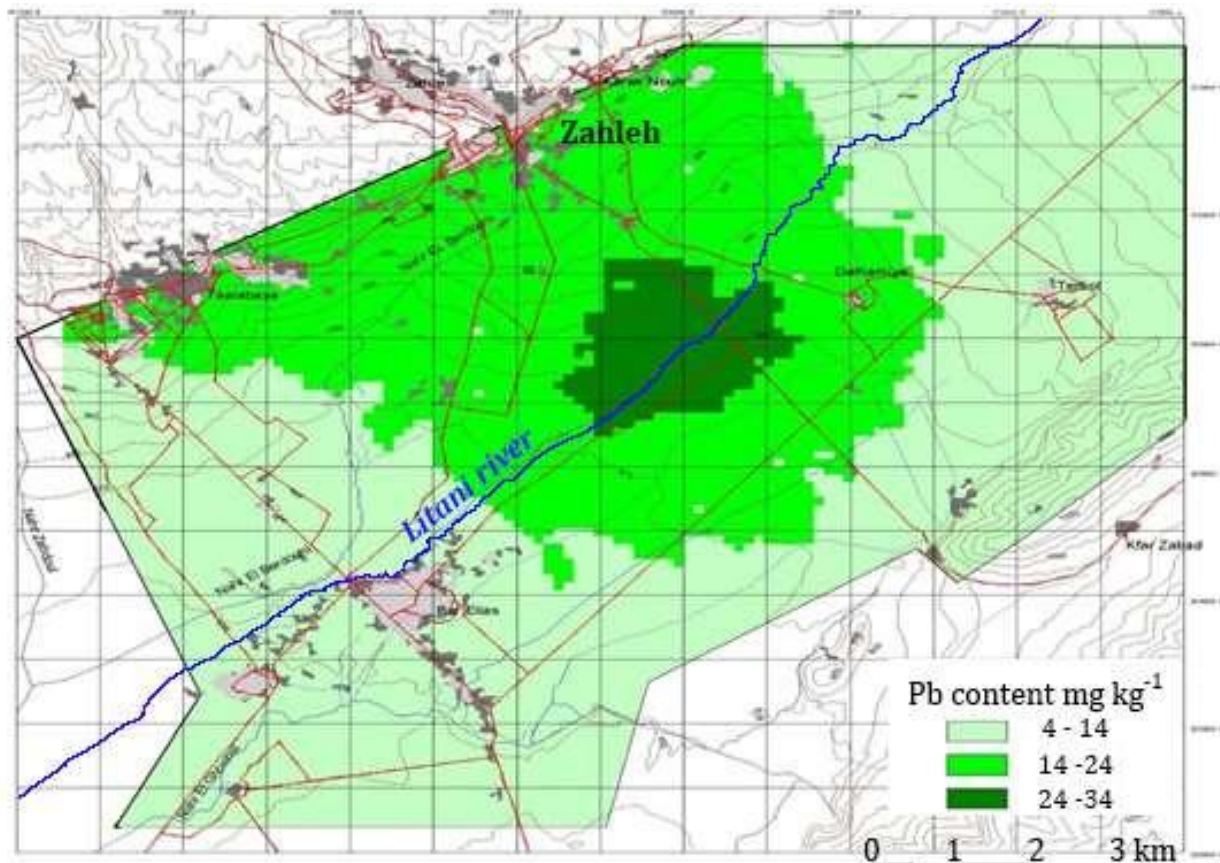


Figure S1. Cartography by universal kriging of topsoil Pb distribution in Central Bekaa plain showing: (i) above a low geogenic background, an anthropic contamination along the roads with heavy traffic including the plume effect was controlled by the main northeast wind and (ii) a surface runoff gradient from the slopes near the town of Zahleh towards the Litani river

- Soil sampling collection

Topsoil samples (0-15 cm depth) were collected from Central Bekaa area (1200 ha) by grid system 1kmx2km, based on topography, lithology and land use. The soil samples were cleaned from roots and gravels, disintegrated, air dried, ground and sieved through 2 mm sieve. Following ISO 11466. Sixty samples were collected in 1997 before the introduction of unleaded fuel in 2000.

- Samples digestion and Pb analyses

The samples digestion was done by aqua regia mixture and Pb was analysed by means of ETA-AAS device.

- Quality control

The applied methodology of soil samples collection and preparation for analyses was quality-controlled, with reproducible results by means of reference samples from “The

International Soil-Analytical Exchange Program” of the University of Wageningen, The Netherlands.

- Spatial analyses and Pb mapping

Spatial analysis and Pb mapping were done using the universal kriging method with a matrix table of 5 m x 5 m resolution.