United Arab Emirates University

College of Science

Department of Biology

THE CHARACTERIZATION OF HEAVY METALS IN THE DIET OF SOCOTRA CORMORANTS (*PHALACROCORAX NIGROGULARIS)* IN THE UNITED ARAB EMIRATES

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Under the Supervision of Prof. Sabir Bin Muzaffar

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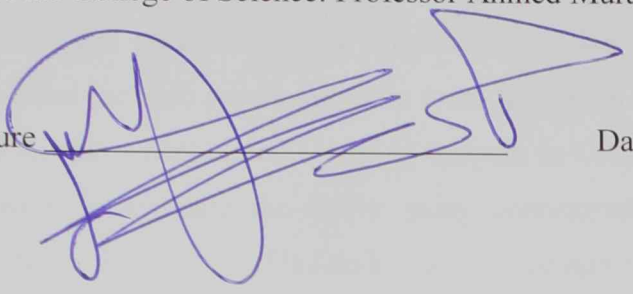
Signature  Date 22/12/2019

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جامعة الإمارات العربية المتحدة
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GROWTH OF MICROALGAE FOR SIMULTANEOUS TREATMENT OF INDUSTRIAL WASTEWATER AND BIODIESEL PRODUCTION

Mohammed Ahmad Abujayyab

This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science in Chemical Engineering

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Abstract

This study was performed to measure the bioaccumulation of Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, P, Pb, V, Zn, Ca, K, Na, Mg, S, Sr, and Hg in the liver, gastrointestinal tract (GI) and muscles of one hundred and five samples of Indian anchovy (*Stolephorus indicus*) purchased from local fisherman at the three main study areas, namely Ajman, Sharjah, and Umm Al Quwain in UAE. The main objectives of this study were to evaluate the heavy metal concentration in the muscle, liver and gastrointestinal tract (GI) of Indian anchovy, to compare the level of heavy metals with conducted limits by international guidelines, and to investigate the effect of heavy metals on the biomass of Indian anchovy and understand the heavy metal distribution in Socotra cormorant (*Phalacrocorax nigrogularis*). The ICP-OES system was used for simultaneous determination of Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, P, Pb, V, Zn, Ca, K, Na, Mg, S, Sr in the tissues of Indian anchovy. To study Hg concentration, simultaneous axial view Varian SpectrAA 220FS, coupled to an on-line continuous vapor generation system was used. Some metals showed the significance of the model to discriminate between study areas. The level of heavy metals varied significantly among tissues of fish. As expected, liver possessed the highest concentration of all metals. Fe, Zn, Cu, Cr and Cd in liver and GI tract were exceeding the maximum permissible limit recommended by international guidelines. Cd and Cu were also found in the edible part (muscle) of Indian anchovy with the concentration slightly exceeding the maximum permissible limit. Thus, the potential danger may emerge in the future, depending on the quality of wastewaters and industrial activities in the region. Furthermore, heavy metal accumulation in lower trophic levels suggest that species at higher trophic levels could have even higher concentrations of heavy metals due to biomagnification. Therefore, further monitoring programs should be conducted to provide spatial and temporal distribution of heavy metals in the Arabian Gulf.

Keywords: Heavy metals, Arabian Gulf, Accumulation, Fish, Tissues, *Stolephorus indicus, Phalacrocorax nigrogularis.*

Title and Abstract (in Arabic)

النمذجة الرياضية توصيف المعادن الثقيلة المستهلكة في النظام الغذائي للغراب السقطري (*Phalacrocorax nigrogularis*) في الإمارات العربية المتحدة

الملخص

أجريت هذه الدراسة لقياس التراكم الأحيائي لـ (Al، As، Cd، Co، Cr، Cu، Fe، Mn، Mo، Ni، P، Pb، V، Zn، Ca، K، K، Na، Mg، S، Sr، Hg) في الكبد والجهاز الهضمي والعضلات لمئة وخمس عينات من الأنشوفة الهندية (*Stolephorus indicus*) التي تم شراؤها من صيادين محليين في مناطق الدراسة الثلاثة الرئيسية: عجمان، الشارقة، وأم القيوين في دولة الإمارات العربية المتحدة. الهدف الرئيسي من هذه الدراسة هو تقييم تركيز المعادن الثقيلة في العضلات والكبد والجهاز الهضمي للأنشوفة الهندية، بالإضافة إلى مقارنة مستويات المعادن الثقيلة مع الحدود المرساة بموجب المبادئ التوجيهية الدولية، وأيضا التحقيق في تأثير المعادن الثقيلة على الكتلة الحيوية للأنشوفة الهندية وفهم توزيع المعادن الثقيلة في الغراب السقطري (*Phalacrocorax nigrogularis*). تم استخدام نظام (ICP-OES) لتحديد المعادن الثقيلة (Al, As Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, P, Pb, V, Zn, Ca, K, Na, Mg, S, Sr) بالتزامن في أنسجة الأنشوفة الهندية. لدراسة تركيز الزئبق، تم استخدام المنظار المحوري المتزامن (Varian SpectrAA 220FS)، إلى جانب نظام توليد البخار المستمر عبر الإنترنت. أظهرت بعض المعادن أهمية النموذج للتمييز بين مناطق الدراسة. تباين مستوى المعادن الثقيلة بشكل كبير بين أنسجة الأسماك. كما هو متوقع، احتوى الكبد على أعلى تركيز لجميع المعادن. تجاوزت المعادن Fe وZn وCu وCr وCd في الكبد والجهاز الهضمي الحد الأقصى المسموح به من قبل الإرشادات الدولية. تم العثور أيضاً على الكادميوم النحاس في الجزء (العضلي) للأنشوفة الهندية الصالح للأكل بتركيز يزيد قليلاً عن الحد الأقصى المسموح به. وبالتالي، قد يظهر الخطر المحتمل في المستقبل، اعتمادًا على جودة مياه الصرف الصحي المحلية والأنشطة الصناعية في المنطقة. علاوة على ذلك، يدل تراكم المعادن الثقيلة في المستويات الغذائية المنخفضة على(Roméo et al., 1999) أن المستويات الغذائية الأعلى قد تحتوي على تراكيز عالية من المعادن الثقيلة بسبب التضخم الأحيائي. لذلك، ينبغي إجراء مزيد من برامج المراقبة لتوفير التوزيع المكاني والزمني للمعادن الثقيلة في الخليج العربي.

مفاهيم البحث الرئيسية: المعادن الثقيلة، الخليج العربي، التراكم، سمك، أنسجة، *Stolephorus indicus*، *Phalacrocorax nigrogularis*.

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Dedication

To my beloved parents

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List of Abbreviations

AAS	Atomic Absorption Spectrometry
AJ	Ajman
EC	European Committee
FAO	Food and Agriculture Organization
GI	Gastrointestinal
MPL	Maximum Permissible Limit
SH	Sharjah
UAQ	Umm Al Quwain
WHO	World Health Organization

Chapter 1: Introduction

1.1 Overview

During the past few decades, heavy metals and other contaminants have been increasingly released into the freshwater and marine environments, resulting in increased contaminants in ecosystem components, including plankton, benthic organisms, fish, seabirds and whales (Eisler, 1985a, 1985b; Nimmo et al., 1998). Heavy metals are generally found in very low concentration in the marine environment, but human activities have increased the concentration of these metal ions. The term "heavy metals" have been called hopelessly imprecise and objectionable by many environmental scientists where there are connotations of toxicity (Ansari et al., 2004; Phipps, 1981; Vanloon Gary and Duffy, 2001; VanLoon and Duffy, 2017; Venugopal, 2013). Some heavy metals are essential to organisms, while other non-essential heavy metals, such as Cadmium (Cd), Mercury (Hg) and Lead (Pb) are extremely toxic (Rajeshkumar and Li, 2018). However, the unique characteristic of essential heavy metals being under certain environmental conditions, may accumulate to toxic concentrations and cause ecological damage to the aquatic organisms and their environment (Sfakianakis et al., 2015). Especially, fish species have been extensively used as a bioindicator of heavy metals in the marine environment, due to its ability to concentrate heavy metals in their tissues (Authman et al., 2015; Cunningham et al., 2019). The two main routes of heavy metal uptake by marine organism are direct consumption of water and food through digestive tract and indirect or non-dietary routes throughout permeable membranes such as muscles and gills (Rajeshkumar and Li, 2018). These chemicals accumulate in tissues at higher concentrations than concentrations in water, possible resulting in biomagnification in

the food webs to levels that cause physiological impairment at higher trophic levels (e. g. in tertiary consumers such as large fish, seabirds or seals) (Raposo et al., 2009). Furthermore, exposure to heavy metals can affect the reproduction efficiency of aquatic biota and their consumers (marine mammals and marine birds) leading to gradual declines in populations in polluted waters (Burger and Gochfeld, 2004; Das et al., 2002). Indeed, in recent years, seabirds are undergoing large-scale and long-term substantial population declines worldwide due to anthropogenic activities such as disturbance, development, habitat loss, pollution and fishing gear entanglement (Whelan et al., 2018). Specifically, the toxic effect of heavy metals in seabirds and fishes occur when the body accumulate high concentration of pollutants, and excretory, metabolic, storage and detoxification mechanisms are no longer able to prevent accumulation. This could lead to eventual physiological, pathological, behavioral changes and mortality (Authman et al., 2015). Thus, to protect seabirds and fishes, it is necessary to determine contamination level of heavy metals in fishes (biomarkers) through evaluation and monitoring, since the fish is an important source of food for seabirds and humans.

Large fishes and seabirds are often at the top of the marine food web and may concentrate large amounts of some metals from the water (Mansour and Sidky, 2002). Research has shown a difference in accumulation of heavy metals in fish. The extent of accumulation of heavy metals in fish depends on intrinsic species differences and extrinsic environmental conditions. The trophic status and feeding strategy (diet), the age of fish, the gender, phase of sexual reproduction, tissue type analyzed, and body size are attributed to intrinsic species differences. Extrinsic environmental conditions include chemical form of the metal, the severity of the contamination, presence of other contaminants in the marine environment, water quality conditions, such as

salinity, temperature, dissolved oxygen concentrations, pH, total suspended solids, and the nature of the bottom sediments (serve as a reservoir for metal adsorption and resuspension) (Cunningham et al., 2019). A high level of heavy metals is usually found in liver, kidney and gill of the fish (Rajeshkumar and Li, 2018). Thus, seabirds are more in danger due to high accumulation than human, since seabirds are consuming liver, kidney, and gill of the fishes (Schreiber and Burger, 2002).

There is no research concerning the impact of heavy metal pollutants on marine birds, including Socotra Cormorant (*Phalacrocorax nigrogularis*) in the Arabian Gulf. The Socotra Cormorant is a regionally endemic marine bird restricted to the Arabian Gulf and Gulf of Oman (Jennings, 2010, 1993). All known subpopulations are declining, and the global population is estimated at 110,000 breeding pairs (Jennings, 2010). Breeding biology, habitat characteristics and ecology of this species is poorly studied. The species is currently categorized as 'Vulnerable' and many colonies have become extinct. Approximately 34% of the global population of Socotra Cormorant breeds in the United Arab Emirates (Aspinal, 1995; Jennings, 2010; Nelson, 2005)and breeding activity is limited to eleven colonies (Jennings, 2010). Siniya Island, in Umm Al Quwain Emirate in the eastern Arabian Gulf is occupied by possibly the largest single colony of Socotra cormorant in the UAE, and with a stable population of approximately 28,000-41,000 breeding pairs (Sabir B. Muzaffar et al., 2017). Siniya Island is known for a high level of heavy metal contamination, although whether this is one of the factors influencing population declines is not known (Muzaffar et al., 2012).

Over the last two decades, Arabian Gulf has experienced substantial increases in economic, social and industrial development. Excessive concentration of heavy metals has been reported from coastal areas experiencing increasing settlement, traffic,

agricultural and industrial activities such as textile, detergent, paint, oil and gas production, plastic industries, etc. (Aonghusa and Gray, 2002; Sarker et al., 2015; Sungur and Gülmez, 2015). In addition to anthropogenic sources, dredging and reclamation, sewage effluents, hypersaline water discharges from the desalination plant, waste disposal are other sources of heavy metals (Sheppard et al., 2010). Reports have shown that the Arabian Gulf became notoriously known with elevated concentration of such heavy metals like Cd, Zn, Pb, Cr, Ni and Cu, accompanied by several harsh environmental challenges (Al Rashdi et al., 2015; De Mora et al., 2004; Sheppard et al., 2010; Sheppard, 1993).The Arabian Gulf is a shallow semi-enclosed sea with a maximum depth of 100 m at Strait of Hormuz (Taher et al., 2012), located in the subtropical zone and characterized by the one of the harshest conditions due to natural stressors such as high levels of salinity and temperature, and reduced levels of pH (H. A. Naser, 2013). Heavy metal concentrations of Arabian Gulf in several Emirates are different. It can be related to the amount and type of industries situated in different Emirates that discharge municipal wastewater into the Arabian Gulf. It was found that Ajman and Sharjah are mostly occupied with textile, detergent and oil and gas industries, which are the main source of Cd, Zn, Pb, Cr, and Cu (Almasoud et al., 2015; Furness, 2017; H. A. Naser, 2013).

This study was designed to (1) evaluate the concentration of Aluminum (Al), Arsenic (As), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Molybdenum (Mo), Nickel (Ni), Phosphor (P), Lead (Pb), Vanadium (V), Zinc (Zn), Calcium (Ca), Potassium (K), Sodium (Na), Magnesium (Mg), Sulfur (S), Strontium (Sr) and Mercury (Hg) in the muscle, liver and gastrointestinal (GI) tract of Indian anchovy (*Stolephorus indicus*) collected from Ajman, Sharjah, and Umm Al Quwain; (2) compare the level of these heavy metals

with permissible international limits in small fish species; and (3) determine the relationship between heavy metal concentrations and fish biomass.

1.2 Statement of the Problem

The Arabian Gulf is of paramount socio-economic importance in the world (Taher et al., 2012). The area is of high value for its globally significant oil resources that is exported worldwide through maritime transportations in the area. The seafood (fish and shrimp) is commercially valuable for both local consumption and export revenue (Vaughan et al., 2019). Besides, 61% of the global seawater desalination capacity is located along the Arabian Gulf coastlines and all Arabian Gulf countries rely on desalinated seawater for most of their drinking water supply (H. A. Naser, 2013).

The Arabian Gulf is a fragile ecosystem that is prone to disturbance from anthropogenic activity (Freije, 2015). It is exposed to increased challenges to environmental management and conservation efforts, as its coastline is shared by several countries. Its ecosystem has been subject to a variety of pollutants, including heavy metals, over the years due to massive infrastructure development and urbanization on an unprecedented scale accompanied by several harsh environmental challenges. Sheppard (1993) describes the Arabian Gulf as a relatively shallow, semi-enclosed sea with immense evaporation rates and faulty flushing characteristics. Consequently, anthropogenic input of pollutants such as heavy metals undergoes more limited dilution and slower dispersion than would occur in open marine systems. As a result, output of pollutants is likely to have severe consequences by adding stress to many species that are already functionally close to their physiological limits (Sheppard, 1993).

Offshore islands that serve as breeding colonies for marine birds within this harsh environment are threatened by disturbance, pollution, construction and oilrig installations (Aspinal, 1995; Gardner and Howarth, 2009; Jennings, 2010). Socotra Cormorant can be recognized as a species among marine birds that is undergoing considerable decline due to anthropogenic factors, such as disturbances on breeding sites, by catch in fish nets and pollution (Sabir B. Muzaffar et al., 2017). They can be exposed to relatively high levels of heavy metals in their prey, as they are often at the top of the food chain (where pollutants may be detectable due to amplification) (Schreiber and Burger, 2002). However, there is limited research concerning the impact of heavy metals on marine birds, especially Socotra Cormorant in the Arabian Gulf. The Socotra Cormorant is a regionally endemic marine bird restricted to the Arabian Gulf and Gulf of Oman (Jennings, 2010). Small forage fish species including anchovy, sardines and flying fish consist of the main diet of Socotra Cormorant (Ksiksi et al., 2015). Fish can accumulate elevated concentration of metals in their tissues, passing these metals up through the food chain to Socotra Cormorant (Authman et al., 2015; Freije, 2015). Considering the fact, that Socotra Cormorant will consume several hundred small fishes daily, they could be exposed to a higher concentration of heavy metals compared to that in the fish.

Thus, heavy metals affecting the single part of the marine system can cause the failure of the whole system as a chain reaction. Therefore, an interrelated marine environment and its dependents (marine organisms) should be monitored as a single system to achieve a healthy marine environment in addition to an adequate management program (such as Integrated Coastal Zone Management).

1.3 Relevant Literature

Widespread pollution from heavy metals may cause substantial problems to fishes and marine birds, especially when they are stressed by changing environmental conditions (Kitaysky and Golubova, 2000) and prey abundances (Furness and Camphuysen, 1997). The most heavily affected marine vertebrate are marine birds as a colony, with regards to heavy metal contamination. They are declining faster than any other group of birds (Butchart et al., 2004; Croxall et al., 2012). The contaminants such as heavy metals can have deleterious effects on breeding success and juvenile survival, which result in high mortality and decline of some marine species, including birds (Levin, 1984; McCluggage, 1991). It must be considered that the level of heavy metals may vary among marine birds, depending on the bird`s feeding ecology, intensity and timing of exposure in feeding habitat, as well as their physiology and biochemical characteristics (Savinov et al., 2003). The fact that seabirds are most often top predators and long-lived species means also that they are useful as biomonitors of heavy metals. Great number of factors such as phylogeny, molt pattern, sex, life span, diet are likely to influence heavy metals bioaccumulation in seabird tissues. Among all these factors, diet was one of the more discriminant factors for the differences in heavy metal concentrations. Thus, Indian anchovy have been determined to be an important vector for the transfer of heavy metals to top marine predators (Socotra Cormorant). Understanding the presence of heavy metals in marine birds requires examination of internal tissues (liver, brain, kidney, and muscle) of adult, young birds and its diet and feathers. However, for understanding the relative role of heavy metals in the internal field requires controlled laboratory experiments on toxicodynamics. Toxidynamic studies have been conducted for mercury, and plastic particles (Braune and Gaskin, 1987; Lewis and Furness, 1991; Ryan, 1988a, 1988b).It has been

suggested that aquatic ecosystems are major repositories of heavy metals and marine bird species, that have a primarily marine-based diet, are at a higher risk of metal exposure in their feeding habitant (Furness, 2017, 2012; Wolfe et al., 1998). Marine fishes, mammals, and marine birds accumulate a large amount of Hg, Cd, Ag in their liver, as they occupy the highest trophic position in the marine food web (Thompson, 1990; Wagemann and Muir, 1984).In all cases, these inorganic elements are evidently toxic in high concentration (Thompson, 1990). They can impact the reproductive output or even cause death in species (Sanpera et al., 2000), and in this sense, heavy metals constitute a serious threat to the survival of seabird populations (Hernández et al., 1999).

The Arabian Gulf has been subjected to heavy metal pollution over the past four decades (Freije, 2015). Heavy metal contamination in the Arabian Gulf was investigated with marine organisms (Fowler et al., 1993; Habashi et al., 1993).Arabian Gulf was the scene of environmental impact from three wars in the past two decades. As a result, Arabian Gulf was subjected to periodic oil spills on a massive scale (6-8 million barrels of Kuwait crude oil is the largest oil spill in 1991), which released heavy metals including Pb, Ni, V, Zn, Cd as the major contaminants into the environments (Kureishy, 1993; Literathy, 1993; Sheppard et al., 2010). An oil spill is the uncontrolled release of crude oil into the environment and a major contributor to the higher levels of heavy metals in oil producing areas of Gulf region (H. A. Naser, 2013). Crude oil and its refined petroleum products have been reported to contain several toxic organic and inorganic components such as polycyclic aromatic hydrocarbon compounds and metals such as Fe, V, Na, Ni, Cr and other metals which constitutes a significant health risk to both marine organisms and people (Abarshi et al., 2017; Nwaichi and Ntorgbo, 2016).

1.4 Overview and Diet of Socotra Cormorants (*Phalacrocorax nigrogularis)*

The Socotra Cormorant is a regionally endemic marine bird that is undergoing rapid population declines and is currently categorized as Vulnerable (Jennings, 2010). The population of Socotra Cormorants in Oman is smaller (10,000 pairs) and is geographically isolated from the Arabian Gulf population (Jennings, 2010). The population of Socotra Cormorants has been reduced by 60% due to substantial declines (Jennings, 2010; Symens et al., 1993). The population`s decline can be explained by two factors: periodic decline caused by major events, such as the oil spill during the Gulf War in 1991 during which tens of thousands of Socotra Cormorant`s died (Symens et al., 1993) and the gradual, long-term decline in populations due to degradation or destruction of breeding sites or accumulation of a high concentration of heavy metals in the body (Jennings, 2010; Symens et al., 1993).

The local population is estimated to be about 110.000 breeding pairs mostly living and breeding within the Arabian Gulf (about 90%) with a global population of 750.000 individuals (Furness, 2012; Jennings, 2010). This is comparable less than historic numbers, some of which showed large populations in the 1970s (e.g. Zarka Island, up to 250,000 breeding pairs).

Among the eleven colonies currently known in the Arabian Gulf, at least seven colonies are extinct due to oil exploitation activities and contaminant disturbances of islands that marine birds used to breed or feed on (Jennings, 2010; Khan et al., 2019). Due to construction work that connected the island to the mainland in Ras Al Aysh, one colony became extinct, allowing ground predators to invade the colony and decimate it (Jennings, 2010).

They are estimated to be 38,000 to 39,000 pairs nesting on nine islands in the United Arab Emirates, eight of which are in the Abu Dhabi Emirate (Table 1) (Aspinal,

1995; Muzaffar, 2014). Due to oil exploitation and persecution, the population breeding in the Abu Dhabi islands (with breeding populations ranging from a few hundred to a few thousand pairs) have suffered significantly over the last three decades, and 12 colony sites have been abandoned completely by breeding birds (Jennings, 2010). However, one recent study shows that islands protected from human activities have substantial populations of breeding birds, that could be increasing (Khan et al., 2019).

Table 1: Breeding pairs and breeding colonies of Socotra Cormorants recorded during 2006–2007 to 2016–2017 in Abu Dhabi Emirate
Source: (Khan et al., 2019)

Site name	Status 2016-17	Status 2006-07	Status 1996 *	Access
Rufayq	14,000	**Unknown** [2]	300	Restricted
Ghagah	11,500	Inactive	6,000	Restricted
Butinah	11,000	**No breeding**	**No breeding**	Restricted
Yasat	7,500	94	2,000-2,200	Restricted
Digala	5,000	**No breeding**	**No breeding**	Open
Dinah	2,400	4,150	8,000-10,000	Restricted
Qasr Salaha	251	287	80	Open
Furaijjidat	161	Inactive	**Unknown** [1]	Open
Muhammaliyah	Inactive	Inactive	Inactive	Open
Umm Qasr	Inactive	4,000	150	Open

*Information in (Aspinal, 1995); [1] breeding recorded but number not known, [2] Not visited

The nesting numbers of Socotra Cormorant at Siniya Island in Umm Al Quwain also increased from 15,500 in the 1990s (Aspinal, 1995) to more than 40,000 pairs in 2014 and stabilized in the last decade (Sabir Bin Muzaffar et al., 2017). The overall nesting population for UAE is estimated to be between 60,000 pairs to 70,000 pairs, which is more than 50% of global breeding population of 110,000 pairs (Sabir Bin Muzaffar et al., 2017; Symens et al., 1993).

The cormorants are surface diving piscivores (Cramp and Simmons, 1977). The diet of Socotra cormorant mainly includes sardines (*Sardinella spp*.), anchovies (*Stolephorus indicus, Encrasicholina spp*), bigeye (*Selar crumenophthalmus*), yellowtail scads (*Atule mate*), silversides (*Atherinomorus lacunosus*), spotted halfbeaks (*Hemiramphus far*) and streaked rabbitfishes (*Siganus javus*) (Jennings, 2010). During the breeding season 2011/2012, the diet was almost entirely (> 90%) sailfin Flying fish (*Parexocoetus mento*). As the chicks became larger, the diet was switched to include other fish, including Blue-stripe Sardine (*Herklostychthyes quadrimaculatus*) (41.5%) and Pink-eared Emperor (*Lethrinus lentjan*) (26.8%), while the Sailfin Flying fish declined in abundance (28%) (Muzaffar et al., 2012). In contrast, the diet for the period between 2012/2013 was different from 2011/2012 with anchovies (*Encrasicholina spp*.) being the main diet throughout the season. Very minor components of the diet formed halfbeaks (*Hyporamphus*), scads (*Selar crumenopthalmus*) in both years (Muzaffar et al., 2013). It appears that the species is highly adaptable and feeds on fish species which are abundant in numbers. The limited studies presented suggest that sardines (*Sardinella sp*.), scads (*S. crumenophthalmus* and *Atule mate*), silverside (*Atherino morphuslacunosus*), spotted Halfbeak (*Hemiramphus far*) and streaked rabbit-fish (*Siganus javus*) form part of the diet in the Hawar Islands colony in Bahrain, although proportions were not reported (Jennings, 2010). The diet study of Siniya Island has shown that some were from the same families (*Hemiramphidae, Clupeidae,* or *Carangidae*). The changes in the diet of Socotra Cormorant can be explained also by distribution pattern, abundance of fish populations or the major preference of fish groups. Marine fish typically show multi-decadal fluctuations in abundance, mainly related to overexploitation, climate, or a combination of both.

1.5 Anchovy: Habitat, Spatial Dynamics and Aggregation Patterns

Anchovies are a group of small fish belonging to the family *Engraulidae* that occur mostly in marine or brackish waters, with some species in freshwater (Gücü et al., 2017). Plankton is the main diet of anchovies. Anchovy populations exist in most temperate and productive coastal regions in the oceans and major seas (Figure 1) (Checkley et al., 2017). Within populations, stocks are usually the target of fisheries (Ganias, 2014; Parrish et al., 1989)

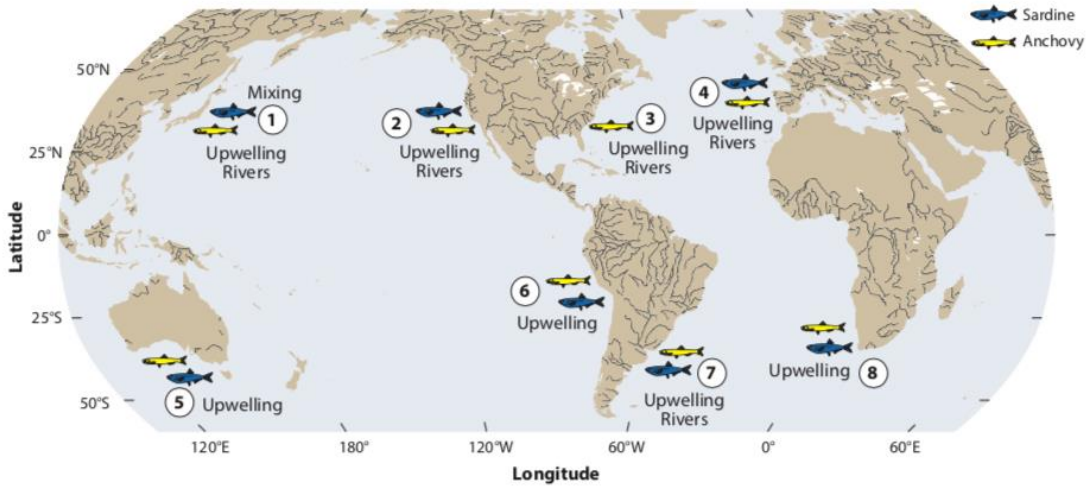

Figure 11: Areal distribution of anchovy (*Engraulis* spp.) and sardine (*Sardinops* spp., except for *Sardinella brasiliensis*)
Source: Checkley et al., 2017
Note: Major rivers are shown as black lines. Anchovy: (1) Japanese anchovy, *E. japonicus*; (2) northern anchovy, *E. mordax*; (3) anchovy, *E. eurystole*; (4) European anchovy, *E. encrasicolus*; (5) Australian anchovy, *E. australis*; (6) Peruvian anchoveta, *E. ringens*; (7) *anchoita*, *E. anchoita*; (8) cape anchovy, *E. capensis*.

Anchovy populations have multiple stocks in all regions (Ganias, 2014; Zarraonaindia et al., 2012). Peruvian anchoveta is the world's largest single-species fishery, with a maximal biomass of 22.8 million tons (MT). Most anchovy stocks are coastal. Anchovy are more closely associated with land, including coasts, capes, orographic features, and rivers, all of which are geographically fixed. Anchovy have different constraints to respond to latitudinal shifts in water properties (such as

warming) and hydrology. Anchovies are present where new nitrogen is supplied primarily by wind-driven upwelling. Once, the surface water is cooled by cold, dry winter winds, the mixing occurs. The convection mixes these cooled waters deep in the water column, bringing nitrate-rich waters to the surface. Rivers also supply nitrate to coastal waters and are particularly important for anchovy.

The species of anchovy that was used in this research as prey of Socotra Cormorant to identify heavy metals is Indian anchovy, *Stolephorus indicus* (Van Hasselt, 1823), which was originally described from India and was caught in the Arabian Gulf (Figure 2). The species is considered to be native to the Red Sea and the Indo-West Pacific from South and East Africa east to Society Islands, north to Hong Kong (China), south to Gulf of Carpentaria (Australia).

Figure 2: Indian anchovy *Stolephorus indicus* (Van Hasselt, 1823), November 20, 2018, Umm Al Quwain

Indian anchovy, *Stolephorus indicus* (Van Hasselt, 1823) is a valuable species for human consumption. It is a small pelagic fish with a key ecological role in the marine food web. Anchovies are preyed on by larger predators and schools over soft bottoms mainly around 20–50 m depth. Thus, it is an important link connecting the

lower and upper trophic levels (Galaţchi et al., 2017). Young fish occur in shallower waters and the biology of the species is poorly studied (Whitehead, 1988). They are generally very accepting of a wide range of temperatures and salinity, which permits their survival in the Arabian Gulf (Checkley et al., 2017).

1.6 Heavy Metals

Being toxic or poisonous even at low concentration, the term "heavy metals" refers to group of metals and metalloids with atomic density greater than 4 g/cm^3, or 5 times or more, greater than water (Battarbee et al., 1988; Garbarino et al., 1995; Hawkes, 1997; Hutton and Symon, 1986; Nriagu, 1989; Nriagu and Pacyna, 1988). The great concern of heavy metals is related to their chemical properties. A pollutant is any substance in the environment, which causes objectionable effects, impairing the welfare of the environment, reducing the quality of life and may eventually cause death (Ansari et al., 2004; Nriagu and Pacyna, 1988). It has to be present in the environment beyond a set or tolerance limit (either a desirable or acceptable limit; (Wang et al., 2005). As natural constituents of the earth`s crust, heavy metals are persistent environmental contaminants, since they cannot be degraded or destroyed (Garbarino et al., 1995). The essential heavy metals are known to be essential to life, namely Fe, Zn, Cu and Mn, and non-essential which are toxic even in low concentration such as Co, Ni, Pb and Cd (Carvalho et al., 2005). Usually, they enter the organisms through food, air, and water and bio-accumulate over a period of time (Costa, 2003).

The production of heavy metals increased steeply for more than 100 years, since the middle of the 19th century, with concomitant emissions to the environment (Figure 3).

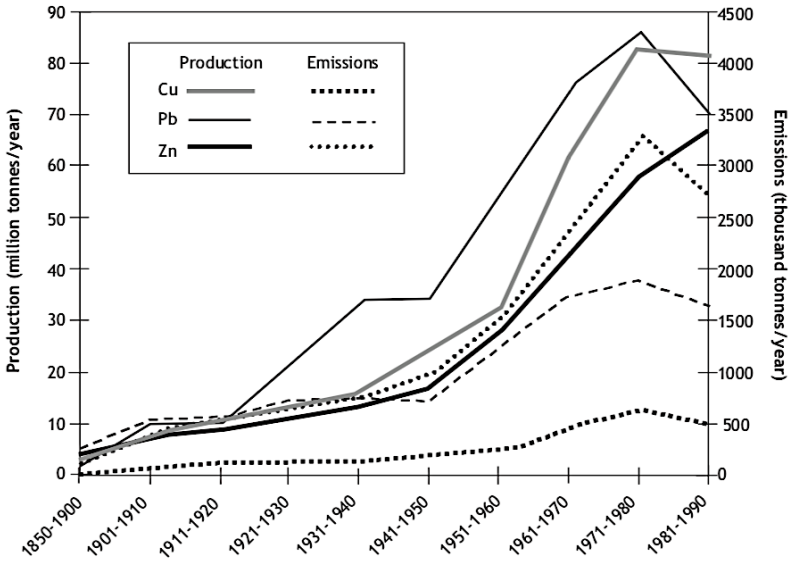

Figure 3: Global production and consumption of selected toxic metals, 1850–1990
Source: Nriagu, 1996

Heavy metals are present in rocks as their ores in different chemical forms, from which they are recovered as minerals (Furness, 2017). Consequently, heavy metals are very prominent in areas of mining and old mine sites and pollution reduces with increasing distance away from mining sites (Peplow, 1999). Thereby, water bodies are most commonly polluted through mining activities (Garbarino et al., 1995). The metals are transported through rivers and streams either in dissolved form in water or as an integral part of suspended sediments. It has been noted that the dissolved form in water has the greatest potential of causing the most deleterious effects (Furness, 2017). They may contaminate water from underground sources by being stored in riverbed sediments or seeped into ground water. The extent of contamination will depend on the nearness of the well to the mining site which has been reported to contain heavy metals at levels that exceed drinking water criteria (Garbarino et al., 1995; Peplow, 1999). Heavy metals are consumed above the bio-recommended limits could cause the biotoxic effects potentially harmful to the organisms.

1.6.1 The Historical Prospect and Source of Heavy Metal in the Arabian Gulf

A variety of studies were carried out on the investigation of heavy metal contamination among different marine organisms in the Arabian Gulf. The highlight of studies was mainly the effect of the Gulf War oil spill in 1991 on the concentration of heavy metals in marine organisms (Freije, 2015). The large extent of this oil spill and the slow dissipation of the environmental pollutants (heavy metals) in the Arabian Gulf waters and the limited dilution of water than would occur in a more open circulating coastal system, resulted in long-term damage to this ecosystem (Cunningham et al., 2019). The metals such as Cu, Pb, Zn, Cd, Fe, Mn, As and Ni were having specific concerns in the Arabian Gulf, as they were determined at higher concentrations in the tissues of fish species such as twobar seabream (*Acanthopagrus bifasciatus*), Indian halibut (*Psettodes erumei*), yellowfin tuna (*Thunnus albacares*), blackspotted rubberlip (*Plectorhinchus gaterinus*) since 1993 (Cunningham, 2019). The same heavy metals were found by Al- Sayed et al. (1994) in seawater and the pearl oyster (*Pinctada radiata*) from two locations around Bahrain between March 1991 and 1992 where the metal concentration in the seawater was less than in the oysters (Al-Sayed et al., 1994). De Mora et al. (2004) conducted an assessment study of heavy metal concentration in two economically important fish species, the orange spotted grouper (*Epinephelus coioides*, hamour) and the spangled emperor (*Lethrinus nebulosus*, sheiry) and various bivalves from the Arabian Gulf and Gulf of Oman during 2000–2001 (De Mora et al., 2004). They found high Cd concentrations in their liver of the fish and this was attributed to food-chain bioaccumulation. On the other hand, certain bivalve species (*Pinus radiata* and *Saccostrea cucullata*) had very high

concentrations of As, which the authors attributed to natural origins rather than to anthropogenic contamination.

The coasts of the Arabian Gulf are undergoing rapid construction activities like intensive dredging and reclamation (Naser, 2011a, 2011b). The prime target for most of the major housing, recreational, and economic developments are coastal and marine environments in the Arabian Gulf (Naser, 2013)and more than 40% of the coasts of the Arabian Gulf have been developed (Cunningham et al., 2019). Dredging and reclamation processes are typically causing short-term and long-term biological, physical and chemical impacts. Physical and chemical alternations may reduce biodiversity, richness, abundance, and biomass of marine organisms due to dredging and reclamation (Smith et al., 2007; Smith and Rule, 2001). Consequently, elevated levels of heavy metals are mobilized during dredging and reclamation activities which may end up in important food web components including fish and shellfish, and ultimately posing threats to human health (Guerra et al., 2009; Hedge et al., 2009).

Sewage discharges are considered to be the major sources of coastal pollution in the Arabian Gulf countries. Despite high standards of sewage treatment, large quantities of domestic effluents are discharged to coastal and marine environments (Freije, 2015; Naser, 2013). The main components of these effluents are high-suspended solid and high load of nutrients such as ammonia, nitrate, and phosphate (Naser, 2011a). Sewage effluents are generally accompanied by biological and chemical pollutants, including heavy metals (Al-Muzaini et al., 1999; Shatti and Abdullah, 1999) that may cause degradation in the receiving coastal and marine environments, and subsequently affect the quality of marine food (Singh et al., 2005).

Due to its location within the richest oil province in the world (67% of the world oil reserve), the Arabian Gulf experiences a stress to its ecosystem (Literathy,

1993). Oil refining, petrochemical, and other industries are going through rapid industrial growth within the Arabian Gulf countries. In addition to other development and anthropogenic activities, the oil-related activities, result in several marine pollution problems. The major contributors to pollution in the Arabian Gulf have been considered oil exploration, production, and transport (Naser, 2013). Offshore oil wells, underwater pipelines, oil tanker incidents, oil terminals, loading and handling operations, weathered oil and tar balls, illegal dumping of ballast water, and military activities are sources of oil spills in the Arabian Gulf (Sale et al., 2011). The Arabian Gulf has been the scene for arguably the mass oil spill incidents. For instance, in the 1991 Gulf War, an estimated 10.8 million barrels of oil were spilled in the Arabian Gulf waters (Massoud et al., 1998) and notably elevated levels in heavy metals were reported after this major oil spill (Naser, 2013). Petroleum refinery wastewaters consist of different chemicals, which include oil and greases, phenols, sulphides, ammonia, suspended solids, and heavy metals like Cr, Fe, Ni, Cu, Mo, Se, V and Zn (Wake, 2005). Consequently, the coastline of the Arabian Gulf is recognized as a hotspot for high concentrations of heavy metals, since coastal and marine environments are receiving intensive industrial effluents (De Mora et al., 2004; Kamal et al., 2015).

There is increased need for fresh water within the Arabian Gulf countries as a result of rapid industrial development and population growth (Smith et al., 2007). Since Arabian Gulf countries have low precipitation and high aridity, most of the freshwater needs are being met from seawater through the various processes of desalination (Hashim and Hajjaj, 2005). The Arabian Gulf countries account for more than 60% of the world's total of desalinated water (Lattemann and Höpner, 2008). Unfortunately, the large quantities of wastewater from desalination plants associated with heavy metals are being discharged to coastal and subtidal areas in the Arabian

Gulf daily. Elevated levels of heavy metals have been reported to be associated with several desalination plants along the coastline of the Arabian Gulf (Naser, 2011a; Rashed, 2001).

The high concentrations of Cd, Cu, Zn, Ni and Pb also can be the result of uncontrolled industrial activities such as textile, detergent, paint, and plastic industries taking place in the coastal areas of Arabian Gulf (Aonghusa and Gray, 2002; Sarker et al., 2015; Sungur and Gülmez, 2015).

1.6.2 Interspecific Variation in Heavy Metal Accumulation in Fish and Seabirds

Heavy metals discharged from industrial and agricultural wastewater enter into rivers and are absorbed by suspended sediment as a primary pollutant (Fang et al., 2016; Xiao-ping, 2001). In case of certain disturbances, heavy metals can be released into the water column as the potential source of secondary pollutants, and this can be a potential threat to ecosystems (Fang et al., 2016; Varol, 2011). Several factors such as mineralogical and chemical compositions of suspended material, bioavailability of metals, anthropogenic influences, deposition, sorption, enrichment in organisms (Jain et al., 2007), and various physicochemical characteristics (Singh et al., 2005) are involved in the distribution of heavy metals. Since the concentration of heavy metals in sediments usually exceeds those in overlying water by between 3-5 orders of magnitude, sediment metals with high concentration can seriously affect benthic organisms. Two different strategies can be adopted by an aquatic organism to counter the toxic impacts of heavy metals through binding of metals to metallothionein or granular form in the tissues (Phipps, 1981):

1. “Regulators” efficiently excrete metals and exhibit low uptake rates for metals.

2. “Non-Regulators” accumulate large concentrations of heavy metals and store them in detoxified form in their tissues.

In general, the whole soft tissue concentration of metals in marine organisms is many times greater than in the surrounding seawater (Ansari et al., 2004). Many variables should be taken into account in the rate of uptake of metals by an organism such as the chemical form of the metal as well as pH, salinity, hardness, and temperature of seawater. Interaction of metals is also possible so that one metal inhibits the uptake of another metal by a particular organism (Das et al., 2002).

In the last decades, the contamination of the marine environment by heavy metals has become a global problem (Minamata and Itai-Itai diseases) (Yilmaz, 2009). Especially, heavy metals such as mercury, plutonium, and lead are highly toxic (Furness, 2017). Their accumulation over a while in the marine organisms can cause serious illness and fatality (Duruibe et al., 2007). Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver and other vital organs (Das et al., 2002; Tchounwou et al., 2002). Long-term exposure may affect the physical, muscular and neurological degenerative processes (Thomas and Mohaideen, 2014). The gradual increase in the levels of such metals in aquatic environments has become a problem of primary concern, especially for aquatic systems that are very sensitive to heavy metal pollutants. One of the aquatic inhabitants highly affected by heavy metals are fishes (Ayas et al., 2007). The existence of metal ion in the environment increases the mucus like secretion from gill and excessive excretion, as well as anorexia and fin movement (Kamal et al., 2015). Most metals in a permissible level are essential for the physiological functions in the fish (Taylor et al., 1985) and are considered as normal constituents of the marine environment (Nieboer and Richardson, 1980). These metals

are a major threat above permissible level, potentially causing fish mortality, and behavioral, biochemical and histological changes in fish (Bu-Olayan and Thomas, 2004; Duruibe et al., 2007).

By nature, heavy metals are inert in the sediment and considered as conservative pollutants (pollutants that are not normally physically or chemically transformed to non-toxic substances in the receiving water). The sediment provides food and habitat for fish and benthos (Yilmaz, 2009). This results in bioaccumulation of heavy metals from water and sediment to marine organisms through the prey relationships in the food webs (Demirak et al., 2006; Vicente-Martorell et al., 2009). Fish is an effective sentinel organism for detection of heavy metal pollution in aquatic environments as its bio-accumulates metals from surrounding water, sediment and from their diet (Jayaprakash et al., 2015; Liu et al., 2018; Yi et al., 2017a). Metal bioaccumulation can produce long-term impacts on biogeochemical cycling of fishes (Ayas et al., 2007; Sapkota et al., 2008; Yi et al., 2017b), where metals accumulate with increasing trophic levels, causing a significant risk to health when consumed in amounts exceeding safe levels. Therefore, it is important to determinate the accumulated chemical content of aquatic organisms, particularly pollutants of concern, such as heavy metal concentrations in fish species widely consumed by seabirds and humans. The heavy metals risk of human health by fish consumption can be carcinogenic or noncarcinogenic (Peng et al., 2016).

Even though important progress was reached in developing countries for environmental management, heavy metals remain a huge issue for human health, flora, and fauna (Teodorof et al., 2009). The metals are not biodegradable (Wepener et al., 2001) and it is difficult to manage and eliminate metals (Furness, 2017). However, these metals depending on the circumstances can be transformed into more toxic forms

or complex forms more stable compounds or more or less toxic combination. In marine ecosystem, the toxicity of the metal can be influenced by the biotic variation of environmental factors such as oxygen, water hardness, pH and transparency which can make them more toxic or less (Ansari et al., 2004).

To assess the influence of physical and chemical factors on the distribution of heavy metals in the muscle tissue of fish, the following physical and chemical indicators should be taken into account: water temperature, water pH and dissolved oxygen (Ansari et al., 2004; Furness, 2017). For example, at low values of pH, the toxicity of heavy metals increases, since the metal concentrations and the values of pH are inversely proportional (negative values of the Pearson coefficient; (Furness, 2017). For the water temperature, it was found that the increase in the temperature has resulted in increase of animal metabolism and oxygen consumption, decrease in gas solubility and oxygen availability in water and hence, increase in gill epithelium permeability and strong absorption of heavy metals. For example, an increase of 10 °C of the water temperature within the limit compatible with life, cause a doubling of metabolic intensity and speed of penetration of heavy metals in the body (Authman et al., 2015; Dallinger et al., 1987).

Heavy metals can be affected by changes in dissolved oxygen in water contents, where they are oxidized (Yi et al., 2017a). The low concentration of dissolved oxygen in water and the high concentrations of metals may adversely affect fish by reducing the length of survival. It can possibly happen through the effect on respiratory organs and gills, reducing gradually their functional capacity, and thus reducing the amount of oxygen absorbed by them. Since the heavy metals are easily oxidized, a high concentration of dissolved oxygen in water can accelerate oxidation of metals and hence, positively effect on fish survival (Authman et al., 2015).

Heavy metal such as Hg can be biomagnified through the food chain, so large predatory fish species tend to have higher levels of mercury. The dominant source of mercury to the aquatic systems is enhanced atmospheric deposition of mercury, which may reflect in fish mercury concentrations (Haakanson et al., 1988; Rolfhus and Fitzgerald, 1995). The most concern has centered on the presence of mercury in fish due to the tragedy of Minamata Bay in Japan (Kureishy, 1993). Fish are acknowledged to be the major largest source of mercury to humans as they can accumulate substantial concentrations of mercury in their tissues (Uchida, 1961).

Fish is the major source of heavy metals for birds and mammals. At a certain stage of the life cycle, the fish accumulates heavy metal at a harmful concentration. As marine birds spend most of their time in marine environments and have a primarily marine-based diet, and they are at a higher risk of metal exposure in their feeding habitat (Wolfe et al., 1998). The primary ways of exposure are external contact, inhalation, and particularly ingestion of food and water that should be considered.

As contaminant levels can be examined in marine birds, they also can be used as proxy for coastal and marine pollution (Furness, 1993; Furness and Camphuysen, 1997; Gochfeld, 1980; Hays and Risebrough, 1972; Peakall, 1992; Walsh, 1990). They have been used to assess pollution over local, regional, or wide-scale geographical areas as well to determine whether levels of contaminants have changed over time (Walsh, 1990).

The potential impact of a pollutant occurs both at the individual and the population level (Furness, 2012). The effects of pollutants can vary, depending on the intrinsic toxicity and exposure level (Michelutti et al., 2010). For exposure to occur, there must be contact with a substance that is readily bioavailable, which must gain access from the external environment to target organ systems, which usually requires

absorption into the bloodstream. Thus, the bioavailability of heavy metals is another essential and interesting aspect of environmental pollution studies. It refers to the concentration of metals that is potentially available for biological action such as uptake by an aquatic organism. Important factors that influence the bioavailability are not only environmental speciation and characteristics of chemical, but also the physiology and behavior of the organism (Rand, 1995).

Widespread pollutants from heavy metals can affect various marine bird species in different ways, regarding breeding schedules, foraging methods, geographical ranges and life history (Schreiber and Burger, 2002). The following graph highlights the effects and damages caused by heavy metal exposure (Figure 4).

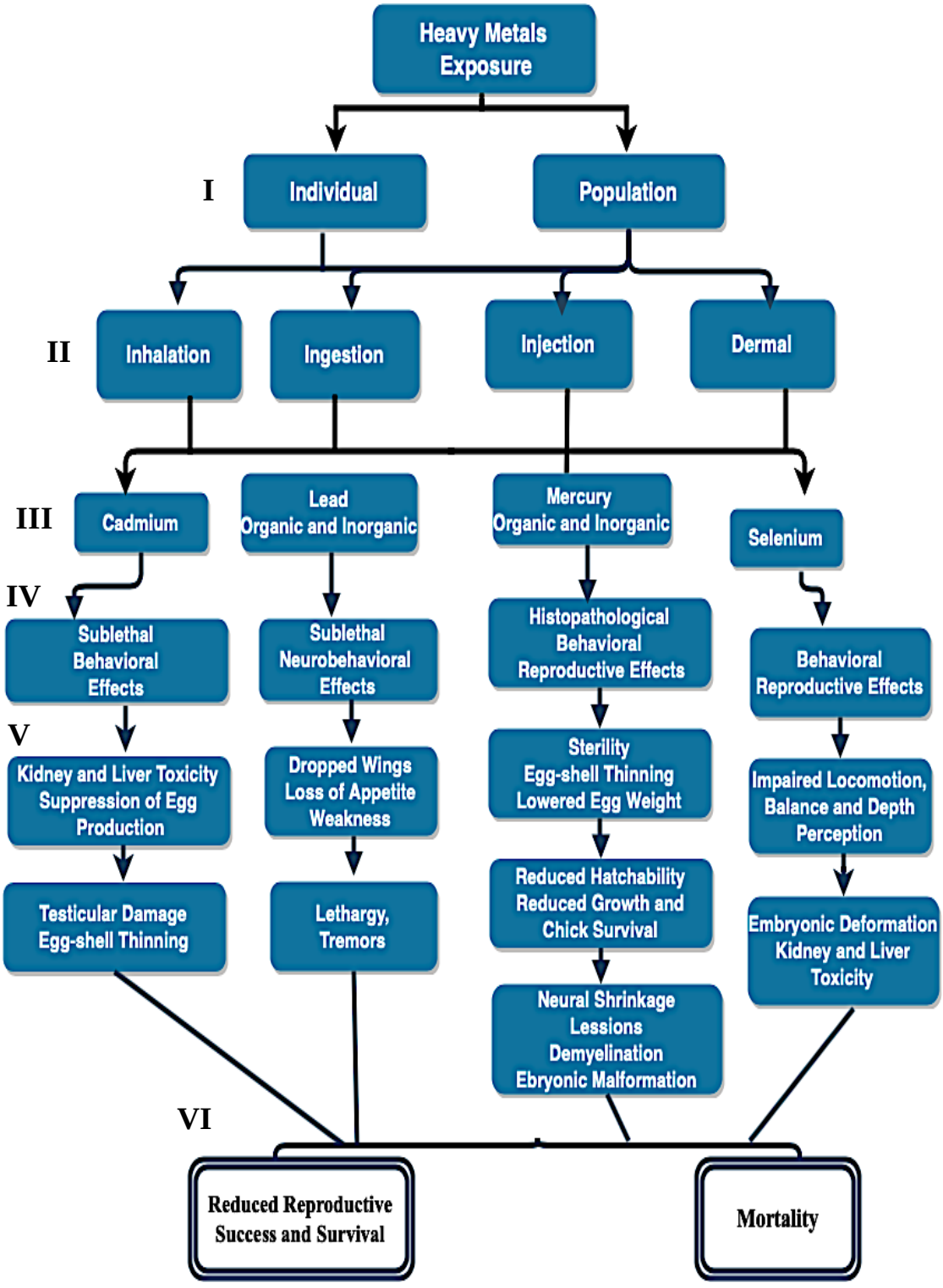

Figure 4: Consequences of heavy metal exposure to marine birds

I-impact level, II- the routes of exposure, III- the primary toxic heavy metals, IV-the toxic effects caused by exposure (III), V-damages caused by effects (IV), VI-general impact

It has been reported that heavy metals such as lead, mercury, and cadmium are primary metals of concern for water environments such as ocean, estuaries, river and they tend to accumulate at higher concentrations in marine mammals and marine birds (Burger and Gochfeld, 2004). Since marine birds are top predators and also accumulate high levels of heavy metals in their tissues, the risk to suffer from lower reproductive success and survival is higher (Burger, 2008; Elliott et al., 1992; Storelli, 2008). Cd can cause sublethal and behavioral effects at lower concentration, which cause kidney toxicity(Eisler, 1985a, 1985b). The high level of Cd has been reported in kidneys and livers of pelagic species, such as petrels, fulmars, albatrosses, penguins, skuas, as compared to the coastal and inshore species (Nisbet et al., 2010). Organo-lead compounds are more toxic, than inorganic lead compounds and can affect all body systems, especially young organisms (Eisler, 1985b). Since even a small concentration of Pb can be toxic for marine birds, so there is no “no effect” level (Franson, 1996). Other metals, such as chromium (Cr) and selenium (Se), are also potentially problematic to marine birds (Eisler, 1985a; Ohlendorf et al., 1986). The most susceptible to monomethyl mercury (CH_3Hg) contamination appears to be aquatic ecosystems, as they are major repositories of natural and pollution-derived Hg and host active populations of Hg methylating bacteria (Fitzgerald et al., 2007). In contrast to Hg, methylmercury has greater toxicity and bioaccumulation. Inorganic Hg exerts its greatest effect on the kidney, whereas CH_3Hg readily penetrates the blood-brain barrier in birds and mammals, producing brain lesions, spinal cord degeneration and central nervous system dysfunction (Wolfe et al., 1998). The main sources of Hg are fossil-fuel-fired power plants, artisanal small-scale gold mining, and non-ferrous metal manufacturing (Pirrone et al., 2010). Nowadays, the Hg concentrations in Arctic marine animals can be estimated to be about 10−12 times higher than those in pre-

industrial times (UNEP, 2013), indicating that Hg accumulation is due to recent anthropogenic sources. The level of Cd that causes renal tubular necrosis is also high in several marine birds (Burger and Gochfeld, 2004; Schreiber and Burger, 2002). However, information on metal accumulation on organisms living in the offshore is scarce (Honda et al., 1990).

1.7 Potential Contributions and Limitations of the Study

This study will provide insight into the pollution of heavy metals and their incorporation into food webs of the marine environment. This work is the first of its kind that quantitatively assesses the impact of major widespread heavy metal pollutants in the diet of marine birds in the Arabian Gulf. Thus, information on heavy metal and other pollutants in the Arabian Gulf will help in the management of the environment better.

Limitations of the study include difficulty in dissection of the fish and identification of fish species. Since anchovy small forage fish, it was difficult to distinguish liver from GI tract during dissection. Also, the identification guides for fish species are not well developed and distinguishing species of anchovies and other small forage fish was a challenge.

Chapter 2: Methods

2.1 Study Area

The area of sample collection is depicted in Figure 5. Samples were collected from the Arabian Gulf, located specifically in three main areas namely, Ajman, Sharjah, and Umm Al Quwain (AJ, SH and UAQ) in the United Arab Emirates. Ajman, Sharjah and Umm Al Quwain Emirates are situated southeast of the Arabian Gulf with the coordinates of 25.400° N, 55.453° E; 25.3495° N, 55.379° E and 25.564° N, 55.553° E respectively.

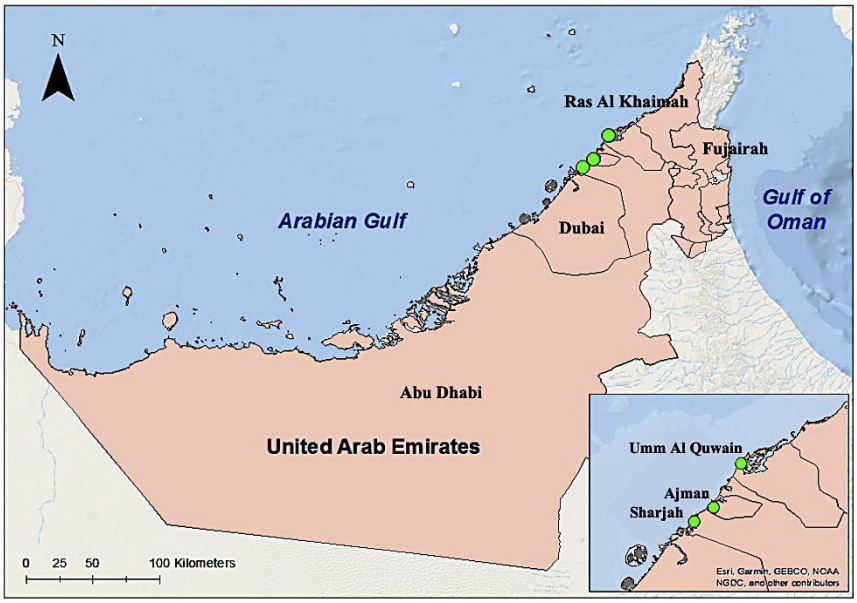

Figure 5: Map of the United Arab Emirates showing individual Emirates and the sampling stations for the collection of fish (inset)

The Arabian Gulf is located in the north-temperate tropical margin and bordered by eight countries; Iran, Iraq, Kuwait, Saudi Arabia, Bahrain, Qatar, United Arab Emirates (UAE), and Oman. It is a partially closed sea covering a surface of 240,000 km^2 and with an average depth of 35 m (Kardovani, 1995). The average seawater temperature of the Arabian Gulf is 28–30 °C, but it can increase up to 35.8 °C (Almasoud et al., 2015). Freshwater is supplied mainly by rivers in the northwest,

but this amount is insufficient to compensate for the evaporation. Water circulation is thus essential in maintaining the ecological balance of the Arabian Gulf. A surface current from the Indian Ocean moves slowly counterclockwise towards the Iranian coast, which plays an important role in salinity and likewise pollutant distributions of the Arabian Gulf. It also has an effect on suspended particles and plankton. According to estimations made by the FAO, potential fish resources in the Arabian Gulf (amount to 550,000 tons) are about eight times more than in the Sea of Oman (Kardovani, 1995).

2.2 Collection of Samples

A total of 105 fish samples of Indian anchovy were collected from three main areas namely, Ajman, Sharjah, and Umm Al Quwain in the United Arab Emirates (Table 2). The samples were purchased from local fish markets (UAQ Fish Market, Ajman Fish Market, and Souq Al Jubail Market) during November 2018.

Table 2: Overview of fish samples and sampling sites

Species Name	Sampling Sites	Coordinates	Number of Samples
Indian anchovy (*Stolephorus indicus*)	Ajman	25.400° N, 55.453° E	35
	Sharjah	25.3495° N,55.379° E	35
	Umm Al Quwain	25.564° N, 55.553° E	35

Samples were packed in ice and brought to the Entomology and Animal Ecology Laboratory in the College of Science on the same day. In the laboratory, their standard length (SL) were recorded. The length of the samples varied within 8.5 cm to 11 cm. Each fish was identified to the lowest possible taxonomic level using FishBase (Froese, 2009). The biomass of each species was estimated using the equation.

$$\text{Biomass} = a\text{SL}^{b}$$

where SL is standard length. Values of fitting parameters a and b were obtained from published references for the species or genus in FishBase (Froese, 2009).

Samples were dissected with clean stainless-steel equipment and were separated and grounded with stainless steel kits and glass equipment to the three tissues types: liver, GI tract, and muscle. They were placed on aluminum foil for each tissue type (Karadede et al., 2004; Papagiannis et al., 2004; Tuzen et al., 2010) and were labelled according to the collectors names, location, species name and number of sample (for example, NN-UAQ-ANCH-001) and were kept frozen at -20 °C until further analysis.

2.3 Analytical Procedures

2.3.1 Digestion Procedure

Analysis for heavy metals was carried out at the Animal Nutrition Laboratory in the College of Food and Agriculture. Once tissues were thawed and measured with microbalance, they were analyzed as it is. After measuring, the dissected samples were transferred to a 75 mL Teflon beaker. Thereafter, 10 mL of 65% nitric acid (HNO_3; Sigma-Aldrich) was added and they were placed into the rotor for 40 samples (Alam et al., 2002). The samples were heated at 200 and 250 °C on a hot plate for 0.5, 0.5, 0.5 and 2 hours with One-touch Heating Digester (Mars 6). The solution was diluted with deionized water and transferred into a 50 mL test tube (Karadede and Ünlü, 2000).

All reagents were of analytical reagent grade. Deionized water was used throughout the study. All the plastics were washed in nitric acid and rinsed with deionized water before use. Instrument calibration Standard solutions were prepared

from commercially available materials. High purity argon was used as inert gas (Karadede et al., 2004).

2.3.2 Determination of 20 Elements

The Varian 710-ES system is a patented compact, bench-mounted simultaneous Inductively Coupled Plasma Optical Emission Spectrometer (Agilent 710 Series ICP-OES; USA) with full PC control of instrument settings and compatible accessories (Figure 6; Agilent, 2010). It was used for the simultaneous determination of Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, P, Pb, V, Zn, Ca, K, Na, Mg, S and Sr in fish tissues (liver, gastrointestinal tract and muscle). It features an innovative megapixel CCD detector designed specifically for ICP-OES and provides complete wavelength coverage from 177-785 nm.

Figure 6: Varian 710-ES (Agilent 710 Series ICP-OES) system used in this study

A portion of homogeneous fish tissue sample is weighed and treated with acids to destroy the organic matter and heated to solubilize the recoverable elements. After cooling, the sample is made up to the volume with deionized water. The sample solution is aspirated through a nebulizer and the resulting aerosol is transported to the plasma torch where excitation occurs. Element specific emission spectra are produced

by radiofrequency inductively coupled plasma. Fitted background correction is used to correct the blank signal and matrix effect (Williams, 1991). Standards calibration curves for the metal analytes already prepared covering the optimum working range stored in the system software was used to produce the computerized analysis report. With MultiCal software, results were monitored at two wavelengths for each element which gives confidence in results. Standard solutions were prepared from stock solutions (Merck, multi-element standard). High purity argon was used as inert gas (Karadede et al., 2004).

2.3.3 Determination of Mercury

Determination of mercury (Hg) in all three tissues was carried out by using a simultaneous axial view Varian SpectrAAS 220FS (Varian, Mulgrave, Australia; Figure 7), coupled to an on-line continuous vapor generation system (Varian, model VGA-77). Vapor generation is often referred to as graphite furnace analysis for Hg because of the improved speed of analysis and the lack of background absorbance signals. Vapor generation Atomic Absorption Spectrometry (AAS) detection limits are usually in the sub-parts per billion (μg/L) range. The cold vapor technique is the most sensitive method available for the detection of ultra-trace levels of mercury by AAS. The improved sensitivity of the vapor generation technique is achieved by the 100% sampling efficiency. All of the analyte in the sample solution used in the reaction is chemically reduced and transported to the sample cell for measurement. This process also effectively separates the analyte element from its chemical matrix, eliminating matrix interference effects in the atomization process and minimizing background absorption (Cresser, 1993; Rothery, 1988; Tsalev et al., 1990).

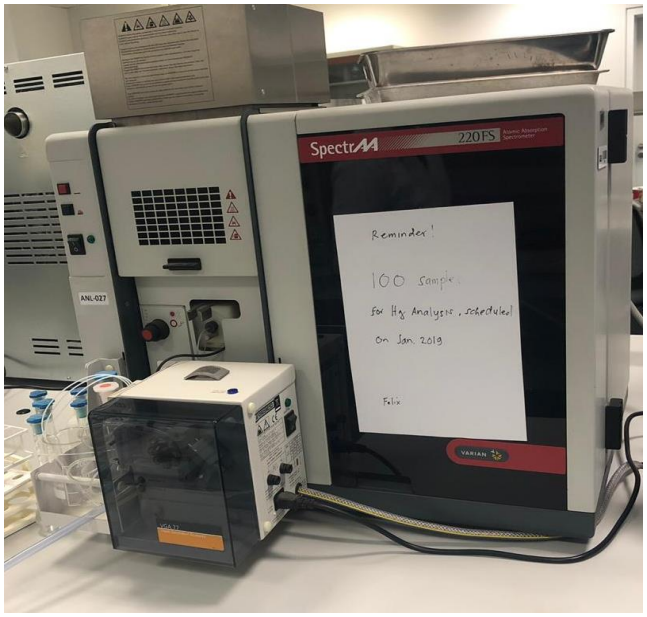

Figure 7: Varian SpectrAAS 220FS coupled to an on-line continuous vapor generation system (Varian, model VGA-7)

Varian's Vapor Generation Accessory employs a peristaltic pump to provide continuous flow vapor generation. This is shown schematically in Figure 8. In this technique, the sample flow is combined with a flow of concentrated acid and stannous chloride solution ($SnCl_2\,2H_2O$), before being pumped into a reaction coil. The mercury in the digested sample solution is reduced to the elemental state using $SnCl_2\ 2H_2O$. The mercury vapor from the solution is brought into a cell positioned in the light path of an AAS and determined by cold vapor AAS. Quantification was performed against simple aqueous calibration solutions containing 1 ppb, 5 ppb and 10 ppb Hg^{2+} mg/l (Rothery, 1988).

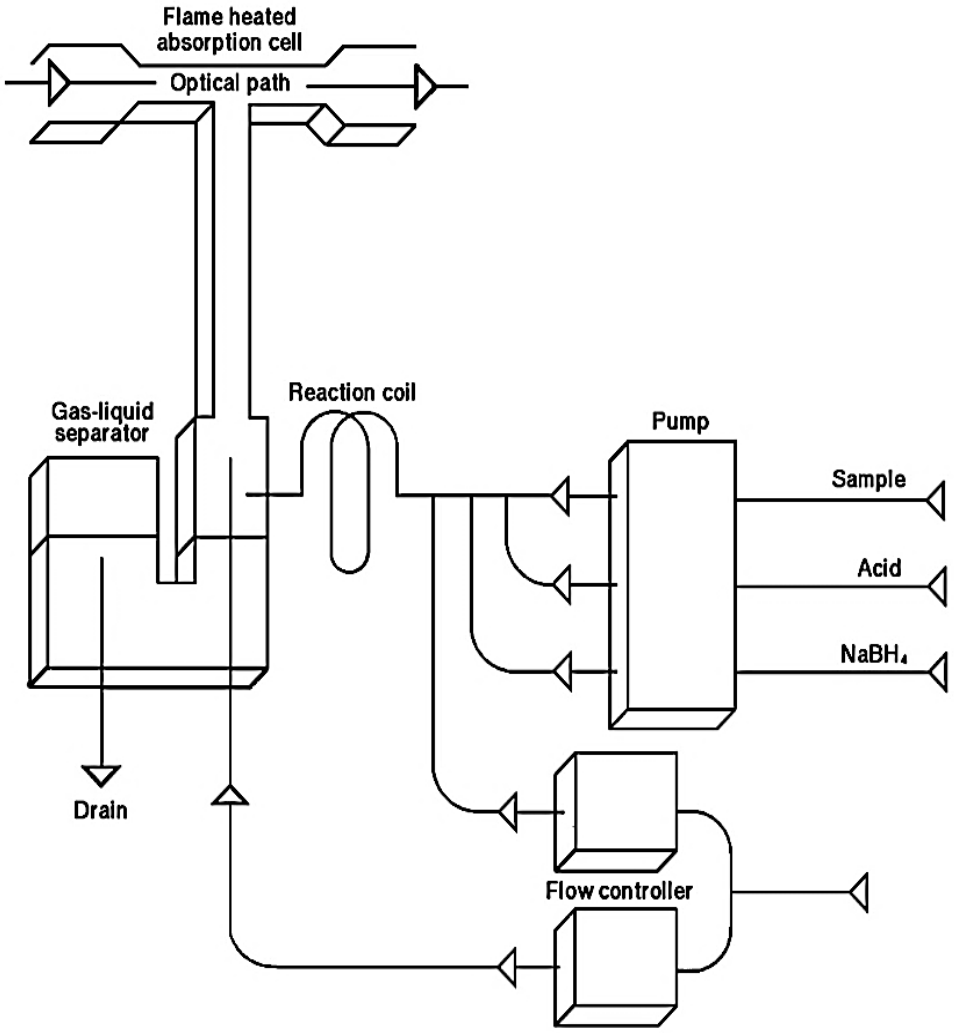

Figure 8: Schematic diagram of the Varian VGA-77 vapor generation accessory configured for hydride generation as three channel systems

Source: Rothery, 1988

2.3.4 Statistical Analysis

In the present study, the SPSS software (version 25) was applied for statistical analysis.

A discriminant analysis was performed to examine whether significant differences existed among the groups, in terms of predictor variables (elements) and to determine which predictor variables contribute the most to the inter-group differences (Arbuckle, 2010). The stepwise multivariate discriminant analysis was

used to assess the significance of contributions from each parameter measured for the three sampling sites (AJ, SH and UAQ) (Sokal and Rohlf, 2012). The analysis included 17 variables (elements) and Pb, As, Co and Mo were removed, since they were below the detection limits (concentrations were below than 0.011, 0.009, 0.003 and 0.018 respectively) in all tissues of sample. Three separate discriminant analyses were generated. First, the analysis included elements-related variables for muscle between the three sampling sites. Second, the analysis included elements-related variables for GI between the three sampling sites. Lastly, we performed the same analysis for elements-related variables in the liver.

The post-hoc MANOVA test were also performed for each significant variables (elements) and effect sizes were calculated for MANOVA (Ott, 2018; Zar, 2013). The aim was to indicate differences of variables between the three different sampling sites and to present the magnitude of the reported effects in a standardized metric (to communicate the practical significance of their results), instead of only reporting the statistical significance. Significance levels for pairwise comparisons and the most widely used effect size (Cohen's *d*) were indicated where appropriate (Sokal and Rohlf, 2012).

Multiple linear regression analysis was used to study the effect of the elements in different tissues on the biomass of fish. The value of α was set at 0.05 for all tests of significance.

Chapter 3: Results

3.1 Metal and Non-metal Concentrations in Tissues

The total mean and standard error (mean ± standard deviation) of Cd, Cr, Hg, Cu, Ni, Fe, Ca, Mn, S, Sr, Zn, P and V accumulated in tissues of Indian anchovy from the three different location was calculated (Table 3). Accordingly, Pb, As, Co and Mo were below the limits of detection (0.011, 0.009, 0.003 and 0.018 respectively) in all tissues of sample and were removed for further analysis. The concentrations of metals and non-metals were detected in ppm (except Hg, which is ppb). The standard deviation was fond to be higher for Cr, Fe, Mn and Ni compare to mean concentration of metals. These differences were explained by the extreme and potential outliers that was found for these metals (see Appendix1, 2, and 3). In addition, the result was compared with international and national guidelines namely with European Union (EC (2005), WHO (2006) and FAO (1983). The values in the Table 3 highlighted in light gray are values exceeding the MPL allowed by international guidelines.

Table 3: Metal and non-metal concentrations (mg/kg; ppm) in the liver, muscle and GI tract of Stolephorus indicus

Elements	Location	Tissue Metal Concentration (mean ± standard deviation)			Reference		
		Stolephorus indicus			Maximum permissible limit in fish		
		Muscle	GI	Liver	EC[1] (2005)	WHO[2] (2006)	FAO[3] (1983);
Cd(ppm)	AJ SH UAQ	0.12±0.08 0.08±0.05 0.12±0.08	6.4±4.8 1.6±1.8 3.3±2.4	3.99±2.04 4.86±6.05 7.99±3.05	0,10	–	0.05
Cr(ppm)	AJ SH UAQ	0.36±0.5 0.18±0.25 0.13±0.68	3.7±7.8 5.5±18.8 9.8±6.3	20±38 24±36 4.6±5.2	1	0.15	1
Hg(ppb)	AJ SH UAQ	0.8±0.13 0.76±0.11 0.67±0.12	0.43±0.05 0.52±0.12 0.36±0.6	1.32±0.14 0.41±0.15 0.73±0.19	500	500	500
Cu(ppm)	AJ SH UAQ	1.7±0.86 1.2±0.71 1.7±0.94	22±16.7 10.1±7.1 18.8±12	17±6.7 12.6±5.9 24±13	1	3	–
Ni(ppm)	AJ SH UAQ	0.61±1.07 0.38±0.86 0.27±0.16	10.8±30.8 20.7±70 24.3±13.9	57.33±85.5 76.54±110 28.1±20.1	–	–	80
Fe(ppm)	AJ SH UAQ	11.1±7.8 11.1±8.9 9.4±4.3	446±364 423±394 1714±1069	2036±2702 2731±3685 413±211	–	–	100
Ca(ppm)	AJ SH UAQ	2193±1562 1237±537 1900±1353	7297±5250 3487±3305 15270±9489	9254±9074 5614±3549 7691±4320	–	–	–
Mn(ppm)	AJ SH UAQ	0.6±0.26 0.4±0.16 0.52±0.2	3.2±2.8 4.9±5.1 23.1±14.5	25.5±34.3 36.2±43.2 6±2.2	–	–	–
S(ppm)	AJ SH UAQ	3002±659 2966±1107 3380±1374	5126±3646 3129±1614 4856±2251	6810±1304 5497±2018 7467±2408	–	–	–
Sr(ppm)	AJ SH UAQ	9.9±5.2 8.3±3.3 11.5±5.5	99±80 61±73 298±280	78.2±74.9 73.7±70.8 85.5±75.7	–	–	–

[1] European Union Commission Regulation (2005). Note: The MAL is 0.10 mg/kg of cadmium (Cd) for anchovy (Engraulis species), bonito (Sarda sarda), common two, banded seabream (Diplodus vulgaris), eel (Anguilla anguilla) and etc.

[2] World Health Organization (2006)

[3] Food and Agriculture Organization of the United Nations (1983)

Table 3: Metal and non-metal concentrations (mg/kg; ppm) in the liver, muscle and GI tract of Stolephorus indicus (Continued)

Elements	Location	Tissue Metal Concentration (mean ± standard deviation)				Reference	
		Stolephorus indicus			Maximum permissible limit in fish		
		Muscle	GI	Liver	EC (2005)	WHO (2006)	FAO (1983)
Zn(ppm)	AJ	9.3±2.7	108±69	139±58	–	–	40
	SH	7.1±2.5	56±35	126±53			
	UAQ	10.4±3.7	128±81	247±85			
P(ppm)	AJ	2882±980	3209±3476	6710±1582	–	–	–
	SH	2430±790	3666±2491	5932±1971			
	UAQ	1942±794	10241±6076	6086±1904			
V(ppm)	AJ	0.3±0.1	3.4±4.2	8±5.2	–	–	–
	SH	0.1±0.1	0.5±0.1	3.7±4.4			
	UAQ	0.2±0.2	27±26	3.4±2.1			

In addition, the mean concentration of Cu from the present study was also compared to the Cu levels in living organisms from the Black Sea, which included relatively similar fish species (*E. encrasicholus*; Table 4).

Table 4: Cu levels (mg/kg) in living organism from the Black Sea
Source: Boran and Altınok, 2010

References / Species	Galatchi et al. (2017)	Durali et al. (2010)	Tüzen (2009)	Nisbet et al. (2010)	Uluozlu et al. (2007)	Tüzen (2003)
Anchovy (E. encrasicholus)	4.58	–	1.96	2.73	0.95	1.96
Whiting (M. merlangus)	–	1.8	1.32	3.72	1.25	–
A. horse mackerel (T. trachurus)	–	2.4	0.65	1.79	0.95	1.55
Atlantic bonito (S. sarda)	–	1.9	1.43	1.74	0.84	1.29
Red mullet (M. barbatus)	–	1.4	0.96	3.14	0.98	–
Flathead mullet (M. cephalus)	–	–	2.14	–	1.26	–
Turbot (P. maxima)	–	–	0.75	2.13	–	–
Bluefish (P. saltor)	–	–	2.78	2.86	1.83	–

3.2 Metal and Non-metal Analysis in Muscle

Stepwise discriminant analysis showed that the significant metals and non-metals in muscle that discriminated sampling sites were K, Na, S, V, Sr, P, and Fe ($p \leq 0.001$) (George and Mallery, 2016; Sokal and Rohlf, 2012). The stepwise methods used in this discriminant analysis assessed these variables using seven steps, adding one variable at each step ($p \leq 0.001$). Therefore, these seven elements in the muscle of *Stolephorus indicus* were included in the Variables in the Analysis and Wilks Lambda table, because each was significant at $p \leq 0.001$ adding some predictive power to the function. The remaining variables (*i.e.*, Al, Cu, Ca, Zn, Cr, Ni, Mn, Cd and Hg) were removed as their addition did not improve the significance of the model to discriminate between the three sampling sites. F ratio from Variables in the Analysis table output was calculated for each predictor and the highest F ratio is the first to be selected for inclusion in the discriminant function, if meets certain significance and tolerance criteria. In the Variables in the Analysis table, K in the muscle was having the highest F ratio. Higher the F ration, higher the ability of predictor to discriminate between the three sampling sites (AJ, SH and UAQ) (Lachenbruch and Goldstein, 1979).

Table 5, the result of univariate ANOVA`s, carried out for each predictor and provides strong statistical evidence of significant differences between means of three sampling sites for K, Na, P, V and Zn variables, except Cd, Cr, Cu, Ni, Ca, Fe, Mg, Mn, S, Sr and Al ($p \geq 0.001$). An F ratio of univariate ANOVA is calculated for each predictor by conducting a univariate analysis of variance in which sampling sites are treated as the categorical variable and the predictors (heavy metals) as the criterion variable. The high value of F supports univariate ANOVA`s significance and indicates that K, Na, P, V and Zn significantly differentiate between three sampling sites (George and Mallery, 2016). In addition, the Pooled Within-Group Matrices indicates

low correlations between the predictors (heavy metal variables) except for correlation between Na and K; Na and Mg; S and Mg; Sr and Ca ($r > 0.95$).

Table 5: Tests of equality of group means (muscle)

	Wilks' Lambda	F	df1	df2	Sig.
Cd (ppm)	.944	2.560	2	87	.083
Cr (ppm)	.911	4.246	2	87	.017
Cu (ppm)	.921	3.737	2	87	.028
Ni (ppm)	.969	1.411	2	87	.249
Fe (ppm)	.987	.574	2	87	.565
Ca (ppm)	.906	4.527	2	87	.013
K (ppm)	.490	45.338	2	87	.000
Mg (ppm)	.950	2.308	2	87	.105
Mn (ppm)	.919	3.826	2	87	.026
Na (ppm)	.807	10.418	2	87	.000
P (ppm)	.827	9.085	2	87	.000
S (ppm)	.970	1.342	2	87	.267
Sr (ppm)	.937	2.932	2	87	.059
V (ppm)	.849	7.727	2	87	.001
Zn (ppm)	.845	7.968	2	87	.001
Hg (ppb)	.872	6.399	2	87	.003
Al (ppm)	.949	2.316	2	87	.105

Box M tests the null hypothesis that the covariance matrices do not differ between sampling sites formed by the dependent. In our result it shows that the log determinants are not equal (Table 6). When tested by Box M, F is 6 with Box M equal to 386.364, which is significant at $p \leq 0.001$ (Table 7). It indicates that sampling areas are differ from each other (Ott, 2018).

Table 5: Log determinants table (muscle)

Location	Rank	Log Determinant
AJ	7	44.766
SH	7	40.626
UAQ	7	41.426
Pooled within-groups	7	46.847

Table 6: Box M test result table (muscle)

Test Results		
Box's M		386.364
F	**Approx.**	6.096
	df1	56
	df2	20837.495
	Sig.	≤ 0.001

Only two discriminant functions were estimated, since there are three sampling sites (groups). The eigenvalue associated with first function is 9.732 and the eigenvalue associated with second function is 5.813 and this accounted for 62.6% and 37.4% of the explained variance in Function 1 and Function 2, respectively (Table 8). An eigenvalue indicates the proportion of variances explained and a large eigenvalue is associated with a strong function, so the first function is likely to be superior. A high canonical correlation indicates a function that discriminates well and the present correlations of Function 1 and Function 2 (0.952 and 0.924, respectively) are extremely high (1.00 is perfect). The square of this correlations, $(0.95)^2$ and $(0.92)^2$ = 0.9025 and 0.8464, indicate that 90% of the variance in the dependent variable (sampling sites) is explained by Function 1 model and 85% of the variance in the dependent variable (sampling sites) is explained by Function 2 model (Ott, 2018).

Table 7: Canonical discriminant function (Eigenvalues; muscle)

Eigenvalues				
Function	**Eigenvalue**	**% of Variance**	**Cumulative %**	**Canonical Correlation**
1	9.732	62.6	62.6	.952
2	5.813	37.4	100.0	.924

The null hypothesis that, in the population, the means of all discriminant functions in all sampling sites are equal can be statistically tested. This test is based on Wilks lambda table (Table 9). The significance level of Wilks lambda is estimated

based on a chi-square transformation of statistic. The difference between the sites based on Function 1 and 2 was significant, since small Wilks lambda (in our case, Function 1) indicates that the sampling sites means differ with highlights significant α values (Ott, 2018; Sachs, 2012).

Table 8: Canonical discriminant function (Wilks Lambda; muscle)

Wilks' Lambda				
Test of Function(s)	**Wilks' Lambda**	**Chi-square**	**df**	**Sig.**
1 through 2	.014	360.536	14	≤ 0.001
2	.147	161.183	6	≤ 0.001

The standardized coefficients indicate a large coefficient for S, K, P and V on Function 1, whereas Function 2 has relatively larger coefficients for Fe and Na (Table 10).

Table 9: Standardized Canonical Discriminant Function Coefficients for all seven elements in muscle used to assess the three sampling sites (Ajman, Sharjah, Umm Al Quwain) in the UAE.

	Function 1	**Function 2**
Fe (ppm)	.357	.519
K (ppm)	-2.519	1.128
Na (ppm)	-2.591	-2.640
P (ppm)	2.413	1.866
S (ppm)	3.461	.604
Sr (ppm)	-2.508	-2.019
V (ppm)	1.530	.671

A different conclusion for some variable is reached by an examination of the structure matrix (Table 11). Many researchers use the structure matrix correlations because they are considered more accurate than the Standardized Canonical Discriminant Function Coefficients (Sachs, 2012). To help interpret the functions, variables with large coefficients for a particular function are grouped together and it presents the individual weights of each predictor variable in the classification function.

These groupings are shown with asterisks. Thus, Na, Al, Hg, Ni, Zn, Ca, Cr, Mn and Sr have asterisks for Function 1 because these variables have coefficients which are larger for Function 1 than for Function 2. These variables are associated primarily with Function 1. On the other hand, K, P, Cu, V, S, Mg, Fe, Cd are predominantly associated with Function 2, as indicated by the asterisks (Ott, 2018; Sachs, 2012).

Table 10: Structure matrix table (muscle)

	Function 1	**Function 2**
Na	-.149*	-.065
Al	-.146*	.006
Hg	.131*	.069
Ni	.126*	-.002
Zn	.115*	-.029
Ca	.105*	.053
Cr	.100*	-.013
Mn	.078*	.072
Sr	.077*	-.040
K	-.237	.292*
P	-.023	.187*
Cu	.054	-.137*
V	.095	.124*
S	.038	-.054*
Mg	-.024	-.052*
Fe	-.023	.037*
Cd	-.002	-.029*

*. Largest absolute correlation between each variable and any discriminant function.

Figure 9 visually summarizes the contributions of Functions 1 and 2 to the model. Function 1 was slightly better in differentiating between the three sampling sites. The AJ and UAQ data are concentrated in the positive side of Function 1 and AJ in the positive side of Function 2. However, the segregation was not adequately clear from the perspective of Function 2. This supports the higher contribution of Function 1 to the model when compared with Function 2 (62.6% *vs.* 37.4%). Figure 9 further indicates that Function 1 tends to separate UAQ (highest value) from SH (lowest

value). This function primarily associated with Na, Al, Hg, Ni, Zn, Ca, Cr, Mn and Sr. Given the positive correlations of Hg, Ni, Zn, Ca, Cr, Mn and Sr variables with Function 1 in the structure matrix table, we expect to find UAQ to be higher with the Hg, Ni, Zn, Ca, Cr, Mn and Sr concentration than in SH. In contrast, Function 2 tends to separate AJ (highest value) from UAQ (lowest value). This function primarily associated with K, P, Cu, V, S, Mg, Fe, Cd. Given the positive correlations of K, P, V and Fe variables with Function 2 in the structure matrix table, we expect to find AJ to be higher with the K, P, V and Fe concentration than in UAQ (Ott, 2018; Sachs, 2012).

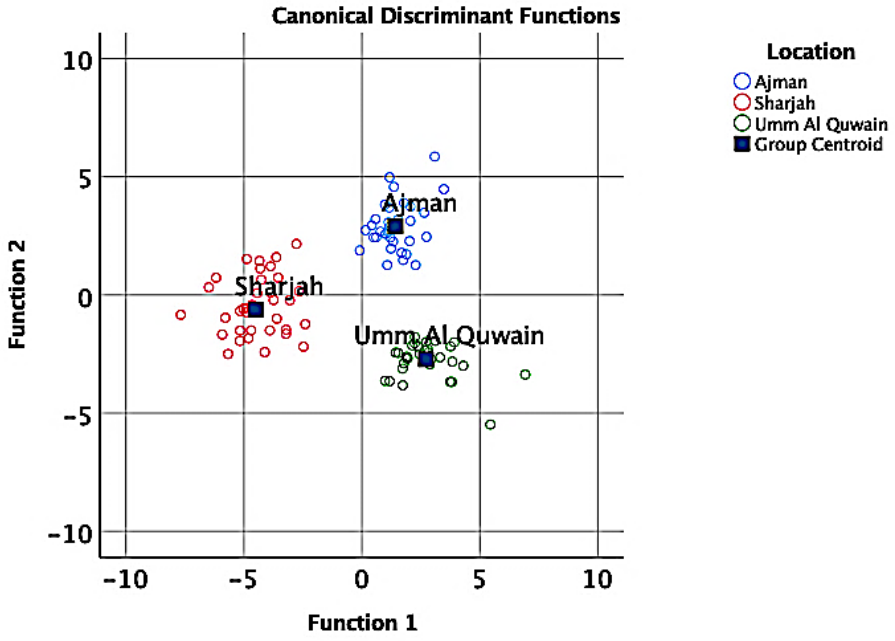

Figure 9: Canonical discriminant functions and their success in separating the three sampling areas (AJ, SH, UMQ) for the significant metal variables in the UAE (muscle)

A same conclusion for some variable is reached by an examination of the Classification Function Coefficients. Table 12 presents the individual weights of each predictor variable in the classification function, providing the reader with insight as to each variable's predictive value in discerning between each group. The classification

function coefficient indicates that Fe and Na have high correlation with Ajman, while K, P, S, Sr and V have with Sharjah.

Table 11: Classification Function Coefficients (using Fisher's linear function; muscle)

	Location		
	AJ	**SH**	**UMQ**
Fe (ppm)	.337	-.204	-.001
K (ppm)	.010	.030	-.007
Na (ppm)	-.014	.011	-.003
P (ppm)	.007	-.017	-.001
S (ppm)	.007	-.014	.008
Sr (ppm)	-1.143	3.412	.547
V (ppm)	12.348	-62.067	.554
(Constant)	-14.276	-20.745	-9.835

The classification results from discriminant analysis revealed that for the all the three sampling sites, 100% of the samples were correctly classified (Table 13). Overall, the model was successful in classifying 100% of the original grouped cases.

Table 12: Classification results of the discriminant model for the three location (Ajman, Sharjah, Umm Al Quwain) in the UAE (muscle)

	Location	**Predicted Group Membership**			**Total**
		AJ	**SH**	**UAQ**	
%	AJ	100.0	.0	.0	100.0
	SH	.0	100.0	.0	100.0
	UAQ	.0	.0	100.0	100.0

In addition to stepwise discriminant analysis, the post hoc test was performed to determine the significant differences lie between sampling sites (i.e., which specific independent variable significantly differs from another) (Tabachnick and Fidell, 2012). The pairwise group comparisons of post-hoc test of MANOVA revealed highly significant differences ($p \leq 0.05$) for K, Na, V, Sr and P between three sampling sites, although S and Fe showed differences that did not agree with the conclusions of discriminant analysis. S and Fe were loading strongly in the discriminant analysis with

Function 1 and 2, while here they seem to have no effect. However, the significance test does not tell the size of a difference between two measures (practical significance). Effect size is a statistical concept that measures the strength of the relationship between two variables on a numeric scale. The greater the effect size, the greater the difference between sampling sites (groups). Cohen's d is used to determine the effect size for the differences between two groups, such as in a t-test or pairwise comparisons (i.e. Pairwise post hoc) and is expressed in standard deviation units (Ott, 2018; Sachs, 2012). To report the p-value of variables from pairwise group comparisons table, t value was calculated, and degree of freedom was obtained from univariate test.

- The calculation of effect sizes for Na are as follows:

AJ (level i) vs. SH (level j) t (87) = 1100/255= 4.3 ($p \leq 0.05$), Cohen`s d= 1.05

AJ (level i) vs. UAQ (level j) t (87) = 172/252= 0.682 ($p = 0.498$), Cohen`s d= 0.22

SH (level i) vs. UAQ (level j) t (87) = 928/263= 3.53 ($p \leq 0.05$), Cohen`s d= 0.79

- The calculation of effect sizes for P are as follows:

AJ (level i) vs. SH (level j) t (87) = 452/222= 2.04 ($p \leq 0.05$), Cohen`s d= 0.51

AJ (level i) vs. UAQ (level j) t (87) = 940/220= 4.27 ($p \leq 0.05$), Cohen`s d= 1.05

SH (level i) vs. UAQ (level j) t (87) = 488/229= 2.13 ($p \leq 0.05$), Cohen`s d= 0.61

- The calculation of effect sizes for V are as follows:

AJ (level i) vs. SH (level j) t (87) = 0.154/0.039=3.95 ($p \leq 0.05$), Cohen`s d= 1.03

AJ (level i) vs. UAQ (level j) t (87) = 0.089/0.039=2.28 ($p \leq 0.05$), Cohen`s d= 0.58

SH (level i) vs. UAQ (level j) t (87) = 0.065/0.041=1.58 ($p = 0.113$), Cohen`s d= 0.33

- The calculation of effect sizes for K are as follows:

AJ (level i) vs. SH (level j) t (87) = 202/139=1.45 ($p = 0.151$), Cohen`s d= 0.33

AJ (level i) vs. UAQ (level j) t (87) = 1058/138=7.6 ($p \leq 0.05$), Cohen`s d= 2.47

SH (level i) vs. UAQ (level j) t (87) = 1260/144=8.62 ($p \leq 0.05$), Cohen`s d= 2.15

- The calculation of effect sizes for Sr are as follows:

AJ (level i) vs. SH (level j) t (87) = 1.53/1.24=1.23 ($p = 0.220$), Cohen`s d= 0.35

AJ (level i) vs. UAQ (level j) t (87) = 1.56/1.23=1.27 ($p = 0.206$), Cohen`s d= 0.29

SH (level i) vs. UAQ (level j) t (87) = 3.09/1.28=2.42 ($p \leq 0.05$), Cohen`s d= 0.68

Therefore, we reject the null hypothesis for some heavy metals claiming that here was a statistically significant difference between the sampling sites ($p \leq 0.05$). Cohen's effect size value (d) suggested that K (Cohen`s d= 2.47) had the highest practical significance to differentiate Ajman from Umm Al Quwain ($p \leq 0.05$), and P (Cohen`s d= 0.51) had the least practical significance to differentiate Ajman from Sharjah, which is in agreement with conclusion of discriminant analysis (Cohen, 1988).

3.3 Metal and Non-metal Analysis in GI Tract

Stepwise discriminant analysis showed that the significant metals and non-metals in GI tract that discriminate between the sampling sites were Cd, Mn, and Hg ($p \leq 0.001$) (George and Mallery, 2016; Sokal and Rohlf, 2012). The stepwise methods used in this discriminant analysis assessed these variables using three steps, adding one variable at each step ($p \leq 0.001$). Therefore, these three elements in the GI tract of *Stolephorus indicus* were included in the Variables in the Analysis and Wilks Lambda table, because each was significant at $p \leq 0.001$, adding some predictive power to the function. The remaining variables (*i.e.*, Al, Cu, Fe, Ca, Na, K, P, S, Sr, Zn, V, Cr and Ni) were removed as their addition did not improve the significance of the model to discriminate between the three sampling sites. F ratio from Variables in the Analysis table output was calculated for each predictor and the highest F ratio is the first to be selected for inclusion in the discriminant function, if meets certain significance and

tolerance criteria. In the Variables in the Analysis table, Mn in the GI tract was having the highest F ratio (Lachenbruch and Goldstein, 1979). Higher the F ration, higher the ability of predictor to discriminate between the three sampling sites (AJ, SH and UAQ).

Table 14, the result of univariate ANOVA`s, carried out for each predictor and provides strong statistical evidence of significant differences between means of three sampling sites for Al, Cd, Fe, Ca, Mn, P, Sr, Zn and Hg variables, except Cr, Cu, Ni, Mg, S, Sr ($p \geq 0.001$). An F ratio of univariate ANOVA is calculated for each predictor by conducting a univariate analysis of variance in which sampling sites are treated as the categorical variable and the predictors (heavy metals) as the criterion variable. The high value of F supports univariate ANOVA`s significance and indicates that Al, Cd, Fe, Ca, Mn, P, Sr, Zn and significantly differentiate between three sampling sites (George and Mallery, 2016). In addition, the Pooled Within-Group Matrices indicates low correlations between the predictors (heavy metal variables) except for correlation between Cr and Ni, Fe and Mn, and P and Ca ($r > 0.95$).

Table 13: Tests of equality of group means (GI tract)

	Wilks' Lambda	**F**	**df1**	**df2**	**Sig.**
Al (ppm)	.505	31.797	2	65	.000
Cd (ppm)	.692	14.472	2	65	.000
Cr (ppm)	.984	.531	2	65	.591
Cu (ppm)	.823	6.995	2	65	.002
Ni (ppm)	.989	.351	2	65	.705
Fe (ppm)	.599	21.718	2	65	.000
Ca (ppm)	.661	16.642	2	65	.000
Mn (ppm)	.500	32.518	2	65	.000
P (ppm)	.751	10.799	2	65	.000
S (ppm)	.882	4.337	2	65	.017
Sr (ppm)	.717	12.838	2	65	.000
Zn (ppm)	.791	8.579	2	65	.000
Hg (ppb)	.756	10.510	2	65	.000

Box M tests the null hypothesis that the covariance matrices do not differ between sampling sites formed by the dependent. In our result it shows that the log determinants are not equal (Table 15). When tested by Box M, F is 9.6 with Box M equal to 132.468, which is significant at $p \leq 0.001$ (Table 16). It indicates, that sampling areas are differ from each other (Ott, 2018; Zou et al., 2003).

Table 14: Log determinants table (GI tract)

Location	**Rank**	**Log Determinant**
AJ	3	-1.884
SH	3	-.288
UAQ	3	.421
Pooled within-groups	3	1.128

Table 15: Box M test result table (GI tract)

Test Results		
Box's M		132.468
F	Approx.	9.626
	df1	12
	df2	1299.137
	Sig.	≤ 0.001

Only two discriminant functions were estimated, since there are three sampling sites (groups). The eigenvalue associated with first function is 1.504 and the eigenvalue associated with second function is 0.855 and this accounted for 63.7% and 36.3% of the explained variance in Function 1 and Function 2, respectively (Table 17). An eigenvalue indicates the proportion of variances explained and a large eigenvalue is associated with a strong function, so the first function is likely to be superior. A high canonical correlation indicates a function that discriminates well and the present correlations of Function 1 and Function 2 (0.775 and 0.679, respectively) are relatively high (1.00 is perfect). The square of this correlations, $(0.77)^2$ and $(0.68)^2 = 0.59$ and 0.46, indicate that 59% of the variance in the dependent variable (sampling sites) is

explained by Function 1 model and 46% of the variance in the dependent variable (sampling sites) is explained by Function 2 model.

Table 16: Canonical discriminant function (Eigenvalues; GI tract)

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	1.504	63.7	63.7	.775
2	.855	36.3	100.0	.679

The null hypothesis that, in the population, the means of all discriminant functions in all sampling sites are equal can be statistically tested. This test is based on Wilks lambda table (Table 18). The significance level of Wilks lambda is estimated based on a chi-square transformation of statistic. The difference between the sites based on Function 1 and 2 was significant, since small Wilks lambda (in our case, Function 1) indicates that the sampling sites means differ with highlights significant α values (Ott, 2018; Sachs, 2012).

Table 17: Canonical discriminant function (Wilks Lambda; GI tract)

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 2	.215	98.306	6	≤ 0.001
2	.539	39.560	2	≤ 0.001

The standardized coefficients indicate a large coefficient for Mn on Function 1, whereas Function 2 has relatively larger coefficients for Cd and Hg (Table 19).

Table 18: Standardized Canonical Discriminant Function Coefficients for all three elements in GI tract used to assess the three location (Ajman, Sharjah, Umm Al Quwain) in the UAE.

	Function 1	Function 2
Cd(ppm)	-.658	.753
Mn(ppm)	1.040	.159
Hg(ppb)	-.091	-.803

A same conclusion for some variable is reached by an examination of the structure matrix (Table 20). As previously mentioned, many researchers use the structure matrix correlations because they are considered more accurate than the Standardized Canonical Discriminant Function Coefficients. To help interpret the functions, variables with large coefficients for a particular function are grouped together and it presents the individual weights of each predictor variable in the classification function. These groupings are shown with asterisks. Thus, Mn, Fe, Al, Ni and Cr have asterisks for Function 1 because these variables have coefficients which are larger for Function 1 than for Function 2. These variables are associated primarily with Function 1. On the other hand, Cd, Hg, Zn, S, Ca, Cu, P and Sr are predominantly associated with Function 2, as indicated by the asterisks.

Table 19: Structure matrix table (GI tract)

	Function 1	**Function 2**
Mn (ppm)	.775*	.339
Fe (ppm)	.647*	.447
Al (ppm)	.526*	.496
Ni (ppm)	-.153*	-.129
Cr (ppm)	-.130*	-.095
Cd (ppm)	-.280	.619*
Hg (ppb)	-.109	-.598*
Zn (ppm)	.040	.573*
S (ppm)	-.043	.551*
Ca (ppm)	.294	.499*
Cu (ppm)	-.058	.486*
P (ppm)	.182	.481*
Sr (ppm)	.353	.419*

*. Largest absolute correlation between each variable and any discriminant function

Figure 10 visually summarizes the contributions of Functions 1 and 2 to the model. Function 1 was slightly better in differentiating between the three sampling sites. The UAQ data are concentrated in the positive side of Function 1and 2 and AJ in the positive side of Function 2. However, the segregation was not adequately clear

from the perspective of Function 2. This supports the higher contribution of Function 1 to the model when compared with Function 2 (63.7% *vs.* 36.3%). Figure 10 further indicates that Function 1 tends to separate UAQ (highest value) from AJ (lowest value). This function primarily associated with Mn, Fe, Al, Ni and Cr. Given the positive correlations of Mn, Fe and Al variables with Function 1 in the structure matrix table, we expect to find UAQ to be higher with the Mn, Fe and Al concentration than in AJ. In contrast, Function 2 tends to separate UAQ (highest value) from SH (lowest value). This function primarily associated with Cd, Hg, Zn, S, Ca, Cu, P and Sr variables. Given the positive correlations of Cd, Zn, S, Ca, Cu, P and Sr variables with Function 2 in the structure matrix table, we expect to find UAQ to be higher with the Cd, Zn, S, Ca, Cu, P and Sr concentration than in UAQ (Ott, 2018; Zou et al., 2003).

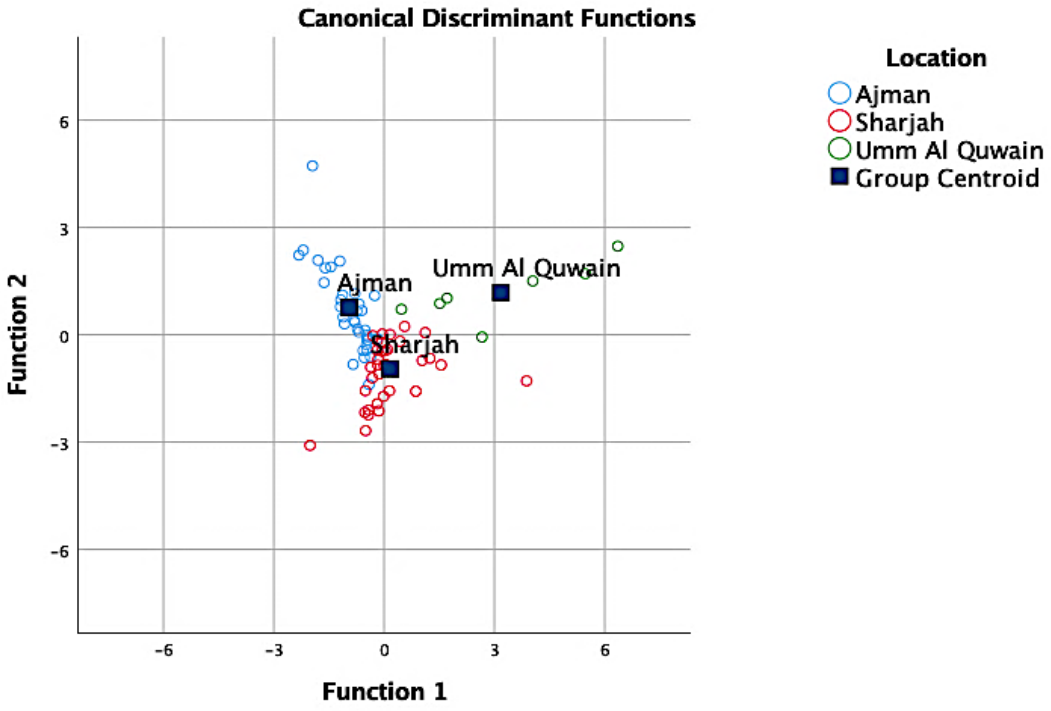

Figure 10: Canonical discriminant functions and their success in separating the three stations (Ajman, Sharjah, Umm Al Quwain) for the significant heavy metal variables in the UAE (GI tract)

A same conclusion for some variable is reached by an examination of the Classification Function Coefficients. Table 21 presents the individual weights of each predictor variable in the classification function, providing the reader with insight as to each variable's predictive value in discerning between each group. The classification function coefficient indicates that Cd and Mn have high correlation with Umm Al Quwain, while Hg have with Sharjah.

Table 20: Classification Function Coefficients (using Fisher's linear function; GI tract)

	Location		
	AJ	**SH**	**UAQ**
Cd (ppm)	.269	-.313	-.421
Mn (ppm)	-.071	.078	.659
Hg (ppb)	46.436	59.954	38.935
(Constant)	-11.832	-16.483	-15.132

The classification results from discriminant analysis revealed that for Ajman, only 71.4% of the samples were correctly classified while the remaining 28.6% was classified as Sharjah. For Sharjah, the discriminant model successful in classifying 94.1% of the sampling points. The rest were incorrectly classified as Ajman (2.9%) and as Umm Al Quwain (2.9%; Table 22). For Umm Al Quwain, the model was properly classified 85.7% of the sampling points. The model was incorrect in classifying 14.3% for Ajman. Overall, the model was successful in classifying 82.9% of the original grouped cases (Ott, 2018; Zou et al., 2003).

Table 21: Classification results of the discriminant model for the three location (Ajman, Sharjah, Umm Al Quwain) in the UAE (GI tract)

		AJ	**SH**	**UAQ**	**Total**
%	**AJ**	71.4	28.6	.0	100.0
	SH	2.9	94.1	2.9	100.0
	UAQ	14.3	.0	85.7	100.0

In addition to stepwise discriminant analysis, the post hoc test was performed to determine the significant differences lie between sampling sites (i.e., which specific independent variable significantly differs from another) (Tabachnick and Fidell, 2012). The pairwise group comparisons of post-hoc test of MANOVA output revealed highly significant differences ($p \leq 0.05$) for Cd, Mn and Hg between three sampling sites, agreed with the conclusion of discriminant analysis. However, the significance test does not tell the size of a difference between two measures (practical significance). Effect size is a statistical concept that measures the strength of the relationship between two variables on a numeric scale. The greater the effect size, the greater the difference between sampling sites (groups). Cohen's d is used to determine the effect size for the differences between two groups, such as in a t-test or pairwise comparisons (i.e. Pairwise post hoc) and is expressed in standard deviation units (Ott, 2018; Sachs, 2012). To report the p-value of variables from pairwise group comparisons table, t value was calculated, and degree of freedom was obtained from univariate test.

- The calculation of effect sizes for Cd are as follows:

AJ (level i) vs. SH (level j) t (65) = 4.8/0.9=5.3 ($p \leq 0.05$), Cohen`s d= 1.32

AJ (level i) vs. UAQ (level j) t (65) = 3.1/1.4=2.06 ($p \leq 0.05$), Cohen`s d= 0.84

SH (level i) vs. UAQ (level j) t (65) = 1.6/1.4=1.1 ($p = 0.261$), Cohen`s d= 0.8

- The calculation of effect sizes for Mn are as follows:

AJ (level i) vs. SH (level j) t (65) = 1.7/1.5=1.13 ($p = 0.271$), Cohen`s d= 0.41

AJ (level i) vs. UAQ (level j) t (65) = 19.9/2.5=7.9 ($p \leq 0.05$), Cohen`s d= 1.96

SH (level i) vs. UAQ (level j) t (65) = 18.2/2.5=7.58 ($p \leq 0.05$), Cohen`s d= 1.7

- The calculation of effect sizes for Hg are as follows:

AJ (level i) vs. SH (level j) t (65) = 0.08/0.02=4 ($p \leq 0.05$), Cohen`s d= 0.88

AJ (level i) vs. UAQ (level j) t (65) = 0.06/0.04=1.5 ($p = 0.103$), Cohen`s d= 1.27

SH (level i) vs. UAQ (level j) t (65) = 0.15/0.03=5 ($p \leq 0.05$), Cohen`s d= 1.47

Therefore, we reject the null hypothesis for some heavy metals claiming that here was a statistically significant difference between the sampling sites ($p \leq 0.05$). Cohen's effect size value (d) suggested that Mn (Cohen`s d= 1.96) had the highest practical significance to differentiate Ajman from Umm Al Quwain ($p \leq 0.05$), and Cd had the least practical significance (Cohen`s d= 0.8) to differentiate Sharjah from Umm Al Quwain, which is in agreement with conclusion of discriminant analysis (Cohen, 1988).

3.4 Metal and Non-metal Analysis in Liver

Stepwise discriminant analysis showed that the significant metals and non-metals in liver that discriminate between the sampling sites were Hg, K, Na and S ($p \leq 0.001$) (George and Mallery, 2016; Sokal and Rohlf, 2012). The stepwise methods used in this discriminant analysis assessed these variables using three steps, adding one variable at each step ($p \leq 0.001$). Therefore, these four elements were included in the Variables in the Analysis and Wilks Lambda table, because each was adding some predictive power to the function. The remaining variables (*i.e.*, Al, Cu, Fe, Ca, Cd, P, Mn, Sr, Zn, V, Cr and Ni were removed as their addition did not improve the significance of the model to discriminate between the three sampling sites. F ratio from Variables in the Analysis table output was calculated for each predictor and the highest F ratio is the first to be selected for inclusion in the discriminant function, if meets certain significance and tolerance criteria. In the Variables in the Analysis table, Hg in the liver was having the highest F ratio (Lachenbruch and Goldstein, 1979). Higher the F ration, higher the ability of predictor to discriminate between the three sampling sites (AJ, SH and UAQ).

Table 23, the result of univariate ANOVA`s, carried out for each predictor and provides strong statistical evidence of significant differences between means of three sampling sites for Al, Cd, Fe, Ca, Mn, P, Sr, Zn and Hg variables, except Cr, Cu, Ni, Mg, S, Sr ($p \geq 0.001$). An F ratio of univariate ANOVA is calculated for each predictor by conducting a univariate analysis of variance in which sampling sites are treated as the categorical variable and the predictors (heavy metals) as the criterion variable. The high value of F supports univariate ANOVA`s significance and indicates that Hg, K, Zn, Na and V significantly differentiate between three sampling sites (George and Mallery, 2016). In addition, the Pooled Within-Group Matrices indicates low correlations between the predictors (heavy metal variables) except for correlation between Cr and N; Fe and Mg ($r > 0.95$) which differ from muscle tissue of fish.

Table 22: Tests of equality of group means (liver)

	Wilks' Lambda	**F**	**df1**	**df2**	**Sig.**
Al (ppm)	.855	4.671	2	55	.013
Cd (ppm)	.890	3.407	2	55	.040
Cr (ppm)	.940	1.751	2	55	.183
Cu (ppm)	.799	6.923	2	55	.002
Ni (ppm)	.937	1.858	2	55	.166
Fe (ppm)	.911	2.694	2	55	.077
Ca (ppm)	.957	1.248	2	55	.295
K (ppm)	.532	24.155	2	55	.000
Mg (ppm)	.961	1.113	2	55	.336
Mn (ppm)	.904	2.916	2	55	.063
Na (ppm)	.746	9.346	2	55	.000
P (ppm)	.831	5.609	2	55	.006
S (ppm)	.861	4.449	2	55	.016
Sr (ppm)	.952	1.374	2	55	.262
V (ppm)	.795	7.092	2	55	.002
Zn (ppm)	.664	13.907	2	55	.000
Hg (ppb)	.195	113.443	2	55	.000

Box M tests the null hypothesis that the covariance matrices do not differ between sampling sites formed by the dependent. In our result it shows that the log determinants are not equal (Table 24). When tested by Box M, F is 3.954 with Box M

equal to 90.899, which is significant at $p \leq 0.001$ (Table 25). It indicates, that sampling areas are differ from each other (Ott, 2018; Zou et al., 2003).

Table 23: Log determinants table (liver)

Location	**Rank**	**Log Determinant**
AJ	4	35.278
SH	4	37.401
UAQ	4	36.935
Pooled within-groups	4	37.794

Table 24: Box M test result table (liver)

Test Results		
Box's M		90.899
F	Approx.	3.954
	df1	20
	df2	4720.575
	Sig.	≤ 0.001

Only two discriminant functions were estimated, since there are three sampling sides (groups). The eigenvalue associated with first function is 7.648 and the eigenvalue associated with second function is 3.896 and this accounted for 66.3% and 33.7% of the explained variance in Function 1 and Function 2, respectively (Table 26). An eigenvalue indicates the proportion of variances explained and a large eigenvalue is associated with a strong function, so the first function is likely to be superior. A high canonical correlation indicates a function that discriminates well and the present correlations of Function 1 and Function 2 (0.940 and 0.892, respectively) are relatively high (1.00 is perfect). The square of this correlations, $(0.94)^2$ and $(0.89)^2 = 0.88$ and 0.79, indicate that 88% of the variance in the dependent variable (sampling sites) is explained by Function 1 model and 79% of the variance in the dependent variable (sampling sites) is explained by Function 2 model.

Table 25: Canonical discriminant function (Eigenvalues; liver)

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	7.648	66.3	66.3	.940
2	3.896	33.7	100.0	.892

The null hypothesis that, in the population, the means of all discriminant functions in all sampling sites are equal can be statistically tested. This test is based on Wilks lambda table (Table 27). The significance level of Wilks lambda is estimated based on a chi-square transformation of statistic. The difference between the sites based on Function 1 and 2 was significant, since small Wilks lambda (in our case, Function 1) indicates that the sampling sites means differ with highlights significant α values (Ott, 2018; Sachs, 2012).

Table 26: Canonical discriminant function (Wilks Lambda; liver)

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 2	.024	200.394	8	≤ 0.001
2	.204	84.977	3	≤ 0.001

The standardized coefficients indicate a large coefficient for Hg on Function 1, whereas Function 2 has relatively larger coefficients for K, Na and S (Table 28).

Table 27: Standardized Canonical Discriminant Function Coefficients for all three elements in liver used to assess the three location (Ajman, Sharjah, Umm Al Quwain) in the UAE.

	Function 1	**Function 2**
K (ppm)	-.829	1.421
Na (ppm)	-.076	-2.274
S (ppm)	.914	1.026
Hg (ppb)	.863	.042

A different conclusion for some variable is reached by an examination of the structure matrix (Table 29). As previously mentioned, many researchers use the

structure matrix correlations because they are considered more accurate than the Standardized Canonical Discriminant Function Coefficients. To help interpret the functions, variables with large coefficients for a particular function are grouped together and it presents the individual weights of each predictor variable in the classification function. These groupings are shown with asterisks. Thus, Hg, K, P, Ca, Sr, Cd, S, Al and Cu have asterisks for Function 1 because these variables have coefficients which are larger for Function 1 than for Function 2. These variables are associated primarily with Function 1. On the other hand, Mg, Na, Mn, Fe, Zn, Ni, Cr and V are predominantly associated with Function 2, as indicated by the asterisks (Ott, 2018).

Table 28: Structure matrix table (liver)

	Function 1	**Function 2**
Hg	.732*	.090
K	-.296*	.231
P	-.268*	.219
Ca	-.263*	-.165
Sr	-.261*	-.204
Cd	.170*	.068
S	.125*	.104
Al	-.082*	-.046
Cu	.058*	.045
Mn	-.058	-.255*
Na	-.115	-.247*
Mg	.031	-.196*
Fe	-.002	-.189*
Zn	.174	.176*
Ni	.069	-.142*
Cr	.020	-.079*
V	.024	.053*

*. Largest absolute correlation between each variable and any discriminant function

Figure 11 visually summarizes the contributions of Functions 1 and 2 to the model. Function 1 was slightly better in differentiating between the three sampling

sites. The UAQ data are concentrated in the positive side of Function 1and 2 and AJ in the positive side of Function 2. However, the segregation was not adequately clear from the perspective of Function 2. This supports the higher contribution of Function 1 to the model when compared with Function 2 (63.7% *vs.* 36.3%). Figure 11 further indicates that Function 1 tends to separate UAQ (highest value) from AJ (lowest value). This function primarily associated with Mn, Fe, Al, Ni and Cr. Given the positive correlations of Mn, Fe and Al variables with Function 1 in the structure matrix table, we expect to find UAQ to be higher with the Mn, Fe and Al concentration than in AJ. In contrast, Function 2 tends to separate UAQ (highest value) from SH (lowest value). This function primarily associated with Cd, Hg, Zn, S, Ca, Cu, P and Sr variables. Given the positive correlations of Cd, Zn, S, Ca, Cu, P and Sr variables with Function 2 in the structure matrix table, we expect to find UAQ to be higher with the Cd, Zn, S, Ca, Cu, P and Sr concentration than in UAQ (Ott, 2018; Zou et al., 2003).

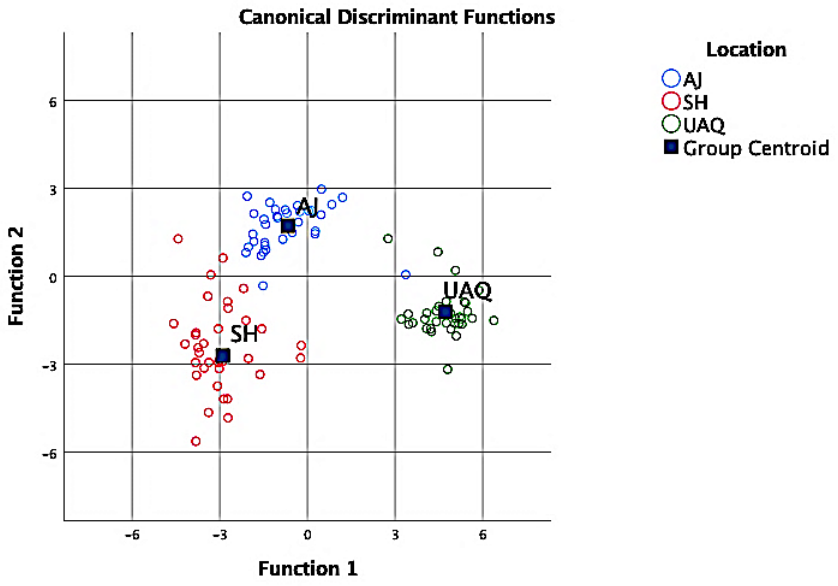

Figure 11: Canonical discriminant functions and their success in separating the three stations (Ajman, Sharjah, Umm Al Quwain) for the significant heavy metal variables in the UAE (liver)

A same conclusion for some variable is reached by an examination of the Classification Function Coefficients. Table 30 presents the individual weights of each predictor variable in the classification function, providing the reader with insight as to each variable's predictive value in discerning between each group. The classification function coefficient indicates that S and Hg have high correlation with Umm Al Quwain, while K and Na have with Sharjah.

Table 29: Classification Function Coefficients (using Fisher's linear function; liver)

	Location		
	AJ	**SH**	**UAQ**
K (ppm)	.005	.001	-.003
Na (ppm)	-.003	.002	.000
S (ppm)	.003	.000	.004
Hg (ppb)	31.628	18.604	59.621
(Constant)	-27.652	-14.995	-54.568

The classification results from discriminant analysis revealed that for the Ajman, the discriminant model successful in classifying 97.1% of the sampling points while the remaining 2.9% was classified as Umm Al Quwain (Table 31). For the Sharjah, only 94.3% of the samples were correctly classified. The rest were incorrectly classified as Ajman (5.9%). For the Umm Al Quwain, the model was successfully classified 100% of the sampling points. Overall, the model was successful in classifying 97.1% of the original grouped cases.

Table 30: Classification results of the discriminant model for the three location (Ajman, Sharjah, Umm Al Quwain) in the UAE (liver)

	Location				**Total**
		AJ	**SH**	**UAQ**	
%	**AJ**	97.1	.0	2.9	100.0
	SH	5.7	94.3	.0	100.0
	UAQ	.0	.0	100.0	100.0

In addition to stepwise discriminant analysis, the post hoc test was performed to determine the significant differences lie between sampling sites (i.e., which specific independent variable significantly differs from another) (Tabachnick and Fidell, 2012). The pairwise group comparisons of post-hoc test of MANOVA output revealed highly significant differences ($p \leq 0.05$) for K, Na, S and Hg, which is agreed with the conclusion of discriminant analysis. However, the significance test does not tell the size of a difference between two measures (practical significance). Effect size is a statistical concept that measures the strength of the relationship between two variables on a numeric scale. The greater the effect size, the greater the difference between sampling sites (groups). Cohen's d is used to determine the effect size for the differences between two groups, such as in a t-test or pairwise comparisons (i.e. Pairwise post hoc) and is expressed in standard deviation units (Ott, 2018; Sachs, 2012). To report the p-value of variables from pairwise group comparisons table, t value was calculated, and degree of freedom was obtained from univariate test.

- The calculation of effect sizes for K are as follows:

AJ (level i) vs. SH (level j) t (55) = 362/321=1.13 ($p = 0.264$), Cohen`s d= 0.36

AJ (level i) vs. UAQ (level j) t (55) = 2267/329=6.89 ($p \leq 0.05$), Cohen`s d= 2.19

SH (level i) vs. UAQ (level j) t (55) = 1904/384=4.95 ($p \leq 0.05$), Cohen`s d= 1.71

- The calculation of effect sizes for Na are as follows:

AJ (level i) vs. SH (level j) t (55) = 2516/598=4.21 ($p \leq 0.05$), Cohen`s d= 1.30

AJ (level i) vs. UAQ (level j) t (55) = 194/613=0.31 ($p = 0.752$), Cohen`s d= 0.10

SH (level i) vs. UAQ (level j) t (55) = 2321/715=3.24 ($p \leq 0.05$), Cohen`s d= 0.93

- The calculation of effect sizes for S are as follows:

AJ (level i) vs. SH (level j) t (55) = 1312/572=2.29 ($p \leq 0.05$), Cohen`s d= 0.77

AJ (level i) vs. UAQ (level j) t (55) = 657/587=1.11 ($p = 0.268$), Cohen`s d= 0.34

SH (level i) vs. UAQ (level j) t (55) = 1970/684=2.88 ($p \leq 0.05$), Cohen`s d= 0.88

- The calculation of effect sizes for Hg are as follows:

AJ (level i) vs. SH (level j) t (55) = 0.32/0.05=6.29 ($p \leq 0.05$), Cohen`s d= 2.19

AJ (level i) vs. UAQ (level j) t (55) = 0.59/0.05=11.13 ($p \leq 0.05$), Cohen`s d= 3.8

SH (level i) vs. UAQ (level j) t (55) = 0.92/0.06=14.9 ($p \leq 0.05$), Cohen`s d= 8.45

Therefore, we reject the null hypothesis for some heavy metals claiming that here was a statistically significant difference between the sampling sites ($p \leq 0.05$). Cohen's effect size value (d) suggested that Hg (Cohen`s d= 8.45) had the highest practical significance to differentiate Sharjah from Umm Al Quwain ($p \leq 0.05$), and S had the least practical significance (Cohen`s d= 0.77) to differentiate Ajman from Sharjah, which is in agreement with conclusion of discriminant analysis (Cohen, 1988).

3.5 The Biomass Response on Metal and Non-metal Concentrations in Tissues of Fish

Multiple linear regression analysis was carried out to investigate the effects of metals and non-metals variables in the muscle, GI tract and liver of fish on biomass of fish (Zou et al., 2003). The scatterplot of standardized predicted values verses standardized residuals, showed that the data met the assumptions of homogeneity of variance and linearity and the residuals were approximately normally distributed (Sachs, 2012). However, the validity of equation test did not approve the significance of the model ($p \geq 0.05$) for the all three tissues of fish. Moreover, the coefficients table showed that there is no linear relationship except for Al in the muscle. The main effect of Al on biomass was significant, $F(17,72) = 1.118$, $MSE = 0.833$, $p \leq 0.05$. The slope coefficient for Al was -0.029, so the biomass of Indian anchovy increased by 0.029 when the Al level decreased by 1%. The R^2 value was 0.20 indicating poor prediction.

Thus, only 20.9% of the variation in biomass can be explained by the differences of Al concentration in the muscle of Indian anchovy. The remaining variation 100 – 20.9 = 79.1% is due to factors other than Al. In terms of GI tract, the coefficients table showed there is no effect of 17 heavy metals (*i.e.*, Al, Cd, Cr, Cu, Ni, Fe, Ca, K, Mg, Mn, Na, P, S, Sr, V, Zn and Hg) in the GI tract on biomass of Indian anchovy, while for the liver, only Al and V in the liver had effect on biomass of Indian anchovy ($F(17,40) = 1.743$, $MSE = 0.682$, $p \leq 0.05$). The slope coefficient for Al was -0.001, so the biomass of Indian anchovy increases by 0.001, when the Al level decreased by 1 percent and the slope coefficient for V was -0.093, indicating that the biomass of Indian anchovy increases by 0.093 when the V level decreased by 1%. The R^2 value was 0.425, thus only 42.5% of the variation in biomass can be explained by the differences of Al and V concentration in the liver of Indian anchovy.

Chapter 4: Discussion

Escalating population, economic and industrial growth in the Arabian Gulf contributed to deterioration in water quality (H. A. Naser, 2013). The excessive concentration of heavy metals has been reported from coastal areas experiencing increasing settlement, traffic, agricultural and industrial activities such as textile, detergent, paint, oil and gas production, plastic industries, etc. Regarding the heavy metal concentrations in Arabian Gulf waters, it was estimated based on the published articles. The annual average values for all elements investigated in the last years did not exceed the proposed target values, except for Cu, Ni, Zn and V, for which a slight exceeding was observed in certain coastal areas such as Kuwait, Iran, Bahrain and UAE (Elshorbagy, 2005). Non-essential metals have been demonstrated to accumulate along the trophic chain in the freshwater ecosystem (Rajeshkumar and Li, 2018).

The results showed that fish exhibited wide range of interspecific variations in metal concentration in muscle, GI tract and liver. *Stolephorus indicus* contained higher concentration of metals in liver and lower in muscle with few exceptions. The bioaccumulation of metals in liver may be linked to its function of metabolism, that is, chemical processes that occur within a living organism in order to maintain life (Bawuro et al., 2018). However, the mean concentrations (the average concentration of Ca and Sr within three sampling sites) of Ca (8685 mg/kg) and Sr (153 mg/kg) in Stolephorus indicus appear considerably higher in the GI tract compare to liver and muscle (Table 43). In general, the highest metal concentrations were found in the liver and GI tract, since the muscle tended to accumulate less metal. Certain fish species may also accumulate higher concentration of heavy metals compared to others (Rajeshkumar and Li, 2018). The observed variability of heavy metal levels depends

on age, size and length of the fish, feeding habits, ecological needs, metabolism, and their habitats (Canli and Atli, 2003; Canli and Furness, 1993; Roméo et al., 1999).

The accumulation patterns of heavy metals in the muscle, GI tract and liver of *Stolephorus indicus* were in the decreasing order of S > P > Ca > Fe > Sr > Zn > Cr > Cu > Mn > V > Cd > Hg; Ca> P > S > Fe > Cr > Sr > Zn > Ni > Cu > Mn > V > Cd > Hg; Ca > S > P > Fe > Zn > Sr > Ni > Mn > Cu > Cr > Cd > V > Hg, respectively. In the present study, Fe had the highest concentration in the liver and GI tract, followed by Zn, Cr, Ni Cu, Cd. Metals such as Fe, Co, Zn and Mg considered as essential metals since they play an important role in biological systems whereas non-essential metals, such as Cr, Ni, Pb, and Cd, are toxic even in trace amounts. However, essential metals can also produce toxic effects at high concentrations (Yilmaz, 2009). The heavy metal concentrations of Cd, Cr, Ni, Cu and Fe in the tissues of *Stolephorus indicus* in 2018 exceeded the allowed levels under the EC, FAO and WHO legislation but for other heavy metal elements, the recorded values were well below the maximum level (Table 3).

The concentration of iron (Fe) was ranging from 413 to 2731 mg/kg in the liver of the *Stolephorus indicus*, followed by GI tract ranging from 423 to 1714 mg/kg and muscle ranging from 9.4 to 11.1 mg/kg, depending on the sampling site. These values are higher than those measured in some edible fish by other authors. Carvalho et al. (2005) obtained Fe values in the order of 7–11 mg/kg and 6–10 mg/kg in the muscle of *Solea vulgaris* and *Lophius piscatorius*, respectively. El-Moselhy et al. (2014) obtained Fe concentration in the order of 3.35 µg/g and 291.76 mg/kg in muscle and liver of *Epinephelus sp.* from Shalateen, respectively. However, Fe concentration in the muscle obtained from present study was lower to *Lepomis gibbosus* and *Triglia*

lucerna, Lophius budegassa, Solea lascaris from Iskenderun Bay (Turkey) and Penaeus semisulcatus from Bushehr (Arabian Gulf) (Heidarieh et al., 2013; Yilmaz, 2009; Yılmaz et al., 2007). Compare to liver, Fe concentration in present study was higher than those mentioned above. The reason of this discrepancy might be because Fe content depends on species, individuals, and sampling period. The differences in Fe also occur between species with more affinity for rocky bottoms and species with more affinity for sandy/muddy bottoms (El-Moselhy et al., 2014). Considering Indian anchovy as pelagic species, the biggest difference occurs between pelagic and benthic species with increased Fe values in benthic species. Individual diet can also play important role in the highest concentrations of Fe. Fe concentration in muscle from present study was generally in agreement with the legislation, while in the liver and GI, Fe values were exceeding the limits allowed by FAO (1983). The concentration of Fe from present study is supported by the Samara et al. (2016) findings for sediment samples obtained from Khalid Khor water, Sharjah. The highest concentrations were found for Fe (5769-12.668 mg/kg) from sediment sample (Ksiksi et al., 2015; Samara et al., 2016). The source of Fe might be sewage disposal from ships, dust depositions, fertilizers and herbicides, in addition to its natural presence sediment (Rajeshkumar and Li, 2018).

Mean concentration of zinc (Zn) ranged between 8.9 and 170.6 mg/kg within three sampling sites with higher concentration in the liver and GI tract which is exceeding the limits allowed by FAO (1983), 40 mg/kg. These results are higher to those reported earlier in fishes from lakes of Turkey (Karadede et al., 2004; Mendil and Uluözlü, 2007; Yilmaz, 2009; Yılmaz et al., 2007) and from Shalateen in Epinephelus sp. (2.42 and 59.89 mg/kg) (Samara et al., 2016). However, the concentration of Zn in the liver of fish obtained from Sharjah was lower than those

reported in the liver of *Epinephelus sp.* from Shalateen (Samara et al., 2016). The concentration of Zn was also lower compared to findings obtained from Ataturk Dam Lake and Lake Kasumigaura (Alam et al., 2002; Karadede and Ünlü, 2000) and in *E.areolatus and P.maculatus* from Arabian Gulf (Kamal, 2015). It was suggested that concentrations of Zn element in fish increase with depth and anchovy as a pelagic species should contain accordingly less Zn concentration (Cronin et al., 1998). The source of Zn in Arabian Gulf can be municipal wastewater discharged from textile industry, detergent industry and from production of crude oil.

Average manganese (Mn) concentration in different tissues of *Stolephorus indicus* varied between 0.51 and 22.57 mg/kg in three different sampling sites. Our results of Mn content were higher than the results reported by Mendil et al. (2005) for fish species in the lakes of Tokat, by Yilmaz et al. (2007) for *Leuciscus cephalus and Lepomis gibbosus* from Saricay lake and by Kamal et al. (2015) for *E. areolatus* from Arabian Gulf. However, the concentration of Mn was lower than those given for *Triglia lucerna, Lophius budegassa, Solea lascaris* from Iskenderun Bay, Turkey (Yilmaz et al., 2009) and for P. maculatus from Arabian Gulf (Kamal et al., 2015).

Copper (Cu) is another metal present in amounts that exceeded the international standard (EC (2005) and WHO (2006)). Cu concentration was ranging 1.53–17.8 µg/kg between three sampling sites for analyzed fish samples with higher concentration recorded in liver and GI tract. Mendil et al. (2010) and Tüzen (2003) found almost close results (1.96 mg/kg) for copper in muscle of anchovy (*E. encrasicholus*) (Mendil et al., 2010; Tüzen, 2003). The mean concentration of Cu present in this study were exceeding concentration reported by Aucoin et al. (1999), Suhendan Mol et al. (2010) and Mendil et al. (2005) for the fish samples from the lake Boeuf, Southeastern Louisiana, Ataturk Dam Lake and Lakes of Tokat, Turkey. As

shown in Table 4, our values of Cu in muscle of anchovy were lower than those reported by Galaţchi et al. (2017) and almost same as those reported by Mendil et al. (2010), Tüzen (2010), Nisbet et al. (2010), Uluozlu et al. (2007), Tüzen (2003). The higher concentration of Cu was found from sediment sample from Sharjah by Samara et al. 2016. The high concentration of Cu in seabird body can lead to gastrointestinal and kidney problems, and liver damages. The concentration of Cu obtained in present study was exceeding the limits allowed by EC (2005) and WHO (2006) which is 1 µg/g and 3 mg/kg, respectively. The highest concentration of Cu is attributed to increased boating activities, recurrent usage of antifouling paint, oil dropping from boats, electroplating, mining and commercial fishing activity in the study area (Rajeshkumar and Li, 2018; Al Rashdi et al., 2015).

Cu and Zn are essential elements enzymes (Bowen and Grant, 1997). However, they are considered as potential hazards that can endanger both animal and human health. Therefore, it is important to define their concentrations in fish with respect to nature management and seabird consumption of fish. Compare values in present study with the Canadian food standards (Cu: 100 mg/kg; Zn: 100 mg/kg), Hungarian standards (Cu: 60 mg/kg; Zn: 150 mg/kg) and the range of international standards (100 mg/kg), EC standards (Cu: 1 mg/kg), WHO standards (Cu: 3 µg/kg) and Turkish acceptable limits (Cu: 20 mg/kg; Zn: 50 mg/kg) (Papagiannis et al., 2004) and FAO/WHO (Cu: 30 mg/kg; Zn: 40 mg/kg) our values for Cu in GI and liver of fish were exceeding permissible level established by Turkey, EC (2005) and WHO (2006), whereas values of Zn were much higher than maximum permissible level established by international guidance. The values of Zn s obtained in the present study was almost same as those reported by Kamal et al. (2015) for *E. areolatus* and *P.*

maculatus from Arabian Gulf and higher than those reported by Galatchi et al. (2017) for anchovy from Romanian Black Sea coast (Table 32) (Galaţchi et al., 2017).

Table 31: Heavy metal concentrations in anchovy (Romanian Black Sea coast) tissue in 2010
Source: Galaţchi et al., 2017

Station	**Cd (0.3 µg/g)**[4]	**Pb (0.3) µg/g**	**Ni**	**Cr**	**Cu**
Cape Midia	0.52±0.07	1.62±0.24	1.13±0.16	1.62±0.27	5.24±0.78
Mamaia	0.35±0.05	1.03±0.17	0.13±0.02	0.32±0.05	5.76±1.03
Costineshti	0.29±0.04	0.35±0.06	0.27±0.04	0.44±0.06	3.96±0.59
2 Mai	0.31±0.05	0.34±0.05	0.21±0.03	0.45±0.08	4.29±0.72
VamaVeche	0.32±0.04	0.29±0.04	0.21±0.03	0.19±0.02	3.63±0.65

Liver and GI tract showed a wide range of cadmium (Cd) concentrations in *Stolephorus indicus* (5.6 mg/kg and 3.8 mg/kg, respectively), a low Cd concentration in muscle (0.11 mg/kg). Concentration of Cd in liver and GI tract was exceeding the limit proposed by EC (2005) and FAO (1983). However, the most concerning part of fish is muscle, since it is edible part consumed by humans and concentration of Cd was exceeding the limit proposed by EC (2005) and FAO (1983). These values are found to be higher than those reported earlier by El-Moselhy et al. (2014) for *Epinephelus sp*. from Suez, by Mendil et al. (2005), by Yilmaz et. al. (2007), by Yilmaz et. al. (2010) in fish from Turkish Lakes and by Galaţchi et al. (2017) in anchovy from Romanian Black Sea coast (Table 32). Cd is a serious contaminant, a highly toxic element, which is transported in the air. The maximum admissible value for fish is 0.05 µg/kg (FAO (1983)). Mormede and Davies (2001) suggested that the liver was the target organ and marine fishes, mammals, and marine birds tend to accumulate a

[4] EC Regulation No. 1881/2006 (amended in 2007, 2008, 2010)

large amount of Hg, Cd, Ag in their liver, as they occupy the highest trophic position in the marine food web (Mormede and Davies, 2001; Thompson, 1990; Wagemann and Muir, 1984). They can impact the reproductive output or even cause death, and in this sense, metal constitute a serious threat to the survival of seabird populations (Sanpera et al., 2007). According to the reports, Cd concentration in fishes from the Iranian coast near Bandar Abbas, including Qeshm Island, Bushehr city, and the area of the Shatt Al-Arab and the Musa Estuary were exceeding maximum permissible limits (Cunningham et al., 2019). The source of this Cd enrichment in Indian anchovy is still conjectural, but mainly Cd is coming from mining, industrial wastes, water pipes and electroplating plants (Al Rashdi et al., 2015). However, monitoring should focus on the industrial facilities associated with combustion of fossil fuels (power plants, waste incineration and disposal sites, manufacturing plants for phosphate fertilizers and smelting operation with non-ferrous metals such as Cu, Pb and Zn) and production of consumer products such as batteries, pigments and as a stabilizer in plastics (Cunningham et al., 2019).

Chromium (Cr) is considered as a heavy metal and pollutant of particular concern, but at the same time as a microelement and its biologically usable form plays an essential role in glucose metabolism. The mean concentration of Cr presents in this study between 4.51 mg/kg and 16.2 mg/kg in the tissues of *Stolephorus indicus*, with the highest concentration in the liver. The value of Cr was higher compared to those reported by De Mora et al. (2004) for fish species from UAE and by Rajeshkumar and Li (2018) for *C. carpio* from the Meiling Bay, China. Cr was also determined in the edible part of the cuttlefish *Sepia pharaonic* from Arabian Gulf (Freije, 2015). Cr is widely used in industrial applications and existing in different oxidation states, Cr has different biological effects on different species. The first 10 m of soil in a coastal area

of UAE is dominated with high levels of Cr and Fe arising from naturally occurring mixed metal oxides of Fe and Cr. Cr (VI) itself is a highly active carcinogen. Cr (VI) can be reduced to Cr (III) which is 500–1000 times less active in living cells because of its poor uptake (Costa, 2003). Cr, As and Ni are hazardous elements notified by the USFDA (1993) even though not covered by USFDA regulations for fish and other aquatic products (US, 1993). Cr was detected in almost all the samples, with the highest concentration (16.2 mg/kg, the average of Cr within three study area) in liver of Stolephorus indicus, but the values were exceeding the acceptable limit proposed by EC (2005) and WHO (1983). Thus, because Cr level is exceeding allowable international standard in the present study and the danger that Cr might expose to, it is highly recommended to find and control the source of Cr in the Arabian Gulf.

The metals Fe, Zn, Cu, Cr and Cd exceeded the maximum permissible limit recommended by international guidelines because of uncontrolled anthropogenic activities takes place in the study area, in addition to its natural presence in high concentration for some metals. These metals can be released to Arabian Gulf from large scale industries such as textile, detergent, paint, oil and gas production, plastic industries, etc. The anthropogenic sources also include dredging and reclamation, sewage effluents, hypersaline water discharges from desalination plants, waste disposal. The large amount of textile, detergent and oil and gas industry are located in Ajman and Sharjah, and uncontrolled discharge of their wastewater to the environment, subsequently release many organic pollutants and heavy metals (Cd, Cu, Zn, Ni and Pb) to Arabian Gulf (Aonghusa and Gray, 2002; Sarker et al., 2015; Sungur and Gülmez, 2015).

Nickel (Ni) contents were detected at the higher concentration in the liver and GI tract in the present study at the concentration ranging between 28.1-57.3 mg/kg and

10.8-24.3 mg/kg, respectively. However, the concentration of Ni didn`t exceed the maximum permissible limit established by international guidelines. Karadede and Unlu (2000) did not determine Ni in some fish samples from Ataturk Dam Lake. Ni values were also reported as 1.2-3.4 mg/kg in fish from lakes of Tokat and 0.56-1.06 mg/kg in *Silurus triostegus* and *Liza abu* from Ataturk Dam Lake (Karadede et al., 2004; Mendil et al., 2005), which are close to our results. Ni in the muscle from present study are also found to be almost close to those reported earlier by Kumar Sarkar et al. (2013) in fish from Southeast Coast of India, by Yilmaz et. al (2010) in fish from Iskenderun Bay, Turkey and by Galatchi et al. (2017) in anchovy from Romanian Black Sea coast (Table 31) and were higher than those reported in fishes from the Atlantic (Galaţchi et al., 2017; Kumar Sarkar et al., 2013; Usero et al., 2005).

The range of total mercury (Hg) levels in Indian anchovy to be between 0.36 µg/kg and 1.32 mg/kg, which were in the acceptable limit proposed by international standards. The level of Hg in our study was considerably less than in several marine fish species from the South China Sea reported by Hajeb et al. (2009) and by Saei-Dehkordi et al. (2010) in several commercially valuable fish species from the Arabian Gulf (Hajeb et al., 2009; Saei-Dehkordi et al., 2010). Concentration of mercury in the Arabian Gulf can increase due to discharge from hydroelectric, mining, pulp, and paper industries. Incineration of municipal and medical waste and emissions from coal-using power plants also contribute to high levels of mercury (Li et al., 2015). However, long-term monitoring of Hg should remain a high priority given the small size of the Arabian Gulf and a tendency to accumulate diverse pollutants in the system.

The mean concentration of vanadium (V) ranged from 0.5-27 mg/kg and 3.4-8 mg/kg, in liver and GI tract, respectively. The concentrations of V were found to be higher in Ajman and Umm Al Quwain. V in the muscle from present study was found

to be less than those reported earlier by Fard et al. (2015) in belanger's croaker *(Johnius belangerii)* fish from the Arabian Gulf and comparable to those reported by Pourang (2004, 2005) in four species of fishes in the Arabian Gulf (Fard et al., 2015; Pourang et al., 2005, 2004). This is also consistent with low concentrations in sediments in Umm Al Quwain's Siniya Island (Ksiksi et al., 2015) and relatively higher concentrations in sediments in Sharjah (Samara et al., 2016). The high V accumulation observed in samples in the present study may have resulted primarily from the pollution caused by petrochemical industrial plants and crude oil transportation activities, combustion of fossil fuels and the residual fuel oil in this region (Monikh et al., 2011). Identifying the association between the concentrations in sediments and in fish tissue will be important in future studies.

Thus, fish may accumulate heavy metals through direct absorption or via their food chain and pass them to seabirds (Socotra Cormorant) and mammals, by consumption, causing chronic or acute disorder. The result in the present study is creating an assumption that non-essential metals that exceed maximum permissible limits can be the main factor affecting the population of seabirds, specifically Socotra cormorant and it will continue to be problematic to the marine biodiversity. Especially, a cationic form of heavy metals is dangerous to living organisms because of their capacity to bind with short carbon chains. Therefore, numerous reports describe metal residues in wild fish from marine species (Canli and Atli, 2003; Mormede and Davies, 2001; Roméo et al., 1999; Storelli et al., 2008). The further research is recommended to find a relationship between heavy metals concentration and their effect on seabirds and marine organisms.

Usually, liver and muscles of marine organism is most exposed part since they are protein-rich tissues (Freije, 2015). In general, the concentration differences

were very significant between muscle, GI tract and liver of *Stolephorus indicus*. The muscle is an important site for the entry of the heavy metals and is the first target organ for exposure in fish. The concentration of metals in the muscle usually reflects the level of the metal in waters where *Stolephorus indicus* live, whereas the concentration in the liver represents the storage of metals. Moreover, the statistical similarity of specific heavy metals in the muscle of *Stolephorus indicus* was found between Ajman and Umm Al Quwain. This may be related to the pollution ratios of the study areas.

Regarding the geographical variation of metals, it was found in the present study that Ajman, Sharjah and Umm Al Quwain could be discriminated based on some heavy metals in liver, GI tract and muscle of Stolephorus indicus ($p \leq 0.001$). Specifically, Na and V in muscle were having strong ability to discriminate between Ajman and Sharjah and K, Sr and P ability to discriminate between Sharjah and Umm Al Quwain. Cd in GI tract of fish was having strong ability to discriminate between Ajman and Sharjah, Mn to discriminate between Ajman and Umm Al Quwain and Hg to discriminate between Sharjah and Umm Al Quwain. The results for liver were slightly different. Hg and S were having strong ability to discriminate between Sharjah and Umm Al Quwain, while K to discriminate between Ajman and Umm Al Quwain, and Na to discriminate between Ajman and Sharjah. Overall, K had the highest discriminant ability in discerning Sharjah from Umm Al Quwain, while for GI tract attributes, Mn had the highest discriminant ability in discerning Umm Al Quwain from Ajman. In terms of liver attribute, Hg had the highest discriminant ability in discerning Sharjah from Umm Al Quwain. Thus, muscle, GI tract and liver characteristics were collectively important in distinguishing areas selected for the feeding area of Socotra Cormorant. Notably limited amount of heavy metals had strong discriminating ability whereas other heavy metals variables did not show clear patterns (data is shown).

The present study also shows that the variation in biomass can be explained by the differences in Al concentration in the muscle and liver of *Stolephorus indicus*. Only a few studies of Al effects on marine organisms have been reported, and our understanding of the role of Al in marine biogeochemistry is limited. (Iwasaki et al., 2009) results suggest, that heavy metal pollution has a negative effect on potential food availability for drift-feeding fish and indirect effects through reduction of food, and that the direct effects should be taken into account for ecological risk assessment of heavy metal pollution on fish populations that largely depend on drifting macroinvertebrates (Iwasaki et al., 2009). Levin (1984) noted that such indirect effects should be considered in ecological risk assessment for chemicals (Levin, 1984).

Chapter 5: Conclusion

The metal and non-metal levels in the muscle, liver and GI tract of *Stolephorus indicus* from the three study locations (Ajman, Sharjah and Umm Al Quwain) in the Arabian Gulf have been investigated. Metal concentrations in *Stolephorus indicus* from Umm Al Quwain was lower compare to Ajman and Sharjah, which can be considered as hotspots. These hotspots were identified in localized areas influenced by textile millers from the textile industry, wastewaters discharged from detergent industries, oil pollution from refiners and dredging and reclamation activities. Thereby, industrial activities and the discharge of municipal wastewater should be controlled and monitored and regular monitoring of heavy metal levels in fish species is necessary to prevent health risks and nutritional safety to seabirds and humans.

The significant differences were identified among muscle, liver, and GI tract of the *Stolephorus indicus* in light of the bioaccumulation of the selected heavy metals in the Arabian Gulf. The metals Fe, Zn, Cu, Cr and Cd in liver and GI were exceeding the maximum permissible limit recommended by international guidelines. Cd was also found in the edible part (muscle) of *Stolephorus indicus* from Ajman, Sharjah and Umm Al Quwain with the concentration slightly exceeding the maximum permissible level established by EC (2005) and FAO (1983). Even though the liver and GI tract of fish are seldom consumed by humans, they are mostly consumed by seabirds and other marine organism, which represents great danger to them. The very high Cd concentration in the liver of *Stolephorus indicus* may result from food-chain bioaccumulation of elevated Cd concentration brought into the productive surface waters by direct discharge of municipal wastewater or by prominent upwelling in the region. Thus, the level of Cd in *Stolephorus indicus* should be continuously monitored

in potential polluted areas since it showed a tendency to accumulate Cd in the muscle from polluted water. However, information available on the levels of heavy metals in fish species is generally patchy and does not provide spatial and temporal distribution of metals in the Arabian Gulf. Overall, this study provides important information on the contamination status of *Stolephorus indicus* from the three study sites and possibility of importance of monitoring these types of sites which are frequently visited for feeding by Socotra Cormorant.

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Appendix 1

Metal and Non-metal Analysis in Muscle

Descriptive statistic was performed for each predictor variables to see mean, standard error, standard deviation, maximum, minimum, interquartile range and skewness of the variables depending on sampling sites. Any data from this appendix may be used only after explicit written permission is obtained from Nuray Alizada (nuray.aalizada@gmail.com) and Sabir Bin Muzaffar (s_muzaffar@uaeu.ac.ae).

Table 32: Overview of descriptive statistic for Cd in muscle

	Location				Std. Error
Cd	Ajman	Mean		.1191	.01396
		95% Confidence Interval for Mean	Lower Bound	.0906	
			Upper Bound	.1475	
		5% Trimmed Mean		.1093	
		Median		.0890	
		Variance		.006	
		Std. Deviation		.08021	
		Minimum		.03	
		Maximum		.41	
		Range		.38	
		Interquartile Range		.06	
		Skewness		2.321	.409
		Kurtosis		5.759	.798
	Sharjah	Mean		.0809	.01029
		95% Confidence Interval for Mean	Lower Bound	.0598	
			Upper Bound	.1020	
		5% Trimmed Mean		.0746	
		Median		.0675	
		Variance		.003	
		Std. Deviation		.05443	
		Minimum		.02	
		Maximum		.25	
		Range		.23	
		Interquartile Range		.03	
		Skewness		2.192	.441
		Kurtosis		4.930	.858
	Umm Al Quwain	Mean		.1186	.01519
		95% Confidence Interval for Mean	Lower Bound	.0875	
			Upper Bound	.1497	
		5% Trimmed Mean		.1063	
		Median		.0960	
		Variance		.007	
		Std. Deviation		.08180	
		Minimum		.05	
		Maximum		.47	
		Range		.42	
		Interquartile Range		.06	
		Skewness		3.165	.434
		Kurtosis		12.211	.845

Table 33: Overview of descriptive statistic for Cr in muscle

	Location				Std. Error
Cr	Ajman	Mean		.3645	.08800
		95% Confidence Interval for Mean	Lower Bound	.1852	
			Upper Bound	.5437	
		5% Trimmed Mean		.2983	
		Median		.1430	
		Variance		.256	
		Std. Deviation		.50554	
		Minimum		.05	
		Maximum		2.09	
		Range		2.04	
		Interquartile Range		.21	
		Skewness		2.184	.409
		Kurtosis		4.044	.798
	Sharjah	Mean		.1785	.04734
		95% Confidence Interval for Mean	Lower Bound	.0814	
			Upper Bound	.2757	
		5% Trimmed Mean		.1347	
		Median		.1010	
		Variance		.063	
		Std. Deviation		.25048	
		Minimum		.04	
		Maximum		1.36	
		Range		1.32	
		Interquartile Range		.06	
		Skewness		4.237	.441
		Kurtosis		19.793	.858
	Umm Al Quwain	Mean		.1274	.01248
		95% Confidence Interval for Mean	Lower Bound	.1019	
			Upper Bound	.1530	
		5% Trimmed Mean		.1228	
		Median		.1020	
		Variance		.005	
		Std. Deviation		.06723	
		Minimum		.04	
		Maximum		.29	
		Range		.25	
		Interquartile Range		.10	
		Skewness		1.150	.434
		Kurtosis		.632	.845

Table 34: Overview of descriptive statistic for Cu in muscle

	Location				Std. Error
Cu	Ajman	Mean		1.7267	.14963
		95% Confidence Interval for Mean	Lower Bound	1.4219	
			Upper Bound	2.0314	
		5% Trimmed Mean		1.6086	
		Median		1.5200	
		Variance		.739	
		Std. Deviation		.85954	
		Minimum		.86	
		Maximum		5.16	
		Range		4.30	
		Interquartile Range		.82	
		Skewness		2.644	.409
		Kurtosis		8.448	.798
	Sharjah	Mean		1.2032	.13430
		95% Confidence Interval for Mean	Lower Bound	.9277	
			Upper Bound	1.4788	
		5% Trimmed Mean		1.0913	
		Median		1.0450	
		Variance		.505	
		Std. Deviation		.71062	
		Minimum		.65	
		Maximum		4.56	
		Range		3.91	
		Interquartile Range		.49	
		Skewness		4.146	.441
		Kurtosis		19.743	.858
	Umm Al Quwain	Mean		1.7324	.17505
		95% Confidence Interval for Mean	Lower Bound	1.3738	
			Upper Bound	2.0910	
		5% Trimmed Mean		1.5920	
		Median		1.4900	
		Variance		.889	
		Std. Deviation		.94269	
		Minimum		.93	
		Maximum		5.94	
		Range		5.01	
		Interquartile Range		.57	
		Skewness		3.454	.434
		Kurtosis		14.523	.845

Table 35: Overview of descriptive statistic for Ni in muscle

	Location				Std. Error
Ni	Ajman	Mean		.6148	.18681
		95% Confidence Interval for Mean	Lower Bound	.2343	
			Upper Bound	.9954	
		5% Trimmed Mean		.4293	
		Median		.2630	
		Variance		1.152	
		Std. Deviation		1.07313	
		Minimum		.08	
		Maximum		6.12	
		Range		6.04	
		Interquartile Range		.49	
		Skewness		4.527	.409
		Kurtosis		22.852	.798
	Sharjah	Mean		.3847	.16205
		95% Confidence Interval for Mean	Lower Bound	.0522	
			Upper Bound	.7172	
		5% Trimmed Mean		.2278	
		Median		.1605	
		Variance		.735	
		Std. Deviation		.85748	
		Minimum		.06	
		Maximum		4.70	
		Range		4.63	
		Interquartile Range		.26	
		Skewness		5.050	.441
		Kurtosis		26.211	.858
	Umm Al Quwain	Mean		.2770	.02956
		95% Confidence Interval for Mean	Lower Bound	.2164	
			Upper Bound	.3375	
		5% Trimmed Mean		.2722	
		Median		.2840	
		Variance		.025	
		Std. Deviation		.15921	
		Minimum		.04	
		Maximum		.62	
		Range		.59	
		Interquartile Range		.25	
		Skewness		.354	.434
		Kurtosis		-.683	.845

Table 36: Overview of descriptive statistic for Fe in muscle

	Location				Std. Error
Fe	Ajman	Mean		11.1018	1.36703
		95% Confidence Interval for Mean	Lower Bound	8.3173	
			Upper Bound	13.8864	
		5% Trimmed Mean		10.1518	
		Median		8.0800	
		Variance		61.670	
		Std. Deviation		7.85301	
		Minimum		3.63	
		Maximum		36.43	
		Range		32.80	
		Interquartile Range		7.83	
		Skewness		1.970	.409
		Kurtosis		3.990	.798
	Sharjah	Mean		11.1639	1.67851
		95% Confidence Interval for Mean	Lower Bound	7.7199	
			Upper Bound	14.6080	
		5% Trimmed Mean		9.7898	
		Median		8.9700	
		Variance		78.887	
		Std. Deviation		8.88185	
		Minimum		4.92	
		Maximum		51.01	
		Range		46.09	
		Interquartile Range		7.59	
		Skewness		3.576	.441
		Kurtosis		15.513	.858
	Umm Al Quwain	Mean		9.3714	.79281
		95% Confidence Interval for Mean	Lower Bound	7.7474	
			Upper Bound	10.9954	
		5% Trimmed Mean		8.9017	
		Median		8.1000	
		Variance		18.228	
		Std. Deviation		4.26944	
		Minimum		4.63	
		Maximum		25.73	
		Range		21.10	
		Interquartile Range		4.03	
		Skewness		2.255	.434
		Kurtosis		6.766	.845

Table 37: Overview of descriptive statistic for Ca in muscle

	Location				Std. Error
Ca	Ajman	Mean		2193.4303	271.96248
		95% Confidence Interval for Mean	Lower Bound	1639.4608	
			Upper Bound	2747.3998	
		5% Trimmed Mean		2103.2850	
		Median		1406.5000	
		Variance		2440798.575	
		Std. Deviation		1562.30553	
		Minimum		780.60	
		Maximum		5257.20	
		Range		4476.60	
		Interquartile Range		2545.55	
		Skewness		.985	.409
		Kurtosis		-.674	.798
	Sharjah	Mean		1236.8464	101.45743
		95% Confidence Interval for Mean	Lower Bound	1028.6730	
			Upper Bound	1445.0199	
		5% Trimmed Mean		1192.5294	
		Median		1075.8500	
		Variance		288221.073	
		Std. Deviation		536.86225	
		Minimum		585.10	
		Maximum		2728.10	
		Range		2143.00	
		Interquartile Range		399.88	
		Skewness		1.598	.441
		Kurtosis		2.019	.858
	Umm Al Quwain	Mean		1899.8172	251.34140
		95% Confidence Interval for Mean	Lower Bound	1384.9677	
			Upper Bound	2414.6668	
		5% Trimmed Mean		1703.3985	
		Median		1640.4000	
		Variance		1832002.456	
		Std. Deviation		1353.51485	
		Minimum		817.00	
		Maximum		7203.60	
		Range		6386.60	
		Interquartile Range		607.90	
		Skewness		2.736	.434
		Kurtosis		8.483	.845

Table 38: Overview of descriptive statistic for Mn in muscle

	Location				Std. Error
Mn	Ajman	Mean		.6000	.04583
		95% Confidence Interval for Mean	Lower Bound	.5067	
			Upper Bound	.6934	
		5% Trimmed Mean		.5849	
		Median		.5490	
		Variance		.069	
		Std. Deviation		.26325	
		Minimum		.24	
		Maximum		1.38	
		Range		1.14	
		Interquartile Range		.42	
		Skewness		.864	.409
		Kurtosis		.749	.798
	Sharjah	Mean		.4466	.03034
		95% Confidence Interval for Mean	Lower Bound	.3843	
			Upper Bound	.5088	
		5% Trimmed Mean		.4341	
		Median		.4140	
		Variance		.026	
		Std. Deviation		.16052	
		Minimum		.19	
		Maximum		1.01	
		Range		.82	
		Interquartile Range		.20	
		Skewness		1.593	.441
		Kurtosis		4.316	.858
	Umm Al Quwain	Mean		.5236	.03763
		95% Confidence Interval for Mean	Lower Bound	.4465	
			Upper Bound	.6007	
		5% Trimmed Mean		.5065	
		Median		.4840	
		Variance		.041	
		Std. Deviation		.20266	
		Minimum		.29	
		Maximum		1.11	
		Range		.81	
		Interquartile Range		.27	
		Skewness		1.144	.434
		Kurtosis		1.278	.845

Table 39: Overview of descriptive statistic for S in muscle

	Location				Std. Error
S	Ajman	Mean		3002.2485	114.78927
		95% Confidence Interval for Mean	Lower Bound	2768.4304	
			Upper Bound	3236.0666	
		5% Trimmed Mean		2921.6278	
		Median		2760.6000	
		Variance		434827.030	
		Std. Deviation		659.41416	
		Minimum		2445.40	
		Maximum		5187.70	
		Range		2742.30	
		Interquartile Range		537.25	
		Skewness		2.121	.409
		Kurtosis		4.277	.798
	Sharjah	Mean		2965.6857	209.15729
		95% Confidence Interval for Mean	Lower Bound	2536.5304	
			Upper Bound	3394.8410	
		5% Trimmed Mean		2774.0127	
		Median		2677.1000	
		Variance		1224909.571	
		Std. Deviation		1106.75633	
		Minimum		2263.50	
		Maximum		7946.30	
		Range		5682.80	
		Interquartile Range		433.07	
		Skewness		3.829	.441
		Kurtosis		16.135	.858
	Umm Al Quwain	Mean		3380.0000	255.12576
		95% Confidence Interval for Mean	Lower Bound	2857.3986	
			Upper Bound	3902.6014	
		5% Trimmed Mean		3158.7481	
		Median		3226.1000	
		Variance		1887585.407	
		Std. Deviation		1373.89425	
		Minimum		2377.00	
		Maximum		9421.70	
		Range		7044.70	
		Interquartile Range		1024.80	
		Skewness		3.429	.434
		Kurtosis		13.858	.845

Table 40: Overview of descriptive statistic for Sr in muscle

	Location				Std. Error
Sr	Ajman	Mean		9.9158	.90470
		95% Confidence Interval for Mean	Lower Bound	8.0729	
			Upper Bound	11.7586	
		5% Trimmed Mean		9.5795	
		Median		7.4900	
		Variance		27.010	
		Std. Deviation		5.19712	
		Minimum		4.11	
		Maximum		22.98	
		Range		18.87	
		Interquartile Range		6.87	
		Skewness		.990	.409
		Kurtosis		-.131	.798
	Sharjah	Mean		8.3846	.62467
		95% Confidence Interval for Mean	Lower Bound	7.1029	
			Upper Bound	9.6664	
		5% Trimmed Mean		8.0213	
		Median		7.1200	
		Variance		10.926	
		Std. Deviation		3.30545	
		Minimum		4.81	
		Maximum		19.76	
		Range		14.95	
		Interquartile Range		1.98	
		Skewness		2.048	.441
		Kurtosis		4.628	.858
	Umm Al Quwain	Mean		11.4790	1.03226
		95% Confidence Interval for Mean	Lower Bound	9.3645	
			Upper Bound	13.5935	
		5% Trimmed Mean		10.8610	
		Median		10.0700	
		Variance		30.901	
		Std. Deviation		5.55890	
		Minimum		5.71	
		Maximum		29.97	
		Range		24.26	
		Interquartile Range		4.59	
		Skewness		1.955	.434
		Kurtosis		4.128	.845

Table 41: Overview of descriptive statistic for V in muscle

	Location				Std. Error
V	Ajman	Mean		.2958	.02681
		95% Confidence Interval for Mean	Lower Bound	.2412	
			Upper Bound	.3504	
		5% Trimmed Mean		.2795	
		Median		.2620	
		Variance		.024	
		Std. Deviation		.15403	
		Minimum		.10	
		Maximum		.82	
		Range		.71	
		Interquartile Range		.16	
		Skewness		1.789	.409
		Kurtosis		3.859	.798
	Sharjah	Mean		.1420	.02663
		95% Confidence Interval for Mean	Lower Bound	.0874	
			Upper Bound	.1967	
		5% Trimmed Mean		.1193	
		Median		.0980	
		Variance		.020	
		Std. Deviation		.14093	
		Minimum		.05	
		Maximum		.78	
		Range		.73	
		Interquartile Range		.07	
		Skewness		3.653	.441
		Kurtosis		15.654	.858
	Umm Al Quwain	Mean		.2071	.03053
		95% Confidence Interval for Mean	Lower Bound	.1446	
			Upper Bound	.2697	
		5% Trimmed Mean		.1848	
		Median		.1620	
		Variance		.027	
		Std. Deviation		.16438	
		Minimum		.04	
		Maximum		.82	
		Range		.78	
		Interquartile Range		.10	
		Skewness		2.642	.434
		Kurtosis		7.677	.845

Table 42: Overview of descriptive statistic for Zn in muscle

	Location				Std. Error
Zn	Ajman	Mean		9.3539	.47829
		95% Confidence Interval for Mean	Lower Bound	8.3797	
			Upper Bound	10.3282	
		5% Trimmed Mean		9.1619	
		Median		8.5200	
		Variance		7.549	
		Std. Deviation		2.74755	
		Minimum		5.40	
		Maximum		17.06	
		Range		11.66	
		Interquartile Range		3.51	
		Skewness		1.160	.409
		Kurtosis		.827	.798
	Sharjah	Mean		7.1329	.48276
		95% Confidence Interval for Mean	Lower Bound	6.1423	
			Upper Bound	8.1234	
		5% Trimmed Mean		6.7756	
		Median		6.3200	
		Variance		6.526	
		Std. Deviation		2.55453	
		Minimum		4.41	
		Maximum		18.17	
		Range		13.76	
		Interquartile Range		1.19	
		Skewness		3.288	.441
		Kurtosis		13.183	.858
	Umm Al Quwain	Mean		10.2424	.68480
		95% Confidence Interval for Mean	Lower Bound	8.8397	
			Upper Bound	11.6452	
		5% Trimmed Mean		9.9103	
		Median		9.6000	
		Variance		13.600	
		Std. Deviation		3.68776	
		Minimum		5.88	
		Maximum		20.62	
		Range		14.74	
		Interquartile Range		4.94	
		Skewness		1.442	.434
		Kurtosis		2.136	.845

Table 43: Overview of descriptive statistic for Hg in muscle

	Location				Std. Error
Hg	Ajman	Mean		.6718	.02149
		95% Confidence Interval for Mean	Lower Bound	.6280	
			Upper Bound	.7156	
		5% Trimmed Mean		.6682	
		Median		.6700	
		Variance		.015	
		Std. Deviation		.12345	
		Minimum		.48	
		Maximum		.94	
		Range		.46	
		Interquartile Range		.15	
		Skewness		.429	.409
		Kurtosis		-.380	.798
	Sharjah	Mean		.7846	.02553
		95% Confidence Interval for Mean	Lower Bound	.7323	
			Upper Bound	.8370	
		5% Trimmed Mean		.7843	
		Median		.8100	
		Variance		.018	
		Std. Deviation		.13511	
		Minimum		.50	
		Maximum		1.13	
		Range		.63	
		Interquartile Range		.13	
		Skewness		-.312	.441
		Kurtosis		1.087	.858
	Umm Al Quwain	Mean		.7476	.02239
		95% Confidence Interval for Mean	Lower Bound	.7017	
			Upper Bound	.7934	
		5% Trimmed Mean		.7498	
		Median		.7800	
		Variance		.015	
		Std. Deviation		.12055	
		Minimum		.48	
		Maximum		.96	
		Range		.48	
		Interquartile Range		.19	
		Skewness		-.582	.434
		Kurtosis		-.250	.845

The boxplot was illustrated in order to see outliers for each predictor variables depending on sampling sites. Extreme outliers were pointed out with stars and potential outliers were depicted as a circle.

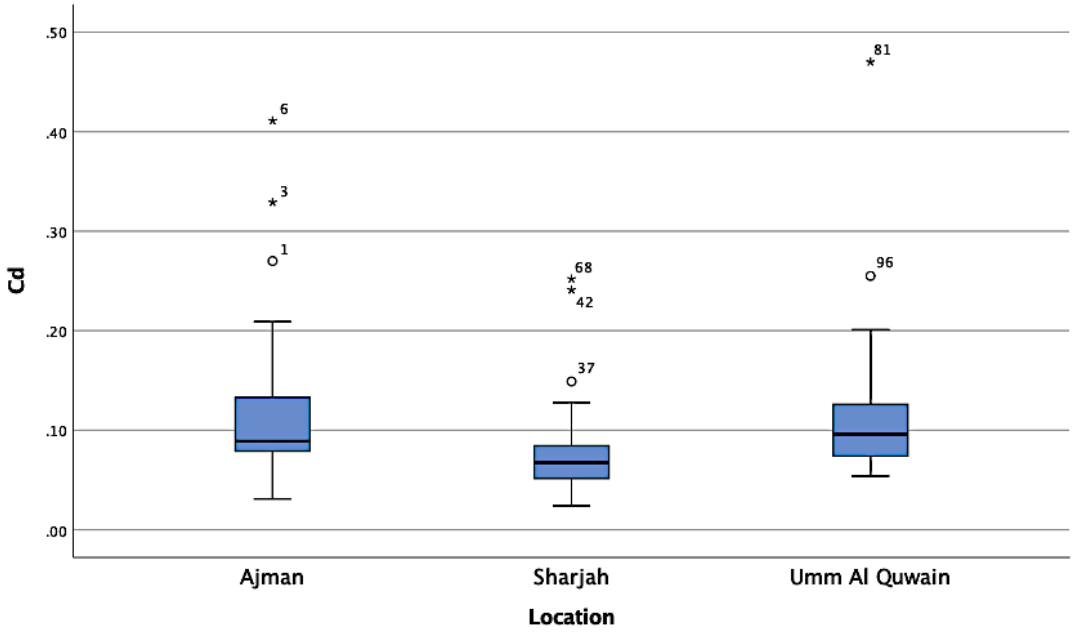

Figure 12: Representation of outliers for Cd in muscle

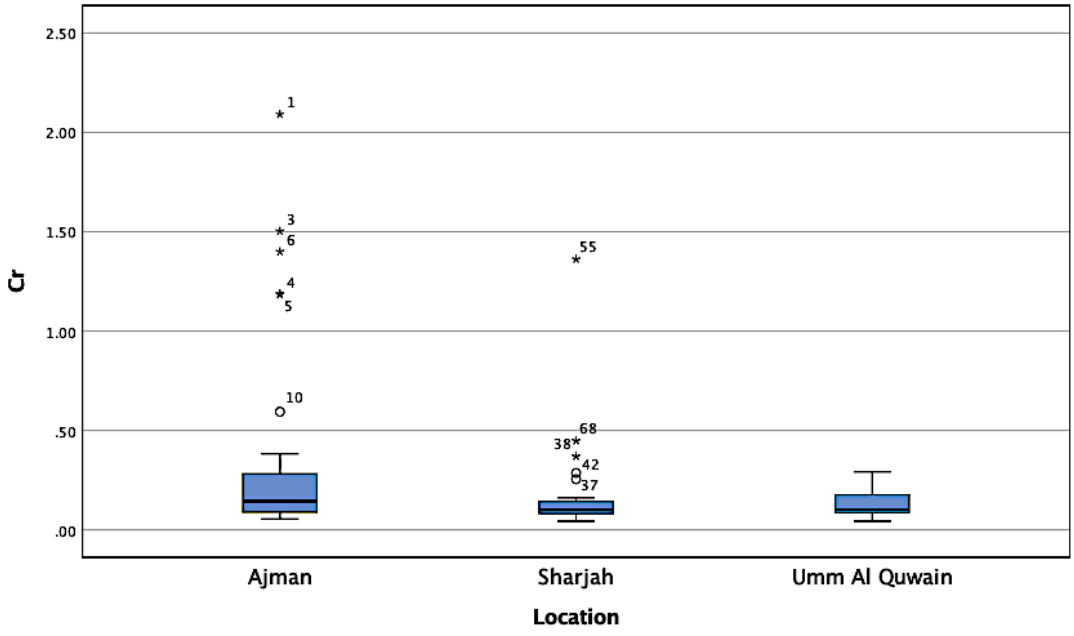

Figure 13: Representation of outliers for Cr in muscle

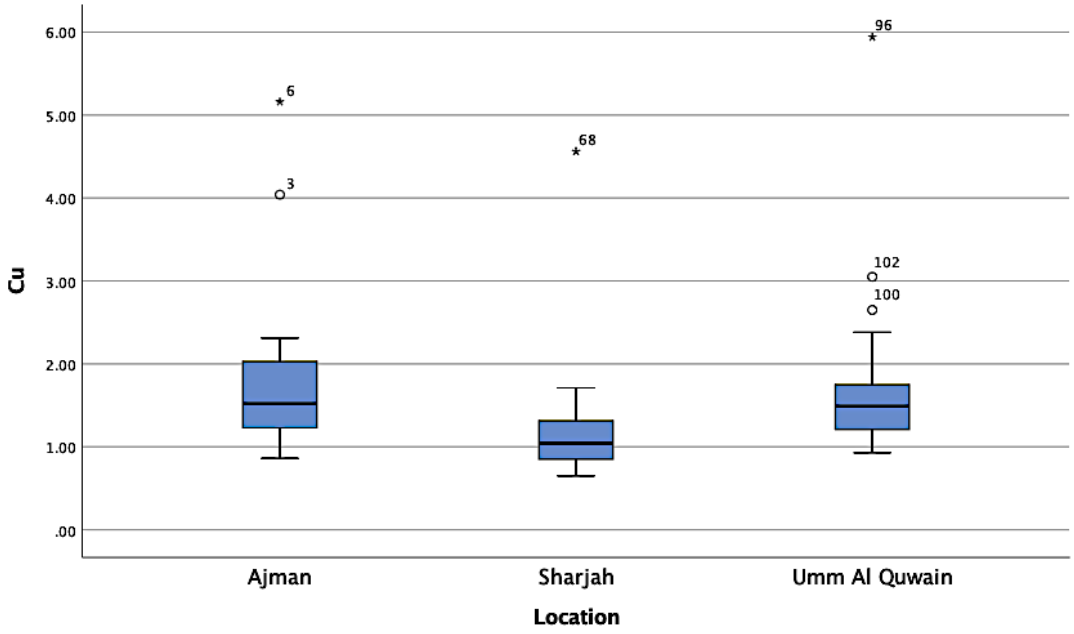

Figure 14: Representation of outliers for Cu in muscle

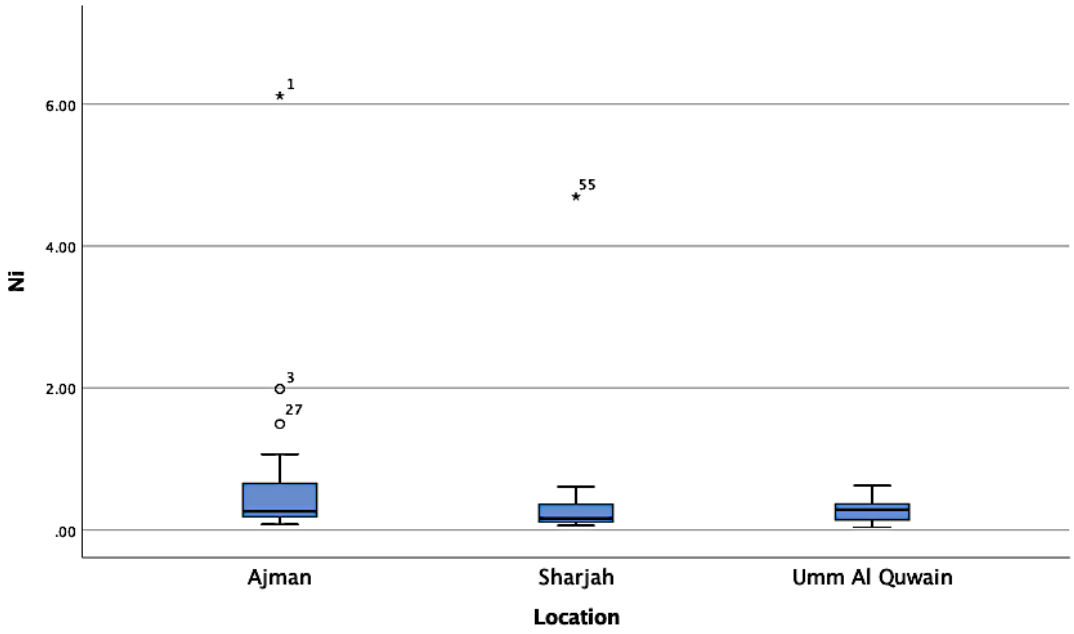

Figure 15: Representation of outliers for Ni in muscle

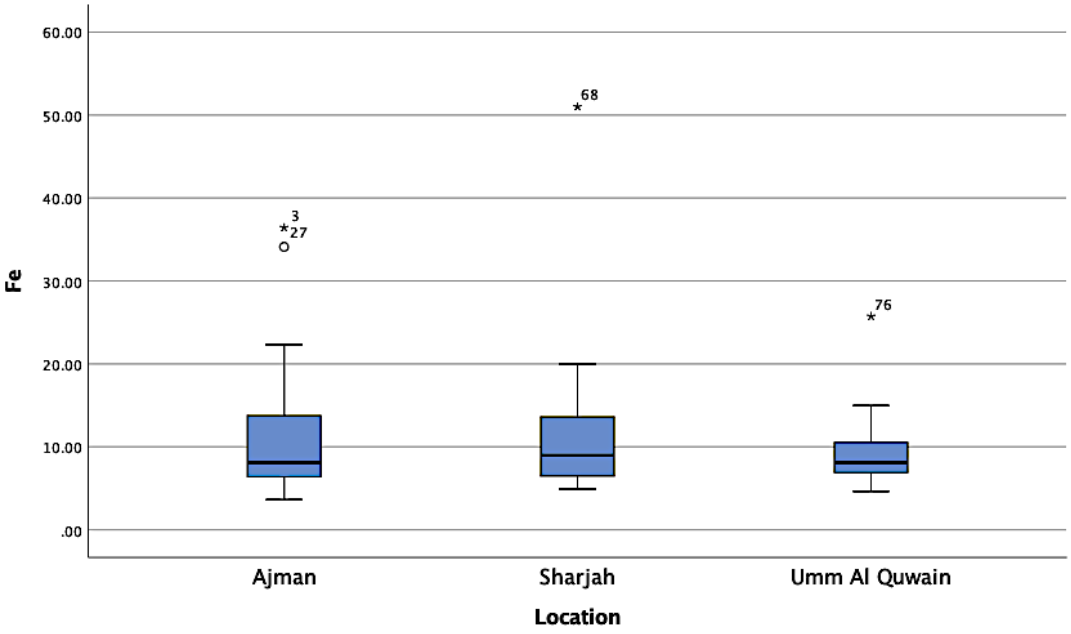

Figure 16: Representation of outliers for Fe in muscle

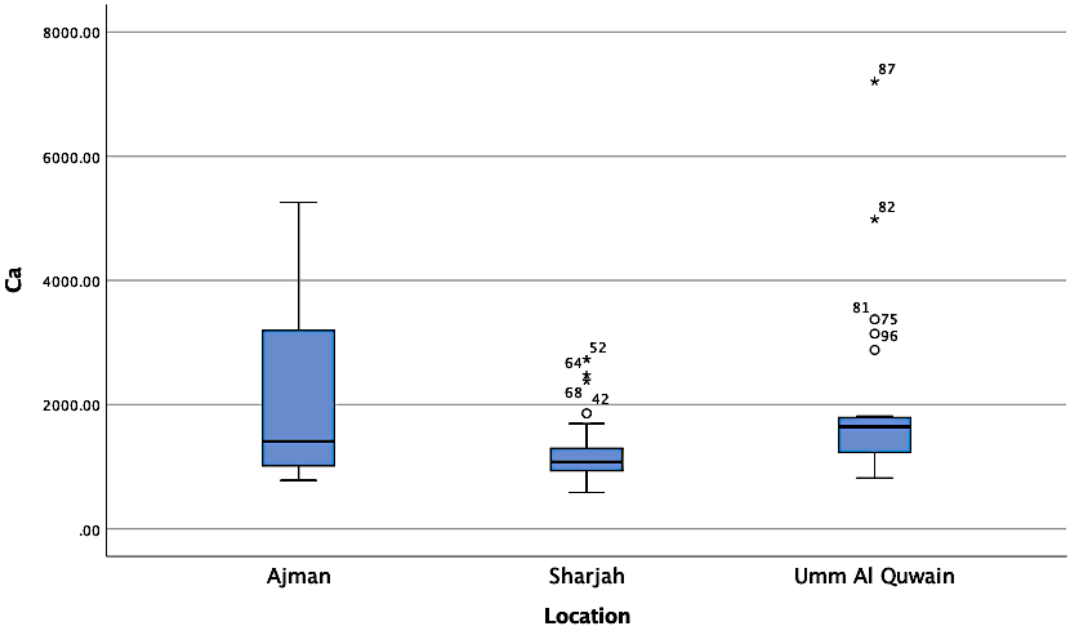

Figure 17: Representation of outliers for Ca in muscle

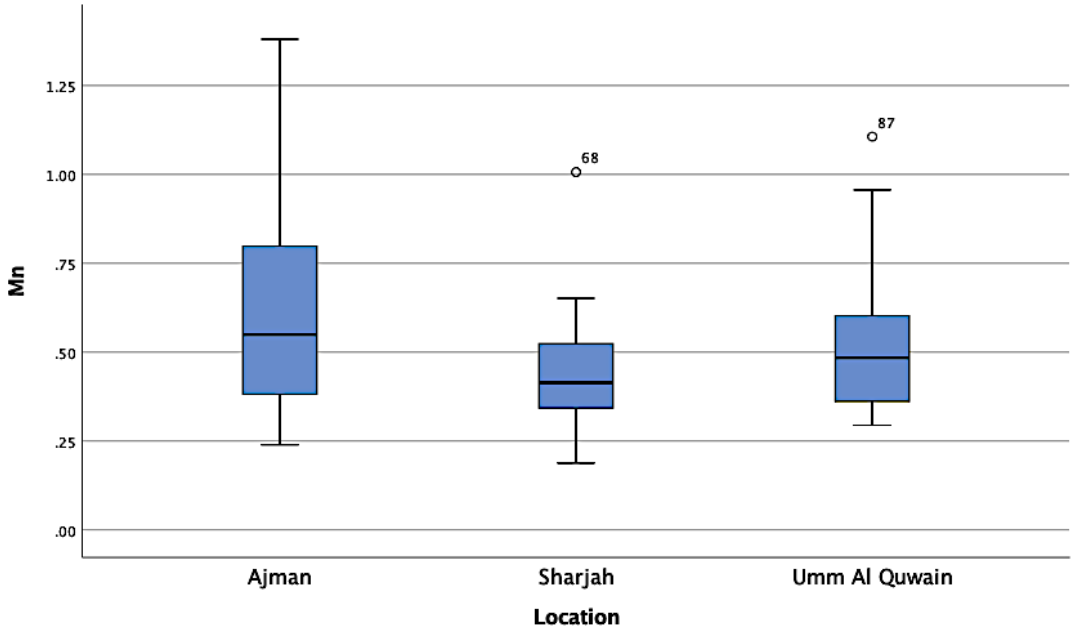

Figure 18: Representation of outliers for Mn in muscle

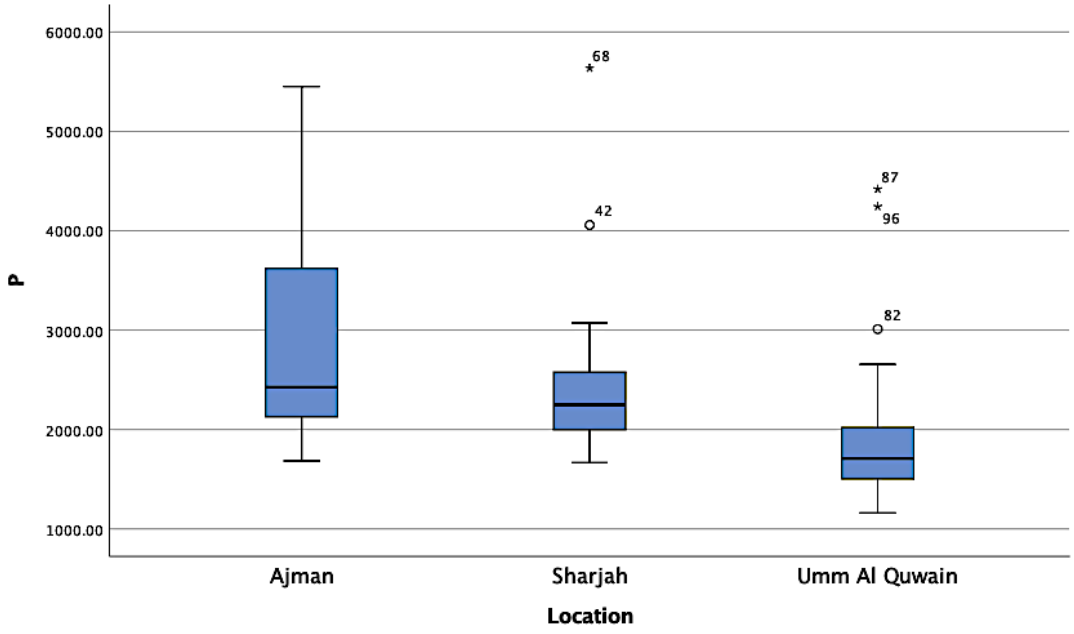

Figure 19: Representation of outliers for P in muscle

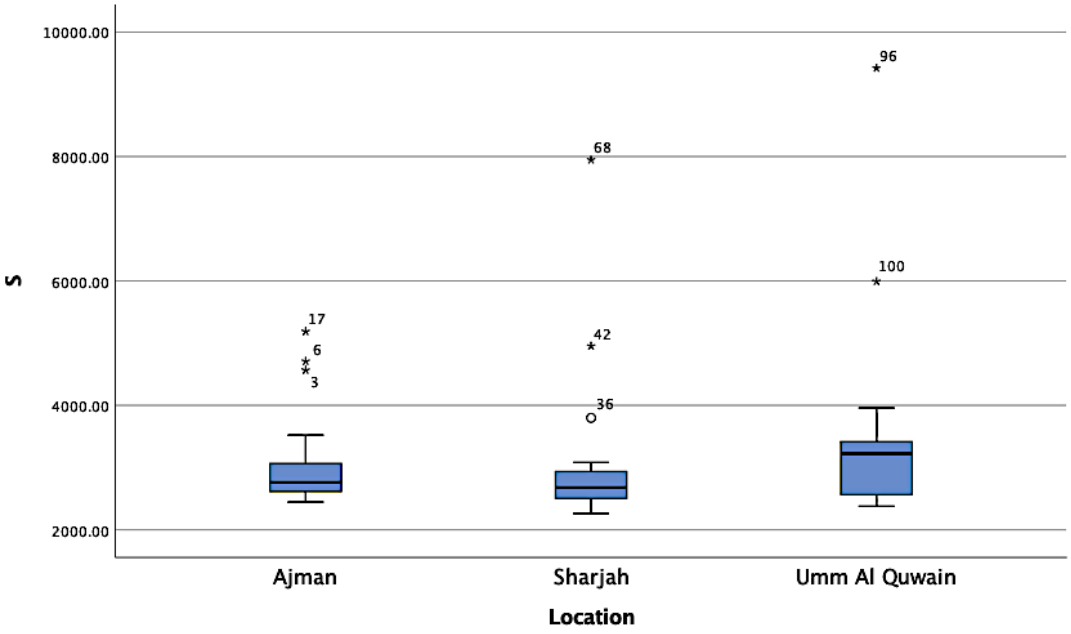

Figure 20: Representation of outliers for S in muscle

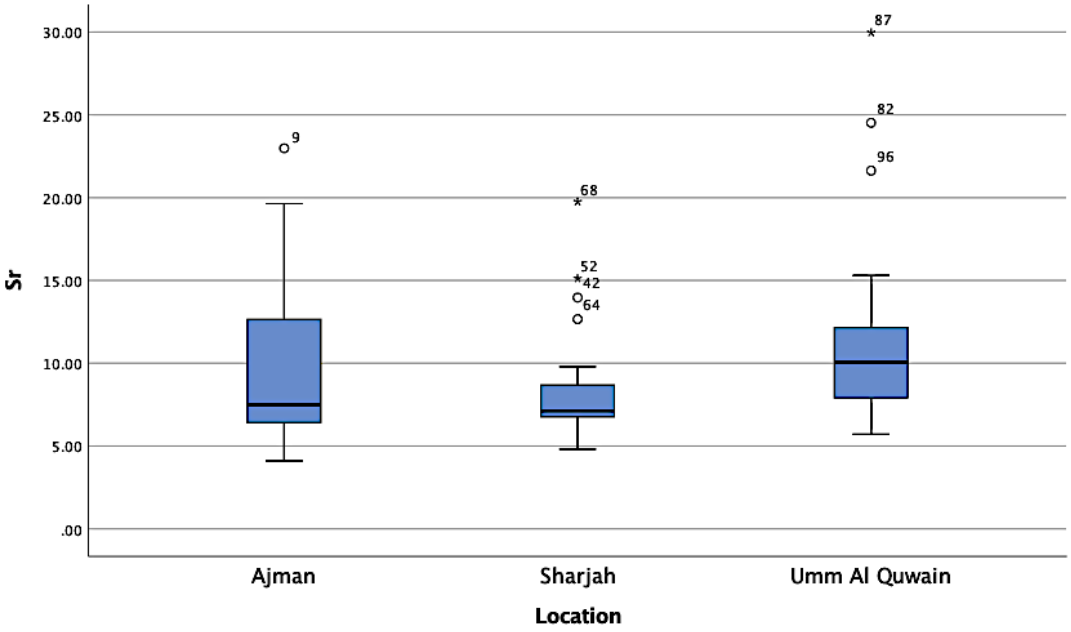

Figure 21: Representation of outliers for Sr in muscle

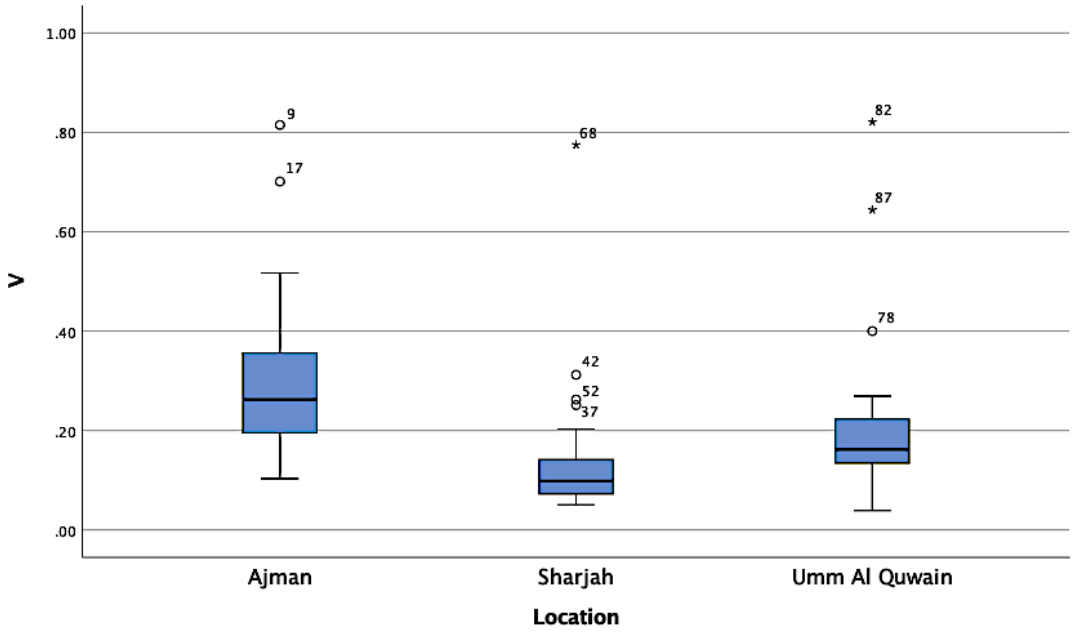

Figure 22: Representation of outliers for V in muscle

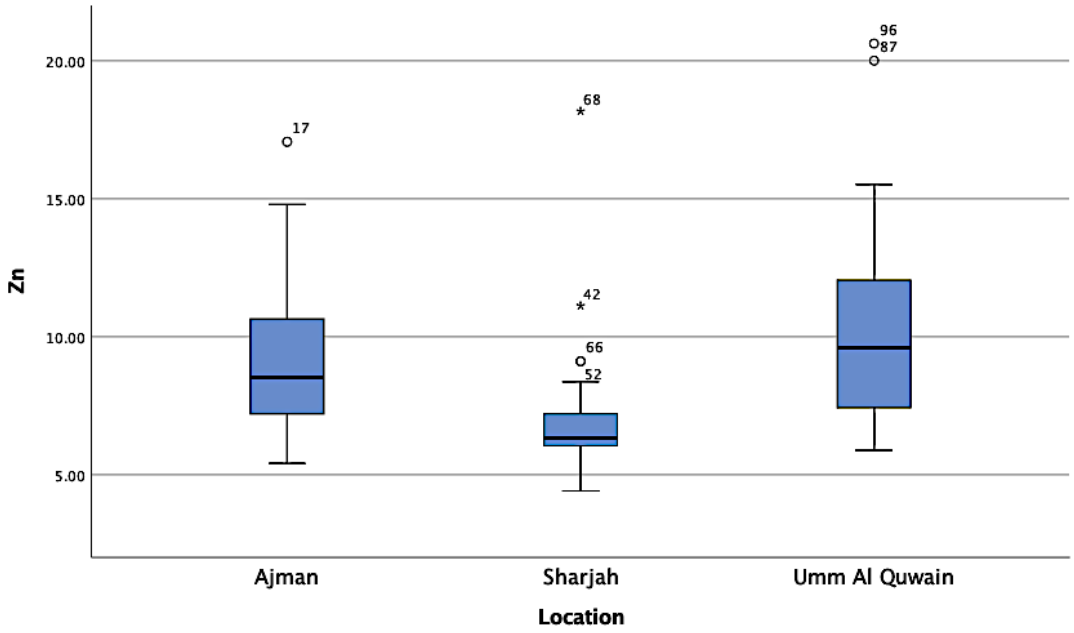

Figure 23: Representation of outliers for Zn in muscle

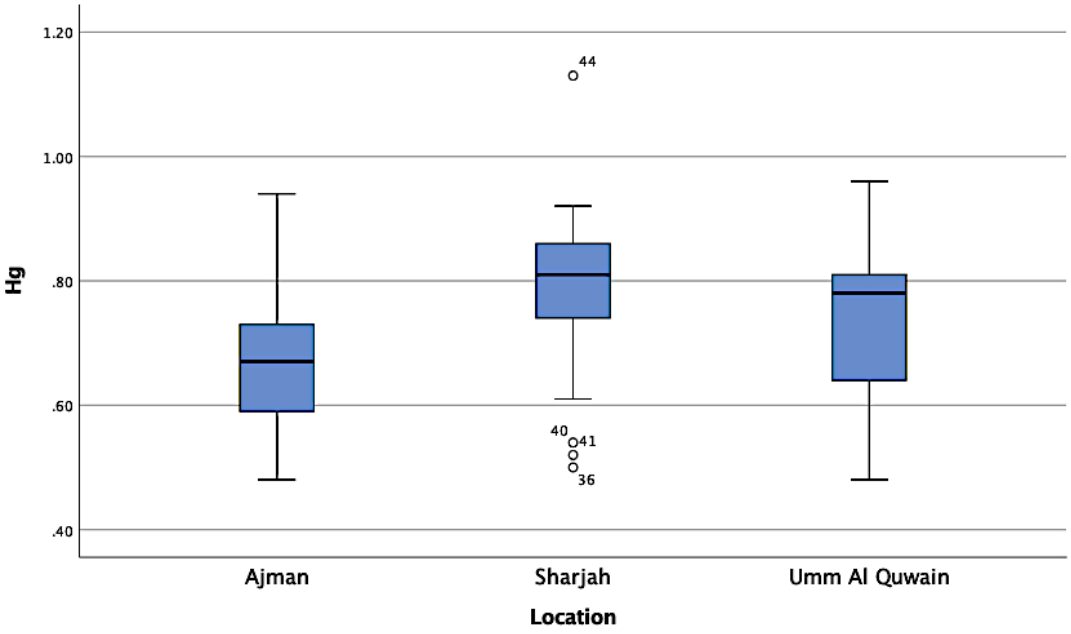

Figure 24: Representation of outliers for Hg in muscle

Appendix 2

Metal and Non-metal Analysis in GI tract

Descriptive statistic was performed for each predictor variables to see mean, standard error, standard deviation, maximum, minimum, interquartile range and skewness of the variables depending on sampling sites.

Table 44: Overview of descriptive statistic for Cd in GI tract

	Location				Std. Error
Cd	AJ	Mean		6.4266	.89213
		95% Confidence Interval for Mean	Lower Bound	4.5991	
			Upper Bound	8.2540	
		5% Trimmed Mean		5.9693	
		Median		5.0200	
		Variance		23.081	
		Std. Deviation		4.80428	
		Minimum		1.44	
		Maximum		22.18	
		Range		20.74	
		Interquartile Range		6.97	
		Skewness		1.516	.434
		Kurtosis		2.700	.845
	SH	Mean		1.6363	.32899
		95% Confidence Interval for Mean	Lower Bound	.9653	
			Upper Bound	2.3072	
		5% Trimmed Mean		1.3386	
		Median		1.1750	
		Variance		3.463	
		Std. Deviation		1.86102	
		Minimum		.04	
		Maximum		10.81	
		Range		10.77	
		Interquartile Range		.96	
		Skewness		4.176	.414
		Kurtosis		20.060	.809
	UAQ	Mean		3.2857	.92445
		95% Confidence Interval for Mean	Lower Bound	1.0237	
			Upper Bound	5.5478	
		5% Trimmed Mean		3.2402	
		Median		1.8800	
		Variance		5.982	
		Std. Deviation		2.44587	
		Minimum		.79	
		Maximum		6.60	
		Range		5.81	
		Interquartile Range		4.53	
		Skewness		.419	.794
		Kurtosis		-2.280	1.587

Table 45: Overview of descriptive statistic for Cr in GI tract

	Location				Std. Error
Cr	AJ	Mean		3.7631	1.46639
		95% Confidence Interval for Mean	Lower Bound	.7593	
			Upper Bound	6.7669	
		5% Trimmed Mean		2.2916	
		Median		1.9160	
		Variance		62.359	
		Std. Deviation		7.89676	
		Minimum		.63	
		Maximum		43.02	
		Range		42.39	
		Interquartile Range		1.80	
		Skewness		4.732	.434
		Kurtosis		23.747	.845
	SH	Mean		5.5222	3.32342
		95% Confidence Interval for Mean	Lower Bound	-1.2560	
			Upper Bound	12.3003	
		5% Trimmed Mean		2.0442	
		Median		1.3370	
		Variance		353.444	
		Std. Deviation		18.80010	
		Minimum		.07	
		Maximum		106.99	
		Range		106.93	
		Interquartile Range		1.89	
		Skewness		5.408	.414
		Kurtosis		29.926	.809
	UAQ	Mean		9.8023	2.38824
		95% Confidence Interval for Mean	Lower Bound	3.9585	
			Upper Bound	15.6461	
		5% Trimmed Mean		9.6035	
		Median		8.8240	
		Variance		39.926	
		Std. Deviation		6.31868	
		Minimum		2.81	
		Maximum		20.37	
		Range		17.56	
		Interquartile Range		10.86	
		Skewness		.899	.794
		Kurtosis		-.336	1.587

Table 46: Overview of descriptive statistic for Cu in GI tract

	Location				Std. Error
Cu	AJ	Mean		22.0252	3.10576
		95% Confidence Interval for Mean	Lower Bound	15.6633	
			Upper Bound	28.3870	
		5% Trimmed Mean		20.3244	
		Median		16.5200	
		Variance		279.726	
		Std. Deviation		16.72500	
		Minimum		4.63	
		Maximum		80.49	
		Range		75.86	
		Interquartile Range		19.28	
		Skewness		1.794	.434
		Kurtosis		4.045	.845
	SH	Mean		10.1091	1.26764
		95% Confidence Interval for Mean	Lower Bound	7.5237	
			Upper Bound	12.6944	
		5% Trimmed Mean		9.2906	
		Median		8.2950	
		Variance		51.421	
		Std. Deviation		7.17088	
		Minimum		.94	
		Maximum		36.16	
		Range		35.22	
		Interquartile Range		3.79	
		Skewness		2.407	.414
		Kurtosis		6.163	.809
	UAQ	Mean		18.6771	4.51456
		95% Confidence Interval for Mean	Lower Bound	7.6304	
			Upper Bound	29.7239	
		5% Trimmed Mean		18.1352	
		Median		15.5700	
		Variance		142.669	
		Std. Deviation		11.94442	
		Minimum		7.44	
		Maximum		39.67	
		Range		32.23	
		Interquartile Range		19.46	
		Skewness		.904	.794
		Kurtosis		-.012	1.587

Table 47: Overview of descriptive statistic for Ni in GI tract

	Location				Std. Error
Ni	AJ	Mean		10.8183	5.71666
		95% Confidence Interval for Mean	Lower Bound	-.8918	
			Upper Bound	22.5283	
		5% Trimmed Mean		5.0440	
		Median		3.5000	
		Variance		947.726	
		Std. Deviation		30.78516	
		Minimum		1.07	
		Maximum		168.60	
		Range		167.53	
		Interquartile Range		5.51	
		Skewness		5.154	.434
		Kurtosis		27.199	.845
	SH	Mean		20.7566	12.36759
		95% Confidence Interval for Mean	Lower Bound	-4.4673	
			Upper Bound	45.9804	
		5% Trimmed Mean		7.8889	
		Median		4.7100	
		Variance		4894.633	
		Std. Deviation		69.96165	
		Minimum		.39	
		Maximum		398.01	
		Range		397.62	
		Interquartile Range		6.17	
		Skewness		5.390	.414
		Kurtosis		29.799	.809
	UAQ	Mean		24.3714	5.23608
		95% Confidence Interval for Mean	Lower Bound	11.5592	
			Upper Bound	37.1837	
		5% Trimmed Mean		24.0294	
		Median		21.8500	
		Variance		191.916	
		Std. Deviation		13.85337	
		Minimum		7.56	
		Maximum		47.34	
		Range		39.78	
		Interquartile Range		24.36	
		Skewness		.716	.794
		Kurtosis		-.258	1.587

Table 48: Overview of descriptive statistic for Fe in GI tract

	Location				Std. Error
Fe	AJ	Mean		446.5621	67.64979
		95% Confidence Interval for Mean	Lower Bound	307.9877	
			Upper Bound	585.1364	
		5% Trimmed Mean		394.0718	
		Median		432.6000	
		Variance		132718.344	
		Std. Deviation		364.30529	
		Minimum		138.70	
		Maximum		1900.60	
		Range		1761.90	
		Interquartile Range		355.85	
		Skewness		2.693	.434
		Kurtosis		9.073	.845
	SH	Mean		423.3625	69.74294
		95% Confidence Interval for Mean	Lower Bound	281.1208	
			Upper Bound	565.6042	
		5% Trimmed Mean		371.3910	
		Median		283.3500	
		Variance		155650.500	
		Std. Deviation		394.52567	
		Minimum		19.70	
		Maximum		2090.30	
		Range		2070.60	
		Interquartile Range		390.15	
		Skewness		2.753	.414
		Kurtosis		9.783	.809
	UAQ	Mean		1714.4571	404.39392
		95% Confidence Interval for Mean	Lower Bound	724.9409	
			Upper Bound	2703.9734	
		5% Trimmed Mean		1684.4302	
		Median		1388.3000	
		Variance		1144741.086	
		Std. Deviation		1069.92574	
		Minimum		515.70	
		Maximum		3453.70	
		Range		2938.00	
		Interquartile Range		1874.90	
		Skewness		.908	.794
		Kurtosis		-.517	1.587

Table 49: Overview of descriptive statistic for Ca in GI tract

	Location				Std. Error
Ca	AJ	Mean		7297.9690	974.90588
		95% Confidence Interval for Mean	Lower Bound	5300.9648	
			Upper Bound	9294.9731	
		5% Trimmed Mean		6732.8358	
		Median		6473.7000	
		Variance		27562802.708	
		Std. Deviation		5250.02883	
		Minimum		1961.10	
		Maximum		23573.60	
		Range		21612.50	
		Interquartile Range		6163.50	
		Skewness		1.685	.434
		Kurtosis		3.205	.845
	SH	Mean		3487.1094	584.42280
		95% Confidence Interval for Mean	Lower Bound	2295.1712	
			Upper Bound	4679.0475	
		5% Trimmed Mean		3036.7153	
		Median		2588.8500	
		Variance		10929600.416	
		Std. Deviation		3305.99462	
		Minimum		404.80	
		Maximum		18662.50	
		Range		18257.70	
		Interquartile Range		2651.88	
		Skewness		3.328	.414
		Kurtosis		14.350	.809
	UAQ	Mean		15270.6143	3586.63095
		95% Confidence Interval for Mean	Lower Bound	6494.4445	
			Upper Bound	24046.7841	
		5% Trimmed Mean		15274.8659	
		Median		19293.5000	
		Variance		90047450.975	
		Std. Deviation		9489.33354	
		Minimum		3076.80	
		Maximum		27387.90	
		Range		24311.10	
		Interquartile Range		17536.70	
		Skewness		-.203	.794
		Kurtosis		-1.937	1.587

Table 50: Overview of descriptive statistic for Mn in GI tract

	Location				Std. Error
Mn	AJ	Mean		3.2310	.52477
		95% Confidence Interval for Mean	Lower Bound	2.1561	
			Upper Bound	4.3060	
		5% Trimmed Mean		2.8393	
		Median		2.4000	
		Variance		7.986	
		Std. Deviation		2.82599	
		Minimum		.84	
		Maximum		14.43	
		Range		13.59	
		Interquartile Range		3.12	
		Skewness		2.597	.434
		Kurtosis		8.377	.845
	SH	Mean		4.9322	.91489
		95% Confidence Interval for Mean	Lower Bound	3.0663	
			Upper Bound	6.7981	
		5% Trimmed Mean		4.2453	
		Median		3.2450	
		Variance		26.785	
		Std. Deviation		5.17542	
		Minimum		.26	
		Maximum		26.69	
		Range		26.43	
		Interquartile Range		4.58	
		Skewness		2.711	.414
		Kurtosis		9.439	.809
	UAQ	Mean		23.1700	5.49196
		95% Confidence Interval for Mean	Lower Bound	9.7317	
			Upper Bound	36.6083	
		5% Trimmed Mean		23.0167	
		Median		17.8800	
		Variance		211.131	
		Std. Deviation		14.53036	
		Minimum		5.11	
		Maximum		43.99	
		Range		38.88	
		Interquartile Range		28.85	
		Skewness		.479	.794
		Kurtosis		-1.184	1.587

Table 51: Overview of descriptive statistic for P in GI tract

	Location				Std. Error
P	AJ	Mean		4530.9966	645.48004
		95% Confidence Interval for Mean	Lower Bound	3208.7906	
			Upper Bound	5853.2025	
		5% Trimmed Mean		4095.2272	
		Median		3735.8000	
		Variance		12082690.049	
		Std. Deviation		3476.01641	
		Minimum		1206.50	
		Maximum		17120.10	
		Range		15913.60	
		Interquartile Range		3828.65	
		Skewness		2.140	.434
		Kurtosis		5.612	.845
	SH	Mean		3666.1813	440.42204
		95% Confidence Interval for Mean	Lower Bound	2767.9346	
			Upper Bound	4564.4279	
		5% Trimmed Mean		3345.7208	
		Median		2841.8500	
		Variance		6207090.420	
		Std. Deviation		2491.40330	
		Minimum		272.40	
		Maximum		14077.60	
		Range		13805.20	
		Interquartile Range		1685.05	
		Skewness		2.781	.414
		Kurtosis		9.737	.809
	UAQ	Mean		10241.3000	2296.63779
		95% Confidence Interval for Mean	Lower Bound	4621.6298	
			Upper Bound	15860.9702	
		5% Trimmed Mean		10188.4111	
		Median		13344.1000	
		Variance		36921815.820	
		Std. Deviation		6076.33243	
		Minimum		2688.30	
		Maximum		18746.30	
		Range		16058.00	
		Interquartile Range		9710.00	
		Skewness		-.048	.794
		Kurtosis		-1.709	1.587

Table 52: Overview of descriptive statistic for S in GI tract

	Location				Std. Error
S	AJ	Mean		5126.7690	677.05827
		95% Confidence Interval for Mean	Lower Bound	3739.8780	
			Upper Bound	6513.6600	
		5% Trimmed Mean		4704.8027	
		Median		4293.6000	
		Variance		13293829.234	
		Std. Deviation		3646.07038	
		Minimum		1675.10	
		Maximum		19592.90	
		Range		17917.80	
		Interquartile Range		4751.30	
		Skewness		2.290	.434
		Kurtosis		7.964	.845
	SH	Mean		3129.2031	285.31245
		95% Confidence Interval for Mean	Lower Bound	2547.3045	
			Upper Bound	3711.1017	
		5% Trimmed Mean		3012.5688	
		Median		2679.6000	
		Variance		2604902.197	
		Std. Deviation		1613.97094	
		Minimum		241.30	
		Maximum		8036.80	
		Range		7795.50	
		Interquartile Range		1518.45	
		Skewness		1.552	.414
		Kurtosis		2.948	.809
	UAQ	Mean		4856.8571	850.97241
		95% Confidence Interval for Mean	Lower Bound	2774.6027	
			Upper Bound	6939.1116	
		5% Trimmed Mean		4881.7468	
		Median		6075.9000	
		Variance		5069078.250	
		Std. Deviation		2251.46136	
		Minimum		2093.90	
		Maximum		7171.80	
		Range		5077.90	
		Interquartile Range		4190.30	
		Skewness		-.340	.794
		Kurtosis		-2.546	1.587

Table 53: Overview of descriptive statistic for Sr in GI tract

	Location				Std. Error
Sr	AJ	Mean		99.4966	14.91476
		95% Confidence Interval for Mean	Lower Bound	68.9450	
			Upper Bound	130.0481	
		5% Trimmed Mean		88.9452	
		Median		81.4000	
		Variance		6451.054	
		Std. Deviation		80.31845	
		Minimum		27.30	
		Maximum		444.20	
		Range		416.90	
		Interquartile Range		86.20	
		Skewness		2.960	.434
		Kurtosis		11.886	.845
	SH	Mean		61.0375	12.85722
		95% Confidence Interval for Mean	Lower Bound	34.8150	
			Upper Bound	87.2600	
		5% Trimmed Mean		49.8299	
		Median		44.6500	
		Variance		5289.859	
		Std. Deviation		72.73142	
		Minimum		5.10	
		Maximum		420.90	
		Range		415.80	
		Interquartile Range		46.72	
		Skewness		4.158	.414
		Kurtosis		20.288	.809
	UAQ	Mean		297.7714	105.88876
		95% Confidence Interval for Mean	Lower Bound	38.6710	
			Upper Bound	556.8719	
		5% Trimmed Mean		281.2738	
		Median		144.7000	
		Variance		78487.002	
		Std. Deviation		280.15532	
		Minimum		58.10	
		Maximum		834.40	
		Range		776.30	
		Interquartile Range		385.60	
		Skewness		1.468	.794
		Kurtosis		1.452	1.587

Table 54: Overview of descriptive statistic for Zn in GI tract

	Location				Std. Error
Zn	AJ	Mean		108.2759	12.91402
		95% Confidence Interval for Mean	Lower Bound	81.8227	
			Upper Bound	134.7290	
		5% Trimmed Mean		100.7845	
		Median		114.8000	
		Variance		4836.385	
		Std. Deviation		69.54412	
		Minimum		35.00	
		Maximum		347.40	
		Range		312.40	
		Interquartile Range		86.95	
		Skewness		1.643	.434
		Kurtosis		3.891	.845
	SH	Mean		56.3656	6.12514
		95% Confidence Interval for Mean	Lower Bound	43.8733	
			Upper Bound	68.8579	
		5% Trimmed Mean		53.4118	
		Median		44.7000	
		Variance		1200.556	
		Std. Deviation		34.64904	
		Minimum		4.70	
		Maximum		164.80	
		Range		160.10	
		Interquartile Range		26.63	
		Skewness		1.749	.414
		Kurtosis		2.935	.809
	UAQ	Mean		129.9714	30.71347
		95% Confidence Interval for Mean	Lower Bound	54.8183	
			Upper Bound	205.1246	
		5% Trimmed Mean		127.8738	
		Median		103.0000	
		Variance		6603.222	
		Std. Deviation		81.26021	
		Minimum		42.00	
		Maximum		255.70	
		Range		213.70	
		Interquartile Range		159.70	
		Skewness		.597	.794
		Kurtosis		-1.151	1.587

Table 55: Overview of descriptive statistic for Hg in GI tract

	Location				Std. Error
Hg	AJ	Mean		.4300	.00931
		95% Confidence Interval for Mean	Lower Bound	.4109	
			Upper Bound	.4491	
		5% Trimmed Mean		.4278	
		Median		.4100	
		Variance		.003	
		Std. Deviation		.05014	
		Minimum		.36	
		Maximum		.54	
		Range		.18	
		Interquartile Range		.06	
		Skewness		.862	.434
		Kurtosis		-.021	.845
	SH	Mean		.5153	.02216
		95% Confidence Interval for Mean	Lower Bound	.4701	
			Upper Bound	.5605	
		5% Trimmed Mean		.5033	
		Median		.5000	
		Variance		.016	
		Std. Deviation		.12536	
		Minimum		.36	
		Maximum		1.00	
		Range		.64	
		Interquartile Range		.17	
		Skewness		1.893	.414
		Kurtosis		6.059	.809
	UAQ	Mean		.3643	.02277
		95% Confidence Interval for Mean	Lower Bound	.3086	
			Upper Bound	.4200	
		5% Trimmed Mean		.3637	
		Median		.3400	
		Variance		.004	
		Std. Deviation		.06024	
		Minimum		.30	
		Maximum		.44	
		Range		.14	
		Interquartile Range		.12	
		Skewness		.306	.794
		Kurtosis		-2.358	1.587

Table 56: Overview of descriptive statistic for V in GI tract

	Location				Std. Error
V	AJ	Mean		3.4086	.78727
		95% Confidence Interval for Mean	Lower Bound	1.7960	
			Upper Bound	5.0213	
		5% Trimmed Mean		2.7217	
		Median		2.2900	
		Variance		17.974	
		Std. Deviation		4.23956	
		Minimum		.49	
		Maximum		24.26	
		Range		23.77	
		Interquartile Range		2.32	
		Skewness		4.518	.434
		Kurtosis		22.590	.845
	SH	Mean		1.4516	.20597
		95% Confidence Interval for Mean	Lower Bound	1.0315	
			Upper Bound	1.8716	
		5% Trimmed Mean		1.3734	
		Median		1.0450	
		Variance		1.358	
		Std. Deviation		1.16513	
		Minimum		.12	
		Maximum		4.19	
		Range		4.07	
		Interquartile Range		1.74	
		Skewness		1.030	.414
		Kurtosis		.280	.809
	UAQ	Mean		26.8286	9.94346
		95% Confidence Interval for Mean	Lower Bound	2.4978	
			Upper Bound	51.1593	
		5% Trimmed Mean		25.4662	
		Median		14.2500	
		Variance		692.106	
		Std. Deviation		26.30792	
		Minimum		4.72	
		Maximum		73.46	
		Range		68.74	
		Interquartile Range		43.25	
		Skewness		1.326	.794
		Kurtosis		.172	1.587

The boxplot was illustrated in order to see outliers for each predictor variables depending on sampling sites. Extreme outliers were pointed out with stars and potential outliers were depicted as a circle.

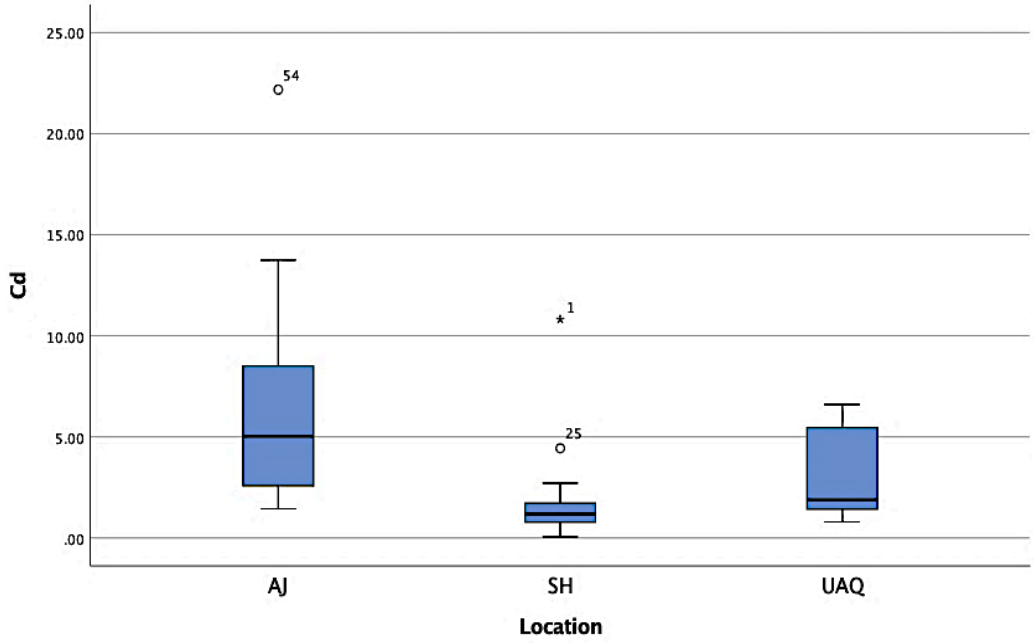

Figure 25: Representation of outliers for Cd in GI tract

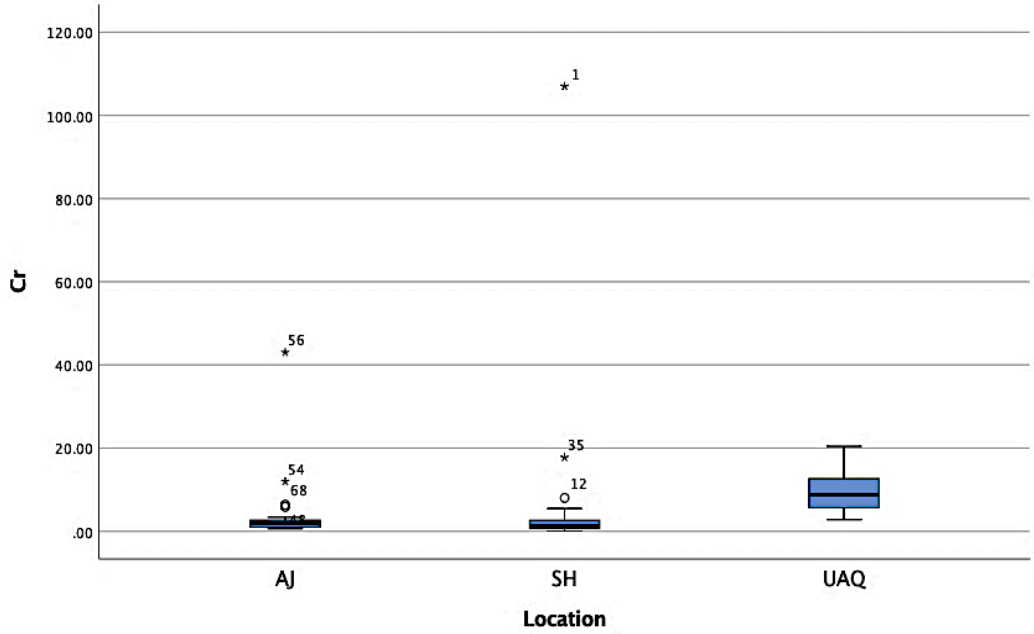

Figure 26: Representation of outliers for Cr in GI tract

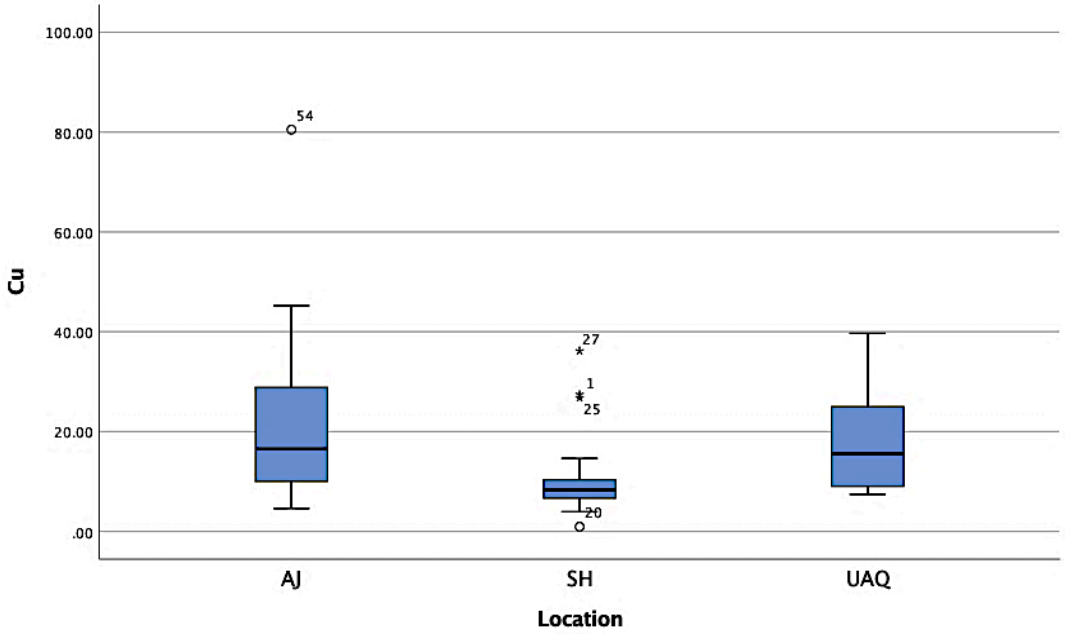

Figure 27: Representation of outliers for Cu in GI tract

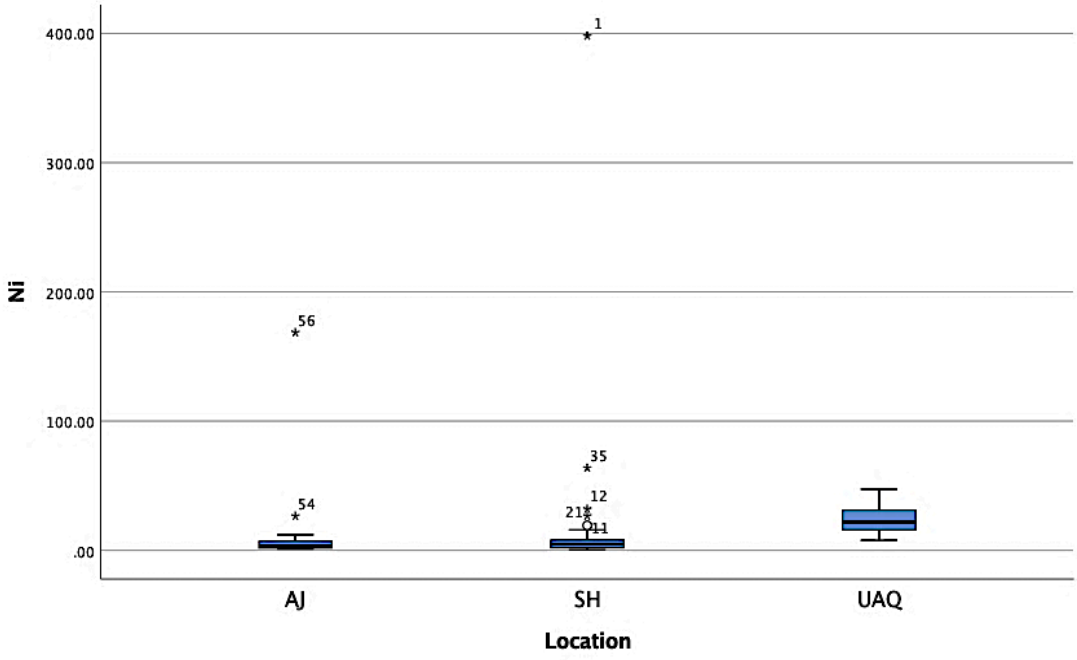

Figure 28: Representation of outliers for Ni in GI tract

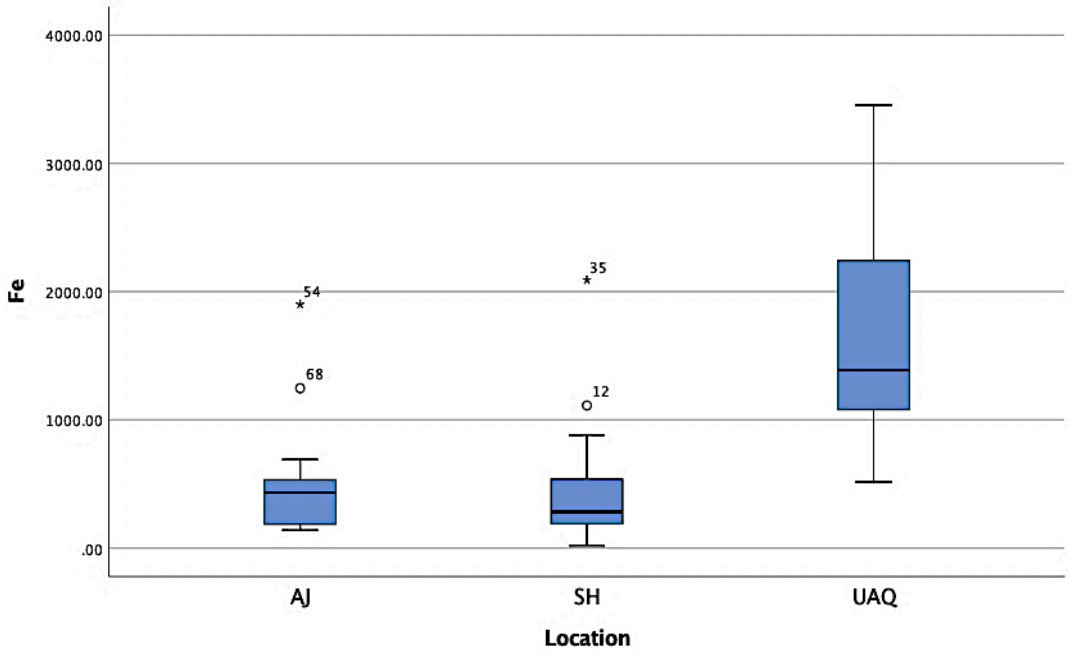

Figure 29: Representation of outliers Fe in GI tract

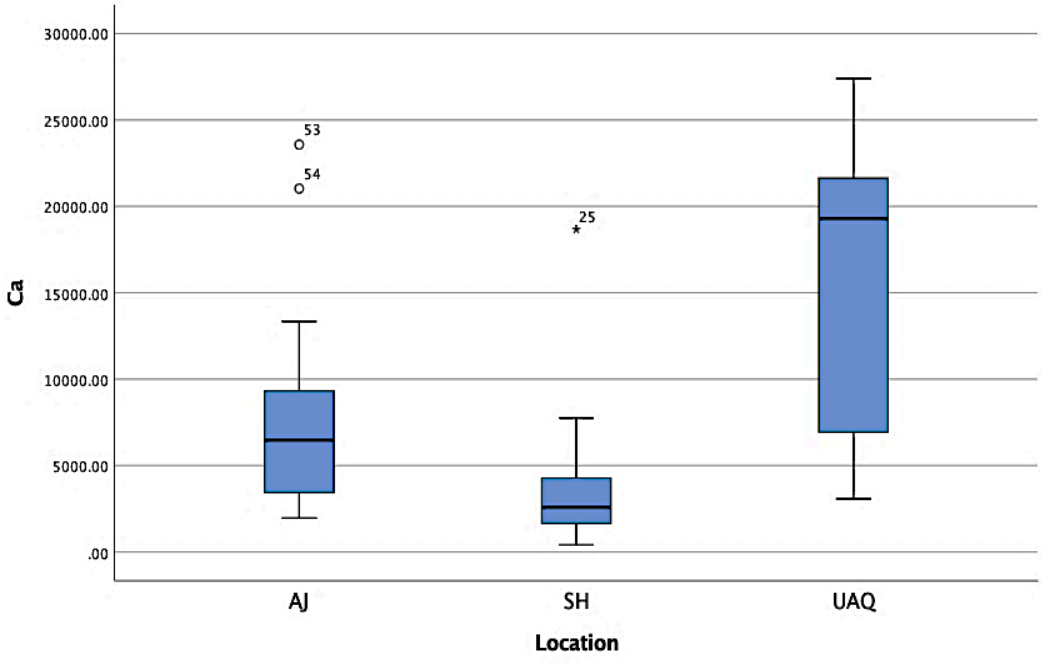

Figure 30: Representation of outliers for Ca in GI tract

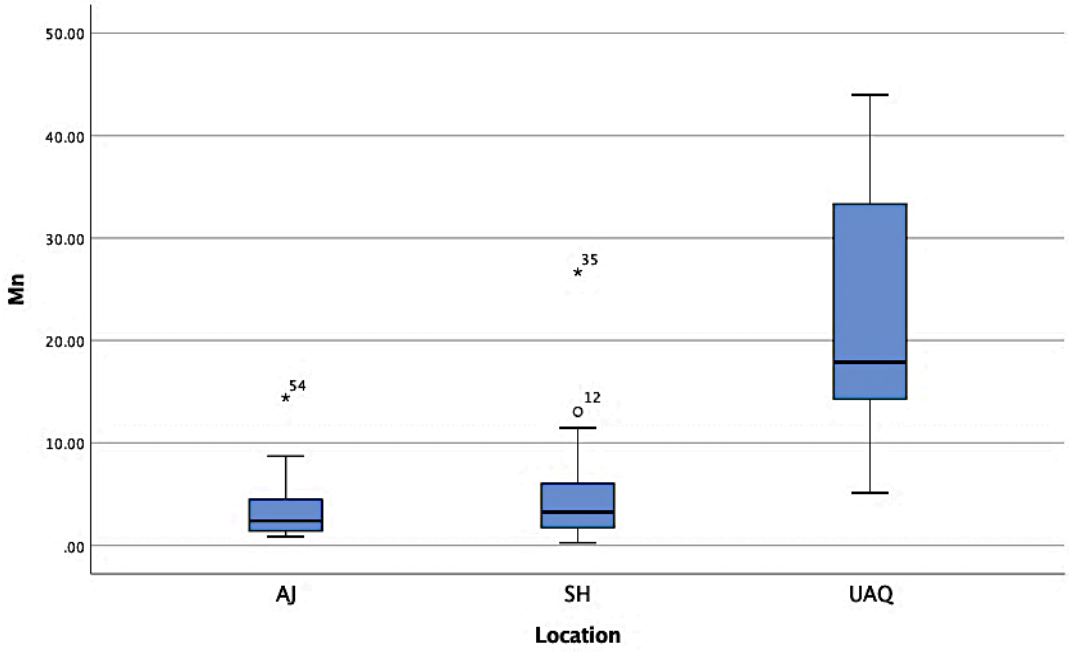

Figure 31: Representation of outliers for Mn in GI tract

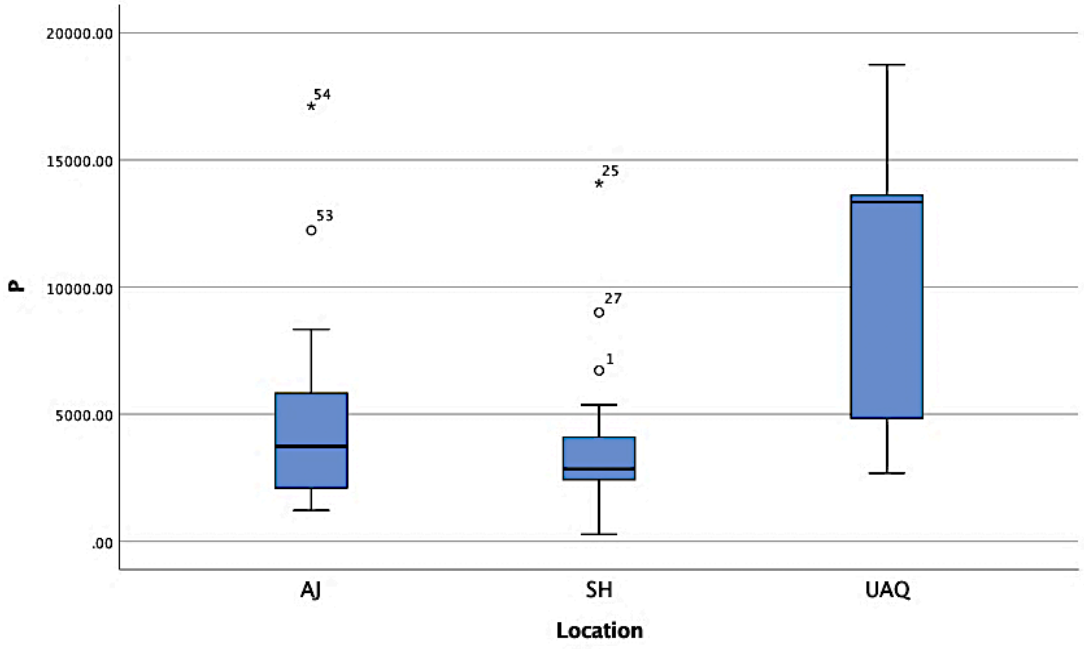

Figure 32: Representation of outliers for P in GI tract

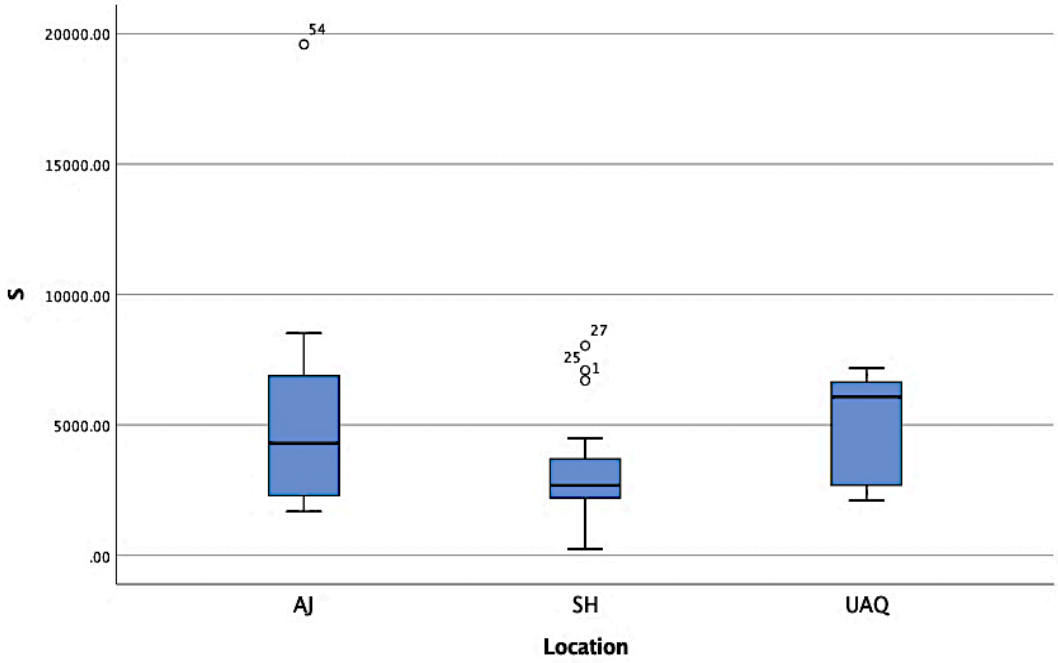

Figure 33: Representation of outliers for S in GI tract

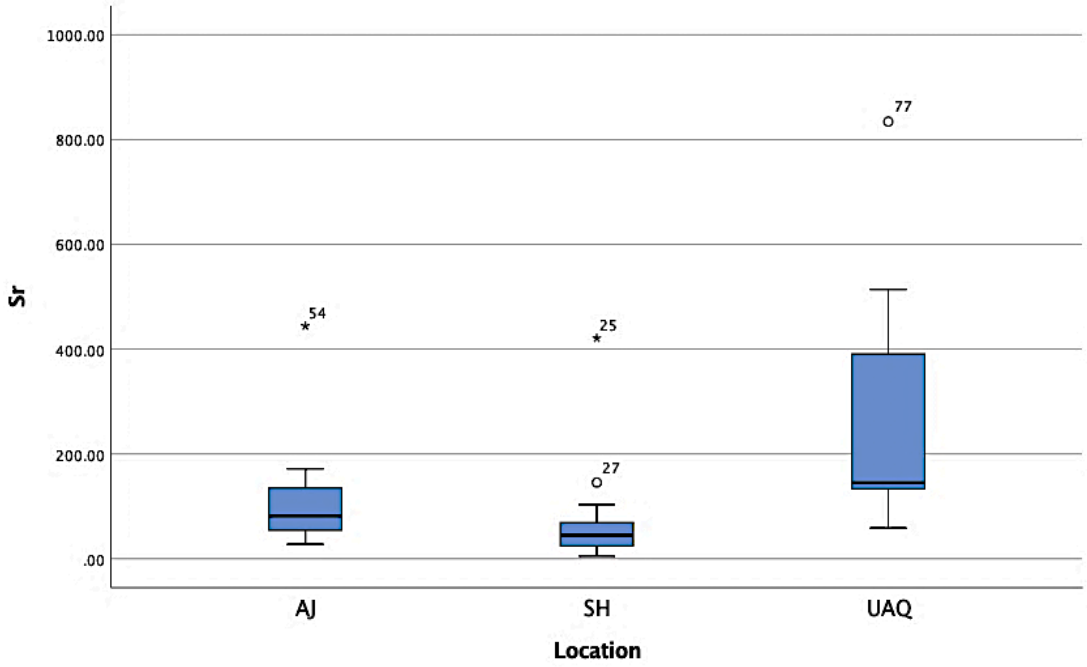

Figure 34: Representation of outliers for Sr in GI tract

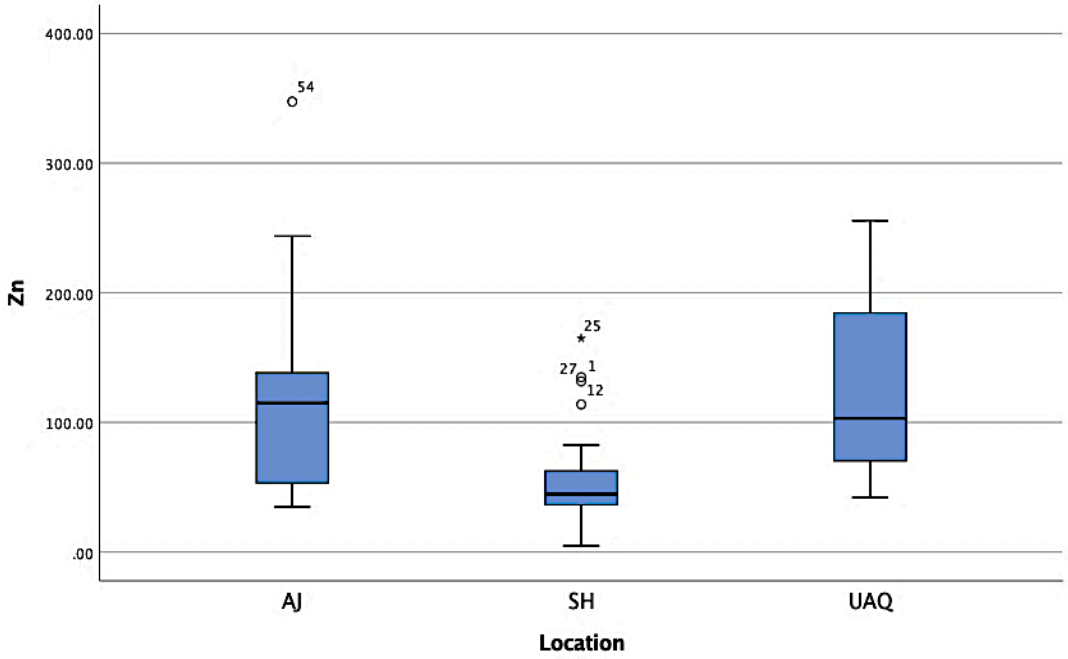

Figure 35: Representation of outliers for Zn in GI tract

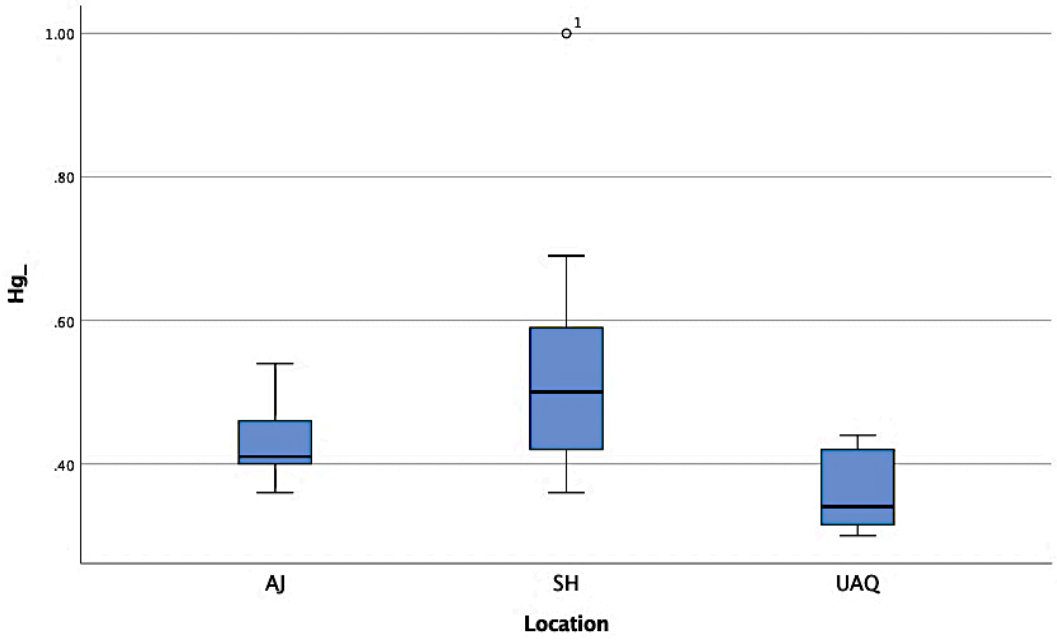

Figure 36: Representation of outliers for Hg in GI tract

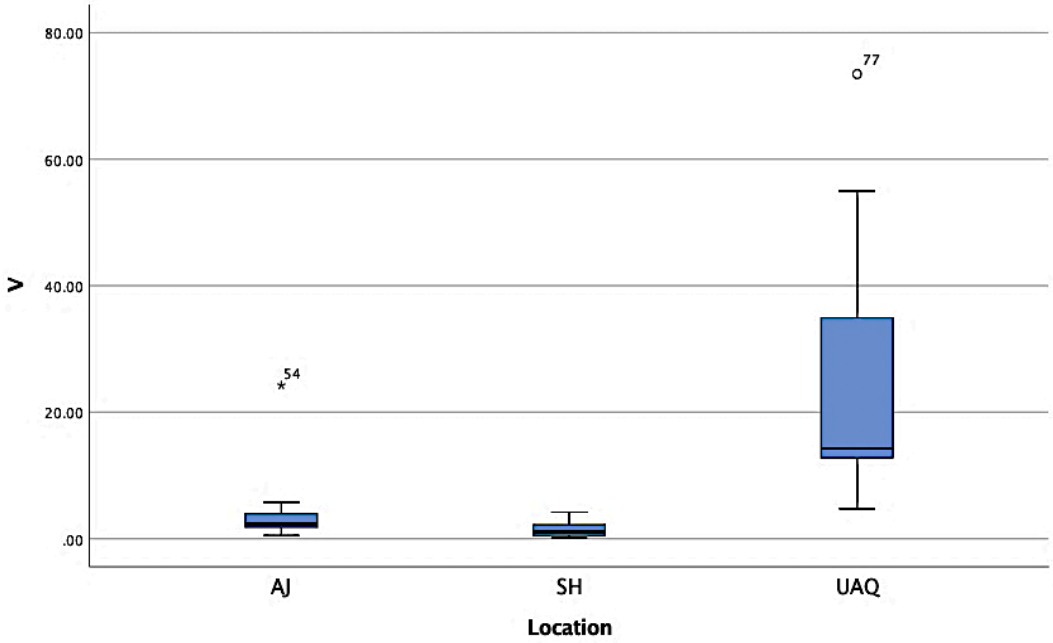

Figure 37: Representation of outliers for V in GI tract

Appendix 3

Metal and Non-metal Analysis in Liver

Descriptive statistic was performed for each predictor variables to see mean, standard error, standard deviation, maximum, minimum, interquartile range and skewness of the variables depending on sampling sites.

Table 57: Overview of descriptive statistic for Cd in liver

	Location				Std. Error
Cd	AJ	Mean		3.9461	.36707
		95% Confidence Interval for Mean	Lower Bound	3.1965	
			Upper Bound	4.6958	
		5% Trimmed Mean		3.8192	
		Median		3.3400	
		Variance		4.177	
		Std. Deviation		2.04378	
		Minimum		.81	
		Maximum		9.86	
		Range		9.05	
		Interquartile Range		2.51	
		Skewness		1.128	.421
		Kurtosis		1.376	.821
	SH	Mean		5.1457	1.94624
		95% Confidence Interval for Mean	Lower Bound	.9411	
			Upper Bound	9.3503	
		5% Trimmed Mean		3.9886	
		Median		3.8950	
		Variance		53.030	
		Std. Deviation		7.28217	
		Minimum		1.16	
		Maximum		29.96	
		Range		28.80	
		Interquartile Range		3.23	
		Skewness		3.494	.597
		Kurtosis		12.695	1.154
	UAQ	Mean		7.4215	.71988
		95% Confidence Interval for Mean	Lower Bound	5.8531	
			Upper Bound	8.9900	
		5% Trimmed Mean		7.4256	
		Median		6.5300	
		Variance		6.737	
		Std. Deviation		2.59555	
		Minimum		2.98	
		Maximum		11.79	
		Range		8.81	
		Interquartile Range		4.08	
		Skewness		.165	.616
		Kurtosis		-.684	1.191

Table 58: Overview of descriptive statistic for Cr in liver

	Location				Std. Error
Cr	AJ	Mean		21.0884	7.04394
		95% Confidence Interval for Mean	Lower Bound	6.7027	
			Upper Bound	35.4740	
		5% Trimmed Mean		14.0057	
		Median		7.6200	
		Variance		1538.130	
		Std. Deviation		39.21899	
		Minimum		1.69	
		Maximum		206.83	
		Range		205.14	
		Interquartile Range		16.10	
		Skewness		4.008	.421
		Kurtosis		17.762	.821
	SH	Mean		26.7629	9.53684
		95% Confidence Interval for Mean	Lower Bound	6.1598	
			Upper Bound	47.3659	
		5% Trimmed Mean		22.6982	
		Median		13.8550	
		Variance		1273.317	
		Std. Deviation		35.68357	
		Minimum		1.08	
		Maximum		125.61	
		Range		124.53	
		Interquartile Range		34.42	
		Skewness		2.025	.597
		Kurtosis		4.061	1.154
	UAQ	Mean		3.7446	.43156
		95% Confidence Interval for Mean	Lower Bound	2.8043	
			Upper Bound	4.6849	
		5% Trimmed Mean		3.7201	
		Median		3.5100	
		Variance		2.421	
		Std. Deviation		1.55601	
		Minimum		1.23	
		Maximum		6.70	
		Range		5.47	
		Interquartile Range		2.36	
		Skewness		.471	.616
		Kurtosis		-.274	1.191

Table 59: Overview of descriptive statistic for Cu in liver

	Location				Std. Error
Cu	AJ	Mean		16.6787	1.20473
		95% Confidence Interval for Mean	Lower Bound	14.2183	
			Upper Bound	19.1391	
		5% Trimmed Mean		16.4872	
		Median		16.8300	
		Variance		44.993	
		Std. Deviation		6.70765	
		Minimum		1.35	
		Maximum		36.30	
		Range		34.95	
		Interquartile Range		7.42	
		Skewness		.504	.421
		Kurtosis		1.683	.821
	SH	Mean		12.6021	1.56894
		95% Confidence Interval for Mean	Lower Bound	9.2126	
			Upper Bound	15.9916	
		5% Trimmed Mean		12.1213	
		Median		12.2950	
		Variance		34.462	
		Std. Deviation		5.87045	
		Minimum		4.33	
		Maximum		29.53	
		Range		25.20	
		Interquartile Range		3.50	
		Skewness		1.865	.597
		Kurtosis		5.365	1.154
	UAQ	Mean		24.4946	3.70815
		95% Confidence Interval for Mean	Lower Bound	16.4153	
			Upper Bound	32.5740	
		5% Trimmed Mean		23.3512	
		Median		19.1400	
		Variance		178.754	
		Std. Deviation		13.36991	
		Minimum		9.64	
		Maximum		59.93	
		Range		50.29	
		Interquartile Range		14.03	
		Skewness		1.718	.616
		Kurtosis		3.413	1.191

Table 60: Overview of descriptive statistic for Ni in liver

	Location				Std. Error
Ni	AJ	Mean		60.3187	15.70650
		95% Confidence Interval for Mean	Lower Bound	28.2418	
			Upper Bound	92.3957	
		5% Trimmed Mean		46.1916	
		Median		31.9000	
		Variance		7647.516	
		Std. Deviation		87.45007	
		Minimum		10.47	
		Maximum		467.04	
		Range		456.57	
		Interquartile Range		43.17	
		Skewness		3.711	.421
		Kurtosis		16.023	.821
	SH	Mean		81.0143	24.94626
		95% Confidence Interval for Mean	Lower Bound	27.1212	
			Upper Bound	134.9074	
		5% Trimmed Mean		70.3937	
		Median		46.1900	
		Variance		8712.423	
		Std. Deviation		93.34036	
		Minimum		3.57	
		Maximum		349.63	
		Range		346.06	
		Interquartile Range		110.15	
		Skewness		2.012	.597
		Kurtosis		4.863	1.154
	UAQ	Mean		23.2654	2.42923
		95% Confidence Interval for Mean	Lower Bound	17.9725	
			Upper Bound	28.5582	
		5% Trimmed Mean		23.5449	
		Median		22.4100	
		Variance		76.715	
		Std. Deviation		8.75871	
		Minimum		7.47	
		Maximum		34.03	
		Range		26.56	
		Interquartile Range		16.19	
		Skewness		-.491	.616
		Kurtosis		-.961	1.191

Table 61: Overview of descriptive statistic for Fe in liver

	Location				Std. Error
Fe	AJ	Mean		2036.1355	485.25063
		95% Confidence Interval for Mean	Lower Bound	1045.1215	
			Upper Bound	3027.1495	
		5% Trimmed Mean		1622.3219	
		Median		960.1000	
		Variance		7299513.316	
		Std. Deviation		2701.76115	
		Minimum		32.80	
		Maximum		13462.90	
		Range		13430.10	
		Interquartile Range		2002.80	
		Skewness		2.983	.421
		Kurtosis		10.604	.821
	SH	Mean		2731.3929	984.84684
		95% Confidence Interval for Mean	Lower Bound	603.7606	
			Upper Bound	4859.0251	
		5% Trimmed Mean		2250.9198	
		Median		1572.6500	
		Variance		13578926.176	
		Std. Deviation		3684.95945	
		Minimum		101.80	
		Maximum		14009.50	
		Range		13907.70	
		Interquartile Range		3860.93	
		Skewness		2.420	.597
		Kurtosis		7.078	1.154
	UAQ	Mean		412.8000	58.58911
		95% Confidence Interval for Mean	Lower Bound	285.1453	
			Upper Bound	540.4547	
		5% Trimmed Mean		394.3944	
		Median		387.6000	
		Variance		44624.897	
		Std. Deviation		211.24606	
		Minimum		155.40	
		Maximum		1001.50	
		Range		846.10	
		Interquartile Range		186.70	
		Skewness		1.862	.616
		Kurtosis		4.907	1.191

Table 62: Overview of descriptive statistic for Ca in liver

	Location				Std. Error
Ca	AJ	Mean		9254.2548	1629.72557
		95% Confidence Interval for Mean	Lower Bound	5925.9112	
			Upper Bound	12582.5985	
		5% Trimmed Mean		8144.2341	
		Median		5091.0000	
		Variance		82336168.171	
		Std. Deviation		9073.92794	
		Minimum		2085.70	
		Maximum		36977.10	
		Range		34891.40	
		Interquartile Range		7743.90	
		Skewness		2.073	.421
		Kurtosis		3.828	.821
	SH	Mean		5614.0357	948.47546
		95% Confidence Interval for Mean	Lower Bound	3564.9791	
			Upper Bound	7663.0924	
		5% Trimmed Mean		5528.8841	
		Median		5752.2000	
		Variance		12594479.822	
		Std. Deviation		3548.87022	
		Minimum		955.50	
		Maximum		11805.30	
		Range		10849.80	
		Interquartile Range		5809.83	
		Skewness		.280	.597
		Kurtosis		-.821	1.154
	UAQ	Mean		7691.3154	1198.18796
		95% Confidence Interval for Mean	Lower Bound	5080.6881	
			Upper Bound	10301.9427	
		5% Trimmed Mean		7242.1115	
		Median		5435.7000	
		Variance		18663507.155	
		Std. Deviation		4320.12814	
		Minimum		4179.20	
		Maximum		19289.10	
		Range		15109.90	
		Interquartile Range		5401.70	
		Skewness		1.800	.616
		Kurtosis		3.581	1.191

Table 63: Overview of descriptive statistic for Mn in liver

	Location				Std. Error
Mn	AJ	Mean		25.5455	6.16433
		95% Confidence Interval for Mean	Lower Bound	12.9563	
			Upper Bound	38.1347	
		5% Trimmed Mean		20.3850	
		Median		11.6500	
		Variance		1177.966	
		Std. Deviation		34.32152	
		Minimum		1.19	
		Maximum		146.49	
		Range		145.30	
		Interquartile Range		24.64	
		Skewness		2.638	.421
		Kurtosis		6.960	.821
	SH	Mean		36.2414	11.56365
		95% Confidence Interval for Mean	Lower Bound	11.2597	
			Upper Bound	61.2232	
		5% Trimmed Mean		31.4766	
		Median		21.9300	
		Variance		1872.052	
		Std. Deviation		43.26722	
		Minimum		1.98	
		Maximum		156.27	
		Range		154.29	
		Interquartile Range		56.40	
		Skewness		1.822	.597
		Kurtosis		3.798	1.154
	UAQ	Mean		6.0985	.61016
		95% Confidence Interval for Mean	Lower Bound	4.7690	
			Upper Bound	7.4279	
		5% Trimmed Mean		6.0166	
		Median		5.8100	
		Variance		4.840	
		Std. Deviation		2.19995	
		Minimum		3.33	
		Maximum		10.34	
		Range		7.01	
		Interquartile Range		3.54	
		Skewness		.522	.616
		Kurtosis		-.592	1.191

Table 64: Overview of descriptive statistic for Na in liver

	Location				Std. Error
Na	AJ	Mean		5334.8097	190.49317
		95% Confidence Interval for Mean	Lower Bound	4945.7707	
			Upper Bound	5723.8486	
		5% Trimmed Mean		5365.3260	
		Median		5326.9000	
		Variance		1124917.122	
		Std. Deviation		1060.62110	
		Minimum		3071.10	
		Maximum		6933.30	
		Range		3862.20	
		Interquartile Range		1843.60	
		Skewness		-.256	.421
		Kurtosis		-.786	.821
	SH	Mean		7850.8571	669.45645
		95% Confidence Interval for Mean	Lower Bound	6404.5844	
			Upper Bound	9297.1299	
		5% Trimmed Mean		7811.2968	
		Median		7807.0000	
		Variance		6274407.143	
		Std. Deviation		2504.87667	
		Minimum		4464.10	
		Maximum		11949.70	
		Range		7485.60	
		Interquartile Range		4806.65	
		Skewness		.223	.597
		Kurtosis		-1.279	1.154
	UAQ	Mean		5529.7846	691.41688
		95% Confidence Interval for Mean	Lower Bound	4023.3167	
			Upper Bound	7036.2526	
		5% Trimmed Mean		5189.7718	
		Median		4913.7000	
		Variance		6214744.875	
		Std. Deviation		2492.93900	
		Minimum		3802.80	
		Maximum		13377.00	
		Range		9574.20	
		Interquartile Range		1646.95	
		Skewness		2.965	.616
		Kurtosis		9.705	1.191

Table 65: Overview of descriptive statistic for P in liver

	Location				Std. Error
P	AJ	Mean		6709.7032	284.14293
		95% Confidence Interval for Mean	Lower Bound	6129.4059	
			Upper Bound	7290.0005	
		5% Trimmed Mean		6674.5029	
		Median		6577.4000	
		Variance		2502853.378	
		Std. Deviation		1582.04089	
		Minimum		3734.10	
		Maximum		10082.20	
		Range		6348.10	
		Interquartile Range		2084.40	
		Skewness		.381	.421
		Kurtosis		-.348	.821
	SH	Mean		5931.6214	526.85341
		95% Confidence Interval for Mean	Lower Bound	4793.4238	
			Upper Bound	7069.8190	
		5% Trimmed Mean		5925.3405	
		Median		5847.2000	
		Variance		3886043.159	
		Std. Deviation		1971.30494	
		Minimum		2930.40	
		Maximum		9045.90	
		Range		6115.50	
		Interquartile Range		3737.47	
		Skewness		-.048	.597
		Kurtosis		-1.332	1.154
	UAQ	Mean		4766.6615	543.68508
		95% Confidence Interval for Mean	Lower Bound	3582.0735	
			Upper Bound	5951.2496	
		5% Trimmed Mean		4622.3184	
		Median		4307.0000	
		Variance		3842715.103	
		Std. Deviation		1960.28444	
		Minimum		2770.70	
		Maximum		9360.80	
		Range		6590.10	
		Interquartile Range		2176.90	
		Skewness		1.520	.616
		Kurtosis		1.634	1.191

Table 66: Overview of descriptive statistic for S in liver

	Location				Std. Error
S	AJ	Mean		6810.4548	234.32899
		95% Confidence Interval for Mean	Lower Bound	6331.8912	
			Upper Bound	7289.0185	
		5% Trimmed Mean		6912.6177	
		Median		7049.3000	
		Variance		1702212.329	
		Std. Deviation		1304.68859	
		Minimum		2546.40	
		Maximum		8860.30	
		Range		6313.90	
		Interquartile Range		1330.50	
		Skewness		-1.437	.421
		Kurtosis		2.771	.821
	SH	Mean		5497.4571	539.27491
		95% Confidence Interval for Mean	Lower Bound	4332.4245	
			Upper Bound	6662.4898	
		5% Trimmed Mean		5485.2913	
		Median		5826.8000	
		Variance		4071444.056	
		Std. Deviation		2017.78196	
		Minimum		2632.90	
		Maximum		8581.00	
		Range		5948.10	
		Interquartile Range		3853.58	
		Skewness		.108	.597
		Kurtosis		-1.313	1.154
	UAQ	Mean		7467.5769	667.82453
		95% Confidence Interval for Mean	Lower Bound	6012.5123	
			Upper Bound	8922.6416	
		5% Trimmed Mean		7197.2577	
		Median		6852.5000	
		Variance		5797864.882	
		Std. Deviation		2407.87560	
		Minimum		5126.10	
		Maximum		14674.80	
		Range		9548.70	
		Interquartile Range		1623.15	
		Skewness		2.504	.616
		Kurtosis		7.373	1.191

Table 67: Overview of descriptive statistic for Sr in liver

	Location				Std. Error
Sr	AJ	Mean		78.1903	13.44832
		95% Confidence Interval for Mean	Lower Bound	50.7252	
			Upper Bound	105.6555	
		5% Trimmed Mean		68.6634	
		Median		54.1000	
		Variance		5606.574	
		Std. Deviation		74.87706	
		Minimum		18.10	
		Maximum		365.90	
		Range		347.80	
		Interquartile Range		88.00	
		Skewness		2.259	.421
		Kurtosis		6.345	.821
	SH	Mean		73.6643	18.92569
		95% Confidence Interval for Mean	Lower Bound	32.7778	
			Upper Bound	114.5508	
		5% Trimmed Mean		65.4381	
		Median		66.2000	
		Variance		5014.546	
		Std. Deviation		70.81346	
		Minimum		11.30	
		Maximum		284.10	
		Range		272.80	
		Interquartile Range		70.88	
		Skewness		2.197	.597
		Kurtosis		6.031	1.154
	UAQ	Mean		115.7923	22.31394
		95% Confidence Interval for Mean	Lower Bound	67.1744	
			Upper Bound	164.4102	
		5% Trimmed Mean		108.4359	
		Median		84.3000	
		Variance		6472.852	
		Std. Deviation		80.45404	
		Minimum		47.60	
		Maximum		316.40	
		Range		268.80	
		Interquartile Range		103.50	
		Skewness		1.533	.616
		Kurtosis		2.091	1.191

Table 68: Overview of descriptive statistic for V in liver

	Location				Std. Error
V	AJ	Mean		8.0616	.92877
		95% Confidence Interval for Mean	Lower Bound	6.1648	
			Upper Bound	9.9584	
		5% Trimmed Mean		7.7384	
		Median		6.4600	
		Variance		26.741	
		Std. Deviation		5.17117	
		Minimum		1.55	
		Maximum		20.49	
		Range		18.94	
		Interquartile Range		6.53	
		Skewness		1.001	.421
		Kurtosis		.152	.821
	SH	Mean		3.7564	1.19049
		95% Confidence Interval for Mean	Lower Bound	1.1845	
			Upper Bound	6.3283	
		5% Trimmed Mean		3.3766	
		Median		2.2200	
		Variance		19.842	
		Std. Deviation		4.45440	
		Minimum		.11	
		Maximum		14.24	
		Range		14.13	
		Interquartile Range		4.24	
		Skewness		1.739	.597
		Kurtosis		2.235	1.154
	UAQ	Mean		3.4385	.59194
		95% Confidence Interval for Mean	Lower Bound	2.1487	
			Upper Bound	4.7282	
		5% Trimmed Mean		3.4255	
		Median		3.5200	
		Variance		4.555	
		Std. Deviation		2.13427	
		Minimum		.57	
		Maximum		6.54	
		Range		5.97	
		Interquartile Range		4.23	
		Skewness		.060	.616
		Kurtosis		-1.663	1.191

Table 69: Overview of descriptive statistic for Zn in liver

	Location				Std. Error
Zn	AJ	Mean		140.6194	10.75953
		95% Confidence Interval for Mean	Lower Bound	118.6455	
			Upper Bound	162.5932	
		5% Trimmed Mean		137.7964	
		Median		134.0000	
		Variance		3588.790	
		Std. Deviation		59.90651	
		Minimum		30.10	
		Maximum		331.90	
		Range		301.80	
		Interquartile Range		83.80	
		Skewness		.866	.421
		Kurtosis		2.138	.821
	SH	Mean		126.3214	14.79804
		95% Confidence Interval for Mean	Lower Bound	94.3522	
			Upper Bound	158.2907	
		5% Trimmed Mean		123.3127	
		Median		109.2500	
		Variance		3065.748	
		Std. Deviation		55.36920	
		Minimum		63.20	
		Maximum		243.60	
		Range		180.40	
		Interquartile Range		93.47	
		Skewness		.985	.597
		Kurtosis		-.104	1.154
	UAQ	Mean		239.7000	21.20691
		95% Confidence Interval for Mean	Lower Bound	193.4941	
			Upper Bound	285.9059	
		5% Trimmed Mean		240.4278	
		Median		261.9000	
		Variance		5846.530	
		Std. Deviation		76.46261	
		Minimum		113.60	
		Maximum		352.70	
		Range		239.10	
		Interquartile Range		137.85	
		Skewness		-.240	.616
		Kurtosis		-1.313	1.191

Table 70: Overview of descriptive statistic for Hg in liver

	Location				Std. Error
Hg	AJ	Mean		.7384	.03478
		95% Confidence Interval for Mean	Lower Bound	.6674	
			Upper Bound	.8094	
		5% Trimmed Mean		.7235	
		Median		.7400	
		Variance		.038	
		Std. Deviation		.19365	
		Minimum		.50	
		Maximum		1.39	
		Range		.89	
		Interquartile Range		.34	
		Skewness		1.212	.421
		Kurtosis		2.685	.821
	SH	Mean		.4107	.02940
		95% Confidence Interval for Mean	Lower Bound	.3472	
			Upper Bound	.4742	
		5% Trimmed Mean		.4063	
		Median		.3900	
		Variance		.012	
		Std. Deviation		.11000	
		Minimum		.28	
		Maximum		.62	
		Range		.34	
		Interquartile Range		.22	
		Skewness		.547	.597
		Kurtosis		-.880	1.154
	UAQ	Mean		1.3331	.03155
		95% Confidence Interval for Mean	Lower Bound	1.2643	
			Upper Bound	1.4018	
		5% Trimmed Mean		1.3390	
		Median		1.3900	
		Variance		.013	
		Std. Deviation		.11375	
		Minimum		1.10	
		Maximum		1.46	
		Range		.36	
		Interquartile Range		.19	
		Skewness		-.795	.616
		Kurtosis		-.477	1.191

The boxplot was illustrated in order to see outliers for each predictor variables depending on sampling sites. Extreme outliers were pointed out with stars and potential outliers were depicted as a circle.

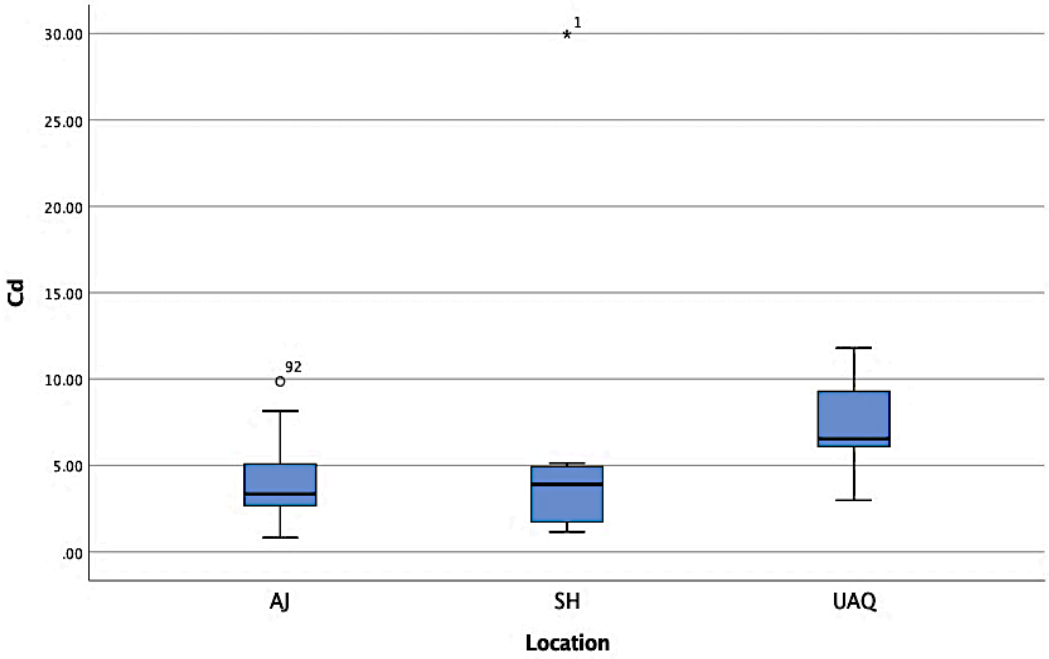

Figure 38: Representation of outliers for Cd in liver

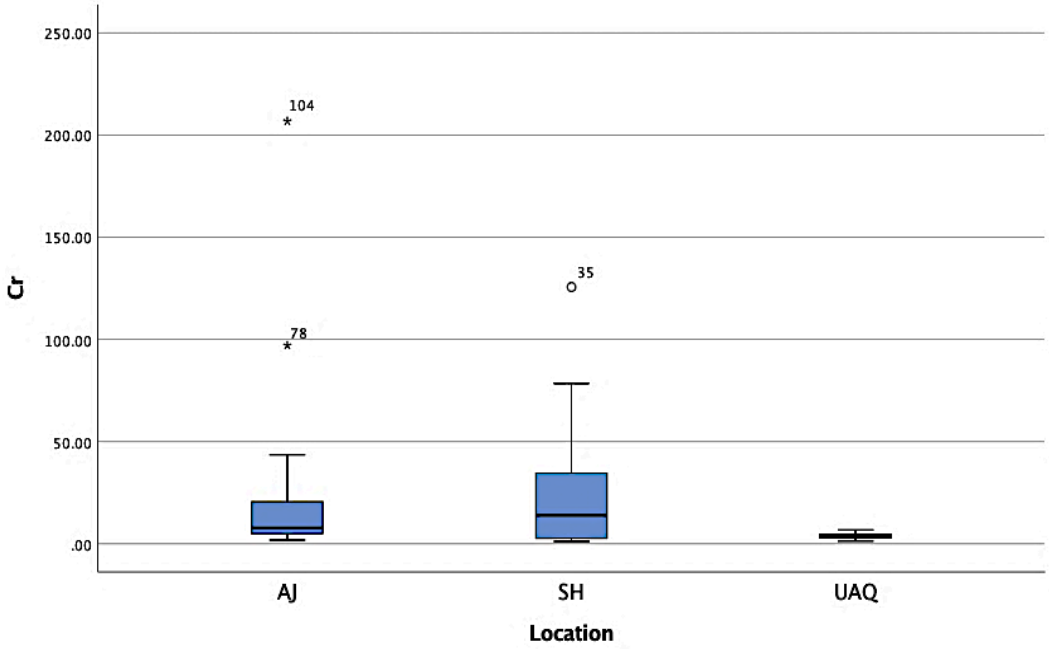

Figure 39: Representation of outliers for Cr in liver

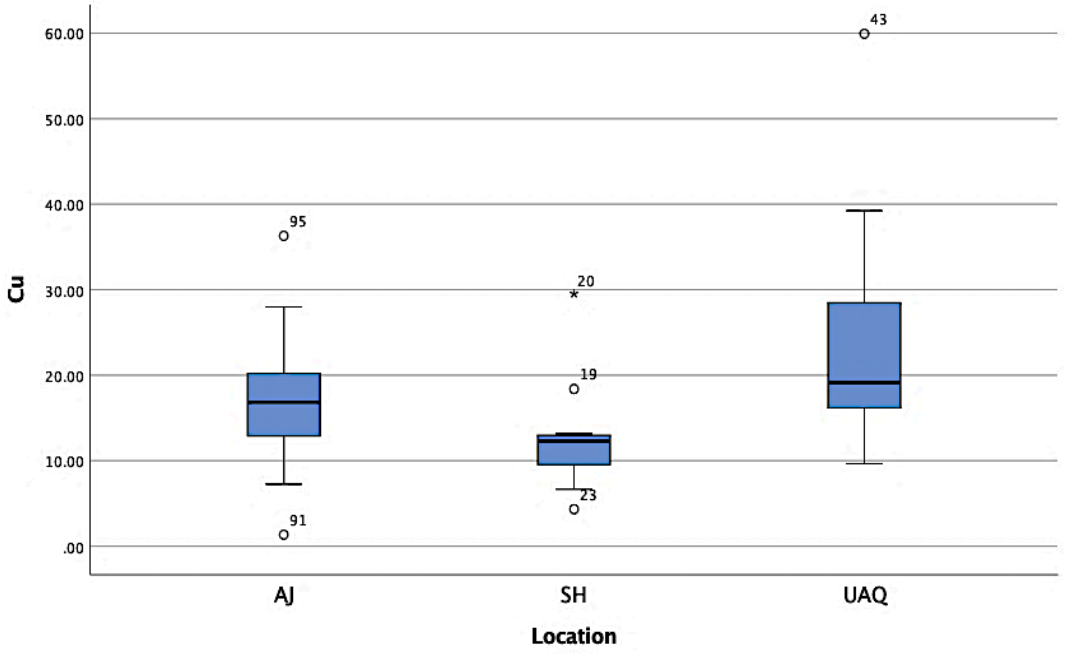

Figure 40: Representation of outliers for Cr in liver

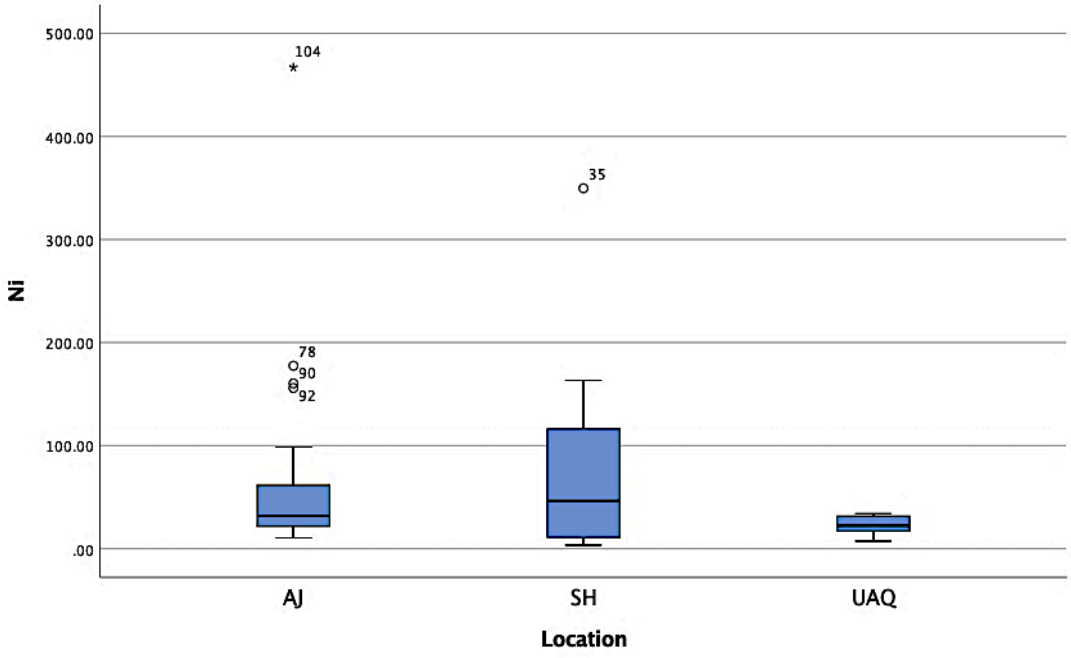

Figure 41: Representation of outliers for Ni in liver

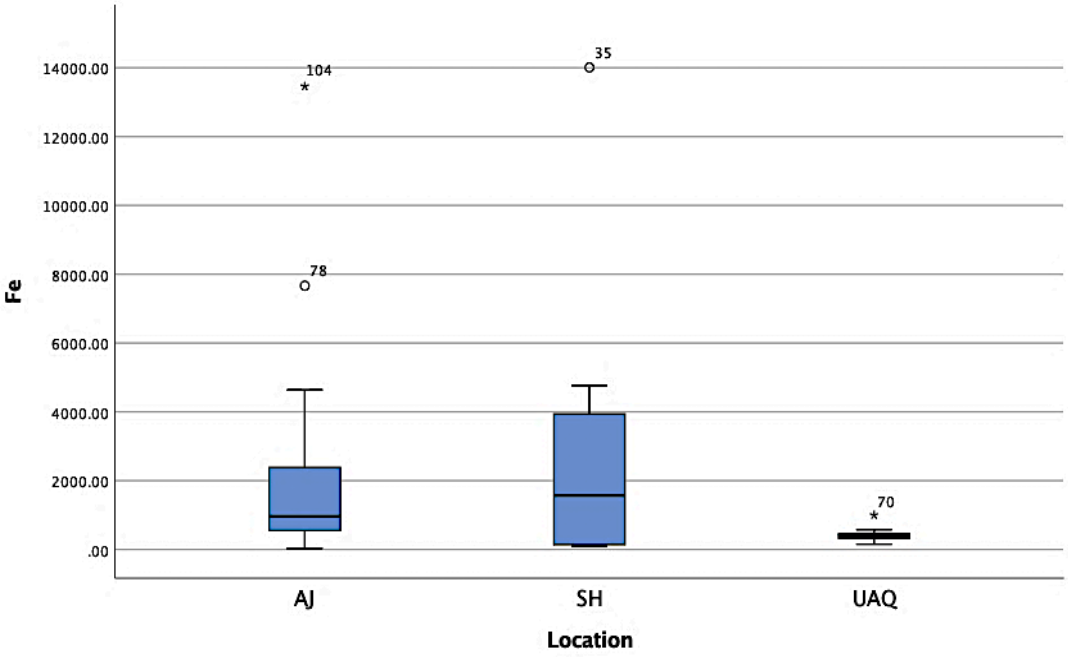

Figure 42: Representation of outliers for Fe in liver

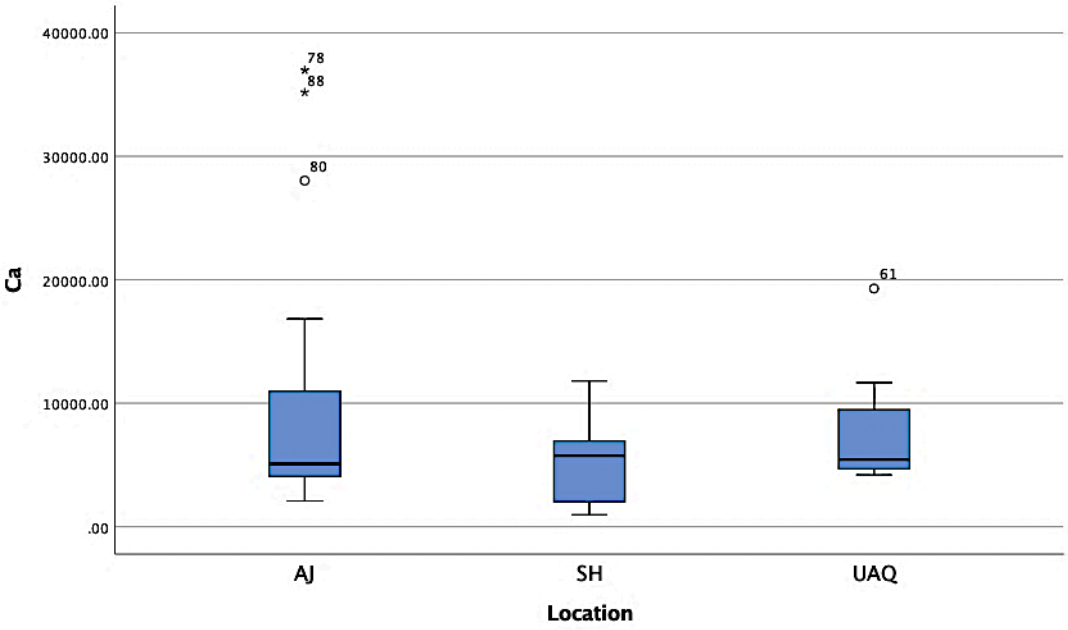

Figure 43: Representation of outliers for Ca in liver

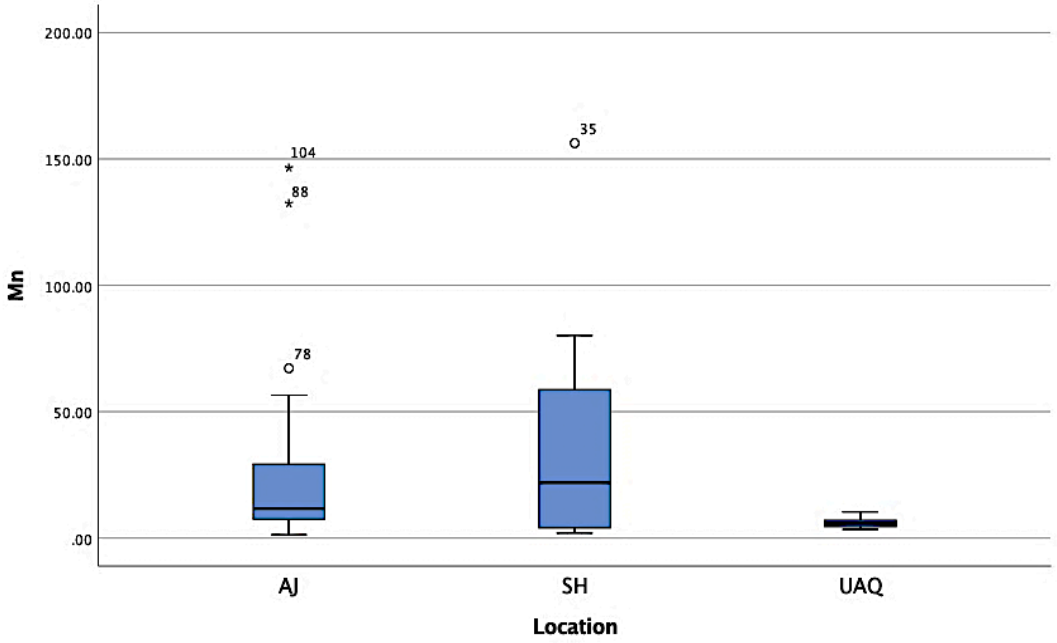

Figure 44: Representation of outliers for Mn in liver

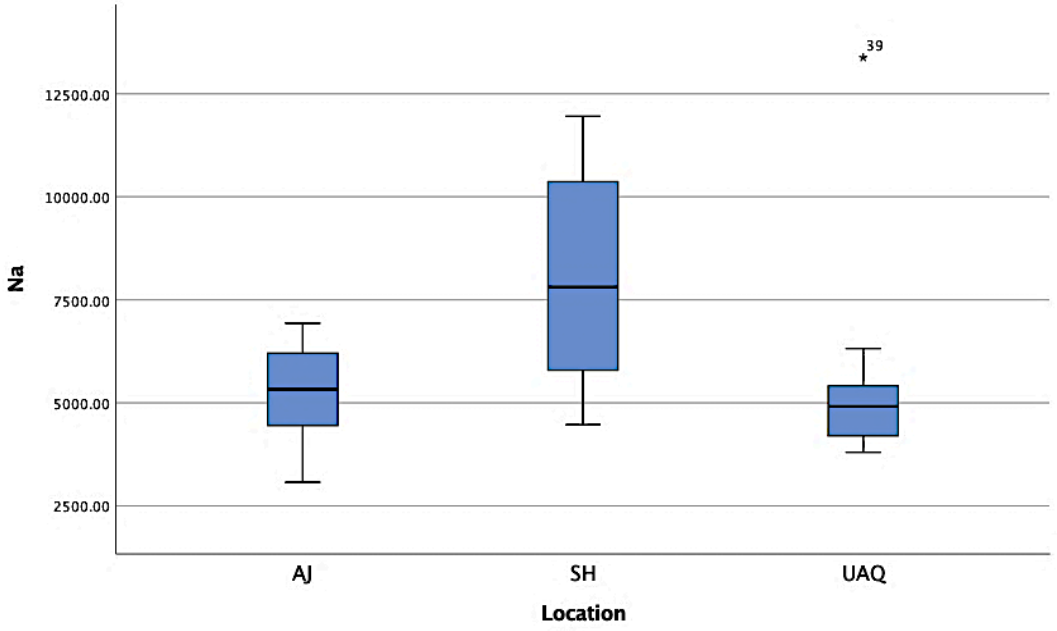

Figure 45: Representation of outliers for Na in liver

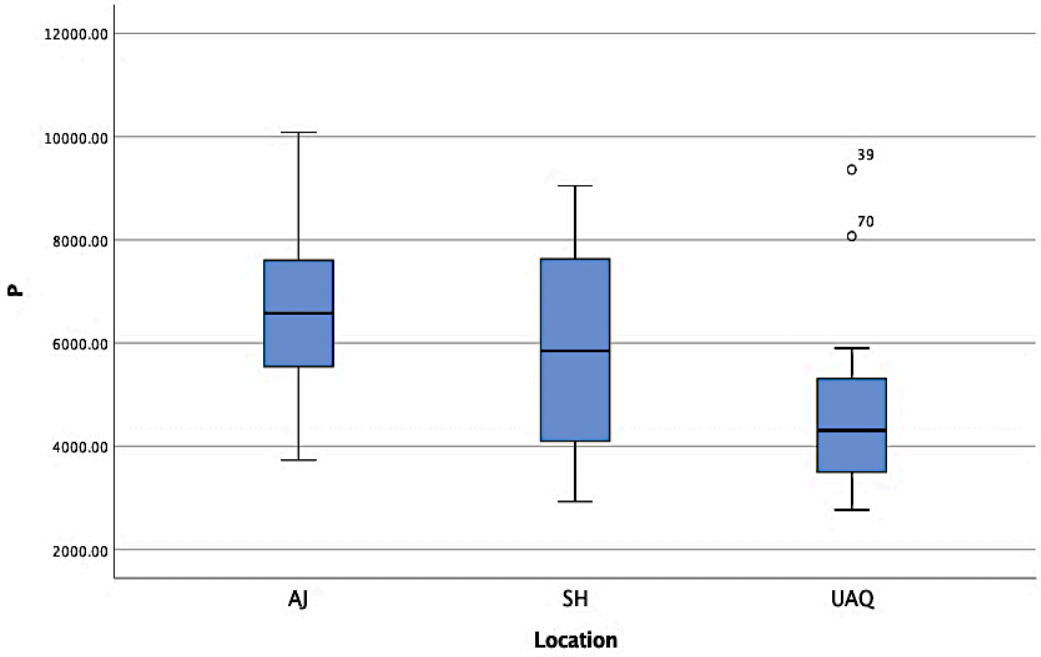

Figure 46: Representation of outliers for P in liver

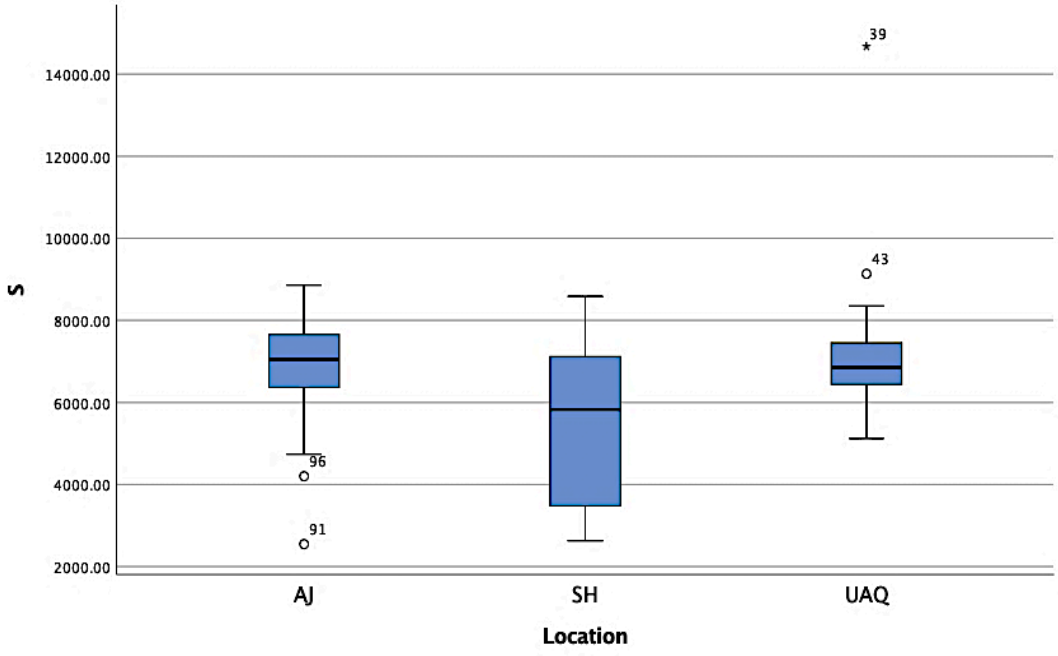

Figure 47: Representation of outliers for S in liver

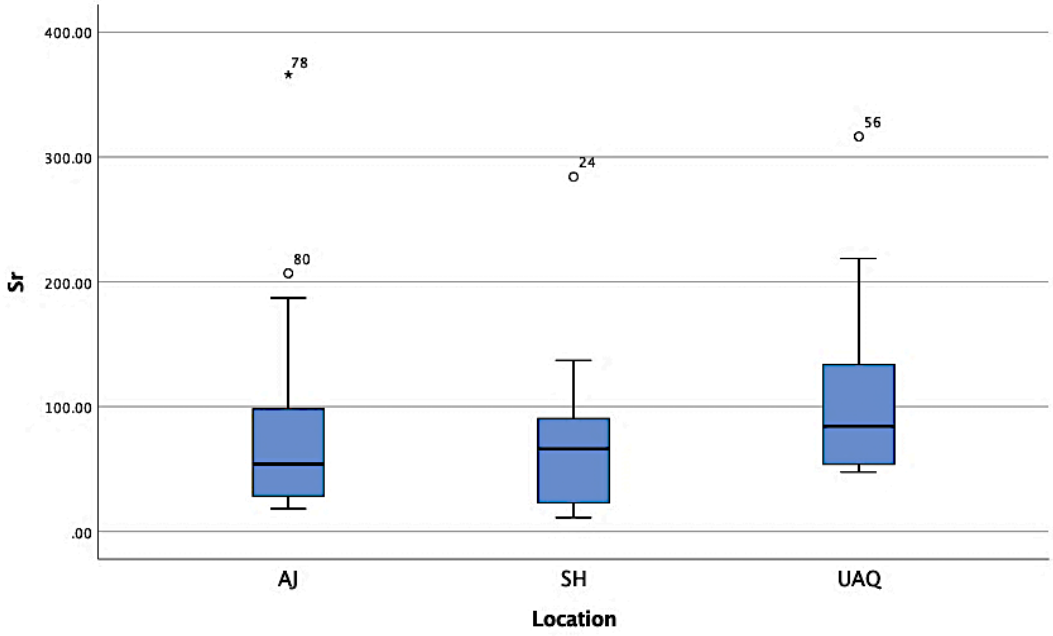

Figure 48: Representation of outliers for Sr in liver

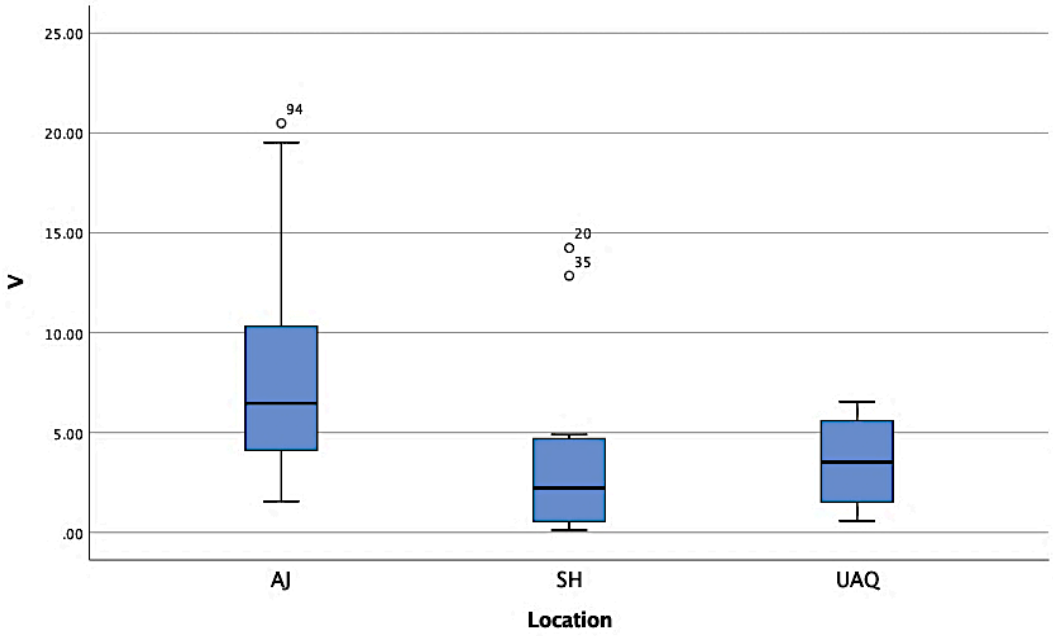

Figure 49: Representation of outliers for V in liver

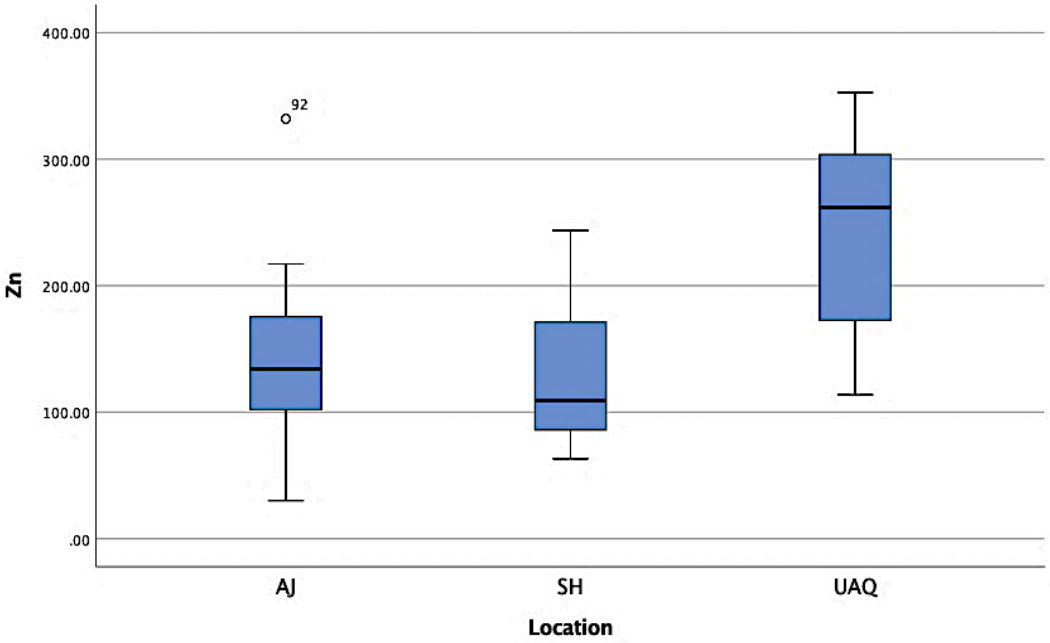

Figure 50: Representation of outliers for Zn in liver

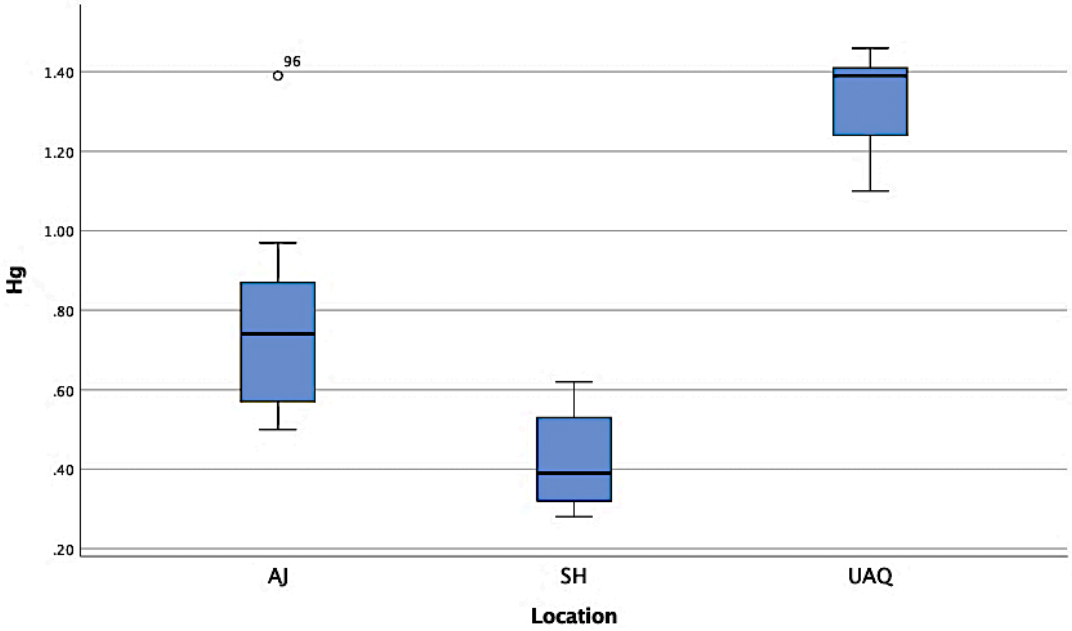

Figure 51: Representation of outliers for Hg in liver