Heavy Metals in Blubber and Skin of Mediterranean Monk Seals, *Monachus monachus* from the Greek Waters

A THESIS SUBMITTED TO THE UNIVERSITY OF NORTH WALES, BANGOR

BY

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ξυπναν οι ναυτες του βυθου ρισαλτο να βαρεσουν
κι απε να σου χτενισουνε για παντα τα μαλλια.
τροχισε κεινα τα σπαθια του λογου που μ΄ αρεσουν
και ξαναγυρνα με τις φωκιες περα στη σπηλια.

νικος καββαδιας

Declaration

Declaration

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

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Statement 1

This dissertation is being submitted in partial fulfilment of the requirements for the degree of Master of Science in Marine Biology at the University of North Wales.

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This dissertation is the result of my own independent work/investigation, except where otherwise stated. Other sources are acknowledged by giving explicit references.

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Abstract

The presence of heavy metals in blubber and skin samples collected from Monk seals, *Monachus monachus*, during the period 1994-1999 in Greece was investigated. The metals examined were Al, As, Cd, Co, Cr, Cu, Fe, Mg, Mn, Pb, Pt, Se, Si and Zn using ICP-AES. Pt and Se were omitted from the study due to a great number of non-detectable concentrations. Cu and Zn were determined using two different methods of ICP-AES. No significant difference was observed in the resulted concentrations of the two methods for Zn while for Cu concentrations differed by a factor of 2. Sex did not account for any significant differences in the metals' concentrations. Tissue type of the samples and stage of development of the animals had the greatest effect on the concentrations with the highest values observed in skin and pups. Year and area of collection did not account for significant differences in the concentrations. Strong interelement association was detected between Cu and Zn. No certain conclusions could be drawn in terms of heavy metal pollution in Monk seals, as there is no background literature. Further research and studies are urgently needed.

1. Introduction

1.1. Heavy metals

Heavy metals are a general collective term applying to the group of metals and metalloids with an atomic density greater than 6 g/cm^3. Although it is only a loosely defined term it is widely recognized and usually applied to the elements such as Cd, Cr, Co, Cu, Fe, Hg, Ni, Pb and Zn which are commonly associated with pollution and toxicity problems. An alternative, theoretically more acceptable, name for this group of elements is 'Trace Metals' but is not as widely used (Alloway & Ayres, 1993). In addition to these, a number of other lighter elements such as aluminum (Al), arsenic (As) and selenium (Se), have most frequently been associated with toxicity from environmental exposures (Freedman, 1995).

Unlike most organic pollutants, such as organohalides, all the above metals are ubiquitous in the environment and occur naturally in rock-forming and ore minerals (Freedman, 1995). Consequently, there is a range of normal background concentrations of these elements in soils, sediments, waters and living organisms (Alloway & Ayres, 1993).

Heavy metals are conservative pollutants. Unlike organic wastes, conservative pollutants are not subject to bacterial attack or, if they are, it is on such a long time scale that for practical purposes they are permanent additions to the marine environment (Clark, 1997).

A great number of them, like copper, iron, zinc and possibly aluminum and selenium, are essential for life. They reach the marine environment and therefore marine mammals, from a vast array of anthropogenic sources (Braham, 1973; Duinker *et al.*, 1979; Hyvärinen & Sipilä, 1984), the main being industrial outflows, sewage effluent and pesticides (Goldblatt & Anthony, 1983), as well as natural geochemical processes like land erosion and volcanic activity (Anas, 1974).

Toxic metals have been observed to increase significantly in the marine biosphere over human history (Boutron & Delmas, 1980). This has occurred especially near polluted coastal, industrialized areas although, atmospheric loading have also been reported offshore over the Atlantic Ocean (Slemr & Langer, 1992).

1.2. Heavy metals in living organisms

Interest in the accumulation of heavy metals in marine species was stimulated around 1970 following observations that relatively high levels of mercury were present in tuna and swordfish (Holden, 1978). During the last few decades, seals and other fish-consuming marine mammals have received attention as indicators of environmental pollution (Holden, 1970; 1975A; 1978; Olsson *et al.*, 1992) as marine mammals retain heavy metals in their tissues to a greater degree than other marine organisms (Jones *et al.*, 1972). Moreover, contaminant concentrations in marine mammals reflect those in their prey species and therefore can be appropriate bioindicator species in the assessment of this kind of environmental problem (Law *et al.*, 1992; Marcovecchio, 1994).

The potential hazard of heavy metals to marine mammals is primarily through biological magnification in the oceans and seas of the world (Boothe & Knauer, 1972; Jones *et al.*, 1972). These aquatic animals occupy a top trophic position in the ecosystem. A higher position in the food chain is not necessarily related to higher concentrations in the organisms (Knauer & Martin, 1972) as abiotic factors play a part as well (Cember *et al.*, 1978). However, heavy metals tend to be greater in the tissues of marine organisms of higher trophic levels as they can be transferred progressively through a food chain, accumulating and even biomagnified at the highest trophic levels (Anas, 1974; Drescher *et al.*, 1977; Holden, 1970; 1978; Holden & Marsden, 1967; Lee *et al.*, 1996). This is also demonstrated by data available on biota from the Dutch, German and Danish Wadden Sea (Reijnders, 1980).

Food ingestion, coupled with metabolism and excretion, and absorption via the water are the dominant pathways for bioaccumulation of heavy metals in tissues of large marine organisms (Beck *et al.*, 1997; Muir *et al.*, 1999).

A number of elements in this group are required by most living organisms in small but critical concentrations for normally healthy growth (Alloway & Ayres, 1993). These essential metals include Al, Co, Cr, Cu, Fe, I, Mn, Se and Zn. Under certain environmental conditions, however, they can bioaccumulate to toxic concentrations and cause ecological damage (Freedman, 1995). Other elements like As, Cd, Hg and Pb, cause toxicity at concentrations greater than the organisms tolerance but do not cause deficiency disorders at low concentrations like the above essential elements. At higher concentrations, when they become toxic, cause multiple symptomatic effects influencing the health and sometimes the survival of these animals (Beck *et al.*, 1997).

All the above mentioned elements are consistently present in the environment. The background concentrations though, meaning the concentrations of metals that occur in the environment in situations that have not been influenced by anthropogenic emissions or by unusual natural exposures, differ between elements (Table 1.1.).

METALS	MARINE FISH	MAMMALS	
		MUSCLE	BONE
Aluminum	20	0.7-28	4-27
Arsenic	0.2-10	0.007-0.09	0.08-1.6
Cadmium	0.1-3	0.1-3.2	1.8
Cobalt	0.006-0.05	0.005-1	0.01-0.04
Chromium	0.03-2	<0.002-0.84	0.1-33
Copper	0.7-15	10	1-26
Iron	9-98	180	3-380
Manganese	0.3-4.6	0.2-2.3	0.2-14
Lead	0.001-15	0.2-3.3	3.6-30
Selenium	0.2	0.4-1.9	1-9
Zinc	9-80	240	75-170

Table 1.1.: Typical background concentrations (μg/g dry wt) of metals in mammals and marine fish. (Source: Freedman, 1995).

Arsenic usually occurs as compounds with sulphur, either alone or in combination with metals. The toxicity of arsenic depends very much upon the nature of the compound it forms and particularly its valency (Clark, 1997). Marine fish and invertebrates can contain quite high concentrations of As, up to 100 μg/g, although the majority is present as organoarsenical compounds and is of relatively low toxicity (Law *et al.*, 1996).

Cadmium is highly toxic and accumulates in the mammalian kidney causing kidney dysfunction (Law *et al.*, 1991). Cadmium is closely related to zinc and will be found wherever zinc is found in nature. Zinc is an essential metal for most life forms (Bowen, 1966; Schroeder *et al.*, 1967) thus, it is probable that no naturally occurring material will be completely free from cadmium (Friberg *et al.*, 1974).

Lead is a cumulative toxin in the mammalian body and toxic concentrations can accumulate in the bone marrow, where red blood corpuscle formation occurs (Alloway & Ayres, 1993). Like Hg is a powerful neurotoxin and a range of pathological conditions are associated with acute Pb poisoning, most characteristic of which is cerebral oedema. Zn has a relatively low toxicity to animals and humans (Alloway & Ayres, 1993). Arsenic, cobalt, mercury, lead and selenium can be methylated in the environment through the action of enzymes secreted by microorganisms and also by abiotic chemical reactions (Matos Salvador, 1999).

However, consideration of total quantity of a metal in the organisms tissues gives little information about its potential toxicity (Freedman, 1995).

1.3. Copper and Zinc

These essential elements serve in the formation or function of many enzymes involved in metabolism and concentrate primarily in the liver. Their concentrations are believed to be homeostatically controlled in marine mammals (Law *et al.*, 1996). They are also closely bioregulated by marine mammals and therefore their concentrations vary little among and between species (Beck *et al.*, 1997).

Copper is plentiful in the environment and essential for the normal growth and metabolism of all living organisms (Eisler, 1998). Despite the existence of a number of detoxifying and storage systems for copper, it is the most toxic metal after mercury and silver to a wide spectrum of marine life (Clark, 1997; Eisler, 1998). It often accumulates and could cause irreversible harm to some species at concentrations just above levels required for growth and reproduction (Eisler, 1998). Copper levels can increase markedly in coastal areas where there is runoff from the land (Osterberg & Keckes, 1977).

Bioavailability and toxicity of copper to aquatic organisms depends on the total concentration of copper and its speciation (Eisler, 1998). Elevated concentrations of copper interfere with oxygen transport and energy metabolism (Hansen, *et al.*, 1992).

In animals, copper interacts with essential trace elements such as iron, zinc, molybdenum, manganese, nickel and selenium and also with nonessential elements like silver, cadmium, mercury and lead. These interactions could be either beneficial or harmful to the organism (Eisler, 1998). In the muscle of Weddell seals, *Leptonychotes weddelli*, copper is positively correlated with iron (Szefer *et al.*, 1994) which is the general case in all tissues of marine vertebrates. Mixtures of copper and zinc are generally acknowledged to be more-than-additive in toxicity to a wide variety of aquatic organisms.

Zinc is a commonly occurring trace metal and is essential to living organisms for enzymatic functions (Schroeder *et al.*, 1967). High levels of zinc are found in coastal areas but biota, dispersion and diffusion (Osterberg & Keckes, 1977) can rapidly remove zinc.

1.4. The Mediterranean Monk Seal, *Monachus monachus*

Monk seals are the most primitives of the seals alive today. Of the three species that survived into the recent era, the Caribbean monk seal, *Monachus tropicalis*, is extinct and the other two, *Monachus schauinslandi* and *Monachus monachus*, have minuscule populations.

The Mediterranean Monk Seal (*Monachus monachus*, Hermann 1779) has been classified by the Species Survival Commission of the World Conservation Union (IUCN) as strongly endangered since 1966. Only a few hundred individuals remain and the species is faced with extinction (Durant & Harwood, 1992; Ronald & Duguy, 1984). It is now listed as one of the worlds six most threatened mammals (IUCN, 1984).

The original range of *Monachus monachus* extended through the whole Mediterranean basin including the Black Sea up to Odessa, the Atlantic coast, the Canaries and Madeira (Panou *et al.*, 1993). At the turn of the century the species was already rare. Today, the world population of the species is estimated at 300 to 500 individuals (Borrell *et al.*, 1997) which strive, fragmented in small and probably isolated subpopulations, in the Greek and Turkish islands, the Mediterranean coast of Morocco and western Algeria, the Desertas Islands in the Madeira archipelago and the Sahara coast (Israëls, 1992). Figure 1.1. illustrates the distribution of the population and its changes during the last 25 years.

Eighty to four hundred individuals are believed to live in Greek waters (Council of Europe, 1991) and about fifty to hundred on the Turkish Mediterranean coast (Berkes *et al.*, 1979; Sergeant *et al.*, 1978). Another fifty to hundred animals may still exist along the North coast of Africa (Avellá & Gonzales, 1984; Maigret *et al.*, 1976; Rosser *et al.*, 1978). In the Black Sea a few animals may still live along the Turkish coast and a small colony perhaps exists in Bulgaria (King, 1983). The species is extinct in Spain, Italy, France, Egypt, Israel, Lebanon and the Canaries (Panou *et al.*, 1993). It is difficult to obtain reliable data from animals that are rarely seen but there is little doubt of the species decline.

The main causes for continuing decline are probably decreased natality due to human disturbance and increased mortality due to deliberate killing, mainly by fishermen, entanglement in nets, dynamite fishing (Yediler *et al.*, 1993) and in part due to exploitation for pelts, skin and oil (Panou *et al.*, 1993). There is also a fragmentation of the population and a general loss of habitat with detrimental consequences for reproduction and survival (Berkes *et al.*, 1979; Ronald & Duguy, 1984; Sergeant *et al.*, 1978). Significance of food shortage through overfishing and pollution is difficult to evaluate but they may contribute to decreased natality as well (Yediler *et al.*, 1993).

Despite the considerable effort devoted in recent decades to its protection, the overall trajectory of the species continues to be a subject of serious concern (Durant & Harwood, 1992; Israëls, 1992; Johnson & Lavigne, 1995).

In 1978 international concern for *Monachus monachus* led to the adoption of an action plan by the First International Conference on Monk Seals in Rhodes, Greece (Ronald & Duguy, 1979). Similar action plans were approved at the Second Conference in La Rochelle, France (Ronald & Duguy, 1984) and a Joint Expert Consultation in Athens (IUCN/UNEP, 1988_A).

Conservation efforts in Greece began in 1976 in the Northern Sporades (Schultze-Westrum, 1976). In 1981, a presidential decree gave complete protection to monk seals. Several studies were carried out in the Northern Sporades and a National Park was founded there in 1986. Another initiative was started in the Ionian Sea where the seal population may have a fair chance of survival (Panou *et al.*, 1993). The Greek government has since declared its intent to establish a protection zone in this region (IUCN/UNEP, 1988_A).

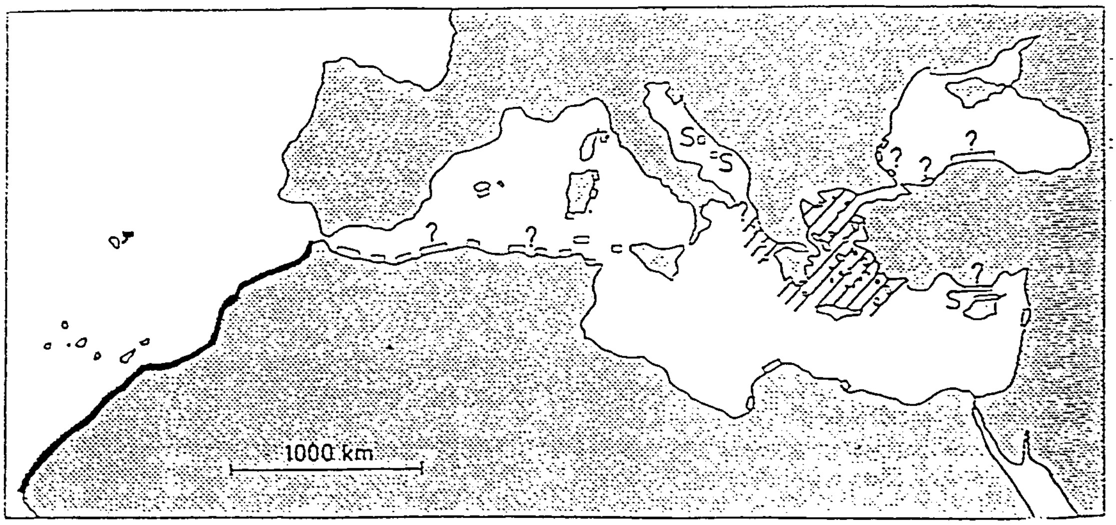


Figure 1.1.: Distribution of the Monk seal, *Monachus monachus* in the Mediterranean from 1970 till present. Solid and open bars and hatched area: estimated distribution 1970-1977 (Sergeant *et al.,* 1978). Solid bars and hatched area: estimated present distribution (Panou *et al.,* 1993). S: scattered sightings; ?: presence uncertain.

1.5. Heavy metals in the Mediterranean monk seal, *Monachus monachus*

Because of the semi-closed nature of the Mediterranean Sea and the relatively large centres of human population that impinge upon its shores, levels of marine contaminants in this ecosystem are considered to be relatively high (Bacci, 1989; Borrell *et al.*, 1997; Kuetting, 1994). As a direct consequence of this and of further human impacts on the Mediterranean, inhabiting populations of certain species, like the Mediterranean monk seal, *Monachus monachus*, have suffered from a dramatic decline (Yediler & Gucu, 1997).

Monk seals occupy a high trophic level in the food web. Their diet consists of fish, cephalopods and marine plants. Therefore they may be considered useful as an indicator species for understanding the biomagnification of heavy metals in the Greek waters and in the East Mediterranean in general.

The IUCN/UNEP (1988_B) Action Plan for the Mediterranean monk seal has stated that pollution from chemical compounds might be an important limiting factor for the species' welfare but its potential impact is difficult to assess at the present time. There are no data from the Mediterranean region about the influence of diet, age, body size or pollution on the heavy metal concentration in the monk seals. Studies on the incidence of metals on the Eastern Mediterranean subpopulations, as well as from the whole Mediterranean region in general, are not existent except one study of mercury concentrations in body hair of *Monachus monachus* (Yediler *et al.*, 1993).

Therefore further research is urgently needed to ascertain the levels of pollutant concentrations in the tissues of the various Mediterranean subpopulations of monk seals and examine the consequences on their health status.

1.6 Objectives of the Study

For the first time the concentrations of metals in the blubber and skin of Monk Seals from the Eastern Mediterranean subpopulation and the Aegean in particular, were studied and analyzed. Given the endangered status of Monk seals and the potential for heavy metals to have detrimental effects upon marine vertebrates (Rawson *et al.*, 1993), there is a clear need to augment the almost inexistent amount of data regarding heavy metal burdens in marine mammals from the Mediterranean Sea.

The aim of this study is to help in this aspect and it is hoped that this work will form an adequate reference for any future work and a motive for future potential researchers. In addition the examination of metals in relation to non-commonly used tissues of the organism is investigated in order to establish their importance in heavy metals studies.

2. Materials and Methods

2.1. Sample collection

All tissues used in this study were derived from animals of the Mediterranean Monk Seal, *Monachus monachus*, Hermann, 1779, species. Samples were obtained from seals either found dead or that died during their stay in the Seal Treatment and Rehabilitation Centre. Alonnisos, North Sporades.

Necropsies of the animals were performed according to Dierauf (1994) and Winchell (1990) and blubber and skin samples were removed for further analysis. All samples were kept immediately in ice until their storage in Mom's sample bank at a temperature of –20 ^{o}C. Tissue collection took place in the period 1994-1999. Characteristics of the animals from which each sample was obtained are listed in Table 2.1. and the exact location where each animal was found in Figure 2.1. Localities were divided into six main areas, Sporades, Cyclades, East Aegean, Southeast Aegean, North Aegean and Saronikos in order to make geographical comparisons possible. Table 2.2. provides details of the geographic division of the samples. Samples were transported the summer of 1999, preserved in dry ice, to the School of Ocean Sciences with appropriate CITES certification.

Sample Code	Date of Collection	Area of Collection	Stage of Development	Sex	State of Decomposition	Tissue Type	Collector
T 94.1	29/10/1994	Skopelos	Pup	♂	Medium	Blubber+Skin	E.Tounta
T 95.1	5/1/1995	Naxos	Juvenile	♀	None	Blubber	E.Androukaki
T 95.2	3/12/1995	N. Evia	Pup	♀	None	Blubber	E.Androukaki
T 96.1	28/1/1996	Psara	Adult	♀	None	Blubber	E.Androukaki
T 96.2	14/3/1996	Milos	Juvenile	♂	Medium	Blubber+Skin	E.Androukaki
T 96.3	15/10/1996	Piperi	Pup	♀	Medium	Blubber+Skin	E.Tounta
T 96.4	13/11/1996	Piperi	Pup	♀	None	Blubber+Skin	E.Tounta
T 96.5	12/12/1996	Fourni	Adult	♂	Advanced	Blubber+Skin	E.Androukaki
T 97.1	20/6/1997	Megara	Adult	♀	None	Blubber	E.Androukaki
T 97.2	24/9/1997	Karpathos	Adult	♂	None	Blubber	E.Androukaki
T 97.3	26/12/1997	Ikaria	Pup	♀	None	Blubber+Skin	E.Androukaki
T 98.1	28/11/1998	Nissyros	Pup	♂	Medium	Blubber+Skin	E.Androukaki
T 98.2	9/12/1998	NE Evia	Pup	♂	Advanced	Blubber+Skin	E.Androukaki
T 99.1	15/4/1999	Chios	Adult	♀	Medium	Blubber	E.Androukaki
T 99.2	2/6/1999	Potidaea	Adult	♀	Medium	Blubber	E.Androukaki
T 99.3	20/6/1999	Pteleos	Juvenile	♂	Medium	Blubber	E.Androukaki
T 99.4	22/8/1999	Milos	Adult	♀	Initial	Blubber	E.Androukaki

Table 2.1.: Information regarding the animals' characteristics and details of the samples' collection.

Figure 2.1.: Location sites where the dead or stranded animals used in the study were found.

Geographic Division	Samples ID Code					
Cyclades	T95.1	T96.2	T99.4			
East Aegean	T96.1	T96.5	T97.3	T99.1		
North Aegean	T99.2					
Saronikos	T97.1					
Southeast Aegean	T97.2	T98.1				
Sporades	T94.1	T95.2	T96.3	T96.4	T98.2	T99.3

Table 2.2.: Allocation of the samples in six geographic areas of the wider Aegean Sea.

2.2. Sample preparation

All samples were removed from the freezer and left to thaw. Porcelaine crucibles, previously washed with 10 % hydrochloric acid, were weighed and adequately labelled. Crucibles plus sample tissues were then weighed and placed in the oven at 60 ^{o}C for the water content to be removed. Crucibles with samples were weighed at certain time intervals till constant weight was achieved. Samples were afterwards placed in a muffle furnace at a temperature of 540 ^{o}C for five hours, until all the organic content was removed and black ash deposits were left. Crucibles with samples were weighed again in order for ash weight to be calculated.

1% nitric acid was used to dissolve the ash and each sample was made up to 25ml solution in acid washed 25ml glass bottles sealed with polythene caps. Solutions were stored in acid washed plastic bottles till their analysis using Inductively Coupled Plasma–Atomic Emission Spectrometry (ICP-AES).

2.3. Metal analysis of the sample solutions

All ICP-AES analyses were performed in the Institute of Environmental Science, Chemistry Tower, Bangor. Simultaneous analysis of 14 metals (Al, As, Cd, Co, Cr, Cu, Fe, Mg, Mn, Pb, Pt, Se, Si and Zn) was performed using an inductively coupled plasma-atomic emission spectrometer, ICP-AES (Jobin Yvon 138 ULTRACE). The wavelenght used for each metal are given in Table 2.3. The method of analysis used was semi-quantitative.

Copper and zinc were afterwards determined more accurately using a set of standard solutions in order to create calibration lines. 0, 2, 4, 6, 8 and 10 ppm (parts per million) Zn and 0, 0.05, 0.1, 0.2, 0.4, 0.6 ppm Cu were made up in 50 ml solutions with 1% nitric acid. The standard solutions produced values against which the concentrations of the two metals in the samples were determined quantitatively. The calibration lines for the two metals are given in Appendix I. The whole procedure was again performed in the ICP-AES. Inductively coupled plasma-atomic emission spectrometry was the method used in the only other related study conducted on this species, for the analysis of metals in hair samples (Yediler *et al.*, 1993).

Care was taken during the whole time to reduce any possible risks of contamination from any metal source that might have resulted in alterations of the original metal composition of the samples. No metal instrument or container was used at any time.

Metal	Chemical Symbol	Wavelength (nm)
Aluminum	Al	396.152
Arsenic	As	189.042
Cadmium	Cd	228.802
Cobalt	Co	228.616
Chromium	Cr	205.552
Copper	Cu	324.754
Iron	Fe	259.940
Magnesium	Mg	279.553
Manganese	Mn	257.610
Lead	Pb	220.353
Platinum	Pt	265.945
Selenium	Se	196.090
Silica	Si	251.611
Zinc	Zn	213.856

Table 2.3.: The wavelengths (in nm) used at the Inductively Coupled Plasma – Atomic Emission Spectrometer (ICP-AES) for the determination of the concentrations of the fourteen studied metals.

3. Results

3.1. Samples description

The weights of the samples, before and after placing them in the oven, are presented in Appendix II. From these values the percentage of water in the samples was calculated (Appendix II). The distribution of the values obtained was not normal (Anderson-Darling normality test; A-squared: 1.708, p-value: <0.001). The sex of the animals and the tissue type of the samples were examined in relation to the percentage water content of the samples. Means and standard deviations of the percentages are presented in Table 3.1. Sex did not appear to cause any differences in the water concentrations, as it is easily observed in Figure 3.1. Tissue type though resulted in significant differences (Mood's Median Test; chi-square: 7.35, p: 0.007) as it is also clearly discernible in Figure 3.1. Skin samples were observed to contain significantly higher concentrations of water in comparison to the majority of the blubber samples and exhibited a constant trend in all of them. The water content in the blubber samples was more variable with a number of observations similar to that of the skin samples. This could only be explained by the fact that the blubber samples were not all obtained from the same part of the animals' body and there were differences in the fat content and type. Some of the blubber samples were derived from the fat layer underlying the skin, which could account for the similarity in the water content values and indicate a possible contamination of the blubber samples with skin.

		Female seals	Male seals
% Water	Blubber	33.94 ± 25.35	36.03 ± 24.48
	Skin	61.20 ± 0.92	60.37 ± 3.37
% Ash	Blubber	1.87 ± 2.45	1.76 ± 1.80
	Skin	4.35 ± 4.13	4.83 ± 1.86

Table 3.1.: Means and standard deviations of the percentage water and ash in the *Monachus monachus* samples.

In the same way the percentage of ash in the samples remained after they were placed in the muffle furnace, was calculated (Appendix II). The distribution of the values was again not normal (Anderson-Darling normality test; A-squared: 1.901, p-value: <0.001). As with water, sex did not account for significant differences in the ash content as can be seen in Figure 3.2. and from the values in Table 3.1. while tissue type did. Skin samples resulted in greater ash concentrations for the majority of the samples with extremely noticeable variability between samples while the ash content in the blubber samples was fairly constant. This presumes that the organic content is significantly greater in the blubber, which would be expected to be the case, as blubber is very rich in fat. In addition an early assumption could be drawn regarding our expecting results in relation to the distribution of the metals in the sampled tissues. Greater metal concentrations would be expected in the skin of the animals, as they are richer in inorganic material.

When plotting percentage water against percentage ash in relation to tissue type the variability of the ash content in the skin samples and of the water content in the blubber samples is more noticeable (Figure 3.3.). The high water content of the skin samples is clearly obvious as well.

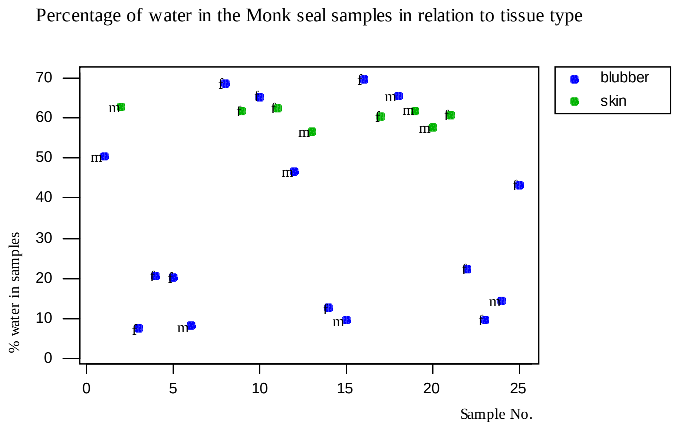


Figure 3.1.: Percentage of water in the Monk seals', *Monachus monachus*, samples in relation to sampled tissue type and sex of the animals. f: female, m: male.

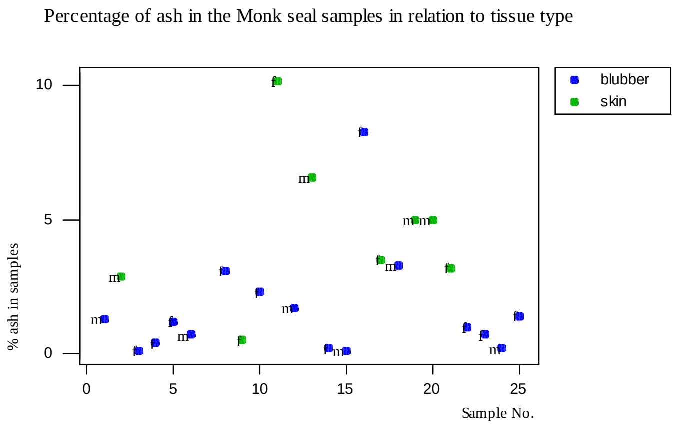


Figure 3.2.: Percentage of ash in the Monk seals', *Monachus monachus*, dry samples in relation to sampled tissue type and sex of the animals. f: female, m: male.

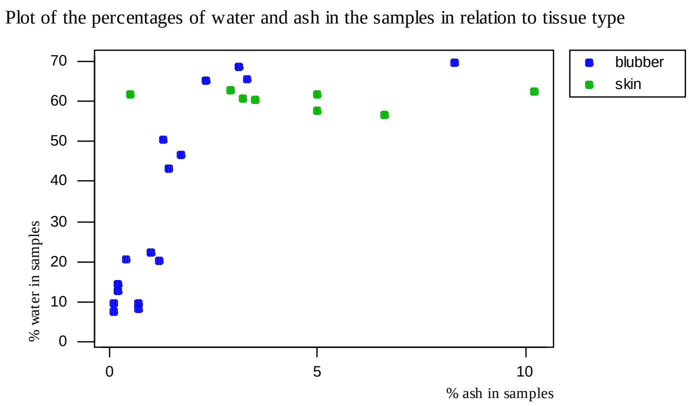


Figure 3.3.: % water against % ash in the *Monachus monachus* samples in relation to the tissue type of the samples.

3.2. Metal concentrations

Details of the concentrations of the 14 examined metals are listed in Table 3.2. Means with their standard deviations and ranges are only reported here. A printout of all concentration values is provided in Appendix III. Platinum and selenium resulted in non-detectable values for 12 of the 25 samples and it was deemed reasonable not to be used in the study.

Concentrations of metals in marine mammals have been proven in the past to be affected by a number of parameters. These could be sex, age, location, tissue type, prey type as well as changes in the environment, which take place during the years. All the parameters, for which sufficient data were available, were taken into consideration in order to determine their influence in the concentrations of metals in the animals' tissues.

All set of data obtained was not normally distributed. Principal Component Analysis (PCA) was carried out for the 12 of the examined metals in order to determine which of the above mentioned parameters poses greater influences in the concentrations of the metals. Medians were also examined in relation to some of the parameters using the Kruskal-Wallis test when significant homogeneity of variance was ascertained.

3.2.1. Sex

Means and ranges of the metals' concentrations when taking sex of the animals into account are listed in Table 3.3. Plots of all the metals in relation to sex are presented in Appendix IV. It is evident from the plots that the sex of the animals did not account for differences in the concentrations. PCA (Appendix V) carried out for the 12 metals resulted that 64% of the variability is accounted to the first two components. Therefore plotting the scores for the data in the first component against those in the second component we could represent 64% of the available information (Figure 3.4.). From the plot it could be concluded that both sexes are spread widely along the two components implying that sex does not account for a great percentage of the variability and that therefore it is not a very influential parameter.

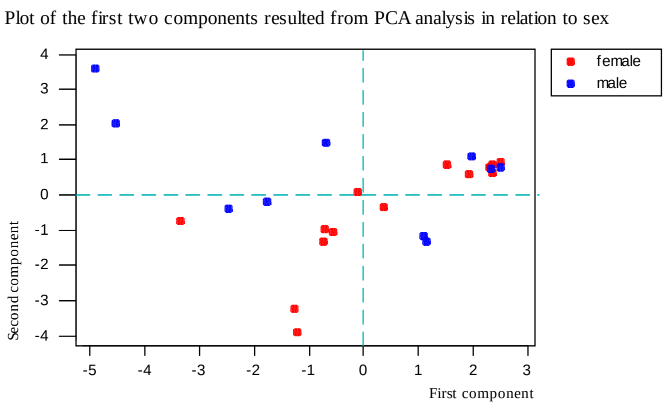


Figure 3.4.: Plot of the scores of the first two principal components derived from the PCA for the concentrations of the 12 metals.

Metal	N	Mean	Standard Deviation	Minimum Concentration	Maximum Concentration
Aluminum	25	45.291	94.005	0.729	407.341
Arsenic	25	0.438	0.466	0.000	2.089
Cadmium	25	0.090	0.107	0.003	0.491
Cobalt	25	0.088	0.134	0.000	0.487
Chromium	25	2.998	3.347	0.165	12.158
Copper	25	2.800	3.404	0.183	15.012
Iron	25	89.775	104.018	5.538	480.092
Magnesium	25	42.219	40.507	1.467	133.357
Manganese	25	2.034	3.927	0.024	16.148
Lead	25	5.875	15.380	0.000	65.319
Platinum	25	0.079	0.134	0.000	0.481
Selenium	25	0.244	0.549	0.000	2.358
Silica	25	61.672	157.949	1.582	799.197
Zinc	25	44.278	38.529	1.567	132.956

Table 3.2.: Means, standard deviations and ranges of the 14 metals in the blubber and skin samples of the Monk seals, *Monachus monachus* sampled in the period 1994-1999. Values in μg/g dry weight.

Metal	Male Seals			Female Seals		
	Mean N: 11	Min. Concentr.	Max. Concentr.	Mean N: 14	Min. Concentr.	Max. Concentr.
Aluminum	90.700	0.700	407.300	9.590	1.320	38.450
Arsenic	0.482	0.000	2.089	0.403	0.000	0.958
Cadmium	0.109	0.006	0.491	0.075	0.003	0.212
Cobalt	0.154	0.000	0.487	0.035	0.000	0.131
Chromium	3.230	0.190	12.160	2.819	0.165	10.308
Copper	2.325	0.183	8.693	3.170	0.210	15.010
Iron	122.900	5.500	480.100	63.800	5.700	144.000
Magnesium	49.300	2.900	133.400	36.650	1.470	111.930
Manganese	3.860	0.040	16.150	0.599	0.024	2.970
Lead	3.250	0.000	33.490	7.940	0.000	65.320
Silica	114.900	3.300	799.200	19.830	1.580	88.900
Zinc	37.490	2.580	77.910	49.600	1.600	133.000

Table 3.3.: Concentrations of the 12 studied metals in tissues of Monk seals in relation to sex. All values in μg/g dry weight. N: number of samples, Min.: minimum, Max.: maximum.

3.2.2. Tissue type

Mean concentration values in μg/g dry weight of all metals in the two different tissue types are presented in Table 3.4.

Levene's Test performed to examine the homogeneity of variance of the concentrations of all 12 metals resulted in significant homogeneity for arsenic (p-value: 0.116), cadmium (p-value: 0.605), cobalt (p-value: 0.341), chromium (p-value: 0.744), copper (p-value: 0.068), iron (p-value: 0.188), magnesium (p-value: 0.634), manganese (p-value: 0.117), lead (p-value: 0.118), silica (p-value: 0.115) and zinc (p-value: 0.920). The Kruskal-Wallis Test of the medians was performed after all these tests for all the above metals. H and P values are presented in Table 3.5. Aluminum was the only of the metals tested that did not exhibit significant homogeneity of variance for its concentrations (p-value: 0.043).

Arsenic, cobalt, chromium, copper, magnesium, manganese and zinc exhibited differences in their concentrations in the two different tissue types. For all of them significantly greater concentrations were reported in the skin samples of the animals sampled in contrast to the blubber samples.

The plot of the scores of the first two components from PCA with information about tissue type is presented in Figure 3.5. It could be easily observed that tissue type is responsible for the variability in the concentration values of the metals. The majority of the skin samples have negative scores for the first principal component and most of them also have negative scores for the second. It could be implied therefore that skin samples are responsible for the highest concentrations in all metals. Although the majority of the blubber samples have positive scores for both components there are a number of them with negative values. This is fairly peculiar and the only possible explanation is that these blubber samples are the same that accounted for the discrepancies in the water content of the samples indicating again contamination of the blubber samples with skin kind of tissue.

From the values of the multipliers of PC1 and PC2 (Appendix V), it could be depicted that on the first principal component the majority of metals except cadmium and lead have equally high negative values while on the second principal component two metal subgroups could be identified. The best suitable assumption that could be drawn from the latter is that metals in the same group correlate with each other. Arsenic, cadmium, chromium, copper, magnesium, lead and zinc form one of the groups and aluminum, cobalt, iron, manganese and silica the other.

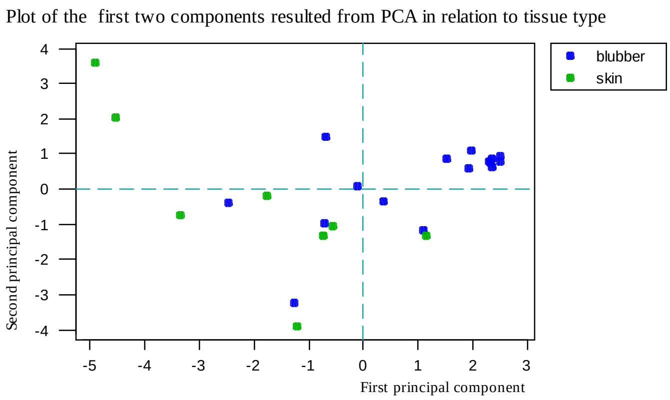


Figure 3.5.: Plot of the scores of the first two principal components derived from the PCA for the concentrations of the 12 metals with distinction between the two different sampled tissue types

Metal	Tissue Type		Stage of Development		
	Blubber N: 17	Skin N: 8	Adult N: 9	Juvenile N: 4	Pup N: 12
Aluminum	19.120	100.900	7.830	108.000	52.600
Arsenic	0.242	0.855	0.273	0.566	0.520
Cadmium	0.085	0.102	0.124	0.042	0.081
Cobalt	0.058	0.151	0.014	0.113	0.134
Chromium	2.294	4.500	1.509	3.310	4.012
Copper	1.730	5.080	1.222	1.420	4.440
Iron	67.100	138.000	39.500	129.000	114.500
Magnesium	29.190	69.900	25.910	32.200	57.800
Manganese	1.138	3.940	0.250	4.180	2.657
Lead	2.540	12.960	3.980	0.164	9.200
Silica	23.190	143.4	11.430	207.000	50.900
Zinc	29.490	75.700	21.310	22.300	68.840

Table 3.4.: Mean concentration values resulted from the *M. monachus* samples in the two tissue types and the three developmental stages. All values in μg/g dry weight. N: number of samples.

Metal	H Value	P Value	Test Assumption
Arsenic	9.90	0.002	Sign. Diff.
Cadmium	1.80	0.180	No Sign. Diff.
Cobalt	4.28	0.039	Sign. Diff.
Chromium	4.65	0.031	Sign. Diff.
Copper	5.16	0.023	Sign. Diff.
Iron	1.10	0.294	No Sign. Diff.
Magnesium	6.57	0.010	Sign. Diff.
Manganese	3.92	0.048	Sign. Diff.
Lead	1.80	0.180	No Sign. Diff.
Silica	3.70	0.055	No Sign. Diff.
Zinc	8.15	0.004	Sign. Diff.

Table 3.5.: Results of the Kruskal-Wallis tests of the medians regarding differences in the concentrations of metals in relation to sampled tissue type of the Monk Seals, *Monachus monachus*. Sign. Diff.: significant difference, No Sign. Diff.: no significant difference.

3.2.3. Stage of Development of the Animals

The exact age of each animal sampled was not provided. Instead they were divided in three developmental stages, pups, juveniles and adults. The stage of development at which each animal was found is reported in Table 2.1. Mean values of all metals studied in the three different stages are presented in Table 3.4.

The homogeneity of variance was examined for the concentrations of every metal in the tissues. All metals exhibited significant homogeneity of variance of their values (p-values**:** Al: 0.164, As: 0.379, Cd: 0.501, Co: 0.162, Cr: 0.364, Cu: 0.200, Fe: 0.258, Mg: 0.694, Mn: 0.181, Pb: 0.555, Si: 0.086, Zn: 0.637). The Kruskal-Wallis Test of the medians being the appropriate test followed the above results. Values obtained and assumptions derived from this test are summarized in Table 3.6.

Principal component analysis was again utilized to eliminate some of the error associated with testing the medians and to allow the best visualization of the differences. From the plot of the scores of the first two principal components is clearly evident that samples derived from pups were responsible for the highest concentrations of all metals (Figure 3.6.).

Metal	H-Value	P-Value	Test Assumption
Aluminum	3.700	0.157	No Sign. Diff.
Arsenic	3.370	0.186	No Sign. Diff.
Cadmium	1.970	0.373	No Sign. Diff.
Cobalt	5.770	0.056	No Sign. Diff.
Chromium	4.280	0.117	No Sign. Diff.
Copper	9.370	0.009	Sign. Diff.
Iron	7.210	0.027	Sign. Diff.
Magnesium	6.010	0.050	Sign. Diff.
Manganese	8.670	0.013	Sign. Diff.
Lead	1.610	0.448	No Sign. Diff.
Silica	6.110	0.047	Sign. Diff.
Zinc	9.690	0.008	Sign. Diff.

Table 3.6.: Statistical results of the Kruskal-Wallis tests of the medians utilized to investigate the potential differences in the concentrations of metals due to the different stages of development of the Monk seals used in our study. Sign. Diff.: significant differences, No Sign. Diff.: no significant differences.

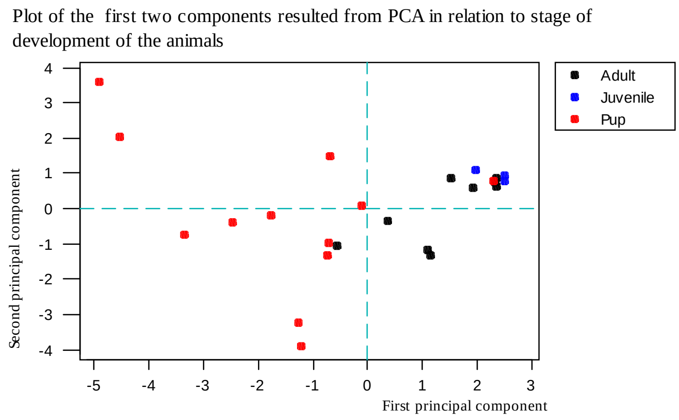


Figure 3.6.: Plot of the scores of the first two principal components derived from the PCA for the concentrations of the 12 metals with distinction between the three different developmental stages of the animals.

3.2.4. Temporal Differences

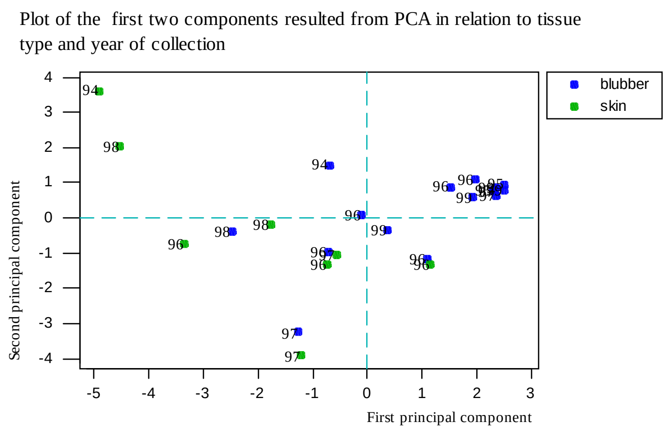
The collection of the samples took place in the period 1994 to 1999. Differences in the metals concentrations in the tissues of the animals during that period were examined and mean concentrations are reported in Table 3.7. Changes in the concentrations of metals form a useful aid in the understanding of the general environmental status, in terms of pollution, of the area, the Aegean Sea in particular in our study. The plot of the first two components of PCA shows that the years are widely spread and there are no distinctive differences in the metals concentrations between them (Figure 3.7). Plots of every metal's concentrations against the different sampling years in relation to the tissue type of the samples are presented in Appendix VI. Some differences in concentrations during the period of collection are observable although the difference in the tissue type could explain the majority of them. The difference in the number of samples obtained every year does not allow very accurate assumptions as well.

Metal	1994 N: 2	1995 N: 2	1996 N: 9	1997 N: 5	1998 N: 3	1999 N: 4
Aluminum	177.100	1.615	58.200	9.240	65.400	2.229
Arsenic	0.313	0.070	0.529	0.539	0.653	0.192
Cadmium	0.059	0.004	0.117	0.115	0.083	0.063
Cobalt	0.174	0.004	0.084	0.030	0.298	0.009
Chromium	0.657	0.186	4.340	3.640	4.310	0.762
Copper	5.530	0.741	4.090	2.610	1.930	0.445
Iron	182.400	11.960	113.500	40.100	144.000	50.500
Magnesium	18.030	3.670	50.500	34.500	104.200	18.200
Manganese	5.330	0.061	2.510	0.426	4.900	0.167
Lead	N.D.	0.066	5.090	19.900	0.281	0.168
Silica	106.200	1.785	116.800	17.430	57.000	4.237
Zinc	49.670	6.690	48.900	61.100	60.030	17.000

Table 3.7.: Mean concentrations of metals in the tissues of Monk Seals reported for the different sampling years. Values in μg/g dry weight. N.D.: Not Detectable.

3.2.5. Geographical Differences

Geographic location might result in differences in pollutant concentrations in marine mammals' tissues. The concentrations of all metals analyzed in our study were examined for any such differences between the six different geographic locations in which the wider area of sample collection was divided (Table 2.2.). The plot of the PCA components (Figure 3.7.) suggests that the Cyclades, North Aegean and the majority of the East Aegean samples contributed the lowest concentrations of all samples. When taking into consideration the type of tissue as well though, it is obvious that all these samples are blubber which explains the lower metal concentrations. Therefore we cannot assume any geographical differences and analysis of more samples of both tissue types from all areas would be helpful in detecting any possible differences.



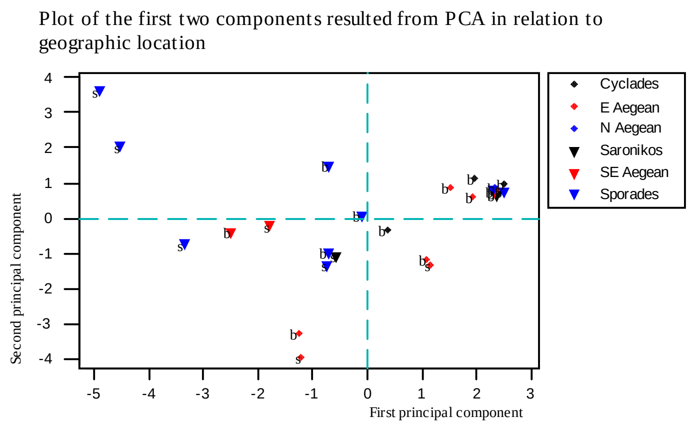


Figure 3.7.: Plot of the scores of the first two principal components derived from the PCA for the concentrations of the 12 metals with distinction between the different sampling years and the tissue type and the different geographic locations and tissue type. b: blubber, s: skin.

3.3. Quantitative Results of Copper and Zinc

3.3.1. Comparison of the two spectrometry methods employed

Copper and zinc were determined practicing two different procedures in the ICP-AES. The first one was the semi-quantitative, which was also used for the determination of all the other metals, and the other was the quantitative where known standard solutions were put to use. Concentrations obtained from both these methods are listed in Appendix VII. These two methods were investigated in terms of their resulted values in order to study the degree of difference, if any, between them.

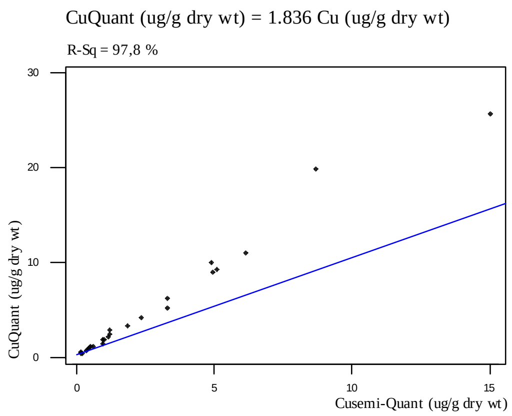
All of the resulted sets of values were not normally distributed. The homogeneity of variance was subsequently examined using Levene's test and concluded in significant homogeneity for both copper and zinc (copper, p-value:0.141; zinc, p-value:0.764). The latter allows for the most powerful non-parametric two-sample test to be used. That is the Mann-Whitney test and outputs of the tests outcomes are presented in Table 3.8. The W values with each associated probabilities state that there is no significant difference between the two methods.

Metal	Method	N	Median (μg/g dry wt)	95% CI H1-H2	W	Probability
Copper	Semi-quantitative	25	1.215	-2.902, 0.175	557.00	0.121
	Quantitative	25	2.418			
Zinc	Semi-quantitative	25	43.960	-14.110, 23.450	662.00	0.642
	Quantitative	25	36.080			

Table 3.8.: Statistical results of the Mann-Whitney test performed to examine any potential differences between the two different methods of analysis. H: Median.

This assumption also renders a significant credibility in the semi-quantitative method that suggests that the results obtained for all the other metals could be considered accurate in the 95% confidence intervals. The one problem though is that the Mann-Whitney test employed is non-parametric and not quite as powerful as the relevant parametric Two-Sample T-Test. Normality of the concentrations would have resulted in more accurate assumptions.

In order to be more certain of the above test's results the semi-quantitative values were plotted against the quantitative ones for both copper and zinc and the equations of the fitted lines were calculated (Figure 3.8.). Quantitative copper values were proven to be greater than the values obtained from the semi-quantitative method by almost a factor of 2. Zinc values on the other hand did not differ between the two methods. It can be concluded therefore that the method of analysis could be important for some metals while it does not have any effect on others. Performing the quantitative analysis on the rest of the examined metals would have been beneficial for the explicitness of the concentrations values resulted from our study.



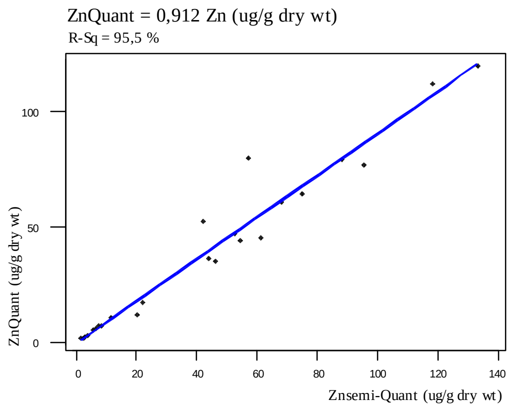


Figure 3.8.: Relationship of the semi-quantitative and quantitative results for copper and zinc. Values in µg/g dry weight. Blue lines indicate the 100% precision between the two methods.

3.3.2. Copper and Zinc Association

The relationship between the two metals was investigated. Firstly, the correlation was determined. The Pearson's correlation coefficient was calculated to be 0.686 with a p-value of <0.001. Both variables though, were dependent and did not fit assumptions for single linear regression. In addition when metals' concentrations plotted against each other there was an obvious curve and great variability. Instead copper and zinc concentrations were transformed to the logarithmic scale and the Geographic Mean Regression was utilized. Graphical presentation of the Log values for the two metals is presented in Figure 3.9. The Pearson's correlation coefficient for the Log values was 0.815 and the p-value <0.001. The plot of the standardized residuals versus the fits postulated a good fit to a straight line and the resulted equation for the geographic mean regression line was

$$\text{LogCu } (\mu g/g \text{ dry wt}) = -0.736 + 0.882 \times \text{LogZn } (\mu g/g \text{ dry wt}).$$

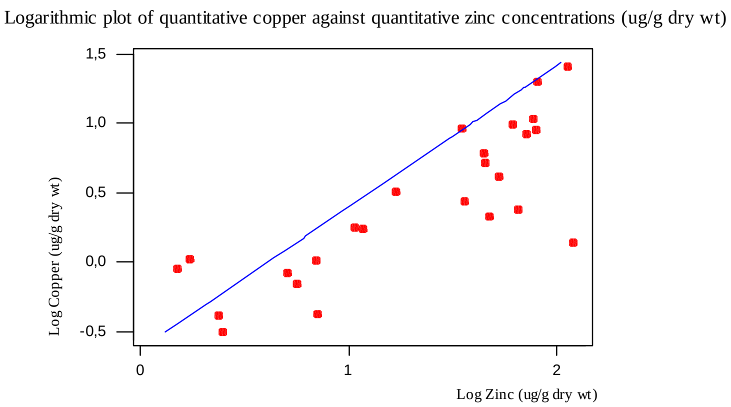


Figure 3.9.: Logarithmic plot of the relationship between quantitative copper and zinc concentrations (µg/g dry weight).

4. Discussion

4.1. Metal Concentrations

In order to establish baseline data on environmental pollutant levels in seal species it is advisable to assess and standardize the ages and sexes of animals being taken, because environmental pollutant residues may be concentrated and metabolized differently depending on age, sex and reproductive condition of individual animals (Shaughnessy, 1993), as well as any additional information regarding the animal itself or the sampling.

Total heavy metal concentrations are by themselves insufficient to assess either the health status of the animals or the effective toxicity of the toxicant in question. Yet, they are important and necessary as they can provide a benchmark for what is to be regarded as a 'normal' or 'abnormal' heavy metal burden for animals (Wagemann & Muir, 1984). The assessment of the toxicity of these metals to marine mammals requires additional information, like consideration of the antagonistic and synergistic influences by other metals and the speciation of metals in tissues. Possible effects from sublethal levels of metals on neurological and immunological functions and behaviour of marine mammals themselves, have not been studied and the health implications for the animals are unknown (Wagemann & Stewart, 1994).

Recent investigations have shown that changed uptake and distribution of essential and non-essential elements occur during an infectious disease such as a common viral infection. Therefore, it seems reasonable to assume that activation of the immune system and associated inflammatory tissue lesions may cause changes in the uptake and distribution of both trace elements and environmental pollutants during the course of infections (Frank *et al.*, 1992).

Extremely limited data regarding metal concentrations in monk seals are available (Borrell *et al.*, 1997; Menchero *et al.*, 1994). As a consequence, data from the Mediterranean region about the influence of diet, age, body size or pollution on the heavy metal concentrations in seals are non-existent. The former render this study very difficult to comment and evaluate and very important in terms of essential information about the species and the area. Larger sample sizes and comparative data from polluted and unpolluted regions are required to help assess the possible contribution of pollution to the vitality of Mediterranean Monk Seals.

4.1.1. Aluminum

Aluminum values in the Monk seals ranged from 0.729 to 407.341 μg/g dry weight with a mean value of 45.291 μg/g dry weight. Reports of aluminum in marine mammals are scarce, so it is not possible to provide comparisons with this study. However samples had been preserved in foil at Mom's sample bank which could account for the highest of the resulted concentrations.

4.1.2. Arsenic

Arsenic has previously been determined in only a few species of marine mammals like harbour seals, common dolphins and pilot whales, with the overall range of concentrations reported being 0.036 to 10.44 μg/g dry weight (Law, 1996). The concentrations of arsenic obtained from our study are towards the lower end of this range with values <0.001 – 2.089 μg/g dry weight and a mean value of 0.438 μg/g dry weight. Greater values have been reported in the blubber of Grey seals, *Halichoerus grypus*, from the Swedish waters with concentrations of 7.56 – 18.36 μg/g dry weight (Frank *et al.*, 1992). Concentrations reported in the liver of whales and dolphins were of the range 2.1-9.0 μg/g dry weight (Kuiken *et al.*, 1994; Law *et al.*, 1997) which are within the above mentioned overall range.

The toxicological importance of arsenic is partly due to its chemical similarity with phosphorus, which means that arsenic can disrupt metabolic pathways involving phosphorus (Alloway & Ayres, 1993).

4.1.3. Cadmium

Cadmium concentrations obtained from our study were very low with a mean concentration of 0.090 μg/g dry weight. However, cadmium concentrates primarily in the kidney (Beck *et al.*, 1997; Goldblatt & Anthony, 1983; Shaughnessy, 1993) and levels would be expected to be very low in blubber (Bard, 1999) and skin.

Some values of cadmium concentrations previously reported in the liver of seals were around 110 μg/g dry weight in Ross seals, *Ommatophoca rossi*, (McClurg, 1984) and 5 μg/g dry weight in Steller sea lions, *Eumetopias jubata*, (Hamanaka *et al.*, 1982). In marine mammals from Cardigan Bay all values were reported to be below 0.36 μg/g dry weight (Morris *et al.*, 1989). Skin values previously reported were 0.007-0.03 μg/g dry weight in Beluga whales, *Delphinapterus leucas*, 0.148 μg/g dry weight in ringed seals, *Phoca hispida* and 0.065 μg/g dry weight in Narwhals, *Monodon monoceros* from the Arctic area (Muir *et al.*, 1999) which are comparably low with the concentrations obtained from our samples.

Cadmium in hair samples of Monk seals from the Ionian Sea was reported to be less than 1 μg/g dry weight (Yediler *et al.*, 1993). Another related study was of marine turtles from Northern Cyprus, Eastern Mediterranean. Median values reported in the muscle of turtles from northern Cyprus were 0.57 μg/g dry wt in Loggerhead turtles, *Caretta caretta* and 0.37 μg/g dry wt in green turtles, *Chelonia mydas* (Godley *et al.*, 1999).

4.1.4. Cobalt

Cobalt concentrations ranged from zero to 0.487 μg/g dry weight with six of the samples resulting in non-detectable values. 50% of the concentrations were below 0.035 μg/g dry weight. No data were found in the literature regarding cobalt concentrations in tissues of marine mammals.

4.1.5. Chromium

Levels of chromium reported in marine mammals are usually below 3.5-3.6 μg/g dry weight (Thompson, 1990). The mean concentration obtained from our samples is 2.998 μg/g dry weight with the 75% of the observations laying between 0.165 and 5.298 μg/g dry weight, which is relatively in accordance with the above values.

The toxicity of Cr is dependent on the speciation of the metal, with hexavalent Cr being much more toxic than trivalent Cr (Law *et al.*, 1992).

4.1.6. Copper

Copper values ranged from 0.183 to 15.012 μg/g dry weight. However, as we concluded that the concentrations obtained from the semi-quantitative method were almost half of these obtained from the quantitative analysis we will discuss only the quantitative values in Chapter 4.3.

4.1.7. Iron

The mean iron concentration obtained from our samples was 89.775 μg/g dry weight with a range of the values of 5.538 to 480.092 μg/g dry weight. Mean concentrations were 67.100 and 138.000 μg/g dry weight in the blubber and skin respectively. Iron concentrations in the blubber, hair and skin of Baikal seals, *Phoca sibirica*, from the Lake Baikal were 7.2, 130.0-200.0 and 150.0-230.0 μg/g dry weight respectively (Watanabe *et al.*, 1996). The latter values though, were derived from only two adult seals which makes the comparison of the results with our values not liable.

Iron levels in marine mammals can be high up to 1000 μg/g, which is apparently a physiological adaptation associated with their deep-diving behaviour (Sydeman & Jarman, 1998). High Fe concentrations have been recorded in Weddell seals, *Leptonychotes weddellii* (Szefer *et al.*, 1994; Wagemann & Muir, 1984), Crabeater seals, (Szefer *et al.*, 1994), ringed seals, *Phoca hispida* (Frank *et al.*, 1992) and Baikal seals, *Phoca sibirica* (Watanabe *et al.*, 1998). All animals belonging to the above species are known to have good diving ability. The relationship between iron concentration and diving ability was also reported in sea birds (Honda *et al.*, 1990). Monk seals are very competent divers that could account for the reasonably high iron concentrations reported in their tissues.

4.1.8. Magnesium

Magnesium concentrations ranged from 1.467 to 133.357 μg/g dry weight with 50% of the reported values below 34.355 μg/g dry weight.

4.1.9. Manganese

Mean manganese concentrations in the blubber, hair and skin of two adult Baikal seals, *Phoca sibirica*, were 0.18, 5.15 and 1.60 μg/g dry weight respectively (Watanabe *et al.*, 1996). In the tissues of Monk seals mean concentrations were 1.138 and 3.940 μg/g dry weight in the blubber and skin respectively. Our sample size though was a lot bigger than the above study's and it consisted of animals of different ages. Younger animals are expected to possess greater concentrations that will also lead in greater mean concentrations.

4.1.10. Lead

The Monk seal samples exhibited concentrations for lead ranging from non-detectable to 65.319 μg/g dry weight. Six of the samples resulted in non-detectable values. 75% of the concentrations were below 0.867 μg/g dry weight. Noticeably high concentrations were reported for two of the animals sampled. These were the seals with the sample codes T 96.5 and T 97.3. Both these animals were found in the sea area of an island complex in the Eastern Aegean. The first was an adult male and the other a pup female. Concentrations obtained from these animals were respectively 0.642 and 33.133 μg/g dry weight in blubber and 33.492 and 65.319 μg/g dry weight in skin. This could imply a problem of lead pollution in this area and further study is of great need. No more samples have been collected from this area since 1997 and therefore it is essential to investigate the present situation of the area and of the inhabiting organisms as lead is a serious cumulative body poison.

Lead values reported for ringed seals, *Phoca hispida*, in the Greenland area range from below 0.004 to 0.015 μg/g dry weight (Dietz *et al.*, 1996) which are lower than our median value of 0.333 μg/g dry weight. Moreover, values reported in Ross seals liver in Antarctica were less than 0.1 μg/g dry weight (McClurg, 1984) while those in California sea lions, *Zalophus californianus* were around 1.5 μg/g dry weight (Braham, 1973).

Concentrations of lead have been reported to decrease with increasing trophic level, thereby reflecting a bio-depletion of lead (Sydeman & Jarman, 1998).

4.1.11. Silica

Silica concentrations ranged from 1.582 to 799.197 μg/g dry weight with the 75% of the concentrations below 39.581 μg/g dry weight. The higher end of the ranges is attributed to three samples that exhibited noticeably higher concentrations in comparison with the rest of the samples. These high concentrations derived from three male seals, two pups and one juvenile. The highest silica value belonged to the juvenile, which was found in the Cyclades area. Silica is a very abundant component of sand and the highest of the concentrations obtained could be attributed to contamination of the samples with sand. It was also observed that in these animals Al, Fe and Mg concentrations also resulted in very high values that indicates a strong correlation between these metals in nature.

4.2. Parameters' Influence

4.2.1. Sex

Sex was not observed to account for any differences in the concentrations as well as in the water and ash percentage of the samples. Usually differences in metal concentrations are related to parturition and lactation in females. These are excretory routes resulting in lower concentrations in females (Watanabe *et al.*, 1996; 1998).

4.2.2. Tissue Type

Concentrations of heavy metals have been measured in a variety of tissues of pinnipeds but primarily in liver, kidney and muscle where they tend to accumulate (Holden, 1978) and less frequently in brain and blubber (Shaughnessy, 1993). In our study though, the blubber and skin was investigated. In the case of blubber, although it consists a great percentage of total body weight, extremely low concentrations of trace metals have been reported in the past (Fujise *et al.*, 1988; Honda *et al.*, 1982; Watanabe *et al.*, 1996).

From the 12 metals examined arsenic, cobalt, chromium, copper, magnesium, manganese and zinc resulted in differences in their concentrations between the two tissues when testing the medians of their values. Concentrations for all of them were considerably greater in the skin. Principal component analysis resulted that tissue type accounted for differences in the concentrations for all metals studied with the highest concentrations observed in the skin.

Lead and zinc are reported in greater quantities in the hard tissues of the animal's body like skin (Muir *et al.*, 1999) and bones. However in other studies, like in ringed seals, *Phoca hispida* from the Greenland area no differences in lead concentrations were reported between the different tissues examined (Dietz *et al.*, 1996).

A large proportion of metals, like Cu, Mn and Zn is reported in the hair of seals (Watanabe *et al.*, 1996). The presence of small hair amounts in some of the skin samples could explain the significantly greater concentrations that resulted for these metals in our skin samples in comparison to the blubber ones. Hair could also retain sand particles, which could account for the extremely high silica and iron concentrations of some samples.

4.2.3. <u>Stage of Development</u>

Copper, iron, manganese, silica and zinc concentrations were significantly greater in pups decreasing as the animals aged in the Monk seals. The same was proven for copper, iron and zinc in the blubber of Weddell seals, *Leptonychotes weddellii* (Yamamoto *et al.*, 1987). Increase in iron concentrations with decreasing age has also been demonstrated in the tissues of marine mammals such as striped dolphins (Honda *et al.*, 1983), northern fur seals (Noda *et al.*, 1995) and Baikal seals (Watanabe *et al.*, 1998). No information regarding manganese and silica concentrations in relation to age was available.

Distribution of heavy metals in newborn seals could be different in some extent from that in adults, rendering the consideration of growth stage of the animals basic in order to understand the bioaccumulation processes of metals and their physiological and toxicological effects on the animals (Yamamoto *et al.*, 1987).

4.2.4. Temporal Differences

No evident differences were found in the metals' concentrations during the different years of collection. However, the differences in sample sizes and the very small sample sizes obtained for some years, renders the comparisons difficult. The influence of the other factors, like tissue type and stage of development, makes even more complicated the analysis. Greater sample number with less variability of the other parameters would be preferable in any future studies.

4.2.5. Geographical Differences

Variations of metal levels in seal stocks are likely to result from differences in their feeding behaviour and their geographic distribution (Heppleston, 1973; Sergeant & Armstrong, 1973).

The conclusions drawn from our results are that the different locations of sample collection could not account for differences in the concentrations of metals and that the environmental state of the wider area could be considered unvarying.

4.3. Quantitative Analysis of Copper and Zinc

Copper and zinc in the tissues of Monk seals, *Monachus monachus* are discussed using the quantitative results.

Copper and zinc concentrations reported in hair samples of Monk Seals in the only relative study ranged from 9.93 to 20.4 μg/g dry weight for Cu and from 100.0 to 170.0 μg/g dry weight for Zn (Yediler *et al.*, 1993). The only other study found in the literature regarding these two metals in seal hair was that of Watanabe *et al.* (1996) with comparable values. However, these data were comparable to Cu and Zn levels in human hair (Wilhelm & Ohnesorg, 1990). This could be due to the fact that both in seals and humans Cu and Zn are regulated in organs as well as in the hair (Wagemann, 1989; Wagemann *et al.*, 1988).

4.3.1. Copper

Concentration values of copper reported in the past in different tissues of marine mammals are presented in Table 4.1. The mean copper concentration obtained from our study is 5.173 μg/g dry weight that is towards the lower end of the ranges tentatively assigned (Law *et al.*, 1991; Law, 1996). The range of our results is 0.312 to 25.710 μg/g dry weight.

Significant differences were observed between concentrations in blubber and skin. Mean and median concentrations of copper are 3.082 and 1.741 μg/g dry weight in blubber and 9.616 and 7.225 μg/g dry weight in skin.

Previous studies have reported a negative correlation between age and copper concentrations in ringed seals, *Phoca hispida*, walruses, *Odobenus rosmarus rosmarus* and narwhals, *Monodon monoceros* (Wagemann, 1989; Wagemann & Stewart, 1994; Wagemann *et al.*, 1983). The same was observed in our study as well, with pups having significantly higher concentrations than adult monk seals. In general, copper concentrations in tissues of marine invertebrates tend to decrease with increasing age of the organism (Law *et al.*, 1992).

Generally copper is found in low abundance in marine mammals with values less than 44 μg/g dry weight in all tissues except liver (Eisler, 1998), but elevated levels have been associated with premature parturition in some sea lion species (Martin *et al.*, 1976). Copper is essential in the reproductive development of mammals and it has been observed that Cu concentrations in the liver of seals decrease with increasing lactation period (Watanabe *et al.*, 1996). No documented report of fatal copper deficiency is available for any species of aquatic organism (Eisler, 1998).

4.3.2. Zinc

Zinc concentrations ranged from 1.503 to 119.892 μg/g dry weight. The mean was 39.929 μg/g dry weight and 50% of the samples resulted in values less than 36.082 μg/g dry weight.

Zinc, as mentioned before, exhibits higher concentrations in the skin, which have been proven in the past about a number of marine mammal species. Some of them are common seals, *Phoca vitulina* (Roberts *et al.*, 1976), Baikal seals, *Phoca sibirica* (Watanabe *et al.*, 1996), Weddell seals, *Leptonychotes weddellii* (Yamamoto *et al.*, 1987), California sea lions, *Zalophus californianus* (Braham, 1973), striped dolphins, *Stenella coeruleoalba* (Honda *et al.*, 1982) and Dall's porpoises, *Phocoenoides dalli* (Fujise *et al.*, 1988). Bard (1999) in her study reports that zinc was concentrated three times higher in the skin of beluga whales, *Delphinapterus leucas* than in other organs.

Zinc concentrations were significantly higher in pups compared to adult and juvenile Monk seals. Previous researchers though, have found no significant correlation between zinc concentrations and age in harbour seals, *Phoca vitulina* (Drescher *et al.*, 1977; Law *et al.*, 1991) and in northern fur seals, *Callorhinus ursinus* (Goldblatt & Anthony, 1983). Most of the previous studies were examining concentrations in the liver of the animals while our study was about blubber and skin. This difference might explain the discrepancy between the conclusions drawn.

Other researchers though have reported zinc values to exhibit positive correlation with age, both in body tissue (Heppleston & French, 1973) and body hair (Wenzel *et al.*, 1993). This positive correlation, that have been observed in other metals as well, indicates that accumulation of these compounds in the tissues and body hair of seals is consistent throughout their life.

The highest zinc concentrations in the samples were reported in the skin of pups which combines the above observations for tissue type and stage of development.

Species	Tissue type	Copper	Zinc	Source
Delphinapterus leucas	Skin	1.8	274.0	Muir *et al.*, 1999
Eumetopias jubata	Liver	-	140.0	Hamanaka *et al.*, 1982
Halichoerus grypus	Liver	6.0-48.0*	64.0-94.0*	Holden, 1975_B
		13.0-94.0	90.0-321.0	Law *et al.*, 1991
		8.0-280.0	54.0-540.0	Law *et al.*, 1992
	Blubber	0.36	17.0	Morris *et al.*, 1989
Monachus monachus	Hair	9.9-20.4	100.0-170.0	Yediler *et al.*, 1993
	Blubber	0.312-10.901	1.504-79.732	This study
	Skin	1.371-25.710	16.928-119.892	
Monodon monoceros	Skin	1.22	233.0	Muir *et al.*, 1999
Ommatophoca rossi	Liver	80.0-90.0	220.0	McClurg, 1984
Phoca hispida	Skin	3.9	97.0	Muir *et al.*, 1999
Phoca sibirica	Blubber	0.12	2.3	Watanabe *et al.*, 1996
	Skin	2.5-3.3	36.0	
	Hair	23.0	125.0	
Phoca vitulina	Blubber	3.2-10.8	10.8-50.0	Duinker *et al.*, 1979
		-	14.0-47.0	Holden, 1975_B
	Liver	9.0-23.0*	43.0-84.0*	
		13.7-93.6	90.0-320.0	Law *et al.*, 1991
		8.0-285.0	54.0-540.0	Law *et al.*, 1992
Phocoena phocoena	Blubber	2.2	14.0	Morris *et al.*, 1989
	Skin	3.5	-	Paludan-Muller *et al.*, 1993
Physeter macrocephalus	Muscle	1.3-4.3	133.0-237.0	Holsbeek *et al.*, 1999
	Liver	5.3-11.9	90.0-125.0	
	Kidney	13.0-44.0	44.0-140.0	
	-	8.28	122.4	Law *et al.*, 1996
Stenella coeruleoalba	Blubber	1.8	60.0	Morris *et al.*, 1989
Tursiops truncatus	Blubber	3.6	72.0	Morris *et al.*, 1989

Table 4.1.: Copper and zinc concentrations in different tissues of marine mammals reported in the literature. All values in µg/g dry wt. (*µg/g tissue)

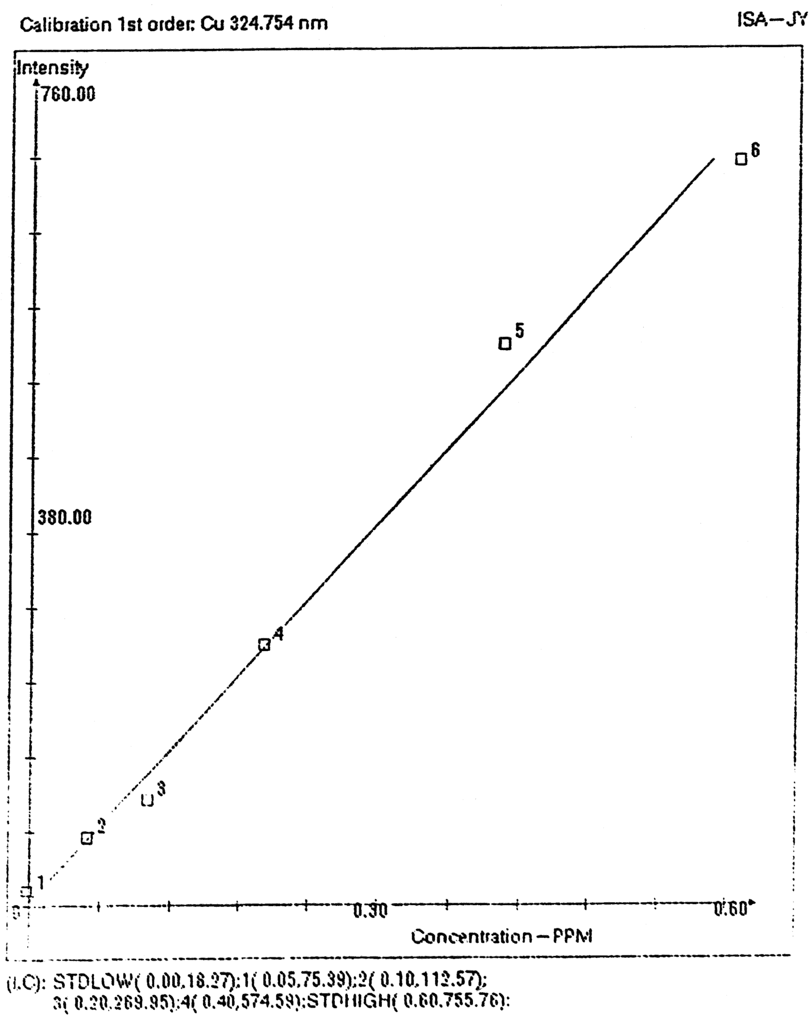
4.3.3. Copper and Zinc Association

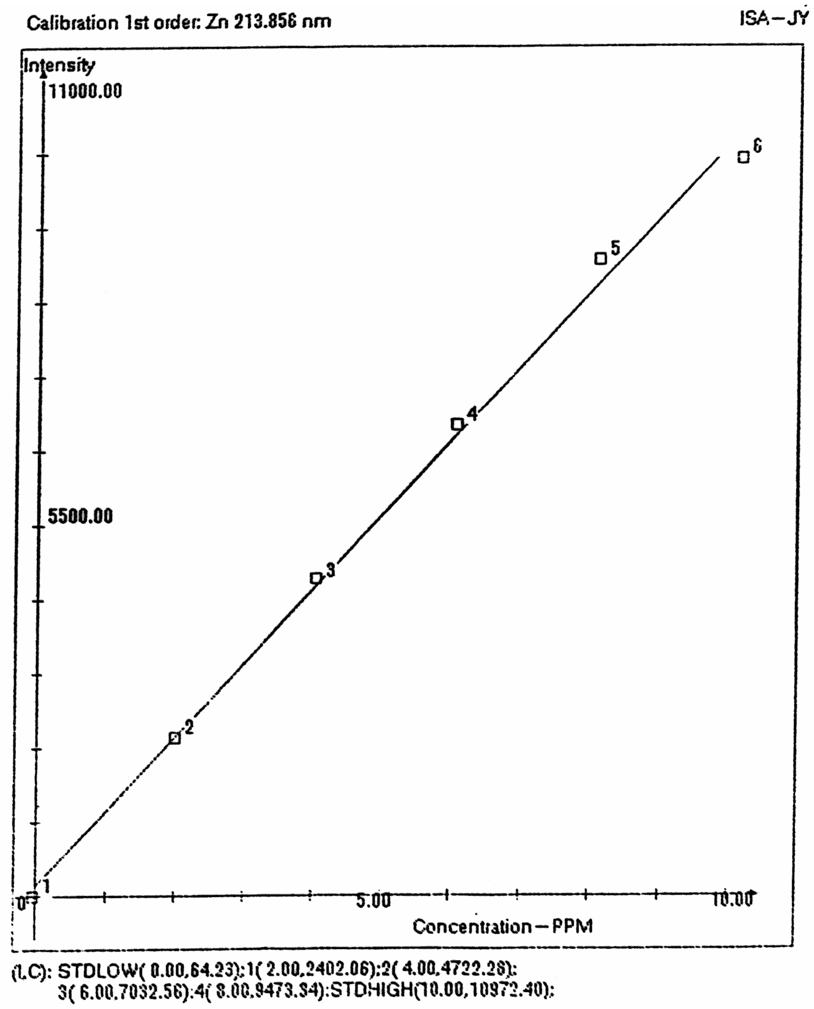
Our values exhibited a significant correlation between the two metals. Significant positive interelement correlation between copper and zinc was also reported in the liver of bowhead whales, *Balaena mysticetus*, from the Alaska region (Krone *et al.*, 1999). The same was the case in livers of Weddell seals, *Leptonychotes weddellii* (Szefer *et al.*, 1994). This could be of importance as changes in the concentrations of one of the metals could reflect the changes that might occur in the other as well. The mechanisms underlying the association of copper and zinc are largely unknown but it is speculated that this association is probably mediated by metallothionein as these metals are inductors and constituents of this metalloprotein (Wagemann & Stewart, 1994).

5. Appendices

5.1. Appendix I

The calibration lines produced by the Inductively Coupled Plasma Atomic Emission Spectrometer for Copper and Zinc. The concentrations are of the standard solutions prepared in the laboratory. The resulted intensity values were used for the quantitative determination of copper and zinc concentrations in the tissue samples under investigation.




Calibration 1st order: Zn 213.856 nm
ISA–JY
Intensity
11000.00
5500.00
0
1
2
3
4
5
6
5.00
10.00
Concentration – PPM
(LC): STDLOW(0.00,64.23):1(2.00,2402.06):2(4.00,4722.28):
3(6.00,7032.56):4(8.00,9473.34):STDHIGH(10.00,10972.40):

5.2. Appendix II

Wet, Dry and Ash Sample Weights (g) and Related Percentages (%) of the Monachus monachus Samples						
Sample Code	**Sex**	**Wet Weight**	**Dry Weight**	**Ash Weight**	**% Water**	**% Ash**
T94.1 b	♂	0.7951	0.3939	0.0053	50.4591	1.3455
T94.1 s	♂	1.5420	0.5746	0.0166	62.7367	2.8890
T95.1 b	♀	12.6056	11.6747	0.0166	7.3848	0.1422
T95.2 b	♀	17.0480	13.5113	0.0489	20.7455	0.3619
T96.1 b	♀	13.9353	11.1222	0.1356	20.1869	1.2192
T96.2 b	♂	15.8160	14.5366	0.1085	8.0893	0.7464
T96.2 s	♂	1.2244	0.6103	0.1017	50.1552	16.6639
T96.3 b	♀	5.2263	1.6477	0.0506	68.4729	3.0709
T96.3 s	♀	1.3476	0.5165	0.0028	61.6726	0.5421
T96.4 b	♀	1.2096	0.4187	0.0096	65.3853	2.2928
T96.4 s	♀	2.4941	0.9401	0.0960	62.3070	10.2117
T96.5 b	♂	0.9658	0.5142	0.0088	46.7592	1.7114
T96.5 s	♂	3.3839	1.4720	0.0966	56.4999	6.5625
T97.1 b	♀	11.1307	9.7290	0.0234	12.5931	0.2405
T97.1 s	♀	5.4968	2.1636	0.0693	60.6389	3.2030
T97.2 b	♂	10.0400	9.0833	0.0066	9.5289	0.0727
T97.3 b	♀	1.1612	0.3541	0.0295	69.5057	8.3310
T97.3 s	♀	2.2567	0.8956	0.0315	60.3137	3.5172
T98.1 b	♂	1.1544	0.3990	0.0137	65.4366	3.4336
T98.1 s	♂	3.2231	1.2271	0.0617	61.9280	5.0281
T98.2 b	♂	3.6365	1.5451	0.0781	57.5113	5.0547
T99.1 b	♀	11.0248	8.5692	0.0837	22.2734	0.9768
T99.2 b	♀	14.2848	12.9149	0.0900	9.5899	0.6969
T99.3 b	♂	5.2070	4.4500	0.0095	14.5381	0.2135
T99.4 b	♀	4.1118	2.3306	0.0332	43.3192	1.4245

b: blubber
s: skin

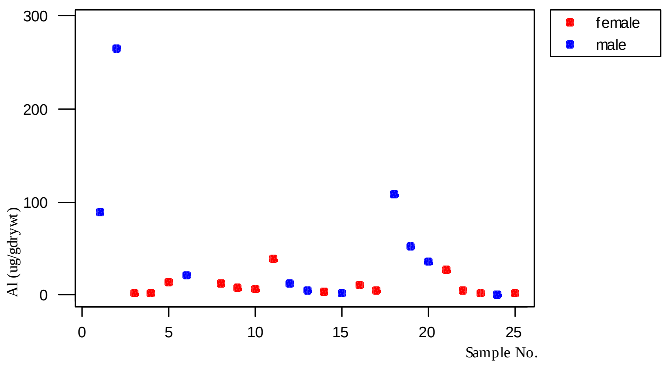
5.3. Appendix III

Minitab printout of the concentrations of the 14 examined metals (μg/g dry wt).

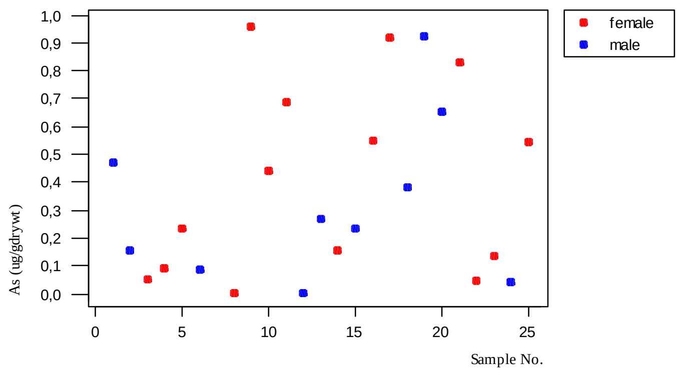
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15
	Code	Al (ug/gdrywt)	As (ug/gdrywt)	Cd (ug/gdrywt)	Co (ug/gdrywt)	Cr (ug/gdrywt)	Cu (ug/gdrywt)	Fe (ug/gdrywt)	Mg (ug/gdrywt)	Mn (ug/gdrywt)	Pb (ug/gdrywt)	Pt (ug/gdrywt)	Se (ug/gdrywt)	Si (ug/gdrywt)	Zn (ug/gdrywt)
1	T94.1b	88,982	0,46966	0,012694	0,126936	0,3998	2,3610	107,134	11,145	2,5006	0,0000	0,000000	0,00000	63,785	42,035
2	T94.1 s	265,184	0,15663	0,104420	0,221893	0,9137	8,6930	257,701	24,922	8,1578	0,0000	0,000000	0,45249	148,625	57,301
3	T95.1 b	1,907	0,05054	0,002784	0,007281	0,1651	0,5064	8,660	1,467	0,0529	0,0221	0,015846	0,00000	1,988	1,567
4	T95.2 b	1,324	0,09029	0,004441	0,000000	0,2061	0,9755	15,267	5,869	0,0690	0,1097	0,000000	0,00000	1,582	11,818
5	T96.1 b	14,069	0,23467	0,044506	0,043157	0,5810	0,5399	66,624	9,124	0,8892	0,3333	0,000000	0,00090	10,430	7,658
6	T96.2 b	20,672	0,08324	0,008083	0,003956	0,3866	0,4470	20,018	5,438	0,4805	0,0000	0,000000	0,05073	24,094	5,680
7	T96.2 s	407,341	2,08914	0,122890	0,442405	12,1580	4,5551	480,092	118,835	16,1478	0,3728	0,012289	0,00000	799,197	77,913
8	T96.3 b	11,317	0,00000	0,013655	0,034897	1,8738	4,9433	135,416	54,045	0,6297	0,9377	0,088001	0,13048	10,706	88,123
9	T96.3 s	7,633	0,95837	0,029042	0,130687	6,6602	3,3204	44,758	51,694	0,4792	3,2720	0,000000	0,00000	30,421	54,259
10	T96.4 b	6,592	0,44184	0,143301	0,053738	6,1858	5,1170	144,017	41,127	0,5911	6,3231	0,244805	2,35849	36,309	46,173
11	T96.4 s	38,453	0,68610	0,029252	0,042549	3,2523	15,0117	87,597	111,930	2,9704	0,4494	0,481332	0,67812	88,900	117,940
12	T96.5 b	12,223	0,00000	0,491054	0,000000	5,1731	1,0064	26,050	24,455	0,1945	0,6418	0,000000	1,49261	34,408	20,512
13	T96.5 s	5,107	0,27004	0,171535	0,000000	2,8278	1,8920	16,523	37,806	0,1749	33,4918	0,149457	0,00000	16,413	22,283
14	T97.1 b	2,798	0,15521	0,038288	0,000000	0,4887	0,4014	6,840	3,998	0,0236	0,0000	0,000000	0,00000	2,123	6,668
15	T97.2 b	1,472	0,23560	0,006055	0,001651	0,1916	0,6454	5,538	4,175	0,0583	0,1417	0,015963	0,00000	3,479	2,585
16	T97.3 b	10,823	0,55069	0,211805	0,000000	10,3078	6,1353	90,441	34,355	0,6495	33,1333	0,000000	0,00000	41,104	95,383
17	T97.3 s	4,519	0,92117	0,167485	0,103283	5,4237	0,9686	59,094	45,919	1,1194	65,3193	0,000000	0,00000	18,033	132,956
18	T98.1 b	108,709	0,38221	0,175439	0,187970	7,4499	3,3271	179,887	95,050	1,5915	0,6955	0,444862	0,35088	38,058	61,153
19	T98.1 s	52,359	0,92494	0,038709	0,220031	2,9358	1,2468	119,672	84,182	2,1759	0,0000	0,000000	0,00000	23,551	74,974
20	T98.2 b	35,273	0,65206	0,035596	0,487023	2,5338	1,2151	132,451	133,357	10,9475	0,1472	0,127823	0,23138	109,249	43,962
21	T97.1 s	26,588	0,83079	0,149057	0,042753	1,7979	4,9154	38,778	83,957	0,2773	0,7996	0,000000	0,22532	22,393	68,046
22	T99.1 b	4,426	0,04785	0,115530	0,023339	0,9969	0,2089	59,895	8,323	0,2459	0,0697	0,040552	0,00000	3,358	8,531
23	T99.2 b	2,245	0,13337	0,007162	0,007937	0,1661	0,2151	5,732	10,097	0,2644	0,0000	0,037166	0,00000	4,032	2,817
24	T99.3 b	0,729	0,04157	0,032584	0,000000	0,5292	0,1826	6,618	2,938	0,0382	0,2596	0,161236	0,06011	3,322	3,964
25	T99.4 b	1,518	0,54707	0,096542	0,006436	1,3548	1,1746	129,580	51,253	0,1180	0,3443	0,152321	0,06007	6,238	52,647

5.4. Appendix IV

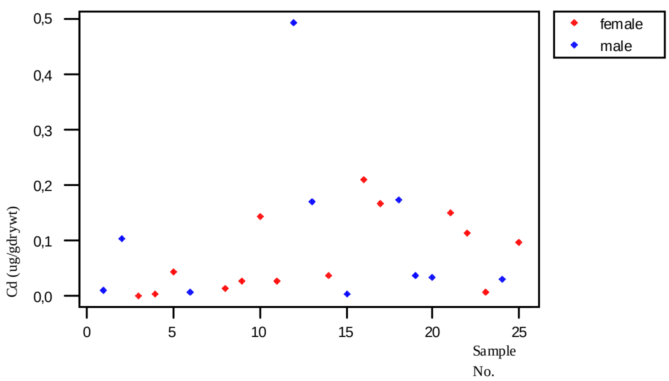
Al concentrations (ug/g dry wt) in relation to sex



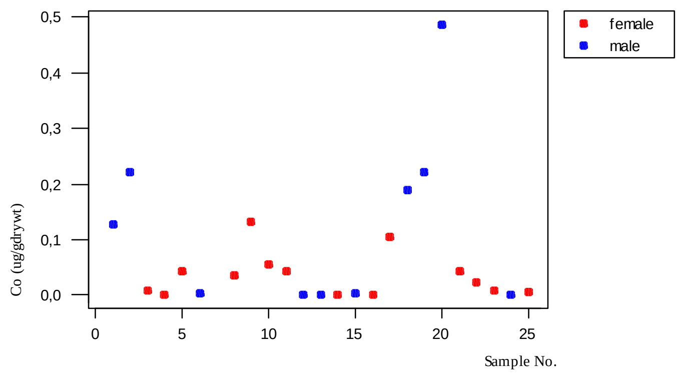
As concentrations (ug/g dry wt) in relation to sex



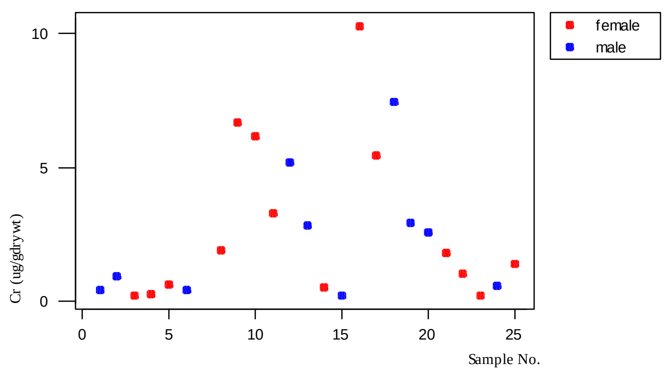
Cd concentrations (ug/g dry wt) in relation to sex



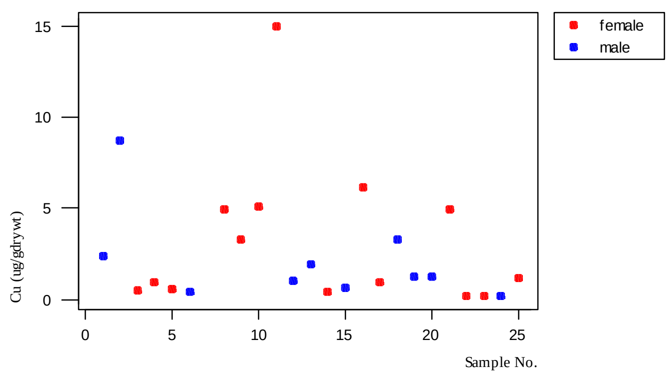
Co concentrations (ug/g dry wt) in relation to sex

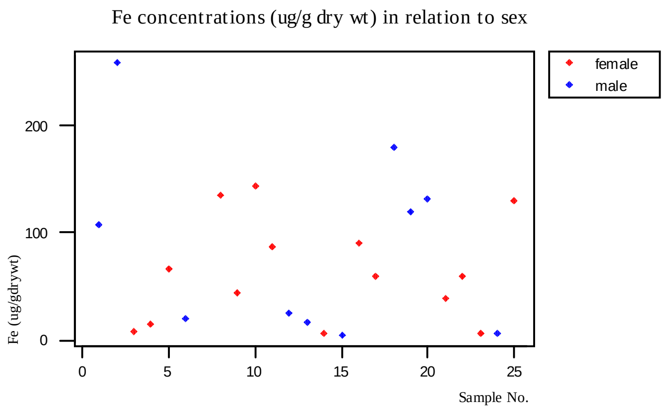


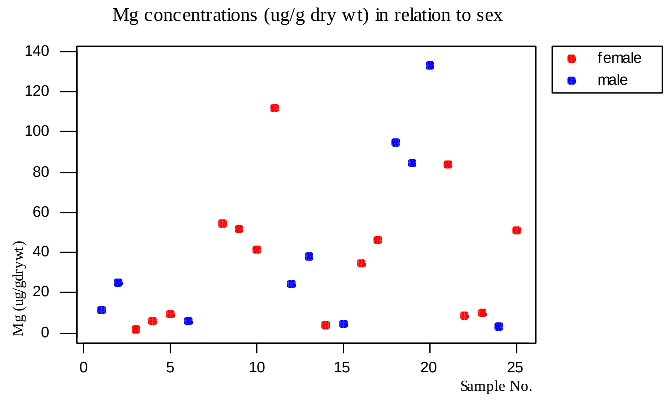
Cr concentrations (ug/g dry wt) in relation to sex

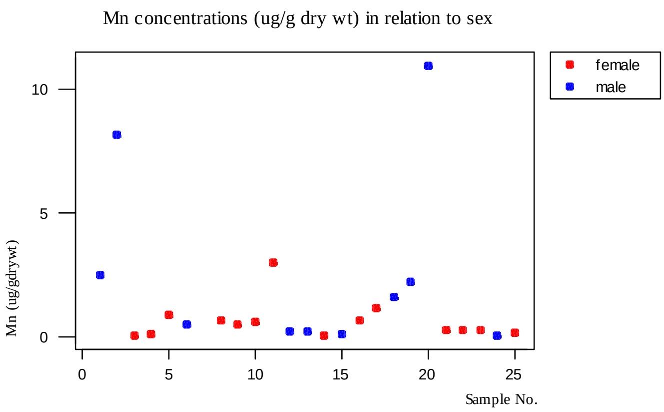


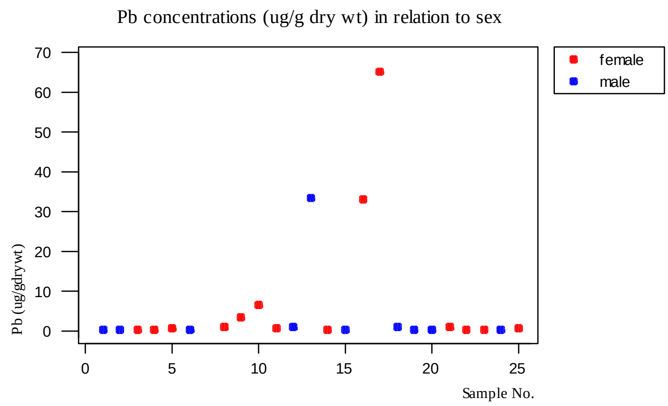
Cu concentrations (ug/g dry wt) in relation to sex

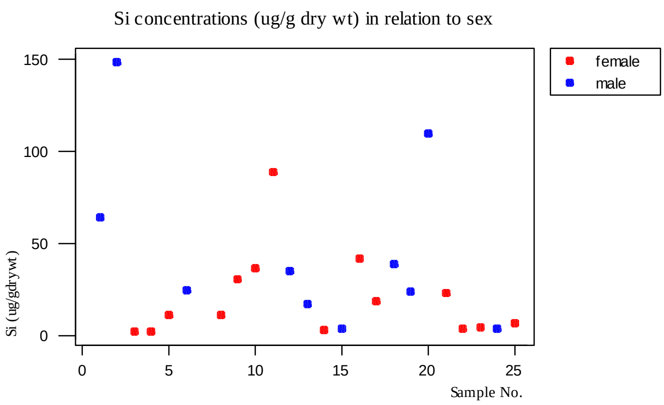


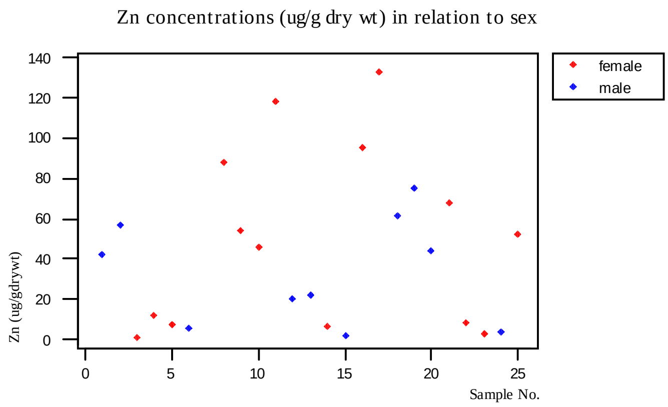

Fe concentrations (ug/g dry wt) in relation to sex
Fe (ug/gdrywt)
Sample No.
female
male


Mg concentrations (ug/g dry wt) in relation to sex
Mg (ug/gdrywt)
Sample No.
female
male


Mn concentrations (ug/g dry wt) in relation to sex
Mn (ug/gdrywt)
Sample No.
female
male


Pb concentrations (ug/g dry wt) in relation to sex
Pb (ug/gdrywt)
Sample No.
female
male


Si concentrations (ug/g dry wt) in relation to sex
Si (ug/gdrywt)
Sample No.
female
male


Zn concentrations (ug/g dry wt) in relation to sex
Zn (ug/gdrywt)
Sample No.
female
male

5.5. Appendix V

Principal Component Analysis

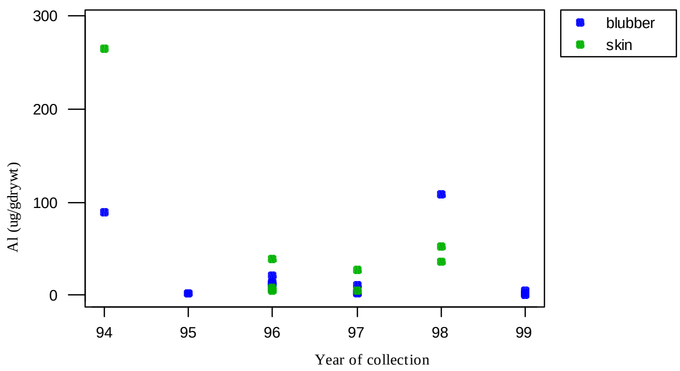
Eigenanalysis of the Correlation Matrix

Eigenvalue	5,0802	2,5888	1,3321	1,0339	0,7366	0,4638
Proportion	0,423	0,216	0,111	0,086	0,061	0,039
Cumulative	0,423	0,639	0,750	0,836	0,898	0,936
Eigenvalue	0,2967	0,2787	0,1017	0,0663	0,0120	0,0092
Proportion	0,025	0,023	0,008	0,006	0,001	0,001
Cumulative	0,961	0,984	0,993	0,998	0,999	1,000

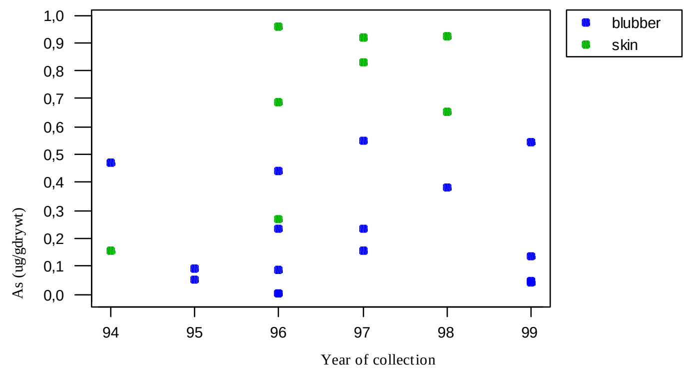
Variable	PC1	PC2	PC3	PC4	PC5
Al (ug/g dry wt)	-0,291	0,298	0,359	-0,013	0,256
As (ug/g dry wt)	-0,252	-0,307	-0,447	-0,045	0,034
Cd (ug/g dry wt)	-0,053	-0,301	0,537	0,436	-0,352
Co (ug/g dry wt)	-0,339	0,184	-0,315	0,416	-0,032
Cr (ug/g dry wt)	-0,195	-0,450	0,189	0,160	-0,229
Cu (ug/g dry wt)	-0,288	-0,065	0,229	-0,644	-0,096
Fe (ug/g dry wt)	-0,360	0,131	0,212	-0,022	0,022
Mg (ug/g dry wt)	-0,342	-0,129	-0,324	-0,024	-0,440
Mn (ug/g dry wt)	-0,347	0,290	-0,106	0,261	0,107
Pb (ug/g dry wt)	-0,050	-0,451	0,036	0,223	0,707
Si (ug/g dry wt)	-0,383	0,206	0,177	0,016	0,082
Zn (ug/g dry wt)	-0,313	-0,350	-0,046	-0,276	0,185

5.6. Appendix VI

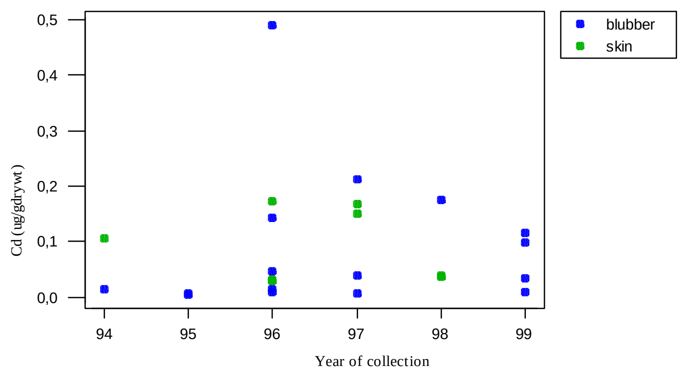
Metal concentrations (ug/g dry wt) in the different sampling years



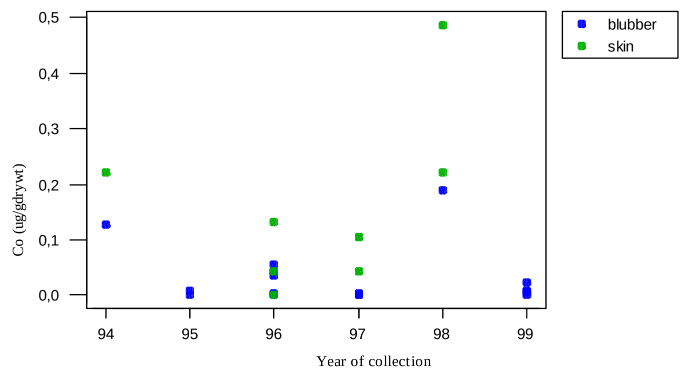
Metal concentrations (ug/g dry wt) in the different sampling years



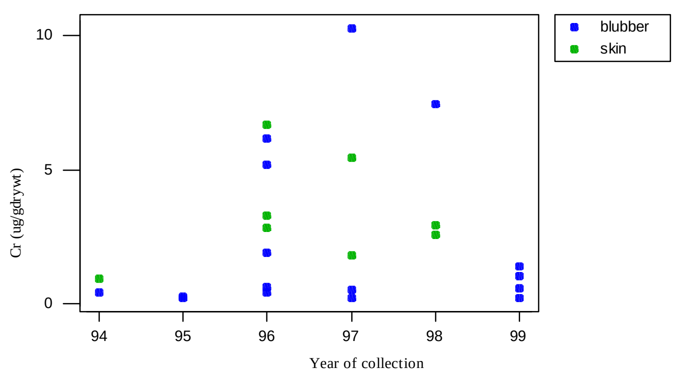
Metal concentrations (ug/g dry wt) in the different sampling years



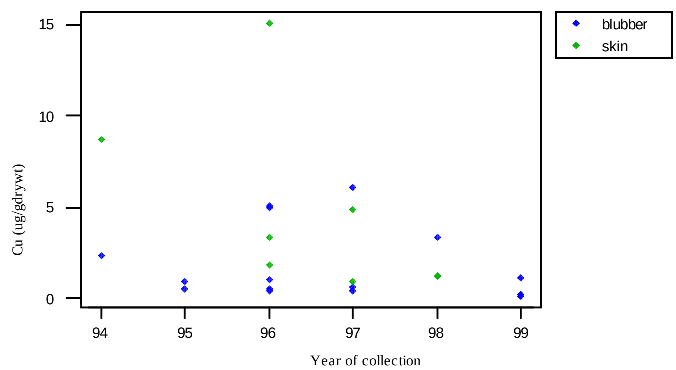
Metal concentrations (ug/g dry wt) in the different sampling years



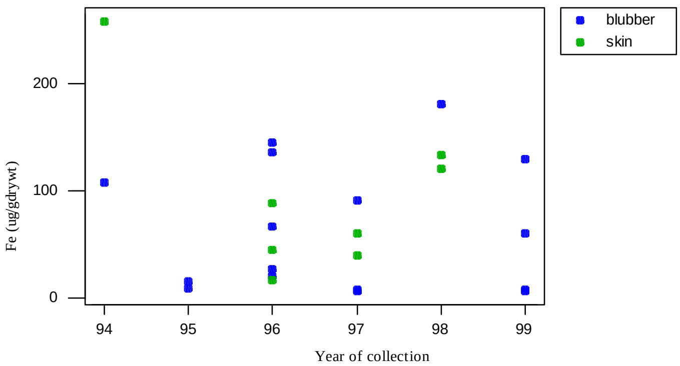
Metal concentrations (ug/g dry wt) in the different sampling years



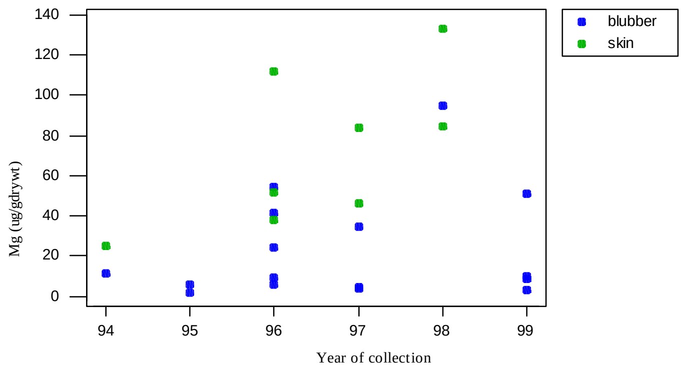
Metal concentrations (ug/g dry wt) in the different sampling years



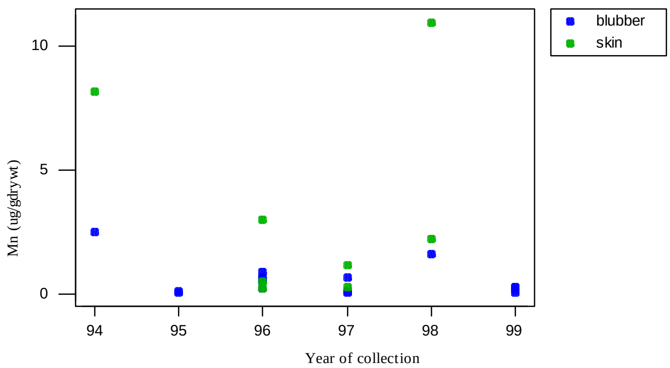
Metal concentrations (ug/g dry wt) in the different sampling years



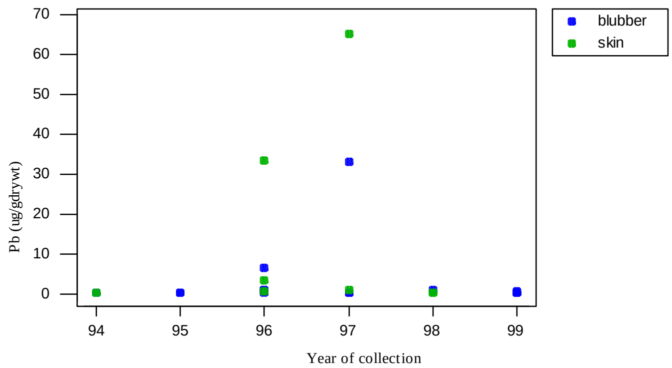
Metal concentrations (ug/g dry wt) in the different sampling years



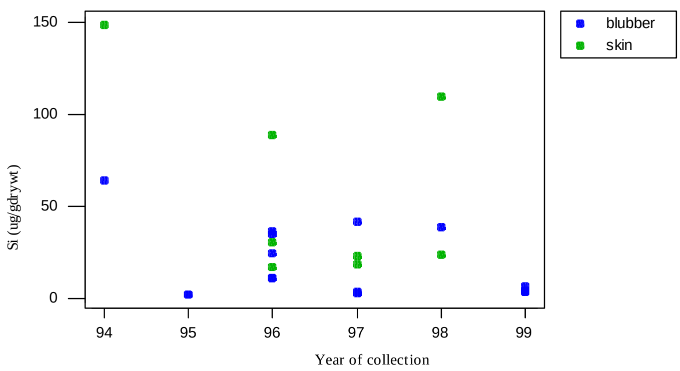
Metal concentrations (ug/g dry wt) in the different sampling years



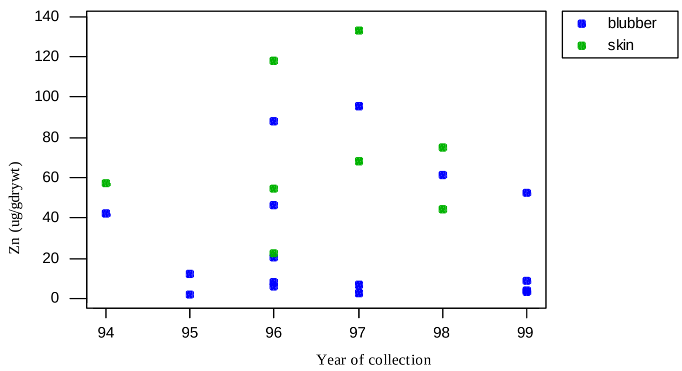
Metal concentrations (ug/g dry wt) in the different sampling years



Metal concentrations (ug/g dry wt) in the different sampling years



Metal concentrations (ug/g dry wt) in the different sampling years



5.7. Appendix VII

Concentrations of Copper and Zinc (µg/g dry weight) obtained from the Two Different Methods of Analysis

Sample Code	Copper (µg/g dry wt)			Zinc (µg/g dry wt)		
	Semi-Quantitative	Quantitative Conc.	St. Dev.	Semi-Quantitative	Quantitative Conc.	St. Dev.
T94.1 b	2.3610	4.1508	0.145976	42.035	52.278	0.55852
T94.1 s	8.6930	19.8007	0.230595	57.301	80.143	2.44083
T95.1 b	0.5064	0.8992	0.036832	1.567	1.503	0.05717
T95.2 b	0.9755	1.7717	0.027755	11.818	10.721	0.35711
T96.1 b	0.5399	1.0234	0.013711	7.658	6.941	0.14341
T96.2 b	0.4470	0.8329	0.003096	5.680	5.042	0.20053
T96.2 s	4.5551	8.3606	0.315419	77.913	70.908	2.14649
T96.3 b	4.9433	8.9185	0.259453	88.123	79.732	2.75687
T96.3 s	3.3204	6.0891	0.203291	54.259	44.153	0.06776
T96.4 b	5.1170	9.2250	0.179126	46.173	35.019	0.52544
T96.4 s	15.0117	25.7100	0.215403	117.940	112.222	1.85619
T96.5 b	1.0064	1.7406	0.063205	20.512	11.771	0.47161
T96.5 s	1.8920	3.2524	0.101902	22.283	16.928	0.65727
T97.1 b	0.4014	0.6894	0.019529	6.668	5.684	0.06296
T97.1 s	4.9154	9.9267	0.285404	68.046	60.801	1.15086
T97.2 b	0.6454	1.0566	0.004679	2.585	1.731	0.03165
T97.3 b	6.1353	10.9009	0.225925	95.383	77.238	4.27139
T97.3 s	0.9686	1.3706	0.142363	132.956	119.892	3.77121
T98.1 b	3.3271	5.1566	0.112782	61,.153	45.482	0.91479
T98.1 s	1.2468	2.4183	0.044821	74.974	64.685	2.69742
T98.2 b	1.2151	2.7555	0.197398	43.962	36.082	0.39965
T99.1 b	0.2089	0.4195	0.022756	8.531	7.119	0.46445
T99.2 b	0.2151	0.4115	0.003871	2.817	2.369	0.10937
T99.3 b	0.1826	0.3118	0.020787	3.964	2.487	0.05506
T99.4 b	1.1746	2.1325	0.033253	52.647	47.295	1.89114

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