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Chemical contamination in different marine organisms along the Lebanese coast : Environmental implications and health risks

Micheline Ghosn

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Discipline : BIOLOGIE, MEDECINE ET SANTE, physiologie, biologie des organismes,
populations, interactions

Chemical contamination in different marine organisms along the Lebanese coast: Environmental implications and health risks

Contamination chimique de différents organismes marins le long du littoral Libanais : Implications environnementales et risques sanitaires

Thèse présentée par

Micheline Ghosn

Pour obtenir le grade de Docteur de l'Université du Littoral Côte d'Opale

Le 12/12/2019

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“All things are poisons, for there is nothing without poisonous qualities. It is only the dose which makes a thing poison.”

Paracelsus

Abstract

Global food fish consumption has been in continuous increase and fishery products have become some of the most traded items in the world. However, the quality of these products is becoming an issue of concern to human health with the increase of anthropogenic activities threatening marine coastal ecosystems. Therefore, despite the well-known health benefits of fishery products, their consumption may be a route of human exposure to different kinds of chemical contaminants including trace elements. In this context, evaluating the contamination levels in these products and the risks they may engender to human health proves to be a necessity in the absence of local guidelines and monitoring programs. Thus, the thesis has 2 main objectives: i) the evaluation of environmental quality through marine organisms and, ii) the assessment of health risks related to the consumption of a selection of fishery products.

The first objective was to study the levels of metallic contaminants (20 trace elements) in six marine species from different trophic levels of a food web (algae, mussel, shrimp and fish), representative of Lebanese coastal waters. The results showed that species accumulated metals differently underlining the importance of a multi-specific approach to reflect the contamination level of a certain site. Inter-site variations have been found mainly during the rainy period of the year while they were less prominent during the dry season, highlighting the effect of land-based sources and riverine effluents on the marine coastal area. Levels of trace elements in the muscles and edible tissues in species from our study, were similar to the ones reported in the Eastern Mediterranean (Levantine Basin) whereas livers showed higher levels compared to other studies suggesting that the Lebanese coast is exposed to strong environmental pressure.

The second objective was to evaluate the health risks related to the consumption of different fishery products sampled along the Lebanese coast. In this prospect, five commonly consumed local species belonging to different compartments of the food chain, (1 bivalve, 1 crustacean and 3 fish species) sampled from three sites with different levels of anthropogenic pressures were selected. The samples were all analyzed for their content of trace elements. Metals such as mercury (Hg) are found in several chemical forms, the most toxic form being methylmercury (MeHg). The main route of human exposure to MeHg is the consumption of fishery products. That's why, a method for the speciation of mercury in fishery products by HPLC-ICP-MS was optimized and validated based on an accuracy profile. The results showed that the levels of trace elements and MeHg in the selected species were all below the allowed maximum levels set by the European commission and so their consumption is not likely to cause any adverse effects to human health.

Keywords: Pollution, trace metals, fishery products, Lebanon

Résumé

La consommation mondiale des produits de la mer est en perpétuelle croissance et les produits de la pêche sont devenus parmi les produits les plus marchandés à travers le monde. Cependant, la qualité de ces derniers soulève une problématique au niveau de la santé humaine avec l'augmentation des pressions anthropiques menaçant les écosystèmes côtiers et les organismes marins. Par la suite, malgré les qualités nutritionnelles des produits de la pêche, leur consommation peut être une voie d'exposition de l'homme à différents types de contaminants chimiques dont les éléments traces métalliques (ETMs). Dans ce contexte, l'évaluation du niveau de contamination dans ces produits et les risques qu'ils peuvent engendrer s'avère être une nécessité surtout en l'absence de normes locales et de programmes de surveillance. Les objectifs de cette étude sont de deux ordres : i) l'évaluation la qualité environnementale du littoral Libanais à travers les organismes marins et, ii) l'estimation du niveau des risques sanitaires liés à la consommation d'une sélection des produits de la pêche.

Le premier objectif, était d'évaluer les niveaux de contaminants métalliques (20 éléments traces) chez six espèces marines de différents niveaux du réseau trophique (algues, moules, crevettes et poissons) représentatif des eaux côtières libanaises. Les résultats ont révélé que les espèces accumulent les métaux différemment soulignant ainsi l'importance d'une approche multi-espèce pour mieux refléter le niveau de contamination du milieu. Des variations inter-sites ont été également trouvées surtout durant la période pluvieuse de l'année indiquant l'effet des apports des rivières vers la zone côtière. Les niveaux des ETMs obtenus dans les muscles et les tissus comestibles dans le cadre de notre étude étaient du même ordre de grandeur que ceux mesurés dans d'autres régions du bassin Levantin. Cependant, les foies ont montré des concentrations supérieures comparées à d'autres études, suggérant ainsi que la côte libanaise est soumise à des pressions environnementales importantes.

Le deuxième objectif, concerne l'évaluation des risques sanitaires liés à la consommation de différents produits de la pêche échantillonnés le long du littoral Libanais. Pour cela, cinq espèces consommées localement (1 bivalve, 1 crustacé et 3 espèces de poisson) et échantillonnés le long du littoral Libanais au niveau de trois sites soumis à différents niveaux de pressions anthropiques ont été sélectionnés. Tous les échantillons ont été analysés pour leur contenu en éléments traces. Des métaux comme le mercure (Hg) se retrouvent sous plusieurs formes chimiques, sa forme la plus toxique étant le méthylmercure (MeHg). La voie principale de l'exposition humaine au MeHg est la consommation de produits de la pêche. Pour cela, une méthode de spéciation du Hg dans les produits de la pêche a été optimisée et validée par profil d'exactitude. Les résultats ont montré que les niveaux des ETMs et du MeHg dans les espèces concernées, étaient largement inférieurs aux limites maximales admissibles fixées par la Commission Européenne et que leur consommation ne présente pas de risques pour la santé humaine.

Mots clés : Pollution, éléments traces métalliques, produits de la mer, Liban

Scientific contributions

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Table of Content

Chapter 1 - General Introduction	28
1. Metals and trace elements: an overview	30
1.1. Sources of metals in the marine environment.....	31
1.2. fate of metals in the marine environment	33
1.3. Metal uptake and accumulation in marine organisms.....	34
1.4. Interspecific variation of metal accumulation: Bioindicator species	37
2. Health risks related to the consumption of contaminated seafood	39
2.1. Regulated toxic metals in seafood	40
3. Trace metals in the Mediterranean sea and seafood consumption health risks	46
3.1. Toxic trace metals in seafood: Are seafood from the Mediterranean safe to eat?	48
4. Objectives of the study.....	52
5. Outline of the study.....	52
Chapter 2 - Strategies, protocols and methodologies	55
1. Presentation of the study area: Geographical and climatic context	55
2. Sampling sites	61
3. Biological models sampled	63
4. Samples collection and preparation	67
5. Analysis of trace elements by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)....	69
6. The use of hyphenated techniques for elemental speciation: HPLC-ICP-MS.....	71
6.1. Methylmercury (MeHg)	73
Chapter 3 - Part 1: Seasonal and spatial variability of trace elements in livers and muscles of three fish species from the Eastern Mediterranean	77
Chapter 3 - Part 2: Assessment of trace element contamination and bioaccumulation in algae (<i>Ulva lactuca</i>), bivalves (<i>Spondylus spinosus</i>) and shrimps (<i>Marsupenaeus japonicus</i>) from the Eastern Mediterranean	92

Chapter 4 - Part 1: Levels of Pb, Cd, Hg and As in fishery products from the Eastern Mediterranean and health risk assessment	119
Chapter 4 - Part 2: Toward a routine methodology of speciation analysis of methylmercury in fishery products following the validation based on the accuracy profile approach.....	140
Chapter 5 – General discussion and perspectives	156
ANNEX	172
References	181

LIST OF FIGURES

Figure 1-1 World fish utilization and apparent consumption (FAO, 2018)	29
Figure 1-2 Essential and non-essential elements, abundant or in traces (Amiard, 2011).....	31
Figure 1-3 Relationship between elemental concentration and health response (Ansari et al., 2004)	40
Figure 1-4 Human threats with potential impact on marine biodiversity in the Mediterranean Sea: (a) coastal-based impacts,(cumulative effects from inorganic and organic coastal pollution, nutrient runoff and hypoxia, aquaculture activities and the presence of invasive species.) and (c) ocean-based pollution (cumulative effects from shipments of toxic substances (e.g. toxic waste, radioactive waste and fertilizers) and other ocean-based pollution from shipping traffic, deposition of heavy metals and inorganic nitrogen. (Coll et al., 2012).....	47
Figure 1-5 Regional cadmium, lead and mercury levels (2009-2015) assessment against European Commission (EC) criteria in bivalve sp. (<i>Mytilus gallo provincialis</i> , <i>Ruditapes decussates</i> , <i>Donax trunculus</i> and <i>Macra corralina</i>) and in fish sp. (<i>Mullus barbatus</i>) for the Mediterranean Sea; BAC: Background Assessment Criteria (UN/MAP, 2017).....	50
Figure 2-1 Geomorphology of Lebanon (El Zaatari, 2018).....	56
Figure 2-2 Rainfall patterns in Lebanon	57
Figure 2-3 General circulation of water in the Mediterranean (Millot and Taupier-Letage, 2005)	59
Figure 2-4 Location of the sampling sites along the Lebanese coast	62
Figure 2-5 The Marbled Spinefoot <i>Siganus rivulatus</i> (Forsskål & Niebuhr, 1775)	64
Figure 2-6 The Striped seabream <i>Lithognathus mormyrus</i> L. 1758.....	64
Figure 2-7 The round herring <i>Etrumeus teres</i> (DeKay, 1842).....	65
Figure 2-8 The Kuruma shrimp <i>Marsupenaeus japonicus</i> (Bate, 1888).....	66
Figure 2-9 The spiny oysters <i>Spondylus spinosus</i> (Schreibers, 1793)	66
Figure 2-10 The sea lettuce <i>Ulva lactuca</i> (Linnaeus, 1753)	67
Figure 2-11 The principle of inductively coupled plasma mass spectrometry	69
Figure 2-12 HPLC-ICP-MS speciation interface – exit of the column connected to the nebulizer	72
Figure 2-13 Example of an accuracy profile with intervals of tolerance and acceptability, upper and lower bias uncertainties (Mermet and Granier, 2012)	74
Figure 2-14 Traditional model of accuracy and precision (Feinberg, 1996).....	75
Figure 3-1 Map showing the sampling sites along the Lebanese coast (WWTP: waste water treatment plant).....	97
Figure 3-2 PCA showing the relationship between species and trace metals concentration (mg kg^{-1})	100

Figure 3-3 PCA showing the relation between sites and trace metals concentration in <i>S. spinosus</i> during the wet (A) and the dry (B) season	112
Figure 4-1 : Map showing the sampling sites along the Lebanese coast (Eastern Mediterranean) ...	123
Figure 4-1 Extraction efficiency of MeHg from CRM ERM CE-464 (0.3 g) for different temperatures (the error bars represent the standard deviation over two replicates).	147
Figure 4-2 Recovery percentage of MeHg for different extraction durations in CRMs DOLT-5 and ERM-CE 464 (0.3 g) (the error bars represent the standard deviation over two replicates).	148
Figure 4-3 Accuracy profile for method validation in terms of MeHg determination in fishery products by HPLC-ICP-MS ($\beta = 85\%$, $\lambda = \pm 30\%$).	150
Figure 5-1 PCA showing the relationship species and elemental concentration (mg kg^{-1} dry weight)	158
Figure 5-2 PCA showing the relationship sites and elemental concentrations in the green alga and the bivalve.....	163
Figure 5-3 Annual lead emissions in different EU countries (Von Storch, 2003).....	166
Figure 5-4 Proportions of three As fraction in three different marine food in comparison with rice (Feldmann and Krupp, 2011).....	169

LIST OF TABLES

Table 1-1 Sources of metallic pollutants present in the environment.....	32
Table 1-2 Maximum allowable levels (MALs) of toxic metals in fish, crustaceans and mollusks emitted by different guidelines	40
Table 1-3 Cases where seafood content in As, Cd, Hg et Pb exceeded the MALs	48
Table 2-1 Average sizes and weights of local species collected per season	68
Table 2-2 Limits of quantification (LOQ) and detection (LOD) expressed in mg.kg ⁻¹ wet weight (ww) for elements determined in the analyzed samples.....	71
Table 3-1 Mean concentrations (C) and standard deviations (SD) of trace elements (mg kg ⁻¹ dw) in sampled marine organisms and sediments	101
Table 3-2 Comparison of trace metals concentration (mg kg ⁻¹ dry weight) measured in marine organisms from the Eastern Mediterranean in this study and other previous studies (minimum and maximum levels in each group are marked in bold)	105
Table 3-3 Mean concentration ± standard deviation (mg kg ⁻¹ dw) of trace elements measured in marine organisms sampled during the wet (S1) and dry (S2) seasons.....	110
Table 3-4 Biota-sediment transfer factors (BSAFs) in different organisms along the Lebanese coast	115
Table 4-1 Species size ± standard deviation (cm) and mean trace elements concentrations ± standard deviation (µg g ⁻¹ ww) in muscle of fish and edible parts of prawns and oysters sampled along the Lebanese coast in 2016 and 2017).....	129
Table 4-2 Total Hazard Quotient (THQ), total THQ (TTHQ), Estimated Monthly Intake (EMI; µg kg ⁻¹ BW) (Cd only) and Estimated weekly intake (EWI; µg kg ⁻¹ BW) related to Cd, Hg, Pb and As through consumption of fishery products from the Lebanese coast.....	133
Table 4-3 Mean trace elements concentrations ± standard deviations (µg g ⁻¹ ww) in fishery products from the Eastern Merditerranean.....	135
Table 4-4 ICP-MS and HPLC optimum operating conditions.....	144
Table 4-5 Results in terms of trueness (recovery factor, %) obtained for the speciation analysis of MeHg in three CRMs (mg kg ⁻¹ wet weight).....	151

Table 4-6 z-scores obtained for the analysis three reference materials provided by Central Science Laboratory-Food Analysis Performance Assessment Scheme (CSL-FAPAS) as part of the laboratory external quality control.....152

Table 4-7 HgT and MeHg concentrations in real fishery products samples ($\mu\text{g kg}^{-1}$ wet weight)....153

RESUME DETAILLE DE LA THESE EN FRANÇAIS

La thèse étant rédigée en anglais, ce résumé présente en français de façon synthétique, les objectifs et les principaux résultats et conclusions de ce travail

De nos jours, les produits de la pêche constituent une partie importante d'un régime alimentaire sain et équilibré ; ils sont source de protéines et d'acides gras comme les omega-3 qui contribuent à la réduction des risques liés aux maladies cardio-vasculaires et inflammatoires (Copat et al., 2012; Dural et al., 2006; FAO, 2016). Durant les dernières décennies, la consommation des produits de la pêche a été en perpétuelle croissance dans le monde entier. Sachant que la consommation était estimée à 9.9 kg/personne/an dans les années 1960, elle a atteint 19.2 kg/personne/an en 2012 et a dépassé les 20 kg en 2016 (FAO, 2016). En effet, les acides gras polyinsaturés présents dans les poissons, ont été associés à des effets préventifs et curatifs pour l'hypertension artérielle, le cancer du sein, l'asthme et les troubles du système immunitaire. De plus, par opposition à la viande, le poisson est facilement digestible grâce à ses longues fibres musculaires (Sidhu, 2003). Cependant, la consommation des produits de la mer peut être une source importante d'exposition de l'homme à divers contaminants chimiques telles que les dioxines et les éléments traces métalliques (Storelli, 2008), comme le mercure qui est connu pour son accumulation dans les poissons et surtout les espèces prédatrices constituant le haut de la chaîne alimentaire (Dang and Wang, 2012). Suite à l'étude de leur toxicité, des contaminants comme le plomb (Pb), le cadmium (Cd) et le mercure (Hg) ont fait le sujet de plusieurs règlements émis par des autorités sanitaires comme l'organisation mondiale de la santé (WHO), la Commission Européenne (EC), et l'Environmental Protection Agency (USEPA) qui ont fixé des seuils réglementaires définissant un niveau maximal permissible de ces éléments dans les produits de la pêche. Cependant dans certains pays en développement, la surveillance de la qualité alimentaire et plus particulièrement celle concernant les produits de la pêche n'ont pas été encore élaborés.

Au Liban, l'environnement marin est soumis à différentes pressions anthropiques, en particulier dans les zones côtières en raison de l'urbanisation, et de la surexploitation des ressources côtières. Les exodes ruraux massifs en direction des côtes, et surtout de Beyrouth et de ses banlieues, les constructions anarchiques, l'absence de réseaux d'assainissement et de stations d'épuration appropriées ont amplement contribué à la dégradation des zones côtières et la détérioration de la qualité des eaux. Les lois concernant la sécurité alimentaire et la protection environnementale sont soit inexistantes soit non appliquées et les violations dans ce secteur sont importantes (MOE/UNDP/ECODIT, 2011). En plus, des dépotoirs à ciel ouvert sont répandus le long du littoral

Libanais et particulièrement au niveau des grandes villes comme Tripoli et Beirut ce qui soulève des questions concernant la qualité des produits locaux de la mer.

Au cours des dernières décennies, plusieurs études concernant la contamination des côtes Libanaises ont été réalisées. La majorité de ces études se sont focalisées sur la contamination des eaux marines (Fakhri and Romano, 2005; Nakhlé, 2003) ainsi que des sédiments (Abi-Ghanem, 2008; Abi-Ghanem et al., 2009b, 2011; Merhaby et al., 2015, 2018), notamment par le mercure, le cadmium et le plomb. Cependant, à notre connaissance, il y a très peu d'études qui se sont focalisées sur la contamination des produits de la pêche par les éléments traces métalliques et encore moins sur les risques sanitaires qu'il peuvent engendrés (Kouyoumjian et al., 1986; Kouyoumjian et al., 2001; Nakhlé et al., 2006; J. Shiber, 1981; J. G. Shiber, 1981a; Shiber and Shatila, 1978). En outre, la plupart de ces études datent des années 80, dans le temps où les pressions environnementales et l'urbanisation sont en perpétuelle croissance au niveau de la côte Libanaise. De ce fait, une étude consacrée entièrement à l'évaluation de la contamination par les éléments traces métalliques du littoral Libanais et des produits de la pêche est apparue nécessaire. C'est dans ce contexte que s'inscrit cette étude qui consiste à évaluer le niveau de contamination par les éléments traces métalliques chez différents organismes marins collectés le long de la côte libanaise. L'étude a été focalisée sur l'évaluation des contaminants métalliques dans des organismes marins appartenant à différents compartiments de la chaîne alimentaire.

Le premier objectif de cette étude était d'évaluer le niveau de contamination ainsi que les variations spatiales et saisonnières de 20 éléments traces métalliques dans une espèce d'algue (*Ulva lactuca*), une espèce de bivalve (*Spondylus spinosus*), une espèce de crustacé (*Marsupenaeus japonicus*) et trois espèces de poisson (*Siganus rivulatus*, *Lithognathus mormyrus* et *Etrumeus teres*). Elles ont été échantillonnées le long du littoral Libanais, au niveau de trois sites présentant différents types et niveaux de pollution. Les éléments trace métalliques ont été mesurés dans la chair des bivalves et des crustacés ainsi que dans les muscles et les foies des poissons ; sachant que le foie est le principal organe accumulateur qui est souvent ciblé pour refléter les conditions environnementales.

Un deuxième objectif consistait en l'évaluation des risques sanitaires liés à la consommation des espèces sélectionnées et cela en termes de concentration en As, Cd, Hg et Pb. Les espèces choisies sont représentatives du milieu marin Libanais et sont couramment consommées par la population Libanaise donc économiquement importantes. Les risques sanitaires liés à la consommation de ces espèces ont été calculés à travers deux indices, notamment le « Target Hazard Quotient » (THQ) et « Estimated Weekly Intake » (EWI).

Finally, **un troisième objectif** a visé l'optimisation et la validation d'une méthode de routine pour la spéciation du mercure dans les produits de la pêche par HPLC-ICP-MS. Des métaux comme le mercure (Hg), peuvent se trouver dans l'environnement sous plusieurs formes, le méthylmercure (MeHg) étant la forme la plus toxique et qui est principalement transmise par l'ingestion des produits de la pêche. Jusqu'à présent, il n'y a pas de réglementations en termes de MeHg dans les matrices alimentaires et particulièrement les produits de la pêche malgré le fait que cette espèce représente la fraction la plus toxique du Hg. Cela est dû aux défis de la chimie analytique, justifiés par le fait que l'analyse de spéciation du MeHg n'est pas encore devenue une approche de routine. Ainsi, une méthode de routine pour l'analyse de spéciation de MeHg par HPLC-ICP-MS a été développée et validée par profil d'exactitude et a été utilisée par la suite pour déterminer les niveaux de MeHg dans les échantillons.

Evaluation du niveau de contamination de la côte Libanaise par les éléments traces métalliques

Cette partie a permis de déterminer le niveau de contamination des différentes espèces par 20 éléments traces métalliques (ETMs) (Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Ag, Cd, Sn, Sb, Ba, Hg, Pb and U). Les teneurs en ETMs ont été déterminées dans les algues, les foies et les muscles de 174 poissons, 180 bivalves, 116 crustacés ainsi que dans des sédiments échantillonnés sur 3 sites le long du littoral Libanais pour 2 saisons (humide et sèche). Ces éléments ont été analysés par ICP-MS (spectrométrie de masse par plasma inductif). Les niveaux de concentration ont été discutés selon les espèces, les variabilités saisonnières et spatiales. En outre, les facteurs de bioaccumulation par les algues, les bivalves et les crustacés à partir des sédiments ont été calculés.

Dans une première partie, les ETMs ont été mesurés dans les foies et les muscles de deux espèces démersales (*Siganus rivulatus* et *Lithognathus mormyrus*) et une espèce pélagique (*Etrumeus teres*) de poisson. Des éléments comme Ni, Sn, Ba et U n'ont pas été détectés dans les muscles ou bien avaient une fréquence de détection inférieure à 10%. L'argent (Ag) a été seulement quantifié dans les foies de *S. rivulatus*. Les concentrations en ETMs étaient plus élevées dans les foies que dans les muscles. Cela est dû au fait que les foies sont des organes métaboliquement actifs qui accumulent plus efficacement les ETMs que les muscles (Bahnasawy et al., 2009; Chovanec et al., 2003a; Rakocevic et al., 2017). Des variations interspécifiques ont été également trouvées.

L'espèce herbivore *S. rivulatus* se nourrissant d'algues a montré des niveaux plus élevés de contamination dans les muscles et les foies pour la majorité des ETMs comparés aux espèces carnivores *L. mormyrus* et *E. teres* qui ont montré des niveaux plus élevés en mercure. Ces observations sont en accord avec celles avancées par d'autres auteurs (Abdallah, 2008; Khaled, 2004;

Skaff, 2014). L'accumulation des contaminants métalliques par les algues rend probablement l'espèce *S. rivulatus* plus exposée aux métaux que les deux autres espèces. Plusieurs études ont démontré que le régime alimentaire, l'âge et la taille de l'organisme sont des paramètres qui affectent l'accumulation des ETMs par les organismes (Akan et al., 2012; Ali and Khan, 2019; Bawuro et al., 2018). Les deux espèces carnivores sont les plus susceptibles d'accumuler le mercure et cela est principalement dû à leur régime alimentaire et leur mode de vie. L'effet de l'habitat sur l'accumulation des ETMs a été également démontré. En effet, les espèces habitant dans les eaux superficielles près de la côte sont plus exposées aux effets des activités anthropiques et donc aux contaminants (Stehr et al., 2004). *S. rivulatus* est une espèce qui vit à des profondeurs entre 1 et 30 m tandis que *L. mormyrus* et *E. teres* peuvent se trouver jusqu'à des profondeurs de 80 et 200 m respectivement (Bariche et al., 2004) rendant potentiellement *S. rivulatus* plus exposé aux perturbations environnementales ayant lieu au niveau de la côte. Des concentrations relativement élevées ont été trouvées dans les muscles et les foies des trois espèces, toutefois les foies de *E. teres* ont montré les niveaux les plus élevés en As et Se. En général, les poissons sont capables d'accumuler l'As naturellement ce qui rend difficile d'associer les niveaux trouvés dans les organismes à des sources anthropiques ou naturelles (Kalantzi et al., 2017; Popowich et al., 2016). Plusieurs études ont montré que parmi les ETMs analysés, l'As avait les niveaux les plus élevés (Leung et al., 2014; Storelli et al., 2005a; Thiyagarajan et al., 2012; Zuluaga Rodríguez et al., 2015a). Dans notre étude, les niveaux les plus élevés ont été trouvés dans les deux espèces carnivores qui se nourrissent près du fond. Cela peut être expliqué par le fait que les sédiments sont plus chargés en As que la colonne d'eau, rendant ainsi les eaux profondes plus riches en As que les eaux de surface (Storelli and Marcotrigiano, 2001a; Tremblay and Gobeil, 1990). Les concentrations en ETMs trouvées dans les muscles correspondent à ceux trouvées dans la région Est Méditerranéenne, par contre les niveaux trouvés dans les foies étaient plus élevés dans notre étude suggérant que la côte Libanaise est soumise à de fortes pressions environnementales. Ça pourrait être lié aux eaux usées domestiques et industrielles, et aux lixiviats des dépotoirs présents le long des côtes qui se retrouvent dans la mer sans aucun traitement (Khalil et al., 2018).

Dans une deuxième partie, les ETMs ont été mesurés dans l'algue verte *U. lactuca*, le bivalve *S. spinosus*, la crevette *M. japonicus* et dans les sédiments de chaque site. Les facteurs de bioaccumulation des ETMs par les organismes en question ont été calculés à partir des sédiments. Les espèces ont montré une différence significative en termes d'accumulation des ETMs soulignant l'importance d'une approche multi-espèces pour établir un programme de surveillance. Des concentrations élevées en Al et Fe ont été trouvées dans les algues tandis que Zn et As étaient les plus élevées dans les bivalves et Cu dans les crevettes. Chaque espèce a montré une affinité pour un certain

groupe d'ETMs et cela est fonction d'un ensemble complexe de paramètres. Les organismes marins absorbent les ETMs sous forme dissoute ou particulaire. Cette dernière voie peut consister en une ingestion de débris en suspension, de proies alimentaires ou même de matières organiques déposées (Amiard et al., 2006; Rainbow and Phillips, 1993). La bioaccumulation de métaux traces dans les organismes marins est également liée à plusieurs paramètres contribuant à la variabilité inter et intraspécifique, à savoir des facteurs biotiques tels que la taille, l'âge, le sexe et le métabolisme, ainsi que la position de l'organisme dans la chaîne alimentaire (Jakimska et al., 2011a). Les algues absorbent principalement les éléments dissous en solution et donc sont de bons bioindicateurs de la qualité des eaux. Ils sont aussi reconnus pour leur capacité d'accumuler les ETMs et cela est dû aux polysaccharides sulfatés présents dans leurs parois cellulaires (Alves et al., 2013; Cunha and Grenha, 2016). Des concentrations élevées en Fe ont été trouvées dans les algues. Sachant que cet élément est métaboliquement actif et naturellement bioamplifié par les algues, les sources anthropiques de cet élément augmentent encore plus sa concentration dans l'environnement et par suite son absorption par les algues (Akcali and Kucuksezgin, 2011). Les crevettes ont accumulé les concentrations les plus élevées en Cu comparés aux bivalves et aux algues. Ces organismes sont connus d'avoir de l'hémocyanine qui est une molécule contenant du Cu et ayant pour fonction de transporter l'oxygène et donc son accumulation est liée à son caractère essentiel (Downs et al., 2016; Rainbow and Luoma, 2011). Quant aux bivalves, les concentrations les plus élevées ont été observées pour le Zn et le Cd. Les bivalves sont connus pour leur accumulation de niveaux importants de métaux de l'eau et le Zn et le Cd en font partie. Ces éléments jouent un rôle dans le métabolisme des bivalves en activant la métallothionéine qui est une protéine complexant les métaux. Par contre le cadmium a un taux d'assimilation important et un taux de dépuración faible dans le bivalve ce qui contribue à son accumulation (Thomann et al., 1995). Les teneurs les plus élevées en As ont été trouvées dans les crustacés et les bivalves. Ces organismes sont incapables de métaboliser l'As et de ce fait, les teneurs en As mesurées dans ces espèces reflètent bien les niveaux de contamination du milieu par cet élément (Rainbow, 2002). Les facteurs de bioaccumulation des sédiments vers les organismes (BSAF) ont été calculés. Une bioaccumulation a été confirmée quand $BSAF > 1$. Les valeurs du BSAF ont montré des transferts limités des ETMs à partir des sédiments. Les BSAFs les plus élevés ont été trouvés chez le bivalve et la crevette. L'algue verte avait des BSAFs < 1 pour tous les éléments indiquant l'absence de transfert à partir des sédiments. Cela est principalement dû au fait que les algues absorbent principalement les ETMs en solution. Plusieurs études ont montré une corrélation entre les concentrations des ETMs dans les algues et ceux dans les sédiments et dans l'eau (Akcali and Kucuksezgin, 2011; Bonanno et al., 2019a; Chakraborty et al., 2014a), tandis que d'autres n'ont pas trouvé de corrélations significatives (Kamala-Kannan et al., 2008; Malea and Haritonidis, 2000). En

effet, malgré le fait que les algues absorbent les métaux dissous, les niveaux des ETMs dans les sédiments peuvent refléter dans une certaine mesure la concentration dans la colonne d'eau à travers des processus de diffusion et de re-mobilisation des éléments à partir des sédiments (Bonanno et al., 2019a; Thomann et al., 1995) ce qui explique l'existence d'une corrélation dans certains cas. Les bivalves ont accumulé Cu, Zn et Cd avec les BSAFs les plus élevés observés pour le Zn et le Cd à Tripoli. Cela est dû au fait que ces éléments sont métaboliquement actifs chez les bivalves comme expliqué plus haut. Pour les autres éléments, les facteurs de bioaccumulation étaient tous inférieurs à l'unité chez le bivalve. Quant aux crevettes, Les BSAFs étaient > 1 pour Cu et Zn avec une valeur plus élevée pour le Cu qui constitue un élément essentiel pour ces organismes. Un BSAF plus élevé pour le Hg à Tripoli a été trouvé dans les crevettes. Cela est probablement lié au fait que la concentration en Hg est plus élevée dans les sédiments de ce site sachant que les crevettes habitent près des fonds et sont donc plus exposés aux contaminants liés aux sédiments (Pinedo-Hernández et al., 2015). A noter que les niveaux des éléments essentiels trouvés dans les organismes ne correspondent pas toujours nécessairement à des besoins métaboliques puisque les invertébrés marins peuvent accumuler les métaux suite à leur concentration élevée dans l'environnement (Rainbow and Luoma, 2011).

Les concentrations de métaux traces dans les organismes marins peuvent être le résultat d'absorption provenant de différentes sources et pas seulement des sédiments. La bioaccumulation résulte de plusieurs facteurs, notamment la biodisponibilité, les multiples voies d'exposition et le métabolisme, qui peuvent varier considérablement d'une espèce à l'autre (Jakimska et al., 2011b). Chaque espèce absorbe les métaux traces différemment et il est donc important de prendre en compte différents taxons et même des tissus d'un même organisme lors de l'établissement de programmes de surveillance et de l'évaluation de la bioaccumulation (Yüzereroğlu et al., 2010).

En outre des variations interspécifiques, des variations saisonnières et spatiales ont été trouvées. Des concentrations plus élevées ($p < 0.05$) en ETMs ont été généralement observées durant la saison humide dans les espèces avec quelques exceptions. Les différentes espèces ont également montré des niveaux et des schémas d'accumulation différents selon les saisons. Les fluctuations saisonnières des ETMs dans les organismes marins ont été largement citées dans la littérature (Abdallah, 2008; Azizi et al., 2018; Bahnasawy et al., 2009; Barua et al., 2011; Jezierska and Witeska, 2006; Khaled, 2004). La saisonnalité est en effet intimement liée au type de contaminant et à d'autres paramètres qui conditionnent la biodisponibilité de l'élément. La biodisponibilité et la toxicité d'un élément sont conditionnées par les caractéristiques physico-chimiques des eaux marines (Chovanec et al., 2003a). D'autres paramètres englobent la charge polluante du site et les cycles internes des organismes marins en question (Yilmaz and Yilmaz, 2007). Le long de la côte Libanaise, des paramètres comme le pH,

la température, la dureté de l'eau, la salinité et la concentration en oxygène montrent des variations saisonnières significatives ce qui peut affecter l'accumulation des ETMs par les différentes espèces (Fakhri and Romano, 2011). Les variations de ces paramètres le long de la côte Libanaise sont entre autres liées à la différence des apports fluviaux entre les saisons. Durant la saison de printemps (entre mars et juin), la fonte des neiges augmente les débits des fleuves et des rivières et cela peut se traduire par une augmentation des apports de certains contaminants dans le milieu marin ou alors contribuer à la dilution d'autres. Des éléments comme Al, As, Mn et Ti ont montré des valeurs plus élevées durant la saison humide tandis que les niveaux du Pb ont été généralement plus élevés durant la saison sèche dans tous les organismes et particulièrement dans ulva. Cela peut être lié à une augmentation des teneurs du Pb dans l'eau durant cette saison (Skaff, 2014). Concernant les variations spatiales, elles ont été principalement attribuées aux différentes sources de contaminants et régimes de pluviométrie au niveau de chaque site, sachant que le Nord du pays reçoit plus de pluie que le Sud. Dans la majorité des cas, le niveau de contamination en ETMs à Tripoli a révélé des valeurs supérieures aux deux autres sites. Au niveau de ce site, les usées sont rejetées dans la mer sans aucun traitement et aussi il se trouve un dépotoir qui libère des lixiviats toxiques dans le milieu marin. Une étude a démontré que ces lixiviats sont chargés de Pb et Cd (Khalil et al., 2018). Cela peut expliquer les niveaux plus élevés de Cd qui ont été trouvés dans les bivalves près de ce site. Le site de Beirut, a été caractérisé par des concentrations plus élevées en Zn et Cu, et parfois en Cr. Au niveau de ce site, plusieurs types d'industries comme des tanneries, des industries de ciment, de métaux, de production de cuir et de galvanoplastie, rejettent des effluents chargés en métaux dans les rivières sans traitement préalable, entre autres le Cu, le Zn et le Cr ; ce qui peut expliquer les niveaux plus élevés de ces éléments à Beirut. Les niveaux des ETMs obtenus dans la présente étude sont du même ordre de grandeur que ceux obtenus dans d'autres études autour de la Méditerranée pour les muscles de poissons. Cependant, les concentrations dans les foies ont révélé des concentrations supérieures à celles documentées en Est Méditerranée surtout en Cu et Fe (Abdallah and Abdallah, 2008; Canli et al., 2001a; Khaled, 2004; Yilmaz et al., 2018). En ce qui concerne les algues, bivalves et crustacés, les concentrations trouvées étaient supérieures à celles trouvées dans les zones considérées « loin des activités anthropiques » et en même temps inférieures à celles trouvées dans les zones considérées très polluées.

Evaluation des risques liés à la consommation des produits de la pêche de la côte Libanaise

Cette deuxième partie aborde l'évaluation des risques sanitaires liés au Cd, Hg, Pb et As à travers la consommation des produits de la pêche du littoral Libanais. Les ETMs ont été analysés dans les muscles des poissons et dans la chair des crevettes et des bivalves. Les niveaux de contamination obtenus ont été comparés aux niveaux maximum admissibles émis par la Commission Européenne. En

outre, les risques ont été évalués en calculant la consommation hebdomadaire (EWI) et la comparant à la dose hebdomadaire tolérable provisoire (PTWI) relative à chaque élément. Un quotient de risque (THQ) a été également calculé en prenant en considération des doses de référence émises par l'Agence Américaine pour la protection de l'environnement (US EPA). Les concentrations trouvées présentent un risque potentiel si le THQ obtenu est supérieur à l'unité.

Les concentrations moyennes en Cd, Hg et Pb mesurées dans les organismes étudiés ont montré des niveaux inférieurs aux seuils règlementaires fixés par la Commission Européenne. Cependant, des niveaux élevés en As ont été trouvés dans tous les organismes et particulièrement dans les bivalves et les crustacés. Jusqu'à présent, il n'existe pas de réglementation concernant le niveau maximal admissible d'As dans les produits de la pêche. Il a été démontré précédemment que les produits de la pêche contenaient les plus fortes concentrations d'As total. Cependant, la forme dominante chez les organismes marins est l'arsénobétaïne, qui est l'espèce d'As la moins toxique comparée à l'As inorganique. L'Organisation des Nations Unies pour l'alimentation et l'agriculture (FAO / OMS) a fixé à un PTWI une valeur de $15 \mu\text{g.kg}^{-1} \text{ BW}$ pour les acides inorganiques. Toutefois, cette PTWI a été supprimée par l'Autorité européenne de sécurité des aliments (EFSA) et le Comité mixte FAO/OMS d'experts sur les additifs alimentaires (JECFA). Par conséquent, la valeur indicative définie pour l'As total n'est plus utilisée pour l'évaluation des risques au niveau international, car cela entraînerait une surestimation considérable des risques pour la santé liée à l'exposition alimentaire à l'arsenic (EFSA, 2014a; Llorente-Mirandes et al., 2017). Les deux espèces de poisson carnivores se nourrissant près du fond avaient les valeurs les plus élevées en Hg total comparés aux crustacés et bivalves. Cela est en accord avec d'autres études qui ont documenté des concentration de Hg plus élevées dans les poissons (Bonsignore et al., 2018; Storelli, 2008). La bioaccumulation du Hg dans les organismes marins est principalement faite sous forme de méthylmercure (MeHg) et dépend du niveau trophique de l'animal, de son habitat, sa taille, son âge et son régime alimentaire. Le Comité d'experts sur les additifs alimentaires (JECFA) a déclaré que la quasi-totalité des expositions humaines au Hg par le régime alimentaire était due à la consommation de produits de la pêche et que le MeHg était de loin la forme de Hg la plus toxique. La fraction du MeHg varie entre 70 et 100% dans les poissons et de 28 à 98% dans les bivalves. Dans la présente étude, la totalité du mercure a été considérée comme MeHg pour l'évaluation des risques et même avec cette supposition, la consommation de Hg à travers les espèces étudiées a été largement inférieure à la PTWI. De même pour le Pb et le Cd, les concentrations trouvées étaient loin de causer des risques pour la santé humaine et en général, les produits de la pêche ont été montré d'être les moindres contributeurs à l'exposition au Pb (Arnich et al., 2012). Cependant, les bivalves et les crustacés ont montré une contribution à la PTWI 100 fois

supérieure à celle des poissons en termes de Cd. Cela est dû aux concentrations relativement plus élevées obtenus dans les bivalves et particulièrement à Tripoli. Les valeurs obtenus dans la présente étude sont du même ordre de grandeur et parfois inférieures comparés à ce qui a été obtenu dans d'autres régions autour de l'Est Méditerranée (Abdallah and Abdallah, 2008; Canli et al., 2001a; Khaled, 2004; Masoud et al., 2007; Türkmen et al., 2005; Turkmen, 2012a). En conclusion, nos résultats montrent que la consommation des espèces analysées dans cette étude n'est pas susceptible de causer des effets négatifs sur la santé humaine malgré la présence de sources de pollution le long du littoral Libanais. En revanche, l'étude peut être complétée par l'analyse d'espèces prédatrices se trouvant à un niveau plus élevé de la chaîne alimentaire tout en prenant en compte les habitudes alimentaires de la population Libanaise.

Développement d'une méthode de routine pour la spéciation du MeHg par HPLC-ICP-MS

Le mercure est l'un des polluants les plus toxiques globalement et sa toxicité est reconnue depuis le 19^{ème} siècle (Tchounwou et al., 2003b). Il est libéré dans l'environnement à partir de sources naturelles comme les éruptions volcaniques et les incendies de forêt. Le mercure provient également de sources anthropiques comme la production d'électricité, l'incinération et le traitement des déchets (Jaishankar et al., 2014). Le mercure existe dans l'environnement sous différentes formes les plus communes étant la forme élémentaire Hg^0 , la forme inorganique Hg^{2+} et la forme organique MeHg, cette dernière étant la plus toxique. Le MeHg est l'un des composés les plus bioaccumulés dans l'environnement marin et par suite sa présence dans la chaîne alimentaire est une problématique (Maia et al., 2019). Les produits de la pêche constituent la voie majeure d'exposition au MeHg (Storelli et al., 2005a). En effet, 90% du MeHg est absorbé par le tractus gastrointestinal et puis est transféré dans la circulation sanguine où il forme des complexes avec les groupements thiol des protéines, ce qui perturbe les activités enzymatiques.

Afin de limiter l'exposition alimentaire des consommateurs au MeHg, la Commission européenne (CE) a fixé des niveaux maximaux admissibles de mercure total dans le poisson (0,50 et 1,0 mg kg⁻¹, respectivement, cette dernière limite étant liée aux poissons prédateurs) (CE, 2014). Jusqu'à présent, il n'y a pas de réglementation en matière de MeHg dans les fruits de mer ou autres types de denrées alimentaires, bien que cette espèce représente la principale menace en termes de toxicité du Hg. Cela est principalement dû aux défis de la chimie analytique, confirmés par le fait que l'analyse par spéciation du mercure n'est pas encore devenue une approche de routine.

L'analyse de spéciation du mercure a principalement été réalisée par chromatographie en phase gazeuse (CPG) en raison de ses limites de détection inférieures à celles des techniques couplées à la

chromatographie en phase liquide (HPLC) (Laffont et al., 2013). Cependant, avec les nouveaux développements de la spectrométrie de masse à plasma à couplage inductif (ICP-MS) en termes de sensibilité, les méthodes HPLC-ICP-MS pour l'analyse de spéciation du Hg sont principalement utilisées de nos jours en raison de leur simplicité par rapport à la méthode GC-ICP-MS, principalement parce que la dérivation n'est pas nécessaire (Cattani et al., 2008; Fang et al., 2016; Grotti et al., 2014; Hight and Cheng, 2006).

Cette partie présente la procédure de développement et de validation d'une approche analytique de routine pour la spéciation du MeHg dans les produits de la pêche pour permettre une meilleure évaluation des risques liés à la consommation de ces produits. La procédure de validation est basée sur le profil d'exactitude. Dans ce contexte, les critères de performance en termes de LQ, de spécificité, d'exactitude, de répétabilité et de précision intermédiaire ont été évalués. La méthode a également été appliquée à l'analyse d'une sélection de produits de la pêche.

Dans cette étude, les conditions de séparation et d'extraction ont été optimisées. Deux colonnes C₁₈ en phase inverse et de petit diamètre ont été également testées. Les colonnes à diamètre réduit présentent certains avantages comparés aux colonnes normales, comme une séparation chromatographique plus rapide et une meilleure résolution avec un débit réduit de phase mobile (Grotti et al., 2014). La colonne sélectionnée et qui a donné une meilleure séparation est une C₁₈ peptide mapping d'Agilent (2.1 x 150 mm, 2.7 µm). La phase mobile qui a donné la meilleure séparation consiste en 5% de MeOH et 95% de L-cystéine.HCl.H₂O à 0.5 g L⁻¹. Plusieurs températures, durées et concentrations de phase extractante ont été aussi testées. Des matériaux de référence certifiés ont été utilisés durant l'optimisation : DOLT-5 (Foie de poisson), ERM-CE 464 (Poudre de Poisson lyophilisée) et TORT-3 (Hépatopancréas de homard). Le critère de validation des résultats a été fixé à un taux de récupération compris entre 80 et 120%. L'extraction a été faite par microondes. Parmi sept températures testées, celle qui a donné les meilleurs rendements (99 % taux de récupération du MeHg) est 60 °C. En ce qui concerne la durée, un temps d'extraction de 15 minutes à une température de 60 °C ont été adoptés. En outre, différentes prises d'essai de matrice lyophilisée et matrice fraîche ont été testées. Pour les matrices lyophilisées, une prise d'essai de 300 mg a été adoptée et de 600 mg pour les matrices fraîches.

La fidélité intermédiaire, l'incertitude, la répétabilité et la reproductibilité de la méthode ont été évaluées par le profil d'exactitude suivant la norme NF VO3-110. Le profil d'exactitude est une expression de l'erreur fixe et de l'erreur aléatoire (répétabilité et fidélité intermédiaire) pour une série de concentrations (domaine de validité) de l'analyse dans différentes matrices. Sept niveaux de concentration en MeHg dans différentes matrices ont été sélectionnés pour la validation. Les résultats

ont montré que les limites de tolérance ont été comprises dans les limites d'acceptabilité fixée à priori sauf pour les deux premiers niveaux. La limite de détection correspondant à $0.038 \mu\text{g L}^{-1}$ a été déterminée à partir de l'intersection des limites de tolérance et des limites d'acceptabilité issues du profil d'exactitude. La méthode a été en plus validée en utilisant des contrôles qualité externe (FAPAS) qui sont utilisés dans le cadre des essais inter-laboratoire. Les z-scores obtenus étaient satisfaisants (compris entre -0.4 et -0.2) prouvant ainsi l'adéquation de la méthode à l'usage pour la spéciation du Hg dans les produits de la pêche. La méthode a été prouvée robuste et appropriée pour les analyses de MeHg de routine.

La présente étude a fourni des données récentes concernant la contamination des produits de la pêche en termes de métaux traces le long du littoral Libanais. Comparé aux autres études de l'Est Méditerranée, les niveaux de contamination trouvés le long du littoral Libanais, dans les organismes analysés, tombent entre les valeurs trouvées dans les zones qui sont relativement épargnées des activités anthropiques et celle qui sont considérés pollués suggérant un niveau modéré de pollution. Par ailleurs, la consommation des espèces analysées dans la présente étude n'est pas susceptible de causer des effets néfastes sur la santé humaine.

CHAPTER 1 – GENERAL INTRODUCTION

Chapter 1 - GENERAL INTRODUCTION

Fisheries and aquaculture constitute important sources of food and income for millions of people around the world as they represent a valuable source of nutrients. During the last decades, food fish consumption per capita has been witnessing a continuous increase worldwide as it grew from 9.0 kg in 1961 to reach more than 20 kg in 2017 while providing about 17% of animal protein consumed by the global population (Figure 1-1). In 2015, it was estimated that fish provided 20% of average animal protein intake per capita for 3.2 billion people (FAO, 2018).

The nutritional benefits of fish are mainly attributed to their content in proteins, vitamins (nicotinamide (B₃), pyridoxine (B₆) and cobalamine (B₁₂)), minerals (calcium, iron, selenium, zinc, etc.) and essential omega-3 polyunsaturated fatty acids (PUFAs) that hold a lot of health benefits (Storelli and Barone, 2013; Wang et al., 2006). Unlike meat, fish is easily digested due to its long muscular fibers (Sidhu, 2003). Many studies have evidenced the *health benefits* of eating *seafood*. For example, omega-3 PUFAs are associated with the reduction of coronary heart disease, the decrease of hypertension, and the prevention of cardiac arrhythmias and sudden death (Hoekstra et al., 2013; Sidhu, 2003). Conversely, fish may be a route of human exposure to harmful chemicals such as dioxins and trace elements, namely methylmercury (MeHg) that is known to accumulate in fish. This accumulation of contaminants in fish may pose potential health risks, especially in the most vulnerable part of the population like pregnant women and children (Mozaffarian et al., 2003).

The last decades witnessed a growing interest to determine contamination levels in seafood especially with the increase of anthropogenic pressures on coastal areas worldwide (Anandkumar et al., 2018; Arnich et al., 2012; Avigliano et al., 2019; Chiesa et al., 2018; Masoud et al., 2007; Storelli, 2008; Storelli and Barone, 2013). Exposure to chemicals and particularly metals at certain levels is responsible for human health disorders including kidney damage, liver dysfunction, hormonal imbalance, cardiovascular diseases and mental health problems (EFSA, 2014b, 2010). Diet is one of the main routes of exposure to these chemicals that's why protection from these hazards reveals to be a necessity and thus regulations have been emitted by different health authorities in order to limit exposure of the populations to dangerous substances. In this context, authorities such as the European Commission (EC) (Regulations No 1881/2006 and No 629/2008) (EC, 2008, 2006), the FAO (Heavy Metals Regulations Legal Notice No 66/2003) (FAO, 2003) and the United States Environmental Protection Agency (USEPA) have established maximum allowed levels for elements such as Hg, Pb and Cd in seafood. However, in some developing countries food surveillance in general and seafood in particular have not been elaborated yet.

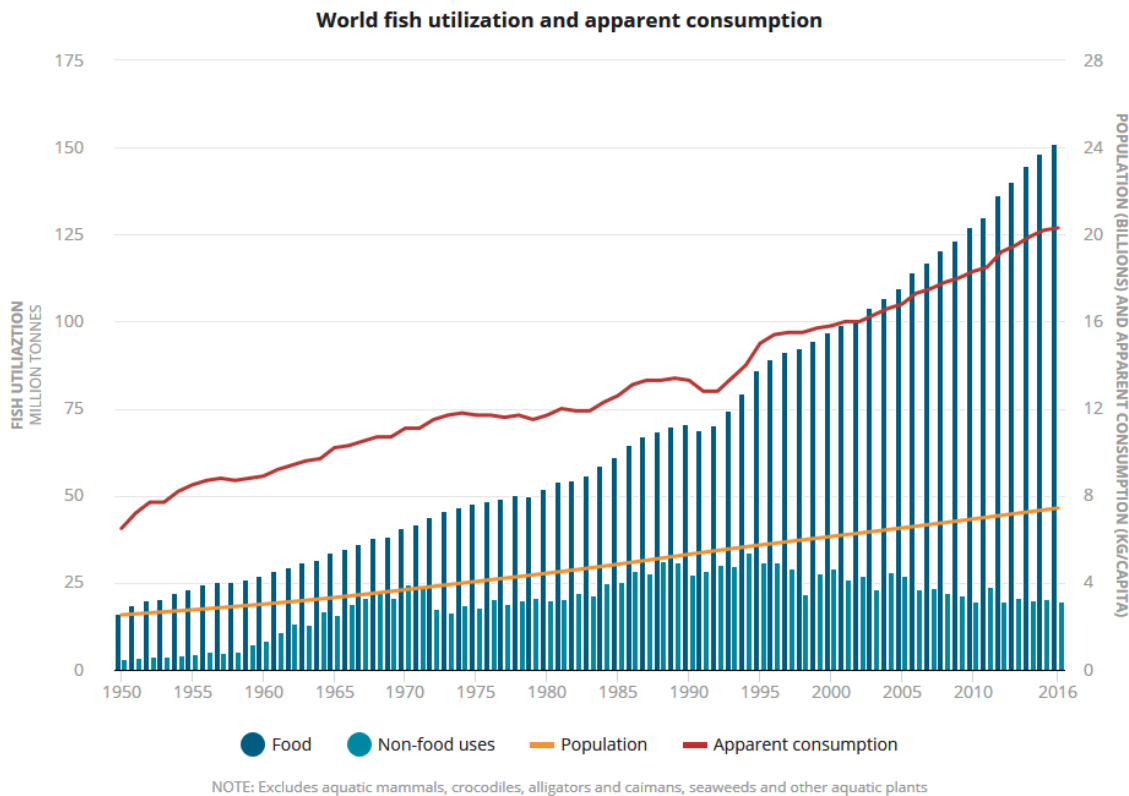


Figure 1-1 World fish utilization and apparent consumption (FAO, 2018)

The long years of war that Lebanon has known since 1975 until the nineties, have left serious consequences on the country and especially on the environmental coastal areas. In fact, the massive rural migration towards the coast, mainly Beirut and its districts, random constructions, the absence of appropriate wastewater treatment plants and management strategies, have contributed to the degradation of coastal environment and the quality of coastal waters. Laws concerning food security or environmental protection are either nonexistent or not applied and violations of the public marine domain are significant (MOE/UNDP/ECODIT, 2011). Moreover, illegal dumpsites are widespread along the Lebanese coast especially in big coastal cities like Tripoli and Beirut.

In the last few decades, various studies concerning the contamination of the Lebanese marine environment have been carried out. Most of these studies focused on the chemical contamination of marine waters as well as marine sediments (Abi-Ghanem, 2008; Abi-Ghanem et al., 2011, 2009b, 2009a; Fakhri and Romano, 2011, 2005; Merhaby et al., 2018, 2015). To our knowledge there are few studies that have dealt with the chemical contamination of fishery products in Lebanon and even less on the health risks they may engender (Kouyoumijian et al., 1986; Kouyoumjian et al., 2001; Nakhlé et al., 2006; J. G. Shiber, 1981a, 1981b; Shiber and Shatila, 1978). Besides, most of these studies date back to the 80's while environmental pressure and urbanization have been in continuous increase along

the Lebanese coast. In this context, a study devoted to the contamination status of Lebanese coastal environment and fishery products reveals to be a necessity.

1. METALS AND TRACE ELEMENTS: AN OVERVIEW

The expression "Heavy metals" defines metallic elements that have a relatively high density compared to water and it is assumed that heaviness is associated to toxicity (Ansari et al., 2004; Tchounwou et al., 2012). Heavy metals are also considered trace elements as they are present in trace concentrations in the ppb range to less than 10 ppm in most environmental matrices (Tchounwou et al., 2012). Previously, scientists used the term "heavy metals" when referring to trace elements. In fact, not all metals are essentially heavy (e.g. Al, Ni) and some elements are not metals (e.g. As, Se). That's why nowadays researchers prefer the term "metallic trace elements" (Duffus and Templeton, 2002).

Metals may be classified as essential and non-essential, depending on whether they exert a function in biochemical and physiological processes (Figure 1-2) (Goyer et al., 2004). According to the World Health Organization (WHO) essential elements are I, Zn, Se, Fe, Cu, Cr and Mo. Other trace elements may/could have beneficial effects or be essential such as Mn, Co, As, Ni and V (Amiard, 2011). In fact, Cu and Fe are incorporated into a number of metalloenzymes involved in hemoglobin formation and carbohydrate metabolism (Henriques et al., 2017; Tchounwou et al., 2012) while Zn plays an important role in cell division and growth, wound healing and breakdown of carbohydrates (Roohani et al., 2013). Non-essential trace elements such as Hg, Pb or Cd play no physiological role and are toxic at very small amounts (Goyer et al., 2004).

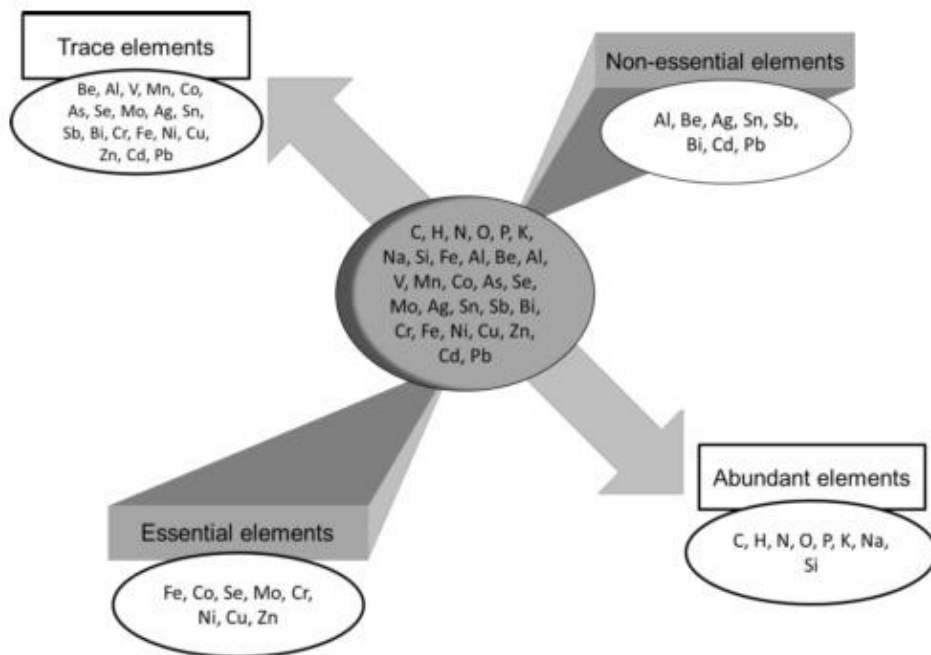


Figure 1-2 Essential and non-essential elements, abundant or in traces (Amiard, 2011)

1.1. SOURCES OF METALS IN THE MARINE ENVIRONMENT

Nowadays, aquatic pollution has become a major concern especially in coastal areas that exhibit high population growth and urbanization (Creel, 2003; Neumann et al., 2015). Various and numerous contaminants are present in the marine environment coming from natural and anthropogenic sources. Metals are naturally found in the earth's crust, but their concentrations are increased due to anthropogenic activities as they reach the marine environment through water or atmospheric ways. Natural sources include geological weathering of underlying bedrocks and volcanic eruptions that have been reported to highly contribute to metal pollution (He et al., 2005; Mohammed et al., 2011). Environmental contamination can also occur through metal corrosion, soil erosion of metal ions and leaching of metals in addition to metal re-suspension and evaporation from water sources (Nriagu, 1989). However, most environmental contamination by metals results from anthropogenic sources including mining, smelting operations, industrial production and use, in addition to domestic and agricultural use of metal and metal-containing compounds (He et al., 2005). Industrial sources such as metal refineries, coal burning in power plants, petroleum combustion, plastics, textiles, microelectronics, wood preservation and paper processing plants are considered important sources of metals (Arruti et al., 2010). Contaminated effluents of wastewater play an important role in metal

transfer into the aquatic environment. In wastewater, metals may be found under particulate (oxides and silicates) or dissolved forms (sulfurs and carbonates), but most of them are associated to suspended particulate matter (INERIS, 2004). Leachate associated to landfills has been proved to contain large amounts of toxic pollutants including metals (Toufexi et al., 2013). An example is elements like Pb and Cd coming from batteries or Hg from instruments such as thermometers and barometers. Table 1.1 provides a summary of the main sources of metals in the environment.

Table 1-1 Sources of metallic pollutants present in the environment

Anthropogenic activity	Metals released
Mines and fonderies	
Soil tips and residues – contamination by lixiviation and aerial erosion	Cd, Hg, Pb
Residues dispersed by water – contamination of soils due to floods	Cd, Pb
Iron and steel industries	Cu, Ni, Pb
Industries	
Plastic	Co, Cr, Cd
Textile	Zn, Al
Tanneries	Cr, Cd, Pb, Zn
Microelectronics	Cu, Ni, Cd, Zn
Wood preservation	Cu, Cr
Refineries	Pb, Ni, Cr
Atmospheric fallouts	
Urban/industrial sources; incinerators and waste elimination	Cd, Cu, Pb
Pyro metallurgical industries	Cd, Cr, Cu, Mn, Ni, Pb, Zn
Exhaust gas	Mo, Pb
Fossil energy burning	Pb, Zn, Cd
Agriculture	
Fertilizers	Cd, Mn and Zn in some phosphate fertilizers

Manures	Cu in pork's manures, Mn and Zn
Pesticides	Cu, Mn and Zn in fungicides, Pb in orchards
Irrigation water	Cd, Pb
Metals corrosion	Fe, Pb, Zn
Waste dumping	
Sewage sludge	Cd, Cr, Cu, Mn, Ni, Pb, Zn
Dumpsite leachate	Cd, Fe, Pb
Fire and ashes	Cu, Pb
Piles of scrap	Cd, Cr, Cu, Pb, Zn
Gold mining activities	Cd, Pb, Hg, Zn

1.2. FATE OF METALS IN THE MARINE ENVIRONMENT

The aquatic environment is very sensitive to environmental disturbances. Metals released in the marine environment are subject to multiple physicochemical factors that influence their forms and distribution between particulate or dissolved phase (Chovanec et al., 2003b). In real environmental conditions, metals have low solubility in water and stay mostly associated to the solid phase that's why metallic elements end up mostly in the sediments (INERIS, 2004).

Once metals reach the marine environment, they exert different behaviors and get distributed between the sediments, the water column, suspended matter and the biota. This distribution varies in space and time and is promoted in spring and summer as metals adsorb on phytoplankton. Phytoplankton is considered one of the most important biotic factors influencing the behavior and distribution of metals in the water column as it shows high metal bioconcentration with uptake mainly occurring from dissolved fractions (Phillips, 1980). On one hand, this adsorption of metals on phytoplankton reduces the amount of dissolved metals in the water, and on the other hand, it leads to contamination along the trophic chain through phytoplankton ingestion. In deep cold waters, due to the absence of primary production and mineralization of suspended matter, the concentration of dissolved metals increases (Morel and Price, 2003). Adsorption of metals on the sediments or suspended particles, is a function of the solid particles in question (Lion et al., 1982). Phytoplankton are also efficient bioaccumulators of trace metals. The incorporation of trace metals by phytoplankton can regulate the form and availability of trace metals. Consequently, the metal contents of phytoplankton reflect the environmental availability and influence the distribution of metals in the ocean (Sanders and Riedel, 1998; Twining and Baines, 2013). Metals can potentially be biomagnified as well as they can be

biodiminished along the food chain. These two phenomena are closely related to the nature of the trace element itself as well as the assimilation efficiency of the organisms in question. Although there is no general rule that predicts whether or not a trace metal is biomagnified along a marine food chain, it is known that the concentration of Cd for example generally decreases at higher trophic levels in classic planktonic food webs (e.g. phytoplankton to zooplankton to fish), whereas Hg concentrations increase at higher trophic levels (Ali and Khan, 2019; Chouvelon et al., 2019; Wang, 2002).

The free ionic metal forms, undergo a solvation process leading to the formation of hydrates that may later on form complexes with organic and inorganic ligands. The complexation process is governed by a multitude of parameters of which pH, redox potential, temperature and eventually the quantity of ligand available (Zhao et al., 2016). Inorganic (major anions such as carbonates, chlorides, and hydroxides) and organic (e.g. dissolved organic matter (DOM), particulate organic matter (POM)) chemical compounds in aquatic environments constitute ligands capable of complexing metal ions (Figure 1-2). Organic matter forms complexes with metals with its principal binding sites being carboxylic, nitrogenous and sulfured ones. Biological effects of metals are mostly related to the concentration of the free metallic ions in the water, thus complexation with DOM and POM reduces bioavailability and toxicity of trace elements to aquatic organisms by decreasing the amount of free ionic metals in the marine environment (de Souza Machado et al., 2016; Guo et al., 2001; Luther et al., 2001). The environmental behavior, bioavailability, and toxicity of trace metals in different matrices (water column, sediments, biota, etc.), are not only correlated with the total concentration of an element, but also with speciation which is an important index of the toxic and ecological risk of trace metals in the environment (Kwon and Lee, 2001; Fan et al., 2008; Ahumada et al., 2011; Nemati et al., 2011). According to IUPAC definition, chemical speciation is defined as the specific form of an element in terms of isotopic composition, electronic or oxidation state, and complex or molecular structure of a certain element (IUPAC, 2000). Consequently, in order to determine the reactivity of trace metals, chemical speciation is essential.

1.3. METAL UPTAKE AND ACCUMULATION IN MARINE ORGANISMS

The major factor governing the uptake of metals by marine organisms is the biological availability of the metal which at its turn is dependent from the physicochemical conditions of the environment (Ali et al., 2019; Chovanec et al., 2003; Rainbow, 1985). Bioavailability of metals in the marine environment is controlled by parameters such as pH, temperature, oxygen concentration, water hardness and salinity.

The influence of the physicochemical parameters of the water on metal speciation, toxicity and uptake is well known and has been widely documented (Ansari et al., 2004; Campbell et al., 2002; Chovanec et al., 2003; Jakimska et al., 2011; Rüdél et al., 2015; Tchounwou et al., 2012). It has been shown that water hardness reduces toxicity of metals and this is due to the competition that takes place between calcium and divalent metals for the binding sites, resulting in reduced uptake and toxicity of metals in hard water. Some studies reported the protective effect of Ca^{2+} ions against dietary and waterborne Cd uptake (Baldisserotto et al., 2004; Brzóška and Moniuszko-Jakoniuk, 1997; Li et al., 2016; Song et al., 2013). Rising temperature, oxygen depletion and metabolic stimulation during reproduction or stress in fish for example, leads to an accelerated gill ventilation and thus increased toxicants' uptake (Chovanec et al., 2003). Similarly to hardness, salinity reduces uptake and accumulation of metals by fish. When salinity decreases, free metal ions and toxicity increase. It has been reported that water acidification also affects metals accumulation rates by marine organisms. The effects can be direct following a damage of tissues which makes them more permeable to metals but at the same time, a competitive uptake of H^+ ions inhibiting metal absorption may occur. In algae, an increase of pH leads to the ionization of the membrane's functional groups making them negatively charged which leads to the attraction of cations. Conversely, a decrease of pH leads to either fast or slow protonation. During a fast protonation, anionic sites are neutralized while in the event of a slow one, metals are released and replaced by protons (INERIS, 2004). In fish, a lower pH leads to a higher accumulation of Cd, Pb and Cu but not Zn (Çoğun and Kargın, 2004; Jezierska and Witeska, 2006).

The accumulation of metals in marine organisms depends on multiple biotic factors such as size, age, sex, diet, metabolism and position in the trophic web of the species. In addition, the same abiotic factors that affect metal bioavailability, uptake and toxicity, affect also the accumulation of metals. Exposure of marine organisms to trace metals happens through direct and indirect or dietary pathways (Jakimska et al., 2011a). Direct pathways such as respiratory and dermal ones, are dependent from elements present in the dissolved phase (Jakimska et al., 2011a). However, feeding remains the major exposure route for metals accumulation in the organism (Jakimska et al., 2011b).

Bioaccumulation is expressed as the ratio between the concentration of the studied metal in the organism and its concentration in the environment (water/sediment) (Arnot and Gobas, 2006; Jayaprakash et al., 2015; Swaleh et al., 2016; Thomann et al., 1995). It considers the uptake by all possible routes occurring in the natural environment and thus including diet. It is calculated using the bioaccumulation factor (BAF) (Arnot and Gobas, 2006). In contrast, **bioconcentration** is the process by which an organism absorbs a chemical substance from the environment only through dermal and respiratory surfaces without including uptake through the diet. The degree to which bioconcentration

occurs is expressed as the bioconcentration factor (BCF) and can only be measured under controlled laboratory conditions in which dietary intake of the chemical is deliberately not included (Arnot and Gobas, 2006). In addition to bioaccumulation and bioconcentration, a process of biomagnification can also take place in the marine food chain. **Biomagnification** is a process in which the thermodynamic activity of the chemical in an organism exceeds that of its diet (Arnot and Gobas, 2006). The potential biomagnification of metals is influenced by metal assimilation or metal efflux rate or both. Bioaccumulation assessment is important in the scientific evaluation of risks that chemicals may pose to humans and the environment. The gender, reproductive status, life-stage or age, size, and lipid content of an organism can influence the BAF (Borga et al., 2005). Organisms with higher lipid contents have a greater capacity to store hydrophobic organic chemicals and therefore can exhibit a higher BAF. Lipid content has been shown to be a governing parameter in a contaminant's uptake as well as its body burden (Akcali and Kucuksezgin, 2011; Campbell et al., 2002). In fact, higher lipid content, allows a better assimilation of lipophilic metallic cations such as the lipophilic complexes of lead and copper (Campbell et al., 2002). Elements such as mercury which accumulates in fatty tissues mainly under the methylated form, is transferred from prey to predator through biomagnification, thus reaching the highest levels and BAFs in terminal predators (Graci et al., 2017; Harding et al., 2018). Trophic position is a key factor influencing the BAF, thus larger organisms have slower elimination rates and may feed at higher trophic levels (Arnot and Gobas, 2006).

BAF may also vary following the species and even the organs of a same individual (Jayaprakash et al., 2015). Variations are related to the different barriers that the contaminant has to cross, to the molecule size, and the physiology of the organ. Tissues that are involved in metal uptake, storage and excretion are capable of synthesizing metallothioneins (MTs) that are non-enzymatic proteins with thiol groups allowing binding to metals (Amiard et al., 2006) and that play an important role in homeostasis, cellular protection and detoxification (Amiard et al., 2006; Wang et al., 2014). MTs are induced by a variety of environmental and physiological factors with metals being the main inducers in aquatic vertebrates especially in organs like livers and kidneys, which explains the organotropism of trace metals and the higher capacity of these tissues for metal accumulation (Wang et al., 2014). This makes livers more sought than muscles in order to monitor chemical contamination in fish while the digestive glands in some gastropods have been shown to accumulate higher metal levels than the muscle tissue (Yüzereroğlu et al., 2010).

1.4. INTERSPECIFIC VARIATION OF METAL ACCUMULATION: BIOINDICATOR SPECIES

While it is known that element concentrations in water and sediment do not necessarily predict the levels of these contaminants in biota (Campanella et al., 2001; Wang et al., 2010), biomonitoring using species from different taxonomic groups with distinct ecological niches or feeding trophic levels enables a fuller assessment of the extent of any contamination (Diop et al., 2016; Pérez-Cadahía et al., 2004). For example, a macrophytic alga responds essentially to dissolved metal sources only, a suspension feeder like a mussel responds to metal sources in dissolved and suspended phases, and a deposit feeder responds to metal available in the sediment (Rainbow, 1995). Organisms uptake metals through several pathways and their accumulation is the result of competing processes between uptake and excretion that also occurs via different routes. Elimination routes include respiratory exchange, fecal egestion, metabolic transformation and to a lesser degree growth dilution. The latter may be considered a pseudo-elimination as it is a process where the chemical concentration is diluted as a function of growth rate of the organism (Arnot and Gobas, 2006; Canli and Atli, 2003). Moreover, the habitat of the species is a very important parameter to take into consideration when studying metal accumulation. For species living in the water column, uptake mainly occurs from water through dermal, respiratory and oral pathways. However, in benthic species, ingestion of contaminated sediments constitutes an important route of metal uptake. It has been shown that sediments can act both as sink and source for metal contaminants and can thus be used as geo-markers for potential pollution sources and conditions (Meng Chuan and Yunus, 2019; Rajeshkumar et al., 2018; Ribeiro et al., 2018). Therefore, species living close to the bottom are more exposed to contaminants than pelagic species (Kerambrun et al., 2012; Rajeshkumar et al., 2018).

Mollusks and crustaceans, and to a lesser extent, algae and fish have been shown to be excellent accumulators of pollutant and have made up the subject of many studies in the past decades (Bonanno et al., 2019b; Campanella et al., 2001; Circuncisão et al., 2018; Conti and Cecchetti, 2003; Cubadda et al., 2017; Kaimoussi et al., 2004; Kontas, 2012; Merciai et al., 2018; Rainbow and Phillips, 1993; Swaleh et al., 2016; Turkmen, 2012a). Aquatic invertebrates are known for their capacity to accumulate high levels of essential and non-essential metals in their tissues and still survive in polluted environments (Rainbow, 2002).

Understanding how metals are bioaccumulated in food webs is essential for elucidating the environmental behaviors of contaminants, evaluating their ecotoxicological risks and assessing their potential hazards to human health (Chen et al., 2000). That's why it is important to establish biomonitoring programs using species belonging to different taxonomic groups (Campanella et al., 2001; Chen et al., 2000; Rainbow, 1995a). Moreover, having knowledge of the accumulation patterns

and kinetics is important for the choice of a bioindicator to monitor certain elements. An element that is high in a species, could be low in another and this is mainly related to different accumulation pattern, metabolic availability and detoxification rates specific to each species and not necessarily to the levels in the surroundings. Consequently, the comparison of relative concentrations is not valid unless done intraspecifically (Rainbow, 2002). It is important to make a distinction between two different components related to accumulated metals, specifically accumulated metals that have been detoxified and metals that are metabolically available to play an essential role in vital functions. If the rate of uptake of a trace metal is faster than the rate of its excretion and detoxification combined, the level of metabolically available metal will exceed a threshold and toxic metals will start interfering with normal metabolic functions. Thus, the onset of toxicity can occur at any total concentration when the accumulation rate exceeds the rates of excretion and metabolic requirements (Rainbow, 2007; Rainbow and Luoma, 2011). For example, mollusks and crustaceans have the mechanisms to regulate essential elements like copper while they are not able to regulate arsenic, thus they accumulate this element and reflect well the levels of its contamination in the environment (Rainbow, 2002). The green alga *Ulva lactuca* widely used as a bioindicator, is known to accumulate essential and non-essential elements at similar concentrations (Bonanno et al., 2019b; Diop et al., 2016; Henriques et al., 2017; Kaimoussi et al., 2004; Kamala-Kannan et al., 2008). Moreover, mussels, bivalves and limpets are also widely used as bioindicators and are considered good integrators of the surrounding contaminations levels. Species used include *Patella caerulea*, *Mytilus galloprovincialis*, *Donax trunculus* and *Brachidontes variabilis* (Abdallah and Abdallah, 2008; Ayas et al., 2009; Campanella et al., 2001; Conti and Cecchetti, 2003; Nakhlé et al., 2006; Yüzereroğlu et al., 2010). Hence, a suitable bioindicator should fulfill certain criteria such as (i) having the capability to accumulate high levels of the targeted contaminant without showing adverse effects; (ii) living in a sessile lifestyle and thus representative of local pollution; (iii) being abundant and widely distributed to assess spatial and temporal variations and (iv) having a reasonable size allowing to sample an adequate tissue amount for chemical analysis (Zhou et al., 2008). The most commonly biomonitoring contaminants in the marine environment include pesticides, anti-foulants, pharmaceuticals and trace metals. These substances have been the subject of biomonitoring programs used in the context of monitoring networks such as the Water Framework and the Marine Strategy Framework Directives adopted in the European Union (EC, 2019a).

1.5. THE MARINE STRATEGY FRAMEWORK DIRECTIVE (MSFD)

The Marine Strategy Framework Directive (MSFD or DCSMM in french) was created and published on June 25th 2008. The directive establishes a community action plan in the field of marine

environment policy and leads each Member State to build a strategy in order to reach a Good Ecological Status (GES). It was transposed into the Environment Code: articles L 219-9 to L 219-18 and R 219-2 to R 219-17 and applies to zones under sovereignty or French jurisdiction, divided into 4 marine sub-regions: the English Channel and the North Sea, the Celtic Seas, the Bay of Biscay and the western Mediterranean Sea. Under the scientific and technical coordination of the Ifremer and the Marine Protected Areas Agency, a set of public establishments (ANSES, BRGM, CNRS, Ifremer, MNHN, SHOM) provide a scientific technical work to define the Good Environmental Status. There are 11 descriptors of good environmental status: Biological diversity, non-indigenous species, commercially exploited species, marine food webs, eutrophication, sea-floor integrity, hydrographical conditions, contaminants, health issues, marine litter and marine energy (MSFD, 2018). The 8th and 9th descriptors are linked and deal with the contamination levels in the biota and the health risks they may engender. The descriptor 8 ensures that the “concentrations of contaminants are at levels that do not give rise to pollution effects” while descriptor 9 aims at ensuring that “contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards” (EC, 2019a; MSFD, 2018). The MSFD program of measures coincides with that of the Water Framework Directive (WFD) that sets environmental targets and deadlines for improving the ecological and chemical status of surface water bodies as well as the quantitative and chemical status of underground water bodies (EC, 2019b). The main objectives of the MSFD are centered on the protection and the prevention of the deterioration of marine ecosystems as well as managing pressures from human activities in order to prevent and progressively eliminate pollution (Ifremer and AFD, 2018).

2. HEALTH RISKS RELATED TO THE CONSUMPTION OF CONTAMINATED SEAFOOD

Major routes of exposure to trace elements for humans are food, water and air. Individual metals have each different degrees of toxicity, that’s why food safety authorities around the world have established maximum allowable levels (MALs) and provisional tolerable weekly intakes for metals in foodstuffs. Marine organisms, especially those that can accumulate high levels of contaminants in their tissues, are a route of human exposure to dangerous compounds. That’s why the numerous health benefits provided by seafood may be compromised by the presence of toxic contaminants, of which metals and metalloids, that can be harmful to human health when consumed in high quantities (Pastorelli et al., 2012; Storelli and Barone, 2013). The toxicity of metals resides in their ability to alter normal biochemical functions by competing with some essential elements (Davidson et al., 2015). A non-essential metal may substitute for an essential one in a metabolic pathway which may block further reactions (Rainbow, 1985). All elements are toxic starting from a certain threshold; even essential

elements when in excess, may become toxic and cause adverse effects. Figure 1-3 shows the relationship between essential and toxic elements in terms of health response.

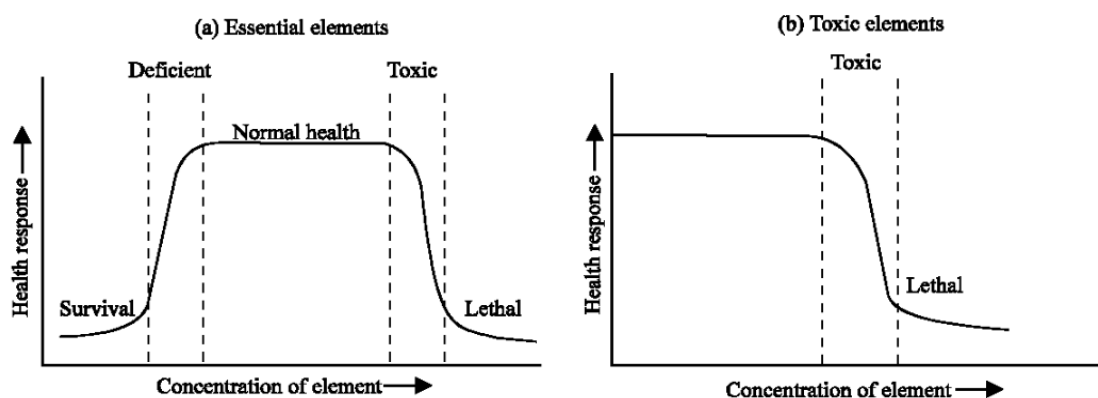


Figure 1-3 Relationship between elemental concentration and health response (Ansari et al., 2004)

2.1. REGULATED TOXIC METALS IN SEAFOOD

As, Cd, Hg and Pb are classified as ones of the most toxic metals and thus their presence in the food chain raises an issue of concern. Seafood tend to accumulate high levels of As, Cd, Hg and Pb, thus, these elements were taken into consideration for health risk assessment related to seafood consumption. Therefore, regulatory limits established by different health authorities and associated specifications are summarized in table 1-2.

Table 1-2 Maximum allowable levels (MALs, mg kg⁻¹ wet weight) of toxic metals in fish, crustaceans and mollusks emitted by different guidelines

Metal	Regulatory body	Country	MALs	Specifications	Reference
As	FAO 1983	Worldwide	0.1 - 4		(FAO, 1983)
	South African Department of Health (DOH)	Africa	3.0	Fish and processed meat	(DOH, 2004)
Cd	European Commission, regulation N° 629/2008	Europe	0.05	Fish meat except for some predatory species	(EC, 2008)
			0.1	Some predatory fish including tuna and horse mackerel	
		Europe	0.3	Muscle meat of swordfish	

	European commission regulation N° 1881/2006		0.5	Crustaceans, with the exception of brown crab meat	(EC, 2006)
			1.0	Bivalve mollusks and cephalopods (without viscera)	
	FAO. Heavy Metals Regulations Legal Notice No 66/2003	Worldwide	0.05	Predatory species including horse mackerel	(FAO, 2003)
Hg	FAO. Heavy Metals Regulations Legal Notice No 66/2003	Worldwide	1.0	Predatory fish including tuna species	(FAO, 2003)
	European Commission, regulation N° 629/2008	Europe	1.0	Predatory fish species including tuna species and swordfish	(EC, 2008)
	FAO. Heavy Metals Regulations Legal Notice No 66/2003		0.5	Edible parts of fishery products except some predatory fish	(FAO, 2003)
	European commission regulation N° 1881/2006	Europe	0.5	Fishery products and fish meat, excluding some predatory species, crustaceans 'muscular flesh of appendages and abdomen	(EC, 2006)
	DOH	Africa	1.0	Predatory fish including swordfish	(DOH, 2004)
			0.5	All other fish and processed fish	
Pb			0.3	Fish meat	
	European commission regulation N° 1881/2006		0.5	Crustaceans: muscle flesh of appendages and abdomen	(EC, 2006)
			1.0	Cephalopods (without viscera)	
			1.5	Bivalve mollusks	
	FAO. Heavy Metals Regulations Legal Notice No 66/2003	Worldwide	0.4	Fish meat	(FAO, 2003)
			0.5	Crustaceans (excluding brown meat of crabs)	
		1	Bivalve mollusks and cephalopods		
	DOH	Africa	0.5	Fish and processed fish	(DOH, 2004)

Arsenic

Arsenic (As), is a widely distributed element in nature due to environmental sources. It is a ubiquitous metalloid present in rocks, soil and natural ground water (EFSA CONTAM Panel, 2009), and can be measured at low concentrations in almost all environmental matrices, but like most contaminants, its levels are being increased due to anthropogenic activities. Anthropogenic sources include smelting activities, glass manufacturing, use of arsenic pesticides, herbicides, fungicides and wood preservatives that constitute a major use (Jaishankar et al., 2014; Tchounwou et al., 2012). As is classified chemically as a metalloid, having both properties of a metal and a nonmetal; however, it is frequently referred to as a metal (ATSDR, 2007). As enters the marine environment through natural and anthropogenic sources. Humans may be exposed to As through several sources including water, soil and air but these sources stay negligible when compared to the average intake brought by food and drinking water (EFSA, 2014a).

Arsenic has a complex chemistry and can be present under various organic and inorganic forms with different degrees of toxicity. Organic arsenic species such as arsenobetaine and different arsenosugars are the most common forms in seafood while inorganic As (iAs), predominates in food from terrestrial origins such as rice (EFSA CONTAM Panel, 2009). Human exposure to As is an issue of concern as it causes several adverse health effects. Inorganic arsenic compounds (iAs) are considered to be more toxic compared to organic ones and seafood including finfish, shellfish, and seaweed constitute great contributors to As exposure in many populations (Feldmann and Krupp, 2011). However, some recent findings have shown that some forms of organic As and their metabolite display cytotoxicity in cell cultures (Leffers et al., 2013; Meyer et al., 2015, 2014; Taylor et al., 2017).

Based on sufficient evidence of carcinogenicity, the International Agency for Research on Cancer (IARC), classified As and iAs compounds as carcinogenic to humans (EFSA CONTAM Panel, 2009). Additionally, As has been shown to be genotoxic as it can inhibit DNA repair and induce chromosomal aberration (Faita et al., 2013; Patra et al., 2004). So far occurrence data of As in the framework of food control, are still being reported as total As. Most of the As present in seafood is in its organic form, consequently a risk assessment that does not take into account different As species, would lead to an over estimation of the health risks related to As exposure (EFSA, 2014a). Nonetheless, regulatory limits of 3.0 mg kg^{-1} were set by the African Department of Health for fish and processed fish (Table 1-2). Currently there are no Maximum Allowable Levels (MALs) established for As in food at a European level. In 2010, the joint WHO/FAO Expert Committee on Food Additives (JECFA) withdrew the provisional tolerable weekly intake (PTWI) of $15 \text{ } \mu\text{g kg}^{-1}$ per body weight (bw).

Cadmium

Cadmium (Cd) is a metal that is widely distributed in the earth crust. The highest levels of Cd, are accumulated in sedimentary rocks and marine phosphates. Natural sources of Cd include volcanic eruptions and rock weathering. Cd is also a product of anthropogenic activities such as smelting, burning fossil fuel, incineration of waste material and the use of certain fertilizers, mainly phosphate (EFSA, 2009). The production of alloys, pigments and batteries are among the major industrial applications of Cd. Cd enters the marine environment through atmospheric depositions as well as waste water and industrial discharges (Jaishankar et al., 2014). This element is commonly found as inorganic compounds (Cd^{2+}) and is mainly present as a complex with chloride in the marine environment.

Cd can readily cross biological membranes and then inside the cells, can bind to ligands and form more stable complexes. It is absorbed into fish body and eliminated at a very slow rate which causes bioaccumulation (EFSA, 2009), thus making fish a major source of Cd (Castro-González and Méndez-Armenta, 2008). However, high salinity in seawater, increases the formation of Cd complexes making it less bioavailable for fish (Ray, 1986).

Human exposure to Cd can occur through inhalation, smoking and ingestion of contaminated food. In humans, Cd absorption occurs in a similar process of that involved in essential elements uptake such as Fe and it is enhanced by calcium deficiency and a low protein diet. Once in the bloodstream, Cd is distributed essentially to the liver and kidneys that are the principal organs of long term storage in the body (Liu et al., 2008). Cd has been shown to affect several organs and tissues such as kidneys, heart, skeletal system, placenta, brain and the central nervous system. The International Agency for Research on Cancer (IARC) classified Cd and its compounds as carcinogenic to humans (EFSA, 2009).

The European Commission has set a PTWI of $7 \mu\text{g kg}^{-1} \text{bw}$ (EFSA, 2009) and species-specific MAL for Cd in different foodstuffs including seafood (Table 1-2). The FAO and the South African Department of health have also set MALs for different species of fishery products. However, in its 73rd report, the JEFCA withdrew the previous PTWI as it was considered no longer appropriate. The committee set a PTMI (Provisional tolerable monthly intake) of $25 \mu\text{g kg}^{-1} \text{bw}$ for Cd (JEFCA, 2011).

Mercury

Mercury (Hg) is a widespread naturally occurring element that is liquid at ambient temperature and pressure. It can be present in the environment most commonly as elemental mercury (Hg^0), inorganic mercury (Hg II) and organic mercury (MeHg) which is the species with the most toxicological concern (Ogrinc et al., 2007; Xia et al., 2007). Mercury is among the most toxic pollutants and is classified

among one of the priority hazard substances by the ATSDR (Agency for toxic substances and Disease Registry) (ATSDR, 1999) and can be released into environment from both natural and anthropogenic sources. This element is largely used in thermometers, barometers, fluorescent lamps and as catalyst. Anthropogenic sources contribute to about 50 – 75% of the yearly input originating mainly from electricity generation, incinerators, waste disposal and industrial minerals (Pacyna et al., 2010; Sundseth et al., 2017). Atmospheric inputs represent the main source of oceanic Hg that is emitted under its gaseous form (Hg^0) (Mason and Sheu, 2002). Other sources include wastewater, surface runoff and rivers. MeHg is the most common form of organic Hg; it is soluble, mobile and quickly enters the aquatic food chain. This form is accumulated to a greater extent in biological tissues than the inorganic forms of Hg (ATSDR, 1999; Harding et al., 2018) and is considered one of the most bioconcentrated trace metals in the marine organisms (Sundseth et al., 2017).

More than 90% of MeHg is absorbed through the gastrointestinal tract and is then rapidly transferred into the bloodstream by forming complexes with thiol groups (Ceccatelli et al., 2010). Consequently, its presence in human food and particularly fishery products is an issue of concern (Maia et al., 2019). Exposure to Hg has been known to be involved in oxidative stress and has been associated to genotoxicity and carcinogenesis (Telahigue et al., 2018). Its toxicity has been known since the 1950's after the Minamata poisoning in Japan that was due to the ingestion of large amounts of fish and shellfish polluted by industrial effluents charged in MeHg resulting in a large number of victims and several hundreds of deaths. Two other massive contamination also occurred in Niigata (Japan) and in Iraq in the 1960's (Eto et al., 2010).

In order to limit consumers' dietary exposure to MeHg, the European Commission (EC) has set maximum permissible levels of Hg in fish (0.50 and 1 mg kg^{-1} particularly for predatory fish) (EFSA, 2014b). Moreover, in 2003, the Joint Committee on Food Additives (JECFA) established a Provisional Tolerable Weekly Intake (PTWI) of 1.6 $\mu\text{g kg}^{-1}$ body weight (bw) for MeHg and 5 $\mu\text{g kg}^{-1}$ bw for total Hg (THg). In 2011, the JECFA emitted a new report where the previous PTWI for THg was withdrawn by the Committee and replaced by a PTWI for inorganic mercury (iHg) of 4 $\mu\text{g kg}^{-1}$ bw (JECFA, 2011). Afterwards, in line with the JECFA, the European Food Safety Authority (EFSA), established a Tolerable Weekly Intake (TWI) of 1.3 $\mu\text{g kg}^{-1}$ bw for MeHg (EFSA, 2012). So far, there is no regulation in terms of MeHg in seafood or other type of foodstuffs, although this species poses the main threat in terms of Hg toxicity. The regulations emitted by different guidelines, consider MALs related to total mercury (Table 1-2).

Lead

Lead (Pb) is a naturally occurring element present at low concentrations in the earth crust, but like all elements, anthropogenic inputs contribute to the release of high levels. Pb is essentially present in mines as PbS where it is also associated to other elements like zinc, antimony, arsenic, copper and silver. Anthropogenic sources of Pb include fossil fuels burning and mining. Lead is also used in the production of lead-acid batteries, ammunitions, metal products such as solder and pipes in addition to house paint and shield devices for X-rays (Jaishankar et al., 2014; Tchounwou et al., 2004). The atmosphere is the main source of Pb deposition in the marine environment. Pb enters the marine environment from urban sources including sewage systems, waste management and households. Leaded petrol was a predominant source of atmospheric Pb until the 70's, before regulations limiting the use of Pb in gasoline were adopted. An important reduction was obvious in seawater lead concentrations during the phase-out leaded gasoline (Reuer and Weiss, 2002; von Storch et al., 2003). In the marine environment, the bioavailability of Pb is increased by the presence of dissolved organic matter (Ebdon et al., 2001). Once in the marine environment, Pb is easily absorbed by fish and accumulates in the gills, kidneys, liver and scales (Akan et al., 2012).

Accumulation of Pb occurs mainly in kidneys followed by the liver and other soft tissues such as the heart and brain but the skeleton remains the major accumulator of Pb in the human body (Flora et al., 2006). The toxicity of Pb depends on its form as organic lead compounds are more toxic than the inorganic ones (Ebdon et al., 2001; Goyer et al., 2004). The marine environment is a substantial source of exposure to Pb in seafood and in humans through consumption (Bosch et al., 2016). Excess exposure to Pb may result in neurological problems, renal failure, hypertension and cancer. High lead exposure has been associated with a decreased sperm count in men and spontaneous abortions in women. Moreover, acute exposure induces brain and kidney damage in addition to gastrointestinal diseases while chronic exposure is associated with adverse effects on the blood, the central nervous system, kidneys and vitamin D metabolism (Andrews et al., 1994; EFSA, 2010; Kaul et al., 1999).

The JEFCA set a PTWI of 50 $\mu\text{g kg}^{-1}$ bw. This value was then withdrawn and replaced by a PTWI of 25 $\mu\text{g kg}^{-1}$ bw in 1993. Afterwards, in 2010, the EFSA CONTAM Panel (Panel on Contaminants in the Food chain) concluded that the PTWI of 25 $\mu\text{g kg}^{-1}$ bw is no longer appropriate and therefore, a margin of exposure approach (MOE¹) was applied to risk characterization because there was no

¹ MOE is calculated based on the BMDL intake value and dietary exposure estimates for the respective endpoints including nephrotoxicity and developmental neurotoxicity. (BMDL: The benchmark dose (BMD) is defined as the dose that corresponds to a specific change in an adverse response compared to the response in unexposed subjects, and the lower 95% confidence limit is termed the benchmark dose level (BMDL) (Dakeishi et al., 2006))

evidence for a threshold related to a number of critical endpoints (EFSA, 2010). However, MALs have been established for Pb in fishery products by the EC and the Food and Agriculture Organization of the United States (FAO) (Table 1-2).

3. TRACE METALS IN THE MEDITERRANEAN SEA: AN OVERVIEW

The Mediterranean Sea is the largest semi-enclosed sea and one of the most affected by pollution. About 300 million people inhabit the Mediterranean basin or discharge their end-product into the Mediterranean and about 100 million tourists “use and live” Mediterranean waters during the summer. All these factors make the Mediterranean Sea particularly susceptible to the impact of most pollutants introduced anthropogenically (Bianchi and Morri, 2000). Due to its relatively reduced dimensions, the contaminants discharged in the Mediterranean will reach a saturation point more quickly than in the oceans (Richir, 2016). Moreover, the almost total absence of tide prevents natural phenomena of depuration and dilution of pollutants like in oceans. The Mediterranean circulation shows deficiency in the movement of deep water masses and surface currents “turn in circles” in a nearly closed basin making it more rapidly responsive to environmental disturbances (Millot and Taupier-Letage, 2005; Robinson et al., 2001).

In the Mediterranean, land-based sources of pollution related to urbanization and industrial activities along with inadequate regulatory mechanisms are considered one of the most important issues. Coastal-based impacts have been shown to be the most pronounced in parts of the Mediterranean Basin with the highest population densities, while the impact of shipping traffic and other ocean-based pollution such as deposition of trace metals and inorganic nitrogen were mainly prevalent in open water (Figure 1-4) (Coll et al., 2012). All around the Mediterranean basin, coastal urbanization takes over and coastal areas host chemical and mining industries that produce large amounts of industrial wastes such as hazardous substances and trace metals (EEA, 2006). The high levels of trace elements currently measured in the Mediterranean indicate non-stationary geochemical cycles which result from an increase of external inputs (Richir, 2016).

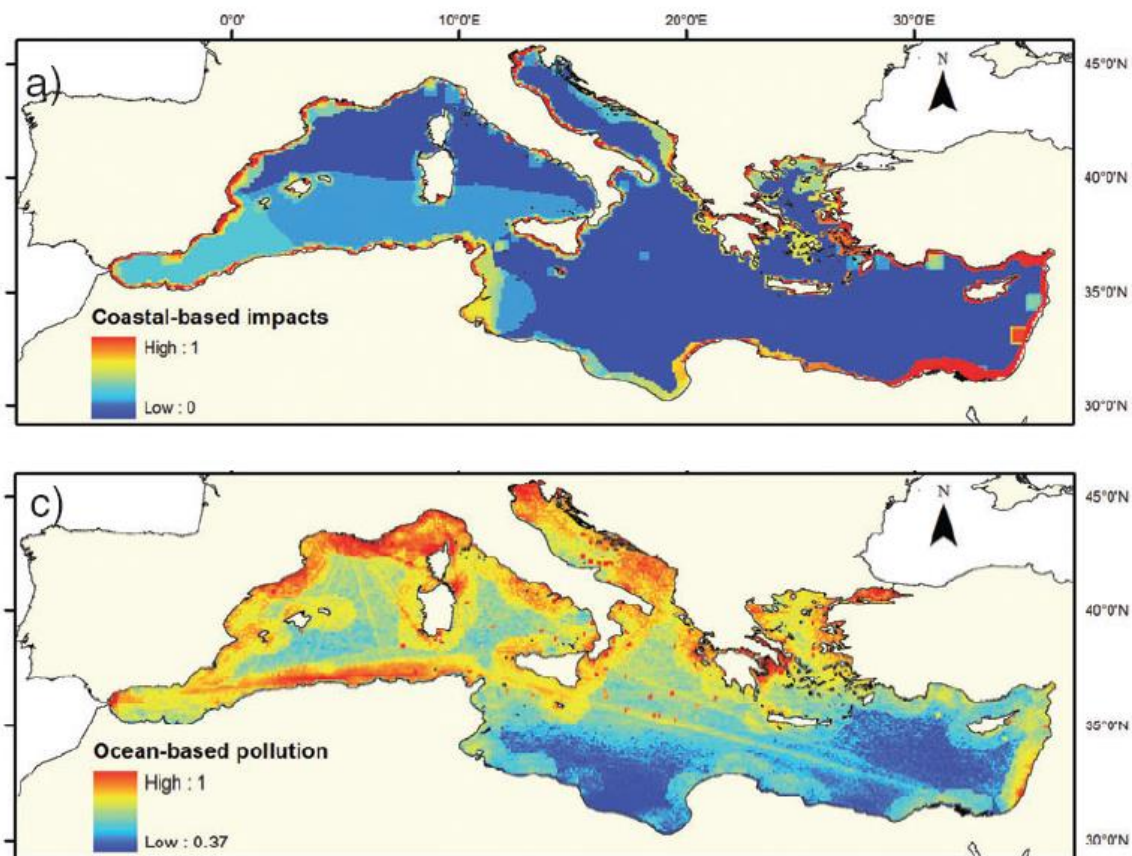


Figure 1-4 Human threats with potential impact on marine biodiversity in the Mediterranean Sea: (a) coastal-based impacts, (cumulative effects from inorganic and organic coastal pollution, nutrient runoff and hypoxia, aquaculture activities and the presence of invasive species.) and (c) ocean-based pollution (cumulative effects from shipments of toxic substances (e.g. toxic waste, radioactive waste and fertilizers) and other ocean-based pollution from shipping traffic, deposition of heavy metals and inorganic nitrogen. (Coll et al., 2012).

In the southern and eastern Mediterranean countries, the major problems revolve around poor wastewater treatment and chemicals' management strategies in addition to the lack of law enforcement compared to northern Mediterranean countries where necessary measures are being implemented in order to reduce environmental problems related to wastewater. This situation shows the need for necessary technical, financial and human resources in southern and northern Mediterranean countries in order to comply with regional regulation (EEA, 2006; Micheli et al., 2013).

After the implementation of environmental measures such as banning leaded gasoline and anti-fouling paints in addition to mercury regulations, the levels of pollutants have decreased significantly in the most impacted areas of the Mediterranean. This was mainly observed in the Western part of the Mediterranean. However, despite those regulations, diffuse sources releasing emerging chemical

contaminants (Pharmaceuticals, personal care products) still exist in the Mediterranean Sea (UN/MAP, 2017).

3.1. TOXIC TRACE METALS IN SEAFOOD: ARE SEAFOOD FROM THE MEDITERRANEAN SAFE TO EAT?

Several studies dealing with the levels of trace elements in fishery products have been conducted along the Mediterranean basin and most of them reported levels below the MALs established by the EC (Abdallah, 2008; Aksit et al., 2013; Masoud et al., 2007). However, some other studies showed levels of these contaminants that exceeded the MALs mainly in Italy (Table 1-3). All the species where levels of toxic metals were exceeded are predators that are naturally known to accumulate higher levels. In a recent report about the state of the Mediterranean, an assessment of Cd and total Hg indicated acceptable environmental conditions as their levels were lower than the EC's MALs and also stated that the situation in terms of metallic pollution is witnessing an improvement as contaminants accumulate and persist more in the sediments. However, levels of Pb at 8% of the stations were above the EC criteria mainly in Italy. Note that Spain and Italy are known to be hotspots for metal pollution (Figure 1-5). Overall, the assessment of trace metals in fish showed acceptable results as there were none over the ECs guidelines. Both Western and Eastern areas showed values even below the BAC² (Background Assessment Criteria) indicating naturally occurring levels of metals in fish (Figure 1-5) (UN/MAP, 2017)

Table 1-3 Cases where seafood content in As, Cd, Hg et Pb exceeded the MALs

Metal > MAL	Species	Concentration (mg kg ⁻¹ ww)	Country	Reference
As	Red mullet	59.91 ± 9.49	Italy	(Perugini et al., 2014)
	European hake	38.70 ± 7.69		
	Blue whiting	35.30 ± 2.82		
	Atlantic Mackerel	30.76 ± 9.95	Jordan	(Ababneh and Al-Momani, 2013)
	Canned tuna	0.06		
	Sardine	8.08 ± 2.43		
	Hake	10.03 ± 0.82		
Hake	23.30 ± 3.56	Croatia	(Jureša and Blanuša, 2003)	
Cd	European conger eel	0.11 ± 0.01	Italy	(Storelli and Barone, 2013)
	Blackbellied angler	0.09 ± 0.02		
	Rosefish	0.10 ± 0.02		
	Brown Ray	0.08 ± 0.04		
	Red mullet	0.08 ± 0.04		

	European pilchard	0.045 ± 0.020	Sicily	(Copat et al., 2012)
	Red mullet	0.084 ± 0.069	Sicily	(Pastorelli et al., 2012)
	Louvar	0.08±0.01	Spain	(Herrerros et al., 2008)
	Grey Mullet	0.10 ± 0.4	Turkey	(Filazi et al., 2003)
	Blue whiting	0.10 ± 0.06		(Perugini et al., 2014)
	European hake	0.05 ± 0.04	Italy	
	Red mullet	0.07 ± 0.05		
Pb	European anchovy	0.32 ± 0.22	Sicily	(Copat et al., 2012)
	European conger eel	1.14 ± 0.46		
	Rosefish	1.04 ± 0.56		
Hg	Brown Ray	1.09 ± 0.39	Italy	(Storelli and Barone, 2013)
	Blackbellied angler	0.96 ± 0.32		
	Red mullet	0.43 ± 0.55		
	Swordfish	0.93 ± 0.07		
	Louvar	0.99 ± 0.06	Spain	(Herrerros et al., 2008)

As a summary, with the exceptions of some identified hotspots, studies dealing with health risk assessment due to the consumption of seafood from the Mediterranean, most often report values that comply with sanitary guidelines established by the EC and show no potential adverse health effects. Moreover, the situation seems to be rather improving with the implementation of environmental measures especially in the Western Mediterranean. In a study made in the Gulf of Catania, Italy, bioaccumulation levels of Hg, Mn, Se and V showed a decrease when compared to data taken previously (Copat et al., 2018). The authors compared datasets from 2012 and 2018 and reported a decreased risk resulting from seafood consumption (THQ³ decreased from 1.1 in 2012 to 0.49 in 2017).

³ Total Hazard Quotient indicates the ratio between exposure and a reference dose (US EPA, 2000)

Regional cadmium assessment in bivalves and fish



Regional lead assessment in bivalves and fish



Regional mercury assessment in bivalves and fish



Figure 1-5 Regional cadmium, lead and mercury levels (2009-2015) assessment against European Commission (EC) criteria in bivalve sp. (*Mytilusgallo provincialis*, *Ruditapes decussates*, *Donax trunculus* and *Mactra corralina*) and in fish sp. (*Mullus barbatus*) for the Mediterranean Sea; BAC: Background Assessment Criteria (UN/MAP, 2017)

Nevertheless, reports dealing with the state of the Mediterranean often identify data gaps in the southern and eastern Mediterranean countries at almost all levels and highlight the need for consistent and reliable investigation programs for these countries (EEA, 2006; UN/MAP, 2017). Figure 1-5 shows data gathered for the regional assessment of Cd, Pb and Hg levels in bivalves and fish (2009-2015) against EC criteria from the following countries: Albania (2005-2006), Bosnia and Herzegovina (2006-2008) Croatia (2009, 2011-2014), Cyprus (1999-2015), Egypt (2009, 2010), France (2009, 2010), Greece (2004-2006), Israel (2001-2012), Montenegro (2008-2012, 2014-2015), Morocco (2006,2007), Slovenia (1999-2013, 2015), Tunisia (2002-2013) and Turkey (2005-2009, 2011, 2013). As can be seen, data from the eastern and southern Mediterranean are scarce highlighting the data gaps and the need for improved monitoring and assessment schemes that will allow better environmental management. The presence of biomonitoring networks such as the MSFD and the WFD cited above, and that apply in the western part of the Mediterranean, helps maintaining a good ecological status of the marine environment. Thus, a better environmental engagement leading to a reliable monitoring network requires an improve of socio-economic capacity in the first place especially in developing countries (EEA, 2006).

4. OBJECTIVES OF THE STUDY

Our study consists in evaluating the level of contamination by metallic trace elements in different marine organisms collected from the Lebanese coast where there is substantial national lack of data concerning the levels of trace elements in fishery products and the risks they may engender through consumption, especially in the shadow of growing environmental pressures.

The **first objective** of this study was to investigate the levels in addition to the spatial and seasonal (wet and dry season) variation of 20 trace metallic elements in six marine species, representative of the Lebanese coastal waters and belonging to different positions in the trophic chain. The species chosen were the green algae *Ulva lactuca*, the spiny oyster *Spondylus spinosus*, the kuruma shrimp *Marsupenaeus japonicus* and three fish species with different habitats and dietary habits: the marbled spinefoot, *Siganus rivulatus*; the striped seabream, *Lithognathus mormyrus* and the round herring *Etrumeus teres*. Samples were taken from different sites with different levels and types of pollution and trace metals were measured in soft tissues of shrimps and bivalves and in muscle and liver of fish species, as livers are the main accumulating organ that reflects the environmental conditions.

The **second objective** of the present study was to evaluate and compare the health risks related to the consumption of local fishery products in terms of As, Cd, Hg and Pb. The bivalve, shrimp and three fish species cited above are commonly consumed by the Lebanese population and thus were used for health risk assessment. Fish muscles and bivalves and shrimps' soft tissues were used for this purpose. The human health risk of each contaminant was assessed using two different methods, namely target hazard quotients (THQs) and estimated weekly intake (EWI).

The **third objective** of the study was to optimize and validate a routine analysis approach for the speciation of Hg in seafood. Metals such as mercury (Hg) can take different forms, with methylmercury (MeHg) being the most toxic for consumption and the main route for human exposure to MeHg is the consumption of seafood. So far there are no regulations in terms of MeHg in seafood or other foodstuffs despite it being the main threat in Hg toxicity. This is due to challenges in analytical chemistry as speciation analysis of MeHg has not become a routine approach yet. Thus, a routine analytical approach for MeHg speciation analysis by HPLC-ICP-MS was developed and validated based on the accuracy profile and was used to determine levels of MeHg in the samples.

5. OUTLINE OF THE STUDY

The thesis consists of five chapters of which chapters 3 and 4 are presented under the form of scientific publications.

Chapter 1 presents an overview about the metals and trace elements, their sources and fates in the marine environment in addition to their uptake and accumulation in marine organisms. Moreover, health risk related to the consumption of contaminated seafood was presented along with the regulations of toxic elements in seafood.

Chapter 2 presents the study zone in addition to strategies, protocols and methodologies adopted in this study. It involves a presentation of the study zone in terms of topography, climate and sources of environmental pressures in addition to sample collection, preparation, analysis and data treatment.

Chapter 3 deals with the contamination level of marine organisms from the Lebanese coast. Levels of 20 trace metallic elements were determined and compared in muscles and livers of three fish species with different habitats and diets. Moreover, metallic elements and bioaccumulation was assessed in the alga, the shrimp and the bivalve. Results were discussed in terms of interspecific, seasonal and spatial variability and compared to other regions in the Mediterranean.

Chapter 4 deals with the sanitary aspect of local fishery products in addition to canned tuna in terms of levels of contamination with toxic trace metals (As, Cd, Hg and Pb). Risk assessment was conducted in order to evaluate the degree of exposure of the population to regulated elements present in these matrices. Moreover, the procedure of optimization of a routine approach for Hg speciation was developed. Speciation analysis of Hg was conducted in a selection of samples that displayed relatively higher levels of total Hg.

Chapter 5 discusses the results obtained in this study and the perspectives for future work.

CHAPTER 2 – STRATEGIES, PROTOCOLS AND METHODOLOGIES

Chapter 2 - STRATEGIES, PROTOCOLS AND METHODOLOGIES

This chapter gives an overview of the Lebanese environment in terms of geography, climate and hydrodynamics as well as anthropogenic activities along the Lebanese coast. The studied sites and selected species are described along with the methods used for the analysis of trace elements.

1. PRESENTATION OF THE STUDY AREA: GEOGRAPHICAL AND CLIMATIC CONTEXT

Lebanon, the cross road of many ancient civilizations, is located in the eastern Mediterranean basin. The country extends over a surface of 10452 km² and is home to more than 6 million inhabitants in 2019 (Worldometers, 2019). Lebanon is located between latitudes 33° 03' 38'' and 34° 41' 35'' North and longitudes 35° 06' 22'' and 36° 37' 25'' East. It is bordered by the Levantine basin in the West, by Syria in the North and East and by occupied Palestine in the South. Lebanon is a mountainous country with rugged topography. Two mountain chains, separated by a fertile valley, dominate its geography and divide the country into four

geographical zones from West to East (FAO, 2009; Walley, 2001) (Figure 2-1):

- A narrow coastal zone (7 km): widens in the North and South resulting respectively in two coastal plains located in Aakar and Tyr.
- Mount Lebanon: the western mountain chain which is more elevated in the North of the country. "Kornet el Saouda" with an elevation of 3008 m, constitutes its highest peak.
- The Beqaa Valley: a fertile land that extends for 120 km and is located between the two mountain chains of Mount Lebanon and Anti-Lebanon at an average altitude of 900 m.
- Anti-Lebanon mountains: divided into two main massifs that are "Talaat Moussa" (2629 m) in the North and "Jabal al Sheikh" (2814 m) in the South. The ridges of this chain constitute the borders with Syria.

The littoral zone

Bordering the eastern shore of the Mediterranean Sea, the Lebanese coast is about 225 km long with SSW-NNE as a general orientation. The coastline extends from Arida (in the North) to Ras el Nakoura (in the south) and is not very indented (Abboud Abi-Saab, 2012; Skaff, 2014). It is characterized by a succession of rocky promontories of which Beirut's is the most important. Despite the mountainous nature of the country, real cliffs are rare and do not exceed 30 m (Nakhlé, 2003).

The Lebanese littoral zone borders the warmest part of the Mediterranean (Abi-Ghanem, 2008). It is separated from the Arab-Syrian desert by a double mountainous partition (Nakhlé, 2003) and is largely

open to the winds and swells of the western sector The coast rises sharply, especially in the center (Faour et al., 2004) making land-sea contact quite brutal. As a result, the coastal plain is reduced to a narrow strip of land except for the plain of Aakar in the North and Tyre in the South. This abrupt termination of the mountain towards the sea is a major and original geographical phenomenon, characteristic of the Lebanese coast (Darwish and Faour, 2008).

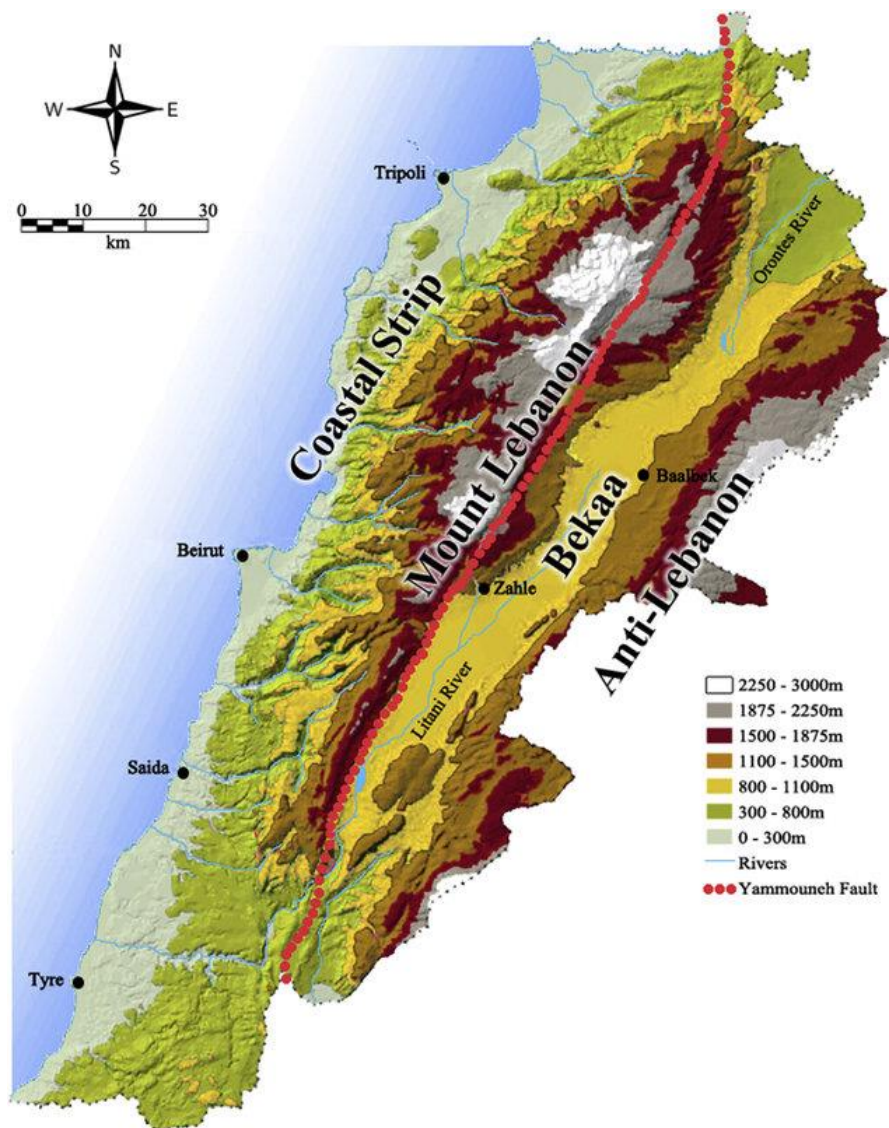


Figure 2-1 Geomorphology of Lebanon (El Zaatari, 2018)

beds are rugged and show sub marine canyons that can reach up to 1500 m depth at only 8 km from the coast (Elias, 2006; Nakhlé, 2003). The most important canyons are found in the South of Lebanon and are supplied by rivers that drain the South of Mount Lebanon region (Abi-Ghanem, 2008).

Climatic conditions

The geographical situation of the Lebanese territory denotes a northern temperate Mediterranean climate divided into two main seasons: a rainy mild winter and a hot dry summer. The two months December and January, witness the lowest temperatures, with a daily of -4°C in the mountains and 7°C in coastal zones whereas July and August are the months with the highest temperatures as the daily average reaches 28°C in the mountains and 33°C in the coastal cities. Lebanon is known as the rainiest country in the Middle East with an annual rainfall average varying between 700 and 1000 mm on the

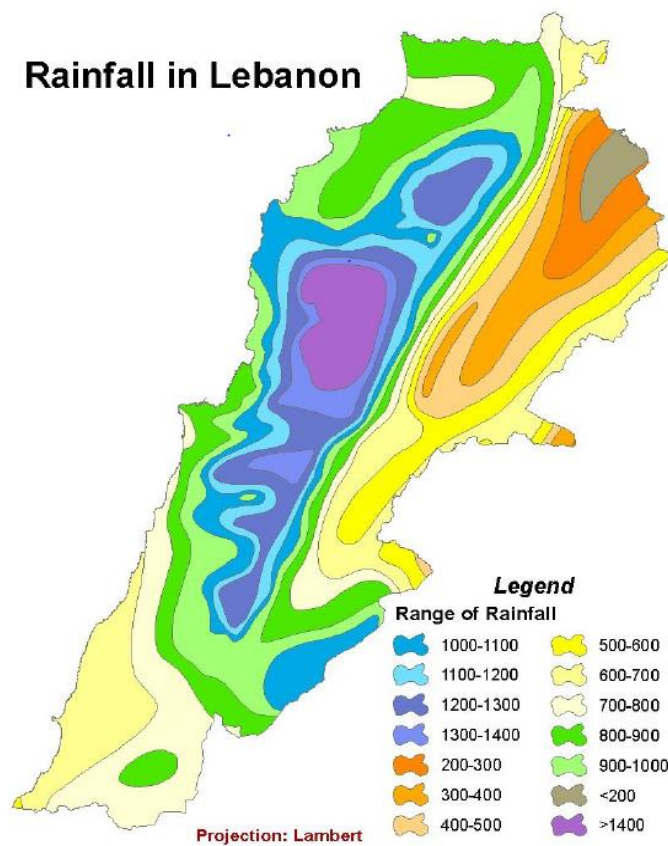


Figure 2-2 Rainfall patterns in Lebanon

coastal zone and between 1500 and 2000 mm on the mountains. Rainfall decreases from North to South (Figure 2-2) and 80 to 90 % of the annual rainfall occurs between November and March and during 80 to 90 days mainly between the months of October and April (CAS, 2008; FAO, 2016).

Currents and water circulation in the Mediterranean

The Atlantic water constitutes the main source of water in the Mediterranean. With a relatively lower density, the Atlantic water travels through the Gibraltar Strait (GS) on the surface passing by the North

African coast, Egypt's Northern coast till it gets to the Easternmost part of the Mediterranean along the Lebanese, Palestinian and Syrian coasts and then continues to the West along the European coasts (Millot and Taupier-Letage, 2005) (Figure 2-3). It is assumed that the eastward flow of Atlantic surface waters that are poor with nutrients, is compensated by a deep counter current of Mediterranean waters that are nutrient-rich (Huertas et al., 2012). The ultraoligotrophic conditions in the Eastern Mediterranean have been attributed to hydrological conditions such as the circulation patterns present through the straits of Sicily and Gibraltar and the higher winter convection in the western basin (Huertas et al., 2012; Moutin et al., 2012). Deep water convection is one of the major processes that is a substantial source of nutrients for the surface water in the North western Mediterranean (Macias et al., 2018). The direction of the current is function of the atmospheric pressure in the western Mediterranean. The gradient of atmospheric pressure between the eastern and western Mediterranean basin, determines the flow rate of water in the direction of the eastern basin and as the atmospheric pressure is always lower in the eastern Mediterranean, a current towards the west is mostly favoured (Millot and Taupier-Letage, 2005). In the Levantine Basin, eddies that are formed, affect salinity and temperature. When they get close to a certain zone, they contribute to pollutants' dispersion. Along the Lebanese coast, a predominance of a northward flowing current is reported from December to July. In the region located in the North of Beirut, particularly the bay of Jounieh, currents go from east to west following the regional topography (Ghorra, 1983). Winds are one of the most important factors that affect the dispersion of contaminants and suspended matter. In Lebanon, south western winds are dominant along the coast all year long. Northern winds come in a second place in terms of frequency, and play an important role in moving water masses and industrial effluents (Abboud-Abi Saab et al., 2004a; Fakhri and Romano, 2005; Taupier-Letage et al., 2003).

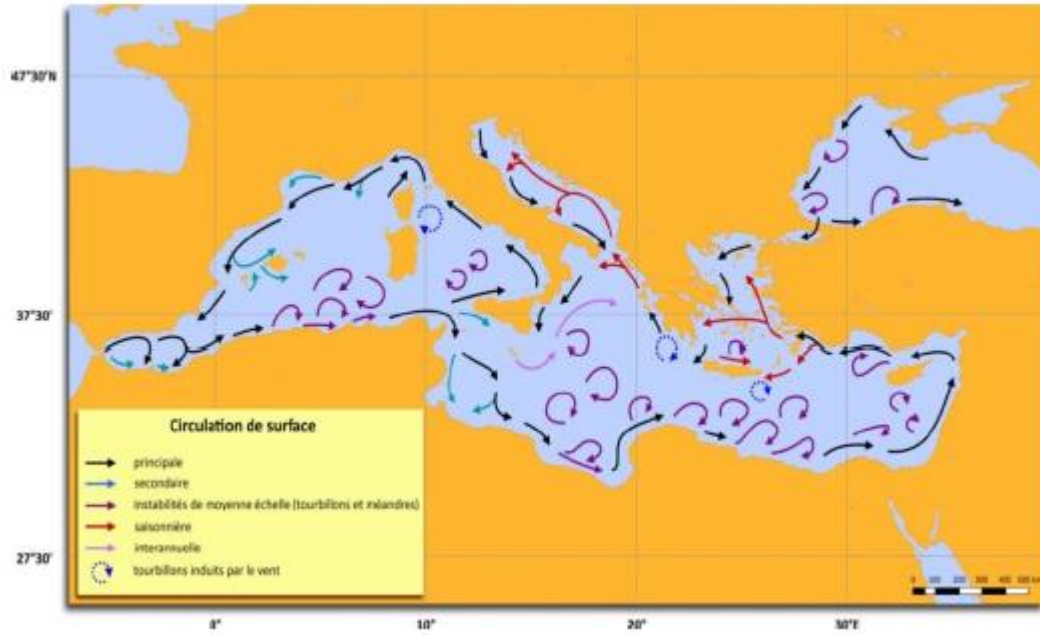


Figure 2-3 General circulation of water in the Mediterranean (Millot and Taupier-Letage, 2005)

Environmental status of the Lebanese Coast

The Lebanese coastal area, much like many other Mediterranean countries, is under continuous pressure from land-based sources and increasing urbanization (Fakhri, 2012). The years of civil war have had deleterious effects on the Lebanese environment and particularly on the coastal area where the largest Lebanese cities are located (Beirut, Tripoli, Saida, and Tyre). The war has destructed public services such as water supply and wastewater treatment facilities. Solid waste collection and disposal, power supply and public transport haven't suffered less (Noujeim, 2012). The Lebanese coastal zone, is a high-density population area with about 82 % of the population inhabiting the coast in 2000 and still in continuous increase (Abu-Jawdeh et al., 2000; MOE/UNDP, 2011). A rapid expansion around the major cities, mediated by rural-urban migration and random space occupation, has been witnessed particularly after the war (Noujeim, 2012).

Until present, wastewater management is one of the major problems facing the Lebanese coast. Organic waste and domestic sewage are discharged directly in the sea without prior treatment resulting in degradation of marine water quality especially with demographic growth (Fakhri, 2012; MOE/UNDP/ECODIT, 2011). The World bank reported that Lebanon generated an estimate of 248 Mm³/year of wastewater, of which 43 Mm³/year are produced by industries (World Bank, 2011). Only a part of this water is treated in the absence of operational wastewater treatment plants (WWTPs), while most wastewater collected is discharged raw in the sea and water courses (UNICEF, 2017). In

the postwar period (early nineties), projects and programs dedicated to the rehabilitation and construction of sewer networks and WWTPs took place, as waste water facilities were limited to old networks. Additionally, in major cities wastewater networks were incomplete and lacked treatment facilities, while they were completely absent in smaller communities. By the year 2007, only one wastewater pre-treatment plant was operational in El-Ghadir (South of Beirut), then Saida WWTP followed (Mouneimne and Gemayel, 2012). Through the last decade, the government has invested in this sector and is still working to improve the wastewater service. Several plants and networks have been completed but not yet operational while some are scheduled for completion few years ahead (CDR, 2017).

Untreated wastewater has serious environmental and sanitary impacts. This is generally due to high loads of ammonia and chlorine and high loads of oxygen demanding material. However, in the absence of wastewater surveys and industrial production statistics, the estimation of the composition and organic load of industrial wastewater, remains difficult to estimate (Mouneimne and Gemayel, 2012). In Lebanon, ground and surface water are polluted with various contaminants including ammonia and other organic and inorganic contaminants such as heavy metals. It has been reported that 90% of water resources below 600 m are contaminated (Fakhri, 2012; Noujeim, 2012).

In addition to poor wastewater management strategies, the Lebanese coast is victim of illegal waste dumping and open dumpsites that are not only source of visual pollution, but also of toxic chemicals. Solid waste production in Lebanon has been a subject of continuous increase especially in the big cities with the rural-urban migration and even more with the Syrian conflict that hit in 2011, leading to a massive influx of refugees towards the country (MOE/EU/UNDP, 2014; Noujeim, 2012). After closing the Naameh landfill and in the absence of long-term planning and waste collection, a solid waste crisis emerged in 2015 eventually leading to unauthorized landfills and open incineration sites near inhabited areas. Landfills have had serious impacts on the environment and public health as they are at the origin of the emergence of infectious diseases and toxic leachate leaking into groundwater and food sources (Hilal et al., 2015). Dumpsites are also sources of toxic trace metals . Field studies made by the Ministry of environment have highlighted several hotspots around Tripoli and its port, the complex of Selaata that manufactures fertilizers and the cement works at Chekka. Moreover, studies made on dumpsites leachate along the Lebanese coast showed high levels of toxic metals such Cd and Pb that showed cytotoxic effects in mice (Khalil et al., 2018).

2. SAMPLING SITES

In order to evaluate the degree of pollution along the Lebanese coast, levels of metals and trace elements were studied in a selection of marine organisms that are both relatively abundant along the coast and economically important for coastal fisheries and consumed by local residents. The sampling sites chosen represent major cities distributed along the Lebanese coast from North to South thus representative of the littoral (Figure 2-4). The sites are also characterized by different levels and types of pollution and different levels of anthropogenic pressures.

Tripoli (TRI)

Tripoli is the second largest city in Lebanon with a total population of 229 398 in 2019 making it the second most populated city in Lebanon after Beirut (Worldometers, 2019). The city is known for its artisanal fishing and boat maintenance activities; the fishing port of Mina-Tripoli is one of the largest in the country accounting for 22% of all the fishing vessels in Lebanon. Along the coastal area of Tripoli, several sewage outfalls discharge polluted waters in the sea with no prior treatments. A dumpsite that was supposed to be closed in 2012 but still receiving waste till present which is leading to increased waste height and difficulties in controlling leachate is located in the north of the port of Tripoli (El-Hoz, 2017). Tripoli's dumpsite leachate is charged with toxic levels of trace metals such as Cd and Pb (Khalil et al., 2018). Main sources of pollution in Tripoli revolve around shipping, ship maintenance activities and sewage outfalls (Merhaby et al., 2018). Effluents are drained into the coastal marine area with no prior treatment causing water quality deterioration and discoloration. Moreover, in the south of Tripoli, a complex that manufactures fertilizers and cement in Selaata, Chekka is known to dump serious amounts of Cd into the sea (Nakhlé et al., 2006).

Beirut (BEY)

Beirut, the capital of Lebanon, is the most densely populated and industrial area in Lebanon. Beirut's port is the main port of the country and receives the largest vessels. Significant oil from vehicles is generated in Beirut, 38% of which are also discharged in the sewer system (Fakhri, 2012). In addition, Beirut River and Antelias River bring in a lot of effluents such as industrial discharges and wastewater from the hill top. Along the coast of Beirut, multiple sewage outfalls are identified. Industrial effluents from tanneries, pesticides, plastics and chemical industries reach the sea either directly or via municipal sewer system. Moreover, special waste from medical facilities and slaughter houses are disposed without pretreatment. Beirut also has high levels of atmospheric pollution that exceed the WHO guidelines (Waked et al., 2013). The capital also suffers from solid waste as there are several dumps located along the coast and that pollute its coastal area. The Dora dumpsite (Figure 2-5) is one

of the country's major dumpsites. The landfill is expected to reach capacity in 2020 and experts state that the toxic leachate released from the landfill is being dumped into the sea. It has also been reported that the leachate is charged with high levels of trace metals (Fakhri, 2012).

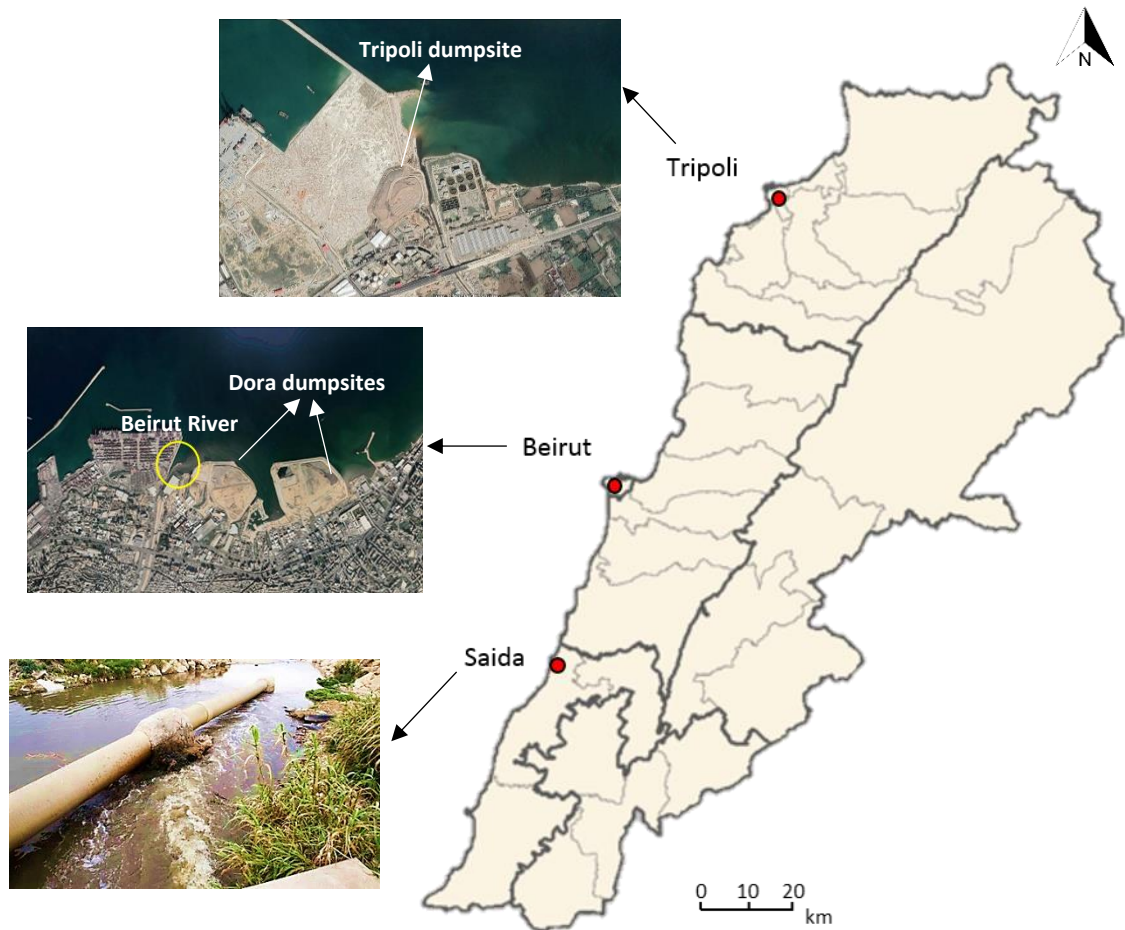


Figure 2-4 Location of the sampling sites along the Lebanese coast

Saida (SYD)

Saida is the third largest city in Lebanon and it is known as "the fishing town". Earlier in the 80's a big dumpsite was located at the southern entrance of the city. It was mainly created to dispose the remains of buildings destroyed during the Israeli air strikes during the 1982 invasion, but then it became the main dump of the city until it became an environmental hazard. In 2014, the dumpsite was rehabilitated into a green space and recently a primary wastewater treatment plant has been established in Saida's coast. Apparently, the WWTP is not working properly and it is pumping polluted water into the coastal area. The South in general accounts for only 2% of the industrial wastewater generated in Lebanon; however, the south of Lebanon is ranked the worst region for discharging oil in the region

as 66% of the oil is disposed in nearby streams and sewer systems or inland (MOE/UNDP/ECODIT, 2011).

3. BIOLOGICAL MODELS SAMPLED

The samples studied in the present work were selected to be representative of different compartments of the food chain thus the species belonged to different habitats and had different feeding behaviors. The choice was also made considering the endemic and/or economically important species with relatively low mobility spread along the Lebanese coast. Moreover, species chosen are appreciated and consumed by local residents, therefore enabling the assessment of human health risk associated to their consumption.

Three fish species (Marbled Spinefoot, Striped seabream, Round herring), one shrimp species (Kuruma shrimp) one bivalve species (Spiny oyster) and one algae species (Sea lettuce) collected along the Lebanese coast were analyzed.

Siganus rivulatus

Siganus rivulatus (Forsskål & Niebuhr, 1775), commonly known as the Marbled Spinefoot, is native to South Africa and the Red Sea (Figure 2-5). Over the last century, this fish along with other Eritrean species, has migrated from the Red sea and invaded the Mediterranean Sea after the opening of the Suez Canal, that's why they are known as Lessepsian species (Bariche, 2006; IUCN, 2009a). This **demersal** fish inhabit shallow waters and is generally found in schools of 50 to hundreds of individuals (Ben Rais Lasram and Mouillot, 2009; Zenetos et al., 2010). They are found in marine brackish waters between 1 and 30 m depth and prefer protected areas such as sheltered bays over rocky bottoms covered with algae (Woodland et al., 1990). It has been reported that this species spwans in waters with temperatures ranging between 24 and 29 °C (Bariche et al., 2003). On the Lebanese coast, these temperatures are reached during the summer season and thus *S. rivulatus* reproduction occurs during this season.

This species is a reef-associated **herbivorous** that grazes on the available macrophytes following the seasons with preference for some species such as *Ulva spp.*, *Jania spp.* and *Halopteris spp.* The siganids family generally have a high market demand, they are meaty, tasty and considered as excellent food fish (Bariche, 2006; Woodland et al., 1990). In Lebanon, this species is widely consumed by local residents and fishermen and is a predominant species in artisanal fishing activities (Barbour et al., 2009).



Figure 2-5 The Marbled Spinefoot *Siganus rivulatus* (Forsskål & Niebuhr, 1775)

Lithognathus mormyrus

Lithognathus mormyrus (Linnaeus, 1758) also known as the Striped seabream or Sand steenbras, is common in the Mediterranean Sea (Figure 2-6). It is a commercially valuable fish widely distributed in the eastern Atlantic Ocean, the Mediterranean, the Red Sea, and the south-western Indian Ocean (Šantić et al., 2010). It is a **demersal** species that inhabits sandy, sand-muddy and rocky bottoms as well as seagrass beds down to 150 m depth in the Atlantic and 80 m in the Mediterranean (IUCN, 2009b; Kallianiotis et al., 2005). It is a **carnivorous** species that feeds on benthic invertebrates such as polychaeta, bivalves, mollusks and small crustaceans. The diet of this species is subject to ontogenic and seasonal changes. *L. mormyrus* spawns between May and September with the maximum gametes emission occurring in June, July and August (Kallianiotis et al., 2005). They tend to become more selective feeders during summer and more generalist in winter depending on the food availability. *L. mormyrus* is considered an important catch for the coastal fisheries in the Mediterranean and is thus a highly prized species (Kallianiotis et al., 2005).



Figure 2-6 The Striped seabream *Lithognathus mormyrus* L. 1758

Etrumeus teres

Etrumeus teres (DeKay, 1842), commonly known as the round herring, is an Indo-Pacific high economic value fish found in the Red Sea, eastern Africa, Japan, southern Australia, eastern Pacific and western Atlantic (Figure 2-7). It is a Lessepsian species, and was first recorded in the eastern Mediterranean in 1961 (Kasapidis, 2007; Osman et al., 2013). *E. teres* is a **pelagic carnivorous** species belonging to the Clupeidae family mainly feeding on small crustaceans, fish larvae, mollusks as well as food remains through the water column. Feeding intensity has been shown to be highest in winter and spring while the lowest values were recorded during summer and autumn (Osman et al., 2013), this related to the fact that the reproductive period of *E. teres* occurs during winter (Plaza et al., 2007).

In a study on fish catches in Lebanese waters, the Clupeidae family was shown to be one of the most represented families in terms of number of catches (Bariche et al., 2007). In Lebanon the Clupeidae family is mostly consumed as juveniles.



Figure 2-7 The round herring *Etrumeus teres* (DeKay, 1842)

Marsupenaeus (Penaeus) japonicus

Penaeus japonicus (Bate, 1888) or the Kuruma shrimp, is a prawn species occurring naturally in bays and seas of the Indo-West Pacific (Red Sea and Eastern Coast of Africa) (Figure 2-8). It is also a Lessepsian migrant and became very abundant along the Levantine coast, outcompeting the native prawn species *Melicertus kerathurus*. This species is **benthic** inhabiting sandy mud and sandy bottoms in littoral and sublittoral zones up to 90 m depth. *P. japonicus* feeds on algae, small fish (squid, sardine fry, sand eel fry) and small bivalves (clams and oysters) making it an **omnivorous** species (Galil, 2006;

Quigley et al., 2013a). The kuruma shrimp is a commercially important and highly prized species in the Mediterranean (Quigley et al., 2013a).



Figure 2-8 The Kuruma shrimp *Marsupenaeus japonicus* (Bate, 1888)

Spondylus spinosus

Spondylus (Linnaeus, 1758), also known as the spiny oysters, is a genus of bivalves that is highly abundant on Mediterranean shores where they live attached to rocky substrates (Aksit et al., 2013) (Figure 2-9). The spiny oyster *Spondylus spinosus* (Schreibers, 1793) is in fact an Indo-Pacific species that has reached the Mediterranean by Lessepsian migration and became well established in there (Mienis et al., 1993). This species of oyster is appreciated and widely consumed among the Lebanese population. It is a **filter feeder** that is observed in many areas at a depth range of 1 - 40 m (Çeviker, 2006). This species has its spawning period that coincides with the seasonal rise of seawater temperatures that occurs during the dry season in the Eastern Mediterranean (Shabtay et al., 2015).



Figure 2-9 The spiny oysters *Spondylus spinosus* (Schreibers, 1793)

Ulva Lactuca

The seaweed used in this study is the sea lettuce, *Ulva lactuca* (Linnaeus, 1753) (Figure 2-10). It is a common and cosmopolitan species with a wide range of distribution. It is found almost worldwide, in the Atlantic Ocean, Mediterranean Sea, Red Sea and the Pacific Ocean.

Macroalgae such as *U. lactuca* are known for their properties as bio-indicator species that are important in assessing the chemical pollution of the aquatic environment as they have a sedentary lifestyle, a high absorptive area due to their thin thallus and they are relatively easy to identify (Kamala-Kannan et al., 2008; Villares et al., 2001).



Figure 2-10 The sea lettuce *Ulva lactuca* (Linnaeus, 1753)

4. SAMPLES COLLECTION AND PREPARATION

The sampling campaigns were realized over 2 periods of time: December to March 2016 (season 1) and considered as the rainy season, June to September 2017 (season 2) and considered as the dry season.

All the samples, except for algae, were caught by local fishermen. Ten individuals (when possible) of each species described above, were collected and transported to the laboratory on ice. Once in the laboratory, fish species were weighed, measured for total length and sexed. Fish's right dorsolateral muscles and livers were removed using stainless steel tools. Shrimps and bivalves' soft tissues (edible parts) were also removed. Shrimps were weighed and decorticated and bivalves' shells were measured for maximum length and width before having the shell removed. All samples were then placed in low density polyethylene vials previously decontaminated by soaking overnight in 10% HNO₃, and stored

at -20 °C. Samples were then freeze dried for 48h then homogenized using an agate mortar and pestle, or an electrical grinder when possible. All the handling (dissection, grinding, drying of materials) was carried out under a laminar flow hood.

In order to limit the individual size effect to a minimum, the samples of each species were chosen in a way to be almost of the same size when possible (Table 2-1).

For canned tuna, the content of each can was homogenized using a food blender after removing excess fluid. Samples were not lyophilized and were stored at -20 °C until analysis.

Table 2-1 Average sizes and weights of local species collected per season.

Species	Season 1			Season 2		
	N	Site	Average weight (g)	Average length (cm)	Average weight (g)	Average length (cm)
<i>S. rivulatus</i>	60	TRI	36.7 ± 4.34	14.03 ± 0.49	37.5 ± 3.28	14.74 ± 0.33
		BEY	65.5 ± 7.73	16.88 ± 0.62	35.8 ± 10.82	14.19 ± 1.45
		SYD	82.9 ± 17.1	19.52 ± 1.12	32.9 ± 5.09	14.8 ± 1.11
<i>L. mormyrus</i>	56	TRI	61.9 ± 11.9	16.9 ± 0.66	68.9 ± 13.8	17.4 ± 1.30
		BEY	76.3 ± 47.2	17.6 ± 2.31	96.2 ± 54.2	19.3 ± 3.68
		SYD	166 ± 10.52	23.5 ± 0.45	52.2 ± 3.7	15.8 ± 0.43
<i>E. teres</i>	60	TRI	100 ± 11.62	22.7 ± 0.62	124.63 ± 17.03	25.61 ± 3.85
		BEY	48.3 ± 7.68	18.2 ± 1.01	37.65 ± 4.93	16.28 ± 0.59
		SYD	139.80 ± 10.76	25.06 ± 0.53	117.15 ± 9.49	23.72 ± 0.64
<i>S. spinosus</i>	60	TRI	-	9.06 ± 0.32	-	10.73 ± 0.88
		BEY	-	6.85 ± 1.12	-	7.26 ± 0.12
		SYD	-	8.99 ± 1.13	-	8.38 ± 0.49

Chemical analyses

The samples were analyzed for their content in trace elements using an accredited method (French Accreditation Comity-COFARC) based on microwave closed vessel acid mineralization (Chevallier et al., 2015). Twenty trace elements (Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Ag, Cd, Sn, Sb, Ba, Hg, Pb, U) were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) Agilent 7700x ICP-MS (Agilent Technologies, Courtaboeuf, France). Moreover, a method for the determination of methylmercury by High Performance Liquid Chromatography coupled to ICP-MS (HPLC-ICP-MS) (Agilent Infinity lab 1200, France) was optimized and validated according to the accuracy profile procedure (AFNOR, 2010).

5. ANALYSIS OF TRACE ELEMENTS BY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS)

ICP-MS technique

This technique uses inductively coupled plasma to ionize the sample (Figure 2-11). It consists of the separation, identification and quantification of elements in a sample based on their mass/charge fraction. Four main steps are involved in the analysis: Introduction-atomization, ionization, separation in mass and detection. The sample is introduced using a peristaltic pump before it reaches the nebulizer where it is atomized in the plasma at very high temperature (6000 – 8000 K).

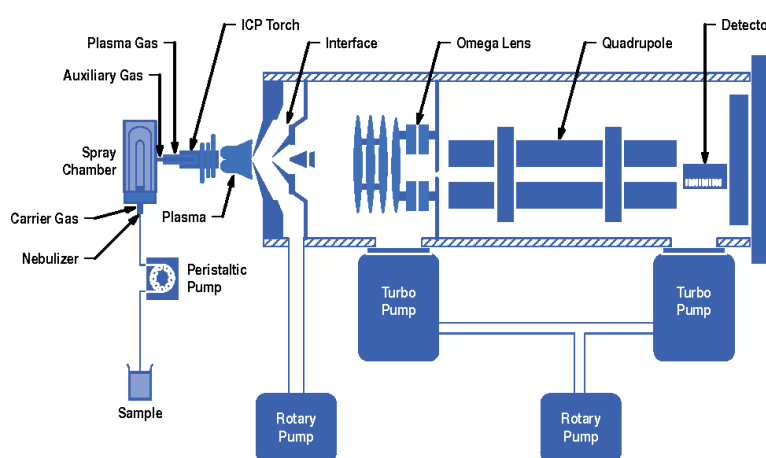


Figure 2-11 The principle of inductively coupled plasma mass spectrometry

The first part of the sample is taken up by the sampler cone through a 1 mm opening and then it is extended under the effect of the moderate vacuum in the interface until it reaches the second opening of the skimmer cone. A system of differential vacuum system accelerates the ions from the plasma towards a system of electrostatic lenses that extracts positively charged ions and transports them towards a quadrupolar filter of mass. The filter of mass only transports ions that have a certain mass/charge ratio that is determined according to the frequency applied to the quadrupole. The principle of the spectrometer is about separating the elements according to their mass. The selection is made according to the frequency applied on the filter. An electron multiplier detector with discrete dynodes allows the quantification of the signal intensity for each mass. The signal is translated into pulse repetition frequency (counts). For a given isotope, the number of ions measured allows a direct calculation of the concentration of the analyzed element. The counts are converted into concentrations after calibration. In the case of the present study, internal standards (ISTD) were used in order to

monitor instrumental drift and matrix effect and thus the ratio of the analyte count/ISTD count was used for calibration. The ratios are then converted into concentrations.

Elevated temperatures used for the ionization of the sample in the plasma are very high thus favoring the combinations between argon with a mass of 40 and atoms of the analyzed matrices such as chlorine 35 and /or carbon 12 resulting in molecules of 75 and 52 mass respectively and thus influencing the measure of As 75 and Cr 52. There are several methods to deal with spectral interferences, ones that are instrumental and others that are mathematical. One of the strategies used to overcome spectral interferences is the collision cell. Gases such as helium collide with the interfering polyatomic species and the target species and as the interfering are larger in size than the target analytes, they will lose kinetic energy and be expelled in the mass analyzer without being detected (Lum and Sze-Yin Leung, 2016).

Analysis method

Between 0.2 and 0.4 g of dry samples and 0.5 to 2 g of fresh samples (canned tuna) were weighed in quartz digestion vessels of 80 ml (operating pressure 80 bar) before adding 3 ml of Suprapur nitric acid HNO₃ 67% (VWR, France). Samples were subject to a pre-digestion process by soaking in nitric acid overnight, the day before analysis. Before use, quartz vessels were decontaminated with 6 mL of 52% HNO₃ and rinsed with ultrapure water then oven-dried at 50 °C. The day of analysis, 3 mL of ultrapure water were added to each sample before placing the digestion vessels in the rotor intended for microwave digestion. A Multiwave 3000 microwave (Anton-Paar, France), equipped with a rotor for eight quartz vessels was used for the mineralization. One of the eight vessels was only filled with reagents and considered as blank through the entire procedure. The resulting sample must be clear and exempt of suspended particles. After cooling down to room temperature, sample digests were transferred into 50 mL polypropylene bottles. Afterwards, 100 µL of an internal standard (IS) solution that contains scandium (Sc), indium (In), bismuth (Bi), rhenium (Re) and yttrium (Y) at a concentration of 1 mg L⁻¹ were added to all the samples and calibration standards with a final concentration of 2 µg L⁻¹. The IS solution also contains gold (Au) (10 mg L⁻¹) which is used to reduce the memory effect of mercury (Hg) during analysis. Au was added to a final concentration of 20 µg L⁻¹.

Quality control procedures

In order to ensure reliability of the results, several internal quality controls (IQC) were used. The data were considered valid when all acceptance criteria were satisfied. IQCs included calibration, blanks and LOQ checking, internal standards, spikes, CRM's and duplicates results were acceptable if the relative standard deviation (RSD) $\leq 20\%$, when mean value $\geq 5 \times \text{LOQ}$ or RSD $\leq 40\%$, when mean value $\geq \text{LOQ}$ and $< 5 \times \text{LOQ}$ (Table 2-2).

Concerning the calibration curves, a determination coefficient $r^2 \geq 0.995$ was considered acceptable using 6 calibration points. The internal standards allowed to correct for instrumental drift and to compensate the matrix effects and their recovery interval was set to 70-130%. A mid-range standard was measured after the calibration curve, every 8 samples and at the end of the sequence in order to monitor the variation of the instrumental response and to assess calibration drift; it's only when the deviation of the control standard was $\leq 20\%$ that results were accepted.

Table 2-2 Limits of quantification (LOQ) and detection (LOD) expressed in mg.kg⁻¹ wet weight (ww) for elements determined in the analyzed samples

	Al	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn
LOQ	0.1667	0.1	0.002	0.02	0.01	0.1667	0.0028	0.1	0.04	0.1
LOD	0.0834	0.05	0.001	0.01	0.005	0.0834	0.0014	0.05	0.02	0.05
	As	Se	Ag	Cd	Sn	Sb	Ba	Hg	Pb	U
LOQ	0.004	0.02	0.1	0.001	0.1667	0.002	0.1	0.0167	0.005	0.001
LOD	0.002	0.01	0.05	0.0005	0.0834	0.001	0.05	0.0084	0.0025	0.0005

With every batch of 8 samples, a method blank, a certified reference material or spiked standard samples on unknown samples were used to control the specificity and trueness while duplicates were used to monitor repeatability. Moreover, sample duplicates were used in some sequences in order to control repeatability. CRM's used include ERM 278K (mussel tissue), DOLT-5 (dogfish liver) and NIST 1566b (oyster tissue).

6. THE USE OF HYPHENATED TECHNIQUES FOR ELEMENTAL SPECIATION: HPLC-ICP-MS

Mercury is present in seafood mainly under the form of methylmercury which is the most toxic form (EFSA, 2014b). Contamination of seafood by methylmercury is one of the most prominent areas of speciation (Kot and Namiesnèik, 2000). Speciation analysis is widely applied in different fields such

as quality control of food products, medicine, dietary supplements or for the monitoring of occupational exposure. The word hyphenation refers to the online combination of a separation technique and one or more spectroscopic detection techniques. Chromatography provides pure or nearly pure fractions of chemical components in a mixture while spectroscopy produces selective information for identification. During the last decades, speciation using hyphenated techniques has received an increasing attention (Marcinkowska and Barańkiewicz, 2016; Patel et al., 2010).

HPLC-ICP-MS is the most widely used technique in multielement speciation analysis as it has valuable advantages. First of all, the ICP-MS detector gives the possibility to analyze many elements in a single run, it also offers very low detection limits, a wide range of linearity in addition to excellent precision and accuracy. Second, the connection between ICP-MS and HPLC is simple (Figure 2-12), as the column is directly connected to the nebulizer (Marcinkowska and Barańkiewicz, 2016). Moreover, until now, the analysis of mercury have been carried out mostly using GC-ICP-MS as it offers low levels of detection compared to HPLC-ICP-MS (Laffont et al., 2013).

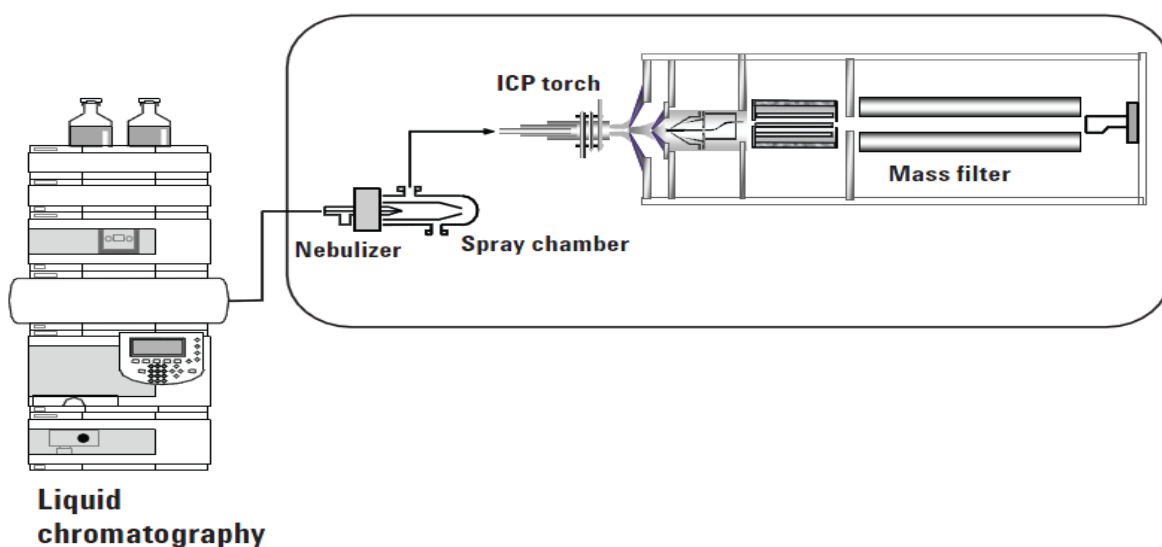


Figure 2-12 HPLC-ICP-MS speciation interface – exit of the column connected to the nebulizer

However, with the development of the ICP-MS in terms of sensitivity, the HPLC-ICP-MS technique has become widely used for elemental speciation and particularly for mercury. Until now, there is no regulation in terms of MeHg as the EC guidelines for Hg in seafood are established in terms of total Hg confirmed by the fact that the analysis of mercury in seafood has not yet become a routine approach.

6.1. METHYLMERCURY (MeHg)

In the present study, a method oriented towards a routine approach for the speciation of mercury in fishery products was optimized and validated using the accuracy profile procedure. The method will be detailed in the chapter X. It is based on microwave assisted extraction and separation of Hg species by HPLC-ICP-MS after complexation by L-Cystein.H₂O.HCl, in order to determine levels of MeHg. The method was validated and has been shown to be adequate for routine analysis of fishery products. Briefly, between 0.3 g of lyophilized samples and 0.5 g of fresh samples were weighed in 50 mL quartz digestion vessels, then 20 mL L-Cystein.H₂O.HCl at a concentration of 5 g/L were added to each sample. The microwave system Novawave from scp science (France) was used for the extraction procedure. The extraction was done for 15 minutes at 60 °C. The resulting samples were transferred to 50 mL polypropylene tubes and then added up with MeOH 5% to a final volume of 50 mL. Samples were then centrifuged at 4000 rpm for 10 minutes. The supernatants were filtrated through a 22 µm and transferred to 1.5 mL ambered vials for injection in the HPLC (Agilent Infinity lab 1200, France). The mobile phase consists of a mix of 5% MeOH and 95 % Cysteine.HCl.H₂O at 0.5 g L⁻¹. The column used is a reversed phase small bore column from Agilent, France (Peptide mapping, 2.1 x 150 mm, 2.7 µm). MeHg was quantified by external calibration.

Method validation: an overview

In analytical chemistry, method validation is required to "confirm the fitness for purpose of a certain analytical method". In others terms, the aim of any analytical method is to produce results that reflect the content of samples with an acceptable accuracy (Feinberg, 2007). So, what is accuracy? Accuracy may be estimated by a proper evaluation of both systematic and random errors and thus shall not be confused with trueness. The main difference between random and systematic error is the consistency. An example of systematic error is a wrongly graduated lab glassware while a random error can be a defectuous machine giving inconsistent results. Trueness is defined as "the closeness of agreement between the average value obtained from a large series of results and an accepted reference", it is ideally verified by using certified reference material and may be expressed as bias or percentage of recovery. Bias is defined as the difference between the expectation of the test results and an accepted reference value (Feinberg, 2007). Precision is a generic term that includes instrumental and method repeatability, intermediate precision, intra-laboratory (internal or within-laboratory) and inter-laboratory (between-laboratory) reproducibility (Mermet and Granier, 2012). The study of accuracy over a large range of concentrations provides an accuracy profile (Figure 2-13). The accuracy profile is a decision-making graphical tool that helps the analyst decide whether an analytical procedure is

valid over a certain domain. That profile allows obtaining experimental tolerance limits that have to be compared with acceptability limits set by the analyst. In fact, the acceptability limits λ (%), are set by the analyst to express trueness. The value of the acceptability limit may be constant over the validity domain or may be variable following the concentration levels, if the domain is large. The acceptability interval is thus calculated from the reference values of the samples used in the validation procedure and the acceptability limit.

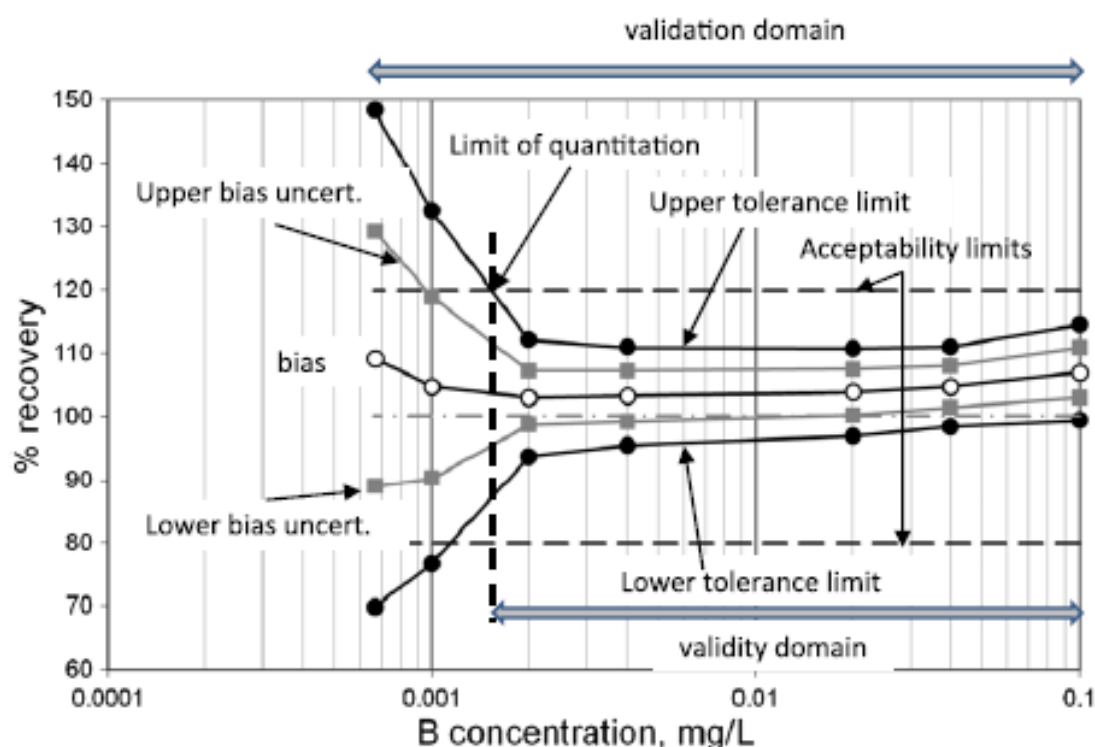


Figure 2-13 Example of an accuracy profile with intervals of tolerance and acceptability, upper and lower bias uncertainties (Mermet and Granier, 2012)

The accuracy tolerance intervals (Lower tolerance limit – Upper tolerance limit) are function of the concentration. For the experimental tolerance intervals, they are equal to reference value + bias \pm intermediate precision (s_R). When the experiments are made within the same laboratory the term intermediate precision is used, and when they are done between laboratories it's called reproducibility (Mermet and Granier, 2012). A method is considered to be valid, when the tolerance intervals are fully included within the acceptability limits, thus having high precision and accuracy with low probability of getting unacceptable results (Figure 2-14). However, when the concentration decreases, accuracy

degrades, thus the tolerance interval becomes larger and intercepts with the acceptability limits. At that intersection, a limit of quantification based on trueness and precision can be deduced (Mermet, 2008).

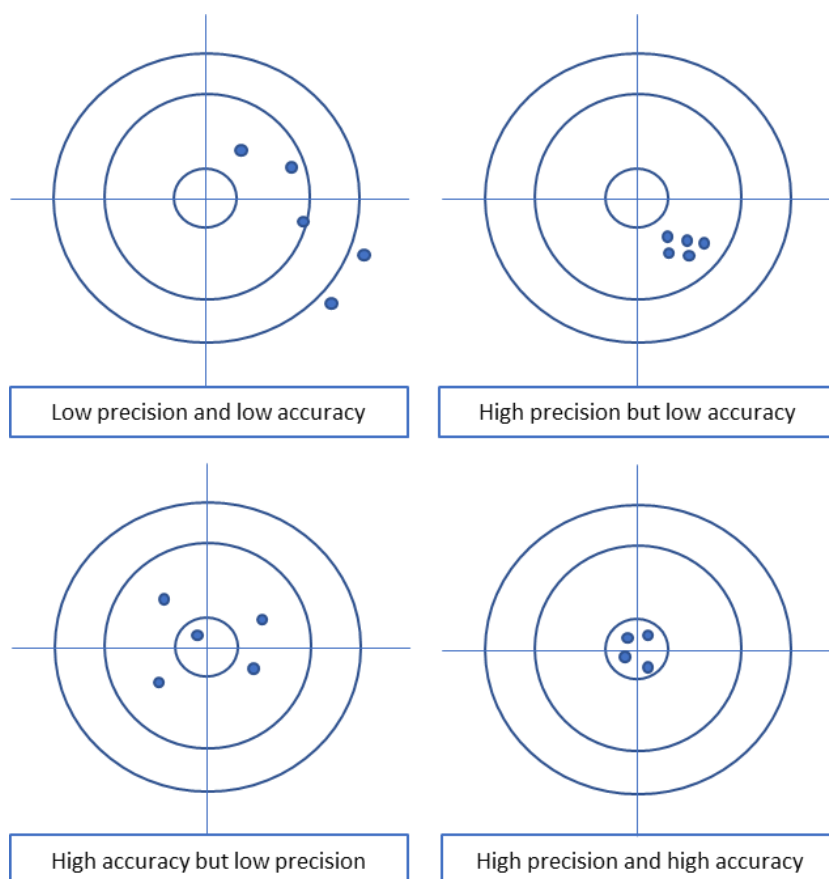


Figure 2-14 Traditional model of accuracy and precision (Feinberg, 1996)

The accuracy profile method has already been used mainly in the field of separation methods such as HPLC. In the present study, the accuracy profile was used to validate the method developed for the speciation of mercury in fishery products, in a perspective of making MeHg determination a routine method.

CHAPTER 3 - METALLIC CONTAMINATION OF MARINE ORGANISMS FROM THE LEBANESE COAST

Chapter 3 - PART 1: SEASONAL AND SPATIAL VARIABILITY OF TRACE ELEMENTS IN LIVERS AND MUSCLES OF THREE FISH SPECIES FROM THE EASTERN MEDITERRANEAN

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Abstract

Levels of 20 trace elements (Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Ag, Cd, Sn, Sb, Ba, Hg, Pb and U) were assessed in livers and muscles of two demersal fish species (*Siganus rivulatus* and *Lithognathus mormyrus*) and one pelagic species (*Etrumeus teres*) from the Lebanese coast located in the Eastern Mediterranean. The samples were collected from three sites along the Lebanese coast during the wet and dry seasons in 2017. The trace elements were more concentrated in livers than in muscles and interspecific differences were also found. The herbivorous species *S. rivulatus* showed the highest levels for most trace elements while the carnivorous species *L. mormyrus* showed the least contamination. Elemental seasonal differences were species dependent and were observed for Ti, Cr, Mn, Fe, Ni, As and Hg with higher values during the wet season. Multivariate analysis showed spatial differences mainly during the wet season being closely related to species that reflected different accumulation patterns in each site. Levels of most elements were higher in livers compared to those reported in other Mediterranean regions (up to 2 to 3 folds) suggesting that the Lebanese marine coastal zone is more exposed to environmental chemical contamination with trace metals. Nevertheless, the levels of Cd, Pb and Hg in fish muscle were below the maximum levels set by the European Commission indicating that the consumption of these fish species is not likely to have adverse effect on human health.

Keywords: trace elements, fish species, Eastern Mediterranean, Lebanon, seasonal variation, spatial variation

1. Introduction

During the last few decades, the marine environment has been witnessing increasing pressure and threats due to demographic growth concentrated essentially on the coastal zones that actually host two thirds of the world's population. The presence of chemical contaminants in the marine environment has been assigned mostly to land-based sources that contribute to about 80% of pollution entering the oceans and seas. Contaminants such as trace metals can enter the marine environment by natural or anthropogenic means. They can be naturally released by geological weathering, erosion and other phenomena such as forest fires and volcanic activities. Anthropogenic activities include industries, untreated sewage, surface runoff and dumpsites leachate which release significant amounts of toxic trace metals that are dangerous for marine ecosystems and ultimately to human health (Fakhri, 2012; Rather et al., 2019; Wright and Mason, 1999).

Trace metals are non-biodegradable and can be accumulated by different aquatic organisms including fish at different levels depending on the species. Therefore, fish have been widely used as bio-indicators of metal pollution status in water as they constitute the top predators in marine food chains (Authman, 2015; Bahnasawy et al., 2009; Canli and Atli, 2003; Dórea, 2008). The main routes of trace elements uptake by fish are the gills and the ingestion of contaminated food (Chovanec et al., 2003; Rakocevic et al., 2017). Tissue concentrations can indicate environmental load of a certain contaminants; however, they do not reflect the ecological and physiological consequences. In fish, concentrations of metals in tissues are the results of several mechanisms such as uptake, storage, and excretion (Chovanec et al., 2003). Muscles have been widely analyzed as they are the main edible part of fish, thus indicating the risk on human health following consumption (Rajeshkumar et al., 2018). However, livers, kidneys and gills accumulate metals more efficiently than muscles as they are metabolically active tissues and are the main organs for the accumulation of most elements (Bahnasawy et al., 2009; Chovanec et al., 2003; Rakocevic et al., 2017). Regarding fish muscles, current regulations established by the European Commission (EC) (EC, 2015) for maximum allowed levels of metals in fish and other seafood products, concern lead (Pb), mercury (Hg) and cadmium (Cd) (Rakocevic et al., 2017).

The Eastern Mediterranean basin is exposed to strong environmental pressures mainly along the coast where most of the population is concentrated. Domestic and industrial wastewater are being discharged along the coastal region without prior treatment, which is the major cause of water quality deterioration. Moreover, numerous dumpsites scattered along the coast release also toxic leachates in the marine environment (Fakhri, 2012). Most of the studies conducted in countries of the Eastern

Mediterranean basin such as Lebanon in terms of marine contamination with trace toxic metals addressed sediment and water contamination with little focus on the contamination of fishery products which ultimately have an impact on human health due to their consumption (Kouyoumjian et al., 1986; Kouyoumjian et al., 2001; Shiber, 1981; Shiber and Shatila, 1978) and so far none have used different fish tissues as indicators of environmental quality. A biomonitoring study conducted in 2003 used only gastropods and sponges as bioindicators to monitor contamination by Cd, Hg and Pb along the Lebanese coast (Nakhlé, 2003). All the above-mentioned studies were carried out over 15 years ago, while environmental pressures have been unceasingly growing in the studied area. Indeed, solid waste production has been in continuous increase in Lebanon during the last few decades and the country has faced a solid waste crisis in 2015. High percentage of Lebanon's solid waste still ends up in landfills and open dumps mainly along the coast. To our knowledge, waste leachate is typically released to the environment without any treatment, thus increasing the risk of environmental and human damages (Khalil et al., 2018).

The main objective of this study was to assess the contamination status of the Lebanese coast by means of three fish species: marbled spinefoot (*Siganus rivulatus*), sand steenbras (*Lithognathus mormyrus*) and red-eye round herring (*Etrumeus teres*) used as bio indicators. For this purpose, the levels of 20 trace elements (Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Ag, Cd, Sn, Sb, Ba, Hg, Pb and U) were determined both in muscles and livers tissues. The spatial and seasonal variation of the levels of these contaminants has been also addressed. Finally, this study also aimed to determine whether the levels of regulated contaminants by EC and the Codex such as Hg, Cd and Pb in these fish species exceed the maximum admissible levels for human consumption.

2. Materials and Methods

Study area and sample collection

Lebanon is an Eastern Mediterranean country with a mild rainy winter and a longer dry hot summer. The rainfall occurs mainly between November and March, peaking in January while the dry season extends from April till October (MOE/UNDP/ECODIT, 2011). Therefore, sampling seasons were chosen in this respect to study the seasonal variabilities in levels of trace elements. Samples for the wet season were taken between November and March 2017, while the dry season's samples were taken between June and September 2017. Samples were collected from three sites characterized by different types and levels of anthropogenic pressures as shown in Figure 3-1.

Starting from the North, Tripoli (site 1) is the second largest city in Lebanon after Beirut. It is essentially recognized for its artisanal fishing and boat maintenance activities as it holds 22% of all fishing vessels in Lebanon. The coastal area in Tripoli is impacted by several sewage outfalls that discharge polluted waters in the sea without prior treatment. A controlled dumpsite is located in the north of the port of Tripoli. It was supposed to be closed back in 2012, but is still receiving waste until present, leading to increased waste height and difficulties in controlling leachate (El-Hoz, 2017). Beirut (the capital of Lebanon, site 2) is a densely populated and highly industrialized area. At least

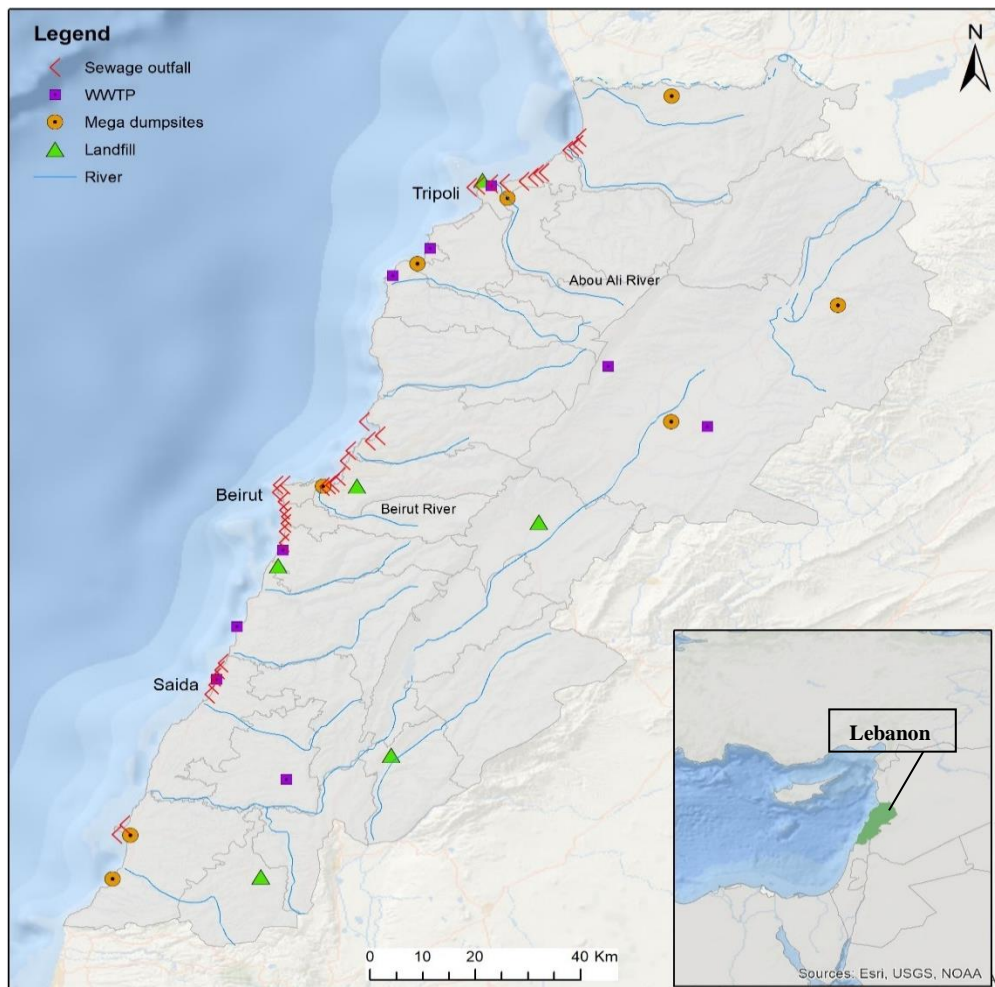


Figure 3-1 Map showing the sampling sites along the Lebanese coast. (WWTP: Wastewater treatment plant)

four sewage outfalls are identified along Beirut’s coastal area. In addition to the Beirut River that brings in a lot of effluents such as industrial discharges and wastewater from the hill top, industrial

complexes such as tanneries, paint manufacturers, detergents, slaughter houses and petrochemical industries discharge their waste without any treatment. Moreover, Dora dumpsite present in Beirut since 1998 is responsible for more than half of Lebanon’s toxic trace metals discharge in the area

(Fakhri, 2012). In the south, Saida (site 3) is less impacted by anthropogenic pressures compared to Beirut despite being the Third densest coastal city in Lebanon. In 2014 a dumpsite in Saida was rehabilitated and recently, a primary wastewater treatment has been established in Saida's coastal area.

Three commercially valuable fish species that are widely distributed along the Lebanese coast were selected according to their dietary habits, their position in the food chain in addition to their behaviour and habitat use. *Siganus rivulatus*, commonly known as the "marbled spinefoot", is an herbivorous/deposit feeder species that feeds mainly on different algae species depending on the season. It is a demersal species widely consumed by fishermen and local residents (Bariche, 2006). The striped seabream, *Lithognathus mormyrus* is a highly prized fish species that is considered as an important catch in Lebanon. It is a carnivorous demersal species that lives close to the bottom, and that feeds on benthic macroinvertebrates (Kallianiotis et al., 2005). The round herring, *Etrumeus teres*, is a pelagic carnivorous species that is widely distributed along the Lebanese coast. *E. teres* feeds on small crustaceans, fish larvae and food remains through the water column (Osman et al., 2013).

When available, ten individuals of each species were purchased from local fishermen over the two seasons (except for *L. mormyrus* on the wet season from Beirut and *E. teres* from Tripoli in the dry season where only 6 and 8 individuals were collected). Fish were then taken to the laboratory on ice and each individual was weighed (W, to the nearest 0.1 g), measured for total length (TL, to the nearest 1 mm) and sexed. The right dorso-lateral muscles and livers were removed and placed in low-density polyethylene vials previously decontaminated by soaking in 10% (m/v) nitric acid (HNO₃) overnight. Samples were stored at -20 °C and afterwards individually lyophilized until constant weight at -100 °C. Dry samples were ground using inox electrical grinders and kept at room temperature until analysis.

Analysis of muscles and livers

Fish muscles and livers were analysed using an accredited method (French Accreditation Comity - COFRAC) described elsewhere (Chevallier et al., 2015). Briefly, 0.3 g of each sample, were weighed in quartz digestion vessels and then 3 ml of 67% Suprapur HNO₃ (VWR chemicals, Prolabo, France) were added to each sample. Three ml of ultrapure water were added afterwards and then mineralization procedure was carried out in closed microwave system (Multiwave 3000 from Aanton Paar, France). Sample digests were then transferred into polypropylene falcons and a solution containing a mix of internal standards, scandium (Sc), indium (In), bismuth (Bi), rhenium (Re) and yttrium (Y) was then added at a final concentration of 2 µg L⁻¹. The mixtures were then added up with ultrapure water to a volume of 50 ml. Moreover, a solution of gold (Au) at a final concentration of 20 µg L⁻¹ was also

added to reduce the memory effect of mercury (Hg) during analysis. Samples were then analyzed using a 7700 Agilent ICP-MS instrument (Agilent, France).

Quality Control

Several internal quality controls (IQC) and associated criteria were used to ensure the reliability of the results. Each run included a standard calibration, three blanks, three CRMs, two different spiked sample solutions, 33 food samples including 2 samples analysed in duplicate and a mid-range standard analysed for every eight samples and at the end of the sequence. The set criteria were as follows: calibration ($r^2 > 0.995$), blanks (values $<$ limit of quantification (LOQ)), internal standards (values within 70 and 130% of the target value), mid-range standards (values within 80 and 120% of the target value), spiked standard solutions (spike recovery within 70 and 130% of the theoretical spiked standard value), CRMs (Z-score $< \pm 2$) and duplicates (acceptable if the relative standard deviation (RSD) $\leq 20\%$ when mean value $\geq 5 \times$ LOQ or RSD $\leq 40\%$ when mean value \geq LOQ and $< 5 \times$ LOQ). When acceptance criteria were not met, the results were discarded and the samples were re-analysed.

Data treatment

Statistical analyses were performed using XLSTAT 2014. The level of significance was set at $\alpha=0.05$. As data were not normally distributed, non-parametric tests namely Kruskal-Wallis and Mann-Whitney U test for pairwise comparisons were used to study inter-specific and spatial differences as well as seasonal variations in elemental concentrations. Principal component analysis (PCA) was used after log transformation of the data to explore relationships between sites, species and fish liver elemental concentration for the two sampling seasons. Left censored data that are $<$ LOD or $<$ LOQ were excluded from statistical analysis (28%). All analytical results were reported in mg kg^{-1} of dry weight. In order to compare with the norms, the levels of Cd, Hg and Pb in wet weight were calculated by dividing the concentration expressed in mg kg^{-1} of dw, by the respective amount of dry matter for each sample determined after lyophilization.

3. Results and discussion

Concentration levels of 17 out of 20 trace elements determined in muscles and livers tissues are reported for each season, species and sampling location in (Annex 1,2,3). Some elements such as Ni, Sn, Sb, Ba and U were not quantified in the muscles or had a very low detection frequency in that tissue (below 10% for both seasons). Ag was quantified only in livers of *S. rivulatus* while Sb was mostly quantified in livers (Annex 1,2,3). Sn, Ba and U levels were not displayed in the tables.

Regardless of the species, livers were more concentrated in elements than muscles as previously described (Bahnasawy et al., 2009; Chovanec et al., 2003; Diop et al., 2016a; Henry et al., 2004; Rajkowska and Protasowicki, 2013). Organs such as livers and kidneys are usually targeted to determine the environmental load of a specific toxicant as they are rich in metallothionein and accumulate metals in higher levels with particularly high affinities to Cd, Hg, Zn and Cu (Chovanec et al., 2003; El-Moselhy et al., 2014; Rajkowska and Protasowicki, 2013; Szarek-Gwiazda and Amirowicz, 2006).

Interspecific variation in muscles and livers

Feeding habits, fish age and size have been shown to influence trace elements accumulation in different species of fish (Bawuro et al., 2018). In the present study, significant variations in element concentrations were found in livers between the three studied species. Fe and Zn had the highest concentrations in all species and tissues. Metals such as Mn, Fe, Cu and Zn are essential elements for biological systems (Maret, 2016). However, even essential elements can be toxic at high concentrations (Brown et al., 2017). In this study levels of Cu were found to be in the same range mostly for muscles or higher than what was reported in other studies especially in livers (Abdallah, 2008; El-Moselhy et al., 2014; Yilmaz et al., 2018). Overall, concentrations in muscles were in the same order of magnitude as levels found in the region but those in the livers were higher in our study. For instance, *S. rivulatus* from El-Mex Bay showed a concentration of $2.7 \pm 1.7 \text{ mg kg}^{-1} \text{ dw}$ in the muscles (Abdallah, 2008) almost similar to the present study ($1.1 \pm 0.48 \text{ mg kg}^{-1} \text{ dw}$ in muscle). However, *S. rivulatus* recorded higher values of Cu in the liver compared to other species ($1.76 \pm 0.52 \text{ mg kg}^{-1} \text{ dw}$ in muscles, $106.7 \pm 22.4 \text{ mg kg}^{-1} \text{ dw}$ livers) from Turkish waters (Ates et al. 2015) which means that in the present study the levels of Cu in *S. rivulatus* are two to three folds higher in livers ($304 \pm 378 \text{ mg kg}^{-1} \text{ dw}$) compared to the study conducted in Turkish waters. Levels in *L. mormyrus* were $0.96 \pm 0.28 \text{ mg kg}^{-1} \text{ dw}$ in the muscle and 18.44 ± 17.96 in the liver $\text{mg kg}^{-1} \text{ dw}$ which is lower compared to the same species sampled in the Aegean Sea ($3.96 \pm 0.8 \text{ mg kg}^{-1} \text{ dw}$ in muscle and $10.88 \pm 1.6 \text{ mg kg}^{-1} \text{ dw}$ in livers) (Tepe, 2009). Levels of most essential elements in the muscles were generally in the same order of magnitude as what was reported in other regions of the Mediterranean however the livers exhibited higher values (Abdallah, 2008; Ateş et al., 2015; El-Moselhy et al., 2014; Yilmaz et al., 2018). This might be related to all the anthropogenic and industrial activities taking place along the Lebanese coast, in addition to inappropriate waste treatment strategies. A study made on the landfill leachates in Lebanon, showed that they presented high levels of Mn, Cr, Ni and Cd posing

health risks to the marine environment and human health due to the lack of efficient waste management and biological leachate treatment (Khalil et al., 2018).

Most elements (Al, Ti, V, Fe, Co, Ni, Cu, Zn, Cd and Pb) were found to be significantly higher ($p < 0.001$) in livers and muscles of *S. rivulatus* for both seasons whereas As and Se were the highest in livers of *E. teres*. Arsenic and Se are naturally occurring elements but may also appear in human discharges such as mining, ground disturbances and fly ash disposal (Selvaraj et al., 2012). The relationship between As and Se has been widely studied in the literature (Chen et al., 2007; Selvaraj et al., 2012; Sun et al., 2014). These two elements have antagonistic effects when it comes to toxicity. A study made on As and Se levels in human blood showed that levels of Se below average, were associated to As-induced skin lesions (Chen et al., 2007). Another study made on fish hepatoma cell lines (PLHC-1), showed that Se had a protective effect against As toxicity suggesting that their co-occurrence in the marine environment may result in lesser toxicity for the biota (Selvaraj et al., 2012). Livers of *L. mormyrus* were found to be the least charged with trace elements compared to other species. However, muscles of *L. mormyrus* and *E. teres* showed higher concentrations of Hg than *S. rivulatus* as they are carnivorous species that are susceptible to bioaccumulate Hg. These results agree with the study made by Skaff (2014) on two fish species sampled from Lebanon. Skaff showed that *S. rivulatus* had higher levels of Cd, Pb, Cu, Ni and Zn than *Diplodus sargus*, which is an omnivorous species (Skaff, 2014). Similar findings were obtained in other studies also reporting higher concentrations of Cd ($2.8 \pm 0.8 \text{ mg kg}^{-1}$), Pb ($1.2 \pm 0.2 \text{ mg kg}^{-1}$) and Zn ($43.9 \pm 13.6 \text{ mg kg}^{-1}$) in *S. rivulatus* muscles compared to other species (Abdallah, 2008; Jezierska and Witeska, 2006; Khaled, 2004). These concentrations are comparable to the levels found in the present study except for Zn that is two folds higher than the results we found (Annex 1). The same observation was presented by Khaled (2004), who also worked on two species, *S. rivulatus* and *Sargus sargus*, the latter being carnivorous. The accumulation of metallic contaminants by algae (INERIS, 2004; Shamsuddoha et al., 2005) probably makes *S. rivulatus*, which is mainly a herbivorous/detritivorous fish, more exposed to metal accumulation than the two other carnivorous fish, although benthic and bottom dwelling species are thought to be more exposed to sediment-related contaminants (Kerambrun et al., 2012; Roméo et al., 1999). Moreover, Cd concentration was found to be highest in algae as it can be readily absorbed by organisms directly from the water in its ionic form Cd (II) (Kumar and Singh, 2010) which may explain the higher level of Cd in the herbivorous *S. rivulatus* that feeds on different algae species depending on the season including *Ulva lactuca* that is known for its use as bio-indicator (Diop et al., 2016b; Kamala-Kannan et al., 2008).

Habitat of the fish has also been shown to affect metal accumulation patterns. Species living in shallow waters closer to the coastal zone, are more exposed to the effects of anthropogenic activities and thus to contaminants (Stehr et al., 2004). This is the case of *S. rivulatus* who lives at a shallow depth from 1 to 30 meters while *L. mormyrus* is found at 80 meters depth and *E. teres* can be found at 200 meters depth (Bariche et al., 2004). Moreover, it has been shown that shallow coastal waters are more affected by anthropogenic activities (Stehr et al., 2004). For instance, along the Lebanese coast, it has been shown that levels of Pb decrease going from the coast to the open sea (Nakhlé, 2003). Some other studies in Lebanon showed that the winds are an important factor for dispersion of contaminants and suspended matter by seaward transportation (Abboud-Abi Saab et al., 2004; Fakhri and Romano, 2011).

Relatively high levels of As have been recorded in this study in the muscles and livers of all species. Levels of As were particularly high in the livers of *E. teres* (68 and 156 mg kg⁻¹ dw). Fish are capable of accumulating As naturally making it difficult to associate As contamination to anthropogenic pressure or naturally occurring levels of this element (Kalantzi et al., 2017; Popowich et al., 2016). Several studies have shown that among analyzed metals, levels of As were the highest in fish muscles (Leung et al., 2014; Storelli et al., 2005; Thiyagarajan et al., 2012; Zuluaga Rodríguez et al., 2015). In the present study, the highest levels of As were found in the muscles of the bottom dwelling *L. mormyrus* followed by *E. teres*. These two species feeds near the bottom, thus they are more exposed to sediment related contaminants than *S. rivulatus*. Sediments commonly contain higher As levels than in the water column and consequently the bottom waters usually contain higher levels of As than shallow waters (Storelli and Marcotrigiano, 2001; Tremblay and Gobeil, 1990). The levels of As found in this study are generally in agreement with the levels found in other studies focused on the same region (Kalantzi et al., 2017; Perugini et al., 2014).

Seasonal and spatial variations

For the three fish species, the levels of trace elements were generally higher in the samples collected during the wet season except for Se in the livers of *S. rivulatus* and Mn in *E. teres* which were significantly higher ($p < 0.05$) during the dry season (Figure 3-2). However, Ti and Cr showed seasonal variation in all species with higher concentrations during the wet season. No significant differences were observed for the rest of the elements. Seasonality is closely related to contaminant type and to a wide range of parameters. Bioavailability and toxicity of trace metals is controlled by physical and chemical characteristics of seawater (Chovanec et al., 2003). Along the Lebanese coast, parameters such as water hardness, pH, temperature, salinity and oxygen concentrations show significant changes

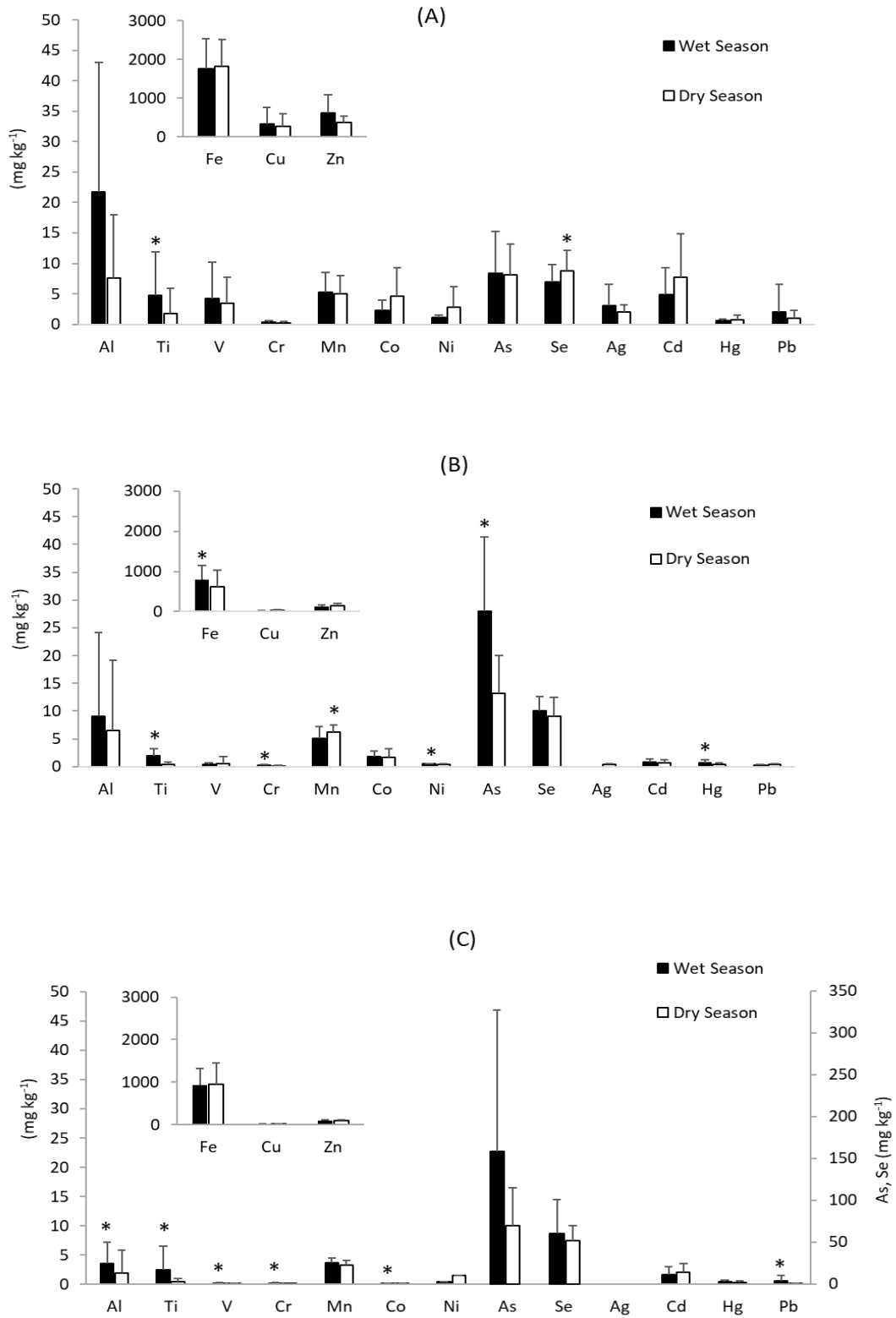


Figure 3-2 Seasonal variation in mean trace element concentration (\pm standard deviation) in livers of *S. rivulatus* (A), *L. mormyrus* (B) and *E. teres* (C). Asterisk denotes significant differences between seasons.

with seasons (Abboud-Abi Saab et al., 2004; Fakhri and Romano, 2005) which might influence the accumulation pattern of trace elements in fish species depending on the season. Previous studies found higher levels of trace elements in the Mediterranean mussel *Mytilus galloprovincialis* in the winter season (Azizi et al., 2018) and As and Pb were found to be higher in fish during the winter season which was attributed to the precipitation of waste caused by rain (Zuluaga Rodríguez et al., 2015). Indeed, for *S. rivulatus* significant seasonal differences were observed for Al, Ti, Cr and Se ($p < 0.05$). Despite not having a statistically significant seasonal variation, some elements such as Cd and Ni showed higher values in summer in *S. rivulatus* and *E. teres*. This agrees with other studies that reported higher Cd values in fish livers and kidneys during summer (Jeziarska and Witeska, 2006). Higher temperatures have been shown to promote Cd uptake in fish especially in organs like livers and kidneys (Jeziarska and Witeska, 2006). Moreover, Cd in livers may be an indicator of a recent accumulation and thus may be a useful indicator of recent exposure (Çoğun et al., 2005). In a previous study made in Lebanon by Kouyoumijian et al. (1986), higher concentrations of Cd were reported during summer in 4 local species (*Mullus barbatus*, *Boops boops*, *Epinephelus alexandrinus* and *Epinephelus aeneus*), which is in accordance with the results obtained in this study.

During the wet season, the results of the PCA (Figure 3A) indicate that the elemental concentration significantly explained 63.2% of spatial variations. Livers of *S. rivulatus* from Saida were associated with higher concentration of Hg, Cd and Fe ($p < 0.05$) while the ones from Tripoli were essentially characterized by higher levels of Al, Mn, Ti and Pb ($p < 0.05$). This may be due to the difference in the patterns of riverine and waste inputs across the sampling sites and industries that take place in the area. The Northern area in Lebanon receives more rainfall than the Southern one, as the mountain chain is much more elevated in the North. Moreover, Tripoli is known for its artisanal fishing port and therefore boat maintenance activities in addition to the presence of an open dumpsite that releases leachate containing all kinds of metals (Khalil et al., 2018). Fish sampled from Beirut generally showed intermediate concentrations for most elements compared to the two other sites. *E. teres* from Tripoli and Saida in this study was associated with high levels of As and Se for both seasons which is reflected by the PCA (Figure 3-3A). In the South of Lebanon multiple agricultural activities take place in the coastal plains thus high levels of As may be associated to the use of arsenical pesticides (Jaishankar et al., 2014). In Beirut, *E. teres* was associated with higher levels of Al, Mn, Cr, Ti and Pb. The livers of *L. mormyrus* were in most cases the less contaminated (except for As and Se) (Figures 3-2 and 3-3). Indeed, in the area, several types of industries including tanneries, cement, metal, leather production and electroplating industries release different kinds of effluents that may be charge in trace elements. Tanneries and cement producing plants are known to release Cr in the environment (Oliveira, 2012).

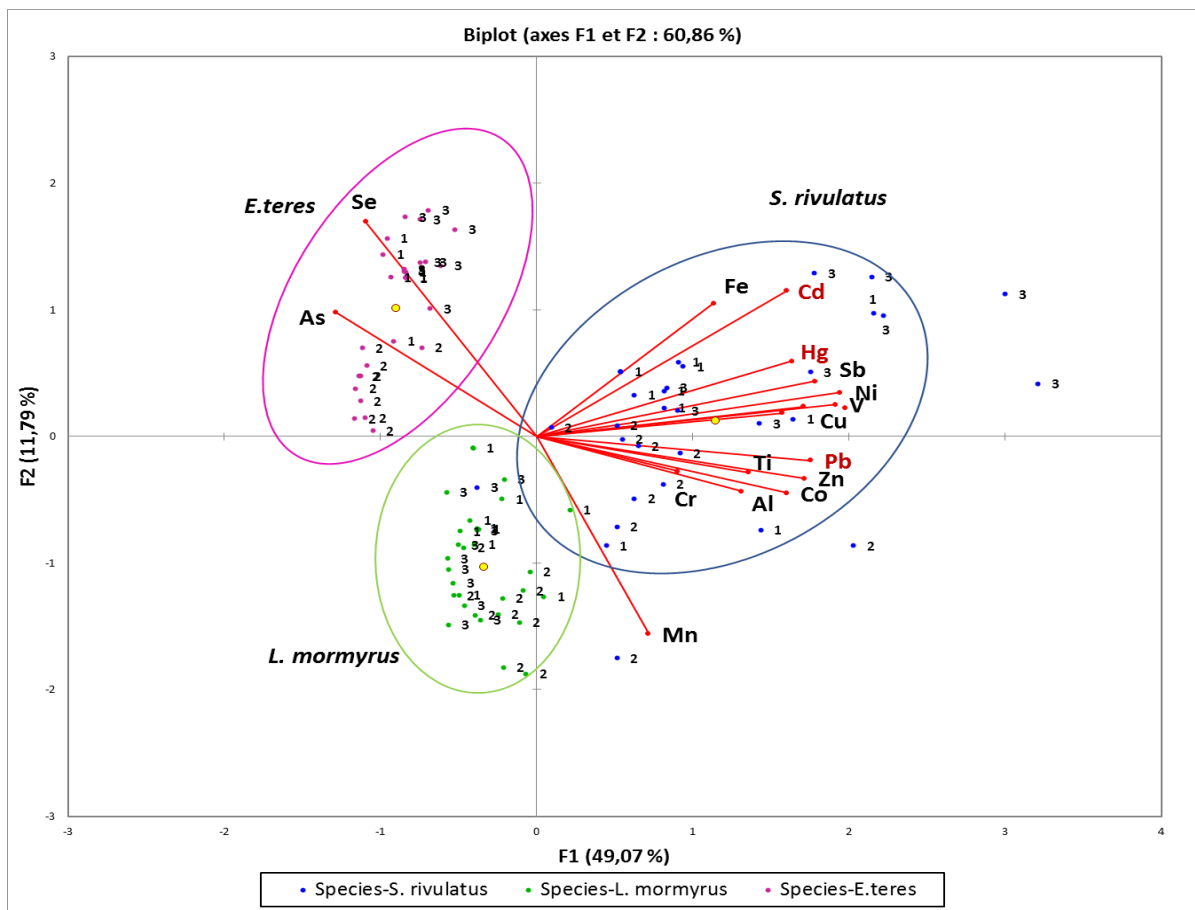
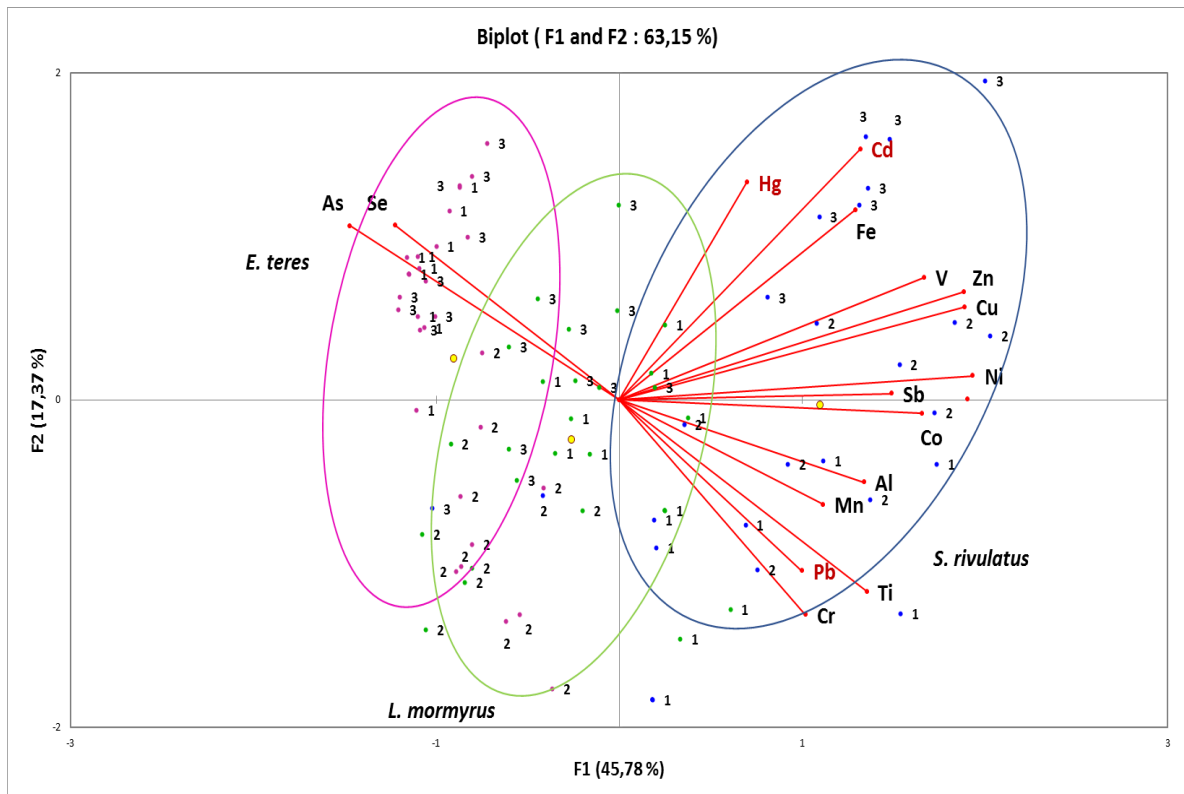


Figure 3-3 PCA showing the relationship between sites (1 = Tripoli, 2 = Beirut, 3 = Saida), species and elemental concentration for the Wet (A) and Dry (B) seasons

Electroplating and metal surface treatment generate effluents that are charged with metals such as Cd, Zn, Pb, Cu, Ti and many others which can explain the high levels of these elements found in *E. teres* from this site.

During the dry season, differences between sites were less pronounced compared to the wet season. For the dry season, observations related to *S. rivulatus* are grouped together with most contaminants reflecting the similarities between the sites with exceptions as levels of Cd ($p < 0.05$), Hg ($p < 0.05$) and Pb ($p < 0.01$) were higher in Saida (Figure 3-3B). Differences between sites concerning the rest of the elements were not statistically significant. *E. teres* from all sites had the highest levels of As and Se and the species *L. mormyrus* had the highest levels of Mn ($p < 0.01$) for the dry season. Overall, *S. rivulatus* had the highest levels of elements in its livers and particularly during the wet season (Figure 3-3A). This may be due to the fact that this species sexual maturation occurs during the wet season and more precisely from March till April which means higher metabolic rate and energy use by the cells and therefore higher metal uptake (Chovanec et al., 2003; Jezierska and Witeska, 2006; Yeldan and Avşar, 2000).

Finally, Hg, Cd and Pb are classified as toxic elements by the European Commission and the WHO (Alexander et al., 2009; EFSA, 2014, 2010; JECFA, 2017) even at trace levels. Despite the high levels of trace elements in the livers compared to other Mediterranean regions, whatever the season or location along the Lebanon coast, Hg, Cd and Pb concentrations in muscles of fish were largely below the limits set by the European Commission (Hg= 0.5 mg kg⁻¹ ww, Cd = 0.25 mg kg⁻¹ ww, Pb = 0.5 mg kg⁻¹ ww) implying that the consumption of the sampled species presents no significant risk to human health. Several studies dealing with health risk assessment due to seafood consumption have been made in the Mediterranean region (Masoud et al., 2007; Pastorelli et al., 2012; Storelli et al., 2005; Storelli and Marcotrigiano, 2001). These studies showed that there was no potential risk associated to the consumption of fishery products from the Mediterranean.

4. Conclusion

The herbivorous *S. rivulatus* fish species showed the highest levels of contamination in most cases. This may be related to many factors such as its diet which is based on algae that are known as bio accumulators of water-related contaminants while *L. Mormyrus* and *E. teres* are mostly exposed to sediments related contaminants. Seasonal differences were observed for certain elements and generally higher levels were reported during the wet season particularly for *S. rivulatus*. Spatial differences were more important during the wet season and were reflected differently depending on the species. This can be explained by the difference in rain patterns and riverine inputs among the three sites. Levels of

most elements in the liver were higher than what was reported in other Mediterranean regions suggesting that the Lebanese marine coastal zone is more largely exposed to environmental chemical contamination. Nevertheless, Hg, Cd and Pb concentrations in fish muscle were in all cases below the levels set by the European Community indicating no potential risk on human health to consume these three fish species. Due to the increasing environmental pressures on the Lebanese coast, more parameters such as landfills lixiviates and riverine water contaminants should be considered in order to assess more accurately the impact of different pollution sources on the coastal zone, and thus establish a regular monitoring program in order to prevent further environmental deterioration.

**Chapter 3 - PART 2: ASSESSMENT OF TRACE ELEMENT
CONTAMINATION AND BIOACCUMULATION IN ALGAE (*ULVA
LACTUCA*), BIVALVES (*SPONDYLUS SPINOSUS*) AND SHRIMPS
(*MARSUPENAEUS JAPONICUS*) FROM THE EASTERN
MEDITERRANEAN**

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Abstract

In this study, levels of 20 trace elements (Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Ag, Cd, Sn, Sb, Ba, Hg, Pb and U) and the respective biota-sediment accumulation factors (BSAF) were assessed in three marine species from the Eastern Mediterranean (Lebanese coast) belonging to different trophic levels of the food chain (algae, bivalves and shrimps). Multivariate analysis showed significant differences in element accumulation between seasons, sites and species thus highlighting the importance of a multi-species and seasonal approach to monitor trace metals in coastal areas. The results of the BSAF showed limited transfer from sediments to the marine organisms suggesting that the bioaccumulation of trace metals by marine organisms should be assessed by considering multiple sources of such contaminants. Pb levels (0.07-4.44 mg kg⁻¹) measured in algae and Cd (0.54-7.74 mg kg⁻¹) and Zn (42.88-972 mg kg⁻¹) levels found in the oyster studied here, were similar and sometimes higher than those found in others polluted areas indicating that the Lebanese coast is subject to environmental pollution.

Keywords: Bioaccumulation, trace elements, marine organisms, Lebanon, Eastern Mediterranean

1. Introduction

The Mediterranean Sea is known for its high biodiversity, however a significant amount of toxic chemicals that are produced by industrial wastes, agricultural and mining activities, taking place mainly in coastal areas are threatening this marine ecosystem (Lafabrie et al., 2008; Ourgaud et al., 2018; Wang et al., 2010). Toxic trace elements that constitute an important part of these discharges, are considered serious pollutants of aquatic environments mainly because of their persistence and tendency to concentrate in organisms (Afshan et al., 2014; Battuello et al., 2016).

Three marine compartments including water column, sediment and biota could be usually used to monitor spatial and temporal trends of marine pollution. Solubility, bioavailability and toxicity of metals in seawater is controlled by several factors namely the pH, the temperature, the salinity, the dissolved oxygen, suspended particles and dissolved organic matter (Chakraborty et al., 2014a; Lim et al., 2012). Moreover, metals concentration in water and sediment tend to be affected by a variety of environmental factors such as tides, winds, currents and fresh water input (Hatje, 2003; Strady et al., 2011). These processes create a stochastic pattern that may complicate the prediction of potential adverse health effects on the surrounding organisms by governing accumulation, suspension and mobilization of sediments (Meng Chuan and Yunus, 2019; Rajeshkumar et al., 2018; Ribeiro et al., 2018). It has been shown in several studies that the sediments can act both as a sink and source of contaminants in the marine environment and are thus used as a geo-marker for potential pollution sources and conditions (Meng Chuan and Yunus, 2019). Moreover, unlike water, sediments can provide reliable measurements that reflect long term conditions (Thomann et al., 1995). However, sediments don't necessarily reflect levels of contaminants in the biota (Meng Chuan and Yunus, 2019; Rainbow, 2007; Wang et al., 2010). Many marine species have been shown to accumulate metals making them good indicators for monitoring local metal pollution and their potential ecological and human risks (Campanella et al., 2001; Conti and Cecchetti, 2003). These species are able to accumulate trace metals, reaching concentration values that are many times higher than the corresponding concentrations in sea water or sediments (Conti and Cecchetti, 2003; Diop et al., 2016).

Accordingly, biomonitoring using species that belong to different taxonomic groups and trophic levels allows a better assessment of the extent of any contamination in terms of presence and relative magnitude (Campanella et al., 2001; Pérez-Cadahía et al., 2004; Rainbow, 1995a). A suitable bioindicator should *(i)* be capable of accumulating high levels of pollutants without death; *(ii)* have a sessile lifestyle in order to reflect the local pollution; *(iii)* be relevant in the food chain and *(iv)* be widespread and abundant (Zhou et al., 2008). Additionally, ease of sampling and identification are

criteria to take into consideration when targeting a bioindicator (Rainbow and Phillips, 1993). So far, mollusks, crustaceans and macrophytes have been shown to be valuable cosmopolitan bioindicators and have been used in several studies (Amiard et al., 2006; Bonsignore et al., 2018; Chakraborty et al., 2014; Chiarelli and Roccheri, 2014; Diop et al., 2015; Rouane-Hacene et al., 2015; Viarengo and Canesi, 1991). Understanding how trace metals are bioaccumulated in food webs is essential for elucidating their environmental behaviors, their ecotoxicological risks and their potential hazards to human health (Chen et al., 2000).

In Lebanon, the substantial development of urban and industrial activities along the coast and the lack of wastewater treatment strategies, result in increased inputs of chemical contaminants in the marine environment (Fakhri, 2012). In addition, waste production in Lebanon has increased significantly during the last decade, and the country has experienced a waste crisis in 2015. The regulated dumpsites could no longer manage the tremendous quantity of municipal garbage generated and, therefore, numerous illegal and open dumpsites were created throughout the country mainly along the coast (Khalil et al., 2018). Leachate effluents originating from these coastal landfills are important source of metal contamination in the surrounding aquatic environment (Stefanakis et al., 2014; Youcai, 2018) and constitute a serious risk to biota (Brand et al., 2018; Toufexi et al., 2013).

Till present, most of the studies conducted along the Lebanese coast focused on the contamination of water and sediments (Abi-Ghanem, 2008; Abi-Ghanem et al., 2011; Merhaby et al., 2018). Few studies dating back mostly to the 1980's have focused on marine organisms (Kouyoumijian et al., 1986; Kouyoumijian et al., 2001; Shiber, 1981; Shiber and Shatila, 1978; Nakhlé, 2003) while environmental pressure along the coast has been in continuous increase. To our knowledge, no studies have assessed the bioaccumulation of trace elements along the Lebanese coast, and particularly organisms belonging to different ecological niches or feeding trophic levels.

The objective of this study was to evaluate the contamination status of 20 trace elements (Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Ag, Cd, Sn, Sb, Ba, Hg, Pb and U) in three benthic marine species from different trophic levels, representative of the Lebanese coast (Eastern Mediterranean). Samples were collected at three sites in both dry and wet seasons during 2017. Moreover, the results of trace elements in sediments were used to calculate the biota-sediment transfer factor (BSAF) from sediments to biota. The species selected were the green algae *Ulva lactuca* that is widely used as a bioindicator of metal pollution, the spiny oyster *Spondylus spinosus* and the kuruma shrimp *Marsupenaeus japonicus* that are commonly consumed among the Lebanese population and that are studied for the first time in order to assess trace metal contamination along the Lebanese coast.

2. Materials and methods

Study area

The study area is located along the Lebanese coast in the Eastern Mediterranean. Three sites representative of the coastline with different levels and types of anthropogenic pressure were selected (Figure 3-4). Tripoli (site 1), is mainly known for its artisanal fishing and boat maintenance activities. Several sewage outfalls that discharge wastewater without prior treatment are identified along its coast and a controlled dumpsite in the north of the port of Tripoli, that is still receiving waste despite a decision to close it in 2012, is leading to increased waste height and uncontrolled leachate (El-Hoz, 2017). Beirut, the capital of Lebanon (site 2) is a densely populated and industrialized area. Along Beirut's coastal area, several sewage outfalls discharge polluted water in the coastal zone in addition to Beirut River that drains all kind of industrial effluents into the sea causing water quality degradation. Moreover, three dumpsites are implemented in this area with the biggest one in Dora responsible for more than half of Lebanon's heavy metals discharge in the coastal area (Fakhri, 2012). Saida (site 3), is less impacted by anthropogenic pressures compared to Beirut despite being the third densest coastal city in Lebanon. In 2014 a dumpsite was rehabilitated and recently a primary wastewater treatment plant has been established in Saida's coastal area.

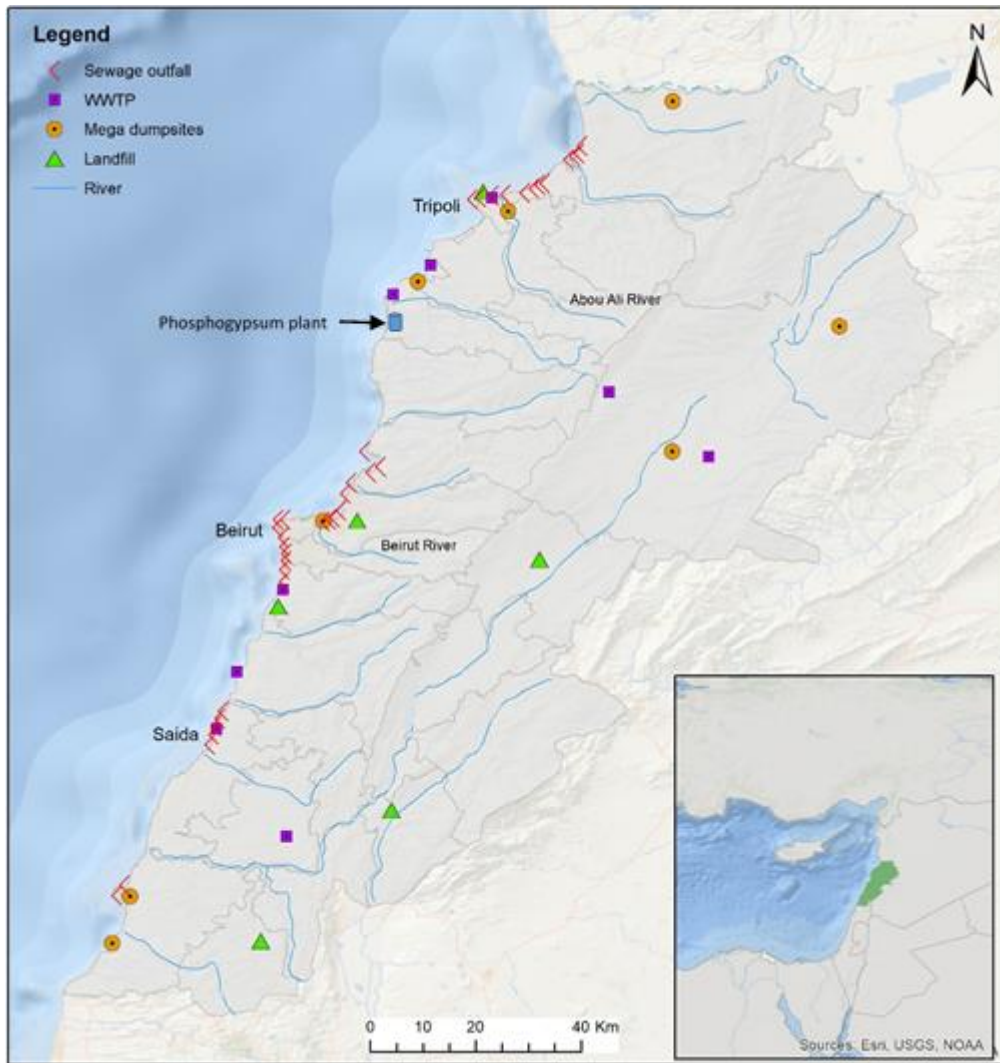


Figure 3-1 Map showing the sampling sites along the Lebanese coast (WWTP: waste water treatment plant)

Samples collection

Three marine species belonging to different positions in the food chain were chosen for this study. The green macroalgae, *Ulva lactuca* is a cosmopolitan species that has been widely used as bioindicator (Diop et al., 2016; Ho, 1990; Kaimoussi et al., 2004; Kamala-Kannan et al., 2008). The spiny oyster, *Spondylus spinosus* is a Lessepsian species that has become very well established in the Eastern Mediterranean, has gained commercial importance and is frequently consumed among the Lebanese population. This bivalve lives attached to rocky substrate and feeds on particles from the water column (Aksit et al., 2013). The kuruma shrimp, *Marsupenaeus japonicus* is also a Lessepsian species that has become highly prized in the Mediterranean. This species inhabits sandy mud and sandy bottoms and feeds on algae, small fish and bivalves (Galil, 2006; Quigley et al., 2013b). These three species were also chosen because they live at different depths and can thus represent the levels of contamination of

different habitats along the coast. Algae and bivalves were collected by hand picking in the subtidal zone at depth between 0.5 and 1 m and between 10 and 20 m respectively. Shrimps were sampled with trammel nets at an average depth of 40 m. The species were collected at each site and at each season (wet: December-March and dry: June-September) except for *U. lactuca* that wasn't found at Beirut and Saida during the dry season and *M. japonicus* that was not found at Beirut during the wet season and at Saida during the dry season. The samples were taken immediately to the laboratory on ice. Each individual was measured (nearest 1 mm) and weighed (nearest 0.1 g) in order to standardize individual size between sites. Ten individuals of each species were selected for the analysis. Bivalves and shrimps' soft tissues were removed under a laminar flow hood using stainless steel scalpel and then stored in low-density polyethylene vials at -20 °C. Algae were rinsed with distilled water to remove substrate residues. Samples were then lyophilized at -100 °C until constant weight and stored at room temperature until analysis. Additionally, sediment samples were collected by diving at each site from the coastal area at depths between 5 and 20 m.

Samples analysis

Bivalves, shrimps' soft tissues and algae were analyzed using an accredited method (French Accreditation Comity-COFRAC) described elsewhere (Chevallier et al., 2015). Samples were lyophilized and their moisture content determined. Aliquots of 0.3 g to 0.5 g of each sample were weighed in quartz digestion vessels before adding 3 mL of HNO₃ 67 % (m/m) (suprapur grade, VWR chemicals, Prolabo, France). Three mL of ultrapure water were added and then the mineralization procedure was carried out using a closed microwave system (Multiwave 3000 from Anton Paar, France). Sample digests were transferred to polypropylene tubes and a solution containing a mix of internal standards, scandium (Sc), indium (In), bismuth (Bi), rhenium (Re) and yttrium (Y) was added at a final concentration of 2 µg L⁻¹. The mixtures were then added up with ultrapure water to a final volume of 50 mL. In order to reduce memory effect of mercury, a solution of gold (Au) was added at a final concentration of 20 µg L⁻¹. Samples were then analyzed using an ICP-MS 7700 (Agilent Technologies, Courtaboeuf, France).

Sediments analysis procedure

Dry sediment (500 mg) was totally digested in a heating block (HotBlock® SC100 Digestion System), with a concentrated acid mixture of (HF/HNO₃/HCl 10:1:3, v/v/v) following a procedure described by Ouddane et al [1999], the sample was weighed into PTFE digestion cups, when the sediments were totally dissolved at 120 °C, the solution was then diluted with ultrapure Milli-Q+ water (18.2 MΩ cm) and filtered through a 0.45 µm cellulose acetate membrane. The metal concentrations were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES, Agilent 5110, dual view) for

the major element (Al, Fe) and by ICP-MS (Varian, 820) for the trace elements (Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, V, Zn). Procedural blanks and certified reference materials (CRMs) MESS-3, HISS-1 and PACS-2 (National Research Council of Canada) were prepared using the same analytical procedure and reagents, and analysed for quality assurance/quality control. All results were >90% comprised in the confidence intervals.

Quality control procedure

In order to ensure the reliability of the results, several internal quality controls (IQCs) and associated criteria were used. Each run included a standard calibration, three blanks, three CRMs, two different spiked sample solutions, 33 samples including 2 samples analyzed in duplicate and a mid-range standard analyzed for every eight samples and at the end of the sequence. The set criteria were as follows: calibration ($r^2 > 0.995$), blanks (values < limit of quantification (LOQ)), internal standards (values within 70 and 130% of the target value), mid-range standards (values within 80 and 120% of the target value), spiked standard solutions (spike recovery within 70 and 130% of the theoretical spiked standard value), CRMs (Z-score < ± 2) and duplicates (acceptable if the relative standard deviation (RSD) $\leq 20\%$ when mean value $\geq 5 \times \text{LOQ}$ or RSD $\leq 40\%$ when mean value $\geq \text{LOQ}$ and < $5 \times \text{LOQ}$). When acceptance criteria were not met, the results were discarded, and the samples were re-analyzed.

Data treatment

Statistics were performed using R studio Setting a level of significance at $\alpha=0.05$. As the data was not normally distributed, Kruskal-Wallis non-parametric test and Mann Whitney U-test for pairwise comparison were used to evaluate spatial and inter-specific differences as well as seasonal variations in elemental concentration. Principal component analysis (PCA) was used after $\log(x+1)$ transformation of the data to explore relationships between sites, species and elemental concentration for the two sampling seasons. Left censored data that are < LOQ and < LOD were excluded from statistical analysis (23 % of the data). All results were reported in mg.kg^{-1} dry weight (dw).

The biota-sediment accumulation factor (BSAF) is defined as the ratio between the concentration of a given element in an organism (C_o) and its concentration in sediment (C_s) (both concentrations are expressed in mg kg^{-1} dry weight) and was calculated as follows (Thomann et al., 1995):

$$BSAF = C_o/C_s$$

Bioaccumulation was confirmed when BSAF was > 1 (Thomann et al., 1995).

3. Results and discussion

The concentrations of 15 trace elements are presented in Table 3-1 and supplementary Annex 4. Ag, Sn, Sb, Ba and U were not detected or showed very low detection frequencies in the tissues of the selected marine organisms (< 20%) and thus were not presented in this study.

Interspecific variability of trace elements concentration

Regardless of the season or site, Al and Fe showed the highest concentrations in algae (378 ± 413 mg.kg⁻¹ dw and 515 ± 473 mg.kg⁻¹ dw, respectively), while Zn (124 ± 162 mg.kg⁻¹ dw) and As (35.2 ± 27.42 mg.kg⁻¹ dw) were the highest in bivalves and Cu was the highest in shrimps (30.91 ± 11.96 mg.kg⁻¹ dw) (Table 3-1). High standard deviations were observed indicating a strong variability among the levels in a same species. Trace element concentrations showed significant differences between all species ($p < 0.001$). As shown in the multivariate analysis (Figure 3-5) the elemental concentration

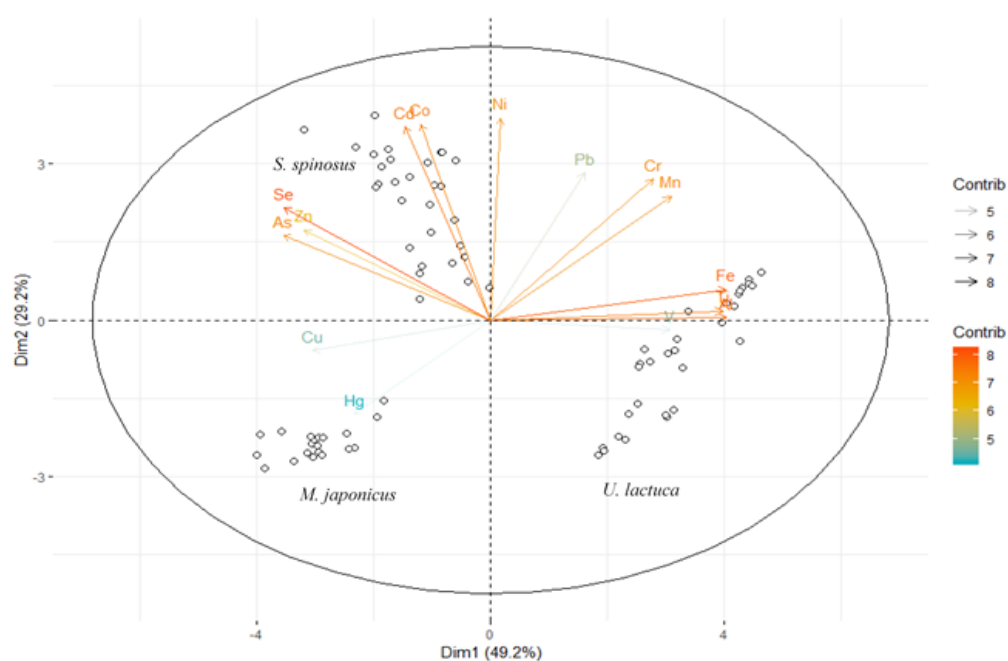


Figure 3-2 PCA showing the relationship between species and trace metals concentration (mg kg⁻¹)

explained 79% of the interspecific variation. Each species showed affinity to a certain group of metals. For instance, *U. lactuca* showed a significantly higher levels of metals with the following order: Fe > Al > Ti > Mn > V > Pb > Cr ($p < 0.001$), while the spiny oyster had the highest levels of Zn > As > Ni > Se > Cd > Co ($p < 0.001$) and the kuruma shrimp had the highest levels of Cu and Hg ($p < 0.001$). Similar trends of concentrations were also recorded in previous studies (Table 3-2) (Akcali and Kucuksezgin, 2011; Circunciso et al., 2018; Kaimoussi et al., 2004; Shams El-Din et al., 2014; Villares et al., 2002).

Table 3-1 Mean concentrations (C) and standard deviations (SD) of trace elements (mg kg⁻¹ dw) in sampled marine organisms and sediments.

Species	n		Al	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Cd	Hg	Pb
<i>U. lactuca</i>	40	C	378	19.4	3.53	1.08	29.4	516	0.34	2.13	2.30	5.12	4.78	0.34	0.05	0.04	1.04
		SD	413	12.7	2.77	0.90	37.8	473	0.38	0.63	1.49	2.29	2.60	0.15	0.03	0.02	1.03
<i>S. spinosus</i>	60	C	11.1	1.43	0.21	0.54	4.51	27.6	0.86	6.47	8.50	124	35.2	3.00	3.00	0.07	0.89
		SD	13.4	1.54	0.18	0.14	2.98	24.5	0.46	3.27	6.45	162	27.4	0.94	1.33	0.04	0.87
<i>M. japonicus</i>	30	C	2.75	0.68	0.05	0.06	0.64	7.77	0.05	0.33	30.9	61.2	28.7	1.77	0.02	0.15	0.04
		SD	2.73	0.69	0.09	0.04	0.38	7.06	0.06	0.40	12.0	9.83	23.6	0.49	0.02	0.15	0.04
Sediments ^a		C (Tripoli)	3403	-	26.6	29.7	228	13287	5.19	12.0	6.29	21.5	-	-	0.32	0.120	17.3
		C (Beirut)	4305	-	38.2	22.9	228	22921	7.13	15.1	3.85	24.3	-	-	0.53	0.07	7.25
		C (Saida)	2592	-	4.75	5.04	175	1550	0.97	3.58	2.02	4.99	-	-	0.13	0.07	3.35

^aSD for sediments analysis is not available (n=1).

Marine organisms uptake trace elements from solution and/or particulate matter. The latter pathway may consist of the ingestion of suspended detritus, food preys or even deposited organic matter (Amiard et al., 2006; Rainbow and Phillips, 1993). The bioaccumulation of trace metals in marine organisms is also related to several parameters contributing to inter and intraspecific variability, namely biotic factors such as the size, age, sex, and metabolism in addition to the position of the organism in the food chain (Jakimska et al., 2011a). For example, macrophytic algae respond essentially to dissolved metal sources and are thus good bioindicators of water quality. They are also known for their exceptional capacity to accumulate essential and trace elements (Farias et al., 2018). That capacity is actually related to the cell walls of green macroalgae and particularly in *Ulva* genus. The cell walls are composed of ulvans that are sulfated heteropolysaccharides with ionic binding properties due to their sulfate groups (Alves et al., 2013; Cunha and Grenha, 2016). More specifically, *U. lactuca* species is known to have a high metal accumulation capacity with particular affinity to Mn, Fe, Cu, Zn, Pb and Cd (Ghoneim et al., 2014; Ho, 1990). It has been used in bioremediation for Cd, Pb and Hg removal from aqueous solutions (Ghoneim et al., 2014; Henriques et al., 2017). Studies dealing with *U. lactuca* usually report higher levels of Fe (Abdallah and Abdallah, 2008) that seems to be dominant in Chlorophyta in general, while Rhodophyta and Phaeophyta (red and brown algae, respectively) tend to accumulate higher levels of Mn (Circuncisão et al., 2018). When comparing to other studies conducted in the Mediterranean area (Table 3-2), levels of trace elements in *Ulva* from our study were generally lower and even closer to values recorded in relatively “uncontaminated” areas, however Fe was higher (Campanella et al., 2001; Conti and Cecchetti, 2003). The dominant pattern of accumulation reported in the green algae such as the higher levels of Fe observed when compared to other essential elements such as Zn and Cu, may be explained by several factors. Fe is actually known to be an essential element for the normal growth of marine plants (Storelli and Marcotrigiano, 2001b). Algal species are able to biomagnify Fe from the surrounding water while industrial effluents continuously increase Fe inputs in the marine environment (Akcali and Kucuksezgin, 2011) which may explain the higher levels observed in our study. It is important to mention that levels of Pb in *U. lactuca* exhibited a decrease when compared to a study made in Beirut in 1977 (Shiber, 1980). Actually, a decreasing trend of Pb in the marine environment has been reported, since a regulation limiting the use of leaded gasoline consumption was established in 1970’s (Storelli, 2008). However, the difference between the techniques of detection used back then and the recent methods used nowadays, should be taken into consideration.

The levels of trace metals in the spiny oyster from the present study were found to be mostly lower than concentrations found in bivalves in other countries around the Mediterranean (Table 3-2). The levels in the spiny oyster were lower than the ones reported from the Iskenderun Bay which is considered as a polluted area in Turkey (Türkmen and Türkmen, 2005) with few exceptions such as Fe, Cu and Zn that are essential elements. Despite the fact that the spiny oyster satisfies all the criteria for a good bioindicator (Aksit et al., 2013), it hasn't been commonly used in biomonitoring studies in the Mediterranean. It must probably be due to the fact that this species lives attached to rocky substrate in the subtidal zone at a depth of more than 10 m and therefore its sampling is difficult. Species that are easy to sample and identify such as *Patella caerulea* and *Brachidontes variabilis* have been the subject of several studies around the Mediterranean (Table 3-2). As a filter feeder, living a little further from the coast compared to other bivalve candidates, *S. spinosus* would provide information about the contamination levels present in the surrounding water in the subtidal zone. Levels of Cd found in the spiny oyster were close to the ones found in *P. caerulea* and *B. variabilis* in a previous study made on the Lebanese coast (Table 3-2; Nakhlé et al., 2006). The concentrations of Cu, Cr, Cd and Pb were lower than the ones found in *M. galloprovincialis* and *P. Caerulea* collected from the Tyrrhenian Sea, Italy and concentrations of Fe, Cu, Cd, Zn and Mn were lower compared to concentrations found in *Donax trunculus* (bivalve) from the Egyptian coast (Table 3-2). Bivalves are known for their ability to accumulate metals in their tissues (Zuykov et al., 2013). They are filter-feeding organisms with low biotransformation capacities that accumulate the bioavailable fraction of the contaminant from water at higher levels than those in the ambient environment and are thus used as sentinel species to monitor pollution in coastal waters (Storelli and Marcotrigiano, 2001b; Türkmen and Türkmen, 2005). High levels of Zn and Cu in mollusks are often attributed to their essentiality. In general, ranges of trace metals in the spiny oyster in this study fall between the levels observed in contaminated and relatively uncontaminated areas suggesting a relatively moderate level of trace metal contamination in this species when compared to oyster, mussels and limpets from other regions around the Mediterranean (Table 3-2). However, caution must be taken as these species do not share neither the same habitat of *S. spinosus* nor the same feeding habits. *P. caerulea* also known as the Mediterranean limpet, is a gastropod that inhabits the medio littoral zone and grazes on phytoplankton and macroscopic algae. *B. variabilis* also known as the variable mussel, inhabits shallow waters (at sea level or just below) and lives attached to rocky substrate (Nakhlé et al., 2006).

The kuruma shrimp had the highest levels of Cu compared to ulva and spondylus. Crustaceans are known to have hemocyanin which is an oxygen carrying molecule containing a copper binding site

found in mollusks and arthropods, which explains higher levels of these molecules in crustaceans compared to algae and bivalves (Downs et al., 2016; Rainbow and Luoma, 2011). Also known for being deposit feeders, crustaceans are more exposed to sediment related contaminants such as As which is more abundant in sediments than in the water column (Kalantzi et al., 2017). Few studies have measured trace element concentration in the shrimp *M. japonicus*, particularly in the Mediterranean Sea (Ayas, 2016). More studies were conducted on the caramote prawn, *Penaeus kerathurus* (Table 3-2). Levels of trace elements in our study are in the same range as the ones found in other studies except for Cu and Zn that were around 2 folds higher than the ones found in species collected from other sites (Table 3-2). These two elements are essential as mentioned above however anthropogenic inputs may increase their levels.

Table 3-2 Comparison of trace metals concentration (mg kg⁻¹ dry weight) measured in marine organisms from the Eastern Mediterranean in this study and other previous studies (minimum and maximum levels in each group are marked in bold).

Species	Location	Al	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Cd	Hg	Pb	Reference
<i>U. lactuca</i>	Ras Beirut (Lebanon)	-	-	-	< 7.5 - 16.3	-	258 - 595	-	9.4 - 43.1	24.4 - 52.5	41.3 - 206	-	-	< 0.8 - 2.3	-	< 7.5 - 37.5	(Shiber, 1980)
	Tyrrhenian Sea (Central Italy)	-	-	-	1.39 - 2.06	-	-	-	-	4.9 - 6.4	-	-	-	0.13 - 0.21	-	1.67 - 2.28	(Conti and Cecchetti, 2003)
	Eastern Harbour (Egypt)	-	-	-	-	73.9 ± 4.8	514.5 ± 2.1	7.08 ± 1.3	-	14.5 ± 4.7	63.1 ± 3.2	-	-	1.84 ± 0.93	-	-	(Abdallah and Abdallah, 2008)
	El Mex Bay (Egypt)	-	-	-	-	33.3 ± 3.9	709 ± 12.7	6.58 ± 1.4	-	7.24 ± 2.30	27.4 ± 8.3	-	-	0.73 ± 0.24	-	-	(Yaich et al., 2011)
<i>U. fasciata</i>	Monastir (Tunisia)	-	-	-	-	13	410	-	-	8	68	-	-	-	-	12.6	(Yaich et al., 2011)
	Abou Qir Bay (Egypt)	-	-	-	-	16.0	65.2	1.12	3.64	2.96	12.6	-	-	1.27	0.06	2.84	(Shams El-Din et al., 2014)
<i>Ulva sp.</i>	Gulf of Thessaloniki (Greece)	-	-	-	16.9 ± 0.69	14.4 ± 3.72	132 ± 48.0	-	1.70 ± 0.12	9.25 ± 2.88	11.3 ± 1.02	116 ± 4.3	0.59 ± 0.41	2.89 ± 0.01	-	6.27 ± 1.79	(Malea and Kevrekidis, 2014)
	Gulf of Thessaloniki (Greece)	-	-	-	15.8 ± 1.09	13.8 ± 2.95	37.3 ± 7.08	-	0.84 ± 0.17	7.41 ± 0.69	9.15 ± 1.73	122 ± 10.3	1.50 ± 0.47	4.83 ± 1.09	0.034 ± 0.004	-	4.62 ± 1.19
<i>U. rigida</i>	Gulf of Thessaloniki (Greece)	-	-	-	16.24 ± 1.19	9.38 ± 1.49	37.92 ± 10.00	-	0.56 ± 0.13	6.44 ± 0.55	7.39 ± 0.89	122 ± 14.11	1.45 ± 0.25	2.71 ± 0.77	-	3.06 ± 0.67	(Malea et al., 2015)
<i>Ulva sp.</i>	Çanakkale/City (Turkey)	-	-	-	0.78 ± 0.20	-	198 ± 19.4	-	-	8.51 ± 0.86	50.1 ± 6.00	-	-	54.2 ± 13.7	53.2 ± 3.17	5.65 ± 0.87	(Akcali and Kucuksegin, 2011)

	Izmir/Foça (Turkey)	-	-	-	1.75 ± 0.42	-	130 ± 26.9	-	-	13.9 ± 3.11	67.8 ± 16.0	-	-	14.9 ± 1.89	48.4 ± 11.8	1.75 ± 0.42	
	Izmir/Bostanlı (Turkey)	-	-	-	1.95 ± 0.51	-	232 ± 28.6	-	-	8.29 ± 0.21	40.4 ± 6.09	-	-	18.7 ± 2.27	59.5 ± 14.4	1.95 ± 0.51	
	Izmir/Narlıdere (Turkey)	-	-	-	4.22 ± 1.53	-	307.5 ± 73.6	-	-	8.21 ± 1.21	61.3 ± 6.52	-	-	22 ± 6.24	199 ± 62	4.22 ± 1.53	
	Izmir/Urla (Turkey)	-	-	-	0.94 ± 0.12	-	146.1 ± 34.2	-	-	7.78 ± 0.35	81 ± 0.47	-	-	24.9 ± 7.88	73 ± 12.9	0.94 ± 0.12	
<i>U. lactuca</i>	Lebanese Coast	66.5 - 1535	2.28 - 43.80	0.25 - 9.54	0.21 - 3.74	1.49 - 128	53.6 - 1714	0.03 - 1.31	1.36 - 3.99	0.24 - 5.64	1.20 - 8.89	1.10 - 10.9	0.17 - 0.72	0.01 - 0.10	0.03 - 0.06	0.07 - 4.44	Present study
	Sicilian Coast (Italy)	-	-	-	0.19 - 0.46	-	-	-	-	1.21 - 2.35	3.5 - 14.6	-	-	3.30 - 6.30	-	0.14 - 1.52	(Campanella et al., 2001)
<i>Patella Caerulea</i>	Sicilian Coast (Italy)	-	-	-	0.10 - 1.01	-	-	-	-	0.47 - 3.79	2.2 - 19.1	-	-	1.7 - 11.8	-	0.06 - 2.18	(Cubadda et al., 2001)
	Tyrrhenian Sea (Italy)	-	-	-	0.72 - 0.96	-	-	-	-	10.2 - 19.2	-	-	-	2.89 - 4.06	-	0.51 - 1.50	(Conti and Cecchetti, 2003)
	Lebanese Coast	-	-	-	-	-	-	-	-	-	-	-	-	0.6 - 8.7	0.14 -0.2	0.5 - 2.6	(Nakhlé et al., 2006)
	Iskenderun (Turkey)	-	-	-	-	-	61.23 - 213	0.15 - 0.33	0.53 - 1.60	1.38 - 5.58	6.50 - 13.71	-	-	0.41 - 0.68	-	0.13 - 0.70	(Yüzereroğlu et al., 2010)
	Yumurtalık (Turkey)	-	-	-	-	-	36.56 - 169	0.05 - 0.14	0.39 - 1.05	1.09 - 2.12	3.70 - 9.84	-	-	0.24 - 0.44	-	0.05 - 0.31	
<i>Mytilus galloprovincialis</i>	Tyrrhenian Sea, Central Italy	-	-	-	0.46 - 1.31	-	-	-	-	5.51 - 11.50	-	-	-	0.32 - 0.49	-	1.67 - 2.49	(Conti and Cecchetti, 2003)

	Cala Iris Al Hoceima, (Morocco)					340 - 673	N.D.	2.03 – 3.82	5.28 – 6.16	160 - 164			0.65 – 0.85		N.D.	(Azizi et al., 2018)	
	Izmir Bay (Turkey)	-	-	-	-	1.23 – 2.0	-	1.09– 1.84	0.71 – 1.72	18.7– 30.0			-	-	-	(Kontas, 2012)	
<i>Monodonta turbinata</i>	Sicilian Coast (Italy)	-	-	-	0.13 – 0.54	-	-	-	-	5.7 – 21.5	14 - 55	-	-	0.58 – 3.35	0.15 – 0.66	(Campanella et al., 2001)	
<i>Brachidontes variabilis</i>	Lebanese Coast												1.08 – 4.0	0.2 – 0.6	1.0 – 2.5	(Nakhlé et al., 2006)	
<i>Donax trunculus</i>	Eastern Harbour (Egypt)	-	-	-	-	21.21 ± 0.23	570.07 ± 8.2	-	-	12.57 ± 0.22	73.99 ± 4.45	-	-	3.93 ± 0.20	-	-	(Abdallah and Abdallah, 2008)
<i>S. spinosus</i>	Iskenderun Bay, (Turkey)	66.0 - 113	-	-	9.78 – 18.1	24.8 – 30.6	64.4 - 251	3.47 – 9.85		14.5 – 20.4	64.8 – 82.8			4.96 – 15.1	-	59.9 – 132	(Türkmen and Türkmen, 2005)
<i>S. spinosus</i>	Lebanese Coast	0.77 – 85.1	0.33 – 7.99	0.04 – 0.67	0.30 – 0.93	0.75 – 14.76	6.78 - 136	0.27 - 2.36	0.86 - 13.9	1.06 - 31.03	42.88 - 972	9.52 - 186	1.42 - 5.25	0.54 - 7.74	0.03 - 0.15	0.12 - 3.54	Present study
<i>Penaeus kerathurus</i>	Izmir Bay, Turkey (Eastern Mediterranean)	-	-	-	-	-	4.45	-	-	6.14	14.62	-	-	0.20	-	0.96	(Turkmen, 2012b)
<i>P. kerathurus</i>	Izmir Bay, Turkey	-	-	-	-	0.57 ± 0.06	6.70 ± 3.63	-	2.11 ± 0.50	4.75 ± 1.29	10.8 ± 4.88	-	-	-	-	-	(Kontas, 2012)
<i>P. semisulcatus</i>	Mersin Bay				2.81 – 5.80									1.05 – 1.40	5.01 – 10.80	(Ayas, 2016)	
<i>P. semisulcatus</i>	Iskenderun Bay	-	-	-	40.75 – 73.3	-	12.6 - 28.5	-	-	21.9 – 57.5	21.5 – 36.6	-	-	10.0 – 25.8	-	-	(Firat et al., 2008)

<i>P. semisulcatus</i>	Iskenderun Bay	-	-	-	-	-	49.7 – 62.2	-	-	30.1 – 31.2	70.2 – 83.6	-	-	1.0 – 1.9	-	5.7 – 8.5	(Kargin et al., 2001)
<i>P. japonicus</i>	Turkish coast (North Eastern Mediterranean)	-	-	-	0.97 – 1.30	-	52.3 – 113	-	2.19 – 3.37	15.71 – 22.6	18.0 – 24.7	-	-	0.64 – 0.82	-	3.70 – 5.40	(Canli et al., 2001b)
<i>Parapenaeus longirostris</i>	Mersin Bay	-	-	-	1.38 – 3.44	-	-	-	-	-	-	-	-	0.44 – 0.67	-	1.99 – 4.40	
<i>Metapenaeus monoceros</i>	Mersin Bay	-	-	-	2.38 – 4.38	-	-	-	-	-	-	-	-	0.55 – 0.97	-	7.29 – 13.4	(Ayas, 2016)
<i>M. japonicus</i>	Mersin Bay	-	-	-	2.40 – 5.37	-	-	-	-	-	-	-	-	0.80 – 1.10	-	5.55 – 8.30	
<i>M. japonicus</i>	Lebanese Coast	0.70- 10.5	0.20- 3.54	0.01- 0.56	0.02 – 0.17	0.34 – 2.57	2.95 – 37.48	0.01– 0.38	0.11 – 1.15	14.20– 80.4	51.39- 116	5.98- 97.41	1.13- 3.83	0.00- 0.10	0.03- 0.79	0.01 – 0.21	Present study

Seasonal and spatial variations

Trace metals levels showed seasonal and spatial variation patterns in all species (Table 3-3 and Annex 4). The sites chosen in this study have a different pluviometry pattern during the year. The North of Lebanon receives more rain than the South and during the wet season surface runoff and riverine inputs increase with rainfall and the snow melting which affects the water's physico-chemical properties. Parameters such as pH, salinity, water hardness and oxygen concentration revealed significant variations with seasons (Abboud-Abi Saab et al., 2004b; Fakhri and Romano, 2005) and these conditions are known to govern the bioavailability of trace metals in the marine environment and thus their bioaccumulation by marine organisms (Rainbow, 1995a).

Levels of Al, V, Cr, Mn, Fe, Co, Ni, As, Se and Pb were significantly higher in algae during the dry season in algae ($p < 0.05$) (Table 3-3). Such pattern was previously observed in *U. lactuca*, with increasing trace metals concentrations from winter to summer (Kaimoussi et al., 2004). Since the intake of metals in algae depends on their concentration in the surrounding water (Ghoneim et al., 2014; Rybak et al., 2012), higher concentration in summer could be attributed to the increased evaporation and decreased rainfall especially for species living in shallow waters like ulva (Rajeshkumar and Li, 2018; Wang et al., 2012). Higher trace metal accumulation during summer can also be explained by the existence of a higher metabolic rate during this season (Villares et al., 2002). Finally concerning the spatial variation, levels of Al ($779 \pm 611 \text{ mg kg}^{-1}$), As ($7.55 \pm 2.15 \text{ mg kg}^{-1}$), Cr ($1.09 \pm 0.13 \text{ mg kg}^{-1}$) and Pb ($1.42 \pm 0.23 \text{ mg kg}^{-1}$) were significantly higher in the algae collected from Tripoli compared to Beirut and Saida ($p < 0.05$) (Annex 4). Indeed, the accumulation pattern of metals by algae depend on the developmental stage and the physiology of the algae (Farias et al., 2018). It has been reported that higher metal levels were found during the growth period of the algae (Kaimoussi et al., 2004; Villares et al., 2002). Trace elements showed significant spatial variations ($p < 0.05$) in the alga. For most elements, the highest levels were found in Tripoli. More particularly, levels of Al ($779 \pm 611 \text{ mg kg}^{-1}$), As ($7.55 \pm 2.15 \text{ mg kg}^{-1}$), Cr ($1.09 \pm 0.13 \text{ mg kg}^{-1}$) and Pb ($1.42 \pm 0.23 \text{ mg kg}^{-1}$) were significantly higher at this site. Pb levels are close to the ones obtained in relatively

Table 3-3 Mean concentration \pm standard deviation (mg kg^{-1} dw) of trace elements measured in marine organisms sampled during the wet (S1) and dry (S2) seasons.

Species	Season	Al	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Cd	Hg	Pb
<i>U. lactuca</i>	S1	245 \pm 214*	18.4 \pm 14.1	3.05 \pm 2.98*	0.63 \pm 0.36*	8.66 \pm 4.92*	359 \pm 274*	0.14 \pm 0.07*	2.35 \pm 0.65*	2.47 \pm 1.72	5.18 \pm 2.65	3.86 \pm 2.09*	0.27 \pm 0.11*	0.06 \pm 0.03	N.D	0.65 \pm 0.60*
	S2	779 \pm 610	22.3 \pm 8.16	4.98 \pm 1.51	2.43 \pm 0.71	91.7 \pm 23.2	2443 \pm 1216	0.95 \pm 0.24	1.72 \pm 0.36	1.82 \pm 0.25	4.92 \pm 0.80	7.55 \pm 2.15	0.52 \pm 0.09	0.04 \pm 0.01	0.04 \pm 0.02	6.87 \pm 12.2
<i>S. spinosus</i>	S1	14.9 \pm 16.4*	2.00 \pm 1.82*	0.23 \pm 0.18	0.54 \pm 0.17	4.72 \pm 2.86	6.98 \pm 5.44*	0.95 \pm 0.48	5.59 \pm 3.16	7.56 \pm 6.75	186.80 \pm 212*	40.98 \pm 32.55*	3.19 \pm 1.03	3.2 \pm 1.51	0.14 \pm 0.02	0.83 \pm 0.72
	S2	7.94 \pm 8.27	1.00 \pm 1.06	0.21 \pm 0.18	0.55 \pm 0.11	4.51 \pm 3.14	19.73 \pm 10.37	0.77 \pm 0.44	7.31 \pm 3.19	9.45 \pm 6.1	62.98 \pm 20.9	31.00 \pm 20.0	2.75 \pm 0.80	2.74 \pm 1.10	0.06 \pm 0.03	1.02 \pm 1.00
<i>M. japonicus</i>	S1	2.93 \pm 2.85	1.27 \pm 1.14*	0.08 \pm 0.12	0.15 \pm 0.03	0.68 \pm 0.49	6.98 \pm 5.44	0.08 \pm 0.08*	N.D	32.72 \pm 14.6	63.4 \pm 12.73	36.0 \pm 25.0*	1.92 \pm 0.54*	0.03 \pm 0.03	0.12 \pm 0.05	0.03 \pm 0.01
	S2	2.59 \pm 9.31	0.48 \pm 0.76	0.02 \pm 0.03	0.05 \pm 0.02	0.60 \pm 0.41	8.55 \pm 10.03	0.03 \pm 0.02	0.17 \pm 0.04	29.1 \pm 9.39	58.9 \pm 6.02	21.43 \pm 17.84	1.61 \pm 0.35	0.01 \pm 0.09	0.17 \pm 1.20	0.05 \pm 0.15

^a the symbol *indicates significant differences between seasons $p < 0.05$

polluted areas (Table 3-2) (Akcali and Kucuksezgin, 2011; Malea et al., 2015; Malea and Kevrekidis, 2014). These levels can be attributed to the leachate from Tripoli's dumpsite, as high levels of these elements were detected in the leachate (Khalil et al., 2018).

The kuruma shrimp showed seasonal variation for Ti, Co, As, and Se with higher levels during the wet season. For the rest of the elements, no seasonal changes were recorded, and values were almost the same in both seasons. *P. japonicus* mainly spawns between spring and summer months (May- July) at temperatures of 24- 28 °C in the Eastern Mediterranean (Pourang et al., 2004; Yilmaz and Yilmaz, 2007) and the increase in body tissue may be involved in the lower levels of elements observed during the dry season. . Significant spatial variation ($p < 0.05$) was only observed for As, Co, Cd, Cu, Pb, Se and V. The shrimps showed less seasonal and spatial variations compared to the alga and the oyster. This could be due to the fact that the shrimp lives further from the coastal zone and thus may be less subjected to dynamics of the coastal area (Stehr et al., 2004) knowing that the kuruma shrimp was caught at an average depth of 40 m but is also known to live down to 90 m of depth (Quigley et al., 2013b).

The spiny oyster was chosen for the multivariate analysis as it was available at all sites and seasons and thus allowing to have a clearer idea about the spatial variations related to each season. For each season, the oyster showed a different spatial accumulation pattern (Figure 3-6). Elemental concentration explained 65% of spatial variability during the wet season (Figure 3-6A) and 73 % during the dry season (Figure 3-6B). Significant seasonal differences in the spiny oyster were found for Al, Ti, Fe, Zn and As ($p < 0.05$) with higher levels found during the wet season. Similar finding with higher values during the winter season were found in the blue mussel *M. galloprovincialis* from the Moroccan coast (Azizi et al., 2018). Higher values recorded during the wet seasons are often associated with increased inputs of trace metals from land based sources and their precipitation in the water due to the increased rain (Zuluaga Rodríguez et al., 2015b). Moreover, the spiny oyster undergoes sexual maturation and spawns between June and August (Shabtay et al., 2015) which means that an increase in body tissues is probably contributing to the lower levels of elements observed during the dry season.

Concerning the spatial variation, *S. spinosus* from Tripoli were characterized by higher levels for most trace elements during the wet season while samples from Saida showed the highest concentrations during the dry season. This is mainly due to the differences in rainfall patterns and the change of

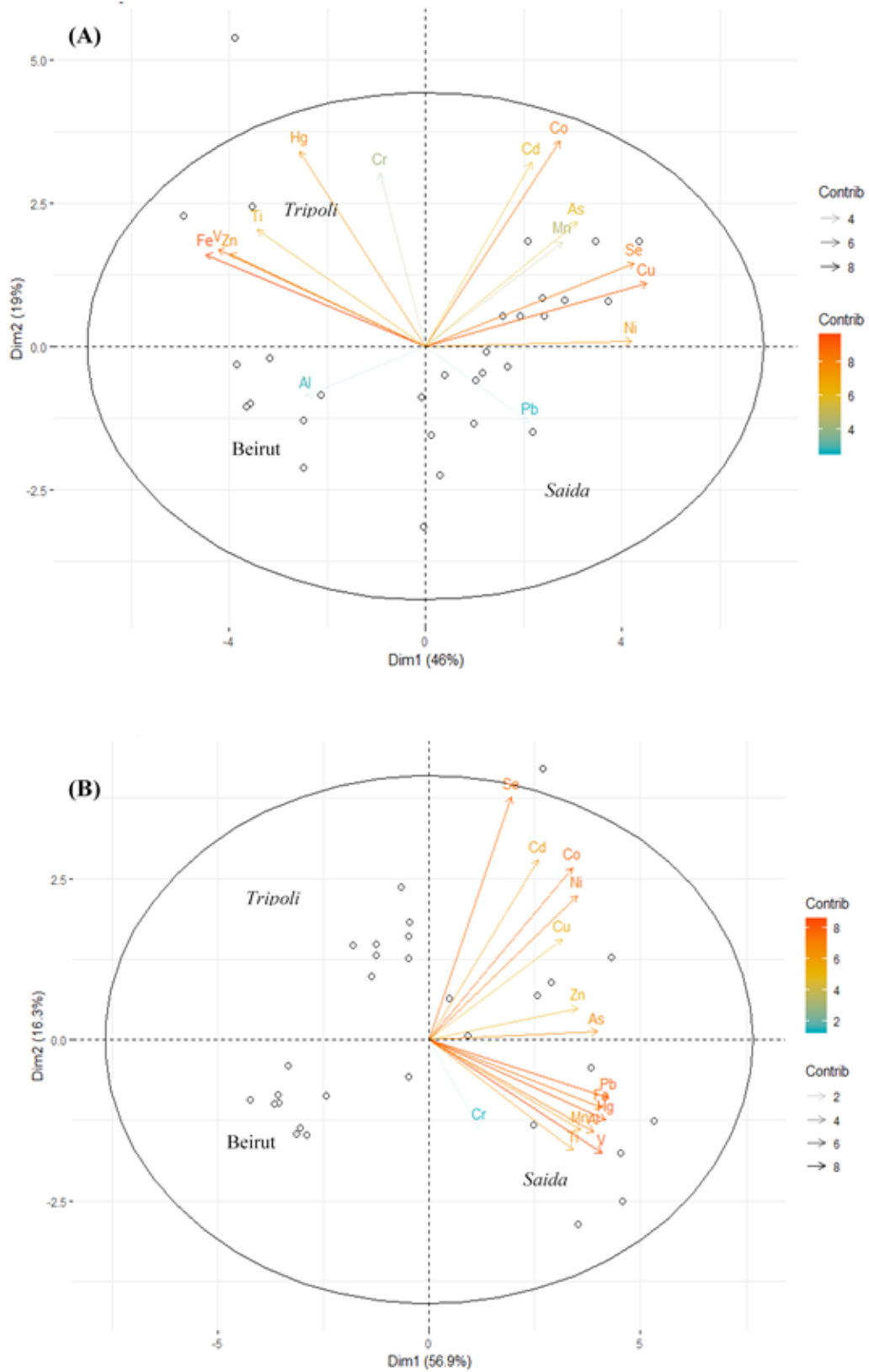


Figure 3-3 PCA showing the relation between sites and trace metals concentration in *S. spinosus* during the wet (A) and the dry (B) season

physico-chemical parameters of the water at different sites, mainly between the North and the South as explained above and also to the sources involved in trace metal input at each site.

More precisely, in Tripoli, Abou Ali river drains all kinds of effluents into the coastal area (Figure 3-4). A wastewater treatment plant was established in the area but hasn't been working properly. In addition, a dumpsite in Tripoli is still receiving waste until present which is leading to increased waste height and a difficulty to control leachate that is drained to sea without treatment (El-Hoz, 2017). A study carried out on the leachate produced by dumpsites in Lebanon showed increased levels of trace metals of Mn, Cr, Ni and Cd due to the lack of efficient waste management (Khalil et al., 2018). For most contaminants, concentrations in dumpsites leachate in Lebanon were higher than the toxicity reference values (TRVs) that are used for ecological risk assessment (Khalil et al., 2018). They reached 12 folds the TRV for Pb and 27 folds for Cd. *S. spinosus* showed relatively high levels of Cd ($p < 0.05$) recorded mainly in Tripoli (Annex 4) for both seasons (4.21 ± 0.77 and 3.80 ± 0.01 mg kg⁻¹ for the wet and dry season respectively), with higher levels ($p < 0.05$) for the wet season. High levels of Cd were also recorded by Nakhlé (2006) in mussel tissue sampled in Selaata (South of Tripoli), (0.6-8.7 and 1.08- 4.03 mg kg⁻¹ in *Patella* and *Brachidontes* respectively, Table 3-2) and a decreasing trend was reported southward as the influence of a phosphogypsum plant in the South of Tripoli (Figure 3-4) decreased highlighting the influence of the plant on Cd levels in the area. Indeed, in a study on the effects of the plant on the marine coastal zone in that area (Fakhri and Romano, 2005), it has been shown that wind regime along the Lebanese coast played a substantial role in spreading various contaminants over a wide area and that the impact of that plant is likely to affect the neighboring northern marine environment especially with a dominant northern marine current along the Lebanese coast (Fakhri and Romano, 2011). Accumulation of Cu, Cr, Ni, Zn, Ti and Cd are more likely to be influenced by anthropogenic inputs due to the presence of industries such as tanneries and electroplating industries, especially in Beirut, releasing those metals in the environment (Fakhri, 2012; Jaishankar et al., 2014; Oliveira, 2012) and studies conducted on the leachate are supportive of this hypothesis.

Biota-sediment accumulation factor (BSAF)

The concentrations of different elements in the sediment are presented in Table 3-1 and the calculated biota-sediment accumulation factor (BSAFs) are shown in Table 3-4.

In the algae *U. lactuca* all the elements had a BSAFs < 1 suggesting no bioaccumulation from the sediment. The same has been observed in a study made along the Senegalese coast. However, they found a higher transfer factor (TF) from water to the green algae suggesting higher accumulation from water rather than from sediments (Diop et al., 2016). Several studies reported significant correlation between the

Table 3-4 Biota-sediment transfer factors (BSAFs) in different organisms along the Lebanese coast.^a

Species	Site	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Hg	Pb
<i>U. lactuca</i>	Tripoli	0.180	0.126	0.059	0.231	0.118	0.109	0.142	0.359	0.281	0.160	0.036	0.239
	Beirut	0.021	0.007	0.006	0.006	0.005	0.005	0.000	0.033	0.043	0.033	0.000	0.009
	Saida	0.022	0.132	0.008	0.022	0.010	0.017	0.115	0.340	0.152	0.133	0.000	0.006
<i>S. spinosus</i>	Tripoli	0.002	0.004	0.018	0.021	0.001	0.231	0.630	1.944	3.162	12.424	0.199	0.027
	Beirut	0.004	0.010	0.019	0.013	0.003	0.111	0.233	0.617	10.495	6.579	0.158	0.015
	Saida	0.004	0.010	0.018	0.026	0.002	0.155	0.750	1.491	3.726	8.898	0.409	0.112
<i>M. japonicus</i>	Tripoli	0.001	0.003	0.001	0.003	0.000	0.014	0.007	4.748	2.776	0.099	1.708	0.003
	Beirut	0.000	0.000	0.001	0.002	0.000	0.002	0.002	1.831	1.386	0.005	0.253	0.000
	Saida	0.000	0.000	0.001	0.001	0.000	0.004	0.000	3.248	1.522	0.008	0.333	0.001

^a the values in bold indicate a transfer of contaminant from sediment to biota (BSAFs >1).

levels of trace metals accumulated by *U. lactuca* and those in the water and sediments (Akcali and Kucuksezgin, 2011; Bonanno et al., 2019a; Chakraborty et al., 2014a) while some others found no significant correlation between trace metal concentrations in algae and water/sediment (Kamala-Kannan et al., 2008; Malea and Haritonidis, 2000). Although the uptake by seaweed is mainly done from solution, levels of trace elements in sediments reflect to a certain extent the degree of pollution in the water column, as concentrations in the water are indirectly related to the sediment through processes of resuspension and interstitial diffusion of metals (Bonanno et al., 2019a; Thomann et al., 1995).

The spiny oyster accumulated Cu, Zn and Cd with the highest BSAF recorded for Zn and Cd in Tripoli compared to the shrimp (Table 3-4). Bivalves are known to accumulate substantial amounts of trace metals from water (Zuykov et al., 2013) and are able to accumulate metals at higher concentrations than the levels in water and sediments (Swaleh et al., 2016). BSAF for Zn was the highest for the oyster compared to the alga and shrimp. Bivalves are high accumulators of Zn as they absorb it readily from water because it is an essential metal for their metabolism as is Cu for shrimps. In fact, Cu, Zn and Cd induce the metal binding protein, metallothionein thus they are metabolically active and that's why they have a high assimilation efficiency and low depuration rates (Thomann et al., 1995). However, the levels of essential metals accumulated by marine organisms do not always reflect a metabolic requirement as marine invertebrates may often accumulate metals due to their high ambient level (Rainbow and Luoma, 2011).

The kuruma shrimp had BSAFs > 1 for Cu and Zn with higher values for Cu (Table 3-4). Cu is widely known to be an essential element in shrimps as it is associated to hemocyanin. Crustaceans take up metals from food and from water in proportion to dissolved concentration of the element (Rainbow, 1995b). In some cases, an increase of uptake is reflected in an elevated concentration such as the case of Cd in decapod crustaceans, however even if the uptake rates of trace metals by crustaceans respond to changes in the total dissolved metal concentration, not all metals are in available form for uptake (Rainbow, 1995b). Concerning Hg, the BSAF was found to be > 1 only in Tripoli. The sediments in Tripoli had a higher level of Hg compared to Beirut and Saida (Table 3-1). The shrimp is known to live close to the bottom and thus may be more exposed to sediment related pollutants than the algae and the bivalve (Pinedo-Hernández et al., 2015).

Finally, levels of trace metals found in marine organisms may be the result of uptake from different sources and not only from sediment. Bioaccumulation is the result of several factors including

bioavailability, multiple routes of exposure and metabolism that can widely vary among species (Jakimska et al., 2011a). Each species uptakes trace metals differently and thus it is important to consider different taxa and even tissues in the same organism when establishing monitoring programs and assessing bioaccumulation (Yüzereroğlu et al., 2010).

4. Conclusion

The present study provided levels of 20 trace elements in three marine species from the Lebanese coast in relation with seasonal and spatial variation and biota-sediment transfer. Significant differences were found in element accumulation between species revealing the importance of using multiple bioindicator organisms to monitor coastal pollution by trace elements. Our results generally fell between values recorded in relatively uncontaminated areas and those recorded in polluted ones suggesting an overall moderate level of contamination in the studied sites with highest levels mostly found in Tripoli. This study reported for the first-time levels of trace elements in the spiny oyster from the Lebanese coast. Seasonal and spatial variations were found and were reflected differently through each species, highlighting the importance of seasonality when it comes to monitoring programs. The sediment-biota transfer study showed limited transfer of trace metals between these environmental compartments. Ecological risks from trace metallic contaminants are not simple to document, as marine organisms accumulate metals through different routes and the responses may vary widely among species. Thus, understanding toxicity requires more than just total metal accumulated in tissues especially when considering the complexity of environmental factors. However, bioindicators remain good integrative indicators of chemical exposure to contaminants in a polluted ecosystem which helps identify research needs as a first step towards further investigation.

CHAPTER 4 – HEALTH RISKS DUE TO SEAFOOD CONSUMPTION

Chapter 4 - PART 1: LEVELS OF Pb, Cd, Hg AND As IN FISHERY PRODUCTS FROM THE EASTERN MEDITERRANEAN AND HEALTH RISK ASSESSMENT

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Abstract

This study reports the contamination assessment of Pb, Cd, Hg and As of 3 fish species, one shrimp and one bivalve sampled from the Eastern Mediterranean (Lebanese coast). The samples were collected from 3 sites characterized by different types and levels of pollution. Pb, Cd, Hg and As levels were measured by Inductively coupled plasma-mass spectrometry following closed microwave (acid) digestion (accredited method). The bivalve species showed the highest concentrations of Cd, Pb and As, whereas fish exhibited higher Hg concentrations compared to crustaceans and bivalves. Trace metals concentrations were found to be below the maximum levels set by the European Commission. The human health risk of each contaminant was assessed using two different methods, namely Target hazard quotients (THQs) and Estimated Weekly Intake (EWI). THQs and EWI indicated that the consumption of fishery products that were analyzed in this study is unlikely to cause adverse effects to human health.

Keywords: Fishery products; Trace elements; ICP-MS; Eastern Mediterranean; Lebanese coast; Health risk assessment

1. Introduction

Fishery products represent a substantial part of a balanced diet for humans as they are a source of proteins and fatty acids such as omega-3 which are known to reduce the risk of inflammatory and heart diseases (Çoğun et al. 2006; Copat et al. 2012; Dural et al. 2006; FAO 2016). Fishery products consumption worldwide has been in continuous increase in the last 5 decades; while it was estimated at 9.9 kg/person/year in 1960, average fish consumption per capita has reached 19.2 kg/person/year in 2012 and has risen above 20 kg in 2016 (FAO 2016). Nevertheless, consumption of fishery products may be an important route of human exposure to chemical contaminants such as heavy metals (Storelli 2008). In fact, anthropogenic activities have considerably increased the pollution of the marine environment having a significant impact on seafood products quality and ultimately on human health. Marine organisms and particularly fish tend to accumulate chemical contaminants such as heavy metals and metalloids that may in some cases exceed the limits set by the European Commission (EC) or other food safety authorities (Olmedo et al. 2013; Pastorelli et al. 2012a). Metals such as lead (Pb), cadmium (Cd) and mercury (Hg) have been classified as toxic to humans by international organizations namely the World Health Organization (WHO) and the United States Environmental Protection Agency (USEPA). These organizations have recommended tolerable intake levels, oral reference doses and health risk factors applicable to a large number of pollutants in order to assess the human health risk of heavy metals through seafood consumption (FAO/WHO 2011; USEPA 2000). Human contamination by Pb, Cd, Hg and As through diet is of main concern nowadays, mainly because of the high toxicity of these elements (and their species), and also due to the fact that they are persistent and bioaccumulate in the environment (Al Sayegh Petkovšek et al. 2012; García et al. 2000; Storelli and Marcotrigiano 2001). Their bioaccumulation in marine biota largely depends on the species, gender and length (Diop et al. 2016; Storelli 2008; Vieira et al. 2011). Thus, assessing human exposure to these elements via diet is very important. Several studies have focused on the health risks generated by the consumption of contaminated seafood around the world in general (Burger and Gochfeld 2005; Castro-González and Méndez-Armenta 2008; Yi et al. 2017; Yi et al. 2011) and along the Eastern Mediterranean basin in particular.

In Lebanon, the marine environment has been facing an increasing anthropogenic pressure particularly at the coastal zone due to urbanization and overexploitation of coastal resources. This is causing deterioration in marine water quality, and increasing releases of urban and industrial effluents, especially in the absence of adequate wastewater management plans (Khalaf 2012) leaving questions about local seafood quality. According to the Food and Agriculture Organization (FAO), total local

fish catches in Lebanon are estimated to be 9000 per year while local consumption is about 35000 tons, making the country more dependent on imported fishery products. To our knowledge, few studies dealt with the chemical contamination of fishery products in Lebanon and even less on the health risks they may engender (Kouyoumjian et al. 2001; Nakhlé 2003; Nasreddine et al. 2006a). To assess potential human health risks, it is recommended to use various fish and seafood products which are representative of different trophic levels in the food chain (Yi et al. 2017). Therefore, the present study aims (i) to assess the contamination of some seafood products by Cd, Hg, Pb and As collected along the Lebanese coast; (ii) to assess the variation of these contaminants between sites and selected marine species; (iii) to compare trace elements levels in seafood between different countries boarding the Eastern Mediterranean basin and finally (iiii) to evaluate the potential human health risk associated to seafood consumption.

2. Materials and methods

2.1. Study area and sample collection

Seafood samples analyzed in this study were collected from three sites: Tripoli, Beirut and Saida, along the Lebanese coast (Eastern Mediterranean Sea) (Figure 4-1). The selected sampling sites are characterized by different types and sources of pollution, namely:

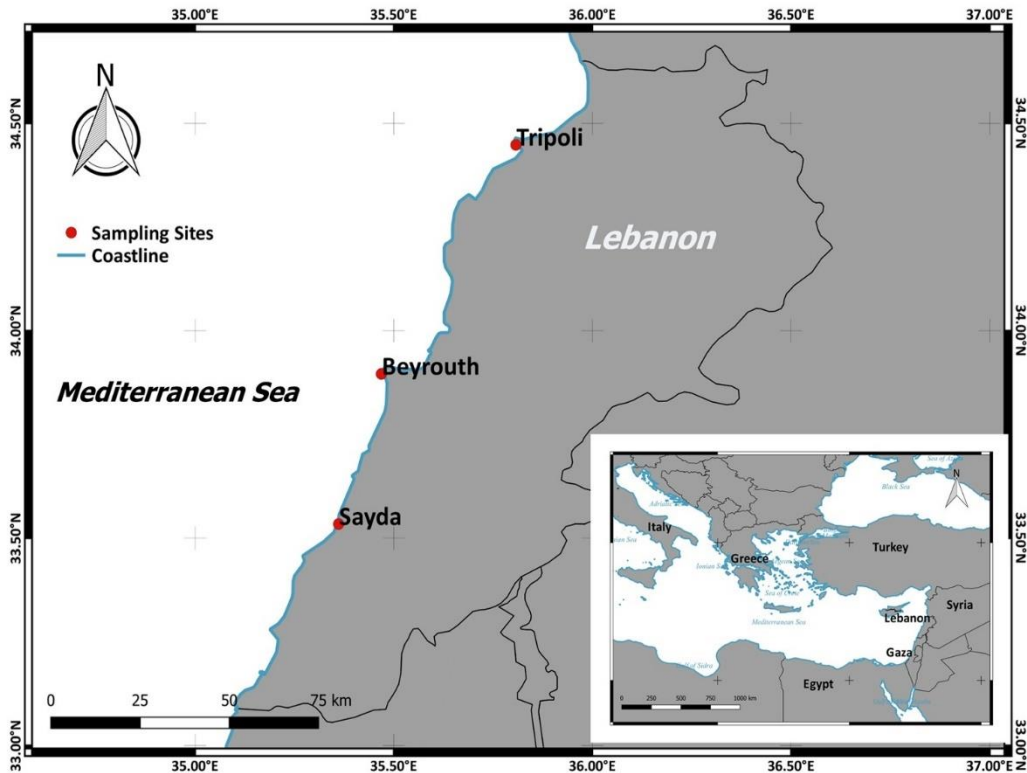


Figure 4-1 : Map showing the sampling sites along the Lebanese coast (Eastern Mediterranean)

Tripoli is characterized mainly by sewage outfalls, industrial fishing and boat maintenance activities. Beirut is a densely populated and highly industrial city with dumpsites along the coast; additionally, *Beirut River* drains urban and industrial effluents to the sea without pre-treatment. Saida site was affected by a waste crisis in the past which led to a rehabilitation plan in 2014, turning the dumpsite into a public garden. A primary wastewater treatment plant was also established at this site, which theoretically contributed to the amelioration of the status of the marine coastal water.

Five marine species, from different trophic levels of the food web, widely distributed along the Lebanese coast and commonly consumed by the local population were collected, such as: three fish species: marbled spinefoot (*Siganus rivulatus*), sand steenbras (*Lithognathus mormyrus*) and the red-eye round herring (*Etrumeus teres*), one shrimp species the kuruma shrimp (*Marsupenaeus japonicus*) and one bivalve species the spiny oyster (*Spondylus spinosus*).

Ten individuals from each species were sampled at each site (except for Beirut area where we only got 6 individuals of *L. mormyrus* and zero individual of *M. japonicus*) and immediately transported to the laboratory on ice.

2.2. Sample preparation and analysis

This study focused on the determination of trace elements in fish muscle and edible parts of shrimps and oyster. Before preparation, each individual was measured for total length and weighed and then the edible parts were removed using stainless steel scalpel. The resulting samples were placed into low density polyethylene containers previously decontaminated by soaking in 10% (m/v) nitric acid overnight and rinsed with distilled water. The samples were lyophilized and ground individually using an agate mortar and pestle and stored at room temperature.

The samples were analyzed using an accredited method (French Accreditation Comity-COFRAC) reported elsewhere (Chevallier et al. 2015). Briefly, between 0.2 g and 0.4 g of the lyophilized and ground sample was weighed in quartz digestion vessels and predigested overnight with 3 ml of ultrapure nitric acid (HNO_3 , 67% v/v, VWR chemicals, Prolabo). Three mL of ultrapure water were added and mineralization was carried out using a closed (high pressure) microwave system (Multiwave 3000 and Multiwave PRO, Anton Paar, Austria). Samples digests were then added up with ultrapure water (18.2 $\text{m}\Omega\cdot\text{cm}$) to 50 ml polypropylene tubes. Finally, concentrations of Cd, Hg, Pb and As were measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS Agilent 7700, Agilent Technologies, France). A solution of internal standards (IS) including scandium (Sc), indium (In), bismuth (Bi), rhenium (Re), yttrium (Y) and gold (Au) was added to all blanks, standards and samples in order to correct for non-spectral interferences and instrumental drift (each IS was at the level of 2 ng mL^{-1} in the analyzed solution). Moreover, a solution of gold (Au) standard was used to reduce the memory effects in terms of Hg analysis. Limits of quantification (LOQ), calculated based on wet weight (ww), were 0.001 $\mu\text{g g}^{-1}$ for Cd, 0.005 $\mu\text{g g}^{-1}$ for Pb, 0.017 $\mu\text{g g}^{-1}$ for Hg and 0.004 $\mu\text{g g}^{-1}$ for As.

Method accuracy and precision were daily assessed by the analysis of three certified reference materials (CRM), namely ERM-278K (European reference material, mussel tissue, European Commission), DOLT 5 (fish liver, Canada) and NIST 1566 (mussel tissue, USA).

2.3. Data treatment

Inter-species and spatial differences of As, Pb, Cd and Hg levels in seafood products in addition to fish length between samples from the three sites were tested using one-way ANOVA, followed by post-hoc Tukey's test since the necessary assumptions for normality and homogeneity of variances for parametric statistics were satisfied. Data analysis was performed using IBM SPSS Statistics 20. The level of significance was set at $\alpha=0.05$.

Uncertainty calculation and quality control

The expanded uncertainty (U_c , $k=2$, see eqn. 1) of the results reported in this study was calculated based on the combined uncertainty (u_c) that was estimated as the standard deviation characterizing the intermediate precision (S_R) during a time span of approximately 3 months, as reported elsewhere (Chevallier et al. 2015); S_R was weighed by the number of analyses ($n=1$ in this case) .

$$U = k \times u_c = 2 \times \frac{S_R}{\sqrt{n}} \quad (1)$$

Several internal quality control (IQC) were set up in this study in order to ensure reliability of the results (Chevallier et al. 2015) and the data were considered valid only when all the acceptance criteria were satisfied. Briefly, an IQC concerning the calibration step relied on the achievement of a correlation coefficient (r^2) ≥ 0.995 when using a 6 point calibration curve. The variation of the IS was also monitored in order to assess the instrumental drift and matrix effects. In most cases IS recovery was between 80% and 120%. A middle-range mix standard solution containing $5 \mu\text{g L}^{-1}$ of each trace element investigated here was also systematically analyzed every 10 samples (and also at the end of the sequence) in order to assess the calibration drift; the data were validated only when the deviation of the concentration of this control standard was $\leq 20\%$ compared to the theoretical value.

Method trueness was also assessed according to the FD V03-115 procedure (Agence Francaise de Normalisation, 1996) by analyzing certified reference materials (CRM) in parallel with each batch of samples. A result was considered valid when its value was comprised within the confidence interval (CI) calculated on the basis of the certified value ($X_{\text{certified}}$) of the CRM as follows (eqn. 2):

$$CI = X_{\text{certified}} \pm \left[k \times \frac{CV_R \times X_{\text{certified}}}{100 \times \sqrt{N}} \right] \quad (2)$$

where CI is the confidence interval, $k=3$ ($p=99\%$) and N is the number of replicate analyses for the CRM.

A mix solution containing each analyte investigated here at a concentration level of the LOQ $+3 \times CV_R$ was also analyzed in parallel with each batch of samples in order to assess data reliability at very low levels. This control was considered satisfactory if the measured concentration for this solution was comprised within the CI obtained during the method validation using a similar equation as eqn. 2. Most

of the data (> 90%) obtained by the analysis of the control standard solution (LOQ level) fell well within the confidence interval for the four trace elements of this study.

2.3.1. Target Hazard Quotient

Target Hazard Quotients (THQ) of the trace elements measured in this study were calculated in order to evaluate the health risk and exposure related to the consumption of marine organisms as described below.

THQ is the ratio of a single substance exposure level over a specified time period (e.g. subchronic) to a reference dose (RfD) for that substance derived from a similar exposure period (USEPA 2000) (eqn. 3).

$$THQ = \frac{EFr \times ED \times FIR \times C_i}{RfDo \times B_w \times ATn} \times 10^{-3} \quad (3)$$

where:

THQ, risk associated with a single element;

EFr, exposure frequency (set as 365 days/year);

ED, exposure duration (70 years) equivalent to the life expectancy at birth;

FIR, seafood ingestion rate in Lebanon (28 g/person/day) (HelgiLibrary - Fish Consumption Per Capita in Lebanon)

C_i, analyte concentration in the sample (μg g⁻¹ ww);

RfDo, oral reference dose: Cd = 1 x 10⁻³ μg g day⁻¹; Hg = 1.6 x 10⁻⁴ μg g⁻¹ day⁻¹; Pb = 4 x 10⁻³ μg g⁻¹ day⁻¹ and As = 3 x 10⁻⁴ μg g⁻¹ day⁻¹ (USEPA 2000)

B_w, average body weight (72.8 kg for adults) based on the dietary survey conducted in 2006 on an adult urban population in Lebanon (Nasreddine et al. 2006b)

A_{tn}, average exposure time for non-carcinogens (365 days/year x 70 years).

A THQ <1 indicates that the daily exposure is unlikely to cause adverse effects to human health during a lifetime, while THQ ≥1 indicates possible adverse effects.

In order to assess the risk related to multiple analytes, total THQ (TTHQ) was calculated by summing up the THQ for each element measured (eqn. 4):

$$TTHQ = THQ(\text{toxicant } 1) + THQ(\text{toxicant } 2) + \dots + THQ(\text{toxicant } n) \quad (4)$$

2.3.2. Estimated Weekly Intake

Consumer's exposure to Cd, Pb, Hg and As was also estimated by calculating the estimated weekly intake according to eqn. 5 (Pastorelli et al. 2012b):

$$EWI = \frac{C \times C_i}{BW} \quad (5)$$

where:

EWI, estimated weekly intake ($\mu\text{g} \cdot \text{kg}^{-1} \text{BW week}^{-1}$);

C, weekly consumption rate of seafood (196 g week^{-1}); (HelgiLibrary 2018)

C_i , level of the contaminant in the food ($\mu\text{g g}^{-1} \text{ ww}$);

BW, average body weight (72.8 kg for this study) (Nasreddine et al. 2010)

The upperbound (UB) approach was adopted following the recommendations of the WHO report (GEMSFoedEuro 1995). Concentrations lower than the limit of detection (LOD), were replaced by LOD and concentrations lower than the limit of quantification (LOQ) were replaced by LOQ.

3. Results and Discussion

3.1. Heavy metals and As levels in studied species

The mean concentrations of Pb, Cd, Hg and As measured in this study are given in Table 4-1. Concentrations obtained are lower or within the range of values reported for various fish, crustaceans and mollusks mentioned in different studies conducted along the Eastern Mediterranean (Abdallah and Abdallah 2008; Abdallah 2008; Ateş et al. 2015; Ayas 2015; Canli et al. 2001; Elnabris et al. 2018; Ersoy and Çelik 2009; Masoud et al. 2007; Türkmen and Türkmen 2005), (Table 4-2).

Cadmium

The highest levels of Cd were found in bivalve *S. spinosus* (range: $0.09\text{-}1.19 \mu\text{g g}^{-1} \text{ ww}$) followed by the crustacean *M. japonicus* (range: $0.001\text{-}0.024 \mu\text{g g}^{-1} \text{ ww}$). The maximum levels of Cd obtained in *S. spinosus* were $\cong 6$ times lower than those reported from other Mediterranean sites such Iskendrun Bay, Turkey (Cd range: $0.13\text{-}7.45 \mu\text{g g}^{-1} \text{ ww}$). This may be related to the different sources of pollution in these 2 sites (Türkmen and Türkmen 2005). Moreover, Cd levels in *M. japonicus* samples analyzed

in a previous study along the Lebanese coast were 17 fold higher in terms of maximum levels compared to the present study (range: 0.1-0.4 $\mu\text{g g}^{-1}$ ww; (Shiber 1981).

The bivalve *S. Spinusus* collected from the region of Tripoli showed significantly higher concentrations of Cd compared to those collected from Beirut and Saida ($p < 0.05$; Table 4-1). Relatively high concentrations of Cd in Tripoli may be related to sewage outfalls, solid waste discharges and boat maintenance activities in the region. Cd have been intensively studied around the region and similar levels were reported in *L. mormyrus* from Iskendrun Bay (1.37-1.57 $\mu\text{g g}^{-1}$ ww; Yilmaz et al. 2010; Table 2). However, all the species analyzed in the present study showed lower concentrations of Cd compared to other Mediterranean regions where the same species were studied (Table 2) (Ateş et al. 2015; Ayas 2015; Canli et al. 2001; Ersoy and Çelik 2009; Masoud et al. 2007; Türkmen and Türkmen 2005).

Mercury

Hg concentrations in fish show a relatively high variation ranging from levels <LD up to 0.29 $\mu\text{g g}^{-1}$ ww. These concentrations are approximately 5 times higher (in terms of maximum levels) than those found in crustaceans (range: 0.02-0.06 $\mu\text{g g}^{-1}$ ww) and bivalves (range: <LD - 0.023 $\mu\text{g g}^{-1}$ ww), which is in agreement with previous studies (Storelli, 2008). Among fish, *E. teres* showed the highest Hg levels (range: 0.02-0.25 $\mu\text{g g}^{-1}$ ww) followed by *L. mormyrus* (range: 0.02-0.19 $\mu\text{g g}^{-1}$ ww) and *S. rivulatus* (range: <LD-0.09 $\mu\text{g g}^{-1}$ ww). Bioaccumulation of Hg in fish notably in the form of methylmercury (MeHg), depends on the trophic level, foraging method/location and age or length of fish (Storelli, 2008; Zhang and Wong, 2007). Although *L. mormyrus* is a demersal species while *E. teres* is a pelagic one, both species are known to feed on bottom dwelling animals such as crustaceans, polychaetes and gastropods (Kallianiotis et al. 2005; Osman et al. 2013). A previous study conducted along the Lebanese coast on *Mullus barbatus* and *Boops Boops* fish species that are also known to feed on crustaceans and polychaetes, reported levels of Hg between 0.02-0.36 $\mu\text{g g}^{-1}$ ww and 0.01-0.04 $\mu\text{g g}^{-1}$ ww, respectively (Kouyoumjian et al. 2001), which is comparable to the levels obtained in the present study.

Table 4-1 Species size \pm standard deviation (cm) and mean trace elements concentrations \pm standard deviation ($\mu\text{g g}^{-1}$ ww) in muscle of fish and edible parts of prawns and oysters sampled along the Lebanese coast in 2016 and 2017).

Region / Species	n*	Size (cm)	Cd	Hg	Pb	As
Tripoli						
<i>Siganus rivulatus</i>	10	14.03 \pm 0.49 (13-15)	0.003 \pm 0.001 (0.002 - 0.003)	0.09	0.08 \pm 0.06 (0.03 - 0.22)	0.45 \pm 0.14 ^h (0.26 - 0.75)
<i>Lithognatus mormyrus</i>	10	16.98 \pm 0.66 ^a (15.4-18.7)	0.007	0.09 \pm 0.03 ^f (0.05 - 0.16)	0.013 \pm 0.008 (0.005 - 0.031)	6.17 \pm 3.84 (2.50 - 15.87)
<i>Etrumeus teres</i>	10	22.68 \pm 0.62 ^c (21.9-23.9)	0.003 \pm 0.001 (0.001 - 0.005)	0.09 \pm 0.02 (0.08 - 0.12)	0.007 \pm 0.002 (0.006 - 0.010)	2.68 \pm 0.63 (1.31 - 3.38)
<i>Marsupenaeus japonicus</i>	10	-	0.010 \pm 0.007 (0.003 - 0.024)	0.03 \pm 0.01 (0.02 - 0.05)	0.006 \pm 0.001 (0.005 - 0.007)	10.48 \pm 7.66 (3.72 - 23.89)
<i>Spondylus spinosus</i>	10	9.06 \pm 0.32 (8-10)	0.86 \pm 0.18 ^{d,e} (0.68 - 1.19)	<LD	0.11 \pm 0.05 (0.05 - 0.19)	13.53 \pm 9.15 ^j (5.02 - 36.76)
Beirut						
<i>Siganus rivulatus</i>	10	16.88 \pm 0.62 (15.6-18.5)	0.002 \pm 0.002 (0.001 - 0.005)	<LD	0.013 \pm 0.04 (0.006 - 0.018)	1.31 \pm 1.16 ^{h,i} (0.37 - 4.49)
<i>Lithognatus mormyrus</i>	6	17.58 \pm 2.31 ^b (15-24)	0.005	0.03 \pm 0.01 ^f (0.02 - 0.04)	0.007	7.73 \pm 5.75 (0.49 - 16.8)
<i>Etrumeus teres</i>	10	18.19 \pm 1.01 ^c (16.5-20.9)	0.001	0.03 \pm 0.01 ^g (0.02 - 0.06)	0.03 \pm 0.01 (0.01 - 0.04)	5.47 \pm 4.77 (1.67 - 17.0)
<i>Marsupenaeus japonicus</i>	-	-	-	-	-	-
<i>Spondylus spinosus</i>	10	6.85 \pm 1.12 (5-9.5)	0.40 \pm 0.31 ^d (0.09 - 1.16)	0.020 \pm 0.002 (0.019 - 0.023)	0.04 \pm 0.01 (0.02 - 0.06)	4.09 \pm 1.93 ^j (1.63 - 7.67)
Saida						
<i>Siganus rivulatus</i>	10	19.52 \pm 1.12 (17.5-20.9)	0.002 \pm 0.001 (0.001 - 0.004)	<LD	0.17 \pm 0.29 (0.006 - 0.506)	2.46 \pm 1.10 ⁱ (1.08 - 4.36)
<i>Lithognatus mormyrus</i>	10	23.51 \pm 0.45 ^{a,b} (22.6-24.4)	0.001	0.10 \pm 0.05 ^f (0.05 - 0.19)	0.009 \pm 0.002 (0.006 - 0.010)	10.00 \pm 3.28 (5.49 - 15.0)
<i>Etrumeus teres</i>	10	25.06 \pm 0.53 ^c (24.2-26)	0.006 \pm 0.005 (0.002 - 0.019)	0.18 \pm 0.05 ^g (0.13 - 0.25)	0.01 \pm 0.01 (0.005 - 0.018)	3.22 \pm 0.68 (2.17 - 4.42)
<i>Marsupenaeus japonicus</i>	10	-	0.002 \pm 0.002 (0.001 - 0.006)	0.03 \pm 0.01 (0.02 - 0.06)	0.007 \pm 0.002 (0.005 - 0.011)	7.36 \pm 3.57 (4.09 - 16.0)
<i>Spondylus spinosus</i>	10	8.99 \pm 1.13 (7.5-12)	0.56 \pm 0.13 ^e (0.32 - 0.70)	<LQ	0.34 \pm 0.13 (0.17 - 0.56)	6.19 \pm 1.59 (4.26 - 9.31)

It is worth to note that the lowest content of Hg was found in *S. rivulatus* (range: <LD-0.09 $\mu\text{g g}^{-1}$ ww) whose diet consists mainly of algae. Therefore, levels of Hg present in the muscle of fish in our study might be related to their foraging method thus indicating a bioaccumulation and biomagnification through the food chain. These results are in agreement with other studies dealing with benthic organisms (Cogun et al. 2017; oğun et al. 2006; Copat et al. 2012; Sirot et al. 2009; Storelli 2008). In addition, taking into consideration the sampling sites, *E. teres* and *L. mormyrus* collected from Saida showed significantly higher concentrations than those collected from Beirut ($p < 0.001$ and 0.05 , respectively) and those collected from Tripoli ($p < 0.05$). This may be explained by the fish size, as *E. teres* and *L. mormyrus* individuals collected from Saida are significantly bigger than those collected from Beirut and Tripoli ($p < 0.001$) and thus, exhibiting significantly higher concentrations of Hg (Table 4-1). Few studies reported the levels of mercury in fishery products from the Eastern Mediterranean (Copat et al., 2013; Jureša and Blanuša, 2003; Masoud et al., 2007). However, the levels of mercury in our study are generally lower than the levels found in the region (Juresa and Blanus 2003), (Table 4-2).

Lead

Pb levels were found to be the highest in *S. spinosus* (range: 0.02-0.19 $\mu\text{g g}^{-1}$ ww) which is similar with Cd patterns. Marine invertebrates and fish are known to bioaccumulate Pb from seawater in proportion to its concentration in solution (Amiard et al. 1987). However, Pb is present in seawater under complexed or microparticulate form, which is scarcely bioavailable or toxic to marine organisms. Therefore, the amount of Pb in tissues of marine animals is not transferred efficiently through marine food webs (França et al. 2005; Neff 2002). Overall, levels of Pb obtained in our study appeared to be lower compared to other species analyzed in adjacent Mediterranean regions (Abdallah and Abdallah 2008; Abdallah 2008; Ateş et al. 2015; Ayas 2015; Canli et al. 2001; Ersoy and Çelik 2009; Kallianiotis et al. 2005; Masoud et al. 2007; Türkmen and Türkmen 2005) (Table 4-3). Other studies reported a decreased trend of Pb levels in the marine environment mainly related to the regulation limiting the use of leaded gasoline consumption (Storelli 2008). Indeed, Pb emissions in Lebanon have been decreasing as a result of the replacement of the leaded gasoline in 1993. For instance, Pb concentrations determined in the bivalve *Brachidontes variabilis* collected between 2002 and 2003 from Lebanese waters ranged between 0.3-0.6 $\mu\text{g g}^{-1}$ ww (Nakhlé 2003), which is 3 folds higher compared to the levels obtained in the bivalve *S. spinosus* exhibiting maximum levels in our study (range: 0.02-0.19 $\mu\text{g g}^{-1}$ ww). Moreover, the crustacean *M. japonicus* collected along the

Lebanese coast in 1978 exhibited higher levels of Pb (range: 1.4-5.3 $\mu\text{g g}^{-1}$ ww; (Shiber 1981) compared to the same species analyzed in the present study (range: 0.005-0.011 $\mu\text{g g}^{-1}$ ww). Only one study reported Pb concentrations in *S. spinosus* collected from Iskenderun Bay ((Türkmen and Türkmen, 2005) and concentrations for samples taken in January are 100 folds higher (192 $\mu\text{g g}^{-1}$ ww; table 4-2) than the concentrations found in the current study.

Arsenic

The highest levels of As were found in the kuruma shrimp (3.72-23.89 $\mu\text{g g}^{-1}$ ww) and the spiny oyster (1.63-36.76 $\mu\text{g g}^{-1}$ ww). Amongst the fish species, the demersal fish *L. mormyrus* showed the highest levels of As (Table 4-1). It has been shown that sediments and bottom waters contain higher As levels compared to shallow waters hence explaining the higher levels of As found in the benthic (*M. japonicus*) and demersal species (*S. spinosus*, *L. mormyrus* and *E. teres*) as they both live in close association to the bottom sediments (Storelli and Marcotrigiano 2001). Taking into consideration the sampling sites, *S. rivulatus* from Saida showed significantly higher As levels compared to those collected from Beirut ($p < 0.05$) and Tripoli ($p < 0.001$). It is, however difficult to directly associate these elevated As levels to anthropogenic sources or naturally occurring elevated As levels as fish may also accumulate As from natural sources like sediment and water (Kalantzi et al. 2017). Finally, As levels found in all the species analyzed in this study are in the same range as the concentrations found in various species collected along the Eastern Mediterranean basin (Table 4-2).

3.1. Health risk assessment

Mean concentrations of trace toxic metals (Pb, Cd, Hg) measured in this study were found to be below the maximum allowed levels (ML) set by the European Commission in edible parts of fish, mollusks and crustaceans (Table 4-1) (EC 2006). It is worth to underline that there is no ML set for As in fishery products. Nevertheless, this contaminant was also considered in this study since the major exposure of humans to total As occurs through diet, mainly by consumption of fishery products (Uneyama et al. 2007). THQ and ED₀₁ for As, Pb, Cd and Hg in the fishery products analyzed in this study are listed in Table 4-3. They provide an indication of the risk level due to these elements but do not serve as a quantitative estimate of the probability of an exposed population experiencing a reverse health effect (Storelli 2008).

THQ was overall < 1 for the regulated trace metals (Pb, Cd, Hg) hence indicating a non-adverse effects of these trace elements at the levels measured in this study. On the other hand, THQ calculated for As ranged between 0.40 and 12.2 which presumably indicate an adverse health effect due to the

consumption of the fishery products investigated here. Nevertheless, it is well known that As is present in fish under various chemical forms with the most abundant being the non-toxic arsenobetaine (EFSA 2009; EFSA 2014b) while it shows its toxicity mainly via the inorganic species such as As(III) and As(V). Therefore, the current guidelines for As exposure exist only for inorganic As as the organic forms of arsenic are considered to be of no toxicological concern (EFSA 2009; Taylor et al. 2017). Therefore, the relatively high THQ obtained for As does not reflect the actual health hazard because the toxic fraction (inorganic As) in fish is generally less than 10% (Sirot et al. 2009). For a more accurate assessment of As health effects associated to the consumption of the fishery products investigated here, speciation analysis of inorganic As should be carried out, but this is beyond the purpose of this study.

TTHQ* was also considered here in order to assess the risks exclusively related to the regulated trace elements (Cd, Hg and Pb) (Table 4-2).

A complementary approach to address the impact on human health of the chemical contaminant investigated here is the Provisional Tolerable Weekly Intake (PTWI) which was established by the joint FAO/WHO Expert Committee on food additives (Joint Expert Committee on Food 2017) as the "safe" weekly intake level. The ratio of the exposure to the PTWI was determined here and multiplied by 100 in order to assess the contribution of mean intake of a trace element to the associated PTWI as described briefly in the next paragraphs.

Table 4-2 Total Hazard Quotient (THQ), total THQ (TTHQ), Estimated Monthly Intake (EMI; $\mu\text{g kg}^{-1}\text{ BW}$) (Cd only) and Estimated weekly intake (EWI; $\mu\text{g kg}^{-1}\text{ BW}$) related to Cd, Hg, Pb and As through consumption of fishery products from the Lebanese coast.

Region/Species	THQ				TTHQ ($\Sigma\text{Cd.Hg.Pb.As}$)	TTHQ As*	EMI Cd	EWI			
	Cd	Hg	Pb	As				Cd	Hg	Pb	As
Tripoli											
<i>S. rivulatus</i>	0.001	0.009	0.005	0.402	0.42	0.015	0.017	0.006	0.04	0.21	1.20
<i>L. mormyrus</i>	0.0003	0.048	0.001	5.564	5.61	0.049	0.009	0.003	0.24	0.03	16.61
<i>E. teres</i>	0.001	0.051	0.0003	2.415	2.47	0.052	0.019	0.006	0.25	0.01	7.21
<i>M. japonicus</i>	0.003	0.016	0.0003	9.453	9.47	0.019	0.081	0.03	0.08	0.01	28.21
<i>S. spinosus</i>	0.233	0.005	0.008	12.200	12.45	0.246	7.000	2.32	0.02	0.31	36.41
Beirut											
<i>S. rivulatus</i>	0.001	0.005	0.001	1.183	1.19	0.006	0.016	0.005	0.02	0.03	3.53
<i>L. mormyrus</i>	0.0003	0.010	0.0002	6.974	6.98	0.010	0.010	0.003	0.05	0.01	20.82
<i>E. teres</i>	0.0002	0.013	0.002	4.934	4.95	0.015	0.005	0.002	0.06	0.07	14.73
<i>M. japonicus</i>	-	-	-	-	-	-	-	-	-	-	-
<i>S. spinosus</i>	0.108	0.006	0.003	3.688	3.81	0.118	3.255	1.08	0.03	0.11	11.01
Saida											
<i>S. rivulatus</i>	0.0003	0.005	0.004	2.214	2.22	0.008	0.010	0.003	0.02	0.14	6.61
<i>L. mormyrus</i>	0.0001	0.052	0.0004	9.029	9.08	0.052	0.004	0.001	0.26	0.01	26.95
<i>E. teres</i>	0.002	0.098	0.0003	2.907	3.01	0.100	0.050	0.017	0.49	0.01	8.68
<i>M. japonicus</i>	0.0004	0.012	0.0004	6.639	6.65	0.013	0.013	0.004	0.06	0.02	19.82
<i>S. spinosus</i>	0.153	0.005	0.023	5.582	5.76	0.180	4.578	1.52	0.02	0.91	16.66

* TTHQ = TTHQ-THQ (As)

Cadmium

The Scientific Panel on contaminants in the Food Chain (CONTAM) established a TWI of $2.5 \mu\text{g kg}^{-1}$ for Cd, which replaced the previous PTWI of $7 \mu\text{g kg}^{-1}$ (EFSA 2009). However, in the last report of the joint FAO/WHO Expert Committee on food additives, the PTWI of $7 \mu\text{g kg}^{-1}$ BW for Cd was withdrawn and replaced by a PTMI (Provisional Tolerable Monthly Intake) of $25 \mu\text{g kg}^{-1}$ BW.

In this study, the mean Cd intake through fish consumption contributed to 0.13% of the PTMI whereas crustaceans and bivalves contributed to 17%. It was noted that a monthly value for health-based guidance for Cd was more appropriate to use owing to cadmium's exceptionally long half-life (EFSA 2011; FAO/WHO 2011). Therefore, Estimated Monthly Intake (EMI) through the consumption of Cd might be more appropriately compared to PTMI. It is worth to note that for Cd, EMI associated to the consumption of fish, was almost 800-fold lower than TMI (see Table 4-2).

Mercury

The Joint Expert Committee on Food Additives stated that almost all dietary exposure of humans to Hg is due to fishery products consumption and that MeHg is by far the most toxic form of Hg. Therefore, the committee adopted a PTWI of $1.6 \mu\text{g kg}^{-1}\text{BW week}^{-1}$ for MeHg to replace the previous PTWI of $3.3 \mu\text{g kg}^{-1}\text{BW week}^{-1}$ of total Hg for general population (FAO/WHO 2011; FAO/WHO 2013). Several studies have shown that MeHg constitutes the major Hg form present in fish and shellfish (Clémens et al. 2011; EFSA 2014a; Sirot et al. 2009). It is worth to note that MeHg fraction vary from 70 to 100% in fish and from 28 to 98% in shellfish. For Hg risk assessment in this study, it has been assumed that 100% of Hg is present as MeHg. Based on this assumption, Hg weekly intake through the consumption of seafood in this study was 14-fold lower than the established PTWI. Mean fish consumption contributed to 12% of the PTWI while crustaceans and bivalves contributed to only 7%. For instance, in France, fish were the main contributors to Hg exposure through the dietary intake (69%) while crustaceans accounted only for 3% which is close to the contribution obtained in the present study (2.2%) (Arnich et al. 2012).

Table 4-3 Mean trace elements concentrations \pm standard deviations ($\mu\text{g g}^{-1}$ ww) in fishery products from the Eastern Merditerranean

Site (year of sampling)	Species	Habitat/Diet	Cd	Hg	Pb	A _{Stot}	Reference
Iskenderun Bay, Turkey (2006)	<i>Triglia lucerna</i>	Bottom-dwelling/carnivorous	0,01 \pm 0,05	–	0,14 \pm 0,15	1,38 \pm 0,72	Yilmaz et al. 2010
	<i>Lophius budegassa</i>	Benthic/carnivorous	0,02 \pm 0,03	–	0,17 \pm 0,02	0,98 \pm 0,07	
	<i>Solea lascaris</i>	Benthic/carnivorous	0,04 \pm 0,01	–	0,39 \pm 0,05	1,74 \pm 0,21	
	<i>Lithognathus mormyrus</i>	Demersal/carnivorous	1,5 \pm 0,11 (1,37-1,57)	–	1,72 \pm 0,06 (1,66-1,78)	–	
Iskenderun Bay, Turkey (2003-2004)	<i>Etrumeus teres</i>	Pelagic/carnivorous	0,07 \pm 0,00	–	0,17 \pm 0,00	–	Ersoy and Çelik 2009
	<i>Scomber japonicus</i>	Pelagic/carnivorous	0,06 \pm 0,00	–	0,14 \pm 0,02		
	<i>Trachurus mediterraneus</i>	Pelagic/carnivorous	0,06 \pm 0,00	–	–		
	<i>Liza aurata</i>	Pelagic/herbivorous	0,07 \pm 0,00	–	–		
Iskenderun Bay, Turkey (2003-2004)	<i>Sparus aurata</i>	Coastal (sandy bottoms)/carnivorous	0,17 \pm 0,04	–	–	–	Ersoy and Çelik 2010
Mersin Bay, Turkey (2013)	<i>Marsupenaeus japonicus</i>	Bottom dwelling/carnivorous	0,2 \pm 0,04	–	1,39 \pm 0,23	–	Ayas et al.2015
	<i>Penaeus semisulcatus</i>	Bottom dwelling/carnivorous	0,26 \pm 0,06	–	1,97 \pm 0,14	–	
	<i>Parapenaeus longirostris</i>	Bottom dwelling/carnivorous	0,12 \pm 0,09	–	0,5 \pm 0,20	–	
Iskenderun Bay, Turkey (2004-2005)	<i>Spondylus spinosus</i>	Benthic/Filter feeder	6,96	–	192	–	Türkmen 2005

Izmir Bay, Turkey (2010-2011)	<i>Penaeus kerathurus</i>	Benthic/carnivorous	0,047	–	0,19	–	Türkmen and Türkmen 2012
Northeast Mediterranean , Turkey	<i>Sparus aurata</i>	Coastal (sandy bottoms)/carnivorous	0,09 ± 0,03	–	1,39 ± 0,25	–	Canli 2003
Iskenderun Bay, Turkey	<i>Sardina pilchardus</i>	Pelagic/ carnivorous	0,315 ± 0,03	–	1,92 ± 0,43	–	Canli et al. 2001
Antalya Bay, Turkey (2004-2005)	<i>Siganus rivulatus</i>	Demersal/deposit feeder	0.06 ± 0.01	–	0.13 ± 0.06	–	Ateş et al. 2015
Mersin Bay, Turkey	<i>Sardina pilchardus</i>	Pelagic/ carnivorous	0,36 ± 0,04	–	0,51 ± 0,04	–	
	<i>Mugil cephalus</i>	Pelagic/omnivorous	0,17 ± 0,02	–	1,33 ± 0,58	–	
Central and North Lebanon	<i>Mullus barbatus</i>	Demersal/carnivorous	–	0.02-0.36	–	–	Kouyoumijian et al. 2001
	<i>Boops boops</i>	Demersal/carnivorous	–	0.01-0.04	–	–	
Central and North Lebanon	<i>Brachidontes variabilis</i>	Medio-littoral zone/ Filter feeder	0.27± 0.06 1 ± 0.17	–	0.25 ± 0.05 0.63 ± 0.18	–	Nakhlé et al. 2006
Beirut, Lebanon (1987)	<i>Patella coerulea</i>	Medio-littoral zone/ Filter feeder	0.1-0.9	–	<1.7 – 12.3	–	Shiber 1987
Gaza strip (2010)	<i>Mugil cephalus</i>	Pelagic/omnivorous	< 0,002 ppm	–	0,172 ± 0,092	–	Elnabris et al. 2013
	<i>Sparus aurata</i>	Coastal (sandy bottoms)/carnivorous	< 0,002 ppm	–	< 0,002 ppm	–	
El-Mex Bay, Egypt (2005-2006)	<i>Siganus rivulatus</i>	Demersal/ deposit feeder	0,71 ± 0,06	–	–	–	Abdallah et al. 2008
	<i>Lithognathus mormyrus</i>	Demersal/ carnivorous	0,41 ± 0,007	–	–	–	
El-Mex Bay, Egypt (2005-2006)	<i>Siganus rivulatus</i>	Demersal/ deposit feeder	0,55-0,94 0,7 ± 0,2	–	0,24-0,325 0,3 ± 0,05	–	Abdallah 2008
El-Mex Bay, Egypt (2000-2001)	<i>Siganus rivulatus</i>	Demersal/deposit feeder	0,22 ± 0,2	–	0.88 ± 0.22	–	Khaled 2014
El-Mex Bay, Egypt (2004-2005)	<i>Siganus rivulatus</i>	Demersal/deposit feeder	0.38 ± 0.20 (0.13 - 0.42)	0.10 ± 0.08 (0.04 - 0.22)	1.69 ± 0.60 (1.10 - 2.48)	–	Masoud et al. 2007

Greece (2013)	<i>Sardina pilchardus</i>	Pelagic/ carnivorous	–	–	–	18,9 ± 1,2	Kalantzi 2017
	<i>Engraulis encrasicolus</i>	Pelagic/ carnivorous	–	–	–	–	
Italy (2012)	<i>Mullus barbatus</i>	Demersal/carnivorous	0,0004 ± 0,0002	–	0,005 ± 0,003	11,024 ± 3,957	Copat et al. 2013
	<i>Engraulis encrasicolus</i>	Pelagic/ carnivorous	0,001 ± 0,0003	–	0,005 ± 0,004	5,275 ± 1,752	
	<i>Scomber scombrus</i>	Pelagic/ carnivorous	0,0013 ± 0,0004	–	0,003 ± 0,001	3,669 ± 0,638	
	<i>Trachurus trachurus</i>	Pelagic/ carnivorous	0,0009 ± 0,0002	–	0,004 ± 0,002	5,409 ± 2,112	
Adriatic Sea, Italy	<i>Pitaria chione</i>	Benthic/filter feeder	0,13 ± 0,022	0,134 ± 0,016	0,121 ± 0,015	8,4 ± 4,6	Juresa and Blanusa 2003
	<i>Mytilus galloprovincialis</i>	Benthic/filter feeder	0,142 ± 0,017	0,182 ± 0,004	0,015 ± 0,009	2,56 ± 0,57	
	<i>Scomber scombrus</i>	Pelagic/carnivorous	0,011 ± 0,005	0,153 ± 0,028	0,015 ± 0,008	1,06 ± 0,29	
	<i>Trachurus trachurus</i>	Pelagic/carnivorous	0,02 ± 0,025	0,218 ± 0,04	0,025 ± 0,009	6,85 ± 6,22	
	<i>Sardina pilchardus</i>	Pelagic/ carnivorous	0,008 ± 0,002	0,208 ± 0,037	0,023 ± 0,007	8,08 ± 2,43	

Lead

Fishery products have been shown to be minor contributor to Pb exposure. Fish, crustaceans and bivalves represent 0.31 and 1.1% of the PTWI, respectively. The contributions of these two categories of fishery products are comparable with those obtained in a French total diet study, for which fish contribution was 0% while crustaceans contributed to 3% of the PTWI (Arnich et al. 2012).

Arsenic

It has been shown previously that fishery products carry the highest concentrations of total As. However, as discussed earlier, the dominant form in marine organisms is arsenobetaine which is the least toxic As species. The Joint Commission Food and Agriculture Organization (FAO/WHO) set a PTWI of 15 $\mu\text{g}\cdot\text{kg}^{-1}$ BW for inorganic As. However, this PTWI value has been withdrawn by the European Food Safety Authority (EFSA) and the Joint FAO/WHO Expert Committee on food additives (JECFA). Therefore, the health-based guidance value defined for total As is no longer used for risk assessment internationally as this would lead to a considerable overestimation of the health risks related to dietary arsenic exposure (Alexander et al. 2009; Llorente-Mirandes et al. 2017).

The Committee on Food Additives noted that populations consuming large quantities of fish that result of organo-arsenic intakes of about 0.05 mg kg^{-1} BW per day do not likely report ill-effects because the organic arsenic species present in fish undergo very little biotransformation to the toxic inorganic form and they are almost entirely excreted (FAO/WHO 2011). The study should be pursued with speciation data in terms of inorganic As in order to better assess the As exposure of the Lebanese population through fishery products consumption.

Conclusion

This study reports the levels of Pb, Cd, Hg and As in some commercially important fishery products from a Mediterranean region (Lebanese coast) allowing a preliminary assessment of the health impact of these contaminants related to the diet habits in Lebanon. Overall, concentrations of these trace elements measured in three fish species, one shrimp and one mollusk were lower than the maximum allowed levels set by the EC. Moreover, their levels were similar or lower compared to the levels found in other studies from the Eastern Mediterranean region. Weekly intake of these contaminants through consumption of the mentioned fishery products did not exceed the PTWI. These findings along with the THQ assessment indicated that the consumption of the selected fishery products is not likely to cause adverse effects on human health despite the relatively important anthropogenic pollution of the investigated sites. This is likely due to the strong coastal currents flowing north-northeastward and to

the relatively narrow (3-18 km) continental shelf off Lebanon as compared to other Mediterranean regions which may lead to fast pollution dispersion by seaward transportation. The study should be pursued by the analysis of top predator fish species such as tuna or swordfish in order to accurately assess the pollution impact on the toxic trace elements bioaccumulation. It can also be extended to a larger number of fishery products and sampling sites in order to obtain a more comprehensive human health exposure to trace inorganic contaminants of the Lebanese population.

**Chapter 4 - PART 2: TOWARD A ROUTINE METHODOLOGY OF
SPECIATION ANALYSIS OF METHYLMERCURY IN FISHERY
PRODUCTS FOLLOWING THE VALIDATION BASED ON THE
ACCURACY PROFILE APPROACH**

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Abstract

A simple, fast and a robust method for routine speciation analysis of methylmercury by reverse phase high-performance liquid chromatography coupled to inductively coupled plasma mass spectrometry was optimized and validated using the accuracy profile approach. An effective separation of inorganic Hg and methylmercury (MeHg) was achieved in less than 3 min. Extraction of MeHg from fishery products was carried out by L-cysteine.HCl.H₂O using closed microwave assisted extraction system. A robust method validation was carried out using the accuracy profile approach. MeHg recovery ranged between 80 and 102%. The limit of quantification was 3.2 µg kg⁻¹ (wet weight) whereas the bias ranged between 0.2 and 2%. The repeatability expressed in terms of relative standard deviation was ≤ 8.3%. This method can be used for the routine determination of MeHg in fishery products with satisfactory analytical performances in order to assess the risks related to the consumption of this type of foodstuffs.

Keywords: Mercury, HPLC-ICP-MS, method validation, accuracy profile

1. Introduction

Mercury is one of the most toxic pollutants on a global scale (Moreno et al., 2013; Zhu et al., 2017) and is considered a priority hazardous substance by the Agency of Toxic Substances and Disease Registry (ATSDR) (Maia et al., 2019). The first disease recognized as being due to exposure to Hg species was known among hat makers and date from the early 19th century. Symptoms include mental confusion, emotional disturbances, and muscular weakness in addition to severe neurological and kidney damages (Tchounwou et al., 2003a).

Hg is released into the environment from both natural and anthropogenic sources. Natural sources include forest fires, volcanoes, water surfaces and soils whereas anthropogenic emissions, contributing to about 50-75% of the yearly input, originate mainly from electricity generation, incinerators, waste disposal and other industrial minerals (Jaishankar et al., 2014). In the environment, Hg exists most commonly as elemental Hg (Hg^0), inorganic Hg (Hg^{2+}) and as methylmercury (MeHg), the latter being the most toxic Hg species (Ogrinc et al., 2007; Xia et al., 2007). The environmental and health interest in Hg and its species started in the 1950s after the MeHg mass poisoning in Minamata, Japan followed by a second epidemic in Niigata, also Japan, due to the ingestion of large quantities of fish and shellfish polluted by industrial effluents charged in MeHg resulting in several hundreds of deaths and a large number of victims (Zhang et al., 2019). MeHg is one of the most bioconcentrated trace metals in the marine environment and hence its presence in human food and particularly in fishery products is still a matter of concern (Maia et al., 2019). Seafood consumption is the major route of human exposure to MeHg (Storelli et al., 2005b). Actually, over 90 % of MeHg is absorbed through the gastrointestinal tract and then transferred into the bloodstream where it can form complexes with thiol groups of several proteins hence disturbing their enzymatic activity.

In order to limit consumers' dietary exposure to MeHg, the European Commission (EC) has set maximum permissible levels of total Hg in fish (0.50 and 1.0 mg kg⁻¹, respectively, the latter limit being related to predatory fish) (EC, 2014). So far, there is no regulation in terms of MeHg in seafood or other type of foodstuffs, although this species poses the main threat in terms of Hg toxicity. This is mainly due to challenges in analytical chemistry being also confirmed by the fact that speciation analysis of Hg has not become yet a routine approach.

Speciation analysis of Hg has been mostly carried out using gas chromatography (GC) due to its lower detection limits compared to liquid chromatography based (HPLC) coupled techniques (Laffont et al., 2013). However, with the new developments of inductively coupled plasma-mass spectrometry (ICP-MS) in terms of sensitivity, HPLC-ICP-MS based method for speciation analysis of Hg are mostly used nowadays due to their overall simplicity compared to GC-ICP-MS, mainly because the

derivatization of Hg species is not required (Cattani et al., 2008; Fang et al., 2016; Grotti et al., 2014; Han et al., 2003; Hight and Cheng, 2006; Sannac et al., 2009; Wang et al., 2007). Nevertheless, the bottleneck of speciation analysis of Hg in fishery products remains the species extraction from the sample matrix. This is still challenging due to the possibility of MeHg degradation to Hg^{2+} but also because of the very low levels of the latter species in such foodstuffs. The most common samples preparation approaches for Hg speciation analysis include microwave assisted acid (Noh et al., 2016) or alkaline extraction (Jiménez-Moreno et al., 2018; Narukawa et al., 2018), solid-phase extraction and solid-phase microextraction (Ferreira et al., 2015; Sannac et al., 2012).

The main aim of this study was to develop and validate a routine analytical approach for MeHg speciation analysis based on the accuracy profile to be used for the risk assessment related to this contaminant in fishery products. To our knowledge, this is the first time that a method for speciation analysis of MeHg by-HPLC-ICP-MS is validated using the robust accuracy profile approach. In this respect, the performance criteria in terms of LOQ, specificity, accuracy, repeatability and intermediate precision were assessed. The method was also applied to the analysis of a selection of fishery products.

2. Materials and methods

2.1. Instrumentation

Extraction of MeHg was carried out using a closed microwave system (Multiwave type, Anton Paar, Courtaboeuf, France). An Agilent ICP-MS 7700x (Agilent Technologies, Courtaboeuf, France) was used for the online detection of Hg species after their separation by using an HPLC bio inert Infinity lab 1260 II (Agilent Technologies). The optimum analytical conditions for the HPLC-ICP-MS method are provided in Table 4-4. Two small-bore reversed-phase (C18) columns were tested, as follows: Agilent poroshell 2.1 x 100 mm, 2.7 μm (column A) and an Agilent peptide mapping 2.1 x 150 mm; 2.7 μm (column B). In each case, an Agilent pre-column with the same composition as the columns (2.1 x 50 mm; 2.7 μm) were used. The chromatographic signals were monitored in the Time Resolved Analysis (TRA) mode of the ICP-MS Software (MassHunter).

2.2. Reagents and standards

All solutions were prepared with analytical reagent grade chemicals and ultrapure water (18 $\text{M}\Omega$ cm) obtained by purifying distilled water with a Milli-QTM PLUS system associated with and Elix 5 pre-system (Millipore S.A., France). MethylHg (II) chloride standard was purchased from VHG Labs (LGC, Molsheim, France) ($\text{CH}_3\text{HgCl} = 1001 \mu\text{g L}^{-1}$), Hg^{2+} (chloride standard, HgCl_2 at $1000 \mu\text{g L}^{-1}$)

Table 4-4 ICP-MS and HPLC optimum operating conditions.

<i>ICP-MS</i>	
Nebuliser	Quartz concentric (Micromist) 400 $\mu\text{L min}^{-1}$
Spray chamber	Scott-type double-pass water cooled
RF power	1400 -1500 W
Reflected power	< 10 W
Plasma gas flow	15 L min^{-1}
Nebuliser gas flow	0.95 – 1.00 L min^{-1}
Auxiliary gas flow	0.99 L min^{-1}
<i>HPLC operating conditions</i>	
Column	Reversed phase C-18, Agilent peptide mapping (2.1 x 150 mm, 2.7 μm)
Pre-column	C18 Agilent infinity lab poroshell (2.1 x 50 mm ; 2.7 μm)
Injection volume	10 μL
Column temperature	Ambient
Mobile phase flow rate	0.25 ml min^{-1}
<i>Mobile phase</i>	
Chanel A	MeOH 5% (v/v)
Chanel B	0.5 g L^{-1} (w/v) L-cysteine.HCl.H ₂ O, pH = 2.3

and the methanol (MeOH) HPLC grade from Sigma-Aldrich, Saint-Quentin Fallavier, France), ethylmercury chloride (EtHg) standard at 83% purity ($\text{C}_2\text{H}_5\text{HgCl}$) from Dr. Ehrenstorfer (Augsburg, Germany), supra pure hydrochloric acid (HCl, 37%) from VWR chemicals, Fontenay-sous-Bois, France) and the L-Cysteine.HCl.H₂O, at 99% purity from Alfa Aesar (Kandel, Germany). A Tuning multi-element solution at a 10 mg L^{-1} (Agilent Technologies) was used to prepare a tuning solution containing several elements such as Li, yttrium (Y), thallium (Tl), Co, cerium (Ce) that made it possible to cover a wide range of masses including Hg.

2.3. Preparation of standard solutions and the mobile phase

MeHg and Hg^{2+} intermediate standards were prepared at 50 $\mu\text{g L}^{-1}$ by diluting the respective stock solutions in 1% v/v HCl. EtHg (50 $\mu\text{g L}^{-1}$), which was tested as internal standard (IS) was prepared by dissolution in 10 ml MeOH (20% v/v) then add up with 50 ml ultrapure water. MeHg and EtHg working solutions were prepared using glass volumetric flasks and then kept in tightly capped glass

tubes as recommended elsewhere (Hight and Cheng, 2006). All intermediate Hg species solutions were kept at 4 °C in the dark. Intermediate standards of MeHg, Hg²⁺ and EtHg at 50 µg L⁻¹ were stable for at least one month in the described conditions, thus these solutions were renewed monthly.

Intermediate standards at a concentration of 1000 µg L⁻¹ were prepared weekly in 0.02 % (w/v) L-cysteine.HCl.H₂O as recommended elsewhere (Hight et al., 2006). The calibration standards were prepared daily by diluting the appropriate amount of the intermediate working solutions (1000 µg L⁻¹) in 0.2 % (w/v) L-cysteine and 5% MeOH in order to match the samples extracts' final concentration. The mobile phase was composed of 5% (v/v) MeOH and 0.05 % (w/v) L-cysteine.HCl.H₂O. L-cysteine (for the mobile phase) was prepared daily by dissolving the solid substance in ultrapure water. Then the pH was adjusted to 2.3 by adding suprapure HCl 37%.

2.4. Analytical procedures

Portions of homogenized edible seafood tissue (0.3 g of lyophilized matrices and 0.6 g of fresh matrices) were weighed in quartz digestion vessels the day of analysis, then 20 ml of 0.5% (w/v) L-cysteine.HCl.H₂O were added. The extraction was carried out using closed microwave system for 15 min at 60 °C. The extract was transferred in to 50 ml polypropylene tubes and made up with 5 % (v/v) MeOH. The extract was centrifuged for 10 min at 4000 rpm and then filtered using 0.22 µm polyvinylidene fluoride (PVDF) syringe filters. The calibration standards were prepared in the same solvent as the sample extracts final composition (2 g L⁻¹ (w/v) L-cysteine.HCl.H₂O and 5% MeOH). The quantification of MeHg was carried out by using the external calibration approach (all results are expressed in mg kg⁻¹ of wet weight).

3. Results and discussion

3.1. Optimization of Hg species separation by reverse phase HPLC

For this study, two reversed-phase (C18) small-bore columns (named here column A and B, respectively, see the Instrumentation section), were compared for the separation of 3 Hg species (Hg²⁺, MeHg and EtHg). These small-bore columns were chosen as they present advantages over normal-bore columns such as lower matrix plasma load, faster chromatographic separation and a better resolution at a considerably lower rate of the mobile phase. An improved sensitivity has also been reported due to the higher sample introduction efficiency and lower solvent load (Grotti et al., 2014). For the same mobile phase composition (3% MeOH and 97% L-cysteine.HCl.H₂O, pH=2.3) the column B provided considerably better resolution and peaks shapes than column A; the resolution factor for the column B was 2.35 whereas the resolution factor obtained with the column A was 0.87.

Thus, column B (peptide mapping 2.1 x 150 mm, 2.7 μm from Agilent) was used throughout the study. To our knowledge, this is the first time that such an RP column (peptide mapping type) was used for the separation of three Hg species.

MeOH concentration was also optimized for optimum HPLC separation. Initially, 3% (v/v) of MeOH was used for columns comparison. An increase to 5% (v/v) led to a slight decrease in the retention time for all species and consequently, separation using MeOH 5% (v/v) was carried out throughout the study.

3.2. Optimization of the extraction

Different extraction temperatures, durations and concentrations of extracting solution were tested using lyophilized and fresh seafood matrices.

The overall analytical process was optimized by the analysis of three certified reference materials (CRMs) : DOLT-5 (Dogfish liver tissue), ERM-CE 464 (Freeze dried fish powder) and TORT-3 (Lobster hepatopancreas) from the National Research Council Canada. For this purpose, 300 mg of the lyophilized CRMs were weighed and analyzed in duplicates with one of the vessels taken as blank to evaluate contamination.

The extraction conditions were based on the studies reported by Hight *et al.* (2006) and Sannac *et al.* (2009) and used L-cysteine as complexing agent for the Hg species. Microwave assisted extraction was optimized using Novawave (SCP science), which is a digestion tunnel system and, in all experiments, 200 mg of dry sample and 20 ml of reagent (5 g L⁻¹ w/v L-cysteine.HCl.H₂O) and 15 min' extraction times were employed.

3.2.1. Extraction temperature

The extraction recovery was initially assessed at the following temperatures: 40, 50, 60, 70, 80, 90 and 100 °C during an extraction time of 15 min. For temperatures > 80 °C (and 15 min' exposure), a poor recovery (< 85%) of MeHg was observed (Figure 4-1). The optimum extraction temperature was obtained at 60°C, providing an extraction recovery of 99% and it was used for the rest of the experiments.

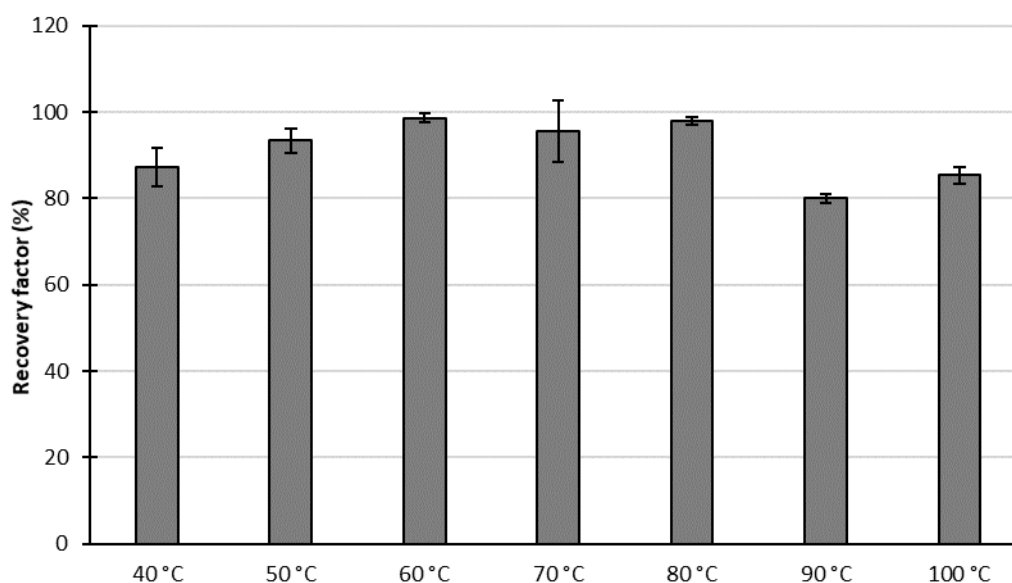


Figure 4-1 Extraction efficiency of MeHg from CRM ERM CE-464 (0.3 g) for different temperatures (the error bars represent the standard deviation over two replicates).

3.2.2. Extraction time

Further, different extraction times (5, 10, 15 and 20 min) were tested. As can be seen in figure 4-2, extraction time of 15 min led to the best recovery of MeHg for both CRMs analyzed for this purpose. An increase of extraction time resulted in a decrease of the extraction efficiency which is most likely due to the degradation (demethylation) of MeHg. For an extraction time < 15 min, the extraction of MeHg was incomplete, with recoveries < 70%. Consequently, 15 min' extraction time at 60 °C was retained for the sample treatment throughout the study.

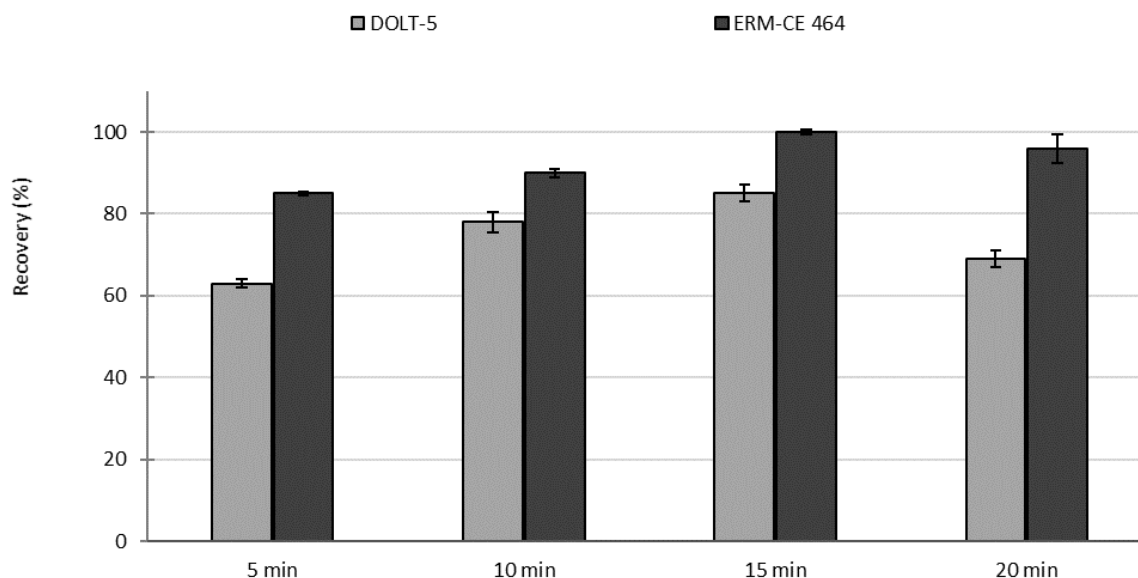


Figure 4-2 Recovery percentage of MeHg for different extraction durations in CRMs DOLT-5 and ERM-CE 464 (0.3 g) (the error bars represent the standard deviation over two replicates).

3.2.3. Sample amount

The influence of the sample weight on the recovery efficiency was also assessed in this study. For dry matrices (ERM-CE 464, DOLT-5 and TORT-3 CRMs), the sample amount ranged between 200 mg and 400 mg while 500 and 800 mg were used for fresh matrices. The latter matrices were reference materials provided by Food Analysis Performance Assessment Scheme (FAPAS, an accredited proficiency testing provider) that were previously analyzed in the laboratory, namely canned tuna fish tissues; both samples were characterized for the MeHg content. Recoveries ranging from 83 to 105% were obtained for the minimum and maximum samples weights used for dry and fresh matrices. assessed here, therefore weight portions of 300 mg for dry matrix and 600 mg for fresh matrix, were used throughout the study.

3.3. The use of ethylHg as an internal standard

The use of EtHg as an IS was also assessed in this study. It showed good sensitivity and separation from MeHg (retention time for MeHg was 2.3 min whereas the retention time for EtHg was 5.7 min). Nevertheless, it was noticed that the signal of EtHg was not stable under the optimum HPLC conditions for MeHg; actually, EtHg degraded rather quickly to Hg^{2+} hence leading to significant positive errors

both for MeHg and Hg²⁺ determination. Consequently, EtHg was not employed as IS for the rest of the experiments.

3.4. Method validation

3.4.1. Assessment of selectivity

The method selectivity was assessed before the determination of the main analytical parameters such as the precision and the uncertainty by means of the spike recovery approach. For this purpose, thirteen samples were spiked with MeHg prior to extraction in order to provide a level of MeHg in the extract between 1 and 10 µg L⁻¹. A calibration curve was constructed in this case and the regression line was tested against the line of unity (slope = 1, intercept = 0) by simultaneously testing the hypotheses of a slope different from 1 and intercept different from 0 using the Student's t-test. According to this test, $t_{\text{obs}} < t_{\text{crit}}$ value, the slope and intercept of this regression line were not different from 1 and 0, respectively. The selectivity of the method was therefore considered acceptable (the recovery factors were between 80 and 120% for all spiking levels).

3.4.2. Determination of intermediate precision and uncertainty by means of the accuracy profile approach

The method was further validated based to the accuracy profile procedure according to the NF V03-110 standard (AFNOR, 2010). A method is validated based on the accuracy profile approach when the β -expectation tolerance interval for a given result is comprised within the a priori set acceptability limit (λ). Basically, the accuracy profile is an expression of the systematic error (trueness) and the random error (repeatability and intermediate precision) for a series of analyte's levels in various matrices within a range of concentrations called validity domain (Mermet and Granier, 2012). Trueness is expressed either through the bias (in case of the analysis of CRM) or by means of the spike recovery factor. In this work, the validity domain was defined between 1/2 of the LOQ assessed in accordance with the NF EN 13804 (AFNOR, 2002) by analyzing 21 reagent blanks spiked at 0.05 µg L⁻¹, and the highest concentration used for the calibration curve.

Seven measurement series were repeated in duplicate on (seven) different days by two different operators, over a timespan of two months for constructing the accuracy profile. Due to the lack of CRM's at very low levels (close to LOQ) the first three levels of the accuracy profile consisted in spiked samples. For the four remaining levels, external quality control (FAPAS) samples and CRMs

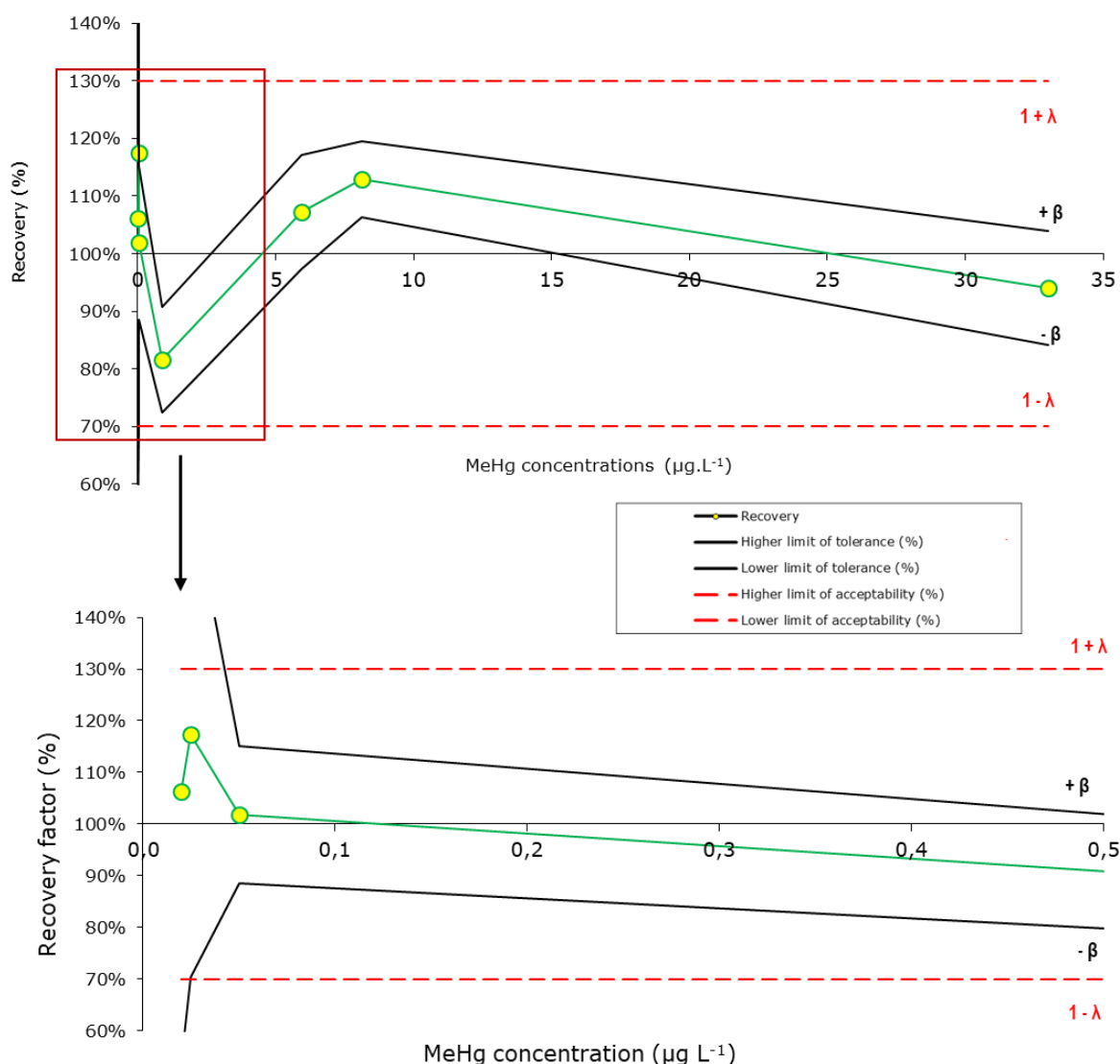


Figure 4-3 Accuracy profile for method validation in terms of MeHg determination in fishery products by HPLC-ICP-MS ($\beta = 85\%$, $\lambda = \pm 30\%$).

(TORT-3, ERM-CE 464) were analyzed for the assessment of the recovery factor. The probability β was set to 85% which means that the risk of future expected results falling outside these limits is below 15%. The acceptance limits (λ) were set to $\pm 30\%$, which corresponds to intermediate precision coefficient of variation estimated at 10% for $k = 3$ ($p = 99\%$).

The accuracy profile obtained for method validation in terms of MeHg determination in fishery products by HPLC-ICP-MS is provided in figure 4-3. As can be seen, β -expectation tolerance intervals were comprised within the acceptability limits except for the two first levels; this corresponds to a validity domain comprised between 0.038 and $33 \mu\text{g L}^{-1}$ or 0.0032 and 2.75 mg kg^{-1} wet weight. The LOQ assessed from the accuracy profile, which corresponds to the intersection of the acceptability and

the tolerance limits was $0.038 \mu\text{g L}^{-1}$ ($3.2 \mu\text{g kg}^{-1}$). The bias corresponding to the validity domain ranged from -0.2 to 2%, whereas the estimated coefficient of variation in terms of repeatability (CV_r) varied from 1.5 to 8.3 % and the coefficient of variation in terms of intermediate reproducibility (CV_R) ranged from 3.6 to 8.3%. For simplicity, it was set at 8.5% for the determination of the expanded uncertainty (U , $k=2$) for the analysis of real-life samples, as shown by eqn. 2:

$$U = \frac{k \times CV_R \times C}{100 \times \sqrt{n}} \quad (2)$$

where:

$k = 2$ ($p = 95\%$);

C , measured concentration (mg kg^{-1});

N , number of independent measurement replicate replicates.

3.4.3. Analytical quality assurance

To ensure the analytical quality assurance, sample extracts were analyzed in batches including several internal quality controls (IQC). Calibration was performed before sample analysis in order to monitor linearity using standard solutions. A determination coefficient of $r^2 \geq 0.995$ was accepted using 5 calibration points (0; 1; 2; 5 and $10 \mu\text{g L}^{-1}$). With every batch of eight samples, one CRM was analyzed in order to assess the method trueness. One of the digestion vessels was filled only with extracting solvent through the whole procedure and the final solution was analyzed as an analytical blank. A mid-range calibration standard was measured every 4 samples and at the end of the sequence in order to assess the stability of instrumental response and the calibration drift (validated when deviation of the control standard $\leq 20\%$). The results were validated when all quality control criteria were met. When acceptability criteria were not met, the results were discarded and the sample(s) re-analyzed. The analysis of CRMs showed satisfactory recovery percentages of MeHg (between 92 and 98%) throughout the study as it is shown in Table 4-5.

Table 4-5 Results in terms of trueness (recovery factor, %) obtained for the speciation analysis of MeHg in three CRMs (mg kg^{-1} wet weight).

CRMs	Concentration $\pm U$ ($k=2$)		
	Certified	Measured	Recovery (%)
ERM CE-464	5.5 ± 0.17	5.4 ± 0.90	98
DOLT-5	0.128 ± 0.063	0.123 ± 0.021	96
TORT-3	0.147 ± 0.013	0.135 ± 0.022	92

3.4.5. External quality control (EQC)

The method was further validated by the analysis of 3 samples that were previously analyzed by the laboratory while participating in proficiency tests; these samples were provided by the Central Science Laboratory-Food Analysis Performance Assessment Scheme (CSL-FAPAS from Sand Hutton, UK) and they are considered as reference materials (they are called here FAPAS samples, see Table 4-6). The z-scores obtained for the analysis of these samples were satisfactory (between -0.4 and -0.2), hence proving the fit for purpose of the method (Table 4-6).

Table 4-6 z-scores obtained for the analysis three reference materials provided by Central Science Laboratory-Food Analysis Performance Assessment Scheme (CSL-FAPAS) as part of the laboratory external quality control.

Sample	Concentration ($\mu\text{g kg}^{-1}$)		Uncertainty	z-score
	Assigned	Measured		
FAPAS 7281	428	413	70.31	-0.2
FAPAS 7305	495	464	78.88	-0.4
FAPAS 7331	496	467	79.39	-0.3

3.4.6. Application of the method to the speciation analysis of MeHg in real life samples

The method was applied to the analysis of a selection of 5 real life samples consisting of fish samples from a previous study (Ghosn et al., 2019). Actually, from 286 samples of fish muscles, bivalves and shrimps' soft tissues analyzed for total Hg (Hg_T) and other trace metals in previous studies, only 5 samples showed a level of total Hg greater than 0.1 mg kg^{-1} as it is the minimum level regulated by the European Commission in edible seafood (EC, 2006), hence being chosen in this study for the speciation analysis of MeHg. It is worth to note that Hg_T in these samples was determined by using an accredited method (French Accreditation Committee-COFRAC) explained elsewhere (Chevallier et al., 2015).

The method was also applied to the analysis of two different brands of canned tuna fish samples purchased in Lebanon. (these samples were also analyzed previously for their Hg_T content). These two canned tuna fish samples were analyzed here out of 15 samples which were previously analyzed for Hg_T as they exhibited levels $\text{Hg}_T > 0.1 \text{ mg kg}^{-1}$.

Between the species analyzed in this study, *N. randalli* had the highest levels of Hg_T and MeHg followed by the tuna and the round herring. All these species are known to be carnivorous; the round herring is a bottom dwelling species feeding on benthic macroinvertebrates and food remains in the

Table 4-7 Hg_T and MeHg concentrations in real fishery products samples ($\mu\text{g kg}^{-1}$ wet weight)

Sample	Hg _T ($\mu\text{g kg}^{-1}$)	MeHg ($\mu\text{g kg}^{-1}$)	MeHg fraction (%)
Round herring (<i>Etrumeus teres</i>) (n=3)	166 130 170	132 111 154	80 85 91
Mean \pm SD (n=3)	155 \pm 22.03	132 \pm 21.50	85 \pm 5.51
Tiger shrimp soft tissue (<i>Marsupenaeus japonicus</i>) (n=1)	210	179	85
Randall's threadfin bream (<i>Nemipterus randalli</i>) (n=1)	853	790	93
Canned tuna fish 1 (n=1)	349	281	81
Canned tuna fish 2 (n=1)	774	710	92

water column, however this species is smaller in size compared to the tuna and *N. randalli*. It is worth to highlight that accumulation of Hg by marine species is influenced by different parameters such as feeding habits, habitat, fish age and size (Bawuro et al., 2018; Dang and Wang, 2012; Hajeb et al., 2009; Merciai et al., 2018). Hg accumulation in fish is positively related to the fish size however, growth rate may lead to less Hg concentration by “growth dilution”. In fact, individuals that grow to a larger size quickly will have lower Hg concentration compared to slow-growing individuals as reported by previous studies (Dang and Wang, 2012; Karimi et al., 2010; Trudel and Rasmussen, 2006; Ward et al., 2010). This might explain the difference of accumulation between the tuna (281 and 710 $\mu\text{g kg}^{-1}$ wet weight of MeHg) and *N. randalli* (790 $\mu\text{g kg}^{-1}$ wet weight of MeHg) which is smaller in size but accumulated higher levels of MeHg when compared to tuna (Table 4-7) which is known for its relatively high growth rate (La Mesa et al., 2005).

The fraction of MeHg/total Hg for the 7 samples analyzed has also been assessed. As can be seen in Table 4-7, MeHg fraction ranged from 80 to 93% of total Hg. These levels were similar (Clémens et al., 2011) or even higher (Hajeb et al., 2009; Kuballa et al., 2009) than those reported in other studies.

These results confirm the present knowledge in terms of predominant MeHg fraction in fish and especially in predatory species (EFSA, 2014b).

Conclusions

A rapid and robust method for the speciation of MeHg in fishery products by HPLC-ICP-MS was optimized and validated using the accuracy profile procedure. Microwave assisted extraction was employed to extract MeHg from fishery products using L-cysteine.HCl.H₂O as a complexing agent. Hg species were efficiently and rapidly separated (< 3 min) using a peptide mapping C18 small-bore column. The method was validated by means of the accuracy profile approach under conditions of intermediate reproducibility. Method accuracy and robustness was also demonstrated by the analysis of external reference materials being used previously for international proficiency tests. Finally, the method was successfully applied to the analysis of a selection of real-life samples (fresh and canned fishery products) hence proving its full capacity for the application to routine speciation analysis of Hg in fishery products.

CHAPTER 5 – GENERAL DISCUSSION AND PERSPECTIVES

Chapter 5 – GENERAL DISCUSSION AND PERSPECTIVES

The present study provided recent data concerning the contamination status of marine organisms from the Lebanese coast in terms of trace metals. There is a substantial lack of data related to this subject and even more to the health risks they may engender. The study of risks related to food consumption in general, and fishery products in particular, is not well elaborated especially under the growing environmental pressures at all levels and particularly in this part of the Mediterranean. Consequently, a study entirely devoted to the assessment of contamination levels in marine organisms reveals to be a necessity. In order to fulfill this objective, two aspects have been investigated along the Lebanese coast: the spatio-temporal variability of contamination level in marine organisms and the health risks they may engender.

1. CONTAMINATION LEVELS IN MARINE ORGANISMS FROM THE LEBANESE COAST

Different anthropogenic activities around the world contributed and are still contributing to the degradation of the marine environment and the Lebanese coast makes no exception. The years of war (1975-1992) have deeply affected the Lebanese environment and since then, environmental matters have not been a priority in the country thus leading to increased problems of habitats degradation and pollution. Sewage, industries and dumpsites are scattered along the Lebanese coast while rivers drain all kinds of land-based pollutants into the coastal area. This situation leaves all the actors in the environmental field as well as the public with questions about the environmental risks that may emerge as a result of all disturbances taking place, mainly on the coastal zone. The coastal zone is particularly touched by human activities, especially zones that are close to rivers' mouths. Are these contaminants susceptible of affecting the ecological status and fisheries quality, to finally present health risks to consumers? This study allowed to have an overview of the contamination levels and the marine environmental quality along the Lebanese coast, through a selection of marine organisms belonging to different habitats, positions in the food chain and that are commonly found, merchandized and consumed in Lebanon.

1.2. A MULTI-SPECIES APPROACH FOR THE EVALUATION OF ENVIRONMENTAL QUALITY ALONG THE LEBANESE COAST

Biomonitoring of the marine environment using species that belong to different taxonomic groups and trophic levels, allows a better assessment of the contamination in terms of presence and relative magnitude (Rainbow, 1995a). This choice is justified by the fact that the levels of contaminants present

in the sediments and the water column do not necessarily reflect the levels in marine organisms (Conti and Cecchetti, 2003). All aquatic organisms accumulate essential and non-essential trace metals at different levels depending on the phase they are in contact with the most, what they feed on and how they uptake metals. Thus, the scale of high or low levels is tightly related to the species and more particularly to the tissues involved (Hamed and Emara, 2006). That's why, biomonitoring using different species that belong to different taxonomic groups and occupying different habitats allows a better assessment of the extent of a certain contamination (Cubadda et al., 2001; Wang, 2002). In order to assess the trace metals pollution status along the Lebanese coast by, six species including algae (*Ulva lactuca*), bivalves (*Spondylus spinosus*), shrimps (*Marsupenaeus japonicus*) and fish (*Siganus rivulatus*, *Lithognathus mormyrus*, and *Etrumeus teres*), belonging to different habitats and compartments of the food chain, have been employed. A maximum number of relevant contaminants was chosen for analysis as the previous scarce studies carried out along the Lebanese coast, were only interested in toxic elements (Cd, Hg, Pb). In this study, 20 trace metals were measured: Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Ag, Cd, Sn, Sb, Ba, Hg, Pb and U using an accredited analysis method and thus allowing a wider screening of trace metal contamination.

The results showed a difference in accumulation patterns between different organisms (Fig. 5-1). The green alga was mostly associated with high levels of Al, Cr, Fe, Mn, Ti and V compared to other species, but exhibited lower levels of Hg. The bivalve *S. spinosus* showed the highest levels of Cd along with As and Zn. The shrimp *M. japonicus* showed high levels of As, Cu, Se and Zn. Fish species were mostly contaminated by Hg and more particularly the carnivorous fish (*L. mormyrus* and *E. Teres*), while the herbivorous *S. rivulatus* species contains high levels for most elements. Following Fig. 5-1, *S. rivulatus* showed tendency to accumulate the same elements that are associated to the green alga that constitutes a part of its diet (Bariche, 2006). Fish species showed variable trace metal concentrations in the livers and levels were generally higher than those found in muscles particularly for the toxic elements such Cd, Pb and Hg. This is the case of the herbivorous *S. rivulatus* of which livers showed the highest levels for most elements at all sites and seasons. The livers of *E. teres* were characterized by high levels of As and Se while those of *L. mormyrus* had the lowest levels of trace elements compared to the two other fish species. All the species employed in this study accumulated the studied trace metals, but each to a certain degree.

The data highlight the importance of a multi-species approach in order to evaluate the contamination status of a site. Some species concentrate contaminants more than others and thus a single species is not enough to comprehensively reflect the contamination status of a site. This interspecific variability has already been described in other studies (Andres and Ribeyre, 2000; Campanella et al., 2001; Canli

and Atli, 2003; Conti and Cecchetti, 2003; Cubadda et al., 2017). However, few studies have compared the accumulation between organisms of a same site while using different trophic levels and habitats.

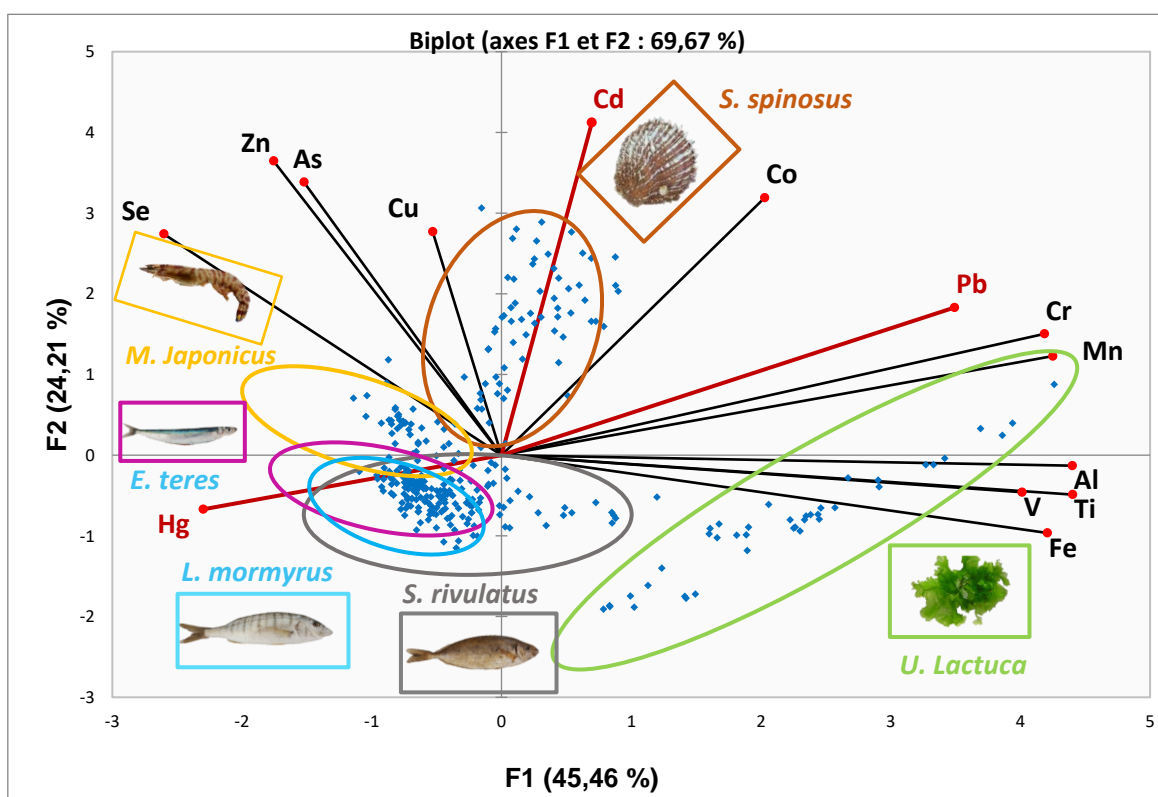


Figure 5-1 PCA showing the relationship species and elemental concentration (mg kg^{-1} dry weight)

Bioaccumulation and bio-amplification of inorganic chemical contaminants in marine organisms are a complex function of multiple parameters and thus may be highly variable even among individuals of a same species. Organisms of a same species can have different responses to contaminants depending on their size, age, sex and their physiological status. The bioaccumulation is a result of imbalance between absorption and detoxification that may occur through excretion or metabolism and it is highly related to bioavailability and the nature of the element (Rainbow, 1995a, 1985). This process is linked to biological functions (diet, respiration, excretion, reproduction), that are species-specific (Rainbow, 1995a; Rainbow and Luoma, 2011). Mollusks, for example, have the capacity to regulate essential elements like Cu and Zn (Swaleh et al., 2016; Zuykov et al., 2013) but they don't have mechanisms to regulate As, which explains the high levels of this contaminant in the bivalve (Azizur Rahman et al., 2012). That is why levels of As measured in these organisms reflect well the contamination of the studied site. Moreover, bivalve mollusks are sedentary species that are widely used as sentinel species.

Oysters such as *S. spinosus* are filter feeders that reflect the conditions of their environment at an adult age (Aksit et al., 2013). Green macroalgae and particularly those from the *Ulva* genus, are composed mainly of ulvans that are polysaccharides with ionic binding properties due to their sulfated structure (Circuncisão et al., 2018). That's why algae are mostly used to monitor levels of trace metals in the water as their physiology and anatomy allow uptake of metals mostly from a dissolved phase (Bonanno and Orlando-Bonaca, 2018; Circuncisão et al., 2018). *U. lactuca* is an alga with high growth rate and high resistance to eco-physiological conditions with high pollution levels which make it a good sentinel species that is widely used to monitor contamination by trace metals (Alves et al., 2013; Diop et al., 2016; Ho, 1990; Kaimoussi et al., 2004; Villares et al., 2001).

Fish have been widely documented as useful indicators of environmental quality because of their differential sensitivity to pollution (Naigaga et al., 2011). Although the sedentarity is one of the ideal criteria for a species to be used for monitoring, which is not the case for most of the fish, these species are used as bioindicators because they are organisms on the top of the aquatic food chain, long living and also easy to collect. Additionally, they are connected to different habitat elements on a broad spatial-temporal scale making them fit for biomonitoring at an individual as well as a population level (Chovanec et al. 2003). Consequently, the knowledge of fish taxonomy, habitat requirements, and physiology is substantial when considering fish as bioindicators. It must be noted that different fish tissues can be targeted for different contaminants. Fish liver accumulates variable levels of elements depending on the species and that includes some toxic elements that are found at higher concentrations compared to other species, as found in the present study.

Interspecific variation may also be the result of habitats and the feeding habits of the organisms in question. Diet is also an important route of fish exposure to chemical contaminants (Jeziarska and Witeska, 2006). Studies have shown that marine organisms having different diets showed different accumulation patterns of trace metals (Abdallah, 2008; Canli and Atli, 2003; Yilmaz et al., 2010). This is the case of the herbivorous *S. rivulatus* that showed higher liver concentrations for most elements analyzed in this study namely toxic element such as Cd, Hg and Pb. The muscles of this species also exhibited higher levels for most elements. While species living close to the sediments are expected to accumulate higher levels, it was not the case in the present study. Such results where herbivorous individuals had higher levels of metals were also documented elsewhere (Abdallah, 2008; Bawuro et al., 2018; Jeziarska and Witeska, 2006; Khaled, 2004). Causes were mainly attributed to the algae-based diet of the marbled spinefoot, and to its habitat near the coastal areas making it more prone to coastal disturbances (Zuluaga Rodríguez et al., 2015b). Moreover, Cd, Cr, Cu and Zn levels have been shown to be generally higher in demersal fish compared to pelagic and benthopelagic fish (Jiang et al.,

2018) which is in accordance with our findings. Carnivorous and omnivorous fish species may be more exposed to MeHg through the diet (Carrasco and Vassileva, 2014) compared to herbivorous species while benthic species are more exposed to bioavailable sediment-related contaminants such as As (Neff, 2002).

The respective bioaccumulation factors (BSAFs) were also calculated in this study through the analysis of sediments from the studied sites. The green algae showed no accumulation from sediments (BSAFs < 1); however, the bivalve and the crustacean both accumulated Cu and Zn that are between 1.5 and 10 times higher than what is found in the sediments. The bivalves had concentrations of Cd up to 12 times higher than the levels found in the sediments of Tripoli. In a study made along the Senegalese coast, the authors have recorded a higher bioaccumulation from water to biota than sediments to biota, as concentrations were 10 or even a 100 times higher than those measured in water while bioaccumulation from sediments was limited (Diop et al., 2016). In the present study, data concerning the concentration of trace metals in water was not available but results showed a low accumulation of metals from sediments to biota for most trace metals except for a few elements. This low bioaccumulation from the sediments, can be explained by the high hydrodynamic conditions which highly contribute to the dispersion of pollutants along the Lebanese coast (Fakhri and Romano, 2011).

Our results showed that species accumulate trace metals with different patterns and levels across tissues based on various parameters that could be intrinsic or extrinsic to the organism or also related to the surrounding environmental conditions. Thus, a multi-species approach allows a better evaluation of the contamination state of an environment at different scales. However, bioaccumulation remains a very complex process that is governed by multiple parameters (Anandkumar et al., 2018; Bawuro et al., 2018).

1.3. A MULTI-SEASON AND MULTI-SITE APPROACH FOR THE EVALUATION OF THE COASTAL ENVIRONMENTAL QUALITY

Seasonal variations

Lebanon is characterized by a Mediterranean climate with a mild rainy winter and a hot dry summer followed by autumn and separated from winter by a spring season. The rainy season extends from November till March with peak rainfall during the month of January. Most of chemical contaminants in the marine environment originate from terrigenous inputs, seasonal differences in rainfall patterns can lead to higher concentrations in contaminants through runoff and leaching of soils as well as through rivers inputs mainly during the rainy season. Conversely, this may lead to a greater dilution of the contaminants in the environment (Azizi et al., 2018; Barua et al., 2011; Çoğun et al., 2005).

Thus, the main driver of the seasonality is the change in hydrodynamic and hydrological conditions of the water, which in turn affect bioavailability of metals and their uptake by marine organisms. In Lebanon, the variation in rainfall patterns and riverine inputs through the year, have an impact on the coastal area and marine waters properties (Fakhri, 2012).

Seasonal variations in trace metals concentrations were found in the species analyzed in this study. Species showed different accumulation patterns following the seasons and the levels were mostly higher during the wet season. The three fish species and the bivalve exhibited significantly higher levels of trace metals mostly during the wet season whereas the green alga had higher concentrations during the dry season. No significant seasonal differences were found in the crustacean except for Se and As for which levels were higher during the wet season.

Seasonal variations with higher levels during the rainy season, have been documented in previous studies (Abdallah, 2008; Ayas, 2016; Bahnasawy et al., 2009; Barua et al., 2011; Khaled, 2004). However, some other studies documented the opposite, as levels of metals were found to be higher during the dry season (Khaled, 2004; Villares et al., 2002; Yilmaz and Yilmaz, 2007). Causes were attributed to increased temperatures during this season. Temperatures affect metabolic activity of the organisms and when the temperature increases, metabolic activity increases which leads to an increased absorption of metals (Chakraborty et al., 2014b; Jezierska and Witeska, 2006; Khaled, 2004; Yeldan and Avşar, 2000). Other causes were attributed to decreased rainfall and an increase in evaporation rates during the dry season especially in shallow waters (Rajeshkumar et al., 2018; Wang et al., 2010; Zuluaga Rodríguez et al., 2015b). Moreover, these variations, can be attributed to internal physiological cycles of the species, to the bioavailability of trace elements in the marine environment, the contaminant concentration in the surroundings as well as the metabolic activity of the organism. In fish, the dynamic of membrane lipids also plays a role in seasonal variations (Maehre et al., 2014). A young individual normally has a higher metabolic activity compared to an older individual, and thus it has been shown that metal accumulation is higher in younger individuals (Canli and Atli, 2003). This fact may also be related to the negative relationship observed between size and metal accumulation (Cubadda et al., 2017; Dang and Wang, 2012; Yi and Zhang, 2012). It has also been reported that some elements are subject to growth dilution (GD) (Dang and Wang, 2012; Karimi et al., 2010; Yi and Zhang, 2012). GD occurs when individual growth causes a gain in biomass that is disproportional to the gain of a specific element and it is mostly observed for elements that have low excretion rates including non-essential metals such as MeHg and essential ones such as Fe (Karimi et al., 2010). In the present study, all the selected species have their spawning period during the summer season, coinciding with a rise in water temperature except for *E. teres* that spawns during the winter

season. Levels of trace metals were predominantly higher during the wet season, could there be an effect of growth dilution for certain elements? Further deeper studies about the life cycle and accumulation rates of each species shall be done in order to better understand the reasons behind trace metals variability.

Bioavailability is the proportion of total trace metals that are available for incorporation into biota and is influenced by the physicochemical conditions in the environment that are also related to seasonality. It is a very important parameter to take into consideration when assessing bioaccumulation (Rainbow, 1995a). It is a function of a lot of parameters including coastal hydrodynamics, that affect the presence of biotic ligands and radicals that are capable of complexing the metals. In certain conditions, high levels of trace metals may be present in the environment under a form that is not readily available for uptake and in such case bioindicators may not fully reflect the levels of contaminants (Rainbow, 1995).

The concentration of trace metals showed significant differences with seasons with few exceptions such as the green alga and the shrimp, and the concentrations measured during the wet season were generally higher than those found during the dry one. Thus, when establishing biomonitoring programs, seasonality is an important parameter to take into consideration as accumulations by organisms may be variable following the climatic conditions.

Spatial variations

Along the Lebanese coast, a significant pollution by toxic elements such as Cd and Pb was recorded mainly in harbors' area (Abi-Ghanem, 2008; Abi-Ghanem et al., 2009a). A significant pollution by Cd, Cr, Pb and Zn has been shown to be localized at Beirut port (Merhaby et al., 2018). In the present study, in order to better evaluate the pollution state of the Lebanese coast, 20 contaminants cited above were measured in marine organisms at 3 sites that are representative of the Lebanese coast. The results showed that spatial difference is expressed differently through the different marine organisms (Fig. 2-5).

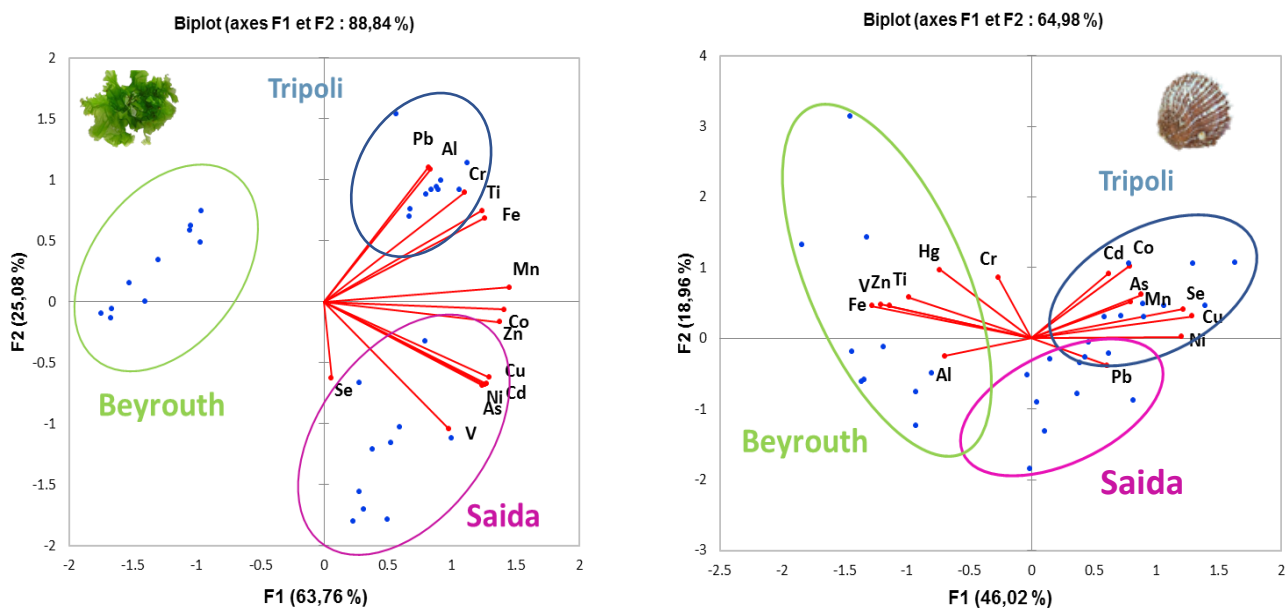


Figure 5-2 PCA showing the relationship sites and elemental concentrations in the green alga and the bivalve

Two sessile and sedentary species that are the green alga and the bivalve were taken as an example to display spatial variability of trace elements. As can be seen in Fig. 2-5, a same site presented a different contamination status depending on the organism in question. Through the green alga, Tripoli was associated to a contamination mainly by Al, Cr and Pb while a contamination by As, Cd, Co, Cu, Mn and Ni was observed through the bivalve at the same site. High levels of Cd were found in the bivalves from Tripoli compared to the 2 other sites. At the South of Tripoli, a phosphogypsum plant release more than 0.7 tons of Cd per year (Nakhlé, 2003) and may thus be at the origin of the Cd levels observed at this site. The same scheme was seen with fish samples. Each fish species was associated with a group of contaminants, and *S. rivulatus* presented the highest levels for most contaminants in their livers. Samples from Tripoli were characterized by higher levels of Al, Mn, Ti and Pb while the ones from Saida had significantly higher ($p < 0.05$) concentrations of Cd, Fe and Hg. Livers of *E. teres* from all sites were associated to a contamination by As and Se while livers of *L. mormyrus* were the least contaminated compared to the two other species. Al and Pb were both found prominent at Tripoli through the macroalga and the livers of the rabbitfish that consumes it. Tripoli is known for its artisanal fishing port and boat maintenance activities in addition to an open dumpsite that releases toxic leachate in the marine environment (Khalil et al., 2018), which may explain the presence of these contaminants. Beirut generally showed an intermediate level of contamination for most elements and levels of trace metals in the sediments were lower than what was previously found by Merhaby et al. (2018) and Abi-Ghanem et al. (2008) in the port of Beirut. This is because samples were not taken from the port but far from the coastal area where fishermen throw their nets.

When compared to other regions, the contamination levels found in the present study, were sometimes higher when considering the livers of fish, thus reflecting the environmental status of the marine ecosystem. Levels of trace metals in algae, muscles of fish and soft tissues of shrimps and bivalves were generally lower than levels found in other polluted areas of the Mediterranean, but sometimes higher than what is found in region that are relatively far from anthropogenic disturbances (Campanella et al., 2001). This suggests that the Lebanese coast faces environmental disturbances that affect the marine biota to a certain extent and places it at a moderate level of contamination compared to the neighboring countries.

The results obtained in the present study, show that the knowledge of the taxonomy, habitat requirements, physiology and feeding behavior of any organism chosen as bioindicator, is substantial to environmental status evaluation as biomonitoring is done through different organisms with different lifestyles. That's why the absolute accumulation of a metal has no significance unless it is associated to a species or even tissue in the species (Rainbow 2002).

2. WHAT CAN BE SUGGESTED IN THE FRAME OF A BIOMONITORING PROGRAM ALONG THE LEBANESE COAST?

Based on our results, we found that organisms accumulate contaminants differently and thus a multi-species approach revealed to be very important in order to evaluate the contamination status of a site at different scales through bioindicators. Accumulation of trace metals was variable with seasons; the highest levels of trace metals were generally encountered during the wet season and causes were on one hand, attributed to increased rainfall that changes the physicochemical properties of the coastal environment and thus affects the levels, forms and bioavailability of different elements, and on the other hand to internal physiological cycles that are species-specific. The spatial variability of trace elements was reflected differently through species which highlights the fact that knowledge of the organisms' lifestyle and physiology is a priority when using them as bioindicators. Moreover, different organs may be targeted; livers, gills and kidneys are often targeted when using fish as bioindicators as they are major accumulators. However, in bivalve mollusks for example, the digestive glands are the parts that accumulate the most as they play a major role in detoxification mechanisms compared to other tissues (Hédouin et al., 2009). In our study, only muscles of fish and bivalves in addition to fish livers were analyzed.

According to our findings, the choice of a bioindicator is tightly related to the contaminant that is being investigated since organisms showed different accumulation patterns. If the monitoring program aim is to monitor contaminants in the water column for example, the green macroalgae is a sessile sedentary

organism that has the capacity to accumulate high amounts of contaminants. In the present study water contaminants haven't been analyzed, but based on previous studies made on *U. lactuca*, it has been shown that this species accumulates metals mostly from dissolved sources and thus is a good indicator of water quality in a certain site (Alves et al., 2013; Bonanno et al., 2019b; Circuncisão et al., 2018; Diop et al., 2016; Ho, 1990; Kaimoussi et al., 2004; Villares et al., 2001). In our study, the green alga didn't show any accumulation of trace metals from the sediments but this species has been shown to accumulate different trace metals and toxic elements such as Al, Cd and Pb as a function of their concentration in the surrounding water (Circuncisão et al., 2018). In the case of the Lebanese coast, *U. lactuca* accumulated relatively high levels of Al, As, Cd, Cr, Cu, Fe and Pb. According to the results obtained, this species can be used to monitor contamination with these elements, however two aspects that haven't been done in the frame of this study, are necessary to be completed before adopting the species as a bioindicator : an assessment of background levels of trace metals in a site that is weakly or less impacted by anthropogenic activities in addition to the analysis of seawater for its content in trace metals. The season is also an important factor to take into consideration. If spatial comparison is targeted for example, the wet season should be chosen as *U. lactuca* is not found at all sites during the dry season.

The bivalve used in our study, is also a sedentary sessile species that has been proved to fulfill all criteria that make a good bioindicator (Aksit et al., 2013). This well-established Indo-pacific species has become widespread in the Eastern Mediterranean and lives at depths ranging from 1 to 40 m. *S. spinosus* is a filter feeder and filter-feeding organism are known to have low biotransformation capacities and to accumulate the bioavailable fraction of the contaminant from water at higher levels than those in the ambient environment and are thus used as sentinel species to monitor pollution in coastal waters (Storelli and Marcotrigiano, 2001b; Türkmen and Türkmen, 2005). The present study showed that this species accumulated a wide range of trace elements and a bioaccumulation from sediments was confirmed for Cd, Cu and Zn that presented bioaccumulation factor greater than the unit. Elements like Cu and Zn are known to be essential elements for bivalves, they are thus metabolically active and may not reflect the concentration in the surroundings (Zuykov et al., 2013). Despite the fact that the spiny oyster satisfies all criteria of a good bioindicator (Aksit et al., 2013), it hasn't been commonly used as a bioindicator probably due to the difficulty of sampling as it lives attached to rocky substrate starting at 1 m of depth and thus is not immediately accessible at the level of the coastal area. However, the range of depth that this species occupies can be interesting as it allows to monitor the presence of contaminants at different depths and distances from the coastal area. The advantages of this species is that it is widespread along the Lebanese coast and present at all seasons,

thus allowing seasonal and spatial comparison of the contamination status. It is also appreciated for consumption by the Lebanese population which would also allow to monitor risks related to human consumption on a regular basis. However, it remains important to choose individuals that are at the same life stage in order to reflect the contamination state of the sites in question and to compare contamination status between sites. In previous studies, species like *Patella caerulea* and *Brachidontes variabilis* were used to assess the pollution state of the Lebanese coast by Cd, Hg and Pb (Nakhlé, 2003; Nakhlé et al., 2006). Levels of trace metals in these two species should be compared with caution to the levels obtained in the spiny oyster since they don't share neither the same diet nor the same habitat. Consequently, establishing trends of trace metal evolution with time is not possible. It was only possible to compare levels of Pb in *U. lactuca* that was analyzed back in 1980 by Shiber et al. (1980). In the present study, the green alga showed levels of Pb that are much lower than what was found few decades ago (37.5 decreased to 4.44 mg kg⁻¹ dry weight at the same site). In fact, Pb emission has shown a decreasing tendency everywhere in the world especially after banning the use of leaded gasoline (Figure 5-3).

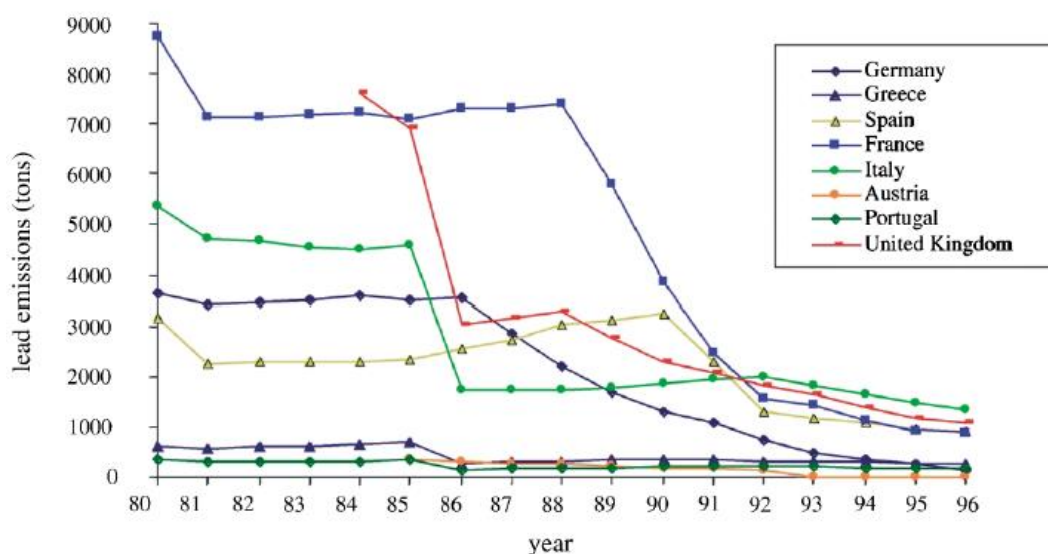


Figure 5-3 Annual lead emissions in different EU countries (Von Storch, 2003)

When comparing levels of trace elements analyzed using modern methods with data of past studies, the difference reveals to be important which makes the results somehow questionable. The historic trend in concentration of trace metals in the ocean shows a decrease across the years (Ansari et al., 2004). It might be because the methods used at present have a greater sensitivity, or because of the

implementation of environmental laws to reduce emissions, thus contributing to lower these levels. There is no doubt that methods developed nowadays have higher detection capacities and sensitivity.

In France, the monitoring of water contamination along the French coast has been performed by the RNO (Réseau National d'Observation), renamed ROCCH (Réseau d'observation de la contamination chimique du milieu marin), in 2008. ROCCH was established by the French Ministry of Environment in 1974 and is coordinated by IFREMER. Its purpose is to assess levels and trends in chemical contamination along the coast. The ROCCH is based on mussels and oysters that are used as quantitative indicator of contamination. These mollusks have the property of concentrating certain contaminants present in the environment in which they live (metals, hydrophobic organic contaminants) (IFREMER, 2020). In 2008, the ROCCH was amended to meet the requirements for monitoring the chemical status of the European Water Framework Directive (WFD) and then the Marine Environment Strategy Directive (DCSMM), and also to respond to European regulations on shellfish growing areas as it also takes into consideration mollusks destined for human consumption (IFREMER, 2020). The existence of such programs allows to obtain trends concerning the evolution of contaminants with time and thus demonstrates the efficiency of regulatory measures that limit the use of a certain contaminants such as Pb. This is not the case in Lebanon due to the absence of a well-established biomonitoring program. At present, with the existence of the DCSMM that applies on the Western Mediterranean part, a lack of data is often encountered in the Eastern Mediterranean due to the absence of such directives in developing countries of the South and Eastern Mediterranean.

In order to establish a biomonitoring program, a knowledge of the physiology of the targeted bioindicators and the behavior of the elements in questions remains substantial. Biomonitoring can be used to establish geographical and temporal variations in the availability of trace metals (Rainbow, 1995a; Rainbow and Luoma, 2011). Based on our results, in order to evaluate the environmental status of a site, it is important to use multiple species that respond to different sources of metals such as the macrophytic alga that responds to dissolved sources and the bivalve that responds to dissolved and suspended metals. *S. spinosus* has all the criteria to make a good biomonitor and may be adopted to assess the extent of contamination at a given site thanks to depth range it occupies.

3. HEALTH RISKS RELATED TO THE CONSUMPTION OF FISHERY PRODUCTS

According the US EPA (US environmental protection agency), risk assessment is the quantitative and qualitative evaluation of the risk posed to human health and or the environment by the actual presence and/or use of a specific pollutant (EPA, 2017). It is commonly known that seafood is a route of human

exposure to harmful chemicals including toxic trace metals. In order to protect the consumers, food safety authorities such as the EC (European commission), the FAO (Food and Agriculture organization) and the US FDA (US food and drug administration) have established maximum allowed levels (MALs) of these contaminants in seafood, especially for the categories that are considered dangerous for human health. Elements such as Cd, Hg and Pb have been shown to be toxic even at very low levels as they don't exert any biological functions in living organisms (Jaishankar et al., 2014; Tchounwou et al., 2012). Those elements have been the subject of many studies (Anandkumar et al., 2018; Avigliano et al., 2019; Barone et al., 2018; Dizman et al., 2017; Sidhu, 2003; Storelli, 2008; Storelli and Marcotrigiano, 2001b). In some cases these elements were found to be above the MALs set by the EC (Copat et al., 2013; Pastorelli et al., 2012; Perugini et al., 2014; Storelli and Barone, 2013). In order to be able to assess human health risk, information must first be gathered in order to identify data gaps, uncertainties and research needs.

In the present study, all species showed levels of metals largely below the regulatory limits set by the EC. The oyster showed the highest levels of Cd while the crustacean had the highest levels of As. It is important to mention that in Lebanon, industries that are susceptible to release As are almost absent. Since 1998, the country has banned the import and use of pesticides containing As (Decree No 94/1/1998) (Skaff, 2014). Therefore the presence of As might be associated to natural sources; especially that marine organisms are natural accumulators of this element making it hard to determine whether it is from an anthropogenic or natural sources (Kalantzi et al., 2017). However studies have shown that Sr and stable isotopes of water can be used as tracers to identify natural and anthropogenic sources of As (Khaska et al., 2015). In seafood, organoarsenicals are the dominant forms including arsenobetaine (AsB) that has been shown to be of no toxicological concern, while the inorganic As (iAs) form is the most toxic one. Regulations exist for iAs levels in rice as it constitutes the dominant fraction in this matrix (EFSA CONTAM Panel, 2009). The different fractions of As are variable following the species and the food matrix in question (Figure 5-1). Arsenosugars represent the main proportion in algae consumed with sushi for example and up to 45% of the As fraction in mollusks. However, very little reliable information exists concerning the toxicity of arsenosugars (Yu et al., 2019). Assessment of exposure to different arsenosugars and more research in regard to As species and their respective toxicities are needed in order to establish clear regulations, highlighting the importance of speciation analysis.

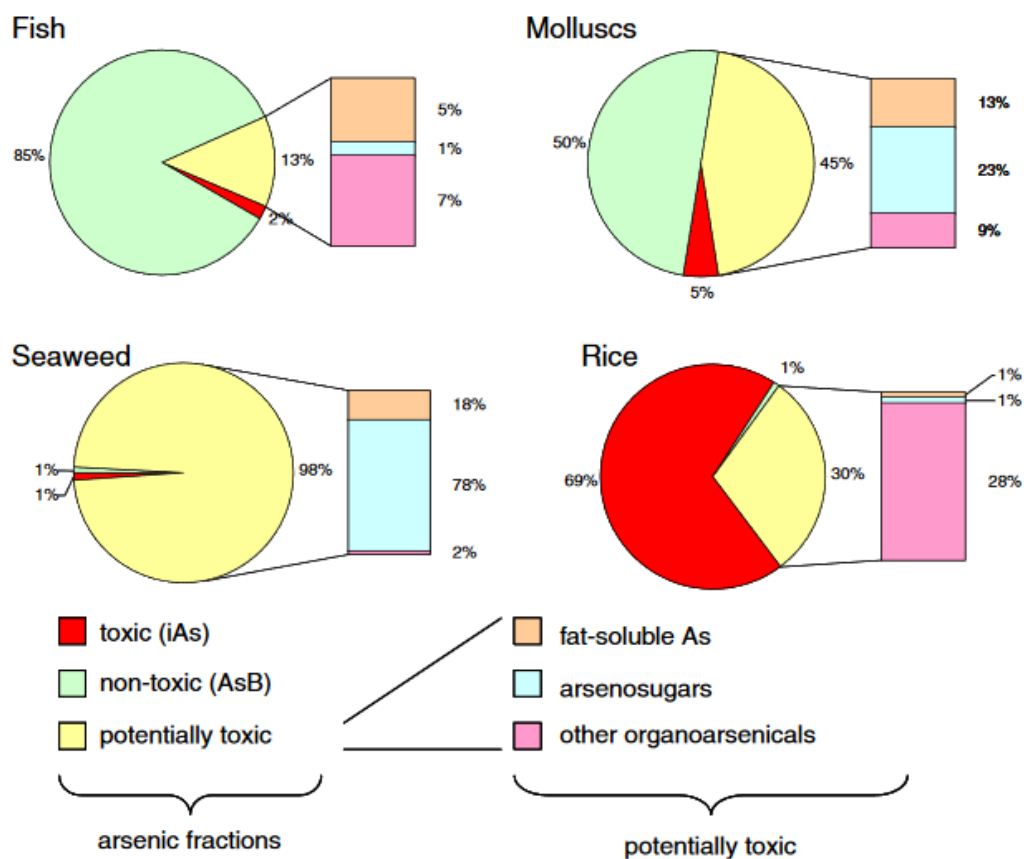


Figure 5-4 Proportions of three As fraction in three different marine food in comparison with rice (Feldmann and Krupp, 2011)

For Hg, it is well known that the most toxic form is methylmercury (MeHg) the dominant fraction in seafood (EFSA, 2014b). The toxicity of mercury has been known since the Minamata poisoning in Japan. Until now, legislation exists in terms of total mercury as MAL, while a tolerable weekly intake (TWI) of $1.3 \mu\text{g g}^{-1} \text{bw}$ per week is adopted at present in terms of MeHg. However, speciation of MeHg has not yet become a routine analysis. In the present study, a method for the speciation of mercury by HPLC-ICP-MS has been optimized as a step towards a routine approach for mercury speciation in seafood. To our knowledge, it is the first time that a mercury speciation method by HPLC-ICP-MS is validated using the accuracy profile procedure. The method showed to be accurate and fit for purpose “as a routine method” for mercury speciation.

Health risks may be determined not only by comparing the obtained levels to the established MALs. One of the used methods is the THQ (Total Hazard Quotient) which is the fraction of the integrated dose as oral reference dose established for each contaminant (US EPA, 2000). The calculated THQ showed that consumption of seafood from the Lebanese coast is unlikely to cause adverse health effects. Even the TTHQs were lower than the unit when As levels were not taken into account. In

addition to the THQ, when provisional tolerable weekly intakes (PTWIs) or Tolerable weekly intakes (TWIs) for the elements exist, the estimated weekly consumption is calculated and then compared to the PTWIs. An important point to take into consideration, is the parts of the organism that are consumed. In distinct parts of the world, people consume marine organisms differently. Some people consume the entire fish while others consume mostly the muscle. Consequently, when establishing regulations, dietary habits of each population should be taken into consideration in order to get the most accurate risk prevention.

Perspectives

Our study has shown that Lebanon's coastal ecosystem is marked to a certain extent by the human footprint in terms of metallic contaminants. From the results obtained in this study, different approaches can be proposed to better understand the pollution of the Lebanese coast and to assess the risks associated with these chemical pollutants. This reveals to be necessary especially in Tripoli that showed the highest levels for most metals. Relatively high concentrations of Cd were found in oysters from this site, but levels remained lower than the maximum permissible levels set by the EC. Moreover, the highest levels of As, Pb and Cr were found in algae from Tripoli. An open dumpsite in Tripoli has been proved to release leachate in the area leading to elevated concentrations of Cd and Pb in the water (Khalil et al., 2018). Additionally, a phosphogypsum plant in the south of Tripoli releases significant amounts of Cd in the coastal area (Nakhlé, 2003). In this scope, controlling and treating dumpsite leachates before they reach the marine environment is a step towards reducing toxic effluents being drained. The bigger problem lies in untreated municipal and industrial effluents that are directly discharged in the coastal area. The use of mussels or oysters engaged at different distances from the pollution sources can be a way to better understand the contamination by Cd in the Northern area in terms of extent and order of magnitude.

In this study, we were interested in sediments contamination by metals and the calculation of bioaccumulation factors in different organism. However, contamination through diet represents an important route of transferring pollutants through the food chain. Thus, this study can be pushed further by evaluating the levels of metallic contaminants as function of size of individuals from different species. In this study, demersal carnivorous species such as *L. mormyrus* and *E. teres* showed the highest levels of Hg. Bioamplification can concern several metals in general, and more specifically MeHg which is the most toxic form of mercury for humans. Indeed, the food chain is known to be the most important route of the process of bioaccumulation of mercury. Thus, a study of Hg levels in

predatory species like *Sarda sarda* that are consumed by the Lebanese population reveals to be necessary.

Nowadays, a problem of actuality, is the presence in the marine environment of emerging contaminants such as pharmaceutical residues, surfactants and surfactant residues, plasticizers and various industrial additives in addition to healthcare products etc. These substances have the capacity to bioconcentrate in aquatic organism and ultimately reach humans through the food chain (Petrovic, 2003). Currently, there are no studies dealing with these contaminants in the Lebanese marine environment. Evaluating the levels of these contaminants in the sediment, the water and the biota would help gain information about the pollution status of the Lebanese marine environment with these contaminants.

In order to identify the extent of any contamination, it is important to identify the sources. In the present study, seawater analysis has been considered, but could not be established. An analysis of effluents before and after dispersion in the marine environment would allow to better localize the sources and extent of contaminants on the Lebanese coastal. Moreover, the speciation of metals in seawater is necessary in order to identify their chemical forms, bioavailability and toxicity to marine organisms (Campbell et al., 2002).

Levels of most trace elements in the liver were higher than what was reported in other Mediterranean regions suggesting that the Lebanese marine coastal zone is exposed to strong environmental pressure. However, Hg, Cd and Pb concentrations in fish muscle were below the levels set by the European Community indicating no potential risk on human health. Due to the increasing environmental pressures on the Lebanese coast, more parameters such as landfills lixiviates and riverine water contaminants should be considered in order to assess more accurately the impact of different pollution sources on the coastal zone, and thus establish a regular monitoring program in order to prevent further environmental deterioration. Environmental chemical contamination does not only affect humans through consumption of fishery products, but has toxic effects on the marine biota. Pollution of water with trace metals, may also adversely affect the immune system of fish leading to decreased production, increased susceptibility to diseases and mortality (Rani et al., 2015).

ANNEX

Annex 1 Trace elements mean \pm SD (mg kg⁻¹ dry weight) in the muscles and livers of *S. rivulatus* collected at the three sites for the wet(S1) and dry (S2) seasons. The minimum and maximum are given into brackets.

Element	Tissue	Tripoli		Beirut		Saida	
		S1	S2	S1	S2	S1	S2
Al	Muscle	69.11 \pm 39.85 (25.54 – 155)	2.49 \pm 2.08 (0.83 – 7.81)	6.46 \pm 7.43 (0.97 – 20.43)	0.92 \pm 0.49 (0.38 – 1.75)	3.88 \pm 3.47 (1.42 – 6.33)	2.2 \pm 1.72 (0.52 – 5.25)
	Liver	37.08 \pm 21.5 (11.07 – 75.18)	11.85 \pm 13.93 (1.49 – 43.01)	25.70 \pm 21.75 (1.91 – 64.68)	2.72 \pm 1.87 (1.16 – 7.02)	5.46 \pm 2.62 (2.09 – 10.70)	7.65 \pm 9.89 (0.79 – 33.94)
Ti	Muscle	5.28 \pm 2.21 (2.38 – 9.85)	0.37 \pm 0.18 (0.19 – 0.76)	1.18 \pm 0.71 (0.47 – 1.80)	0.47 \pm 0.42 (0.23 – 1.65)	0.79 \pm 0.06 (0.74 – 0.83)	0.64 \pm 0.36 (0.32 – 1.57)
	Liver	9.02 \pm 10.29 (2.02 – 33.79)	1.25 \pm 0.80 (0.44 – 2.26)	2.70 \pm 5.56 (0.44 – 8.30)	2.78 \pm 6.56 (0.30 – 19.01)	1.45 \pm 1.69 (0.45 – 5.20)	0.91 \pm 0.99 (0.31 – 3.07)
V	Muscle	0.2 \pm 0.09 (0.1 – 0.41)	0.04 \pm 0.0 (0.01 – 0.09)	0.08 \pm 0.05 (0.02 – 0.17)	0.02 \pm 0.02 (0.01 – 0.05)	0.10 \pm 0.14 (0.03 – 0.51)	0.05 \pm 0.02 (0.01 – 0.07)
	Liver	1.07 \pm 0.76 (0.31 – 2.37)	1.25 \pm 0.8 (0.44 – 2.26)	3.46 \pm 4.60 (0.38 – 16.13)	2.78 \pm 6.56 (0.3 – 19.01)	8.66 \pm 7.91 (0.73 – 25.07)	0.91 \pm 0.99 (0.31 – 3.07)
Cr	Muscle	1.06 \pm 0.76 (0.31 – 2.37)	3.33 \pm 2.69 (0.17 – 8.10)	0.12 \pm 0.03 (0.11 – 0.16)	1.16 \pm 0.86 (0.21 – 2.71)	0.18 \pm 0.03 (0.14 – 0.20)	5.91 \pm 6.03 (0.09 – 17.83)
	Liver	0.46 \pm 0.41 (0.09 – 1.49)	0.13 \pm 0.07 (0.06 – 0.26)	0.21 \pm 0.13 (0.07 – 0.47)	0.29 \pm 0.48 (0.07 – 1.16)	0.16 \pm 0.06 (0.09 – 0.29)	0.14 \pm 0.11 (0.07 – 0.37)
Mn	Muscle	1.86 \pm 0.68 (0.96 – 3.12)	0.73 \pm 0.36 (0.36 – 1.37)	0.65 \pm 0.31 (0.31 – 1.22)	0.47 \pm 0.17 (0.28 – 0.75)	0.43 \pm 0.15 (0.26 – 0.76)	0.77 \pm 0.80 (0.38 – 3.02)
	Liver	3.09 \pm 0.99 (0.64 – 4.22)	4.01 \pm 1.58 (2.37 – 7.96)	6.55 \pm 2.33 (4.34 – 11.43)	6.37 \pm 4.09 (3.07 – 14.15)	3.09 \pm 0.99 (0.64 – 4.22)	4.75 \pm 2.35 (1.74 – 10.52)
Fe	Muscle	112.71 \pm 45.9 (50.43 – 215.44)	21.28 \pm 8.01 (10.68 – 39.04)	26.59 \pm 19.78 (10.10 – 71.58)	14.90 \pm 6.37 (9.83 – 29.85)	14.52 \pm 2.55 (10.74 – 18.52)	23.57 \pm 5.85 (15.79 – 32.45)
	Liver	1091 \pm 413 (568 – 1672)	1846 \pm 803 (902 – 3250)	1621 \pm 482 (485 – 2227)	1847 \pm 749 (849 – 3320)	2566 \pm 594 (1563 – 3140)	1759 \pm 553 (875 – 2451)
Co	Muscle	0.32 \pm 0.17 (0.09 – 0.63)	1.16 \pm 0.84 (0.31 – 2.80)	0.25 \pm 0.09 (0.09 – 0.38)	0.20 \pm 0.21 (0.03 – 0.77)	0.13 \pm 0.04 (0.06 – 0.18)	0.58 \pm 0.54 (0.08 – 2.00)
	Liver	3.22 \pm 2.20 (0.43 – 6.51)	7.1 \pm 6.54 (1.51 – 20.14)	1.5 \pm 0.6 (0.44 – 2.73)	2.63 \pm 3.01 (0.52 – 10.80)	2.31 \pm 1.04 (0.19 – 3.93)	4.09 \pm 3.03 (0.25 – 9.30)
Ni	Muscle	N.D	N.D	N.D	N.D	N.D	N.D
	Liver	0.71 \pm 0.3 (0.40 – 1.31)	1.77 \pm 1.19 (0.63 – 4.49)	1.36 \pm 0.45 (0.68 – 2.23)	0.66 \pm 0.32 (0.31 – 1.29)	1.13 \pm 0.35 (0.74 – 1.76)	5.91 \pm 4.14 (0.78 – 11.25)
Cu	Muscle	1.47 \pm 0.62 (1.01 – 3.16)	0.70 \pm 0.09 (0.59 – 0.87)	1.28 \pm 0.34 (1.01 – 2.13)	1.06 \pm 0.74 (0.63 – 3.07)	0.97 \pm 0.27 (0.72 – 1.61) v	1.12 \pm 0.37 (0.76 – 1.96)
	Liver	123 \pm 89 (24 – 356)	125 \pm 72 (38 – 221)	224 \pm 225 (30 – 758)	328 \pm 201 (38 – 606)	668 \pm 571 (2.32 – 2022)	357 \pm 529 (4.35 – 1810)
Zn	Muscle	24.72 \pm 2.1 (21.77 – 27.50)	21.04 \pm 5.53 (13.7 – 29.59)	24.41 \pm 3.74 (18.54 \pm 30.01)	23.87 \pm 3.79 (18.41 – 31.13)	14.95 \pm 1.64 (12.14 \pm 18.18)	28.22 \pm 5.93 (16.27 – 35.59)
	Liver	309 \pm 110 (182 – 533)	321 \pm 126 (181 – 652)	516 \pm 285 (195 – 1061)	505 \pm 189 (269 – 844)	1111 \pm 499 (549 – 2049)	294 \pm 104 (120 – 434)
As	Muscle	2.07 \pm 0.21 (0.72 – 1.3)	5.74 \pm 2.81 (3.18 – 10.84)	6.11 \pm 5.65 (1.57 – 21.58)	4.38 \pm 2.93 (0.88 – 9.87)	12.05 \pm 5.68 (5.45 – 21.13)	3.97 \pm 1.67 (1.03 – 7.02)
	Liver	3.86 \pm 1.80 (1.68 – 8.15)	6.3 \pm 2.09 (3.37 – 9.6)	7.41 \pm 5.9 (2.17 – 23.47)	10.19 \pm 7.46 (2.06 – 26.24)	13.7 \pm 8.02 (8.13 – 34.03)	7.92 \pm 3.72 (1.37 – 14.36)

Se	Muscle	1.02 ± 0.21 (0.72 – 1.3)	1.15 ± 0.56 (0.50 – 2.40)	0.97 ± 0.22 (0.54 – 1.24)	1.12 ± 0.78 (0.49 – 2.51)	1.49 ± 0.37 (1.11 – 2.20)	1.17 ± 0.29 (0.54 – 1.44)
	Liver	5.84 ± 1.33 (3.65 – 8.18)	8.83 ± 3.63 (4.74 – 14.89)	5.79 ± 1.93 (2.35 – 7.69)	8.47 ± 3.91 (4.51 – 16.23)	9.33 ± 3.24 (2.51 – 13.34)	9.21 ± 5.59 (3.79 – 13.68)
Ag	Muscle	N.D	N.D	N.D	N.D	N.D	N.D
	Liver	1.06 ± 0.80 (0.44 – 2.47)	1.40 ± 0.54 (0.62 – 1.87)	1.99 ± 1.72 (0.31 – 5.63)	2.24 ± 1.35 (0.75 – 4.39)	5.43 ± 4.7 (1.52 ± 17.9)	2.19 ± 1.39 (0.65 – 4.78)
Cd	Muscle	0.01 ± 0.003 (0.009 – 0.014)	0.019 ± 0.01 (0.004 – 0.029)	0.01 ± 0.01 (0.004 – 0.02)	0.003 ± 0.002 (0.001 – 0.006)	0.01 ± 0.01 (0.01 – 0.02)	0.02 ± 0.01 (0.004 – 0.03)
	Liver	2.03 ± 1.50 (0.07 – 4.9)	9.23 ± 5.31 (0.89 – 19.32)	3.37 ± 2.52 (0.22 – 6.52)	2.95 ± 2.55 (0.62 – 8.3)	9.27 ± 4.48 (0.02 – 15.5)	11.03 ± 9.61 (0.08 – 27.02)
Sb	Muscle	N.D	N.D	N.D	N.D	N.D	N.D
	Liver	0.02 ± 0.01 (0.01 – 0.03)	0.03 ± 0.03 (0.01 – 0.1)	0.02 ± 0.01 (0.01 – 0.05)	0.03 ± 0.03 (0.01 – 0.09)	0.02 ± 0.01 (0.01 – 0.04)	0.09 ± 0.06 (0.02 – 0.20)
Hg	Muscle	N.D	0.05 ± 0.01 (0.04 – 0.07)	N.D	N.D	N.D	N.D
	Liver	0.41 ± 0.15 (0.27 – 0.69)	0.80 ± 0.69 (0.25 – 2.62)	0.42 ± 0.25 (0.05 – 0.82)	0.39 ± 0.22 (0.17 – 0.90)	0.86 ± 0.40 (0.40 – 1.53)	1.2 ± 0.77 (0.24 – 2.7)
Pb	Muscle	0.36 ± 0.26 (0.13 – 1.02)	0.06 ± 0.02 (0.04 – 0.08)	0.06 ± 0.02 (0.03 – 0.08)	0.04 ± 0.01 (0.04 – 0.08)	0.90 ± 1.5 (0.03 – 2.63)	0.43 ± 0.43 (0.05 – 1.52)
	Liver	4.4 ± 7.62 (0.4 – 25.74)	0.39 ± 0.23 (0.05 – 0.76)	0.99 ± 0.97 (0.09 – 3.44)	0.49 ± 0.45 (0.15 – 1.45)	0.52 ± 0.37 (0.04 – 1.40)	2.22 ± 1.56 (0.25 – 5.14)

Annex 2 Trace elements mean \pm SD (mg kg⁻¹ dry weight) in muscles and livers of *L. mormyrus* collected at the three sites for the wet(S1) and dry (S2) seasons. The minimum and maximum are given into brackets.

Element	Tissue	Tripoli		Beirut		Saida	
		S1	S2	S1	S2	S1	S2
Al	Muscle	2.18 \pm 0.91 (1.17 – 4.04)	0.28 \pm 0.12 (0.19 – 0.42)	N.D	1.31 \pm 1.08 (0.36 – 3.52)	N.D	0.39 \pm 0.26 (0.19 – 1.05)
	Liver	16.83 \pm 19.14 (1.28 – 57.53)	1.13 \pm 0.72 (0.56 – 2.35)	6.08 \pm 5.08 (1.00 – 11.17)	13.75 \pm 17.50 (0.56 – 2.35)	1.08 \pm 0.31 (0.68 – 1.70)	1.4 \pm 1.11 (0.31 – 3.96)
Ti	Muscle	N.D	0.23 \pm 0.14 (0.11 – 0.45)	N.D	0.37 \pm 0.19 (0.13 – 0.62)	N.D	0.34 \pm 0.25 (0.20 – 0.31)
	Liver	2.08 \pm 1.31 (0.55 – 4.07)	0.42 \pm 0.30 (0.11 – 0.98)	N.D	N.D	N.D	N.D
V	Muscle	N.D	0.02 \pm 0.03 (0.01 – 0.11)	N.D	0.03 \pm 0.04 (0.004 – 0.13)	N.D	0.004 \pm 0.001 (0.003 – 0.004)
	Liver	0.34 \pm 0.22 (0.11 – 0.73)	0.42 \pm 0.3 (0.11 – 0.98)	0.11 \pm 0.11 (0.02 – 0.35)	N.D	0.55 \pm 0.46 (0.13 – 1.66)	N.D
Cr	Muscle	N.D	0.04 \pm 0.01 (0.02 – 0.06)	N.D	0.03 \pm 0.01 (0.02 – 0.06)	0.32 \pm 0.24 (0.09 – 0.57)	0.07 \pm 0.02 (0.04 – 0.07)
	Liver	0.19 \pm 0.09 (0.09 – 0.40)	N.D	0.24 \pm 0.11 (0.09 – 0.36)	0.14 \pm 0.07 (0.06 – 0.21)	N.D	N.D
Mn	Muscle	0.69 \pm 0.44 (0.25 – 1.82)	0.53 \pm 0.15 (0.37 – 0.84)	0.37 \pm 0.08 (0.22 – 0.47)	0.87 \pm 0.42 (0.46 – 1.60)	0.37 \pm 0.23 (0.16 – 0.94)	0.67 \pm 0.12 (0.51 – 0.67)
	Liver	6.55 \pm 2.33 (4.34 – 11.43)	6.51 \pm 1.32 (5.08 – 8.93)	3.11 \pm 0.30 (2.72 – 3.51)	6.24 \pm 1.26 (4.17 – 7.96)	4.92 \pm 1.2 (3.03 – 6.49)	5.71 \pm 1.29 (3.86 – 7.49)
Fe	Muscle	22.50 \pm 13.75 (7.45 – 38.97)	10.91 \pm 3.02 (7.5 – 16.75)	15.09 \pm 14.9 (7.01 – 45.38)	16.01 \pm 6.49 (6.86 – 23.91)	10.79 \pm 2.77 (6.80 – 155.42)	10.49 \pm 1.63 (7.99 – 12.50)
	Liver	974 \pm 272 (611 – 1521)	739 \pm 419 (142 – 1696)	396 \pm 104 (207 – 544)	529 \pm 67 (428 – 652)	867 \pm 313 (450 – 1337)	594 \pm 563 (224 – 2126)
Co	Muscle	0.03 \pm 0.02 (0.01 – 0.06)	0.54 \pm 0.08 (0.08 – 2.00)	0.04 \pm 0.02 (0.02 – 0.07)	0.04 \pm 0.01 (0.02 – 0.06)	0.03 \pm 0.01 (0.02 – 0.05)	0.02 \pm 0.01 (0.01 – 0.02)
	Liver	1.44 \pm 0.59 (0.81 – 2.36)	3.24 \pm 2.02 (1.40 – 7.92)	0.77 \pm 0.26 (0.49 – 1.14)	1.16 \pm 0.36 (0.65 – 1.82)	2.68 \pm 0.89 (1.86 – 4.42)	0.58 \pm 0.19 (0.33 – 0.92)
Ni	Muscle	N.D	N.D	N.D	N.D	N.D	N.D
	Liver	0.52 \pm 0.08 (0.44 – 0.64)	0.34 \pm 0.02 (0.31 – 0.35)	0.44 \pm 0.03 (0.41 – 0.48)	0.36 \pm 0.11 (0.21 – 0.53)	0.39 \pm 0.07 (0.31 – 0.44)	0.40 \pm 0.16 (0.24 – 0.62)
Cu	Muscle	0.61 \pm 0.12 (0.47 – 0.91)	1.3 \pm 0.51 (0.65 – 2.25)	0.88 \pm 0.22 (0.67 – 1.27)	0.91 \pm 0.17 (0.68 – 1.15)	0.81 \pm 0.14 (0.65 – 1.06)	1.29 \pm 0.23 (0.95 – 1.32)
	Liver	17.13 \pm 4.13 (10.74 – 22.95)	32.48 \pm 52.43 (11.99 – 181.47)	9.49 \pm 1.56 (7.78 – 12.23)	17.89 \pm 10.09 (10.69 – 39.55)	16.35 \pm 6.22 (11.14 – 32.84)	15.08 \pm 5.98 (10.63 – 29.35)
Zn	Muscle	18.02 \pm 3.26 (14.62 – 26.18)	22.89 \pm 3.98 (17.62 – 29.18)	25.67 \pm 5.75 (16.67 – 30.73)	21.68 \pm 3.56 (17.47 – 28.05)	17.26 \pm 2.76 (13.45 – 21.15)	23.66 \pm 5.01 (17.04 – 22.18)
	Liver	142 \pm 13.56 (119 – 161)	152 \pm 43 (108 – 256)	101 \pm 26 (52 – 141)	129 \pm 19 (95 – 156)	129 \pm 24.55 (82 – 163)	168 \pm 62 (67 – 310)
As	Muscle	26.61 \pm 15.95 (11.34 – 67.56)	16.93 \pm 14.45 (4.25 – 58.11)	32.48 \pm 24.68 (2.05 – 71.25)	10.38 \pm 4.22 (4.3 – 15.16)	43.84 \pm 14.84 (23.48 – 66.29)	8.24 \pm 6.29 (3.57 – 4.31)
	Liver	22.5 \pm 9.75 (12.99 – 45)	17.79 \pm 6.12 (8.72 – 25.26)	20.87 \pm 10.06 (9.69 – 38.99)	10.57 \pm 2.76 (6.86 – 16.71)	37.11 \pm 12.02 (23.98 – 55.22)	11.27 \pm 8.35 (5.54 – 33.58)
Se	Muscle	1.76 \pm 0.15 (1.61 – 2.01)	1.58 \pm 0.20 (1.29 – 1.83)	1.26 \pm 0.31 (0.92 – 1.74)	1.23 \pm 0.26 (0.91 – 1.63)	2.29 \pm 0.76 (1.47 – 3.64)	1.74 \pm 0.21 (1.56 – 1.68)

Ag	Liver	11.02 ± 1.39 (8.61 – 12.80)	11.05 ± 1.89 (7.57 – 14.27)	7.09 ± 0.94 (5.26 – 8.20)	6.26 ± 0.70 (5.1 – 7.11)	10.80 ± 3.08 (6.26 – 15.89)	10.04 ± 4.07 (5.08 – 18.30)
	Muscle	N.D	N.D	N.D	N.D	N.D	N.D
	Liver	N.D	N.D	N.D	N.D	N.D	N.D
Cd	Muscle	N.D	0.001 ± 0.001 (0.001 – 0.003)	N.D	0.002 ± 0.001 (0.001 – 0.002)	N.D	0.003 ± 0.002 (0.001 – 0.006)
	Liver	0.90 ± 0.29 (0.38 – 1.37)	0.94 ± 0.81 (0.44 – 3.18)	0.18 ± 0.14 (0.04 – 0.46)	0.37 ± 0.32 (0.06 – 1.03)	1.21 ± 0.67 (0.34 – 2.38)	0.62 ± 0.67 (0.32 – 2.53)
	Muscle	N.D	N.D	N.D	N.D	N.D	N.D
Sb	Liver	0.01 ± 0.004 (0.01 – 0.02)	N.D	N.D	0.01 ± 0.005 (0.006 – 0.009)	0.02 ± 0.01 (0.01 – 0.04)	0.009 ± 0.005 (0.004 – 0.02)
	Muscle	0.39 ± 0.13 (0.23 – 0.67)	0.25 ± 0.1 (0.12 – 0.41)	0.12 ± 0.06 (0.08 – 0.18)	0.23 ± 0.09 (0.11 – 0.39)	0.42 ± 0.22 (0.11 – 0.82)	0.20 ± 0.06 (0.07 – 0.26)
	Liver	0.88 ± 0.39 (0.39 – 1.52)	0.57 ± 0.35 (0.29 – 1.49)	0.22 ± 0.11 (0.09 – 0.37)	0.28 ± 0.12 (0.12 – 0.47)	0.76 ± 0.64 (0.14 – 1.99)	0.30 ± 0.08 (0.18 – 0.48)
Hg	Muscle	0.056 ± 0.04 (0.02 – 0.14)	0.01 ± 0.004 (0.006 – 0.01)	N.D	0.05 ± 0.08 (0.01 – 0.25)	0.04 ± 0.01 (0.03 – 0.06)	0.05 ± 0.01 (0.02 – 0.07)
	Liver	0.32 ± 0.11 (0.17 – 0.50)	0.14 ± 0.06 (0.08 – 0.23)	0.20 ± 0.07 (0.11 – 0.29)	0.38 ± 0.11 (0.24 – 0.62)	0.17 ± 0.18 (0.04 – 0.66)	0.51 ± 0.22 (0.11 – 0.75)
	Muscle	0.056 ± 0.04 (0.02 – 0.14)	0.01 ± 0.004 (0.006 – 0.01)	N.D	0.05 ± 0.08 (0.01 – 0.25)	0.04 ± 0.01 (0.03 – 0.06)	0.05 ± 0.01 (0.02 – 0.07)
Pb	Liver	0.32 ± 0.11 (0.17 – 0.50)	0.14 ± 0.06 (0.08 – 0.23)	0.20 ± 0.07 (0.11 – 0.29)	0.38 ± 0.11 (0.24 – 0.62)	0.17 ± 0.18 (0.04 – 0.66)	0.51 ± 0.22 (0.11 – 0.75)
	Muscle	0.056 ± 0.04 (0.02 – 0.14)	0.01 ± 0.004 (0.006 – 0.01)	N.D	0.05 ± 0.08 (0.01 – 0.25)	0.04 ± 0.01 (0.03 – 0.06)	0.05 ± 0.01 (0.02 – 0.07)
	Liver	0.32 ± 0.11 (0.17 – 0.50)	0.14 ± 0.06 (0.08 – 0.23)	0.20 ± 0.07 (0.11 – 0.29)	0.38 ± 0.11 (0.24 – 0.62)	0.17 ± 0.18 (0.04 – 0.66)	0.51 ± 0.22 (0.11 – 0.75)

Annex 3 Trace elements mean \pm SD (mg kg⁻¹ dry weight) in muscles and livers of *E. teres* collected at the three sites for the wet(S1) and dry (S2) seasons. The minimum and maximum are given into brackets.

Element	Tissue	Tripoli		Beirut		Saida	
		S1	S2	S1	S2	S1	S2
Al	Muscle	N.D	0.45 \pm 0.16 (0.21 – 0.66)	3.23 \pm 4.24 (0.77 – 13.92)	0.34 \pm 0.16 (0.19 – 0.76)	1.61 \pm 1.05 (0.76 – 3.38)	0.54 \pm 0.21 (0.31 – 0.99)
	Liver	2.01 \pm 1.23 (0.59 – 4.03)	0.78 \pm 0.41 (0.33 – 1.25)	5.11 \pm 4.86 (0.92 – 13.92)	N.D	N.D	2.29 \pm 5.03 (0.27 – 16.55)
Ti	Muscle	N.D	0.21 \pm 0.06 (0.11 – 0.28)	0.95 \pm 0.73 (0.48 – 1.79)	0.20 \pm 0.09 (0.13 – 0.43)	N.D	0.22 \pm 0.06 (0.17 – 0.37)
	Liver	0.44 \pm 0.05 (0.39 – 0.50)	N.D	4.11 \pm 4.92 (0.63 – 12.02)	0.56 \pm 0.74 (0.16 – 1.67)	N.D	N.D
V	Muscle	0.13 \pm 0.09 (0.06 – 0.36)	0.09 \pm 0.08 (0.02 – 0.23)	0.02 \pm 0.01 (0.01 – 0.04)	0.02 \pm 0.003 (0.01 – 0.02)	0.12 \pm 0.09 (0.04 – 0.35)	0.03 \pm 0.02 (0.01 – 0.07)
	Liver	0.21 \pm 0.09 (0.07 – 0.38)	0.12 \pm 0.05 (0.03 – 0.19)	0.11 \pm 0.09 (0.03 – 0.31)	0.02 \pm 0.005 (0.01 – 0.03)	0.09 \pm 0.06 (0.04 – 0.22)	0.09 \pm 0.04 (0.03 – 0.18)
Cr	Muscle	N.D	N.D	N.D	0.14 \pm 0.15 (0.04 – 0.54)	N.D	N.D
	Liver	N.D	N.D	0.21 \pm 0.16 (0.10 – 0.60)	N.D	0.10 \pm 0.01 (0.08 – 0.12)	0.04 \pm 0.01 (0.03 – 0.04)
Mn	Muscle	1.11 \pm 0.27 (0.78 – 1.61)	1.12 \pm 0.89 (0.52 – 3.24)	2.00 \pm 0.60 (1.08 – 3.18)	1.17 \pm 0.12 (1.02 – 1.41)	1.27 \pm 0.34 (0.88 – 1.89)	1.03 \pm 0.28 (0.71 – 1.61)
	Liver	3.40 \pm 0.64 (2.51 – 4.54)	2.12 \pm 0.26 (1.77 – 2.50)	4.16 \pm 0.96 (2.90 – 6.32)	4.03 \pm 0.58 (3.47 – 5.16)	3.38 \pm 0.62 (2.44 – 4.44)	3.11 \pm 0.33 (2.67 – 3.5)
Fe	Muscle	32.00 \pm 10.47 (22.31 – 56.86)	32.88 \pm 7.46 (22.95 – 44.31)	46.36 \pm 10.21 (29.89 – 64.37)	22.63 \pm 5.16 (17.32 – 31.11)	34.11 \pm 17.01 (19.99 – 78.97)	39.87 \pm 21.93 (26.95 – 100.53)
	Liver	949 \pm 310 (548 – 1452)	1013 \pm 288 (692 – 1323)	795 \pm 271 (386 – 1218)	476 \pm 141 (322 – 729)	1070 \pm 547 (483 – 1948)	1395 \pm 395 (835 – 2044)
Co	Muscle	0.019 \pm 0.01 (0.01 – 0.03)	0.02 \pm 0.004 (0.01 – 0.02)	0.03 \pm 0.004 (0.02 – 0.04)	0.02 \pm 0.005 (0.01 – 0.03)	0.03 \pm 0.02 (0.02 – 0.07)	0.02 \pm 0.01 (0.02 – 0.05)
	Liver	0.09 \pm 0.02 (0.06 – 0.15)	0.07 \pm 0.01 (0.05 – 0.09)	0.18 \pm 0.03 (0.12 – 0.22)	0.08 \pm 0.01 (0.07 – 0.10)	0.11 \pm 0.04 (0.07 – 0.19)	0.12 \pm 0.01 (0.09 – 0.14)
Ni	Muscle	N.D	N.D	N.D	N.D	N.D	N.D
	Liver	N.D	N.D	N.D	N.D	N.D	N.D
Cu	Muscle	2.5 \pm 0.73 (1.73 – 3.49)	2.48 \pm 0.47 (1.82 – 3.19)	3.29 \pm 0.50 (2.70 – 4.30)	2.31 \pm 0.64 (1.72 – 3.95)	2.76 \pm 1.23 (1.59 – 5.86)	3.16 \pm 1.27 (2.05 – 6.33)
	Liver	12.02 \pm 9.95 (4.07 – 38.56)	10.56 \pm 1.48 (8.07 – 12.45)	19.15 \pm 7.98 (12.04 – 34.74)	18.13 \pm 5.23 (14.78 – 32.49)	14.05 \pm 4.38 (8.32 – 19.81)	26.25 \pm 17.33 (11.97 – 55.24)
Zn	Muscle	14.98 \pm 1.86 (12.26 – 19.01)	15.86 \pm 2.23 (14.12 – 21.00)	19.60 \pm 2.73 (16.10 – 24.42)	19.38 \pm 1.68 (16.41 – 22.16)	21.36 \pm 3.05 (15.95 – 24.68)	18.66 \pm 1.49 (16.03 – 21.18)
	Liver	82.47 \pm 8.96 (66.93 – 95.09)	83.70 \pm 7.55 (76.18 – 98.97)	91.30 \pm 23.46 (76.01 – 147.64)	87.65 \pm 21.77 (72.37 – 148.35)	112 \pm 26.58 (89.22 – 167.50)	112.9 \pm 34.40 (77.74 – 168.41)
As	Muscle	11.06 \pm 2.55 (5.35 – 13.93)	7.99 \pm 1.43 (6.09 – 10.21)	22.89 \pm 20.20 (6.84 – 71.01)	10.87 \pm 1.84 (8.57 – 13.65)	13.94 \pm 2.96 (9.02 – 18.34)	14.21 \pm 2.06 (11.69 – 18.71)
	Liver	281 \pm 89 (149 – 415)	19.79 \pm 6.24 (13.51 – 32.88)	33.45 \pm 21.07 (12.94 – 74.77)	114 \pm 36.24 (47.46 – 181.93)	103 \pm 68.13 (47.60 – 268.51)	56.66 \pm 12.21 (43.02 – 78.11)
Se	Muscle	3.74 \pm 0.69 (2.73 – 4.90)	3.95 \pm 0.87 (2.93 – 5.17)	1.82 \pm 0.82 (1.02 – 3.21)	1.77 \pm 0.25 (1.48 – 2.23)	5.42 \pm 1.23 (4.43 – 8.58)	3.64 \pm 1.08 (2.83 – 6.04)

Ag	Liver	93.98 ± 39.87 (42.13 – 171.36)	60.63 ± 12.56 (38.38 – 77.16)	25.39 ± 20.37 (9.22 – 71.00)	30.33 ± 5.31 (21.65 – 38.66)	58.27 ± 20.45 (37.83 – 98.22)	63.68 ± 6.97 (51.16 – 77.96)
	Muscle	N.D	N.D	N.D	N.D	N.D	N.D
	Liver	N.D	N.D	N.D	N.D	N.D	N.D
Cd	Muscle	0.01 ± 0.005 (0.004 – 0.02)	0.04 ± 0.02 (0.02 – 0.08)	N.D	0.04 ± 0.01 (0.02 – 0.06)	0.03 ± 0.02 (0.01 – 0.09)	0.03 ± 0.01 (0.01 – 0.05)
	Liver	1.77 ± 0.76 (0.98 – 3.75)	2.00 ± 0.69 (1.35 – 3.32)	0.47 ± 0.49 (0.06 – 1.42)	0.71 ± 0.15 (0.55 – 0.96)	2.73 ± 1.51 (1.36 – 5.58)	3.37 ± 1.34 (1.84 – 6.20)
Sb	Muscle	N.D	N.D	N.D	N.D	N.D	N.D
	Liver	0.01 ± 0.004 (0.01 – 0.02)	N.D	N.D	N.D	N.D	0.004 ± 0.00 (0.003 – 0.004)
Hg	Muscle	0.39 ± 0.08 (0.31 – 0.54)	0.60 ± 0.21 (0.33 – 0.88)	0.12 ± 0.07 (0.07 – 0.28)	0.08 ± 0.01 (0.06 – 0.09)	0.79 ± 0.22 (0.55 – 1.14)	0.43 ± 0.09 (0.31 – 0.55)
	Liver	0.37 ± 0.09 (0.25 – 0.51)	0.14 ± 0.06 (0.07 – 0.23)	0.14 ± 0.06 (0.07 – 0.23)	0.11 ± 0.02 (0.09 – 0.15)	0.74 ± 0.32 (0.51 – 1.52)	0.50 ± 0.15 (0.26 – 0.75)
Pb	Muscle	0.03 ± 0.08 (0.31 – 0.54)	0.60 ± 0.21 (0.33 – 0.88)	0.12 ± 0.07 (0.07 – 0.28)	0.08 ± 0.01 (0.06 – 0.09)	0.79 ± 0.22 (0.55 – 1.14)	0.20 ± 0.06 (0.07 – 0.26)
	Liver	0.03 ± 0.01 (0.025 – 0.04)	0.04 ± 0.06 (0.01 – 0.18)	0.11 ± 0.04 (0.06 – 0.18)	0.013 ± 0.01 (0.01 – 0.03)	0.05 ± 0.03 (0.02 – 0.08)	0.02 ± 0.02 (0.01 – 0.05)

Annex 4 Mean concentration \pm SD of trace elements (mg kg⁻¹ dw) measured in marine organisms' samples in wet (S1) and dry (S2) seasons. Different letters indicate statistically significant difference between sites per row (^{abc} S1, ^{def} S2), absence of letters indicates no significant difference between sites.

Analyte	Species	Tripoli		Beirut		Saida	
		S1	S2	S1	S2	S1	S2
Al	<i>U.lactuca</i>	444 \pm 271 ^a	779 \pm 611	140 \pm 43 ^b	-	149 \pm 71 ^b	-
	<i>S. spinosus</i>	7.38 \pm 5.34 ^a	4.27 \pm 2.83 ^d	28.05 \pm 24.18 ^b	2.49 \pm 1.50 ^d	10.77 \pm 6.09 ^{ab}	15.51 \pm 9.96 ^e
	<i>M. japonicus</i>	2.46 \pm 2.17	2.35 \pm 3.08	-	2.83 \pm 1.50	3.36 \pm 3.43	-
Ti	<i>U. Lactuca</i>	36.74 \pm 5.26 ^a	22.29 \pm 8.16	6.28 \pm 3.15 ^b	-	12.11 \pm 4.61 ^b	-
	<i>S. spinosus</i>	1.09 \pm 0.36 ^a	0.64 \pm 0.32 ^d	3.36 \pm 2.53 ^b	0.45 \pm 0.09 ^d	1.35 \pm 0.42 ^{ab}	1.77 \pm 1.57 ^e
	<i>M. japonicus</i>	2.00 \pm 2.17	0.55 \pm 0.32	-	0.40 \pm 0.22	0.97 \pm 0.62	-
V	<i>U. Lactuca</i>	1.72 \pm 0.12 ^a	4.98 \pm 1.51	0.39 \pm 0.12 ^b	-	7.03 \pm 1.11 ^c	-
	<i>S. spinosus</i>	0.13 \pm 0.03 ^a	0.09 \pm 0.02 ^d	0.45 \pm 0.14 ^b	0.07 \pm 0.02 ^d	0.11 \pm 0.03 ^a	0.43 \pm 0.14 ^e
	<i>M. japonicus</i>	0.13 \pm 0.16 ^a	0.02 \pm 0.01	-	0.02 \pm 0.01	0.02 \pm 0.02 ^b	-
Cr	<i>U. Lactuca</i>	1.09 \pm 0.13 ^a	2.43 \pm 0.71	0.34 \pm 0.11 ^b	-	0.46 \pm 0.18 ^b	-
	<i>S. spinosus</i>	0.54 \pm 0.11	0.50 \pm 0.09	0.58 \pm 0.24	0.55 \pm 0.09	0.49 \pm 0.11	0.59 \pm 0.14
	<i>M. japonicus</i>	N.D.	0.04 \pm 0.01	-	0.06 \pm 0.03	0.15 \pm 0.03	-
Mn	<i>U. Lactuca</i>	13.59 \pm 1.29 ^a	91.66 \pm 23.24	2.58 \pm 0.82 ^b	-	9.82 \pm 2.47 ^a	-
	<i>S. spinosus</i>	6.74 \pm 3.75 ^{ab}	2.76 \pm 1.09 ^d	3.20 \pm 1.16 ^a	2.54 \pm 1.35 ^d	4.22 \pm 1.88 ^b	7.62 \pm 3.24 ^e
	<i>M. japonicus</i>	0.68 \pm 0.67	0.46 \pm 0.09 ^d	-	0.74 \pm 0.24 ^e	0.67 \pm 0.24	-
Fe	<i>U. Lactuca</i>	701 \pm 132 ^a	2443 \pm 1217	123 \pm 67 ^b	-	254 \pm 126 ^b	-
	<i>S. spinosus</i>	16.38 \pm 4.72 ^a	15.61 \pm 2.92 ^d	69.64 \pm 33.75 ^b	11.09 \pm 2.56 ^d	22.19 \pm 7.34 ^{ab}	30.54 \pm 9.84 ^e
	<i>M. japonicus</i>	5.50 \pm 2.49	5.42 \pm 5.92	-	11.68 \pm 10.98	8.47 \pm 7.17	-
Co	<i>U. Lactuca</i>	0.19 \pm 0.02 ^a	0.95 \pm 0.24	0.37 \pm 0.02 ^b	-	0.22 \pm 0.04 ^a	-
	<i>S. spinosus</i>	1.45 \pm 0.35 ^a	0.95 \pm 0.51 ^d	0.76 \pm 0.37 ^b	0.39 \pm 0.18 ^e	0.64 \pm 0.22 ^b	0.97 \pm 0.30 ^d
	<i>M. japonicus</i>	0.11 \pm 0.10 ^a	0.04 \pm 0.02	-	0.02 \pm 0.01	0.05 \pm 0.02 ^b	-

Ni	<i>U. Lactuca</i>	1.87 ± 0.14 ^a	1.72 ± 0.36	N.D.	-	2.78 ± 0.63 ^b	-
	<i>S. spinosus</i>	6.81 ± 1.41 ^a	8.36 ± 2.17 ^d	1.64 ± 0.79 ^b	3.96 ± 1.63 ^e	8.32 ± 1.56 ^a	9.75 ± 2.46 ^d
	<i>M. japonicus</i>	N.D.	0.15 ± 0.04	-	0.18 ± 0.06	N.D.	-
Cu	<i>U. Lactuca</i>	2.71 ± 0.35 ^a	1.82 ± 0.25	0.42 ± 0.13 ^b	-	4.28 ± 0.99 ^a	-
	<i>S. spinosus</i>	15.27 ± 5.50 ^a	9.18 ± 5.37 ^{de}	1.63 ± 0.57 ^b	6.13 ± 4.39 ^d	5.77 ± 2.72 ^a	12.98 ± 6.89 ^e
	<i>M. japonicus</i>	24.57 ± 6.18 ^a	35.16 ± 7.82 ^d	-	23.04 ± 3.53 ^e	40.86 ± 16.25 ^b	-
Zn	<i>U. Lactuca</i>	7.16 ± 0.87 ^a	4.92 ± 0.80	1.85 ± 1.36 ^b	-	6.55 ± 1.11 ^a	-
	<i>S. spinosus</i>	74.88 ± 10.62 ^a	61.19 ± 13.47 ^{de}	401 ± 261 ^b	50.27 ± 9.21 ^d	84.16 ± 19.97 ^a	76.17 ± 28.01 ^e
	<i>M. japonicus</i>	61.31 ± 2.47	58.14 ± 3.86	-	59.65 ± 6.14	65.51 ± 18.06	-
As	<i>U. Lactuca</i>	4.14 ± 0.33 ^a	7.55 ± 2.15	1.29 ± 0.14 ^b	-	6.14 ± 0.84 ^c	-
	<i>S. spinosus</i>	66.34 ± 46.22 ^a	20.79 ± 11.34 ^d	26.25 ± 11.56 ^b	14.98 ± 4.24 ^d	30.33 ± 7.94 ^b	52.32 ± 15.66 ^e
	<i>M. japonicus</i>	43.26 ± 32.05	33.72 ± 22.50 ^d	-	9.13 ± 2.71	28.83 ± 13.43 ^e	-
Se	<i>U. Lactuca</i>	0.21 ± 0.06 ^a	0.52 ± 0.09	0.27 ± 0.04 ^{ac}	-	0.36 ± 0.15 ^c	-
	<i>S. spinosus</i>	4.49 ± 0.41 ^a	3.51 ± 0.74 ^d	2.21 ± 0.27 ^b	2.05 ± 0.47 ^e	2.88 ± 0.37 ^b	2.85 ± 0.45 ^d
	<i>M. japonicus</i>	1.93 ± 0.34	1.88 ± 0.34 ^d	-	1.34 ± 0.17	1.90 ± 0.71 ^e	-
Cd	<i>U. Lactuca</i>	0.07 ± 0.00 ^a	0.04 ± 0.01	0.02 ± 0.00 ^b	-	0.09 ± 0.01 ^a	-
	<i>S. spinosus</i>	4.21 ± 0.77 ^a	3.80 ± 0.01 ^d	2.66 ± 2.16 ^b	1.58 ± 0.86 ^e	2.76 ± 0.66 ^b	2.97 ± 0.68 ^d
	<i>M. japonicus</i>	0.04 ± 0.03 ^a	0.02 ± 0.01 ^d	-	0.004 ± 0.003 ^b	0.01 ± 0.01 ^e	-
Hg	<i>U. Lactuca</i>	N.D.	0.04 ± 0.02	N.D.	-	N.D.	-
	<i>S. spinosus</i>	N.D.	0.048 ± 0.01 ^d	0.14 ± 0.02	0.038 ± 0.01 ^d	N.D.	0.09 ± 0.02 ^e
	<i>M. japonicus</i>	N.D.	0.28 ± 0.23	-	0.06 ± 0.02	N.D.	-
Pb	<i>U. Lactuca</i>	1.42 ± 0.23 ^a	2.52 ± 1.08	0.32 ± 0.30 ^b	-	0.20 ± 0.07 ^b	-
	<i>S. spinosus</i>	0.56 ± 0.23 ^a	0.39 ± 0.13 ^d	0.27 ± 0.08 ^a	0.26 ± 0.19 ^d	1.65 ± 0.64 ^b	2.21 ± 0.73 ^e
	<i>M. japonicus</i>	0.02 ± 0.00	0.09 ± 0.05 ^d	-	0.01 ± 0.01	0.03 ± 0.01 ^e	-

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