Heavy metals in tissues of demersal fish from the Thermaikos Gulf: detection of changes with trophic level

University of Crete
Biology Department
Postgraduate Programme in Environmental Biology
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Evelyn Vetsis

Evaluation committee

Prof. I Karakassis (supervisor)

Prof. M. Pavlidis

Dr. S. Somarakis

Heraklion, Crete
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Μέτρηση βαρέων μετάλλων σε βενθικά είδη ψαριών του Θερμαϊκού κόλπου: μεταβολές σε διαφορετικά τροφικά επίπεδα

Πανεπιστήμιο Κρήτης
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Πρόγραμμα Μεταπτυχιακών Σπουδών
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Ευανθία Βέτση

Επιτροπή

Δρ. Ι. Καρακάσης (επιβλέπων)

Δρ. Μ. Παυλίδης

Δρ. Σ. Σωμαράκης

Ηράκλειο, Κρήτη
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Abstract

Heavy metals were investigated in twenty-nine demersal marine fish species collected by trawl from the Thermaikos Gulf. Metal concentrations were analyzed in the muscle, gills, liver, skin and scales. Metals accumulated mostly in the gills, followed by the liver and finally the muscle. Patterns of biomagnification were evident in this demersal food web for some species and metals. Concentrations of some metals in the edible portion of the fish exceeded limits provided by food safety authorities. The presence of contaminants in the sediment and water of the gulf of the Thermaikos could be potential contributors to the accumulation of metals in fish species. The degree to which accumulation occurs is dependent on fish swimming activity, feeding behaviour, physiology, and water quality.

Keywords: heavy metals, fish, trophic level, bioaccumulation, biomagnification

Περίληψη

Στην μελέτη αυτή αναλύθηκαν βαρέα μέταλλα σε είκοσι εννέα είδη βενθικών θαλάσσιων ψαριών, τα οποία συλλέχθηκαν με τράτα από τον Θερμαϊκό κόλπο. Για την μέτρηση των συγκεντρώσεων των βαρέων μετάλλων ελήφθησαν δείγματα από τους μύες, τα βράγχια, το συκώτι, το δέρμα και τα λέπια. Τα αποτελέσματα έδειξαν πως περισσότερα μέταλλα συσσωρεύτηκαν στα βράγχια, μετά στο συκώτι και τελευταία στους μύες. Ορισμένα μέταλλα παρουσίασαν βιομεγέθυνση. Οι συγκεντρώσεις ορισμένων μετάλλων στο βρώσιμο σημείο του ψαριού ξεπέρασαν τα θεσπισμένα όρια. Η παρουσία ρύπων στο ίζημα και στο νερό του Θερμαϊκού κόλπου θα μπορούσε να εξηγήσει τη συσσώρευση των μετάλλων στα ψάρια. Ο βαθμός συσσώρευσης στα ψάρια εξαρτάται από την κολυμβητική ικανότητα, την τροφική συμπεριφορά, την φυσιολογία των ψαριών και την ποιότητα του νερού.

Λέξεις κλειδιά: βαρέα μέταλλα, ψάρια, τροφική αλυσίδα, βιοσυσσώρευση, βιομεγέθυνση

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Table of Contents

Introduction

Fish species are considered to be among the top consumers in aquatic food webs, and thus can be endangered by contaminants transferred along the food chain (Moriarty, 1984). Accumulation of heavy metals in fish species can occur through the uptake of contaminated food and water. The primary cause for exposure (i.e. diet or water) is high controversial in the literature (Dallinger et al., 1987; Rejomon et al., 2009; Wang, 2002; Gray, 2002; Connell and Miller, 1984), and depends on the questions scientists are looking to answer.

Metals in the environment

The entry of metals into the environment is caused both by anthropogenic factors and by natural processes. Human activities such as mining, fossil fuel burning, agriculture and urbanization, are responsible for metal inputs. Natural sources such as chemical weathering and volcanic activity are also contributors (Connell and Miller, 1984, Chp.10).

Heavy metals, regardless of their source, end up in the sea. Metals enter the atmosphere through natural processes or anthropogenic activities as gasses (Hg, Se, and B) or aerosols (most other metals). They are then deposited by gas exchange on the sea surface through dry or wet deposition. The sea releases air bubbles to the atmosphere containing salt particles that have the potential to interact with other contaminants, thus working as a source for contaminants in the atmosphere, and also as a sink for atmospheric contaminants (Clark, 2001). Figure 1 displays the cycling of metals from the atmosphere to the sea and vice versa.

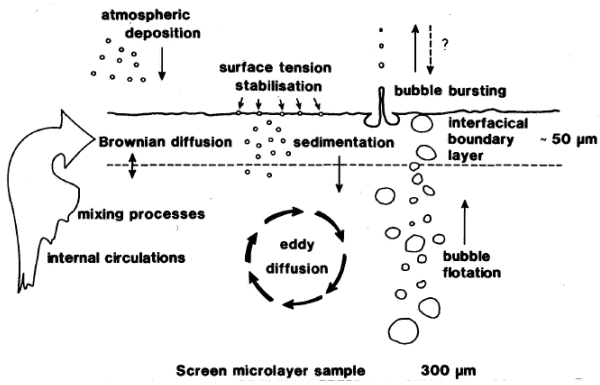

Figure 1. Cycling of trace metals from atmosphere to sea and from sea to atmosphere (Hunter, 1980).

Rivers that run through urban areas become polluted with human wastes and discharge that contain elevated metal content. In estuaries, sediment particles absorb metals and are carried to the bottom. When dredging of shipping channels occurs large amounts of contaminated dredging spoil are dumped at sea (Clark, 2001).

Metals can also be introduced to the sea by direct discharge of sewage sludge and industrial wastes. Through the input of small quantities, effects can be devastating effects on seas with limited water circulation. (Clark, 2001).

The term conservative pollutant is associated with metals for the reason that once they are added to the marine environment they become permanent (Clark, 2001).

Availability and accumulation of metals

Marine organisms differ in their ability to regulate metal content. Metals that cannot be excreted continue to remain in the body and are perpetually being added over the life span of an organism. This addition can occur through passive or active uptake by the organism, and is known as bioaccumulation (Clark, 2001). Figure 2 illustrates the potential pathways for bioaccumulation in an aquatic organism. Through the first pathway, uptake and retention of chemicals by aquatic biota occurs from the food, where metals are deposited in the lipoid tissues of the stomach wall. The second pathway involves the direct transfer of metal from water through the gills to the lipoid tissues. In both cases, the xenobiotic substances enter the circulatory fluid through the walls of the gastrointestinal tract, bathing most body tissues and becoming deposited. A similar term but slightly different in meaning is bioconcentration, which refers only to transfer of chemicals through to the gills (Connell, 1989; Streit, 1992).

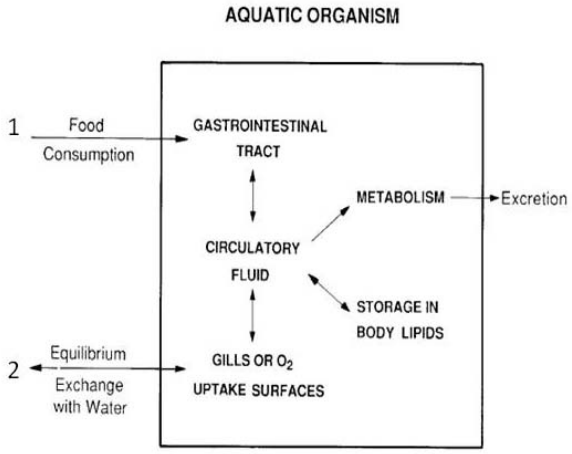

Figure 2. An illustration of the potential pathways of bioaccumulation in aquatic organisms. (Connell, 1989)

In a trophic chain, bioaccumulators have the capacity to expose other members to greater concentrations of metals via the diet. Animals occupying higher levels of the food chain are subject to greater concentrations of a particular metal in their diet. This is termed as biomagnification (Connell, 1989).

Essential and non-essential metals

Both man and animals are exposed to different forms of elements in the environment through diet and water. These elements can be grouped into two categories, essential and non-essential. Essential elements play a particular role in the physiology of an organism. Major essential elements include C, H, O, N, S, Ca, P, K, Na, Cl, and Mg. Furthermore, Fe, Zn, Cu, Co, Mn, I, Mo, Cr, Se, and F also meet the pre-requisites for essential elements in animals. Non-

essential elements in cases of human toxicity include Pb, Cd, Hg, As, Al, Ba, Li, Pt, Te, Ti, Sb, Be, Ga, In, V, Ni, Sr, Sn, Ge, Ag, Au, Bi, Tl, and U (FDA, 2005).

Both essential and non-essential elements can be considered toxic. Under conditions of homeostasis where levels of absorption, storage in the body, and excretion are regulated, essential elements possess a lower toxicity. The features that define an element's toxicity are the degree of exposure, the form of the element, and the physiology of the host along with the dietary status. The most toxicologically important essential elements are F, Co, Fe, Mo, Cu, Mg, Se, Cr, Mn, and Zn, with Se being the most toxic (FDA, 2005).

Not all chemical elements are subject to bioaccumulation, and the extent to which those that are vary greatly. An important factor that dictates this process is membrane permeability and is generally dependent on electrical charges, particle diameters, and chemical interactions. Moreover, the rate at which bioaccumulation occurs is associated with binding constants of substances to living substrates, particularly the binding of different types of molecular structures in organisms (Streit, 1992).

Metal tolerance

Organisms that are considered to be metal-tolerant can have concentrations of metals two or three orders of magnitude higher than normal. Mechanisms of detoxification can consist of the temporary or permanent storage of metals in inactive sites within the organism. The temporary storage can be facilitated through the binding of metals to proteins, polysaccharides, and amino acids in soft tissues or body fluids. Metallothionein is a protein that can effectively store cadmium in the liver and kidneys. Storage in the bone can eliminate some metals (e.g. Pb, Cd, and Hg) (Connell and Miller, 1984).

Fish are able to regulate essential metals such as Zn and Cu, but are not so successful when it comes to non-essential metals such as Hg and Cd. Fish and sea mammals occupying higher levels of trophic chain tend to have lower concentrations of cadmium, at most a few ppm in the kidneys, and are able to detoxify by producing metallothioneins. Copper does not necessarily accumulate in food chains, although fish tend to have higher concentrations of copper than those from uncontaminated areas (Clark, 2001).

Study aim

Heavy metals were measured in all demersal marine fish removed by successive hauls of a commercial trawling vessel in the Thermaikos Gulf, in order to test the following three hypotheses:

- That there is no change in metal concentrations along the food chain,
- That there is no change in metal concentrations between tissues of different species,
- That there is no difference in patterns of bioaccumulation among trace elements, according to fish trophic levels.

Materials and method

Study site and species

In mid-November, 2009, demersal fish were collected by bottom trawl in the eastern region of the Thermaikos Gulf. Figure 3 displays the followed path of the trawl for sampling.

Figure 3. Map of sampling zone in Thermaikos Gulf.

Background information on the Thermaikos Gulf

The coastal area of the Thermaikos Gulf is located in the Northern Aegean Sea of Greece, and the northern part is named after the city of Thessaloniki as Thessaloniki Bay. The catchment of the Gulf is approximately 40,000km^2, and the main rivers are Aliakmon, Axios, and Loudia. The water depths in the gulf of Thermaikos are between 20-60m. The Thermaikos gulf forms a wide continental shelf (Karageorgis et al., 2005).

The Aegean waters are drawn in and transported through the gulf from the deep layers along the eastern coast, moving counter clockwise toward the gulf of Thessaloniki. Water temperatures lie between 10 and 28 degrees Celsius and salinity between 33 and 39psu (Karageorgis et al., 2005).

Riverine waters empty into the gulf of the Thermaikos, introducing grave amounts of pollution, carrying domestic, industrial and animal wastewaters, along with agricultural runoff. The rivers discharge an estimated 0.628-25 x 10^6 tonnes of solid particulate matter per year. Discharges affect the north-western open continental sector of the gulf (Karageorgis et al., 2005), the sample site of this study.

On a daily basis, the wastewater treatment plant from the municipality of Thessaloniki discharges 150,000m^3 of sewage effluents into the gulf. To add to this, roughly 60,000 types of untreated or semi-treated industrial wastes also end up in this coastal system. The surrounding area of the gulf is made up of about one million inhabitants with a growing industrial area and economy. In the harbour of Thessaloniki, both port and commercial activities take place, such as maritime traffic, ship discharging, bunkering, storing hazardous cargo, dredging and disposal of dredging materials. Moreover, mussel farming is practiced in the coastal area, reaching 30,000t/y and representing 85% of the total Greek production (Karageorgis et al., 2005).

Fish sampling and preparation

A total of 115 fish were sampled, placed immediately in ice barrels and transferred to the Technological Educational Institute (T.E.I) of Thessaloniki. Listed in table 1 are the scientific and common names of the species caught, along with the number of individuals per species, total length, weight, mean trophic level, and details on habitat and diet. The fish were sorted, measured, weighed, photographed, and then rinsed with distilled water for dissection. The gills, liver, muscle, skin and scales were removed and placed in separate polyethylene bags. White muscle was sampled in the abdominal area, on the left side. Samples were transported to the Marine Ecology laboratory at the University of Crete in coolers and frozen at -20 degrees Celsius. Samples underwent freeze drying at -45°C for a few days, and were then homogenized.

Table 1. List of the twenty nine species collected by trawl along with details of their total length, weight, trophic level, habitat and diet.

Species	Common name (in English and Greek)	n	Total length (cm) Mean Min-max	Weight (gr) Mean Min-max	Mean Trophic level	Habitat	Diet
Mullus barbatus (Linnaeus, 1758)	Striped mullet Κουτσομούρα	5	16.58 15.50-17.70	48.24 35.44-61.40	3.27	Demersal	zoobenthos
Merluccius merluccius (Linnaeus, 1758)	European hake Μπακαλιάρος	5	24.24 22.90-25.00	105.68 86.02-118.04	4.45	Demersal	nekton
Citharus linguatula (Linnaeus, 1758)	Spotted flounder Ζαγκέτα	5	18.84 18.00-19.00	48.87 39.00-57.80	4.34	Demersal	nekton-zoobenthos
Merlangius merlangus (Linnaeus, 1758)	Whiting Νταούκι του Ατλαντικού	5	16.32 15.50-16.70	43.73 35.21-49.50	4.38	Demersal	zoobenthos
Lophius budegassa (Spinola, 1807)	Black-bellied angler Πεσκαντρίτσα	5	24.26 20.00-29.00	215.28 133.44-354.76	4.54	Demersal	nekton
Trachurus trachurus (Linnaeus, 1758)	Scad Σαυρίδι	5	14.80 14.00-15.50	27.50 22.35-31.80	3.58	Pelagic	zoobenthos
Engraulis encrasicolus (Linnaeus, 1758)	European anchovy Γαύρος	5	12.20 12.00-13.00	10.83 9.35-11.90	3.38	Pelagic	zooplankton
Spicara flexuosa (Linnaeus, 1758)	Blotched picarel Τσέρουλα	5	16.32 15.50-17.70	49.58 40.34-63.61	3.24	Demersal	zooplankton
Pagellus acarne (Risso, 1826)	Axillary seabream Μουσμούλι	5	13.10 12.50-13.50	30.14 26.28-35.23	3.84	Demersal	zoobenthos
Conger conger (Linnaeus, 1758)	European conger Μουγγρί	5	55.10 45.50-69.60	234.43 134.15-396.97	4.18	Demersal	nekton
Gobius niger (Linnaeus, 1758)	Black goby Γωβιός	5	15.04 13.40-17.20	37.41 27.13-51.57	3.32	Demersal	zooplankton
Solea spp.	Sole Γλώσσα	5	22.18 18.70-25.90	86.99 50.26-127.42	3.32	Demersal	zooplankton
Cepola macrophthalma	Red bandfish Κορδέλλα	5	49.10 34.50-62.00	44.18 28.40-62.10	3.13	Demersal	zoobenthos-zooplankton
Serranus cabrilla (Linnaeus, 1758)	Comber Χάνος	5	10.56 10.00-11.30	18.35 15.62-20.95	3.9	Demersal	nekton

Serranus hepatus (Linnaeus, 1766)	Brown comber Χανάκι	5	16.03 15.00-17.10	47.41 43.62-51.79	3.77	Demersal	zoobenthos
Uranoscopus scaber (Linnaeus, 1758)	Stargazer Λύχνος	5	18.34 16.50-20.50	111.14 79.10-168.86	4.43	Demersal	nekton-zoobenthos
Trachinus draco (Linnaeus, 1758)	Greater weever Δράκαινα	5	23.40 18.50-28.20	89.33 39.21-143.11	4.19	Demersal	nekton-zoobenthos
Chelidonichthys lucerna (Linnaeus, 1758)	Tub gurnard Χελιδονάς	5	22.96 21.00-24.00	114.84 78.91-137.27	3.64	Demersal	zoobenthos
Blennius ocellaris (Linnaeus, 1758)	Butterfly blenny Σαλιάρα	5	13.28 12.50-14.90	36.89 29.46-47.50	3.26	Demersal	zoobenthos
Scomber scombrus (Linnaeus, 1758)	Atlantic mackerel Σκουμπρί	5	16.64 13.70-24.00	40.82 17.88-107.88	4.37	Pelagic	nekton
Ophidion spp.	Σαλούφαρδος	4	20.88 19.50-22.00	44.07 37.83-53.23	3.52	Demersal	zoobenthos
Torpedo torpedo (Linnaeus, 1758)	Common torpedo Μαυρομουδιάστρα	2	25.25 23.00-27.50	362 252.70-473.10	4.5	Demersal	nekton
Gaidropsarus spp.	Rockling Γαϊδουρόψαρα Σαλούφαρδος 2	2	32.50 32.50-32.50	79.88 79.65-80.11	3.5	Demersal	zoobenthos-zooplankton
Pomatomus saltatrix (Linnaeus, 1766)	Bluefish Γοφάρι	2	21.60 21.20-22.00	100.63 93.26-108.00	4.5	Demersal	nekton-zoobenthos
Phycis blennoides (Brünnich, 1768)	Greater forkbeard Σαλούβαρδος	3	21.33 18.00-24.00	72.73 45.82-97.50	3.55	Demersal	zoobenthos
Sygnathus acus (Linnaeus, 1758)	Great pipefish Σακοράφα	3	29.33 27.50-32.50	12.97 7.70-18.00	3.39	Demersal	
Mullus surmuletus (Linnaeus, 1758)	Red mullet Μπαρμπούνι	2	21.00	21.15	3.19	Demersal	zoobenthos
Gaidropsarus mediterraneus (Linnaeus, 1758)	Shore rockling Σαλούφαρδος 3	3	11.71 11.00-12.13	17.40 15.00-20.70	3.95	Demersal	zoobenthos
Scyliorhinous canicula (Linnaeus, 1758)	Dogfish Σκυλοψαράκι	1	35.00	137.90	4.41	Demersal	nekton

Scientific and common names retrieved from the EC Dictionary of Aquatic Animals and Plants (1993). Mean trophic levels taken from fishbase.org and Stergiou & Karpouzi (2002).

Biological digestion

The concentration of metals was determined using the EPA method 3052 for digestion and trace metal analysis.

The accuracy of applied analytical procedure was assessed using the following certified reference materials (CRMs), fish protein (DORM-3 Fish) from the National Research Council, Canada, mussel tissue (BCR-668) and Aquatic plant - Lagarosiphon major (BCR-060) both from the European Commission Joint Research Centre. Non-defatted lobster hepatopancreas (LUTS-1) from the National Research Council, Canada was also used in the digestion process and trace element measurements, but was excluded from data as results supported evidence for contamination.

Polypropylene volumetric flasks and sample tubes were rinsed with tap water, soaked in a 10% nitric acid bath for at least 24 hours, rinsed with nanopure water and left to dry in a sterile room.

All digest samples were prepared in a fume cupboard. Each batch for digestion consisted of approximately 0.25g of reference material, 6 samples weighing up to 0.25g and one blank. The nitric acid ($HN0_3$), Fisher Scientific-Trace metal grade, and hydrogen peroxide (H_2O_2), Fluka Analytical – for trace analysis, used were of suprapure quality. Details of the protocol are represented in the tables 2, 3, and 4 below.

Table 2. Protocol for muscle, gills, skin, and scales weighing 0.04-0.25 grams.

Volume	**Chemical**
3mL	HNO_3
1mL	H_2O_2
3mL	Deionized water
50mL	Volumetric flask

Table 3. Protocol for liver samples weighing 0.04-0.25 grams.

Volume	**Chemical**
3mL	HNO_3
2mL	H_2O_2
3mL	Deionized water
50mL	Volumetric flask

Table 4. Protocol for all samples weighing less than 0.04 grams.

Volume	**Chemical**
1mL	HNO_3
1mL	H_2O_2
6mL	Deionized water
25mL	Volumetric flask

For samples weighing less than 0.04g, 1mL of $HN0_3$ was added, 1mL of H_2O_2 and 3mL of deionized water. For all liver samples, regardless of weight, 3mL of $HN0_3$ was added, followed by 2mL of H_2O_2 and 3mL of deionized water. For samples weighing between 0.04-0.25g, 3mL $HN0_3$ was added, 1mL of H_2O_2 and 3mL of deionized water.

Digestion procedure

Name of MW model: Multiwave 3000 – Microwave sample preparation, Anton Paar, Austria.

For a clear solution, the method of digestion utilized consisted of $HN0_3$ and H_2O_2 in a closed high pressure microwave system. 3mL of $HN0_3$ was added to weighed samples in a perfluoroalkoxy polymer (PFA) vessel and pre-digested for an hour on a sand bath at 25W. 3mL of deionized water were added, followed by 1mL of H_2O_2. Vessels were sealed and placed in microwave and heated according to the procedure shown in table 5.

Table 5. Program settings for biological digestion in microwave.

Phase	Power (W)	Time (minutes)
1	1000	5
2	800	5
3	600	15
4	0	20

Analytical instrumentation

ICP-MS model: Thermo Fischer Scientific, Winsford, United Kingdom; Plasma lab software

An Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) machine was used to measure trace metal concentrations in the digests. Each sample contained a dilution of 400, and was analyzed three times in order to determine effects of drifts. A standard was run every 10 samples. Argon gas was used for the ICP-MS machine.

Standard solutions for calibration were prepared by diluting multi-element stock solutions (CPI International) with deionized water and 2% nitric acid.

The following isotopes analyzed were: 7Li, 9Be, 23Na, 24Mg, 27Al, 31P, 39K, 43Ca, 44Ca, 45Sc, 46Ca, 48Ca, 51V, 52Cr, 53Cr, 55Mn, 56Fe, 57Fe, 59Co, 60Ni, 61Ni, 62Ni, 65Cu, 66Zn, 67Zn, 68Zn, 69Ga, 70Ge, 71Ga, 72Ge, 73Ge, 74Ge, 75As, 76Ge, 77Se, 78Se, 82Se, 85Rb, 86Sr, 88Sr, 89Y, 90Zr, 91Zr, 92Zr, 92Mo, 94Mo, 94Zr, 95Mo, 97Mo, 98Mo, 105Pd, 106Pd, 107Ag, 108Pd, 109Ag, 111Cd, 112Cd, 114Cd, 115In, 133Cs, 137Ba, 138Ba, 138La, 139La, 140Ce, 141Pr, 142Nd, 142Ce, 143Nd, 144Nd, 145Nd, 146Nd, 147Sm, 149Sm, 151Eu, 152Eu, 153Eu, 154Sm, 156Gd, 157Gd, 158Gd, 159Tb, 160Gd, 161Dy, 162Dy, 163Dy, 164Dy, 165Ho, 166Er, 167Er, 168Er, 169Tm, 171Yb, 172Yb, 173Yb, 174Yb, 175Lu, 176Lu, 185Re, 187Re, 198Hg, 199Hg, 200Hg, 201Hg, 202Hg, 203Tl, 205Tl, 206Pb, 207Pb, 208Pb, 209Bi, 232Th, 238U.

Preliminary data handling

The concentrations of the blanks were averaged and then subtracted from the measured concentrations of the samples.

The limit of detection (LOD) was calculated by multiplying 3 times the standard deviation of the concentrations of the blanks for each element. The relative standard deviations were calculated for each metal.

Recoveries for the references were calculated by taking the measured value and dividing it by the expected, according to the certified reference materials, and then multiplying by 100. The acceptable range was between 80-120%. Table 6 shows recoveries for all three reference materials. The metals selected for this study were based upon acceptable recoveries and limits of detection.

Table 6. Recoveries for reference materials.

BCR-060	**Recovery %**	**DORM-3**	**Recovery %**	**BCR-668**	**Recovery %**
55Mn	120	27Al	127	56Fe	98
59Co	113	52Cr	96	59Co	98
60Ni	121	56Fe	113	68Zn	107
68Zn	118	60Ni	99	75As	100
109Ag	84	65Cu	115	89Y	106
114Cd	109	68Zn	111	97Mo	103
208Pb	110	75As	108	114Cd	94
		78Se	95	139La	107
		114Cd	101	140Ce	111
		208Pb	93	142Nd	94
				154Sm	99
				153Eu	114
				157Gd	102
				159Tb	100
				162Dy	99
				165Ho	102
				166Er	100
				232Th	118
				238U	101

Concentration levels of contaminants were expressed in µg/g (parts per million, ppm) and µg/kg (parts per billion, ppb). Concentrations can be calculated on the basis of wet (fresh) weight and dry weight. Wet weight represents the weight of a sample of whole tissue removed from the body, whereas dry weight represents the weight after drying, for the removal of unbound water (Clark, 2001). For this study metal concentrations were expressed in parts per million and were calculated both in wet and dry weight.

Data analysis

Statistical analyses were performed using the software Statistica 7. The significance differences between sample means were determined using the Kruskal-Wallis test for multiple independent variables along with post hoc test analyses. A p-value of 0.05 or less was considered to be statistically significant. PRIMER 6 was also used to perform principal component analysis (PCA).

Results

Changes in metal concentrations along the food chain

For the testing of the first hypothesis, metal concentration levels were compared for three tissues (muscle, liver and gills) between species in order to determine whether metals were transferred along this demersal food chain. Tables 7, 8, and 9 list species that showed statistically significant differences for certain metals. Species with greater concentrations of a particular metal were found on higher concentration levels, represented by Greek letter where alpha (α) was the lowest and delta (δ) the highest. Indicated next to each species name is the corresponding trophic level, written in parenthesis.

Table 7. Metal concentrations in the muscle for all species.

Metal	**Species and trophic levels - MUSCLE**	**Concentration levels**
Cr	*L. budegessa* (4.54)	β
	M. merluccius (4.45)	α
Mn	*P. acarne* (3.84)	β
	Ophidion sp. (3.52)	α
Cu	*E. encrasicolus* (3.38)	γ
	M. merlangus (4.38), *T. trachurus* (3.58), *S. scombrus* (4.37)	β, γ
	Solea sp. (3.32), *B. ocellaris* (3.26), *Gaidropsarus sp.* (3.5), *S. acus* (3.39), *G. mediterraneus* (3.95)	α, β
	C. macrophthalma (3.13)	α
As	*L. budegessa* (4.54)	γ
	B. ocellaris (3.26)	β, γ
	P. acarne (3.84), *S. acus* (3.39)	α, β
	S. hepatus (3.77)	α
Se	*L. budegessa* (4.54)	β
	P. acarne (3.84), *G. mediterraneus* (3.95)	α
Ag	*M. merlangus* (4.38)	β
	M. barbatus (3.27), *M. merluccius* (4.45), *C. lucerna* (3.64)	α
Cd	*E. encrasicolus* (3.38)	γ
	S. flexuosa (3.24)	β, γ
	L. budegessa (4.54), *Solea sp.* (3.32)	α, β
	C. lucerna (3.64)	α
Fe	*E. encrasicolus* (3.38)	β
	M. merluccius (4.45)	α
Co	*E. encrasicolus* (3.38), *P. acarne* (3.84), *S. cabrilla* (3.9)	β
	M. merluccius (4.45)	α
Ni	*P. acarne* (3.84)	γ
	E. encrasicolus (3.38), *S. cabrilla* (3.9)	β, γ
	M. merluccius (4.45)	α, β
	L. budegessa (4.54)	α
Zn	*E. encrasicolus* (3.38)	β
	C. lucerna (3.64), *P. blennoides* (3.55), *G. mediterraneus* (3.95)	α

Pb	*S. acus* (3.39), *T. trachurus* (3.58)	γ
	E. encrasicolus (3.38), *P. acarne* (3.84)	β, γ
	U. scaber (4.43)	α, β
	L. budegassa (4.54)	α
U	*S. acus* (3.39)	δ
	T. trachurus (3.58), *E. encrasicolus* (3.38), *P. acarne* (3.84)	γ, δ
	S. flexuosa (3.24)	β, γ
	C. lucerna (3.64)	α, β, γ
	U. scaber (4.43)	α, β
	L. budegassa (4.54)	α

The metal concentrations in the muscles for Cr, Mn, Cu, As, Se, Ag, and Cd displayed patterns of biomagnification, where levels increased going up the food chain. Fe, Co, Ni, Zn, Pb, and U showed an opposing behaviour where levels decreased going up the food chain. The remaining metals: Al, Y, Mo, La, Ce, Nd, Eu, Sm, Gd, Tb, Dy, Ho, Er and Th, did not show statistically significant differences in metal concentrations in the muscle.

Table 8. Metal concentrations in the liver for all species.

Metal	**Species and trophic levels - LIVER**	**Concentration levels**
Al	*C. linguatula* (4.34)	β
	M. barbatus (3.27)	α
Cr	*E. encrasicolus* (3.38)	γ
	C. lucerna (3.64)	β, γ
	Solea sp. (3.32)	α, β
	M. barbatus (3.27)	α
Mn	*L. budegassa* (4.54)	β
	E. encrasicolus (3.38), *U. scaber* (3.77)	α
Co	*L. budegassa* (4.54)	δ
	C. macrophthalma (3.13)	γ, δ
	C. lucerna (3.64)	β, γ, δ
	Solea sp. (3.32), *S. hepatus* (3.77)	α, β, γ
	M. merluccius (4.45)	α, β
	E. encrasicolus (3.38)	α
Cu	*Solea sp.* (3.32), *L. budegassa* (4.54)	β
	E. encrasicolus (3.38), *S. cabrilla* (3.9), *S. hepatus* (3.77), *B. ocellaris* (3.26)	α
Zn	*L. budegassa* (4.54)	γ
	S. flexuosa (3.24)	β, γ
	G. niger (3.32), *U. scaber* (4.43), *B. ocellaris* (3.26)	α, β
	E. encrasicolus (3.38)	α
Se	*L. budegassa* (4.54)	γ
	S. flexuosa (3.24), *C. conger* (4.18), *C. macrophthalma* (3.13), *C. lucerna* (3.64)	β, γ
	M. merluccius (4.45), *G. niger* (3.32), *B. ocellaris* (3.26), *G. mediterraneus* (3.95)	α, β
	E. encrasicolus (3.38)	α

Mo	*L. budegassa* (4.54), *Solea sp*. (3.32) *E. encrasicolus* (3.38), *U. scaber* (4.43), *M. merluccius* (4.45)	β α
Fe	*Solea sp*. (3.32) *E. encrasicolus* (3.38), *M. merluccius*(4.45)	β α
As	*C. macrophthalma* (3.13) *E. encrasicolus* (3.38), *P. acarne* (3.84), *S. hepatus* (3.77)	β α
Ag	*C. macrophthalma* (3.13) *M. merlangus* (4.38), *L. budegassa* (4.54), *C. conger* (4.18), *Solea sp*. (3.32), *S. cabrilla* (3.9), *S. hepatus* (3.77) *M. barbatus* (3.27), *M. merluccius* (4.45)	γ β, γ α, β α
Cd	*C. macrophthalma* (3.13) *S. flexuosa* (3.24) *U. scaber* (4.43), *B. ocellaris* (3.26), *G. mediterraneus* (3.95) *Gaidropsarus sp*. (3.5)	γ β, γ α, β α
La	*Solea sp*. (3.32) *G. mediterraneus* (3.95)	β α
Nd	*Solea sp*. (3.32) *G. mediterraneus* (3.95)	β α
Sm	*Solea sp*. (3.32) *S. cabrilla* (3.95)	β α
Pb	*P. acarne* (3.84) *M. barbatus* (3.27) *T. torpedo* (4.5), *G. mediterraneus* (3.95) *U. scaber* (4.43), *B. ocellaris* (3.26)	γ β, γ α, β α
U	*S. flexuosa* (3.24) *U. scaber* (4.43)	β α

The concentration of metals in the livers for Al, Cr, Mn, Co, Cu, Zn, Se, and Mo displayed patterns of biomagnification where levels increased going up the food chain. Conversely, Fe, As, Ag, Cd, La, Nd, Sm, Pb, and U showed decreasing levels going up the food chain. The remaining metals: Ni, Y, Ce, Eu, Gd, Tb, Dy, Ho, Er, and Th, did not have statistically significant differences in metal concentrations in the gills.

Table 9. Metal concentrations in the gills for all species.

Metal	**Species and trophic levels - GILLS**	**Concentration levels**
Zn	*U. scaber* (4.43) *B. ocellaris* (3.26), *G. mediterraneus* (3.95)	β α
Ag	*M. merlangus* (4.38) *T. trachurus* (3.58) *S. cabrilla* (3.9), *C. lucerna* (3.64), *B. ocellaris* (3.26) *M. barbatus* (3.27), *M. merluccius* (4.45)	γ β, γ α, β α
Cd	*L. budegassa* (4.54)	β

	C. lucerna (3.64), B. ocellaris (3.26)	α
Mn	*C. macrophthalma* (3.13), *C. lucerna* (3.64)	β
	S. cabrilla (3.9)	α
Se	*S. flexuosa* (3.24)	γ
	M. barbatus (3.27)	β, γ
	G. niger (3.32), *C. linguatula* (4.34), *Ophidion sp*. (3.52)	α, β
	E. encrasicolus (3.38)	α
U	*S. flexuosa* (3.24)	γ
	S. scombrus (4.37)	β, γ
	M. merluccius (4.45), *U. scaber* (4.43)	α, β
	B. ocellaris (3.26)	α

For metal concentrations in the gills, Zn, Ag, and Cd showed patterns of biomagnification, whereas Mn, Se and U revealed a decrease in concentration going up the food chain. Species did not show statistically significant differences for the following metals: Al, Cr, Fe, Co, Ni, Cu, As, Y, Mo, Cd, La, Ce, Nd, Eu, Sm, Gd, Tb, Dy, Ho, Er, Pb, and Th.

Changes in tissue metal concentrations among species

For the testing of the second hypothesis, metal accumulations between tissues of different species were considered. The following three tables 10, 11, and 12 show in which tissues metal concentrations were highest. The tissues that will be represented in this section consist of the muscle, gills and liver. The scales and skin could not be included in the statistical analysis for all species as samples were not available. It is worth mentioning that the muscle, gill and liver samples were available for all species, with the exclusion of *Sygnathus acus*, a small fish difficult to dissect. Thus, in this case a portion of the mid- body was cut and considered to be muscle tissue. The species *Scyliorhinous canicula* was represented by only one individual and was therefore excluded from any statistical analyses performed.

Metals were divided into three groups, the first represented a group that highly regulated by food safety authorities in most countries, the second by lanthanides, and the third by the remainder of the metals in this study.

Table 10. Comparison among As, Cu, Zn, Pb, Cr, and Cd concentrations in the muscle, gills, and liver. Tissues in parenthesis did not have statistically significant differences between each other. (M-muscle, G – gills, and L- liver; ns – not significant).

Species	Cr	Cu	Zn	As	Cd	Pb
M. barbatus	G>L	ns	G>M	M>L	L>M	ns
M. merluccius	G>M	L>M	G>M	M>(G, L)	L>M	G>L
C. linguatula	G>L	L>M	L>M	M>G	L>M	G>L
M. merlangus	G>L	L>G	ns	M>G	L>M	M>L
L. budegasa	G>L	L>M	L>M	M>G	L>M	G>M
T. trachurus	ns	L>M	G>M	M>G	L>M	(G, M)>L
E. encrasicolus	ns	M>G	M>L	M>L	G>(L, M)	(G, M)>L

S. flexuosa	G>L	L>(M, G)	G>M	M>G	L>(G, M)	G>L
P. acarne	G>L	L>M	G>M	M>L	L>M	L>M
C. conger	G>L	L>M	ns	ns	L>M	G>L
G. niger	G>L	L>M	G>(L, M)	M>L	L>M	ns
Solea sp.	G>L	L>M	G>M	ns	L>M	ns
C. macrophthalma	ns	ns	ns	ns	L>M	ns
S. cabrilla	G>L	ns	G>M	ns	L>M	G>L
S. hepatus	G>L	ns	ns	ns	L>M	G>L
U. scaber	G>L	L>M	G>L	ns	ns	G>L
T. draco	ns	L>M	ns	ns	ns	G>L
C. lucerna	G>M	L>M	(G, L)>M	ns	L>M	G>L
B. occelaris	G>L	L>M	ns	M>(G, L)	L>(G, M)	G>L
S. scombrus	ns	ns	G>M	ns	ns	G>M
Ophidion sp.	G>L	L>M	G>M	M>G	L>M	ns
P. blennoides	ns	L>M	G>M	ns	L>M	ns
G. mediterraneus	G>L	ns	ns	ns	L>M	ns
Commonly found in:	G	L	G	M	L	G

From table 10, one can see that most species contained higher concentrations of Cr, Zn, and Pb in the gills. Moreover, assimilation of Cu and Cd was in the liver of the majority of the species, and shockingly, As concentrations were highest in the muscle tissue.

According to results shown in table 11, the lanthanides showed greatest concentrations in the gills for most species.

Concentrations of Al, Mn, Fe, Co, Ni, Y, Th, and U, represented in table 12, were greatest in the gills, and Se, Mo, and Ag in the liver

Five species: *G. niger, Solea sp., S. cabrilla, S.hepatus*, and *U. scaber* had replicas for all five tissues (gills, muscle, liver, skin and scales). Statistical analyses were performed to determine in which tissues metals bioaccumulated most. The next three tables 13, 14, and 15 display metal accumulation in tissues for the five species mentioned. Metals were grouped in accordance to the previous section.

As can be seen in table 13, Cr, Zn, and Pb had high concentrations in the gills, but also with the addition of high concentrations in the skin for Zn and in the scales and skin for Pb. No changes were seen in Cu and Cd with greatest accumulation in liver, and As remaining to be concentrated in the muscle tissue.

In considering all five tissues, concentrations of the lanthanides slightly changed, accumulating both in gills and scales (table 14).

Shown in table 15, concentrations of Al and Y remained greatest in the gills, whereas Mn, Co, and Ni had higher concentrations in both the gills and the scales, and Fe had higher concentrations in the gills and the liver. No changes were seen in Se, Mo, and Ag, that continued to be highest in the liver.

Refer to appendix A for graphical plots of all metal concentrations (ppm dry weight) in tissues (gills, liver, muscle, and scales) for all trophic levels.

Table 11. Comparisons of lanthanide concentrations in the muscle, gills, and liver. Tissues in parenthesis did not have statistically significant differences between each other. (M-muscle, G – gills, and L- liver; ns – not significant).

Species	**Y**	**La**	**Ce**	**Nd**	**Eu**	**Sm**	**Gd**	**Tb**	**Dy**	**Ho**	**Er**
M. barbatus	G>L	G>(M, L)	G>(M, L)	G>(M, L)	G>L	G>(M, L)	G>(M, L)	G>L	G>L	G>L	G>L
M. merluccius	G>L	G>(M, L)	G>L	G>(M, L)	G>L	G>L	G>(M, L)	G>(M, L)	G>(M, L)	G>(M, L)	G>L
C. linguatula	G>M	G>(M, L)	G>(M, L)	G>(M, L)	G>M	G>M	G>M	G>(M, L)	G>M	G>M	G>M
M. merlangus	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L
L. budegasa	G>(M, L)	G>(M, L)	G>(M, L)	G>L	G>(M, L)	G>L	G>(M, L)	G>(M, L)	G>L	G>(M, L)	G>L
T. trachurus	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L
E. encrasicolus	G>L	G>(L, M)	G>(L, M)	G>(L, M)	G>L	G>L	G>(L, M)	G>(L, M)	G>(M, L)	G>(M, L)	G>(M, L)
S. flexuosa	G>M	G>M	G>M	G>M	G>(M, L)	G>(M, L)	G>M	G>M	G>M	G>(M, L)	G>M
P. acarne	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L
C. conger	ns	G>M	G>M	G>M	ns	ns	ns	ns	ns	ns	ns
G. niger	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L
Solea sp.	G>L	G>M	G>M	G>M	G>M	G>M	G>M	G>(L, M)	G>(M, L)	G>L	G>L
C. macrophthalma	G>(M, L)	G>M	G>M	G>M	G>(M, L)	G>(M, L)	G>M	G>M	G>(M, L)	G>(M, L)	G>(M, L)
S. cabrilla	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L
S. hepatus	ns	ns	ns	ns	ns	ns	ns	ns	G>L	G>L	G>L
U. scaber	G>L	G>(M, L)	G>L	G>L	G>L	G>(M, L)	G>L	G>(M, L)	G>(M, L)	G>L	G>L
T. draco	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
C. lucerna	G>(L, M)	G>M	G>(L, M)	G>(L, M)	G>L	G>(L, M)	G>(L, M)	G>L	G>(L, M)	G>(L, M)	G>L
B. occelaris	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L
S. scombrus	ns	G>M	G>M	ns	ns	ns	ns	G>L	ns	ns	ns
Ophidion sp.	G>M	G>M	G>M	G>M	G>M	G>M	G>M	G>M	G>M	G>M	G>M
P. blennoides	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
G. mediterraneus	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	ns	G>L	G>L
Commonly found in:	G	G	G	G	G	G	G	G	G	G	G

Table 12. Concentrations of Al, Mn, Fe, Co, Ni, Se, Mo, Ag, Th, and U in the muscle, gills, and liver. Tissues in parenthesis did not have statistically significant differences between each other. (M-muscle, G – gills, and L- liver; ns – not significant).

Species	Al	Mn	Fe	Co	Ni	Se	Mo	Ag	Th	U
M. barbatus	G>L	G> (L, M)	G>M	(G, L)>M	G>L	G>M	L>M	ns	G>L	G>(M, L)
M. merluccius	G>L	G>M	G>M	G>M	G>(M, L)	G>L	L>M	ns	G>(M, L)	G>L
C. linguatula	G>M	G>M	G>M	(G, L)>M	G>L	(L, M)>G	L>M	ns	G>(M, L)	G>M
M. merlangus	G>L	G>L	ns	G>M	G>M	ns	L>(G, M)	L>M	G>L	G>L
L. budegasa	G>L	G>M	G>M	L>M	G>M	L>G	L>M	L>M	G>L	G>M
T. trachurus	G>L	G> (L, M)	G>M	G>M	G>(M, L)	ns	L>(G, M)	L>M	G>L	G>L
E. encrasicolus	G> (L, M)	G>L	G>L	ns	G>M	M>(G, L)	L>G	ns	G>(M, L)	G>M
S. flexuosa	G> (L, M)	G>M	G>M	G>M	G>L	L>M	L>M	ns	G>(M, L)	G>(M, L)
P. acarne	G>L	G>L	G>M	(G, L)>M	G>L	ns	L>M	L>M	G>L	G>L
C. conger	ns	G>M	ns	ns	G>L	ns	ns	L>M	ns	ns
G. niger	G>L	G>L	G>M	G>M	G>L	ns	L>M	L>(G, M)	G>L	G>L
Solea sp.	G>L	G>M	L>M	G>M	G>L	L>M	L>M	L>M	G>L	G>(M, L)
C. macrophthalma	G>M	G>(L, M)	L>M	L>M	G>L	L>M	L>(G, M)	L>M	G>M	ns
S. cabrilla	G>L	G>L	G>M	ns	G>L	ns	L>M	ns	G>L	G>L
S. hepatus	ns	ns	L>M	ns	G>L	ns	L>M	ns	G>L	ns
U. scaber	G>L	G>L	G>M	G>M	G>(L, M)	ns	L>M	L>M	G>L	G>(M, L)
T. draco	ns	G>M	ns	L>M	ns	ns	L>M	ns	ns	G>M
C. lucerna	G>(L, M)	G>M	(G, L)>M	L>M	G>L	L>M	L>M	L>M	G>(L, M)	G>(L, M)
B. occelaris	G>L	G>L	L>M	ns	G>L	ns	L>(G, M)	L>M	G>L	G>L
S. scombrus	G>L	G>M	G>M	G>M	G>L	ns	L>M	L>M	ns	G>L
Ophidion sp.	ns	G>M	L>M	G>M	G>L	ns	L>M	L>M	G>M	G>L
P. blennoides	ns	G>M	G>M	G>M	ns	ns	M>L	ns	ns	G>L
G. mediterraneus	G>L	G>L	G>M	ns	ns	ns	ns	ns	G>L	G>L
Commonly found in:	G	G	G	G	G	L	L	L	G	G

Table 13. Concentrations for Cr, Cu, Zn, As, Cd, and Pb in the muscle, gills, liver, skin and scales of *G. niger, Solea sp., S. cabrilla, S.hepatus*, and *U. Scaber* . (M-muscle, G – gills, and L- liver, Sc.-scales, Sk.-skin ; ns – not significant).

Species	**Cr**	**Cu**	**Zn**	**As**	**Cd**	**Pb**
G. niger	G>L	L>(M, Sk.)	ns	M>Sc.	L>Sk.	Sc.>L
Solea sp.	G>L	L>(M, Sk., Sc.)	(G, Sc.)>M	M>Sc.	L>(M, Sk.)	Sc.>(L, M)
S. cabrilla	G>L	L>Sk.	ns	M>Sc.	L>Sc.	Sc.>(L, M)
S. hepatus	ns	L>M	G>L	ns	L>M	Sc.>L
U. scaber	ns	L>M	G>L	ns	ns	(Sk., G)>L
Commonly found in	G	L	Sk and G	M	L	Sc, Sk, and G

Table 14. Lanthanide concentrations in the muscle, gills, liver, skin and scales of *G. niger, Solea sp., S. cabrilla, S.hepatus*, and *U. Scaber* . (M-muscle, G – gills, and L- liver, Sc.-scales, Sk.-skin ; ns – not significant).

Species	**Y**	**La**	**Ce**	**Nd**	**Eu**	**Sm**	**Gd**	**Tb**	**Dy**	**Ho**	**Er**
G. niger	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L
Solea sp.	(G, Sc.)>L	G>M	G>M	G>(M, Sk.)	(G, Sc.)>M	(G, Sc.)>M	(G, Sc.)>M	G>M	G>(L, M)	(G, Sc.)>L	G>(L, M)
S. cabrilla	(G, Sc.)>L	Sc.>L	(G, Sc.)>L	(G, Sc.)>L	(G, Sc.)>L	(G, Sc.)>L	(G, Sc.)>L	(G, Sc.)>L	(G, Sc.)>L	(G, Sc.)>L	(G, Sc.)>L
S. hepatus	ns	ns	ns	ns	Sc.>L	ns	ns	ns	ns	Sc.>L	(G, Sc.)>L
U. scaber	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L	G>L
Commonly found in:	G	G and Sc	G and Sc	G and Sc	G and Sc	G and Sc	G and Sc	G and Sc	G and Sc	G and Sc	G and Sc

Table 15. Concentrations of Al, Mn, Fe, Co, Ni, Se, Mo, Ag, Th, and U in the muscle, gills, liver, skin and scales of *G. niger, Solea sp., S. cabrilla, S.hepatus*, and *U. Scaber* . (M-muscle, G – gills, and L- liver, Sc.-scales, Sk.-skin ; ns – not significant).

Species	**Al**	**Mn**	**Fe**	**Co**	**Ni**	**Se**	**Mo**	**Ag**	**Th**	**U**
G. niger	G>L	G>L	G>M	G>(M, Sk.)	(G, Sc.)>L	ns	L>(M, Sk.)	L>G	G>L	(G, Sc.)>L
Solea sp.	(G, Sc.)>L	(G, Sc.)>M	L>(M, Sk.)	(G, Sc.)>M	(G, Sc.)>L	L>(Sk., Sc.)	L>(M, Sk.)	L>(M, Sk.)	G>L	Sc.>(L, M)
S. cabrilla	(G, Sc.)>L	(G, Sc.)>L	(G, L)>M	Sc.>(M, Sk.)	Sc.>(M, L)	L>Sc.	ns	ns	(G, Sc.)>L	(G, Sc.)>L
S. hepatus	ns	ns	ns	Sc.>M	Sc.>L	L>Sc.	L>M	ns	ns	Sc.>L
U. scaber	G>L	G>(L, M)	G>M	G>M	G>(L, M)	L>Sc.	L>(M, Sk.)	L>M	G>L	Sk.>(L, M)
Commonly found in:	G	G and Sc	G and L	Sc and G	Sc and G	L	L	L	G and Sc	G and Sc

Bioaccumulation patterns in metals

A PCA analysis was performed on normalized metal concentrations to test for changes in bioaccumulation patterns among trace elements in the muscle, gills and liver. To correct strongly skewed distribution all data was z-transformed. The overall multivariate patterns (Figures 4, 5, and 6) did not appear to be related to the position of species in the trophic level. Refer to the table in appendix B for the naming of species according to the numbers provided in the PCA figures 4 to 6.

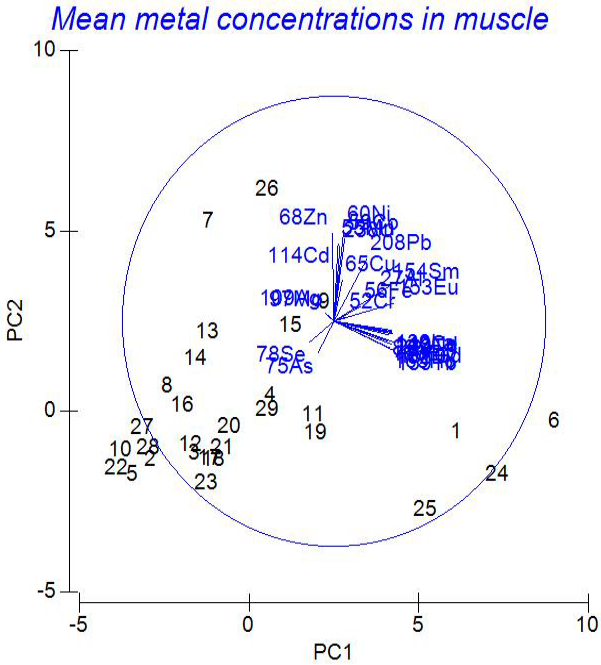

Figure 4. PCA analysis results of metal concentrations in muscle tissues for all twenty-nine species.

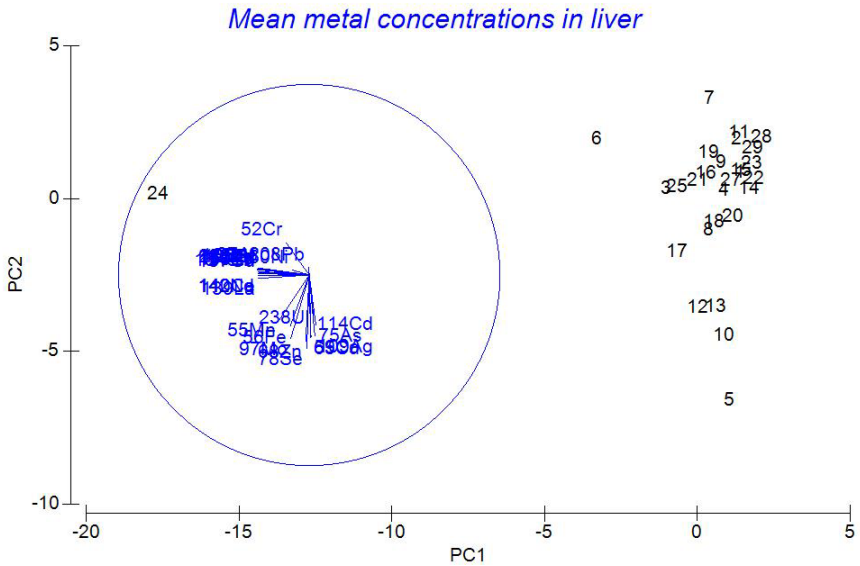

Figure 5. PCA analysis results of metal concentrations in liver tissues for all twenty-nine species.

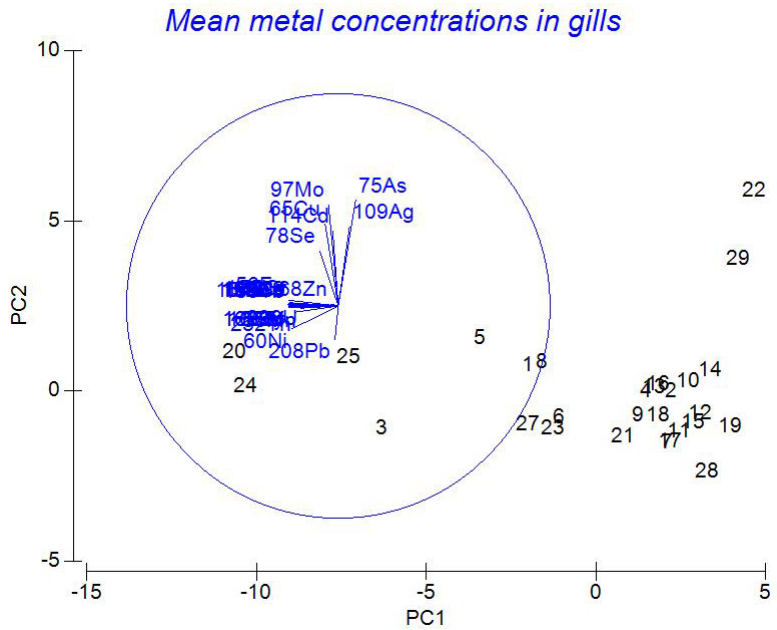

Figure 6. PCA analysis results of metal concentrations in gills for all twenty-nine species.

Discussion

Changes in metal concentrations along food web

Metal accumulation patterns in tissues varied along the trophic chain. Uranium was the only the metal that showed inverse biomagnification in all three tissues. (Note: what is meant by the term 'inverse biomagnification' in this text is an increase in metal concentration in tissues of species occupying lower trophic levels with respect to those of higher trophic levels) Concentrations of Fe and Pb also decreased going up the food chain for muscle and gills only.

Biomagnification did not occur for a specific metal in all three tissues but rather two tissues at a time. For example, Cr, Mn, Cu, As, and Se appeared to be transferred in increasing amounts along trophic levels in only the muscle and liver of species. A similar behaviour was determined for Ag and Cd in the muscle and gills, and for zinc in the liver and gills.

Species played different roles in the transfer of metals along the food chain. In figure 7, species involved in biomagnification and inverse biomagnification patterns are represented, along with the overall order from lowest to highest (trophic level) member of this demersal food web.

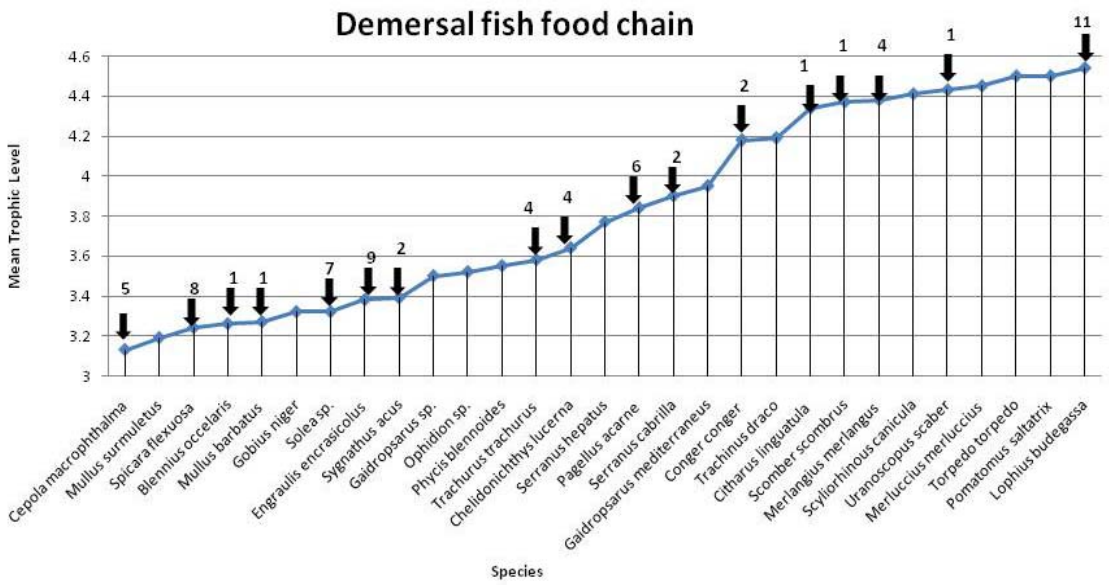

Figure 7. Species classified according to mean trophic level. Species indicated with an arrow were among those that had a top position on the 'concentration levels' for muscle, gills and liver. The digits above the arrows represent the number of times the name of the species appeared at the top of the concentration levels.

As can be seen in figure 7, species *C. macrophthalma*, *S. flexuosa*, *Solea sp*., *E. encrasicolus*, *P. acarne*, and *L. budegassa* were shown repeatedly as having top concentration levels. Note that these species occupied different levels of the food chain, differed in swimming activity and feeding habits (Fishbase, 2010). The species containing high digits played a role in both the biomagnification of metals and the inverse biomagnification of metals. Names of species

that appeared fewer times played a role in inverse biomagnification, as was the case for *S. cabrilla*, *S. acus*, and *M. barbatus* and biomagnification for *B. ocellaris*, *C. linguatula*, and *U. scaber*.

Correlations between elements of the same tissues of fishes may be in part similar to accumulation behaviours of trace elements in fishes and their interactions (Kojadinovic et al., 2007). Perhaps it is difficult to deduce a concrete answer for the presence or absence of biomagnification from metal concentrations in three tissues of different species. According to Bryan (1979), conclusions concerning biomagnification can be best described by comparing whole individual bodies of species. The comparison of different tissues can mistakenly be interpreted because metals are known to accumulate in special organs or tissues such as the liver (Wang, 2002). Although, the usage of same tissues in different trophic levels can help in reducing the variability of heavy metal concentrations within organisms (Gray, 2002).

According to a review written by Gray (2002), there is no substantial evidence to support the biomagnification of metals, with the exception of mercury. Capturing such a phenomenon in a marine food chain is rather difficult as the marine environment, being much larger than the terrestrial environment, represents a vast area where organisms can feed on a variety of available food, and energy can be passed alternately down and up the food web. Thus, the marine food web does not have a particular structure, and can be viewed as a pair of interwoven pyramids for example, comprising an infinite number of steps. Each step can be represented by material and energy resulting from the previous step, with living material in one pyramid and non-living but usable in the other. Marine organisms have the potential to occupy large sections of this unstructured food web, making it difficult to track predator-prey relationships and to explain varying trace element concentrations between and among species. Moreover, in the marine environment predators feed on a large range of prey typically smaller in size. When contaminants differ between species and among age groups within species, biomagnification is less likely to occur (Isaacs, 1973). Thus, in the case of this demersal food chain, exact relationships between pyramids and levels were not well established, making it difficult to say for certain that biomagnification occurred, even though there was evidence.

L. budegassa, the species highest on the trophic level was revealed to be of the top (11 times) on the species 'concentration levels' list. According to Leblanc (1995) species of higher trophic levels can show higher concentrations of trace elements for the reason that once taken up via passive diffusion through the body surface, elimination rates of metals begin to decrease with body size. Not only are elimination rates decreased with the increasing mass of an organism, but also are the depuration rates, (i.e. cleansing) of lipophilic substances (Gray, 2002).

Conversely, *C. macrophthalma*, the species occupying the lowest level of this food chain, also displayed high levels of metal concentrations. Sediments and detritus of polluted waters usually contain elevated levels of metal concentrations, directly increasing bioaccumulation of metals in

fish that feed on sediment and detritus. Thus, fish species of lower trophic levels contain higher metal concentrations than fish of higher trophic levels (Connell and Miller, 1984).

Metal concentrations can increase along a food chain up to the level where the organism on which the fish species feed, however, it should be noted that this is not always the case (Dallinger et al., 1987). The reasoning is that heavy metals are more available at lower trophic levels, and that fish have the capacity to reject large amounts of ingested heavy metals (Tarifeno-Silva et al., 1982). Moreover, metal concentrations in organisms should not be dependent on corresponding trophic levels, but should be based on physiological characteristics of the fish species and the biological role of the trace element (Amiard-Triquet et al., 1993). However, according to more recent findings, species of higher trophic levels can have greater concentrations due to a greater ability to accumulate metals from the surrounding aqueous phase in comparison to species of lower trophic levels, rather than assuming metal uptake resulted from diet (Wang et al., 2002).

Although evidence of biomagnification was apparent in some cases in this work, results were not completely consistent with the findings of Wang (2002), but were more related to Dallinger (1987) that suggested that no one particular pattern (i.e. biomagnification or inverse biomagnification) can be expected due to the complexity of the trophic web.

Changes in metal concentration between tissues

The results showed assimilation of metals in all three tissues. Metals Cr, Zn, Pb, Al, Mn, Fe, Co, Ni, and all of the lanthanides had accumulated in the gills. Fish are in constant contact with the water, whether passing through the mouth and/or over the gills (Pentreath, 1977). The gills thus function as a site for transient metal accumulation (Dallinger et al., 1987). The metal levels measured in the gills mirror metals present in the water (Roméo et al., 1999). As for the lanthanides, according to Kameda (1962) the accumulation of these metals is common in the bone structure of marine organisms (e.g. bone, scales, gills and dorsal fins).

In the liver of fish species high levels of Cu, Cd, Se, Mo and Ag were revealed. The liver is a site for metal storage, and detoxification. Storage occurs by the binding of metallothioneins to heavy metals, protecting the fish from toxicity. Increased metal concentrations in this tissue can suggest the sequestration of metallothionein-heavy metal complex (Roméo et al., 1999). The liver has a tendency of accumulating larger concentrations of metal than the muscle tissue (Yilmaz et al., 2010).

The muscle is a tissue of particular interest as it is the portion of the fish that is consumed by humans. Bioaccumulation of arsenic was found in the muscle. Similar results were presented by Yilmaz et al. (2010) and Mormede & Davies (2001), supporting the fact that arsenic accumulates in muscle organs, such as the flesh and heart.

The comparison of metal concentrations in similar tissues of different species poses a bit of a difficulty due to the differences in feeding, aquatic environment (Yilmaz et al., 2010) and

complex food chain (Isaacs, 1973). However, in this particular study all necessary steps were taken to minimize variability from other sources. For example, species of demersal fish were removed by trawl from the same benthic environment, in a particular space and at a particular time.

Food safety authority standards

Metal concentrations of As, Pb, Cr, Zn, Cd, and Cu in the muscle tissues (µg/g wet weight) were compared with statutory levels recognized as safe for human consumption. It should be noted that standards are different for each country, and are generally difficult to find for fish muscle. The purpose of implementing these standards is to ensure food safety and the prevention of dietary uptake of contaminants (FDA, 2005). It would seem fit for practical reasons to create standards that could be applied on a global scale thereby making it easier for research, and for the exchange of food products between countries. Table 16 lists the standards from various food safety authorities for Cu, Zn, Pb, Cr, Cd and As; heavy metals considered to be a hazard to human health. The limits are provided in µg/g wet weight.

Table 16 . A list of the food safety standards for Cu, Zn, Pb, Cr, Cd, and As in edible fish tissue (i.e. muscle). Underlined numbers were used for comparison of data.

Source	**Cu**	**Zn**	**Pb**	**Cr**	**Cd**	**As**
EC			**0.3**		**0.05 fish muscle**	
					0.10 *Trachurus sp.*	
					0.30 *Engraulis sp.*	
Canada[2]		100	0.5			**3.5**
Australia and New Zealand[3]			0.5			2
China[4]	10	50	1	**0.5**	0.1	0.5
Hungary[5]		150				
International[5]		40-100				
FAO[6]	**30**	**30**	0.5		0.5	
FDA[7]				12-13		
MAFF[8]	20	50	2		0.2	
Turkish standards[8]	20	50	0.3		0.1	

1. European Commission, 2006.
2. Canadian Food Inspection Agency, 2009.
3. Food Standards Australia and New Zealand, 2010.
4. Zhang et al., 2007.
5. Zhang et al., 2007.
6. Zhang et al., 2007.
7. Tuzen, 2009.
8. Tuzen, 2009

The food safety limits used to compare measured metal concentration for Cu, Cr, Zn, Pb, Cd and As in the edible part of the fish (i.e. muscle), are underlined and bolded in table 16. Certain species were above the threshold for Zn, Cr, Pb, and As (table 17), whereas none were above standards for Cu and Cd.

Table 17. List of species that had concentrations of Zn, Cr, Pb, and As in the muscle above food safety authority limits. Average metal concentrations are expressed in μg/g wet weight.

Metal	Species	Average metal concentration (ppm wet weight)
Zn	*S. canicula*	32.19019
Cr	*Gaidropsarus sp.*	0.6608
	S. scombrus	0.5184
	S. canicula	1.0219
Pb	*C. macropthalma*	0.419196
	S. flexuosa	1.757902
	B. occelaris	0.350728
	G. niger	0.342932
	E. encrasicolus	0.345005
	S. acus	0.352841
	Gaidropsarus sp.	0.38528
	P. blennoides	0.789153
	T. trachurus	1.599188
	S. hepatus	1.197304
	P. acarne	1.2817
	S. cabrilla	0.328528
	T. draco	8.108883
	M. merluccius	2.273987
As	*G. mediterraneus*	5.476038
	S. canicula	87.75261
	T. torpedo	14.26045

As shown in table 17, a large number of the species in this demersal food chain exceeded the limits for metal concentration for As, Pb, Cr, and Zn. If a lower standard were to be used for arsenic, for example that given by the Food Standards of Australia and New Zealand (2ppm), the other species that would have exceeded the limits would have been *B. ocellaris*, *M. barbatus*, *C. conger*, *U. scaber*, *P. saltatrix*, and *L. budegassa*. As previously mentioned, it is imperative for the monitoring of these six metals as they can have detrimental effects on human health (FDA, 2005).

It should be noted that in the Thermaikos Bay marine sediments were reported to have traces of Zn, Cu, Pb, As, and Ag resulting from anthropogenic perturbations, notably at the inner part of the bay. According to the sites investigated in the Thermaikos Bay by Violintzis et al. (2009), measured metal concentrations could have a negative effect on marine biota and were considered to be of medium-low and medium-high priority for toxicity. This could be among the reasons explaining why species in this study contained levels exceeding the statutory limits.

Comparison of results with other studies

In this section, the concentrations of different metals (Al, Pb, Cd, Cr, Fe, Mn, Ni, Zn, As, Cu, Se and Co) (μg g^{-1}, wet weight) for ten fish species (*E. encrasicolus*, *M. merlangus*, *M. barbatus*, *T. trachurus*, *S. scombrus*, *P. saltatrix*, *L. budegassa*, *Solea sp*., *M. surmuletus*, and *P. blennoides*) in the edible portion of the fish were compared with the findings of other papers.

Concentrations of the trace element aluminum in species *E. encrasicolus* and *M. merlangus* in this study were revealed to be lower than measurements reported by Turan et al. (2009) from the Black and Mediterranean Sea, whereas levels for *M. barbatus* were impressionably higher.

In the case of lead for *E. encrasicolus* , *M. barbatus*, *T. Trachurus,* and *M. merlangus* concentrations in this work revealed to be within the ranges reported in Black Sea and Mediterranean Sea (Turan et al, 2000; Tuzen, 2009; Mendil et al., 2010). Measurements of Pb in this study were lower than those found for *M. barbatus*, *M. merlangus*, *S. scombrus*, *P. saltatatrix*, *L. budegassa*, *Solea lascaris* and *M. surmuletus*, in the Black Sea, the Mediterranean Sea, Iskenderun Bay, and Catalonia Spain (Turan et al., 2009; Tuzen, 2009, Yilmaz et al., 2010; Castro-González and Méndez-Armenta, 2008). Measurements for *M. barbatus*, S. lacaris, and *P. blennoides* were reported to be higher than those provided by Storelli (2008), Mendil et al. (2010), and Castro-González & Méndez-Armenta (2008).

Compared cadmium concentrations in this study were lower than those reported for *E. encrasicolus*, *M. barbatus*, *M. merlangus*, *T. trachurus*, *S. scombrus*, *P. saltator*, *P. blennoides*, and *M. surmuletus* (Storelli, 2008; Turan et al., 2009; Tuzen, 2009; Mendil et al., 2010; Castro-González and Méndez-Armenta, 2008). Conversely, higher were the levels in this study for M. barbatus and M. merlangus (Storelli, 2008; Castro-González and Méndez-Armenta, 2008). *L. budegassa* and *S. lascaris* were reported to be within measured ranges (Yilmaz et al., 2010; Castro-González and Méndez-Armenta, 2008).

Chromium concentrations of this study, were found to be within ranges for *T. trachurus* and *S. scombrus* (Mendil et al., 2010; Tuzen, 2009). The remaining species, *E. encrasicolus*, *M. barbatus*, *M. merlangus*, *P. saltatrix* and *S. lascaris* had higher concentrations than those revealed for this study (Turan et al., 2009; Tuzen, 2009; Mendil et al., 2010; Yilmaz et al., 2010).

Iron levels reported in this study for *E. encrasicolus*, *M. merlangus*, and T. *trachurus* were below those reported in other papers (Tuzen, 2009; Mendil et al., 2010; Yilmaz et al., 2010). Concentrations measured for *M. barbatus*, and *S. scombrus* in this study were within rages of those provided in other studies (Storelli, 2008; Turan et al., 2009; Tuzen, 2009).

Measured manganese concentrations in this work were within ranges with other reported experiments for *E. encrasicolus*, *M. barbatus*, *T. trachurus*, *S. scombrus, P. saltatrix*, *L. budegassa* and *S. lascaris* (Turan et al., 2009; Tuzen, 2009; Mendil et al., 2010; Yilmaz et al., 2010).

Nickel concentrations were higher for *E. encrasicolus*, *M. merlangus*, *T. trachurus*, *S. scombrus*, *P. saltatrix*, and *L. budegassa* in comparison to those measured in this study (Turan et

al., 2009; Tuzen, 2009; Yilmaz et al., 2010), and were also found to be within ranges for *M. barbatus* and *S. lascaris* (Turan et al., 2009; Tuzen, 2009; Yilmaz et al, 2010).

In most cases, zinc concentrations were lower in this study in comparison to other works for *E. encrasicolus*, *M. barbatus*, *M. merlangus*, *T. trachurus*, *S. scombrus*, *P. saltatrix*, and *S. lascaris* (Turan et al., 2009; Tuzen, 2009; Mendil et al., 2010; Yilmaz et al., 2010) and only in the case of *L. budegassa* were concentrations within range (Yilmaz et al. 2010).

In this study, arsenic levels were impressively high, but interestingly were surpassed by levels reported in *S. lascaris*, and *M. surmuletus* (Yilmaz et al., 2010; Castro-González and Méndez-Armenta, 2008). Measured arsenic levels in this study for species *E. encrasicolus*, *T. trachurus*, *S. scombrus*, and *L. budegassa* were within ranges, whereas *M. barbatus*, *M. merlangus* and *P. saltatrix* showed higher concentrations than those provided in other works (Tuzen, 2009; Yilmaz et al., 2010).

In this study, copper concentrations were below those measured for *E. encrasicolus*, *M. barbatus*, *M. merlangus*, *T. trachurus*, *S. scombrus*, *P. saltatrix* and where within ranges for species *L. budegassa* and *S. lascaris* (Tuzen, 2009; Mendil et al., 2010; Yilmaz et al., 2010).
Reported levels for selenium were in the ranges for those measured in this study for *M. barbatus*, *M. merlangus*, *T. trachurus*, *S. scombrus* and *P. saltatrix*, and finally below levels reported by Tuzen (2009) for *E. encrasicolus* (Tuzen, 2009).

Cobalt concentrations in this work were lower than those for *M. barbatus*, *M. merlangus*, *T. trachurus*, and *S. lascaris*, whereas *L. budegassa* revealed to be within ranges (Mendil et al., 2010; Yilmaz et al., 2010).

In the comparison of results from this study to those of others, it is evident that metal concentrations varied greatly between species, and in many cases measured values were greater, lesser or within certain ranges. Factors responsible for this variability can be both abiotic and biotic. For example, fish habitat, forms of water contaminants, and fish physiology can determine the degree to which a metal may bioaccumulate (Has-Schön et al., 2006).

Metals in sediments

The monitoring of chemical contaminants in coastal areas is extremely important in providing information about the quality or 'state of health' of an aquatic environment. Emphasis should be placed on metals as they are environmentally persistent and can pose a large ecological risk (Clark, 2001).

On their route to sea, contaminants can become permanently or temporarily stored in sediments of rivers, lakes, estuaries, and coastal waters. The building of a harbour, as in the case of the Thermaikos Bay, increases the release of contaminants into the aquatic medium through sedimentation. This resultant build up of sediment in a particular area requires for its removal and dumping to an alternate area, directly interrupting natural processes, placing sediments where they

would never reach. Over time, the accumulation of toxic elements becomes one of the many sources of pollutants to an aquatic environment (Salomons et al., 1987).

Process of contaminant release from particulates

When sediments are deposited in the water column, contaminants can be released during their fall. Sediments can be divided into two parts, the oxic surface layer and the anoxic sediment. The oxic layer consists of the water above the sediment and can extend into the sediment. Here, particles are degraded. This layer is rich in organic matter and houses a large bacterial population (Salomons et al., 1987).

The anoxic layer consists of the sediment and a portion of the water column above the sediment. In this layer contaminants can be transformed to new species by sulphate reduction. These redox reactions create changes in concentrations from the sediment to the surface water, resulting in upward (or downward) diffusion, i.e. the transport of components (Salomons et al., 1987).

Metals such as zinc, cadmium and copper are present as sulphides. Important reactions concerning quality of surface water occur in the oxic surface layer. The movement of deposited sediment and changes in surface water composition can affect the removal of dissolved metals from particulates. Similar results also arise when the sediment is taken from an anoxic to oxic environment, as in the case of dredging (Salomons et al., 1987), a process undertaken in the Thermaikos Bay.

The oxic-anoxic interface in the sediment determines the release process. The behaviour of the chemicals is dependent upon the process of transport and the changes in the chemical environment (Salomons et al., 1987). As previously mentioned, the Thermaikos Gulf is heavily burdened with different pollutants (Violintzis et al., 2009). The potential for the existence of oxic-anoxic zones in the site where specimens were trawled could theoretically explain the high metal concentrations found in these demersal fish.

Conclusion

Patterns of biomagnification were evident in this study, but one could not say with certainty that metals were transferred along this demersal food chain. Bioaccumulation of metals varied in tissues with most metals accumulating in the gills, followed by the liver, and then in the muscle for arsenic only. According to research in this field, it is difficult to test for biomagnification in a trophic chain due to the complex nature of aquatic food web (Isaacs, 1973), the diverse and wide range of fish habitats, the differences in feeding behaviours, physiological responses to metals, and the actual forms of metals in the water (Has-Schön et al., 2006).

Metal concentrations in the edible portion of the fish species exceeded permissible limits provided by food safety authorities. These elevated metal concentrations could be explained by anthropogenic activities that take place in or near the bay of the Thermaikos, pumping large amount of contaminants into the gulf, and having negative effects on the marine biota (Violintzis et al. 2009)

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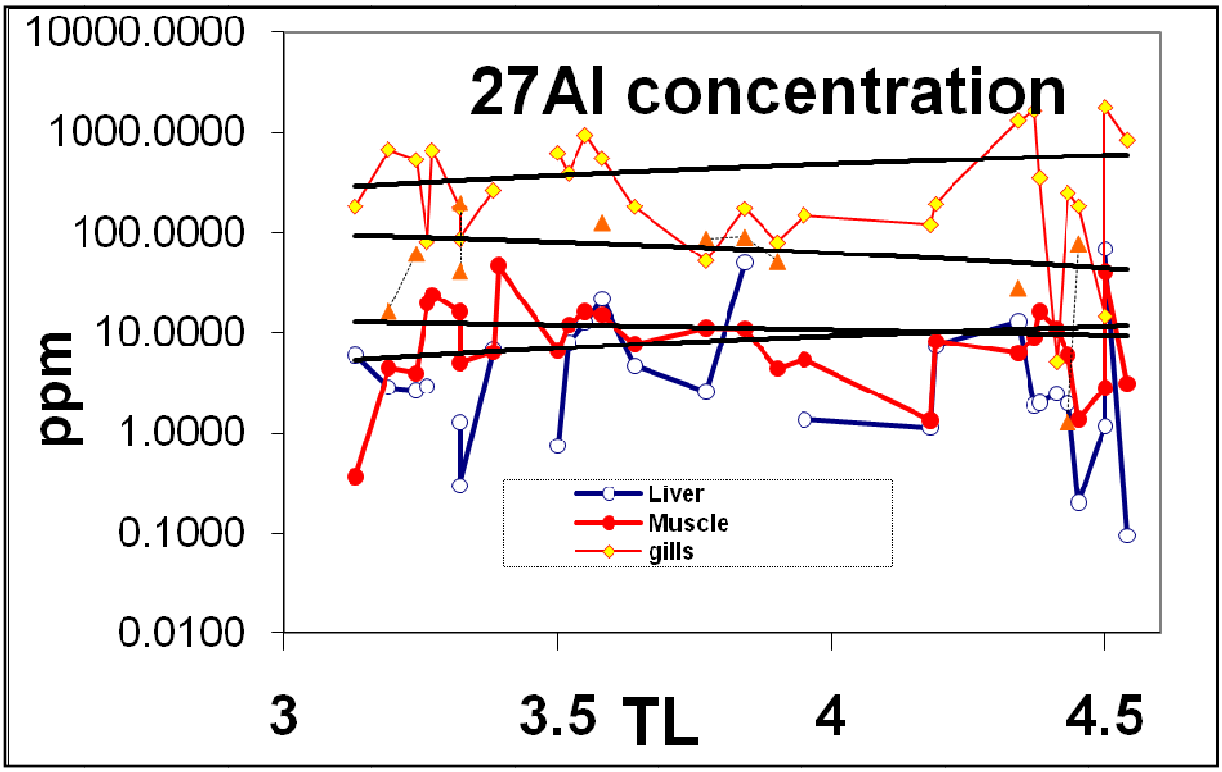
Appendix A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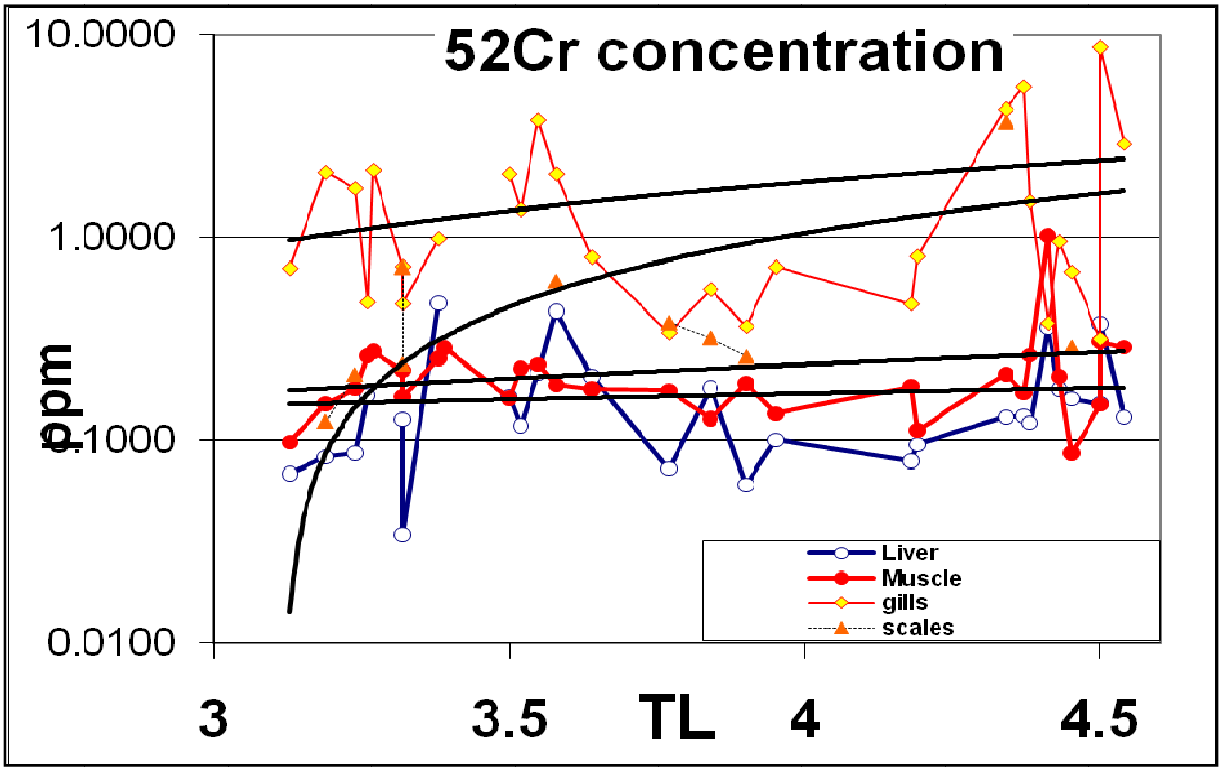
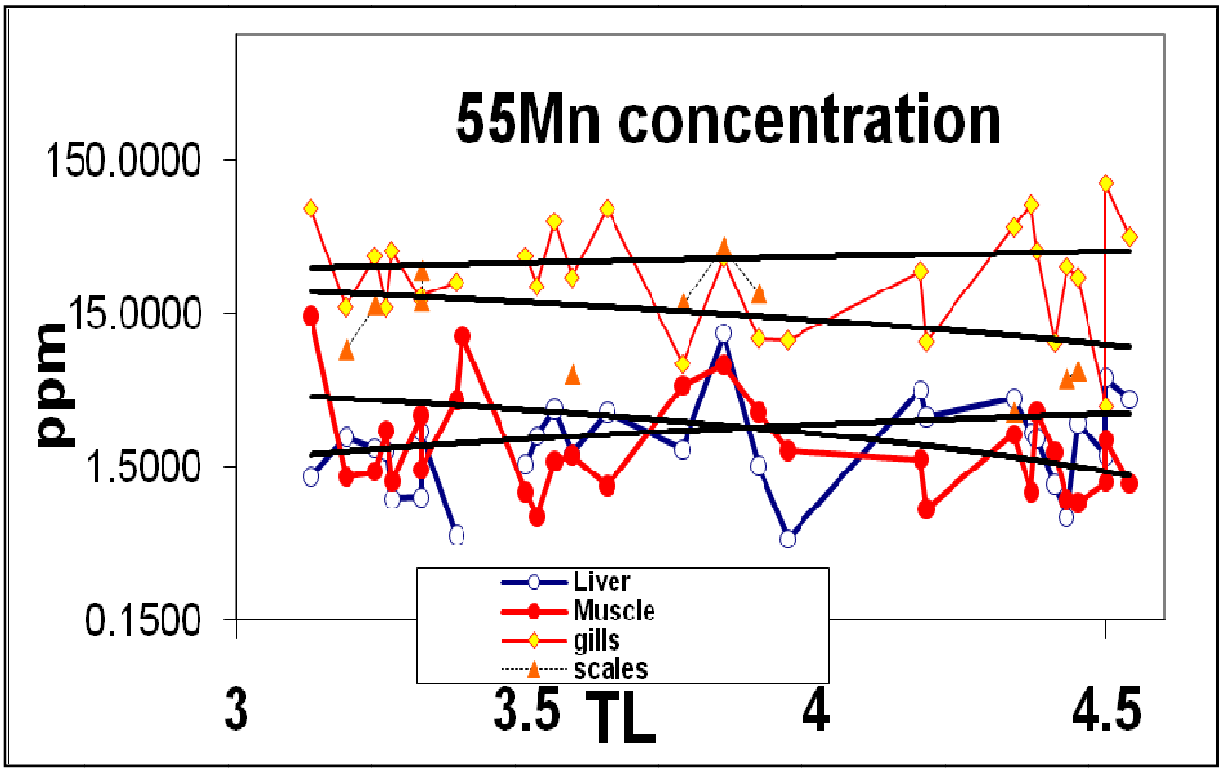
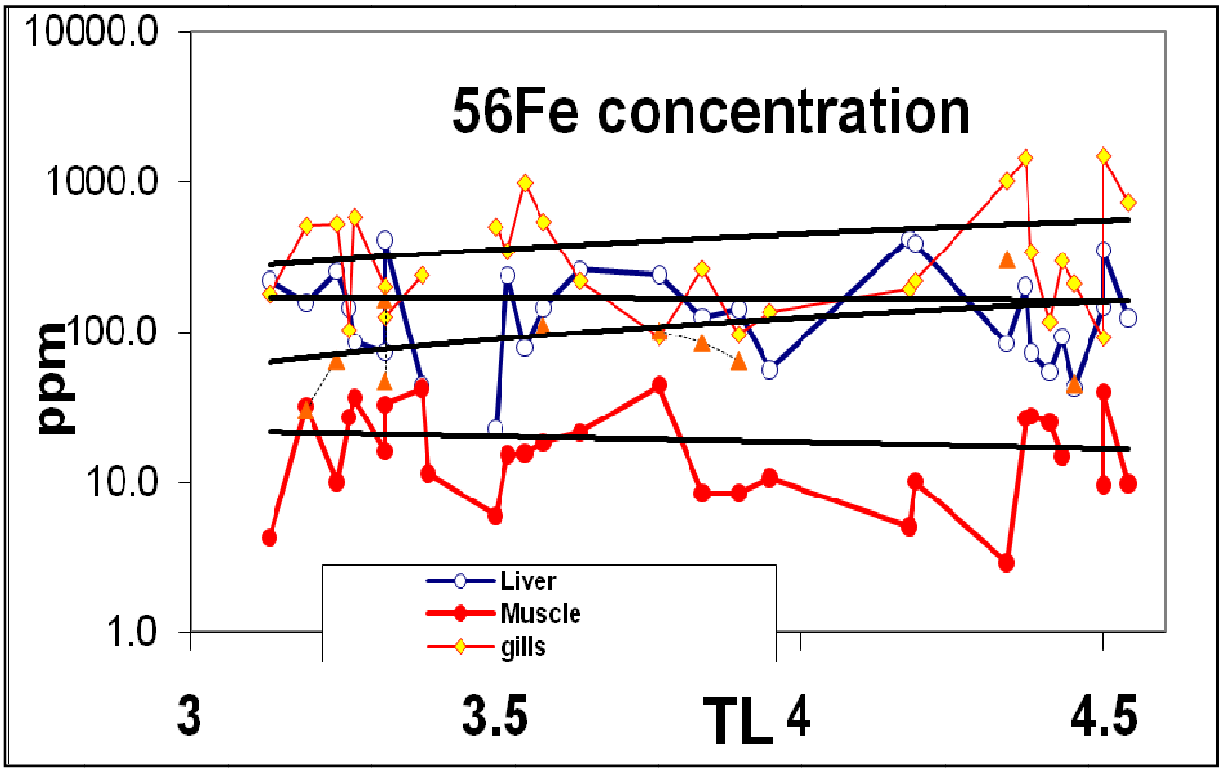
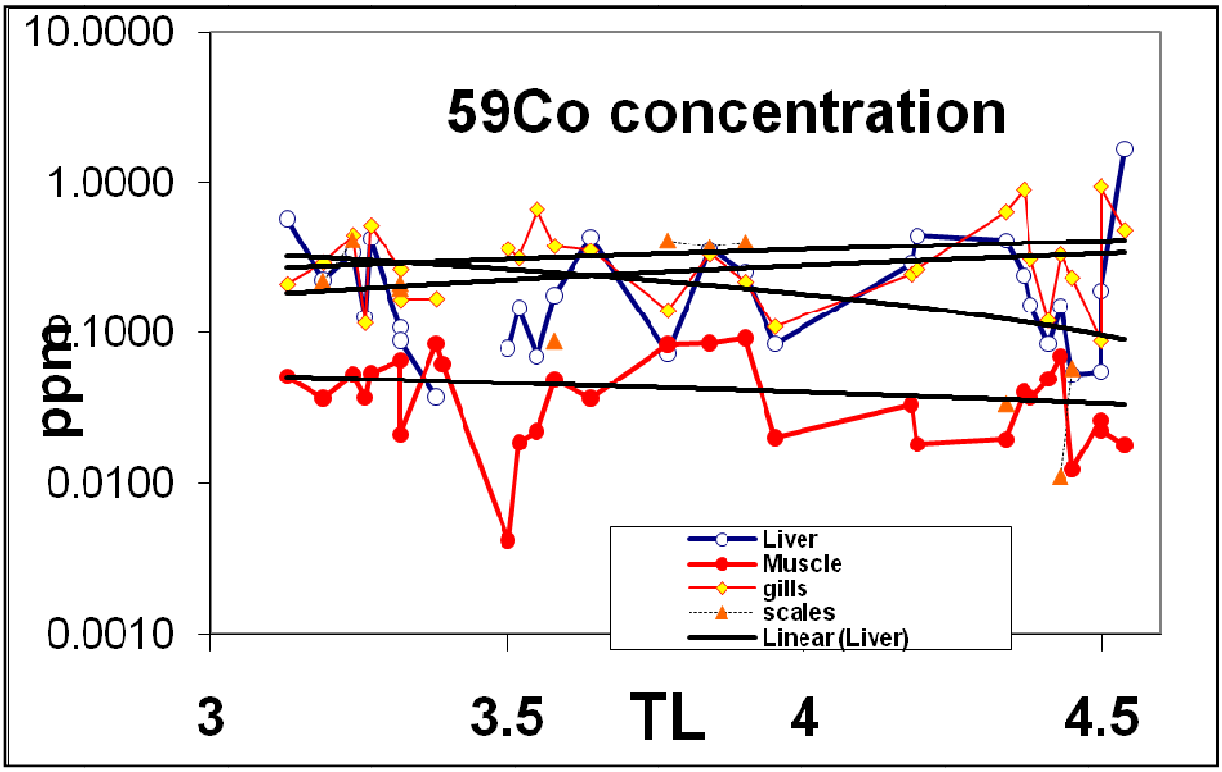
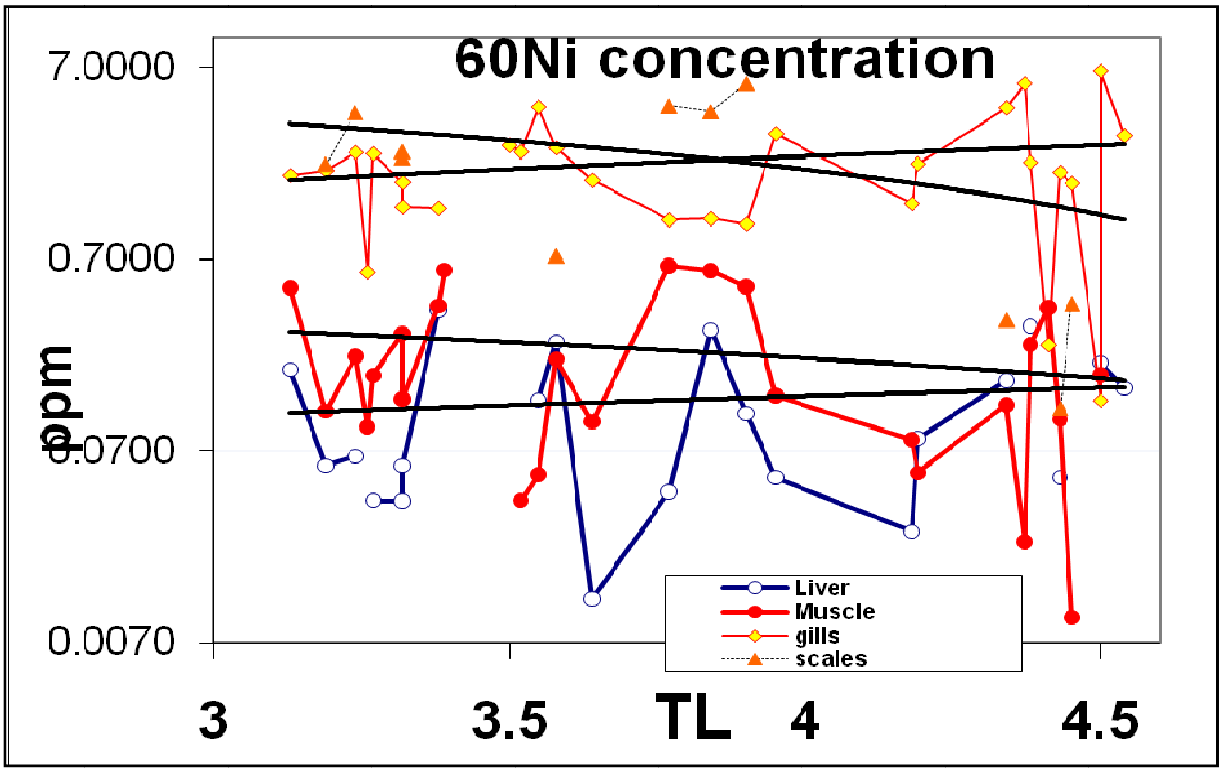
1000.0000
100.0000
10.0000
1.0000
0.1000
0.0100
ppm
3
3.5
TL
4
4.5
Liver
Muscle
gills

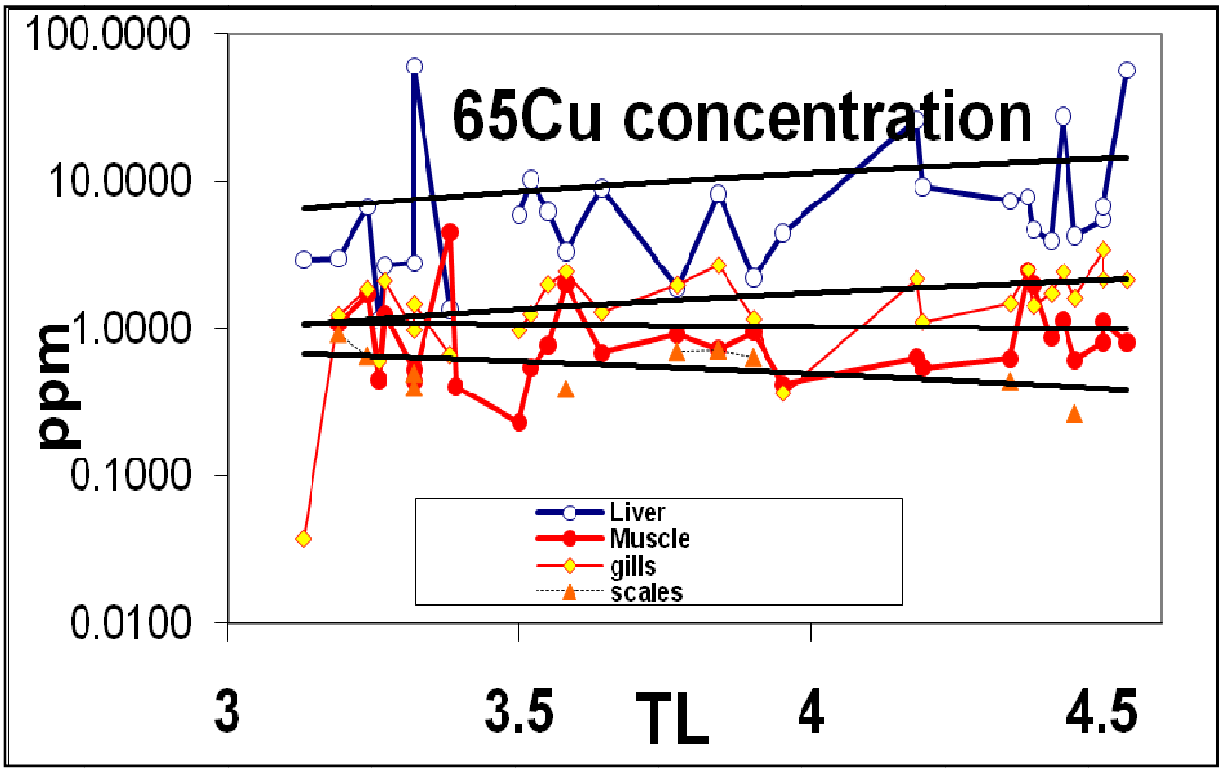

52Cr concentration
10.0000
1.0000
0.1000
0.0100
ppm
3
3.5
TL
4
4.5
Liver
Muscle
gills
scales

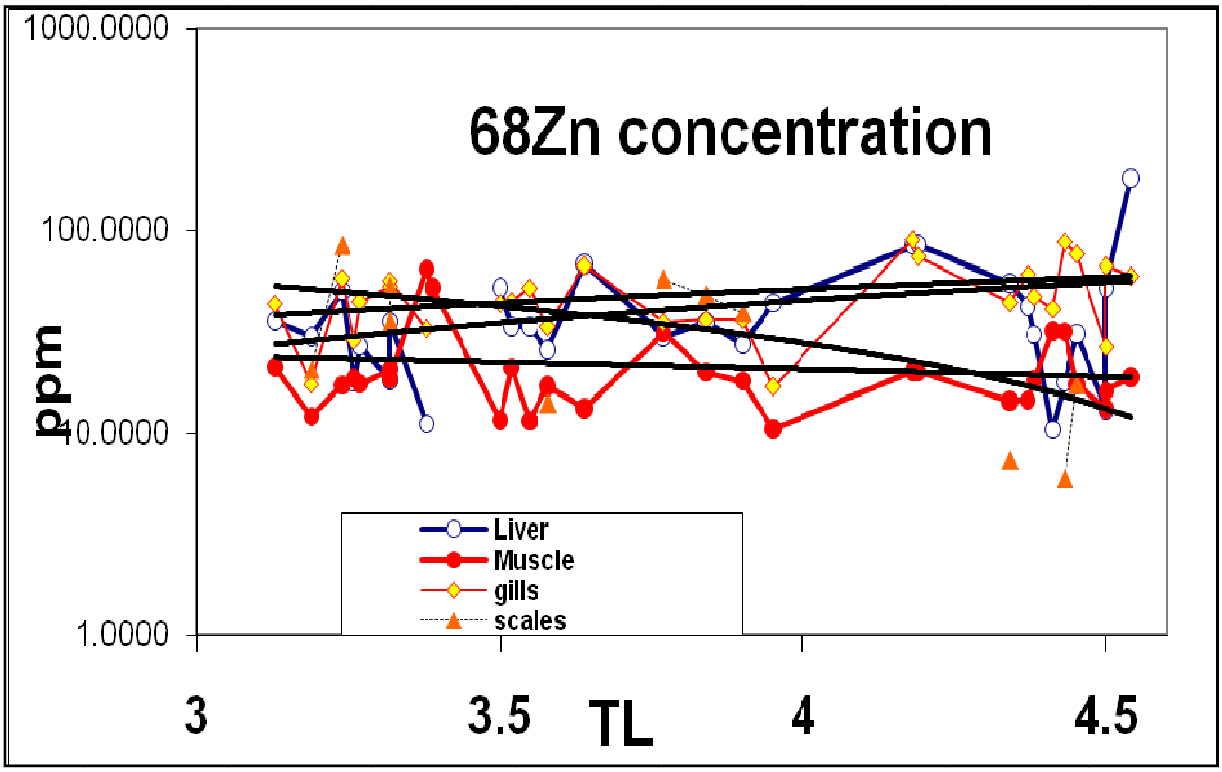

55Mn concentration
150.0000
15.0000
1.5000
0.1500
ppm
Liver
Muscle
gills
scales
3
3.5
TL
4
4.5

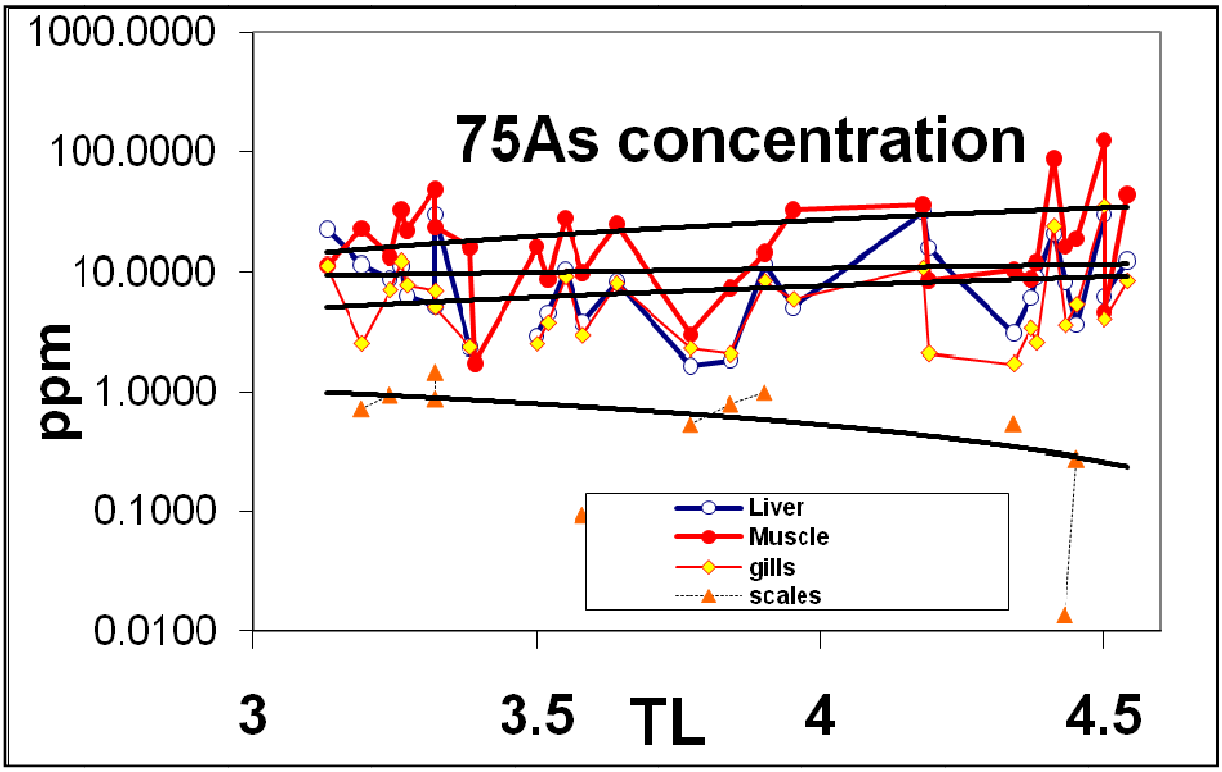

56Fe concentration
10000.0
1000.0
100.0
10.0
1.0
ppm
Liver
Muscle
gills
3
3.5
TL
4
4.5

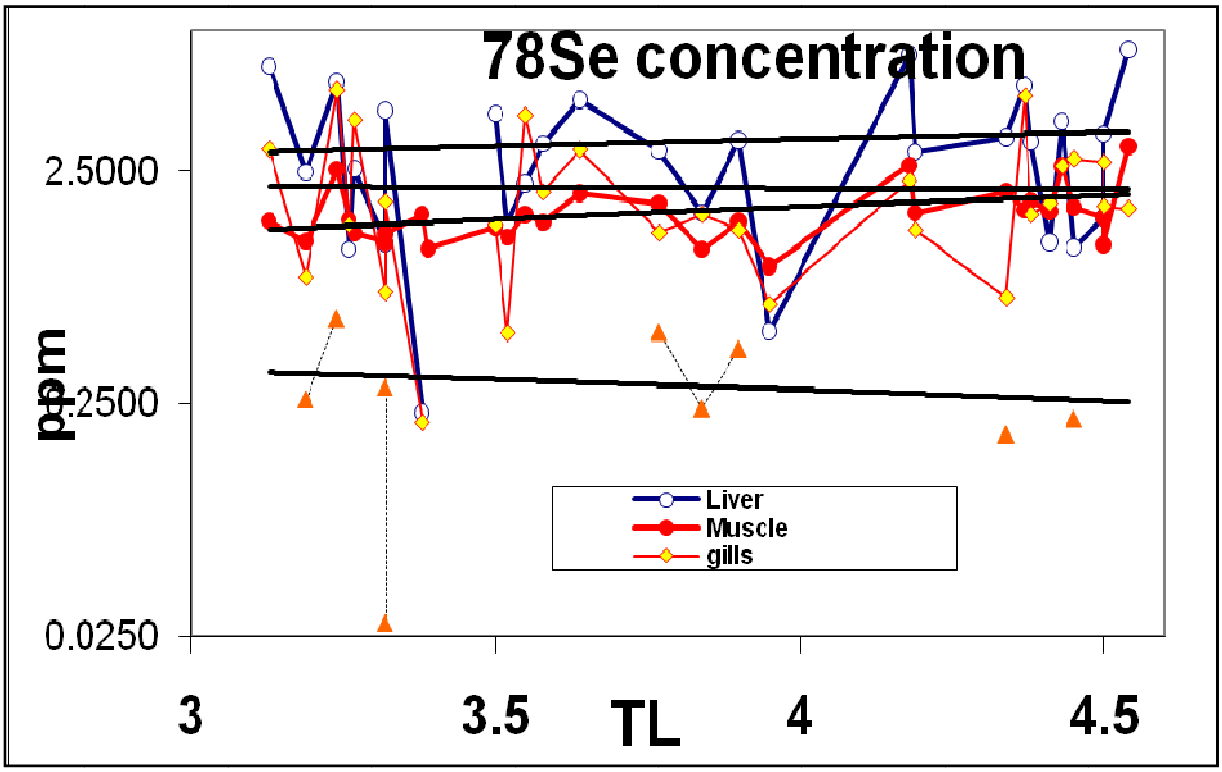

59Co concentration
10.0000
1.0000
0.1000
0.0100
0.0010
ppm
3
3.5
TL
4
4.5
Liver
Muscle
gills
scales
Linear (Liver)

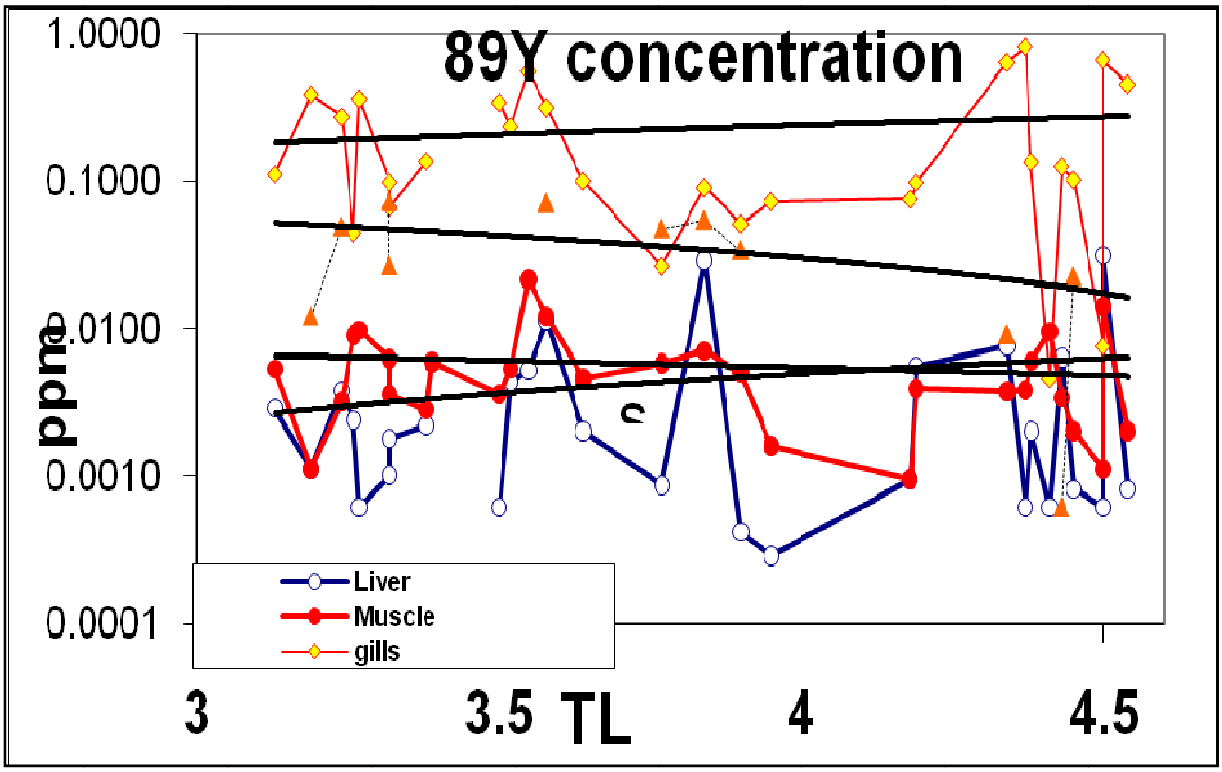

60Ni concentration
7.0000
0.7000
0.0700
0.0070
ppm
3
3.5
TL
4
4.5
Liver
Muscle
gills
scales

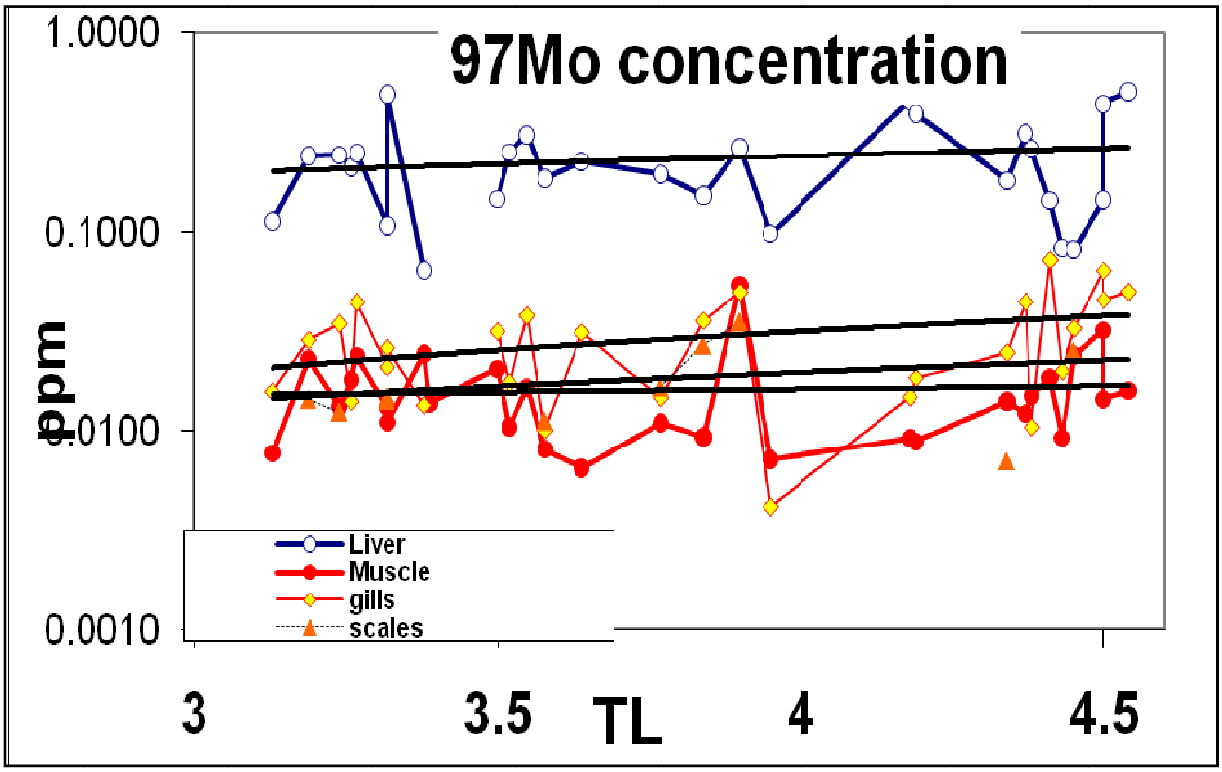

65Cu concentration
100.0000
10.0000
1.0000
0.1000
0.0100
ppm
3
3.5
TL
4
4.5
Liver
Muscle
gills
scales

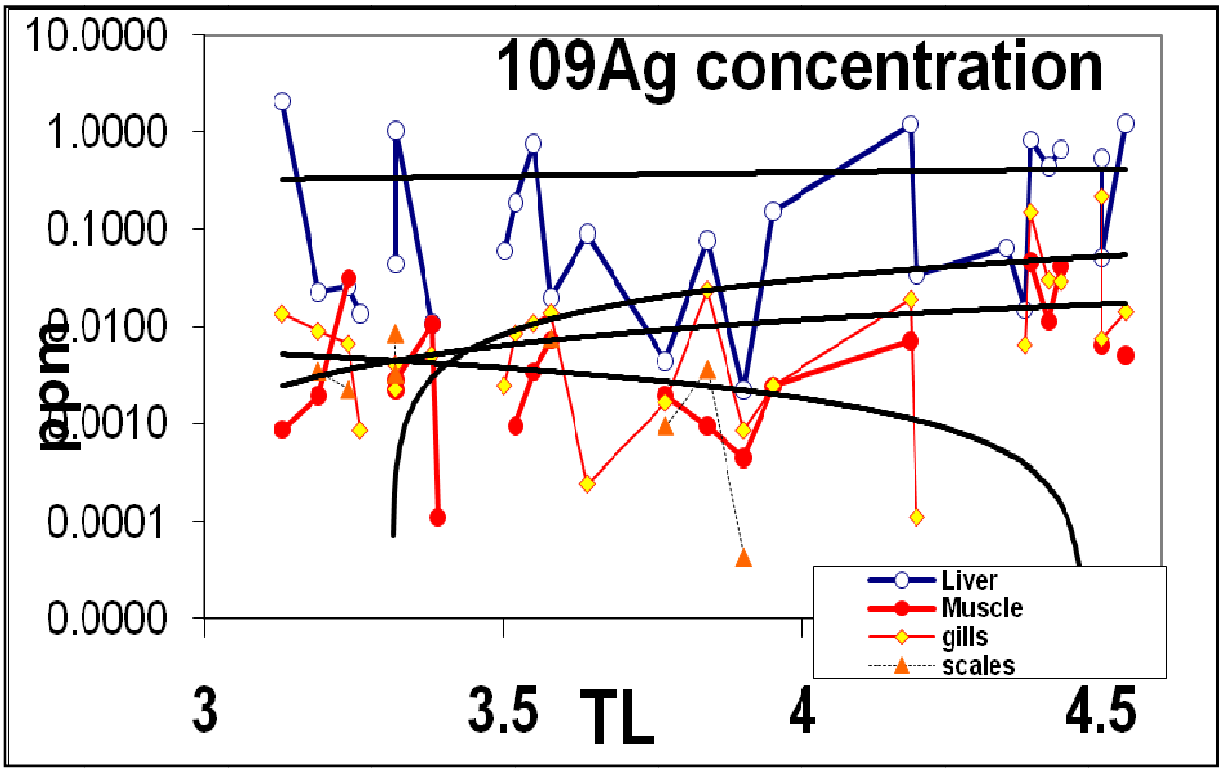

68Zn concentration
1000.0000
100.0000
10.0000
1.0000
ppm
3
3.5
TL
4
4.5
Liver
Muscle
gills
scales

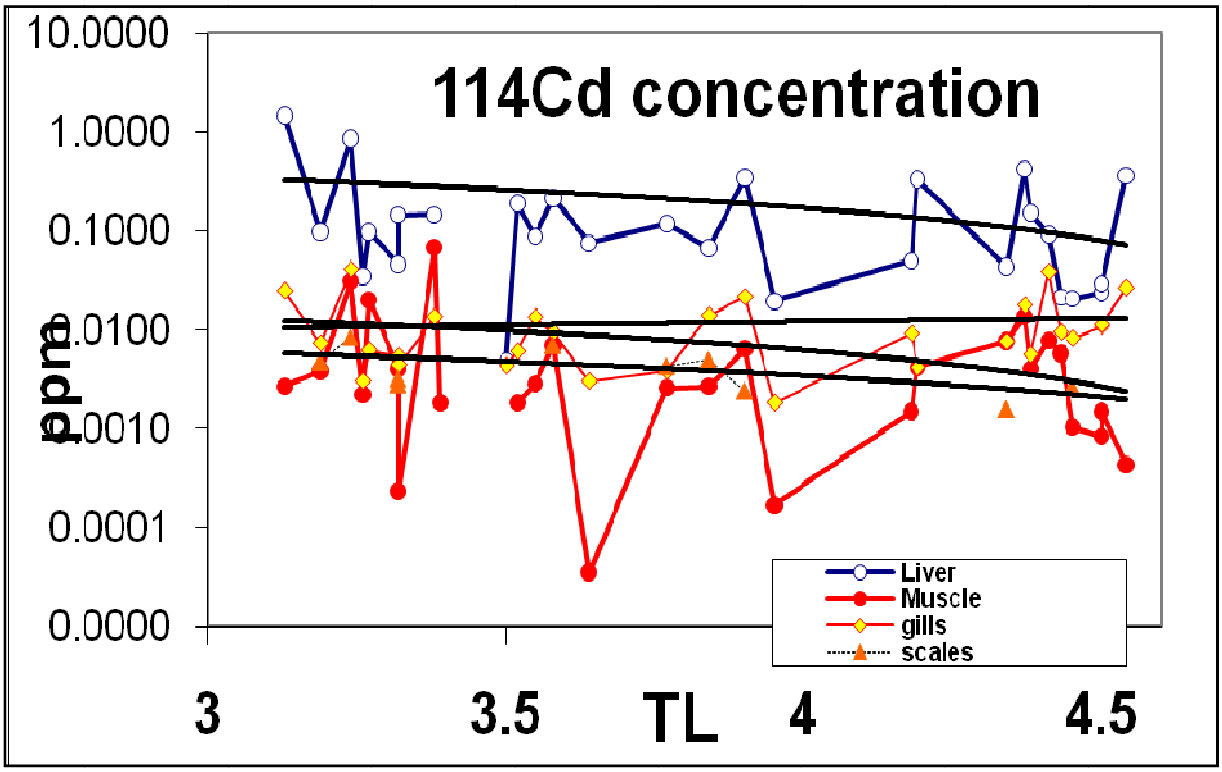

75As concentration
1000.0000
100.0000
10.0000
1.0000
0.1000
0.0100
ppm
3
3.5
TL
4
4.5
Liver
Muscle
gills
scales

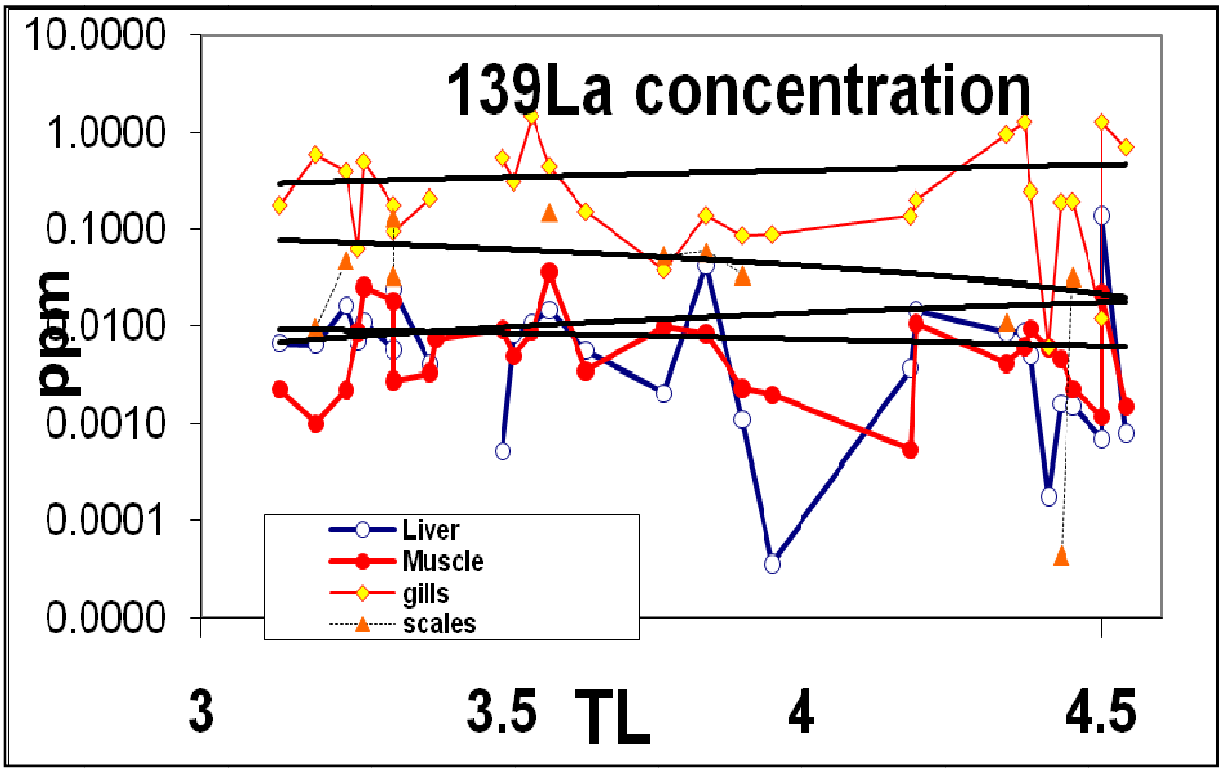

78Se concentration
2.5000
0.0250
ppm
3
3.5
TL
4
4.5
Liver
Muscle
gills

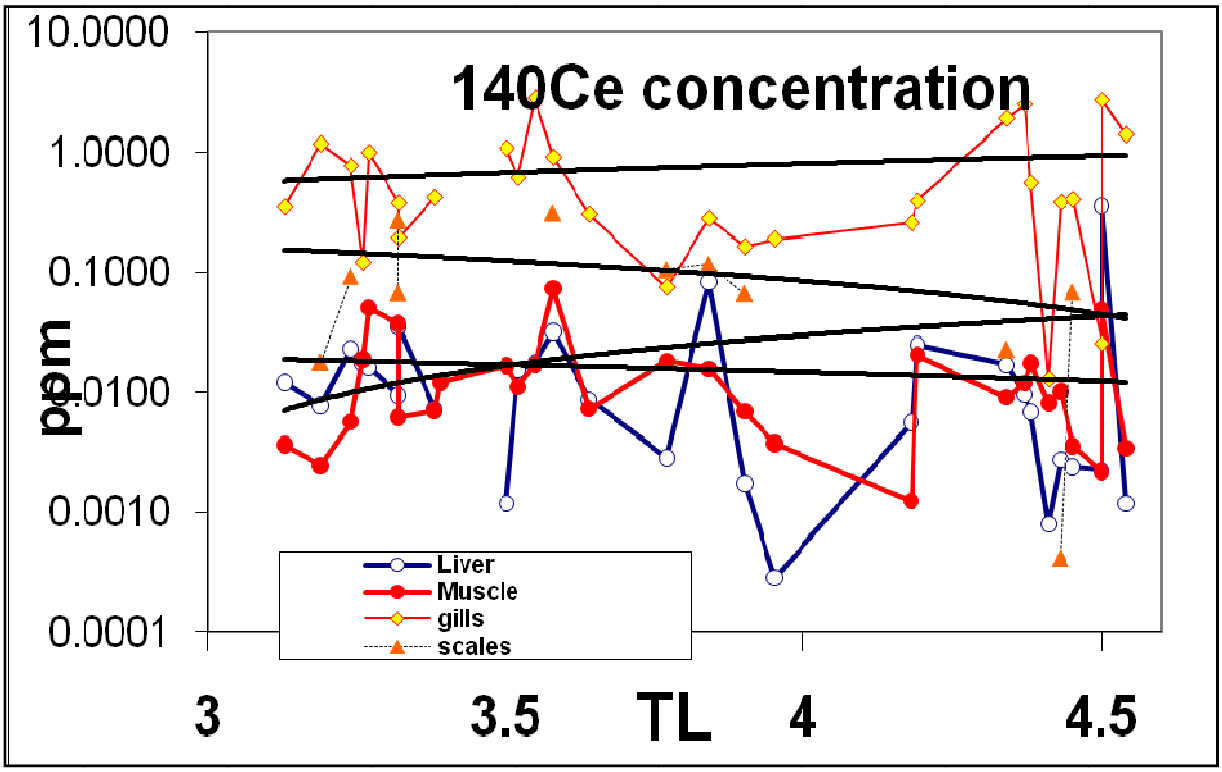

89Y concentration
1.0000
0.1000
0.0100
0.0010
0.0001
ppm
Liver
Muscle
gills
3
3.5
TL
4
4.5

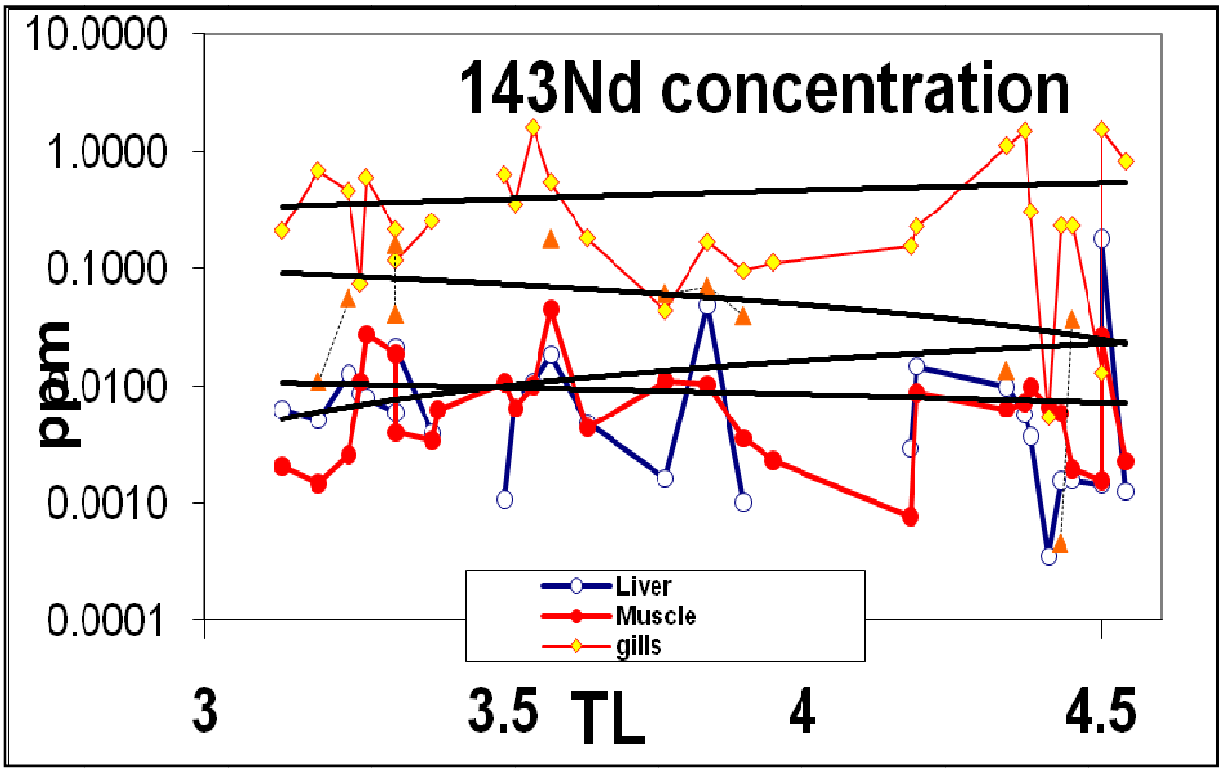

97Mo concentration
1.0000
0.1000
0.0100
0.0010
ppm
Liver
Muscle
gills
scales
3
3.5
TL
4
4.5

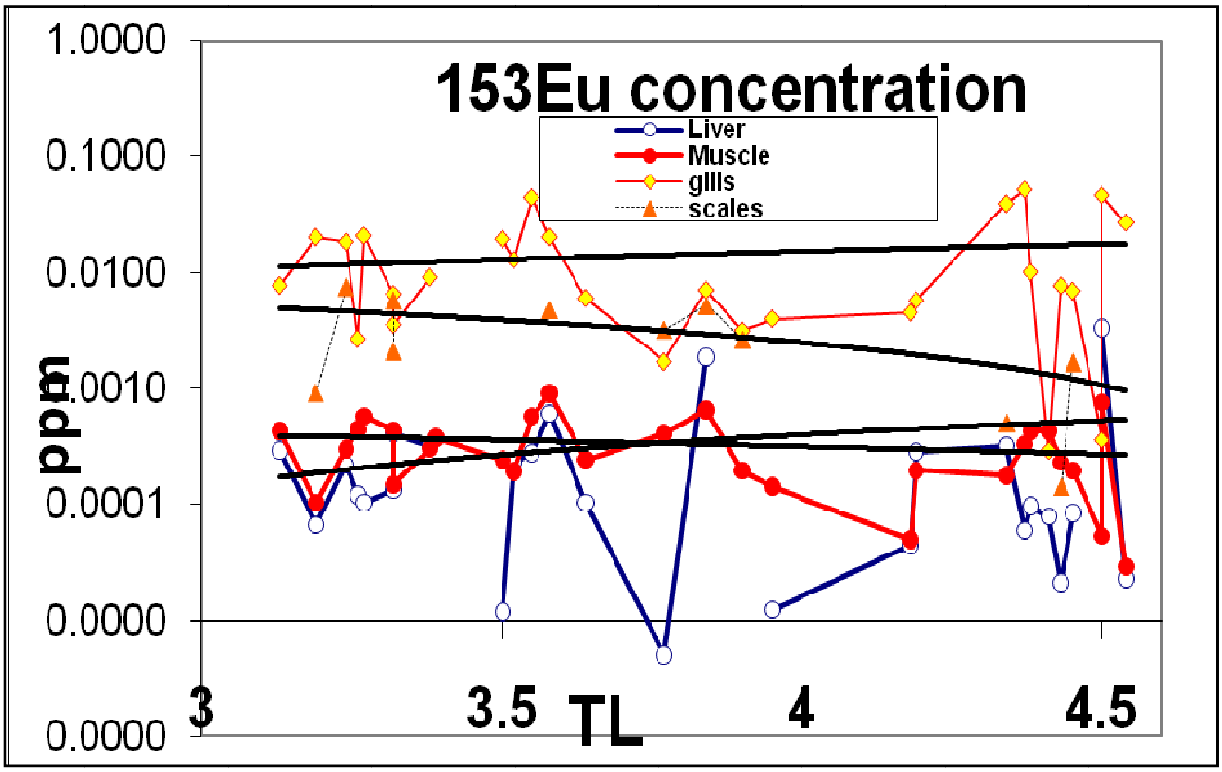

109Ag concentration
10.0000
1.0000
0.1000
0.0100
0.0010
0.0001
0.0000
ppm
3
3.5
TL
4
4.5
Liver
Muscle
gills
scales

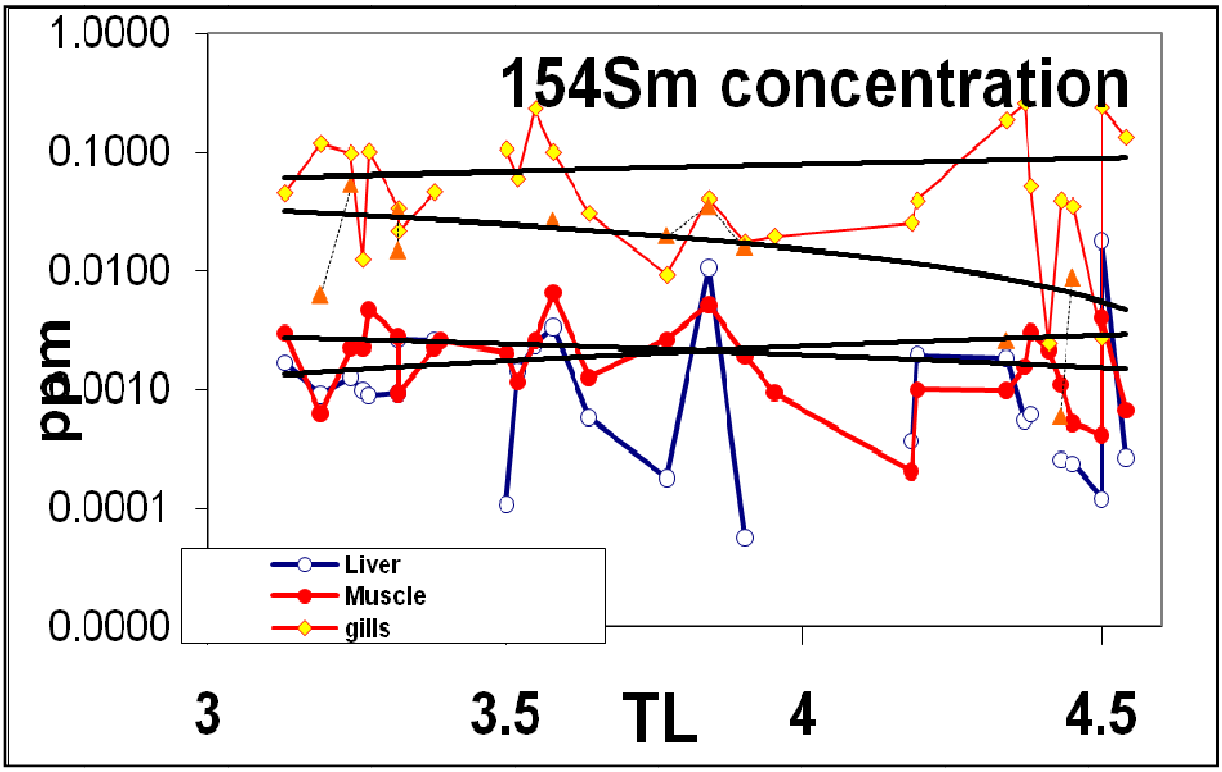

114Cd concentration
10.0000
1.0000
0.1000
0.0100
0.0010
0.0001
0.0000
ppm
3
3.5
TL
4
4.5
Liver
Muscle
gills
scales

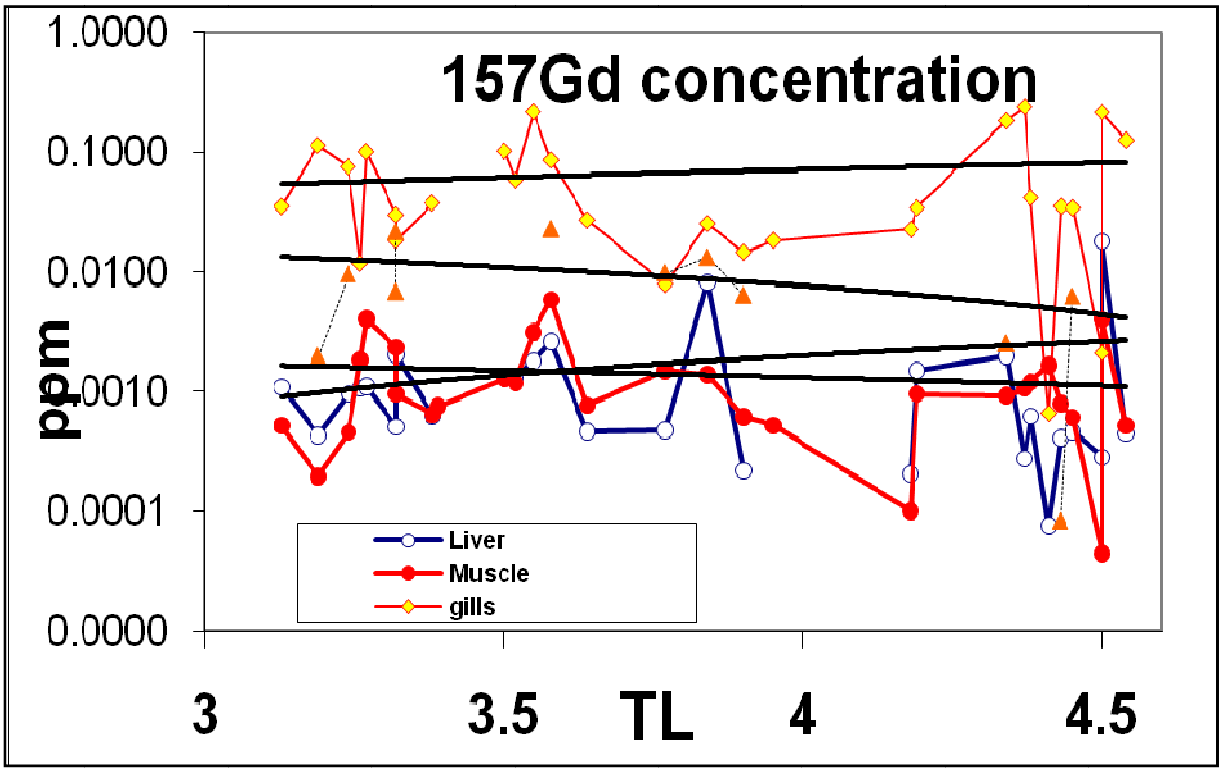

139La concentration
10.0000
1.0000
0.1000
0.0100
0.0010
0.0001
0.0000
ppm
Liver
Muscle
gills
scales
3
3.5
TL
4
4.5

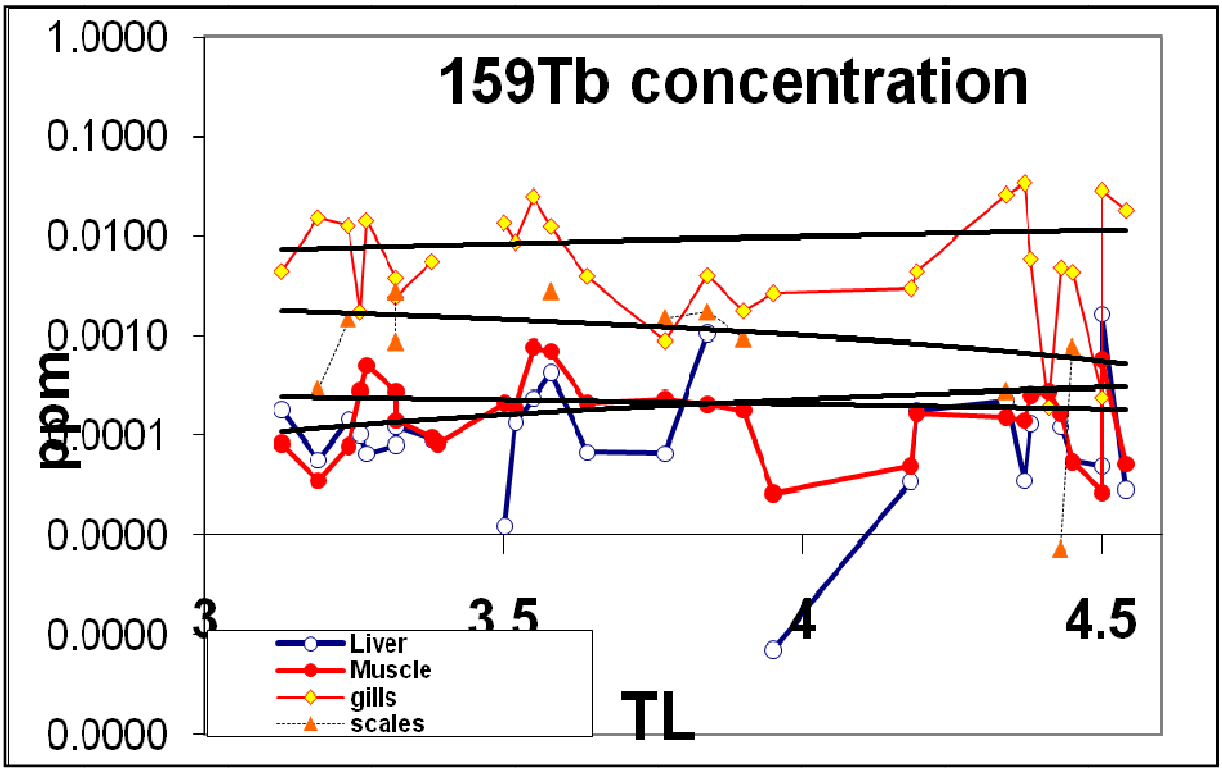

140Ce concentration
10.0000
1.0000
0.1000
0.0100
0.0010
0.0001
ppm
Liver
Muscle
gills
scales
3
3.5
TL
4
4.5

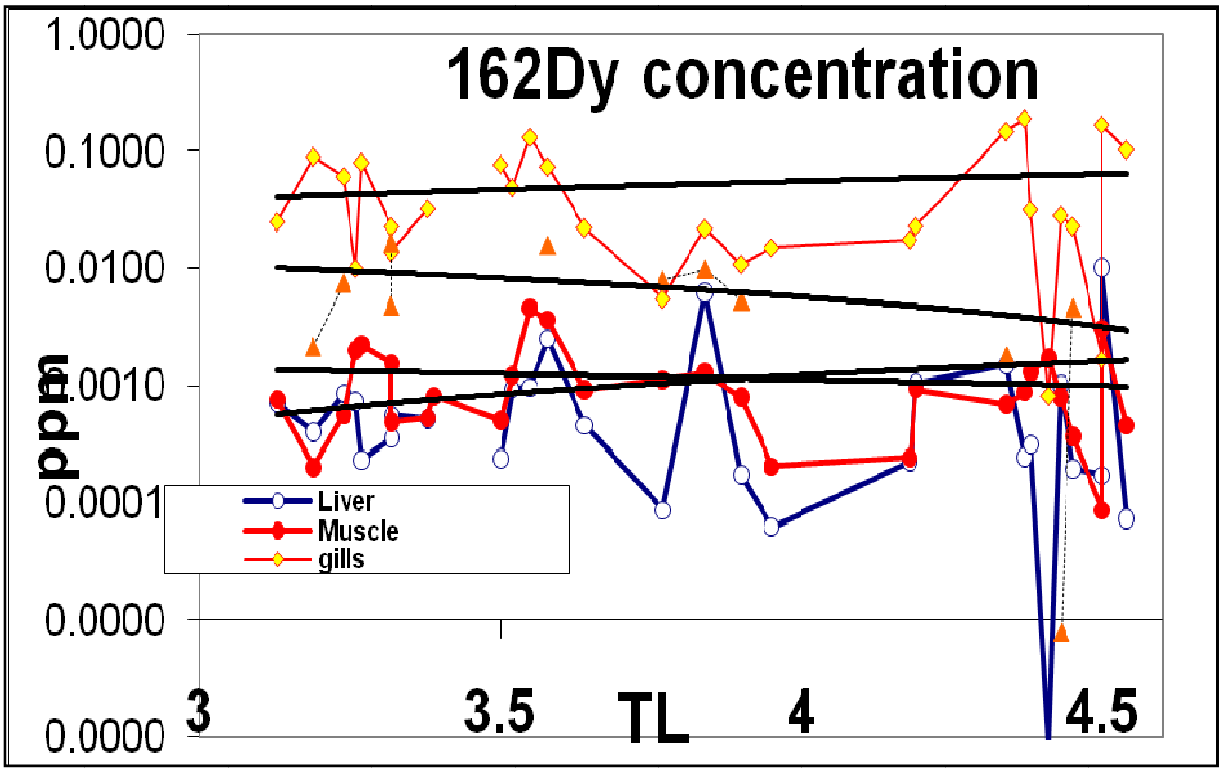

143Nd concentration
10.0000
1.0000
0.1000
0.0100
0.0010
0.0001
ppm
Liver
Muscle
gills
3
3.5
TL
4
4.5

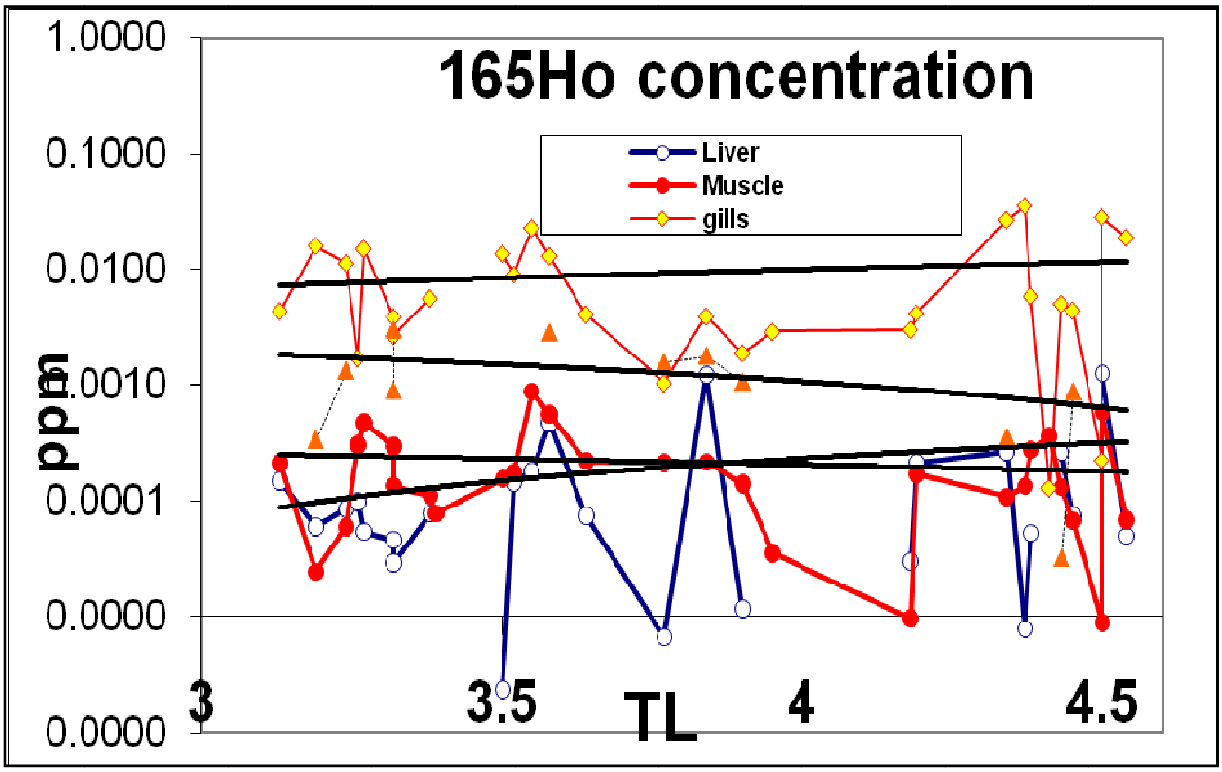

153Eu concentration
1.0000
0.1000
0.0100
0.0010
0.0001
0.0000
0.0000
ppm
Liver
Muscle
gills
scales
3
3.5
TL
4
4.5

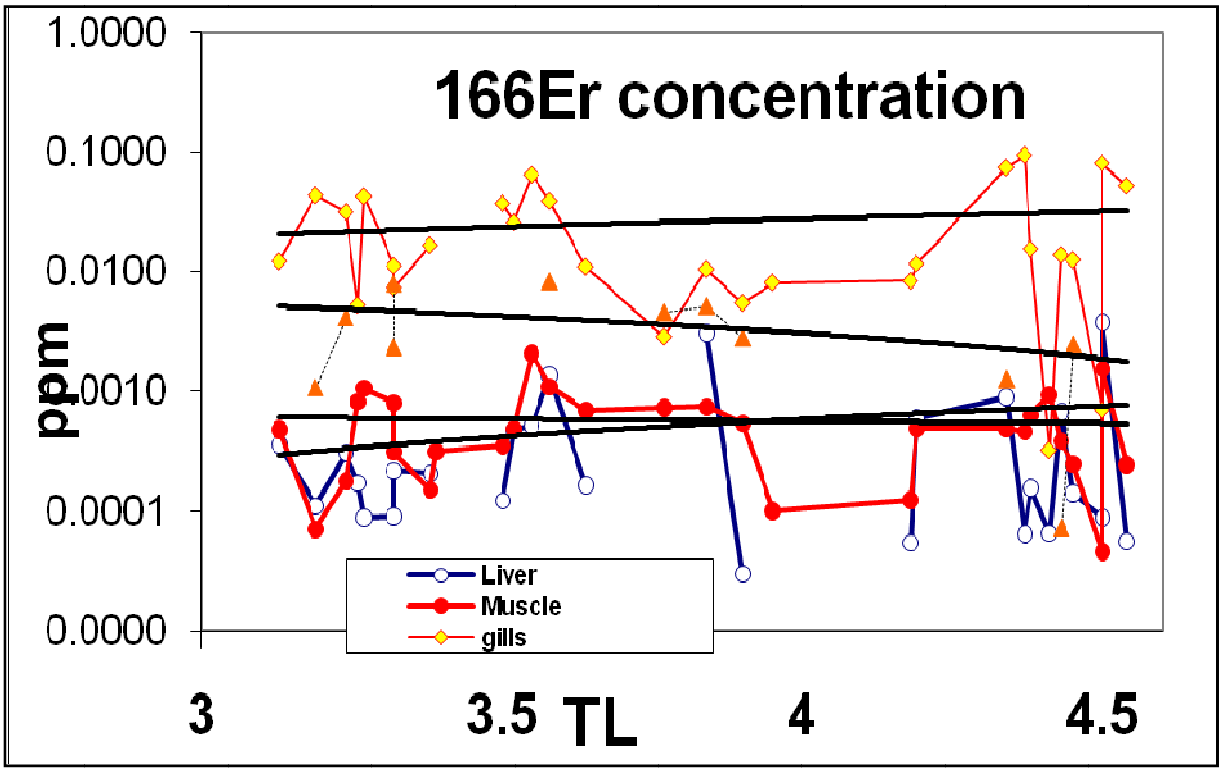

154Sm concentration
1.0000
0.1000
0.0100
0.0010
0.0001
0.0000
ppm
Liver
Muscle
gills
3
3.5
TL
4
4.5

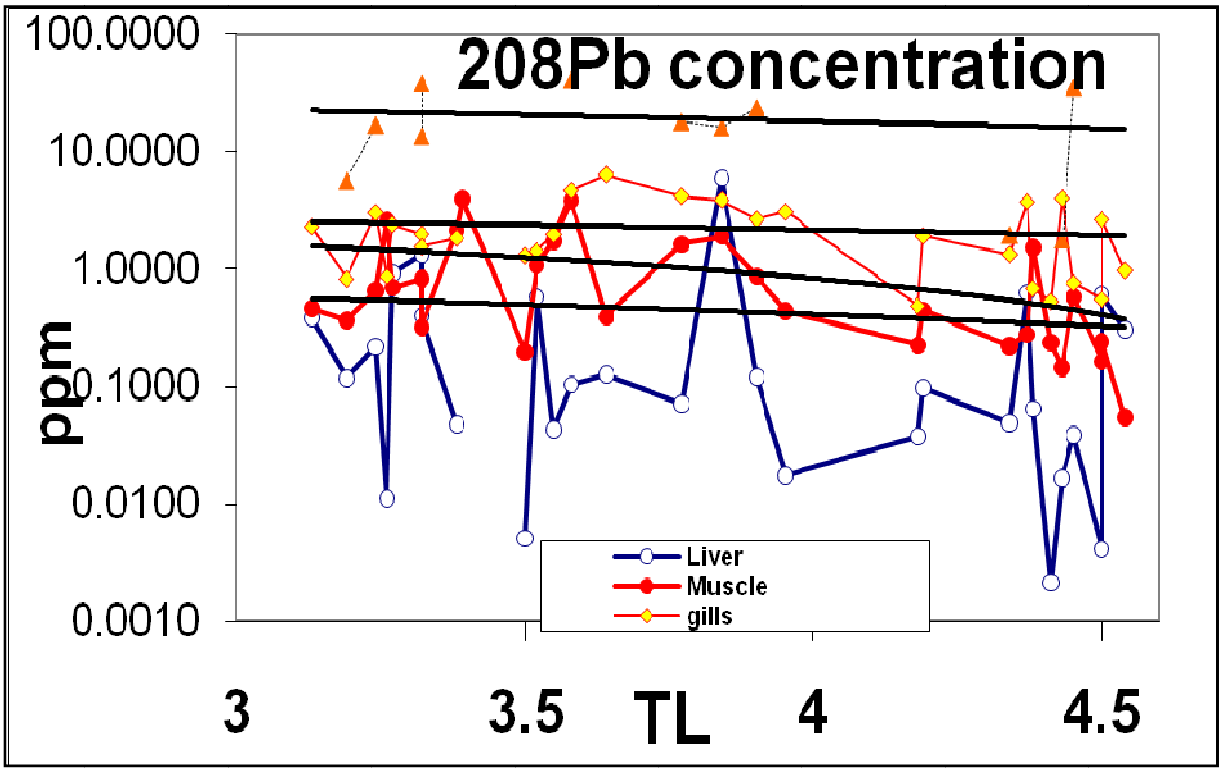

157Gd concentration
1.0000
0.1000
0.0100
0.0010
0.0001
0.0000
ppm
Liver
Muscle
gills
3
3.5
TL
4
4.5

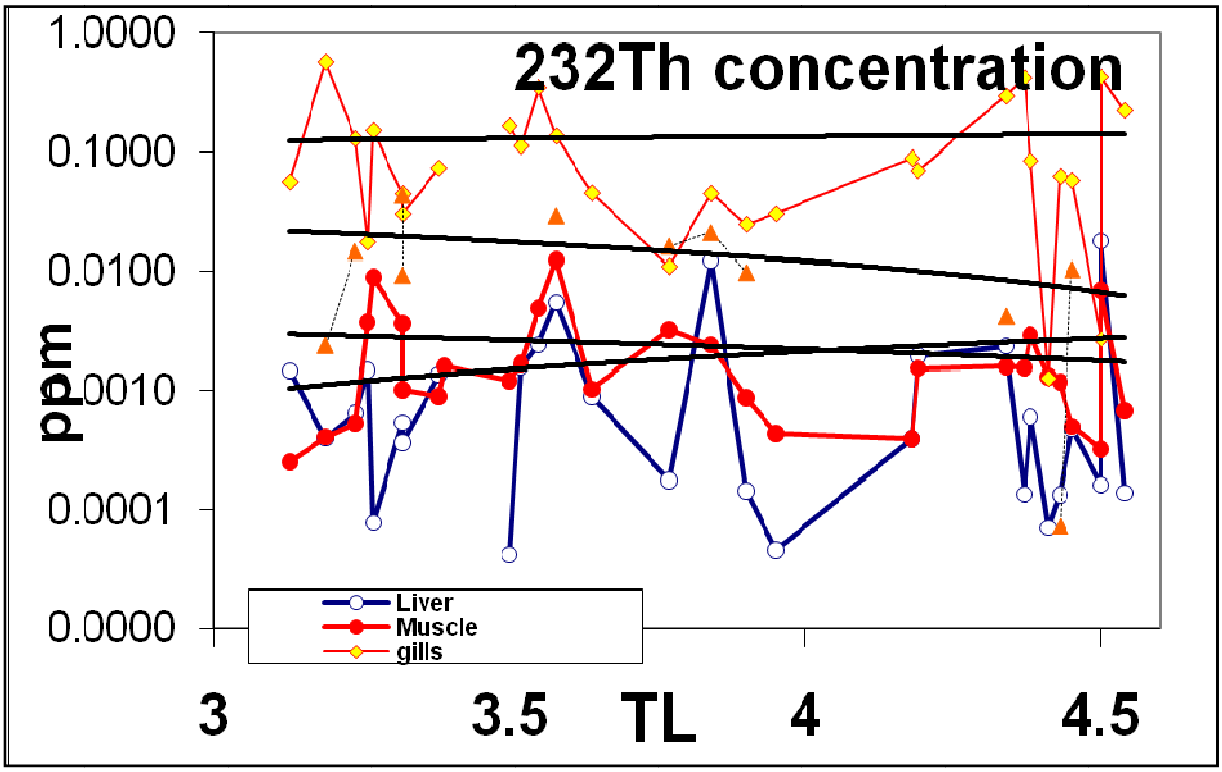

159Tb concentration
1.0000
0.1000
0.0100
0.0010
0.0001
0.0000
0.0000
0.0000
ppm
3
3.5
4
4.5
TL
Liver
Muscle
gills
scales

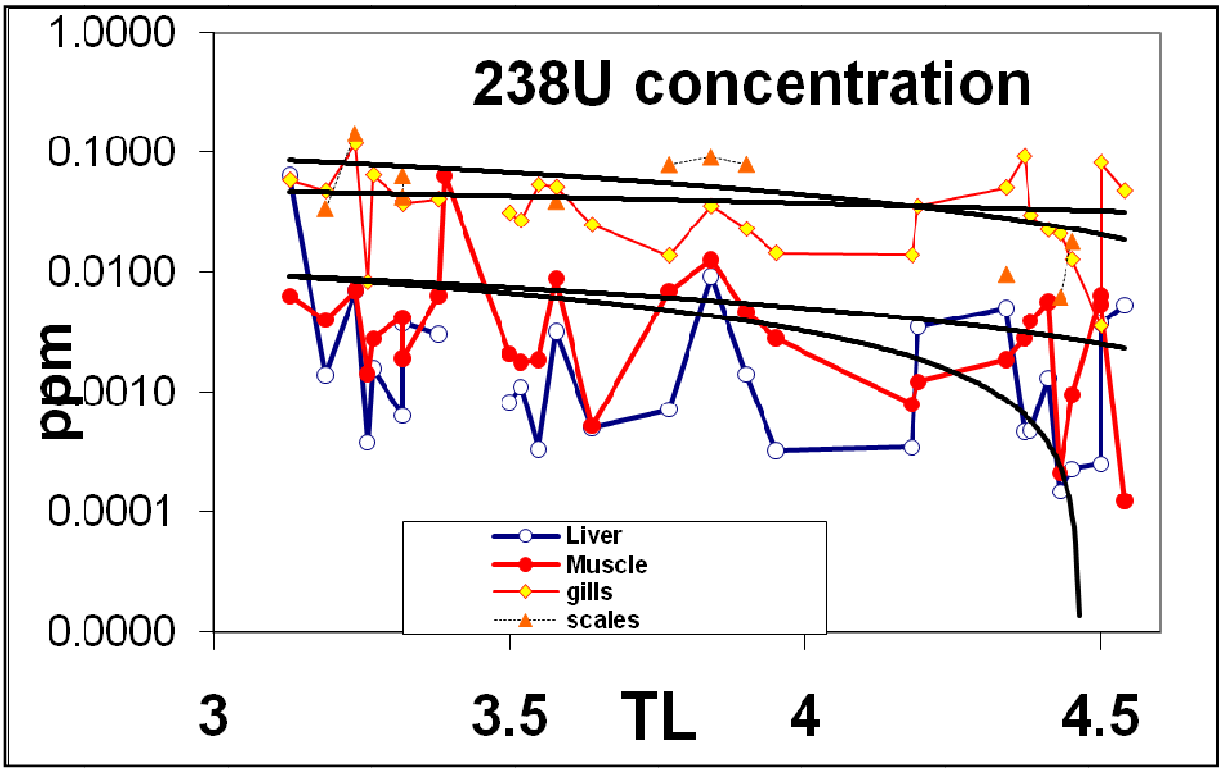

162Dy concentration
1.0000
0.1000
0.0100
0.0010
0.0001
0.0000
0.0000
ppm
3
3.5
TL
4
4.5
Liver
Muscle
gills


165Ho concentration
Liver
Muscle
gills
1.0000
0.1000
0.0100
0.0010
0.0001
0.0000
0.0000
ppm
3
3.5
TL
4
4.5


166Er concentration
Liver
Muscle
gills
1.0000
0.1000
0.0100
0.0010
0.0001
0.0000
ppm
3
3.5
TL
4
4.5


208Pb concentration
100.0000
10.0000
1.0000
0.1000
0.0100
0.0010
ppm
Liver
Muscle
gills
3
3.5
TL
4
4.5


232Th concentration
1.0000
0.1000
0.0100
0.0010
0.0001
0.0000
ppm
Liver
Muscle
gills
3
3.5
TL
4
4.5


238U concentration
1.0000
0.1000
0.0100
0.0010
0.0001
0.0000
ppm
3
3.5
TL
4
4.5
Liver
Muscle
gills
scales

Appendix B

Species were given the following numbering for the PCA analysis.

Number	Scientific name
1	*Mullus barbatus*
2	*Merluccius merluccius*
3	*Citharus linguatula*
4	*Merlangius merlangus*
5	*Lophius budegassa*
6	*Trachurus trachurus*
7	*Engraulis encrasicolus*
8	*Spicara flexuosa*
9	*Pagellus acarne*
10	*Conger conger*
11	*Gobius niger*
12	*Solea sp*
13	*Cepola macrophthalma*
14	*Serranus cabrilla*
15	*Serranus hepatus*
16	*Uranoscopus scaber*
17	*Trachinus draco*
18	*Chelidonichthys lucerna*
19	*Blennius ocellaris*
20	*Scomber scombrus*
21	*Ophidion sp.*
22	*Torpedo torpedo*
23	*Gaidropsarus sp.*
24	*Pomatomus saltatrix*
25	*Phycis blennoides*
26	*Sygnathus acus*
27	*Mullus surmuletus*
28	*Gaidropsarus mediterraneus*
29	*Scyliorhinous canicula*