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Bioaccumulation of arsenic, cadmium, mercury, lead and selenium in the benthic and pelagic food chain of Lake Baikal

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Abstract

Increased anthropogenic release of potentially toxic trace elements such as arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb) and selenium (Se) into freshwater ecosystems over the past century has caused much concern. These elements are well known toxicants in aquatic ecosystems and may exert toxic effects even if present at relatively low concentrations in organisms. In this study, bioaccumulation of As, Cd, Hg, Pb and Se in the pelagic and benthic food chain of Lake Baikal have been investigated, with focus on the benthic and pelagic fish species. Concentrations of the selected trace elements have been analyzed in samples of water, plankton, benthic invertebrates and fish by High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS). Concentration differences in the benthic and pelagic food chain of Lake Baikal have been investigated, with focus on benthic and pelagic fish species. In addition, spatial differences in the concentration of these potentially toxic elements between Selenga Shallows (possibly polluted site) and Listvyanka Bay (reference site) were included in the analysis to reveal potential anthropogenic impact on the lake.

The comparative study revealed some concentration differences in water and biotic components at the two sampling locations. The concentration of Pb was significantly higher in water from the Selenga Shallows. However, several of the biota samples had highest concentrations of trace elements at Listvyanka Bay. This can be related to higher bioavailability of trace elements at Listvyanka Bay, possibly caused by lower abundance of natural ligands at this location.

In both the pelagic and benthic food chain of Lake Baikal, Hg showed indication of biomagnification while As, Cd and Pb seemed to be biodiluted. When comparing fish inhabiting the pelagic and benthic food chain, differences in concentrations of As and Hg were identified. The As concentrations were twice as high in pelagic fish, while the benthic fish had seven times higher Hg content compared to pelagic fish. The observed concentration differences of As and Hg may be related to such as different complexity of the two food chains, different feeding strategies and habitat. Based on the tissue Se:Hg molar ratio there is no risk of Hg induced toxicity in the endemic fish species of Lake Baikal at the present. In general, the present study confirms low concentrations of potentially toxic chemical elements (As, Cd, Hg, Pb and Se) in Lake Baikal in comparison to other freshwater ecosystems.

Abbreviations

B	Benthic
BLM	Binding Ligand Model
CF	Conversion factor
D.w	Dry weight
FIAM	Free Ion Activity Model
HR-ICP-MS	High Resolution Inductively Coupled Plasma Mass Spectrometry
IDL	Instrumental detection limit
KW	Kruskal-Wallis test
L.B	Listvyanka Bay
MDL	Method detection limit
MeHg	Methylmercury
NTNU	Norwegian University of Science and Technology
P	Pelagic
S.S	Selenga Shallows
TL	Trophic level
W.w	Wet weight

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1. Introduction

1.1 As, Cd, Hg, Pb and Se in fresh water ecosystems

Elements such as arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb) and selenium (Se) are often referred to as trace elements since they are present in low concentrations in the environment. The relative abundance of trace elements may vary geographically. Erosion of bedrock and soil, and passage of ground water through aquifers are some of many natural processes that govern their release into aquatic environments. In addition, anthropogenic activity related to industry, agriculture, urban settlements, geochemical structures and mining can be potential pollution sources of these trace elements in fresh water ecosystems. The increased anthropogenic release of As, Cd, Hg and Pb have especially caused concern, considering that these elements are well known toxicants in aquatic ecosystems and may exert toxic effects even if present at relatively low concentrations in organisms. The various toxic effects of these elements on aquatic organisms have been extensively described in literature (Mason, 2002; Merian, 2004). Analysis of ice cores suggests that emission of trace elements such as Cd, Hg and Pb have increased at least one order of magnitude in the industrial era compared to pre-industrial times. Thus, efforts to control, asses, and monitor such metal contaminants have increased in the developed countries over the past decades (Likens, 2010).

Metals and metalloids can be taken up by aquatic organisms through a variety of pathways: directly from solution across the entire body surface, via specialized respiratory structures (e.g. gills) or across the digestive epithelium with water or ingested food. The direct uptake from solution tends to be highest in primary producers and organisms at lower trophic levels, as these organisms usually have a large surface to volume ratio and less developed methods for excretion. In contrast, direct uptake from water may be of minor importance for organisms at higher trophic levels such as fish, mammals, and some crustaceans. For such organisms, food is usually the main route of exposure (Merian, 2004). Bioaccumulation is a process in which a chemical substance reaches higher concentrations in an organism compared to the concentrations of that chemical in its food or in the surrounding environment. Bioaccumulation is affected by elemental speciation, active and passive uptake mechanisms, transport and distribution between tissues, growth dilution and excretion (Arnot and Gobas, 2006).

The uptake of nonessential trace elements (As, Cd, Hg and Pb) by organisms is often related to the competitive uptake of resembling nutrient ions, and is highly dependant on bioavailability (Merian, 2004). The bioavailability of an element refers to the proportion of the element which is available for uptake. In general, only a small fraction of the dissolved pool of any trace metal is available for uptake by organisms. The Free Ion Activity Model (FIAM) and the Biotic Ligand Model (BLM) consider metal uptake as a function of the amount of free metal ions in water. This is particularly related to the above mentioned ability of free metal ions to bind to membrane carrier proteins (Likens, 2010). Changes in pH, salinity, content of organic matter and temperature can significantly influence the speciation and thus the bioavailability of metals and other chemical elements (Merian, 2004). Consequently the bioavailability of metals can vary significantly with the biogeochemistry in different fresh waters. For example, oligotrophic waters may be more susceptible to trace metal stress compared to eutrophic waters or waters more heavily affected by anthropogenic discharges (Luoma, 1983). This is because more organic matter and particles may be present in the latter environments. Particles and organic matter can serve as ligands and form complexes with trace elements in water. Such complexiation reduces the concentration of free ions in water and, according to the FIAM and BLM, reduce the metal bioavailability (Likens, 2010). In general, significant proportions of As, Cd and Se are present as free ions in natural waters. In contrast, Pb is generally found to be associated with particulates and only very low concentrations remain in solution (Luoma, 1983).

The bioavailability of trace metals is of great importance in regards to biomagnification. Biomagnification is the transport of chemicals along the successive links in food chains, with increasing concentrations at each trophic level (TL). In contrast, biodilution refers to decreasing concentrations of a chemical with increasing TL. Mercury is well known to biomagnify in aquatic food chains (Mason, 2002; Merian, 2004). The biomagnification of Hg is related to the high bioavailability of methylmercury (MeHg). Methylmercury is produced by microbial methylation of Hg under the anoxic conditions found in aquatic sediments, wetlands, and other hydric soils. The assimilation efficiency of MeHg is high and it also has a long biological half life. As a consequence, top predators in aquatic food chains can acquire high levels of Hg which can result in severe toxicological effects (Mason, 2002; Merian, 2004). Many reports during the past few decades have given evidence for the antagonistic effects of Se on Hg induced toxicity. Therefore, the risk of Hg exposure to animal life cannot be accurately assessed without considering the moderating effects of Se. A tissue Se:Hg molar ratio greater than one is suggested as a threshold for the protecting action against Hg toxicity (Pelletier, 1986; Peterson, et al., 2009).

1.2 Lake Baikal and its ecosystems

Lake Baikal is located in eastern Siberia, in the centre of a vast mountain region. It is the deepest (1642 m), the most voluminous (23,615 km^3) and the oldest lake in the world. The lake is divided into 3 basins: the northern basin with depths ranging from 800 to 900 m, the central basin (1200 to 1620 m) and the southern basin (1300 to 1400 m). The Selenga River is the major tributary, accounting for 50% of all the water inflow, while the Angara River is the only river flowing out of Lake Baikal (Kozhova and Izmest'eva, 1998). Although Lake Baikal is the deepest lake in the world, life can be found all the way from the water surface down to its greatest depths. This is remarkable in comparison to many other great lakes in the world, many of which are anoxic 200 meters below the surface. Lake Baikal is also unique considering the high degree of endemism: two thirds of the more than 2000 species inhabiting the lake are endemic. So it is not without reason that Lake Baikal was declared a UNESCO World Heritage Site in 1996. The diverse assemblage of endemic, deepwater sculpins (*Cottoidei*) is also unique for Baikal. In fact, two of the all together 11 families of sculpins in the world can only be found in Lake Baikal (Sideleva, 2003).

Most of these endemic sculpins are benthic and belong to the family *Abyssocottidae* (23 species) and *Cottidae* (9 species). In general, the diet of the benthic fish species consists of various types of amphipods, molluscs and fish. However, the diet can vary greatly among different benthic species and also among different individuals of the same species (Sideleva, 2003). Yoshii (1999) studied the benthic food chain of Lake Baikal, and found the complexity of the food web structure to decrease with increasing water depth. Sideleva et al. (2003) suggests that the different species of sculpins inhabit separate vertical zones of the lake. Thus, feeding behavior and trophic level might also be correlated with habitat in the benthic food chain of Lake Baikal (Sideleva, 2003).

The fish species in the pelagic food chain of Lake Baikal are outnumbered by the benthic fish species, and the pelagic fish only represent 4% of the total number of fish in the lake. However, they dominate in biomass and constitute 80% of the total fish production (Sideleva, 2003). In general, the trophic links in the pelagic food chain are fewer and more straight forward compared to the benthic food chain (Yoshii, 1999). The trophic relations in the pelagic and benthic food chain of Lake Baikal are illustrated in Figure 1. The pelagic zone is inhabited by six fish species: *Comephorus baicalensis, Comephorus dybowski, Cottocomephorus grewinkii*, *Cottocomephorus alexandrae*, *Cottocomephorus inermis* and *Coregonus autumnalis migratorius*. The *Coregonus* and the three species of *Cottocomephoridae* are benthopelagic. Most of the year, they inhabit a zone from the middle of the water column and almost down to the lake bottom. However, during spawning season they are closely connected to the lake bottom where they lay eggs underneath rocks (Sideleva, 2003). The benthopelagic fish feed mostly on meso-and macrozooplankton, such as the copepod *Epischura baicalensis* and the amphipod *Macrohectopus branickii,* respectively. However, benthic gammarids and small fish are also included in their diet. In contrast, the two species of *Comephoridea* are exclusively pelagic as they live permanently in the open water column. They do not require contact with the bottom during spawning, as they are ovoviviparous. The main diet of these two sculpins is meso-and macrozooplankton, in addition to juvenile *Comephoridae* (Sideleva, 2003). The two species of *Comephoridae* are also the major food sources of the Baikal seal (*Phoca sibirica*), which is the final link in the pelagic food chain of Lake Baikal (Kozhova and Izmest'eva, 1998).

Several reports have described the concentrations of trace elements in fish species of the pelagic food chain of Lake Baikal (Ciesielski, et al., 2006; Ciesielski, et al., 2010; Grosheva, et al., 2000). However, few studies have focused on concentrations of trace elements in the benthic food chain. Considering that the selected benthic fish species in this study are endemic and have adapted to inhabiting greater depths than known for other species of freshwater fish in the world, it is especially interesting to investigate their bioaccumulation patterns.

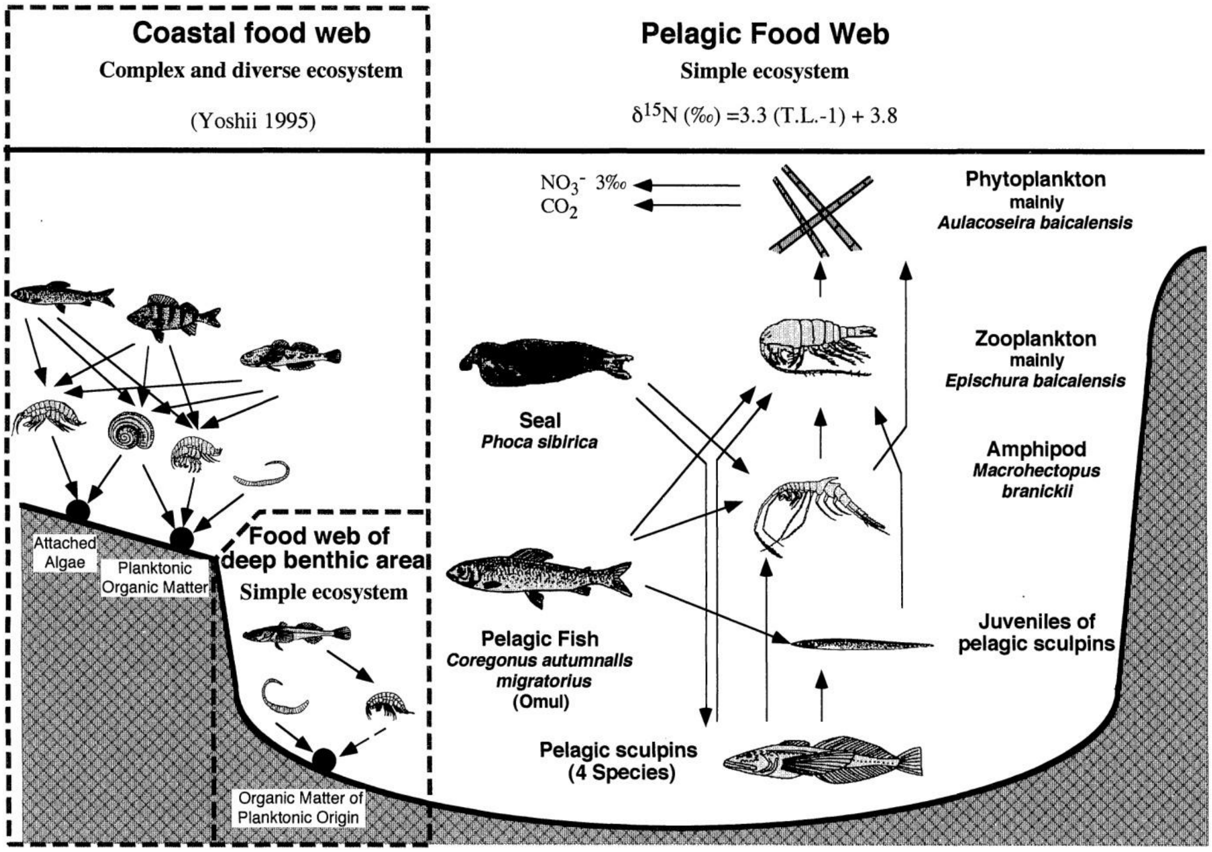


Figure 1. Trophic relations in the pelagic and benthic food chain of Lake Baikal, as described by Yoshii et al. (1999).

1.3 Major anthropogenic pollution sources in the vicinity of Lake Baikal

Industrial and agricultural activities in the immediate vicinity of Lake Baikal are of considerable significance. The major sources of anthropogenic pollution around the lake are indicated in the map in Figure 2, and have been described in more detail in Ciesielski et al. (2006). In the southern basin, the Irkutsk-Cheremkhovo industrial zone (commonly referred to as TPK) and the Baikalsk Pulp and Paper Plant (BPPP) are two of the major sources of anthropogenic pollution. In addition, considerable amounts of pollutants enter through the Selenga River. The catchment area of Selenga is heavily influenced by the industrial region of Ulan-Ude, in addition to discharge from agricultural activities. According to Kozhova and Izmest'eva Selenga is the main source of pollution in the lake, followed by atmospheric precipitation, the region of the Baikal-Amur railway and the region of the BPPP (Kozhova and Izmest'eva, 1998).

Although industrial and agricultural activities are of considerable significance around the lake, concentrations of most metals and other trace elements in the biotic and abiotic compartments are generally found to correspond to natural background levels (Ciesielski, et al., 2006; Ciesielski, et al., 2010; Falkner, et al., 1997; Grosheva, et al., 2000; Koval, et al., 1999; Mackay, et al., 1998; Perrot, et al., 2010). This is partially due to the fact that the water of Lake Baikal is well mixed. In addition, any contaminants reaching the lake will be diluted in the great volume of water. Furthermore, Grosheva et al. suggested self-purification processes which remove contaminants to sediment deposits as a possible explanation for the low water concentrations of pollutants compared to the higher atmospheric concentrations (Grosheva, et al., 2000). However, there have been reports indicating possible local contamination in areas adjacent to pollution sources. For instance, Mackay et al. (1998) found indications of anthropogenic pollution of Pb and carbonate particles in sediment cores from Lake Baikal. This was especially prominent in cores from the southern basin of the lake (Mackay, et al., 1998). In addition, elevated Hg concentrations have been reported for rivers, shallows and semi-enclosed bays of the lake (Meuleman, et al., 1995). Mackay et al. (1998) concluded that: "Lake Baikal is one of the world's unique ecosystems, in terms of its biology, physics and history. Global environmental concern is, therefore, justified on the basis of uncontrolled pollution and disruption of the catchment area".

1.4 Aims of study

The aim of the present study was to examine concentrations of arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb) and selenium (Se) along sequential trophic levels of the pelagic and benthic food chain of Lake Baikal. Bioaccumulation of the selected elements in endemic fishes of Lake Baikal was the main focus of the study. In addition, spatial differences in concentration of these potentially toxic elements between Selenga Shallows (possibly polluted site) and Listvyanka Bay (reference site) were included in the analysis to reveal potential anthropogenic impact on the lake.

2. Materials and methods

Samples of water, plankton, planarians, molluscs, crustaceans and fish were collected during scientific expeditions in southern Baikal in spring 2009 and 2010. The sample material was collect at two main locations: the Selenga Shallows, a possibly polluted site, and Listvyanka Bay, the reference site (Figure 1, S.S and L.B, respectively).

The Selenga Shallows (S.S) is a characteristic underwater elevation on both sides of the Selenga River Delta. The water chemistry at the Selenga Shallows is highly influence by water from the Selenga River (Chebykin, et al., 2010; Kozhova and Izmest'eva, 1998). As indicated in Figure 2, the catchment area of this river is highly influences by anthropogenic activity.

Listvyanka Bay (L.B) is located by the outlet of the Angara River, the only river flowing out of Lake Baikal. The residence time of the lake water is 330 years, which means that the water has circulated for a long time before reaching the river outlet. In addition, a geographical feature at the river outlet results in up-welling of lake water from depths of 1000 meters (Kozhova and Izmest'eva, 1998).

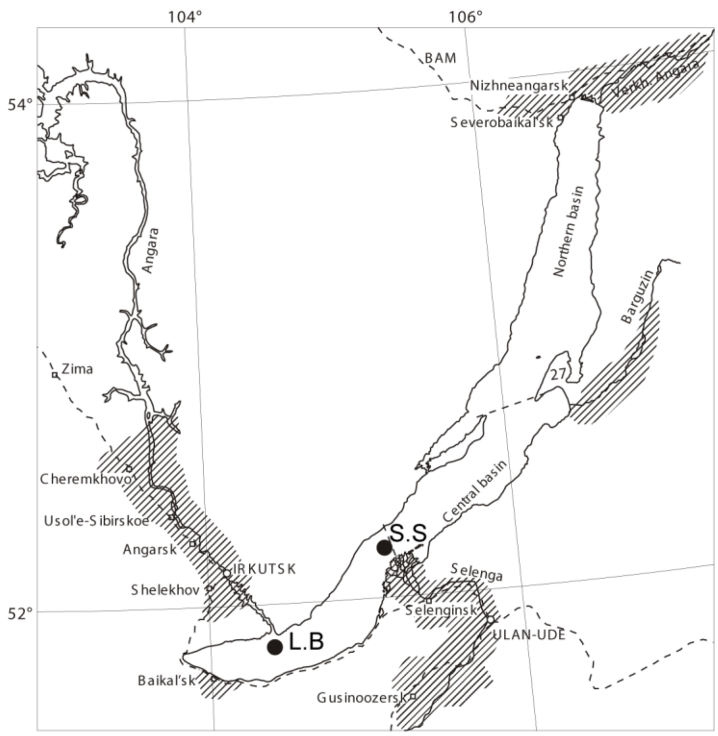


Figure 2. Map of Lake Baikal marked with sampling sites at the Selenga Shallows (S.S) and Listvyanka Bay (L.B). Samples of water, plankton, benthic invertebrates and fish were collected at both sampling sites. Shaded areas on the map indicates major anthropogenic emission sources (based on Ciesielski, et al., 2006).

2.1 Sample collection

Water samples were collected with a bathometer at the Selenga Shallows and Listvyanka Bay (Figure 2, S.S and L.B respectively) at depths of approximately 1, 100, 200 and 300 meters. The samples were filtered (0.45 µm) and collected in 11 ml polypropylene vials. The vials were pre-rinsed with concentrated nitric acid (HNO_3, Scanpure, equal to ultrapure grade, Chemscan, Elverum, Norway). The vials were flushed twice with lake water prior to sampling, and three droplets of Scanpure HNO_3 were added immediately after sampling. Two parallels were collected at each depth.

Plankton was collected at the Selenga Shallows and Listvyanka Bay (Figure 2, S.S and L.B, receptively). Mesozooplankton and phytoplankton were collected at depths down to 200 m with plankton nets (mesh size 67 µm). Subsequently, the mesozooplankton was separated from the phytoplankton with mesh bags of 600-300 µm. The macrozooplankton was collected at depths down to 200-300 meters in nets with mesh sizes of 1000, 600 and 300 µm (upper, medium and bottom parts of the net, respectively).

Fish were caught in gillnets at both sampling locations. Zoobenthos, molluscs and planarians were collected as by-catch in gillnets. In addition, littoral fish were caught using hook and line at Listvyanka Bay. An overview of the biota samples which were selected for further analysis in this project is given in Table 1.

2.2 Sample preparation

Freeze-drying and preparation of biota samples were preformed at the Institute of Geochemistry of the Siberian Branch of the Russian Academy of Science in Irkutsk and at the Department of Biology at The Norwegian University of Science and Technology (NTNU).

The biota samples were stored at -20 °C prior to analysis. Fish age was determined based on age-to-length relationships (personal communication, Pastuhov) with the length of fish defined as the length from the lower yaw to the tail fork. For the dissection of fish, titanium knifes were used. The knifes were rinsed in diluted Scanpure HNO_3 for 24 hours prior to use. All cutting surfaces were wrapped in plastic foil, which was exchanged after each specimen and the titanium knifes were flushed with ultrapure water **(**Q-option, Elga Labwater, Veolia Water Systems LTD, UK**)**. Muscle samples from individual fish were taken for further analysis. However, due to difficulties in obtaining sufficient amount of muscle tissue from small specimens of Comephorus, some samples were pooled. Each pooled sample consisted of muscle tissue from five individuals of similar length (± 0.5 mm).

The samples were lyophilized for 24-36 hours. Lyophilized samples of pooled fish muscle, molluscs (without shell), planarians and large specimens of crustaceans (*Acanthogammarus godlewskii*, *Acanthogammarus victorii* and *Parapallase logowskii*) were homogenized. To avoid possible contamination of samples during homogenization, a mill with zirconballs and zirconcovered chambers was used (Mixer Mill MM 400, Retsch, Haan, Germany).

Table 1: List of species and number of individuals (n) analyzed from the pelagic and benthic food chain of Lake Baikal. Samples of plankton and crustaceans were analyzed as 'batch samples' (b) consisting of multiple individuals.

Sample material	Habitat	Trophic level	Selenga Shallows	Listvyanka Bay
Pelagic food chain				
Plankton				
Phytoplankton	Pelagic	1.0*	b=2	b=1
Zooplankton	Pelagic	1.8*	b=1	b=1
Macrohectopus branickii	Pelagic	2.0*	b=1	b=1
Fish				
Comephorus dybowski	Pelagic	3.4*	n=8	n=20†
Comephorus baicalensis	Pelagic	3.3*	n=21	n=10 + 35†
Cottocomephorus alexandrae	Benthopelagic	NA	n=3	n=6
Cottocomephorus inermis	Benthopelagic	3.2*	n=9	n=10
Cottocomephorus grewingkii	Benthopelagic	3.2*		n=7
Coregonus autumnalis migratorius	Benthopelagic	3.3*	n=7	n=5
Benthic food chain				
Crustaceans				
Acanthogammarus godlewskii	Benthic	2.3**	b=1	b=1
Acanthogammarus victorii	Benthic	2.3**	b=1	
Ommatogammarus albinus	Benthic	3.6**	b=1	b=1
Ommatogammarus flavus	Benthic	4.2**		b=1
Parapallase logowskii	Benthic	2.9**		b=1
Eulimnogammarus sps.	Benthic	1.6**	b=5	
Planarians				
Sorocelis hepatizon	Benthic	NA	n=5	
Molluscs				
Benedictia fragilis	Benthic	1.8**	n=3	n=2
Fish				
Asprocottus abyssalis	Deepwater benthic	NA	n=4	
Batrachocottus baicalensis	Littoral	3.6**		n=5
Batrachocottus multiradiatus	Deepwater benthic	4.3**		n= 5
Batrachocottus nikolskii	Deepwater benthic	3.9**	n= 5	n= 2
Limnocottus bergianus	Deepwater benthic	4.2**	n= 5	n= 5
Leocottus kesslerii	Littoral	NA		n=1
Procottus major	Deepwater benthic	3.8**		n= 11
Paracottus knerii	Littoral	3.1**		n=1

* = Values obtained from literature (Yoshii et al. 1999) ** = Values obtained from literature (Yoshsii 1999).

† = pooled muscle samples. The numbers of individuals in all the pooled samples are given in the table.

2.3 Analytical procedure

Concentrations of trace elements in biota and water samples were determined by High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS). Prior to the HR-ICP-MS analysis, samples were digested as follows: approximately 0.5 gram of lyophilized sample was weighed and transferred to PTFE-Teflon vials (18 mL). Subsequently, 3.25 mL ultrapure water and 3 mL Scanpure HNO_3 was added to the vials. Digestion was carried out in a high-pressure microwave system (Milestone UltraClave, EMLS, Leutkirch, Germany) according to a temperature profile which increased gradually from room temperature up to 250 °C within 1 hour. After cooling to room temperature, the digested samples were diluted with ultrapure water to achieve a final acid concentration of 0.6 M. Finally, the samples were transferred to 11 ml polypropylene vials, which were rinsed twice with sample solution before adding the final volume. HR-ICP-MS analyses were performed using a Thermo Finnigan model Element 2 instrument (Bremen, Germany). The radio frequency power was set to 1400 W. The samples were introduced using a SC-FAST flow injection analysis system (ESI, Elemental Scientific, Inc. Ohama, USA) with a peristaltic pump (1 mL/min). The instrument was equipped with a concentric PFT-ST nebulizer, spray chamber (PFA Barrel 35 mm), demountable torch, quarts standard injector as well as Al sample skimmer and X-skimmer cones. The nebulizer argon gas flow rate was adjusted to give a stable signal with maximum intensity for the nuclides lithium (^{7}Li), indium (^{115}In) and uranium (^{238}U). Methane gas was used in the analysis to minimize interferences from carbon and to provide enhanced sensitivity, especially for Se and As. The instrument was calibrated using 0.6 M Scanpure HNO_3 solutions of matrix-matched multielement standards.

A calibration curve consisting of 5 different concentrations was made from these standards. To check for instrumental drift, one of these multielement standards was analyzed for every 10 sample. The accuracy of the method was verified by analyzing the certified reference materials of Dogfish muscle DORM-2 (Certified Reference Material for Trace Metals, National Research Council of Canada) and Oyster tissue NIST 1566b (National Insitute of Standards and Technology, Gaithersburg, MD). The concentrations were found within 62 – 130% and 103 – 122% for the certified values for the selected trace elements in DORM-2 and NIST 1566b, respectively (Appendix B). To assess possible contamination during sample preparation, blank samples of Scanpure HNO_3 and ultrapure water were prepared using the same procedure as for the samples.

The method detection limits (MDLs) for biota and water samples are given in Appendix A. The MDLs were calculated as follows: depending on which method resulted in higher values, the MDLs were either based on 3 times the standard deviation of the blanks, or on the instrument detection limits (IDLs). The IDLs were estimated from the subsequent analysis of solutions, containing decreasing, low concentrations of the element. Finally, the concentration resulting in a relative standard deviation of approximately 25 % (n=3 scans) were selected as IDL with baseline corrections applied for these values.

2.4 Calculations and statistical analysis

Since the dataset in this study was not normally distributed even after transformation, non-parametric statistics have been applied. The Kruskal-Wallis test (KW) was used to evaluate concentration differences in fish species collected at the Selenga Shallows and at Listvyanka Bay and to determine whether length differed intra-specifically in specimens from the two sampling sites. Furthermore, the KW was also applied to investigate spatial differences in elemental concentrations in water samples from the two sampling sites.

The Spearman's Rank Order Correlation was used to identify correlations between fish length and elemental concentrations in fish muscle.

The KW was used to compare elemental concentrations in pelagic and benthic fish. Linear regression analysis was used to identify significant relationship between concentrations of elements and trophic level (TL) in the benthic and the pelagic food chain.

All statistical analysis and graphing were performed in Statistica10.0 (Statsoft, Inc.) with statistical significance level $p < 0.05$ unless stated differently. All concentrations are given based on dry weight (d.w) unless other is stated. To facilitate comparison with literature values, concentrations converted to wet weight (w.w) are given in Appendix E.

Concentrations in wet weight were calculated as follows:

$$[w.w] = [d.w] / CF$$

Conversion factors (CF) have been estimated from literature studies on the % water content in different organisms (Håkanson and Boulion, 2002). A conversion factor (CF) of 5 was applied for all organisms except zooplankton, for which a CF of 10 was used.

3. Results

3.1 Water, plankton and benthic invertebrates

The average concentrations of arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb) and selenium (Se) in water samples collected at the Selenga Shallows and Listvyanka Bay are given in Table 2. A detailed table with individual concentrations for replicate samples are given in Appendix F. Concentrations were in the range of 0.34 – 0.38, 0.0025 – 0.15 and 0.057 – 0.076 µg/L for As, Pb and Se, respectively. Most of the concentrations of Cd and Hg were below the method detection limit (MDL, Appendix A, Table A2). No significant difference in the concentration of As and Se was found between samples collected at the Selenga Shallows and Listvyanka Bay. In contrast, the average Pb concentration was one order of magnitude higher at the Selenga Shallows compared to at Listvyanka Bay ($KW_{1,6}$ = 9.28, p = 0.0023, Appendix G).

Table 2. Concentrations [µg/L] of As, Cd, Hg, Pb and Se in water samples from the Selenga Shallows (S.S) and Listvyanka Bay (L.B), Lake Baikal. Values are given as average concentrations of eight parallels collected at four depths (0, 100, 200 and 300 m). Two replicates were collected at each depth.

Element	Selenga Shallows			Listvyanka Bay		
As	0.360	±	0.011	0.366	±	0.012
Cd	<0.0020			<0.0020		
Hg	<0.0010			0.0019	±	0.0016
Pb	0.033	±	0.048	0.0040	±	0.0015
Se	0.066	±	0.0067	0.064	±	0.0047

Figure 3 gives an overview of concentrations of As, Cd, Hg, Pb and Se selected in pelagic plankton (P) and benthic invertebrates (B) from the pelagic and benthic food chain of Lake Baikal. Concentrations of As, Cd and Pb were higher in pelagic plankton than in most of the benthic invertebrates. However, some of the herbivores benthic species (*Eulimnogammarus* sps., *Acanthogammarus victorii* and *Benedictia fragilis*) had levels similar to those found in pelagic plankton. On the other hand, the Hg concentrations were higher in several of the benthic invertebrates, such as *Ommatgammarus albinus* and *Ommatgammarus flavus,* compared to Hg levels in pelagic plankton.

Since the species composition of plankton samples was similar at both locations, this allowed to investigate potential differences in bioaccumulation of elements at Selenga Shallows and Listvyanka Bay. In contrast, differences in species composition of benthic invertebrates at the two locations make comparison difficult. The concentration of Cd, Hg and Se was significantly higher in pelagic plankton samples collected at Listvyanka Bay, compared to samples from the Selenga Shallows. This difference was statistically significant for Cd and Se ($KW_{1,6}$ = 3.86, p = 0.049 for both Cd and Se, Appendix H). Because the concentration of Hg in pelagic plankton samples from the Selenga Shallows were below the MDL, no statistical test was applied to test spatial variability of Hg levels. However, all the pelagic plankton samples from Listvyanka Bay had Hg concentrations well above the MDL. Thus, Hg concentrations were assumed to be higher in pelagic plankton at collected at Listvyanka Bay compared to the Selenga Shallows.

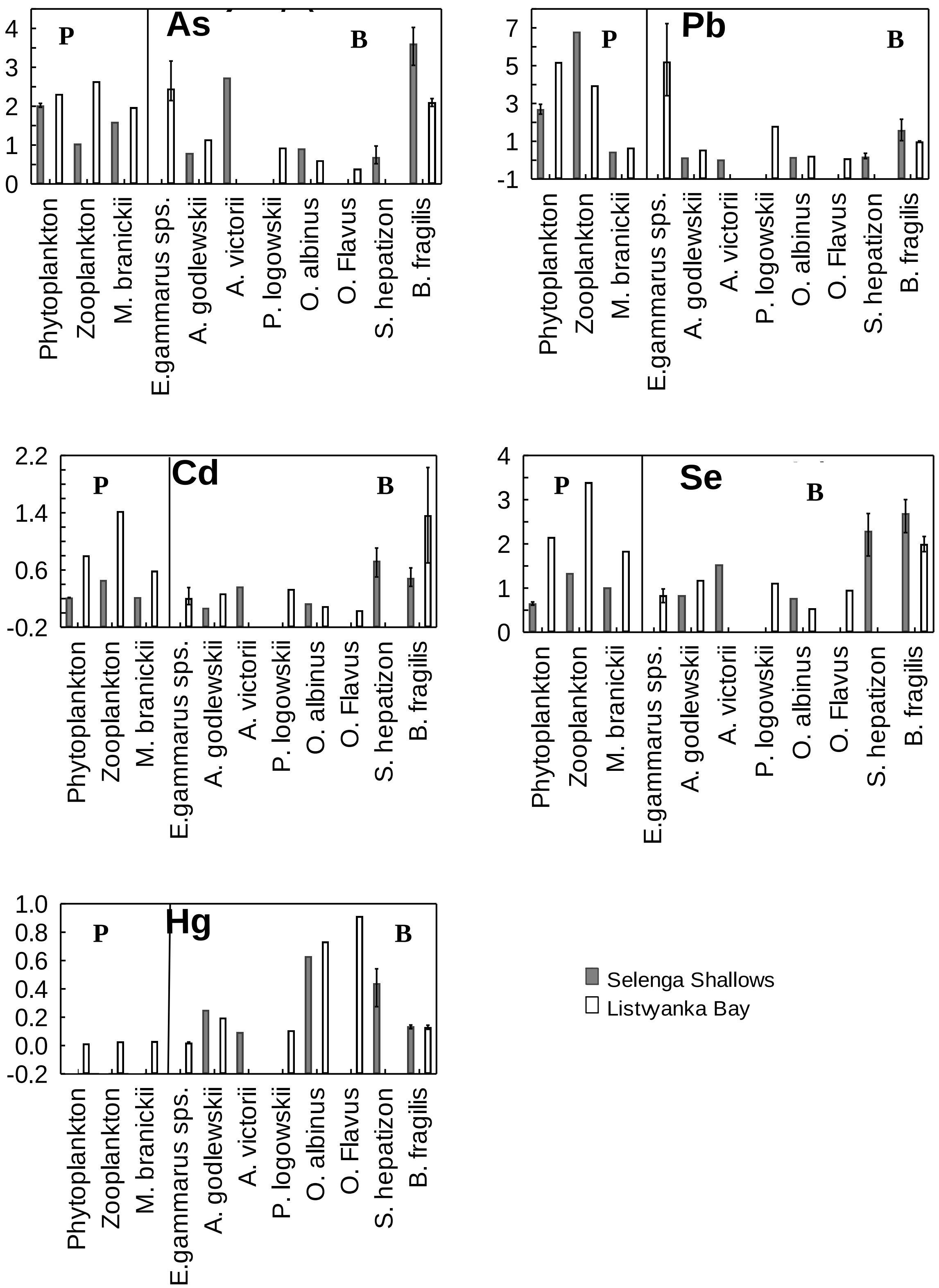


Figure 3. Concentration [µg/g] of As, Cd, Hg, Pb and Se in pelagic plankton and benthic crustaceans, mollusks and planarians collected by the Selenga Shallows and at Listvyanka Bay. Pelagic (P) and benthic species (B) are separated by a vertical line in the diagram.

3.2 Fish

Average concentrations in all fish species were 1.16 ± 0.40 µg/g for Se, 0.011 ± 0.010 µg/g for Cd, 0.31 ± 0.43 µg/g for Hg, 0.032 ± 0.056 µg/g for Pb and 0.36 ± 0.16 µg/g for As. The comparison of results between different species is presented in Figure 4, Figure 5 and Figure 6. As shown in Table 3, some trace elements were found to correlate with fish length. The concentration of Se was found to be negatively correlated with length in two pelagic fish species: *C. baicalensis* and *C. inermis*. However, in the benthic fish *P. major*, the Se concentration was positively correlated to length. Hg was positively correlated with length in several of the pelagic and benthic fish species.

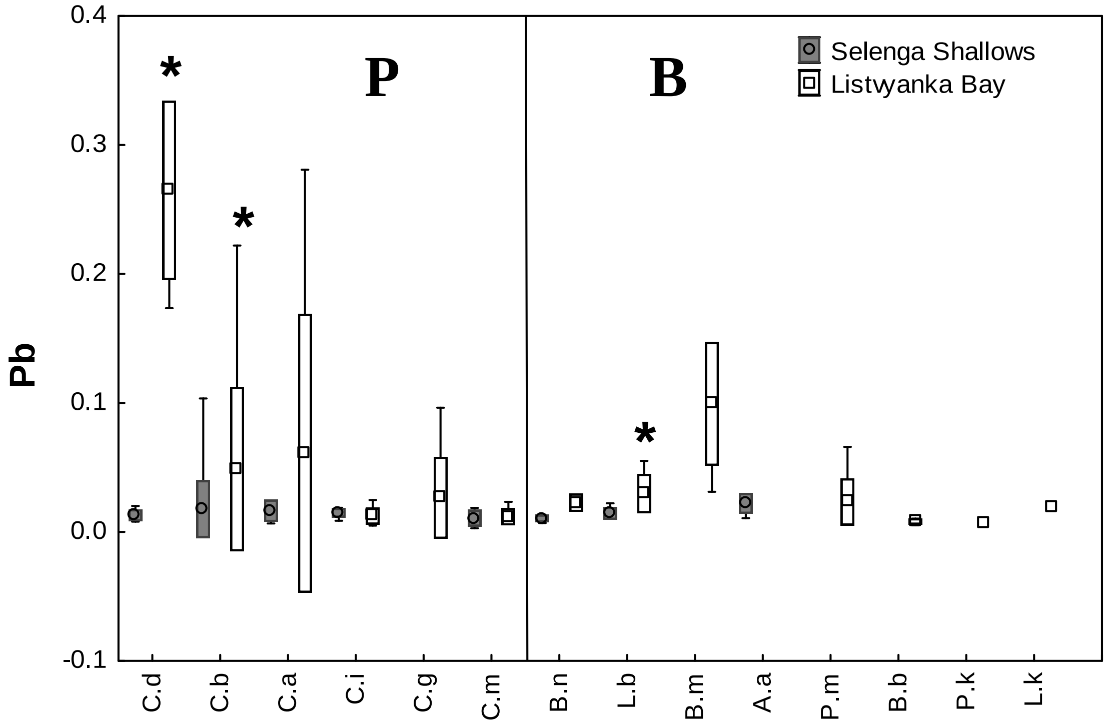
3.2.1 Spatial variation

In order to investigate possible pollution of Lake Baikal by the Selenga River, the average muscle concentrations of As, Cd, Hg, Pb and Se have been compared between individual fish caught at the Selenga Shallows and the reference site (Listvyanka Bay). Only fish species with more than 3 samples available from both location were compared; *C. dybowski, C. baicalensis, C. alexandrae, C. inermis, C. migratorius* and *L. bergianus*. Five fish species showed significant differences in the concentration of at least one element in comparison between these two sampling location (Figure 4, Figure 5 and Figure 6). The concentration of Pb was significantly higher in muscle samples of *C. dybowski* ($KW_{1,12}$ = 7.39, p = 0.0066), *C. baicalensis* ($KW_{1,38}$ = 3.93, p = 0.048) and *L. bergianus* ($KW_{1,10}$ = 4.81, p = 0.028) caught at Listvyanka Bay. The concentration of Hg was also significantly higher in *C. baicalensis* ($KW_{1,38}$ = 8.19, p = 0.0042) and *C. alexandrae* ($KW_{1,9}$ = 5.40, p = 0.021) from Listvyanka Bay. However, for *C. dybowski* the Hg concentration was highest at the Selenga Shallows ($KW_{1,12}$ = 4.15, p = 0.042). The concentration of Cd were significantly higher in *C. migratorius* and *L. bergianus* caught by the Selenga Shallows ($KW_{1,12}$ =4.12, p = 0.042 and $KW_{1,10}$ = 5.77, p = 0.016, respectively). In contrast, individuals of *C. baicalensis* had higher Cd concentration at Listvyanka Bay ($KW_{1,38}$ = 6.90, p = 0.0086). Because Hg and Se were found to correlate with the size of some of the fish species (Table 3), differences in fish length of individuals from Selenga Shallows and Listvyanka Bay were studied. Individuals of *C. baicalensis* and *C. alexandrae* caught at Listvyanka Bay were significantly longer than those from the Selenga Shallows ($KW\text{-}W_{1,38}$ = 10.63, p = 0.0011 and $KW\text{-}H_{1,9}$ = 5.40, p = 0.020, respectively). None of the other fish species differed significantly in length at the two sampling stations.

Table 3: Statistical significant correlations between concentrations of As, Cd, Hg, Pb and Se and length in pelagic and benthic fish species from Lake Baikal.

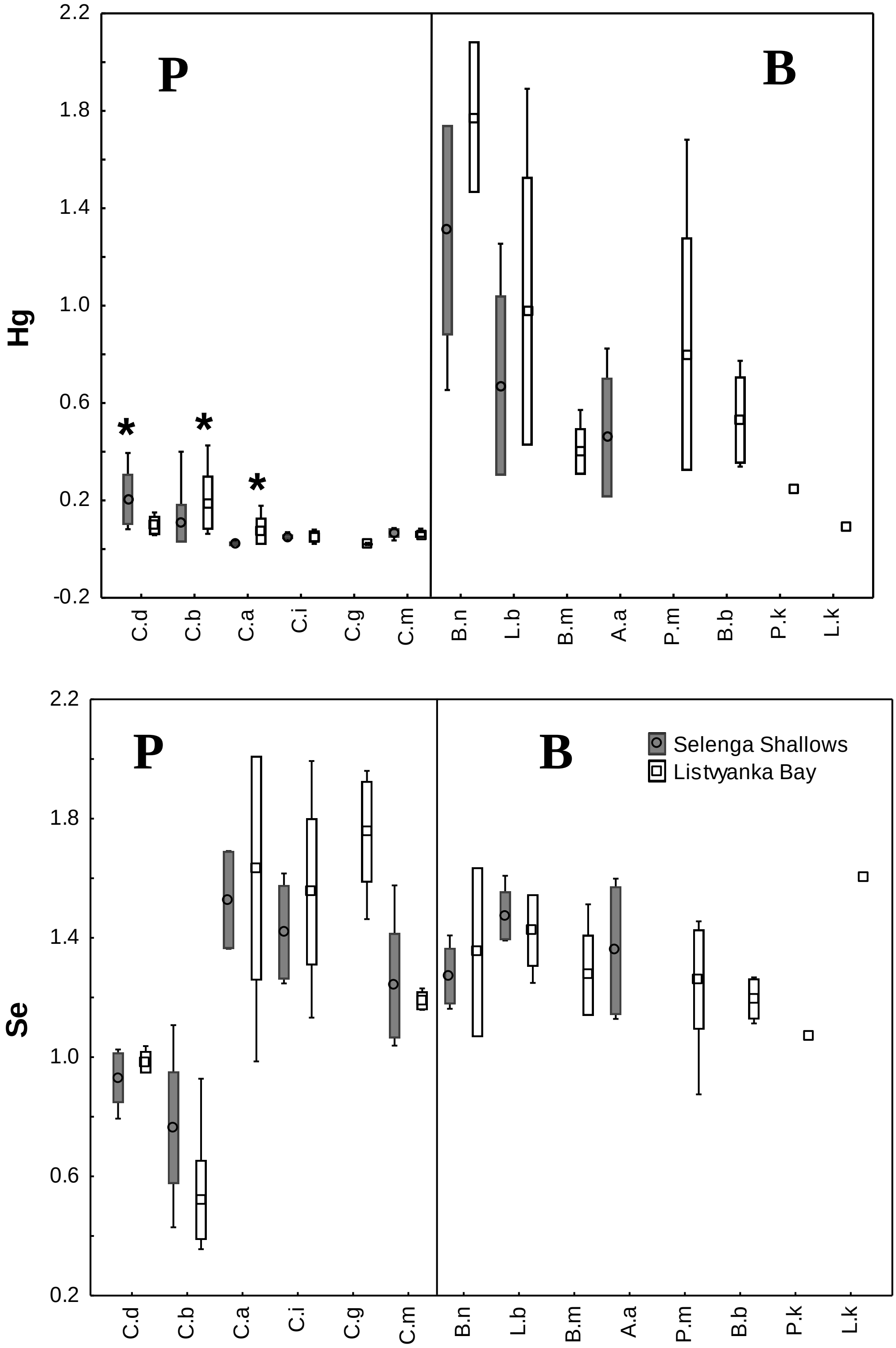
Species	No. of samples	Length
C. baicalensis	n_M=38	Hg(+)**, Se(-)**
C. dybowski	n_M=12	Hg(+)**
C. inermis	n_M=19	As(-)**, Hg(+)**, Se(-)**
C. migratorius	n_M=12	Pb(+)*
L. bergianus	n_M=10	Hg(+)**
P. major	n_M=11	Hg(+)*, Cd (-)*, Se(+)*

*p < 0.05, **p < 0.01; significance level (Spearman's Rank Order Correlation).
(+) = positive correlation, (-) = negative correlation



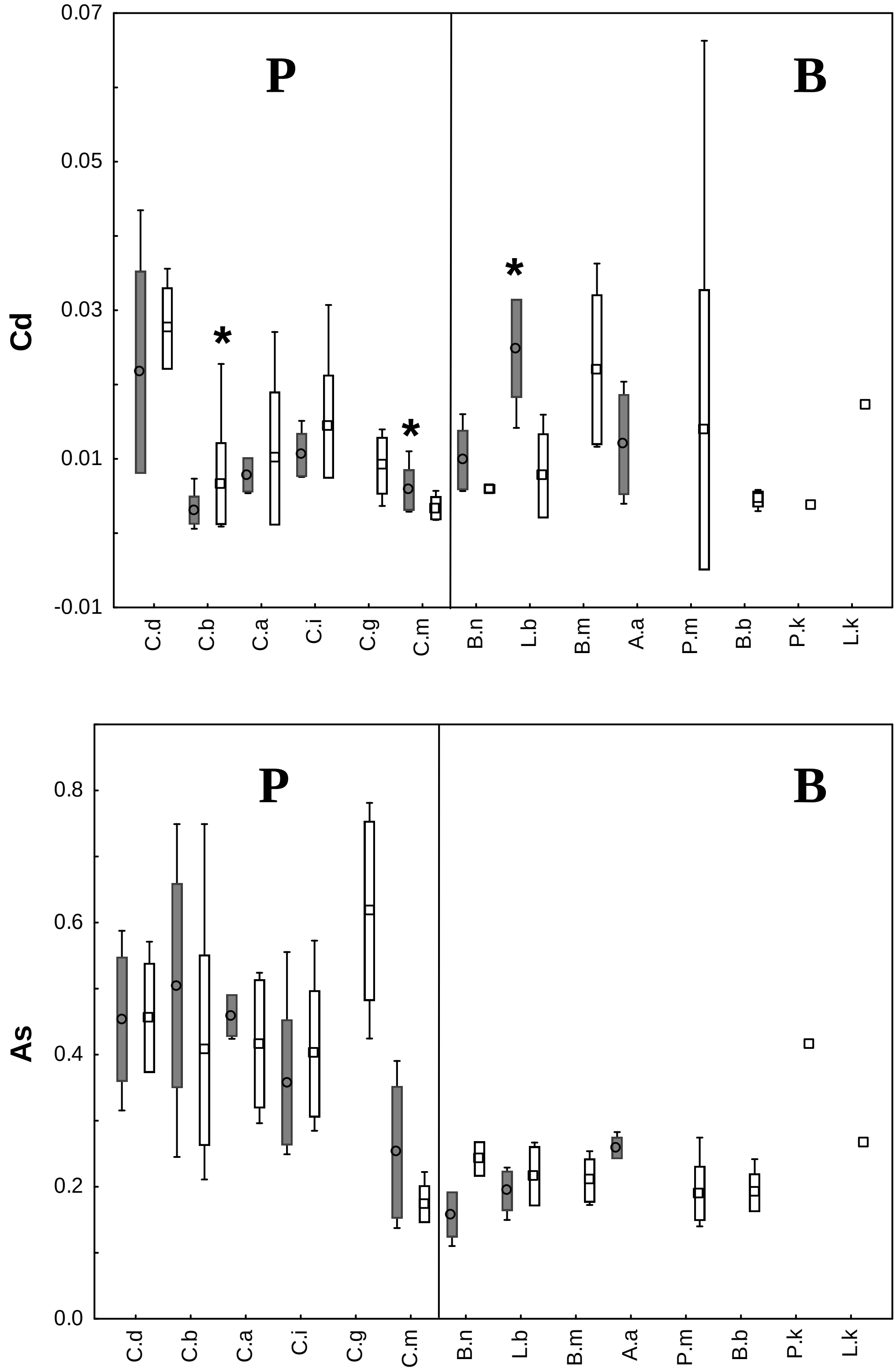
C.d: *C. dybowski*, C.b: *C. baicalensis*, C.a: *C. alexandrae*, C. i: *C. inermis*, C.g: *C. grewingkii*, C.m: *C. migratorius*, B.n: *B. nikolskii*, A.a: *A. abyssalis*, P.m: *P. major*, B.b: *B. baicalensis*, P.k: *P. knerii*, L.k: *L. kesslerii*

Figure 4. Concentrations of Pb in muscle [µg/g dry weight] in pelagic and benthic fish species caught at the Selenga Shallows and Listvyanka Bay, Lake Baikal. The vertical line separates the pelagic (P) and benthic (B) fish species. Asterisk indicate significant (p < 0.05) difference between sampling locations. Boxes represent mean values ± standard deviations, whiskers represents maximum and minimum values.



C.d: *C. dybowski,* C.b: *C. baicalensis,* C.a: *C. alexandrae,* C. i: *C. inermis,* C.g: *C. grewingkii,* C.m: *C. migratorius,* B.n: *B. Nikolskii,* A.a: *A. abyssalis,* P.m: *P. Major,* B.b: *B. baicalensis,* P.k: *P. knerii,* L.k: *L. kesslerii*

Figure 5. Concentrations of Pb in muscle [µg/g dry weight] in pelagic and benthic fish species caught at the Selenga Shallows and Listvyanka Bay, Lake Baikal. The vertical line separates the pelagic (P) and benthic (B) fish species. Asterisk indicate significant ($p < 0.05$) difference between sampling locations. Boxes represent mean values ± standard deviations, whiskers represents maximum and minimum values.



C.d: *C. dybowski,* C.b: *C. baicalensis,* C.a: *C. alexandrae,* C. i: *C. inermis,* C.g: *C. grewingkii,* C.m: *C. migratorius,* B.n: *B. Nikolskii*, A.a: *A. abyssalis*, P.m: *P. Major,* B.b: *B. baicalensis*, P.k: *P. knerii*, L.k: *L. kesslerii*

Figure 6. Concentrations of Pb in muscle [µg/g dry weight] in pelagic and benthic fish species caught at the Selenga Shallows and Listvyanka Bay, Lake Baikal. The vertical line separates the pelagic (P) and benthic (B) fish species. Asterisk indicate significant ($p < 0.05$) difference between sampling locations. Boxes represent mean values ± standard deviations, whiskers represents maximum and minimum values.

3.2.2 Comparison between benthic and pelagic fish species

The concentration differences in muscle tissue of pelagic and benthic fish are illustrated in Figure 4, Figure 5 and Figure 6. Significant differences (Appendix L) were found only for Hg and As at both sampling locations. Only differences that were significant at both sampling locations have been discussed.

As illustrated in Figure 5, the benthic fish had significantly higher (ca. 7 times) Hg concentrations in muscle tissues compared to the pelagic fish ($KW_{1,62} = 30.49$, $p < 0.001$ at the Selenga Shallows and $KW_{1,79} = 49.57$, $p < 0.001$ at Listvyanka Bay). In average, the benthic fish had Hg concentration (d.w) of 0.77 ± 0.51 µg/g, compared to 0.10 ± 0.090 µg/g in pelagic fish. In contrast, the pelagic fish had significantly higher ($KW_{1,62} = 23.67$, $p < 0.001$ at Selenga Shallows and $KW_{1,79} = 35.16$, $p < 0.001$ at Listvyanka Bay) As concentrations (0.42 ± 0.15 µg/g, d.w) compared to benthic fish (0.21 ± 0.05 µg/g, d.w).

To assess the risk of Hg exposure in the endemic fish species of Lake Baikal, tissue molar ratios of Se and Hg were calculated. The Se:Hg molar ratios ranged from 1.47 – 264 in all the fish species analyzed. The fish in the pelagic food chain had Se:Hg molar ratio ranging from 3.4 to 264, while the benthic fish species had lower molar ratios, in the range of 1.5 – 43. No consistent pattern with respect to different molar ratios at the two sampling locations was found.

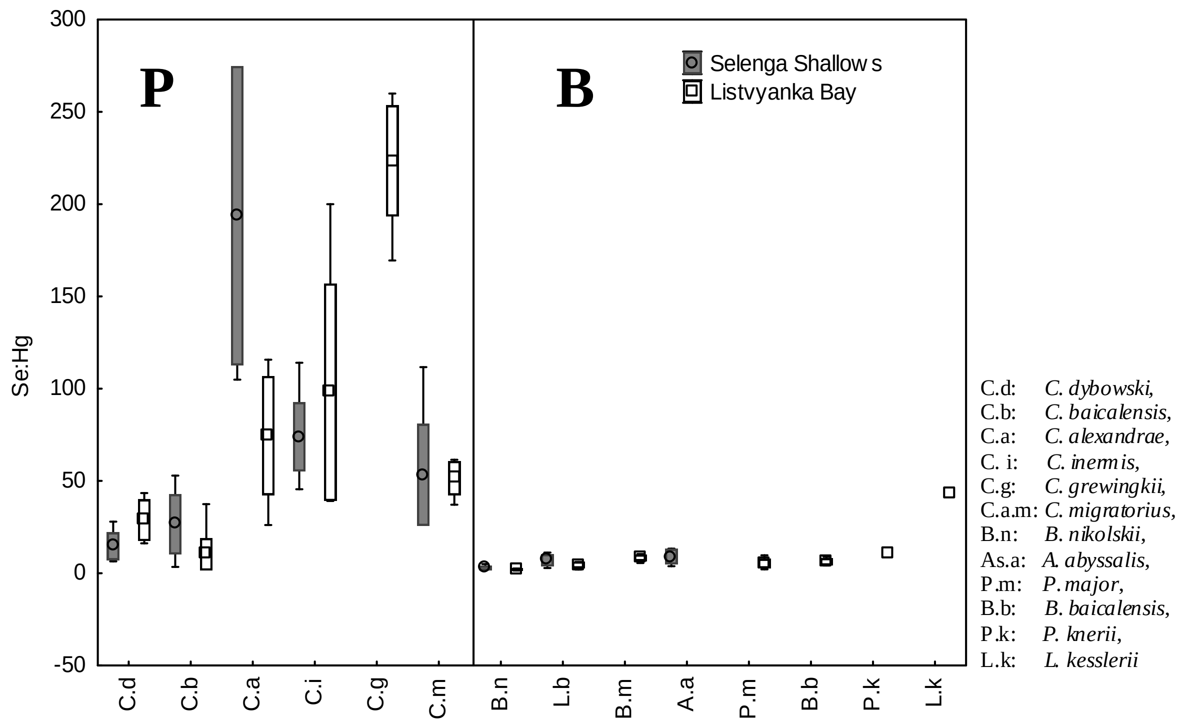


Figure 7. The molar relationship between Se and Hg in muscle tissue of various fish species from the Selenga Shallows and Listvyanka Bay, Lake Baikal. Boxes represent mean values ± standard deviations, whiskers represents maximum and minimum values.

3.2.3 Concentrations of trace elements in the benthic and pelagic food chain

Figure 8 shows the relationship between trophic level (TL) and concentrations of elements in organisms from the pelagic and benthic food chain. Literature data for TL based on stable isotope studies of the pelagic and benthic food chain of Lake Baikal have been used for calculations (Yoshii, 1999; Yoshii, et al., 1999). Mercury was found to correlate positively with TL in both the benthic and pelagic food chain of Lake Baikal. In contrast, As, Cd and Pb was found to decrease with increasing TL (Figure 8).

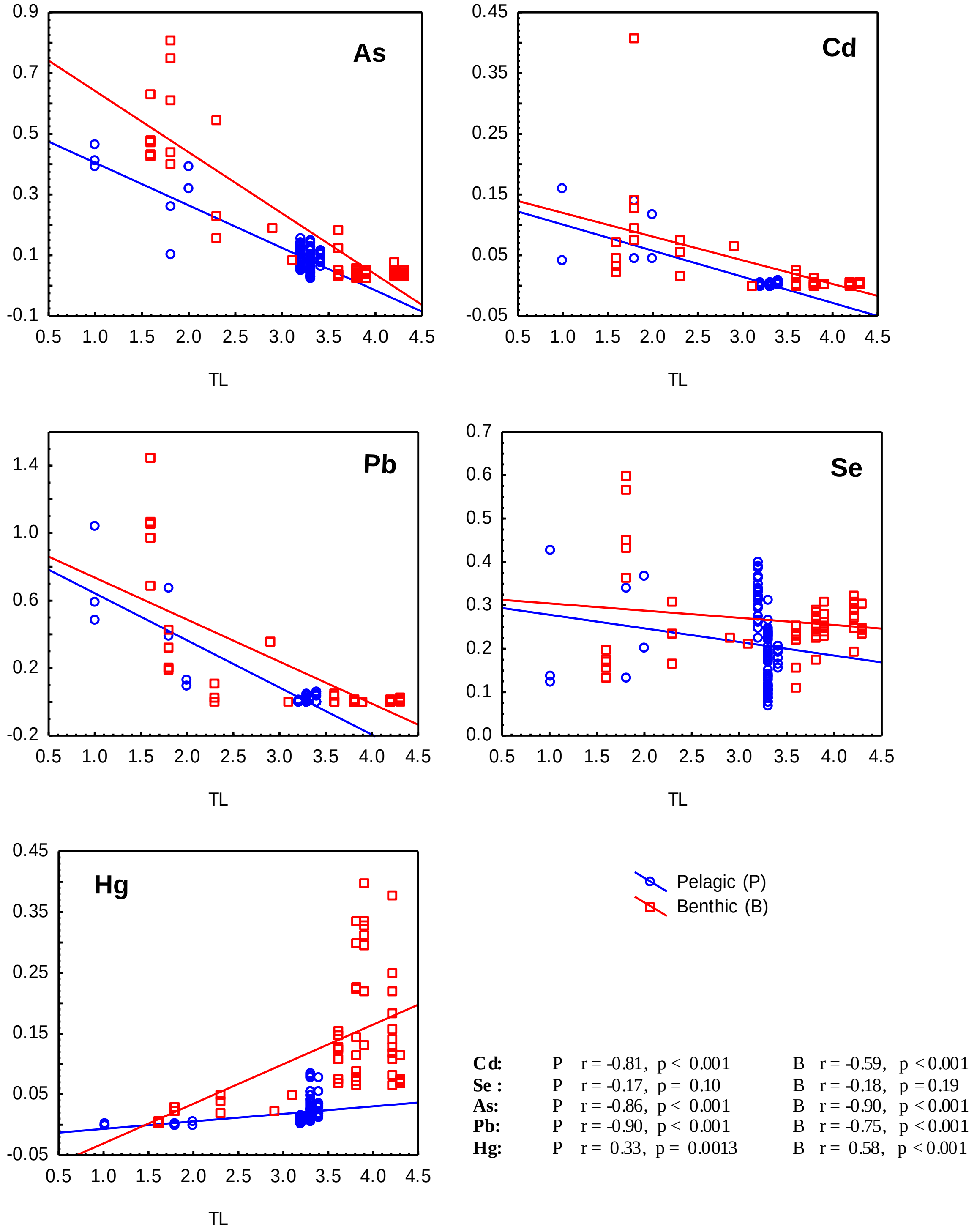


Figure 8. The relationship between trophic level (TL) and concentration of As, Cd, Hg, Pb and Se in plankton and fish belonging to the pelagic (P) food chain (blue) and species of invertebrates and fish from the benthic (B) food chain (red). The given p-values indicate the significance of the linear relationship between TL and concentrations of trace elements. Concentrations of elements are given in µg/g wet weight.

4. Discussion

4.1 Spatial variability in the concentration of trace elements

Low concentrations have previously been reported for the biotic and abiotic compartment of Lake Baikal (Ciesielski, et al., 2006; Ciesielski, et al., 2010; Falkner, et al., 1997; Grosheva, et al., 2000; Koval, et al., 1999; Mackay, et al., 1998). However, further monitoring of trace elements in Lake Baikal is of importance, since some studies have found indication of pollution related to anthropogenic activities. For instance, Mackay et al. (1998) found indications of anthropogenic pollution of Pb in upper layers of sediment cores from Lake Baikal. This was especially prominent in cores from the southern basin and at the very north of the lake (Mackay, et al., 1998). In addition, elevated Hg concentrations have been reported for rivers, shallows and semi-enclosed bays of the lake (Meuleman, et al., 1995). According to Kozhova and Izmest'eva, the Selenga River is the main source of pollution in the lake (Kozhova and Izmest'eva, 1998). Since the chemical composition of the water at the Selenga Shallows is affected by the Selenga River (Chebykin, et al., 2010; Kozhova and Izmest'eva, 1998) it is possible that the freshwater communities at the Selenga Shallows will have higher contaminant burdens compared to 'reference' locations such as Listvyanka Bay.

The higher average water concentration of Pb at the Selenga Shallows (0.033 µg/L) compared to Listvyanka Bay (0.0040 µg/L) seems to confirm this hypothesis. The catchment area of this the Selenga River is characterized by high anthropogenic activity related to agriculture, the industrial region of Ulan-Ude and various cities on Russian and Mongolian territories (Figure 2). Accordingly, increased contents of mineral compounds, suspended matter, oil products, phenols and heavy metals have been recorded in this river (Kozhova and Izmest'eva, 1998). However, besides the anthropogenic revierine input of Pb from the Selenga River, higher Pb concentrations in the water at the Selenga Shallows may be related to natural loads of Pb from the Selenga River. In general, river water has a natural higher content of terregenous elements (such as Pb) compared to lake water (Likens, 2010).

Although the Pb levels in water were higher at the Selenga Shallows, concentrations of Pb were significantly higher in fish from Listvyanka Bay. This difference was found for the species *C. dybowski, C. baicalensis* and *L. bergianus*. The spatial concentration difference was especially pronounced in the case of *C. dybowski*, which had average dry weight Pb concentration of 0.265 µg/g at Listvyanka Bay, compared to 0.013 at the Selenga Shallows.

A possible explanation for these results might be different bioavailability of Pb at the two sampling locations. Most of the Pb in lakes is generally found to be associated with organic matter of terrestrial origin (Likens, 2010). It is reasonable to assume that the amount of ligands in form of particles and organic matter is relatively low at Listvyanka Bay, as the water has circulated in the lake before reaching this location, and emerges from depths of 1000 meters (Kozhova and Izmest'eva, 1998). In contrast, the water chemistry at the Selenga Shallows is greatly influenced by riverine input from the Selenga River (Chebykin, et al., 2010; Kozhova and Izmest'eva, 1998). The concentration of organic and particulate matter is generally much higher in river water compared to lake water (Likens, 2010). According to bioavailability models such as the Free Ion Activity Model (FIAM) and the Biotic Ligand Model (BLM), a reduction of free ions in water due to binding by organic matter is generally expected to reduce uptake of metals in organisms (Batley, et al., 2004). Thus, lower abundance of ligands available for binding and complexiation of trace metals may explain the tendency towards greater bioavaiability of Pb at Listvyanka Bay. Possible higher bioavailability of Pb in more pelagial parts of Lake Baikal has also been reported by Chebykin et al. (2010). They found a 35 times enrichment of Pb in the fine suspension of the pelagial parts of Lake Baikal compared to the fine suspension of the Selenga River Delta. Because the fine suspension in this study also refers to biogenic components like plankton, Chebykin et al. (2010) suggested that Pb might be more bioavailable in the pelagial parts of the lake, compared to areas more influenced by riverine input from Selenga (Chebykin, et al., 2010).

The higher concentration of Cd, Hg and Se in plankton from Listvyanka Bay compared to plankton from the Selenga Shallows seems to confirm the theory about higher bioavailability at this location. The water concentration of these elements did not differ significantly between the two locations.

The spatial pattern of Cd and Hg concentrations in fish is not as clear. For some species of fish, the levels of Hg and Cd were highest at the Selenga Shallows, while others had higher concentrations at Listvyanka Bay. For example, *C. migratorius* and *L. bergianus* caught at the Selenga Shallows had higher Cd levels than speciemens caught at Listvyanka Bay. In contrast, Cd levels in *C. baicalensis* were significantly higher in individuals caught at Listvyanka Bay. Thus, no clear trend in spatial variability of Cd concentrations was observed in the fish species compared. Similarly, an inconsistent spatial pattern was also found as regards to Hg levels in fish. The Hg levels were significantly higher in *C. alexandrae* and *C.*

baicalensis caught at Listvyanka Bay, while specimens of *C. dybowski* had higher Hg levels from the Selenga Shallows. Furthermore, the higher Hg concentrations in specimens of *C. alexandrae* and *C. baicalensis* at the Selenga Shallows could be related to the larger size of fish caught at this location. Positive correlations between Hg levels and size of fish have been reported in several other studies (Allen-Gil, et al., 1997; Mason, 2002; Vieira, et al., 2011). A positive correlation between Hg and fish size was also found for most of the fish species considered in this study. Therefore, it is possible that the apparent spatial variability in Hg levels of *C. alexandrae* and *C. baicalensis* are caused by the significant difference in fish size at the two sampling locations. Individuals of the same species living at two different locations may also differ as regards to diet and TL. However, the higher Pb levels in fish from Listvyanka Bay, and higher Cd, Hg and Se concentrations in plankton from the same location does indicate that some trace elements can be more bioavailable at Listvyanka Bay since that the water concentrations did not differ between the two locations.

It is noteworthy to mention that in recent papers the applicability of the FIAM and BLM as predictors for trace element bioaccumulation in aquatic organisms has been discussed. Both enhanced and reduced uptake of trace elements as a result of increased organic matter in water has been documented (Batley, et al., 2004; Lamelas, et al., 2005; Mylon, et al., 2003; Stemberger and Chen, 1998). For example, Pb uptake have been found to increase in algae's and diatoms in the presence of riverine organic matter (Mylon, et al., 2003). Similarly, when comparing the amount of Pb in fish from several north eastern U.S. lakes, a positive correlation was found between Pb levels in fish and content of dissolved organic matter in lakes (Stemberger and Chen, 1998). In contrast, Lamelas et al. (2005) found decreased Pb uptake in algae in the presence of several types of organic matter which supports the FIAM and BDL models (Lamelas, et al., 2005). In addition, applicability of the FIAM and BDL models have been confirmed by Stemberg and Chen (1998), who found lower uptake of Cd in lakes with higher content of organic matter (Stemberger and Chen, 1998). Similarly, Mylon et al. (2003) found Cd uptake in algae to increase when the amount of organic matter decreased in water (Mylon, et al., 2003). The ongoing debate emphasizes the importance of taking into account site-specific water characteristics, such as different types of organic matter, when estimating possible bioaccumulation of trace elements in aquatic organisms (Lamelas, et al., 2005). However, most experiments confirm the FIAM and exceptions to the model are relatively rare (Batley, et al., 2004). Therefore, the FIAM and BLM are still valuable for explaining the observed higher concentrations of Pb in fish and Cd, Hg and Se in plankton at Listvyanka Bay.

4.2 Bioaccumulation in the pelagic and benthic food chain of Lake Baikal

In both the pelagic and benthic food chain of Lake Baikal, potential prey organisms had higher concentrations of As, Cd and Pb compared to fish. In the pelagic food chain the main food sources for fish is meso-and macrozooplankton (Sideleva, 2003). The meso-and macrozooplankton had average concentrations of As, Cd and Pb (wet weight, w.w) that were 3, 46 and 47 times higher than in pelagic fish, respectively. In the benthic food chain, various species of benthic invertebrates such as gammarids and mollusks are potential prey organisms for fish (Sideleva, 2003). The benthic gammarids as a group had average concentrations of As, Cd and Pb (w.w) that were 8, 15 and 87 times higher than in benthic fish, respectively. In addition, based on literature values for TL, an inverse relationship between As, Cd and Pb was found in organisms of both food chains (Figure 8). This indicates biodilution of As, Cd and Pb in both food chains. Biodilution of trace elements such as As, Cd and Pb in aquatic food chains have also been reported in several other studies (Chen and Folt, 2000; Chen, et al., 2000; Mason, et al., 2000; Stemberger and Chen, 1998). In general, primary producers are vulnerable to the effects of metal excess, and for certain elements they show some of the highest levels of accumulation in food chains. This is related to their large surface area to body volume, which means that metals and other chemicals dissolved in the surrounding water have access to the entire surface of each cell. In contrast, organisms at higher trophic level often have more developed physiological mechanisms for excretion of metals. In addition, compartmentalization of metals within organisms at lower trophic level can make metals unavailable for absorption in the digestive systems of top predators. Thus, most trace metals are considered to have low bioavailability for aquatic organisms at higher trophic level (e.g., fish) and usually do not biomagnify along aquatic food chains (Mason, 2002; Merian, 2004).

A well know exception from this rule is Hg. Several studies have confirmed the biomagnification of Hg in freshwater ecosystems (Chen, et al., 2000; Mason, 2002; Mason, et al., 2000; Merian, 2004). This is also in agreement with the results of this study: the positive linear relationship between TL and Hg concentration in Figure 8 suggests biomagnification of Hg in both the pelagic and benthic food chain of Lake Baikal. Biomagnification of Hg in the pelagic food chain of Lake Baikal have also been reported by Ciesielski et al. (2010). Perrot et al. (2010) also found evidence of bioaccumulation and biomagnification of Hg in Lake Baikal, although the endemic fish species were not included in this study (Perrot, et al., 2010). Biomagnification of Hg is of great concern due to the potential of Hg reaching toxic levels in top predators of food chains, such as predatory fish, birds and mammals (Mason, 2002; Merian, 2004).

4.2.1 Comparison between benthic and pelagic fish species

When comparing fish inhabiting the pelagic and benthic food chain of Lake Baikal, differences in concentrations of As and Hg were identified. The benthic fish had seven times higher concentrations of Hg compared to pelagic fish, while pelagic fish had two times higher As concentrations compared to benthic fish.

The higher Hg levels in benthic fish compared to pelagic fish can be related to differences in habitat, diet and TL. It can also be related to differences in physiology, age and size. The effects of diet and habitat on Hg accumulation in benthic organisms are closely related, as choice of habitat determines which species of prey are available and vice versa. Benthic fish pray on a variety of organisms, and the predator-prey relationships in the benthic food chain in Lake Baikal are generally more complex than those in the pelagic food chain (Sideleva, 2003; Yoshii, 1999; Yoshii, et al., 1999). Since the major food sources of benthic fish are organisms such as gammarids, molluscs and other fish (Sideleva, 2003), the benthic invertebrates included in this study (Table 1) gives a general impression of metal concentrations in potential prey organisms for benthic fish. The fish belonging to the pelagic food chain consume mainly meso-and macrozooplankton, such as *E. baicalensis* and *M. branickii,* in addition to varying amounts of juvenile fish (Sideleva, 2003). The average Hg concentration in potential prey organisms of benthic fish (0.28 µg/g d.w in benthic invertebrates) was about 18 times higher than the Hg content in potential prey organisms of pelagic fish (0.0156 µg/g d.w in meso-and macrozooplankton). In addition, the benthic fish species of Lake Baikal are more piscivorous and than the pelagic fish species (Sideleva, 2003) which means they might be feeding at higher TL than the pelagic fish. By using literature values for TL (Yoshii et al 1999 and Yoshii 1999) the average TL for the benthic fish have been estimated to 3.9 compared to 3.3 for pelagic fish. Considering that Hg was found to increase with higher TL in both food chains, this could also explain the higher Hg content in benthic fish.

The regression line in Figure 8 relating TL to Hg concentrations in the benthic food chain has a steeper slope than the regression line for the pelagic fish. This indicates that the benthic organisms' bioaccumulates Hg at higher rates than the pelagic organisms. Thus, animals in the benthic food chain seem to have higher Hg levels compared to pelagic animals of similar trophic level. For instance, *P. knerii* have an estimated TL around 3.1, and this benthic fish has about 1.8 times higher Hg concentrations compared to *C. baicalensis* (TL 3.3). Similarly, the benthic fish *B. baicalensis* have TL close to that of *C. dybowski* (3.6 and 3.4, respectively). At the same time, *B. baicalensis* had about three times higher Hg levels than *C. dybowski*. This indicates that additional factors besides TL can be important for the observed differences in Hg content between benthic and pelagic fish. Fish belonging to distinct species may also differ in physiology and morphology (Merian, 2004). However, all of the fish species analyzed in this project are sculpins in the superfamily of *Cottoidea*, except *C. migratorius*. It is therefore reasonable to assume that the fish species of the superfamily *Cottoidae* have similar physiological traits.

The higher Hg levels in benthic organisms compared to pelagic organisms at the same trophic level may also emphasize the importance of habitat when it comes to bioaccumulation of Hg. Benthic organisms live in close association with sediments, and total Hg concentrations are generally higher in sediments than in water (Luoma, 1983; Luoma, 1989; Merian, 2004). Furthermore, the microbial production of MeHg in sediments means that benthic organisms are exposed to a more bioavailable form of Hg in their habitat. Thus, benthic organisms are exposed to both higher concentrations and more bioavailable forms of Hg in their habitat compared to pelagic organisms. Luoma et al. (1989) investigated the importance of ingested sediment particles as regards to metal accumulation in benthic organisms. They found that although metal assimilation through ingested particles is inefficient, it is importance because the source (sediment) is highly concentrated (Luoma, 1989), which emphasize the importance of habitat in relation to bioaccumulation of Hg.

Apart from Hg, the pelagic and benthic fish species exhibited significant differences in the concentrations of As. The pelagic fish species had two times higher concentrations of As compared to the benthic fish species. This difference can be only partially explained by the variations of As in the diet of benthic and pelagic fish. Potential prey organisms of pelagic fish such as meso-and macrozooplankton had higher concentrations of As compared to benthic gammarids such as *Ommatogammarus* sps., *A. godlewskii* and *P. logowskii*. However, other species of benthic invertebrates like *Eulimnogammarus* sps., *A. victorii* and the benthic mollusc *B. fragilis* had As concentrations similar to those in plankton. This is probably related to the herbivorous feeding strategies of these species, compared to more omnivorous feeding strategies in species such as *Ommatogammarus* sps (Kozhova and Izmest'eva, 1998). It is possible that the benthic fish species analyzed in this study does not feed on the herbivorous benthic invertebrates such as *Eulimnogammarus* sps., *A. victori* or *B. fragilis.* If the benthic fish feed preferentially on species such as *Ommatogammarus* sps., *A. godlewskii* and *P. logowskii,* diet could explain the higher As levels in pelagic fish (plankton had higher As values than these particular species of gammarids). In addition, most of the benthic fish species analyzed do not feed exclusively on benthic invertebrates, but also feed on fish. Since fish were found to have several times lower As levels than meso-and macrozooplankton (the major prey organisms for pelagic fish) this feeding differences explains the higher As levels in the pelagic fish. Evidence of As being related to feeding strategy in fish have also been reported by Chen and Folt (2000). They found higher levels of As in fish feeding mostly on zooplankton compared to fish feeding on priscivorous fish in the freshwater ecosystem of Upper Mystic Lake in the US. Furthermore, in another study where levels of trace elements were compared in 38 northeastern U.S lakes, Stemberger and Chen (1997) found higher As levels in fish inhabiting lakes with simple food chains with fewer trophic links. They further suggested that As concentrations may decrease at slower rates in simplified food webs, compared to higher rates of As loss in more complex food webs. This may also an explain the observed higher As content in pelagic fish of Lake Baikal: studies by Yoshii et al. (1999) and Yoshii (1999) have shown that in general, the pelagic food chain consists of fewer and more simplified trophic links compared to the benthic food chain of Lake Baikal. Thus, the higher content of As in pelagic fish may be explained by differences in structure of the benthic and the pelagic food chain of Baikal.

4.3 As, Cd, Hg, Pb and Se in Lake Baikal ecosystems

In general, relatively low concentrations of the selected trace elements were found in water and biota of Lake Baikal. This is in agreement with previously reported data (Ciesielski, et al., 2006; Ciesielski, et al., 2010; Falkner, et al., 1997; Grosheva, et al., 2000; Koval, et al., 1999; Mackay, et al., 1998) The concentrations of Cd, Hg, Pb and Se in analyzed Baikal water samples (Table 2) are low compared to other freshwater and marine ecosystems. For example, the maximum value of 0.0042 µg/L for Hg and 0.0042 µg/L for Cd in Baikal water is low compared to maximum values in northeastern US lakes (0.017 and 0.065 µg/L, respectively). However, the concentration of As (0.34 – 0.38µg/L) is comparable to the concentration ranging (0.038 – 0.587 µg/L) of these lakes (Chen, et al., 2000). Nevertheless, the content of As in Baikal water is well below concentrations reported for known As-contaminated lakes such as Upper Mystic Lake in the US, which have As levels in the range of 0.6 – 1.2 µg/L (Chen and Folt, 2000). The average concentration of Pb in water from the Selenga Shallows (0.033 µg/L) is well below the general Pb content in lakes and rivers (0.1 to 10 mg/L) estimated by the World Health Organization (Mason, 2002).

The levels of As, Cd, and Pb in meso- and macrozooplankton from Baikal were in the range of levels found in northeastern US lakes. The values of Hg are well below those reported for plankton from these lakes (Chen, et al., 2000). Furthermore, the average As, Cd, Hg, Pb and Se concentration in Baikal fish is generally lower than the concentrations found in fish from northeastern US lakes (Stemberger and Chen, 1998; Yeardley, et al., 1998), oligotrophic arctic lakes in the U.S (Allen-Gil, et al., 1997) the Atlantic Ocean (Vieira, et al., 2011) and the Kerguelen Islands (Bustamante, et al., 2003). The concentrations of the selected trace elements in Baikal fish are also well below the critical values for human consumption, assessed by Yeardley et al. (1998).

4.3.1 The Se:Hg molar relationship in Baikal fish

Biogeochemistry of aquatic ecosystems is of great importance when considering Hg concentrations in biota. This is because bioaccumulation of Hg does not only depend on factors previously discussed such bioavailability. In oligotrophic nutrient deficient lakes such as Baikal, it is important to consider that depaupuration of certain elements may lead to enhanced risk of toxicity of others (Likens, 2010; Luoma, 1983). One good example is the antagonistic effects of Se on Hg induced toxicity. Because Se is known to reduce Hg toxicity by several different mechanisms, the concentration of Se in relation to the concentration of Hg is critical. First of all, Se can sequester Hg and thus reduced oxidative stress which otherwise might have been generated by free Hg ions in the organism. Secondly, Hg has strong affinity for Se and may bind to and inhibit important selenoenzymes. If Se is in excess of Hg in the cell, it will sequester Hg and thus the inhibition of selenoenzymes is not as likely to occur (Peterson, et al., 2009). A tissue Se:Hg molar ratio greater than one is suggested as a threshold for the protecting action of Se against Hg toxicity (Peterson, et al., 2009; Ralston, et al., 2007). In addition, Se is also believed to reduce the bioavailability of Hg in aquatic ecosystems. This is probably related to the formation of Se-Hg complexes which are not very bioavailable to biota. For example, several field test in Sweden have revealed that addition of sodium selenite (Na_2SeO_3) to Hg contaminated lakes have reduced Hg concentrations with 70-80% in fish tissue (Peterson, et al., 2009). Thus, in Se depauperated environments such as Lake Baikal, Hg may pose an increased risk for wildlife even if present at relatively moderate concentrations. However, as evident in Figure 8, Se was found to be in great excess over Hg in muscle tissue of the endemic fish species of Lake Baikal. The Se:Hg ratios ranged from to 1.47 to 264, which is well above the suggested protective ratio of 1. These molar ratios are much higher than previously reported values for freshwater fish, reviewed by Pelletier (1986). For example, freshwater fish from Northern Canada had molar Se:Hg ratios in a range of 0.8 – 9.8 (Pelletier, 1986). In a more recent review of Se and Hg concentrations in fish tissue, Peterson et al. (2009) discussed several studies which have reported large Se molar excess in relation to total Hg concentrations in fish. These authors further concluded that the excess of Se over Hg may be more widespread in freshwater organisms than expected. In this study, the great molar excess of Se over Hg in the endemic fish species of Lake Baikal indicates that although Baikal belongs to a Se deficient region, the Se levels in fish are high enough to prohibit Hg induced toxicity. This is probably related to the low Hg concentrations in both the abiotic and botic compartments of the lake. Based on the Se:Hg molar relationship, there is no evident risk of Hg induced toxicity in the endemic fish species of Lake Baikal at the present.

5. Conclusion

The present study confirms generally low concentrations of potentially toxic chemical elements (As, Cd, Hg, Pb and Se) in Lake Baikal in comparison to other freshwater ecosystems. However, the comparative study of water and biotic components in the ecosystem of Baikal revealed some concentrations differences between locations with riverine and possible anthropogenic influence (Selenga Shallows) and the reference site (Listvyanka Bay). The concentrations of Pb were significantly higher in water samples from the Selenga Shallows in comparison to Listvyanka Bay, while concentrations of Pb were significantly higher in fish from Listvyanka Bay. This may be related to greater bioavailability of Pb for fish at Listvyanka Bay caused by lower abundance of natural ligands at this location. Furthermore Cd, Hg and Se were also higher in plankton at Listvyanka Bay. Since the water concentration of Cd, Hg and Se did not differ significantly between the two locations, greater bioavailability at Listvyanka Bay can also be responsible for the observed concentration differences in plankton.

In both the pelagic and benthic food chain of Lake Baikal, Hg showed indication of biomagnification while As, Cd and Pb showed indications of biodilution. When comparing fish inhabiting the pelagic and benthic food chain of Lake Baikal, differences in concentrations of As and Hg were identified. The As concentrations were twice as high in pelagic fish, while the benthic fish had seven times higher Hg content compared to pelagic fish. The observed concentration differences of As and Hg in benthic and pelagic fish may be related to such as different complexity and structure of the two food chains, different feeding strategies and habitat. The Se:Hg molar ratio revealed that although Baikal is located in a Se deficient region, Se is still in significant excess over Hg. Based on this relationship there is no risk of Hg induced toxicity in the endemic fish species of Lake Baikal at the present.

6. References

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Appendix A: Detection limits

The method detection limits (MDLs) for biota and water samples are given in Table A1 and Table A2, respectively. In order to obtain accurate MDLs, the average weight for different types of samples were calculated, and 3 different MDLs were calculated based on these weight groups (Table 1).

Table A1: Method detection limits (MDL) in µg/g for different weight groups of biota samples from Lake Baikal. MDL 3 have been used for most of the organisms. Due to low sample weight, MDL 2 has been used for plankton, and MDL 1 has been used for one zooplankton sample with especially low sample weight. The Se isotope 82 was used for most organisms, except for in plankton and crustaceans for which the Se isotope 78 was used.

Element	Mass number	Resolution	IDL-25%	MDL 1 *Zooplankton 0.0194 g*	MDL 2 *Plankton AVG. 0.195 g*	MDL 3 *Other samples AVG. 0.4 g*
As	75	Hr	0.025	0.0773	0.0077	0.0038
Cd	114	Lr	0.002	0.0062	0.0006	0.0003
Pb	208	Lr	0.002	0.0062	0.0006	0.0003
Hg	202	Lr	0.03	0.0928	0.0092	0.0045
Se	78	Hr	0.15	0.4639	0.0462	0.0225
Se	82	Lr	0.05	0.1546	0.0154	0.0075

Table A2: Method detection limits **(**MDL) were for water samples from Lake Baikal.

Element	Isotope	Resolution	IDL-25%
As	75	Hr	0.025
Cd	114	Lr	0.0020
Pb	208	Lr	0.0020
Hg	202	Lr	0.0010
Se	82	Lr	0.05

Appendix B: Quality assurance

Table B: Reference material of Dogfish muscle (DORM-2, Certified Reference Material for Trace Metals, National Research Council of Canada) and Oyster tissue (NIST 1566b) was used to evaluate the accuracy (%) of the HR- ICP-MS analysis for the biota samples. Given certified values (CV) are divided by measured average values (AVG) based on a number of n samples. The values are given in µg/g dry weight, ± the standard deviation (SD) for the AVG values.

Element	Isotope	Scallop CV	Scallop (n=7) AVG ±SD	Accuracy	Dogfish liver CV	Dogfish muscle (n=3) AVG ±SD	Accuracy
Se	82	1.50	1.56 ± 0.029	104	7.06	7.81 ± 0.10	111
Se	78	1.50	1.51 ± 0.043	101	7.06	7.48 ± 0.27	106
Cd	114	1.06	1.31 ± 0.027	123	19.40	19.90 ± 0.29	103
Hg	202	0.04	0.052 ± 0.0096	130	3.37	3.91 ± 0.11	116
Pb	208	0.12	0.074 ± 0.0010	62	0.32	0.39 ± 0.075	122
As	75	3.60	3.96 ± 0.062	110	10.20	10.55 ± 0.20	103

Appendix C: Biometric measurements of fish

Table C: Average age (years), length (millimeters) and weight (grams) ± standard deviation (STD) for fish species collected by either the Selenga Shallows (S.S) or at Listvyanka Bay (L.B) in Lake Baikal. The letter 'n' corresponds to the number of muscle samples analyzed by HR-ICP-MS. Data for trophic level (TL) have been taken from literature.

Species	**Location**	**n**	**TL***	**Age**		**Length**		**Weight**	
				AVG	STD	AVG	STD	AVG	STD
C. dybowski	S.S	8	3.4	6.1	0.8	141	8	11	3.3
C. dybowski	L. B	4	3.4	4.5	1.3	125	17	8	2.8
C. baicalensis	S.S	21	3.3	3.3	1.0	141	25	14	8.0
C. baicalensis	L. B	17	3.3	4.6	1.0	173	24	26	9.7
C. alexandrae	S.S	3				129	18	15	6.6
C. alexandrae	L. B	6		4.3	0.5	168	19	44	15.5
C. inermis	S.S	9	3.2	3.2	0.4	175	24	55	29.2
C. inermis	L. B	10	3.2	2.9	0.7	166	28	49	28.8
C. grewingii	L. B	7	3.2	2.4	0.5	125	9	18	3.6
C. migratorius	S.S	7	3.3	4.1	1.6	278	49	168	74.9
C. migratorius	L. B	5	3.3	4.6	0.9	296	18	212	34.2
B. nikolskii	S.S	5	3.9	4.0	1.0	178	17	94	37.0
B. nikolskii	L. B	2	3.9	4.5	0.7	190	16	93	25.2
L. bergianus	S.S	5	4.2	4.4	1.3	206	16	91	26.0
L. bergianus	L. B	5	4.2	5.0	1.6	206	22	112	52.0
B. multiradiatus	L. B	5	4.3	3.8	1.3	114	9	15	4.3
A. abyssalis	S.S	4		5.0	0.0	88	3	7	0.3
P. major	L. B	11	3.8	5.9	3.7	175	71	127	135.4
B. baicalensis	L. B	5	3.6	5.2	0.4	144	3	57	5.0
P. knerii	L. B	1	3.1	5.0		121		25	
L. kesslerii	L. B	1		4.0		115		16	

*Literature values from Yoshii et al. 1999 and Yoshsii 2000.

Appendix D: Concentrations in biota samples, dry weight

Table D: Average concentrations (µg/g, dry weight) of As, Cd, Hg, Pb and Se in all the organisms analyzed. Average values have been calculated for all the fish species and separately for pelagic and benthic fish, plankton, benthic invertebrates and gammarids.

	Se		Cd		Hg		Pb		As	
	AVG	STD	AVG	STD	AVG	STD	AVG	STD	AVG	STD
Fish										
All fish sps.	1.16	0.40	0.011	0.010	0.31	0.43	0.032	0.06	0.36	0.16
Max	1.99		0.066		1.99		0.33		0.78	1.99
Min	0.36		0.0006		0.0169		0.0015		0.11	0.36
All pelagic sps.	1.09	0.45	0.009	0.009	0.10	0.09	0.035	0.06	0.42	0.15
Max	1.99		0.043		0.43		0.331		0.78	
Min	0.36		0.001		0.02		0.002		0.14	
All benthic sps.	1.32	0.17	0.013	0.012	0.77	0.51	0.028	0.03	0.21	0.05
Max	1.61		0.066		1.99		0.142		0.42	
Min	0.88		0.001		0.10		0.0051		0.11	
Plankton, benthic invertebrates										
Plankton	1.73	0.98	0.62	0.45	0.013	0.014	3.29	2.52	1.93	0.56
Max	3.39		1.42		0.030		6.80		2.64	
Min	0.65		0.22		0.002		0.45		1.03	
Plankton, S.S	1.00	0.35	0.30	0.14	0.002	0.000	3.32	3.22	1.55	0.50
Plankton, L.B	2.46	0.82	0.94	0.43	0.024	0.009	3.27	2.34	2.31	0.33
Benthic invertebrates	1.34	0.70	0.37	0.39	0.332	0.302	1.00	1.53	1.49	1.06
Max	2.69		1.37		0.913		5.22		3.61	
Min	0.54		0.04		0.020		0.03		0.39	
Gammarids	0.97	0.30	0.19	0.13	0.37	0.34	1.03	1.79	1.25	0.86
Planarians	2.30	0.35	0.73	0.17	0.44	0.10	0.18	0.11	0.69	0.19
Mollusks	2.34	0.49	0.93	0.62	0.13	0.00	1.29	0.43	2.85	1.07

Appendix E: Concentrations in biota samples, wet weight

Table E: Average concentrations (µg/g, dry weight) of As, Cd, Hg, Pb and Se in all the organisms analyzed. Average values have been calculated for all the fish species and separately for pelagic and benthic fish, plankton, benthic invertebrates and gammarids.

	Se	Cd	Hg	Pb	As
Fish					
All Fish sps	0.23	0.0021	0.062	0.0065	0.071
Max	0.40	0.013	0.40	0.066	0.16
Min	0.071	0.00012	0.0033	0.00030	0.022
Pelagic fish	0.22	0.0019	0.020	0.0069	0.085
Benthic fish	0.26	0.0026	0.154	0.0056	0.041
Plankton, benthic invertebrates					
Plankton	0.25	0.085	0.0018	0.49	0.34
Max	0.43	0.16	0.0061	1.04	0.46
Min	0.12	0.043	0.00016	0.091	0.10
Benthic invertebrates	0.32	0.093	0.054	0.33	0.35
Max	0.60	0.41	0.18	1.44	0.81
Min	0.11	0.0075	0.0029	0.0068	0.078
Gammarids	0.19	0.039	0.050	0.49	0.33
Max	0.31	0.074	0.18	1.44	0.63
Min	0.11	0.0075	0.0029	0.0068	0.078

Appendix F: Concentrations in water

Table F: Concentrations (µg/L) of As, Cd, Hg, Pb and Se in water samples from the Selenga Shallows (S.S) and Listvyanka Bay (L.B), Lake Baikal. Samples were collected at different depths (0, 100, 200 and 300 m) with two replicates at each depth. Average values for each depth are given ± the standard deviation (SD). Single values without SD means that only one replicate was above the method detection limit (MDL, Table A2), and <MDL means that both replicates were below the MDL.

Locality	Depth	As	Cd	Hg	Pb	Se
L.B	0	0.3785 ± 0.0031	<0,002	0.0042 ± 0.0001	0.0027 ± 0.0003	0.0668 ± 0.0077
L.B	100	0.3605 ± 0.0203	<0,002	0.0014 ± 0.0002	0.0045 ± 0.0008	0.0647 ± 0.0059
L.B	200	0.3585 ± 0.0120	<0,002	0.0014	0.0029 ± 0.0003	0.0625 ± 0.0012
L.B	316	0.3647 ± 0.0002	<0,002	0.0022	0.0059 ± 0.0010	0.0599 ± 0.0031
S.S	0	0.3617 ± 0.0078	0.0042 ± 0.0001	0.0012	0.0170 ± 0.0049	0.0634 ± 0.0056
S.S	100	0.3600 ± 0.0097	<0,002	<0,001	0.0255 ± 0.0003	0.0700 ± 0.0033
S.S	200	0.3473 ± 0.0101	<0,002	0.0024	0.0767 ± 0.1024	0.0589 ± 0.0022
S.S	300	0.3714 ± 0.0031	<0,002	<0,001	0.0132 ± 0.0062	0.0727 ± 0.0051

Appendix G: Statistical analyses: water

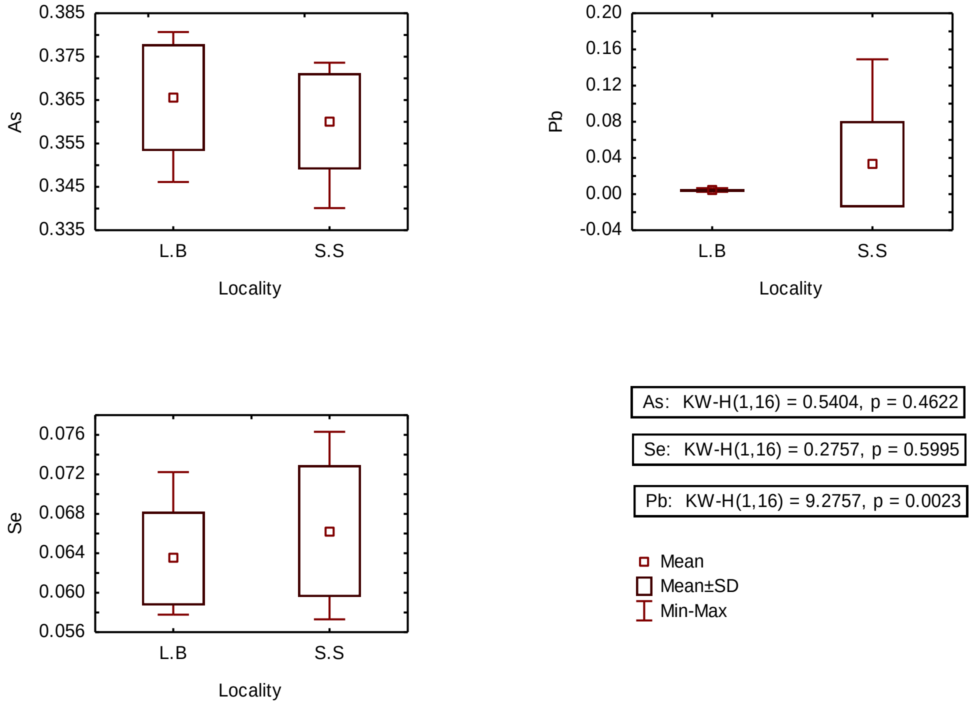

Figure G. Box plots comparing the water concentration (µg/L) of As, Se and Pb at Listvyanka Bay (L.B) and the Selenga Shallows (S.S). A total of eight samples have been collected at depths of 0, 100, 200 and 300 meters, with two replicates at each depth. The Kruskal-Wallis test (KW-H) was applied to study spatial differences between concentrations of elements with all samples above the method detection limit (significance levels $p < 0.05$).

Appendix H: Statistical analyses: plankton

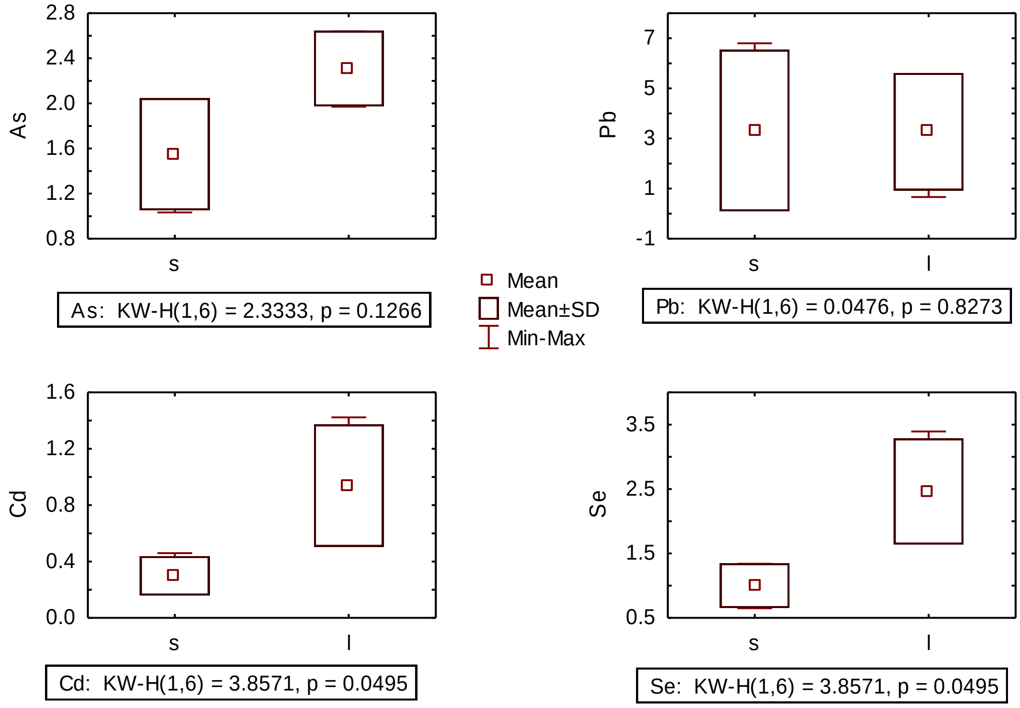

Figure H. Box plots comparing concentration (µg/g, dry weight) of As, Cd, Pb and Se in plankton samples from Listvyanka Bay (l) and the Selenga Shallows (s). Three samples were collected at each location. The Kruskal-Wallis test (KW-H) was used to determine whether the concentrations differed significantly at the two sampling locations at significance levels $p < 0.05$.

Appendix I: Statistical analyses: spatial variability of fish length

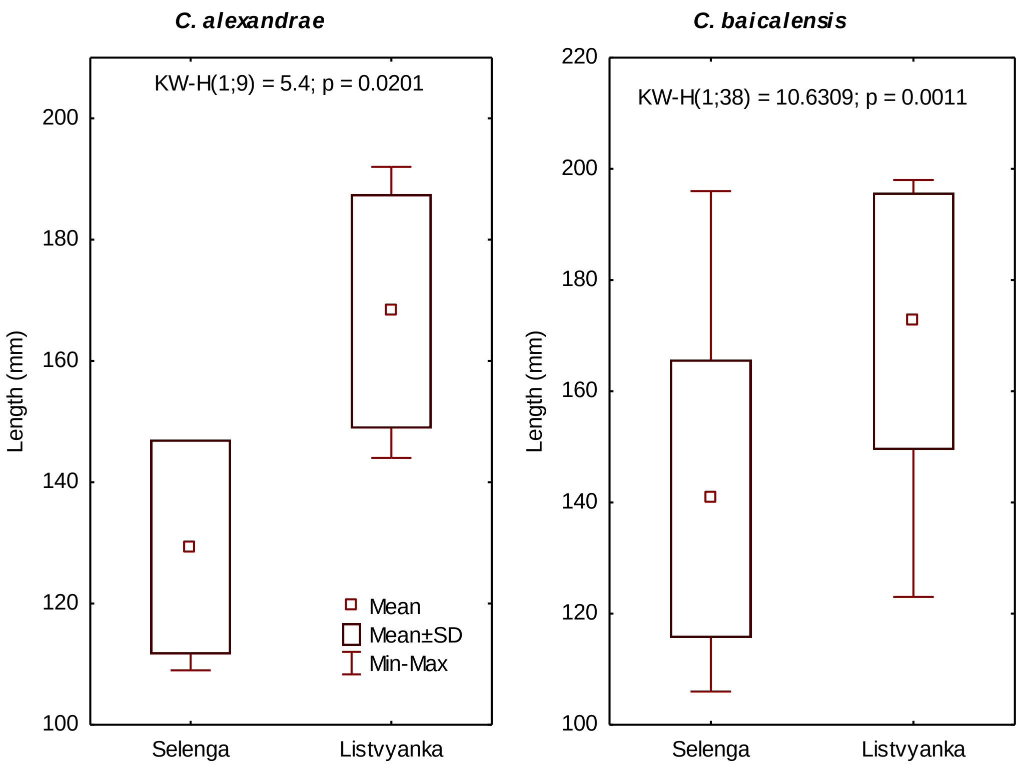

Figure I. Comparison of fish length (mm) of specimens collected at the Selenga Shallows and at Listvyanka Bay. The Kruskal-Wallis test was used to determine whether the length differed significantly at significance level $p < 0.05$. Only species with significant differences in length at the two locations are showed.

Appendix J: Statistical analysis: concentration differences in fish

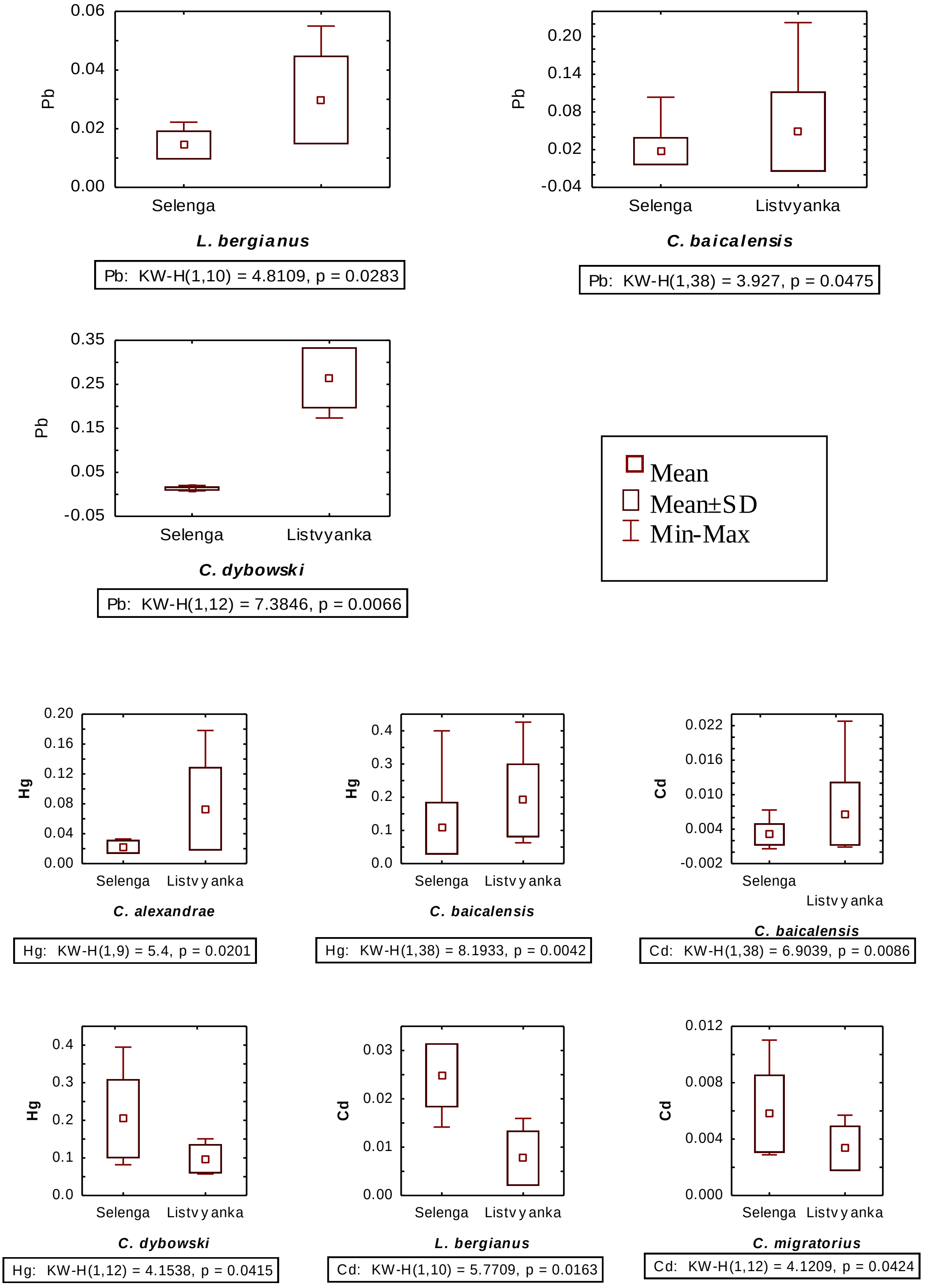

Figure J. Comparison of concentrations of Cd, Hg and Pb (µg/g, dry weight) in individuals of the same species caught at either the Selenga Shallows or Lake Baikal. The Kruskal-Wallis test was used to determine whether elemental concentrations differed significantly at the two locations ($p < 0.05$). Only trace elements that differed significantly in more than one fish species is shown.

Appendix K: Statistical analyses; pelagic and benthic fish

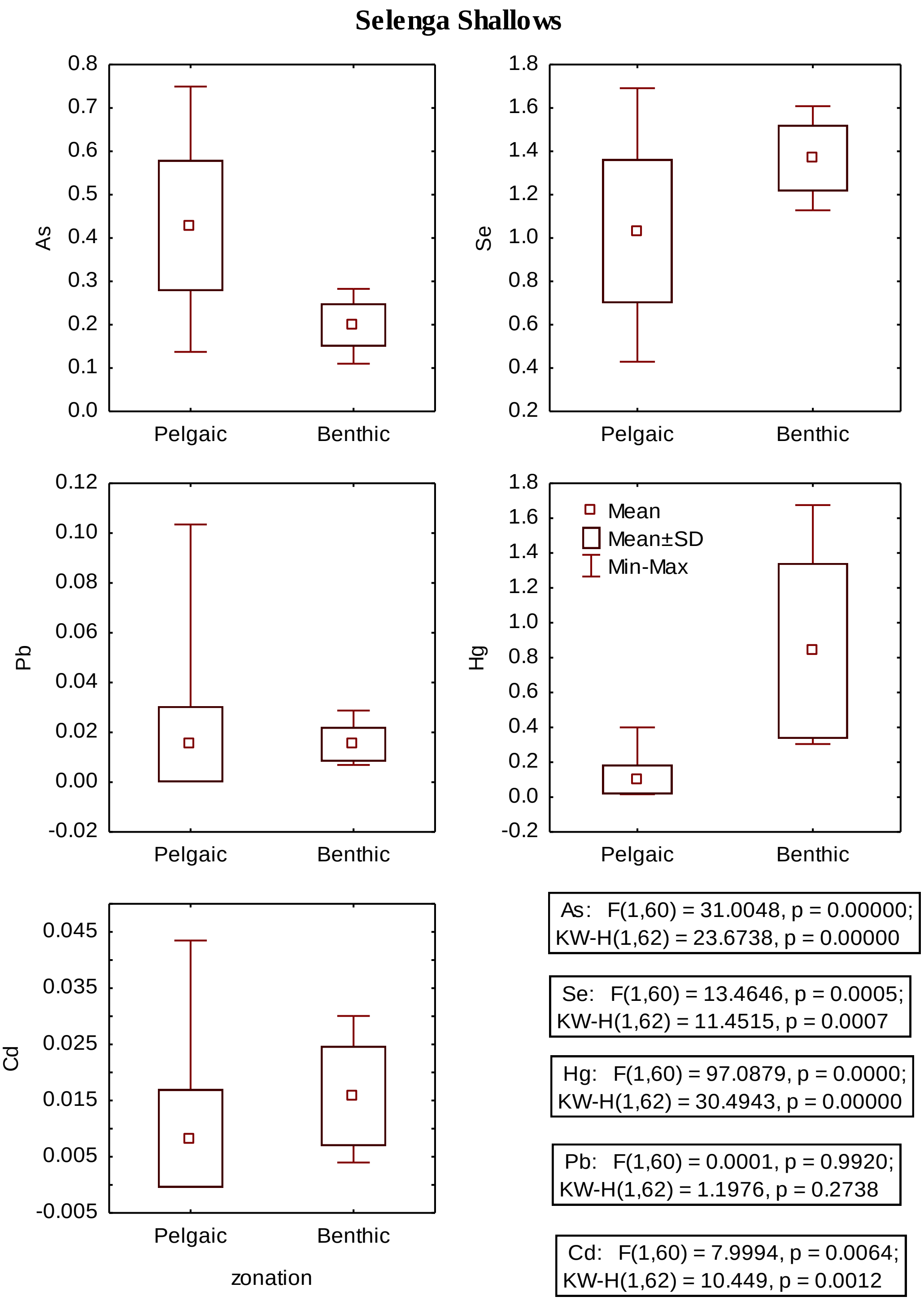

Figure K1. Comparison of As, Cd, Hg, Pb and Se concentrations (µg/g, dry weight) in pelagic and benthic fish species from the Selenga Shallows in Lake Baikal. The Kruskal-Wallis test (KW-H) was applied to test the concentration differences (significance level $p < 0.05$).

Listvyanka Bay

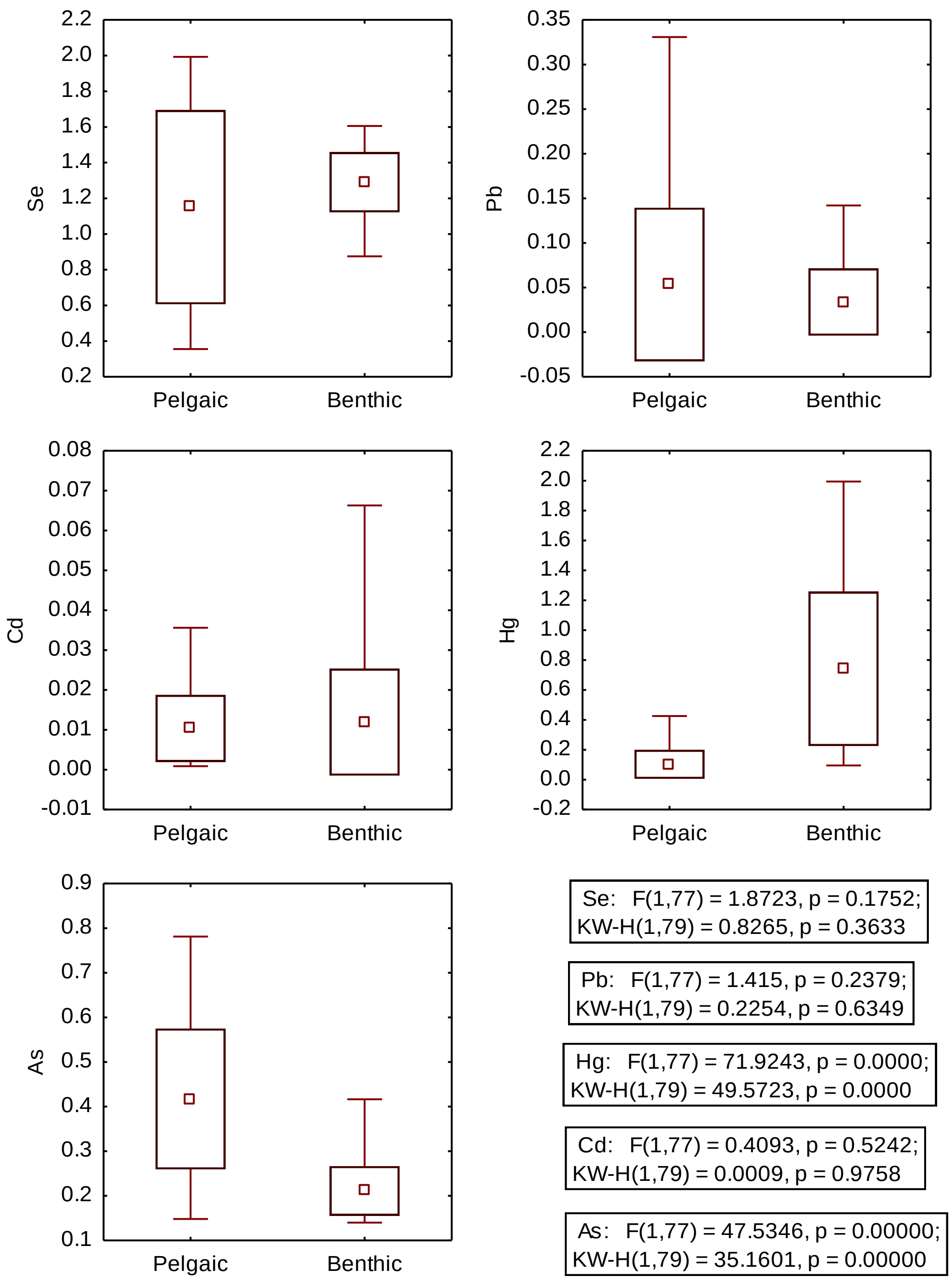

Figure K2. Comparison of As, Cd, Hg, Pb and Se concentrations (μg/g, dry weight) in pelagic and benthic fish species from the Selenga Shallows in Lake Baikal. The Kruskal-Wallis test (KW-H) was applied to test the concentration differences (significance level $p < 0.05$).