NTNU – Trondheim
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Environmental Toxicology and Chemistry
Submission date: May 2013
Supervisor: Bjørn Munro Jenssen, IBI
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Trondheim, May 2013

Jiawen Li

Abstract

Concentrations of As (arsenic), Cd (cadmium), Hg (mercury), Pb (lead) and Se (selenium) were determined by ICP-MS in the muscles, brain, adrenal and thyroid glands, gonads, liver, kidney and hairs of 16 pups and 2 adult females of Baikal seals from the southern Lake Baikal. Low concentrations of these five elements were found in Baikal seals in comparison with those published for other marine mammals. Significant correlation of Hg and Se concentrations in kidney of Baikal seal pups were explained by the formation of Hg-Se complexes which have an important function for the detoxification of Hg. Absence of the significant correlations of Hg concentrations between hair and kidney and between hair and liver in Baikal seal pups indicated that the use of hair for monitoring Hg levels in organs and tissues might be inappropriate for Baikal seal pups. The high Se:Hg molar ratios observed in all the organs and tissues indicates that even in a Se deficiency environment as Lake Baikal, Se concentrations were high enough to prohibit Hg induced toxicity, and thus indicates there is no risk of the Hg toxicity in Baikal seals at present.

Results from this study showed that the main route of maternal transfer of Hg is likely via parturition, whereas that of Cd and Pb is via lactation. Maternal transfer of the selected chemical elements was found to be low, thus probably constitute low potential risk for pups due to the low concentrations of the elements in both adult females and pups. Further study is required on the investigation of element concentrations in plasma and milk, which will provide a better understanding of the mechanism of maternal transfer of potentially toxic elements in Baikal seals. Contamination of the selected elements was found to be low in Lake Baikal in this study and thus these elements were unlikely to exert toxic effects on Baikal seals.

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1. Introduction

1.1 Lake Baikal and its ecosystem

Lake Baikal is located in south-east Siberia in the center 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 (Ciesielski et al. 2006). Lake Baikal consists of three separate basins: the northern (depth ranging from 800 to 900m), the central (depth ranging from 1200 to 1620m) and the southern (depth ranging from 1300 to 1400m) basin, and contains nearly 20% of the world´s unfrozen freshwater reserve. The river Angara is the lake`s only outflow, whereas more than 300 rivers and streams supply the lake with water. The major tributary of the Lake Baikal is the Selenga River, which provides ca. 50% of all the inflow water (Granina 1997).

Lake Baikal is unique also regarding its rich and uncommon freshwater life. Among the more than 2000 species of plants and animals inhabiting the lake, 3/4 are recognized as endemic species (Sideleva 2003). One of these is the Baikal seal (*Phoca sibirica*). The Baikal seal is the only seal species living exclusively in fresh water, and represents the top level in the Baikal pelagic food chain (Yoshii et al. 1999). Like the Caspian seal, the Baikal seal is one of the smallest of the true seals (Ciesielski et al. 2006). Due to the fact that marine mammals have high trophic level and long lifespan, they can accumulate a considerable amount of toxic compounds, including trace elements, in their tissues and organs. In the past few decades, mass mortalities took place in several marine mammal populations including the Baikal seal. Such events might be related to the impact of toxic contaminants (Watanabe et al. 1996). Nowadays, the contamination of Lake Baikal is of concern since it was declared as a UNESCO World Heritage Site in 1996. The increase of human and industrial activities leads to elevated inputs of various chemical elements, particularly toxic chemical elements, to the Baikal ecosystem.

Several studies have reported element concentrations in Baikal seals and compared them with other seals living in the marine environment (Ciesielski et al. 2006, Ikemoto et al. 2004, Miyazaki 2001, Watanabe et al. 1996). In general, Baikal seals have relatively low concentrations of toxic elements in their organs and tissues compared to seals inhabiting other environments. This is likely due to the low concentrations of those elements in Lake Baikal (Grosheva, Voronskaya and Pastukhove 2000). However, Ciesielski et al. (2010) found a high potential of bioaccumulation of Hg in the Lake Baikal pelagic food chain and the highest Hg biomagnification at the ‘fish-seal’ trophic relation. In addition, despite the low concentrations of toxic elements, such as Hg and Cd, in the muscle tissue of the seals, the body burdens are still high due to the high muscle mass of marine mammals (Krishna et al. 2003).

At present, studies on trace element levels of Baikal seals were mainly conducted on mature seals, and data on chemical element levels are limited to concentrations in muscles, liver, kidney and hair (Ciesielski et al. 2006, Ikemoto et al. 2004, Watanabe et al. 1996, Watanabe et al. 1998). In this study the distribution of selected element in other organs and tissues such as brain, adrenal and thyroid glands and gonads was investigated, with emphasis on Baikal seal pups. Pups might be more vulnerable to toxic elements, and in a relatively clean environment such as Lake Baikal, toxic elements in pups will mainly derive from their mothers and be transferred during parturition and lactation. Therefore, a maternal transfer study might provide a further understanding of the potential risks caused by the anthropogenic contaminants on Baikal seals.

1.2 Dominant anthropogenic pollution sources of the Lake Baikal

Industrial and agricultural activities around Lake Baikal are of special concern because it will elevate the inputs of various chemical elements, including toxic chemical elements, to Lake Baikal. The major sources of anthropogenic pollution of the lake are shown in Figure 2.1. and have been described in detail by Ciesielski et al. (2006). Abundant energy and mineral resources in the drainage area are the two main sources of pollution to Lake Baikal, and anthropogenic emissions mainly derive from the developing energy-consuming industries. Dust and soot, nitric and sulfur oxides and heavy metals are the typical discharged contaminants. In the southern basin where the samples of this study originate from, the Irkutsk-Cheremkhovo industrial zone and the Baikalsk Pulp and Paper Plant are the major sources of anthropogenic pollution. Besides, considerable amounts of pollutants enter the lake through the Selenga River, which is heavily influenced by the industrial region of Ulan-Ude (Kozhov et al. 1998).

Even though industrial and agricultural activities are of sepcial concern regarding the contamination of Lake Baikal, concentrations of most metals and other trace elements in the biotic and abiotic environments were until now found to correspond to natural background levels (Anokhin and Izrael 2000, Ciesielski et al. 2006, Ciesielski et al. 2010, Falkner et al. 1997, Flower et al. 1995, Grosheva et al. 2000). This is likely due to the huge amount of water in Lake Baikal, which will dilute the contaminants entering the lake. In addition, Grosheva et al.(2000) suggested that a self-purification process takes place in Lake Baikal, which can remove contaminants to sediment deposits. Thus the contaminant concentrations in the lake remain low, compared to the higher concentrations of atmospheric contamination. However, elevated Hg concentrations have been reported for rivers, shallows and semi-enclosed bays of Lake Baikal (Meuleman, Leermakers and Baeyens 1995). Perrot et al. (2010) found bioaccumulation and biomagnification of Hg through food webs in both the Hg contaminated man-made Bratsk Water Reservoir and the non-contaminated Lake Baikal. Lead was also found in sediment cores from Lake Baikal (Mackay et al. 1998). Therefore, since Lake Baikal is one of the world's unique ecosystems, attention should be paid to the impacts of contaminants on the biotic and abiotic components of the lake.

1.3 Toxicities of As, Cd, Hg, Pb and Se

The elements arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb) and selenium (Se) investigated in this study are often referred to as trace elements, since they are present in low concentrations in the environment. These elements can be released into aquatic environments by natural processes such as erosion of bedrock and soil, and passage of ground water through aquifers. In addition, they might also derive from anthropogenic activities related to industry, agriculture, urban settlements, geochemical structures and mining (Merian 2004). It is well known that As, Cd, Hg and Pb are toxicants in aquatic ecosystems and may exert toxic effects on aquatic organisms even in relatively low concentrations (Mason 2002). Therefore, toxicities of these elements on aquatic organisms, especially on mammals, are of special concern.

The accumulation of Hg and Cd in organs and tissues of marine mammals with increasing age and trophic level is well-studied (Krishna et al. 2003), and this might be the major risk for Baikal seal as it is the top predator in the Lake Baikal pelagic food chain. This implies that, even though the concentrations of toxic elements in the environment are below the threshold level for direct toxicity, the accumulation of such elements may result in toxic concentrations in organisms (secondary poisoning) (Nendza et al. 1997). Mercury is bioaccumulating in food chain mainly in its organic form, methylmercury (MeHg). In mammals, the toxic mode of action of MeHg is mainly the damage to central nervous system (Krishna et al. 2003). MeHg can also easily transfer across the placenta (Wagemann et al. 1988) and can thus concentrate in the brain of fetuses (Wolfe, Schwarzbach and Sulaiman 1998). MeHg may also cause developmental alterations that result in behavioral deficits after birth, impaired fertility and fetal death.

Unlike Hg, it was reported that Cd couldn't cross the placenta (Wagemann et al. 1988). However, Cd is regarded as one of the most toxic metals. High dietary concentrations of cadmium in humans can lead to serious detrimental effects, such as extensive skeletal deformities (Krishna et al. 2003). The renal concentrations can reach levels as high as 2000 $\mu g \cdot g^{-1}$ dry weight in some Arctic ringed seals (Dietz, Nørgaard and Hansen 1998). However, no obvious Cd toxic effect has been registered in marine mammals, and Dietz et al. (1998) suggested that this might be due to highly efficient detoxification mechanisms.

Selenium differs from the mercury, cadmium, lead and arsenic due to its role as an essential micronutrient in organisms. Concentrations of Se are of importance because of its role in the detoxification of Hg. This mechanism occurs throughout the animal kingdom from oysters, shrimps to marine mammals and humans. Se:Hg molar ratio of approximately 1 suggesting the Hg detoxification take place (Krishna et al. 2003). However, high environmental and dietary concentrations may also lead to increased Se body burdens and a potential toxicity of Se (Walker 2006). Since Lake Baikal is a Se deficient region, Se is not likely to accumulate over toxic threshold levels. But it also raises the concern that Hg cannot be detoxified because of the Se deficiency.

It was reported that As concentrations in marine organisms were much higher compared to those in organisms inhabiting terrestrial environments (Lunde 1977). Anthropogenic emissions of As into aquatic ecosystems have been estimated to be as high as 41 000 metric tons per year, which is about five times more than the global emissions originating from natural sources (Nriagu and Pacyna 1988). Considering the situation of Baikal seals, Kubota et al. (2001) reported that Baikal seals had lower As concentrations in the livers than other marine mammals because of the landlocked water environment in Lake Baikal. There is only a limited amount of data available on the toxicity of As in marine mammals. For instance, Freeman and Sangalang (1977) reported that exposure to As would cause alterations of the *in vitro* biosynthesis of steroids in the adrenals and testes of grey seals. There have been various investigations on As accumulation in lower trophic marine organisms. For example, marine algae were reported to accumulate inorganic arsenic from seawater and convert it to organic form, and as a result the concentration of arsenic in marine algae could be 1000- 50 000 times higher than that in ambient seawater (Francesconi and Edmonds 1993). High retention efficiency of organic arsenic was also reported for fish (Francesconi, Edmonds and Stick 1989, Shiomi et al. 1996), mussel (Francesconi et al. 1999), and shrimp (Hunter, Goessler and Francesconi 1998). However, information about As accumulation in higher trophic marine animals is still limited.

Several studies have investigated Pb levels in marine mammals (Agusa et al. 2011, Holmes et al. 2008, Ikemoto et al. 2004, Julshamn and Grahl-Nielsen 2000). However, none of these studies further discussed the toxicity of Pb due to the low levels found in those works. In mammals, after being taken up, lead is initially distributed to soft tissues such as kidney and liver, and then redistributed to skeleton and hair. Lead released from bones is of great importance since it is a significant source of endogenous exposure, especially for females due to bone resorption during pregnancy, lactation, menopause and from osteoporosis (Klaassen, Casarett and Doull 2008). Lead can cross the placenta and accumulate in fetal tissues, including the brain, and the Pb level in fetal tissues are proportional to maternal blood Pb concentrations (Klaassen et al. 2008). To our knowledge there is no study investigating Pb concentration in both adult and pups of Baikal seals.

1.4 Aims of study

The aim of the present study is to examine the organ and tissue distribution and maternal transfer of arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb) and selenium (Se) in Baikal seals. To achieve this goal concentrations of selected elements are analyzed in muscles, brain, adrenal and thyroid glands, gonads, liver, kidney and hairs of Baikal seal pups and adult females. In addition, intra- and inter-correlations of elements in organs and tissues are also included in order to reveal the interactions and distribution patterns of the elements in Baikal seal pups. This work should provide knowledge for a better understanding of the contamination status and physiology of the Baikal seals.

2 Material and Methods

2.1 Sample collection and preparation

Tissues and organs of the Baikal seals (*Phoca sibirica*) were collected from animals obtained in the Mishikha region which is located at the southern Lake Baikal (Fig. 2.1) from hunters during the official cull and with a special permit in April 2011. A total of 18 individuals, (16 pups and 2 females) were included in the study. Among the seal samples there were two mother–fetus pairs. The pups were between 1 and 2 months old. Adrenal and thyroid glands, kidney, liver, brain, gonads, muscles and hairs were collected. All samples were preserved and transported to Norway, frozen (-20 ºC) until analysis. Biometrical data for seals are given in Appendix 1.

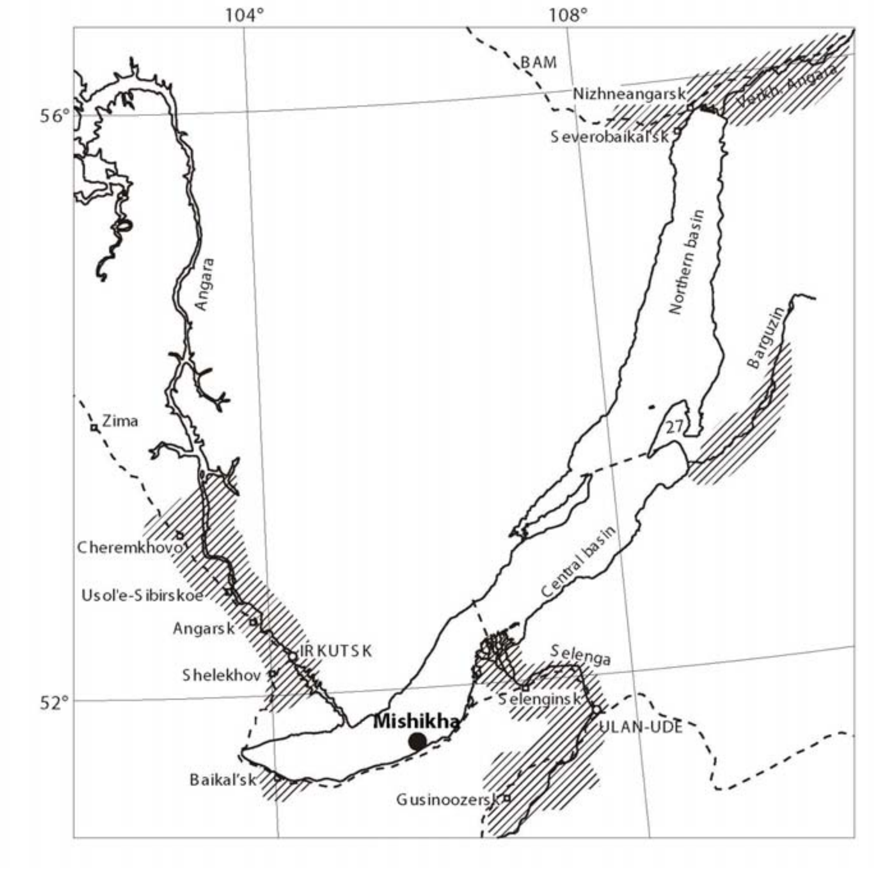


Figure 2.1. Map of Lake Baikal marked with sampling site in the Mishikha area. Shaded areas on the map indicate major anthropogenic emission sources (Ciesielski et al. 2006).

2.2 Chemical element analysis

Samples of tissues and organs were excised from larger organ samples when necessary to ca. 3 g using titanium knife. Then the samples were dried in a freeze dryer (Christ LDC-1 Alpha 1-4, B. Braun, Melsungen, Germany) for about 20 hours. Hair samples were carefully removed from the skin with clean ceramic knife, washed in acetone, rinsed twice with double distilled water and washed again in acetone. The hair samples were subsequently rinsed and air dried over night (Wenzel et al. 1993). Approximately 0.4 g of the sample materials were weighed on a 4 digit balance scale and transferred to a PTFE-Teflon vial (18 mL). Then 6 mL 50% v/v nitric acid (HNO_3, Scan Pure, Chemscan, Elverum, Norway) were added to the vials. Digestion of the samples was performed in a high-pressure microwave system (Milestone UltraClave, EMLS, Leutkirch, Germany). The temperature for digestion would increase gradually to 240 ºC within one hour, and then returned to the original temperature also within one hour. After digestion the samples were diluted with ultra pure water to a final acid concentration of 0.6 M, and transferred to polypropylene vials for further detection.

Concentrations of selected elements (As, Cd, Hg, Pb, Se) were determined by an Element 2 High Resolution Inductively Coupled Mass Spectrometry (HR-ICP-MS) (Thermo Finnigan, Bremen, Germany). The radio frequency power was set to 1400 W. Samples were introduced using a SC-FAST flow injection analysis system (ESI, Element Scientific, Inc. Omaha, USA) with a peristaltic pump (1 mL/min). The instrument was equipped with a PFA-ST nebulizer, spray chamber (PFA Barrel 35 mm), demountable torch, quarts standard injector and 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 ^{7}Li, ^{115}In and ^{238}U. Methane gas was used in the analysis to minimize interference from carbon and to provide enhanced sensitivity, especially for Se and As. The instrument was calibrated using 0.6 HNO_3 solutions of matrix-matched multi-element standards. A calibration curve consisting of 5 different concentrations was made from these standards. To check for instrument drift one of these multi-element standards were analyzed for every ten samples. The accuracy of the method was verified by analyzing the certified reference material DOLT-3 (Dogfish liver, National Research Council Canada) and Chicken GBW 10018 (General Administration of Quality Supervision, Langfang, China) (Appendix 2).

Method detection limits (MDL) were determined by comparing the instrument detection limits (IDL) with the blank detection limit (3 times the mean standard deviation for all blank samples). The higher value was chosen as the MDL (Appendix 3). The calculations of IDL were done by analyzing solutions of decreasing concentrations of each chemical element. The concentration resulting in a relative standard deviation of approximately 25% (n=3 scans) was chosen as the IDL with baseline corrections applied for these values. In the case that results were below the determined detection limit, the concentration of the element was taken to be half of the detection limit. Chemical elements which more than 50% of the samples of a specific organ and tissue were below the detection limit were omitted from further statistical analysis.

2.3 Statistics

Linear correlation analysis (Pearson's r) was used to investigate the intra- and inter-elemental correlations of selected element in organs and tissues. T-test was used to review the relationships between selected element concentrations and sex of the Baikal seal pups.

Principal component analysis (PCA) was used to investigate the relationships between the trace elements and different organs and tissues. The PCA can extract the most important information and pattern from the data set, so that much of the noise can be excluded and the data set can be simplified while retaining the maximum level of variation. The simplified information is then expressed as a set of new variables called principal components, which the first will contain the highest level of variance and the second will contain the highest possible level of variance under the constraint of being orthogonal to the first principal component (Abdi and Williams 2010). Two plots are created in the PCA. One is the score plot which represents the observations or samples. The other one is the loading plot which represents the variables. Samples that are grouped or closely associated in the score plot can be expected to show similar variable compositions. Variables that gather together or are near each other in the loading plot are likely correlated with each other.

The linear correlation analysis and t-test were performed using STATISTICA (Version 10 for Windows, StatSoft Inc., Tulsa, OK, USA). PCA was performed using SIMCA P+ (Version 12, Umetrics, Umeå, Sweden).

The significance level for all the analyses was set to $p<0.05$. All concentrations were given based on dry weight (d.w.) except those were specially stated.

3. Results

3.1 Organ and tissue distributions of chemical elements

Concentrations of As, Cd, Hg, Pb and Se in adrenal and thyroid glands, kidney, liver, brain, gonads, muscles and hairs of the 18 Baikal seals are shown in Figure 3.1-3.3 and Appendix 4. With the exception of Cd, all the trace elements were detected in the samples. Concentrations of Pb and Cd were transformed into $\log_{10}$ scale due to the great varieties in different organs and tissues.

Mercury and selenium were most abundant in kidney (2.83±1.15 $\mu g \cdot g^{-1}$ for Hg and 5.16±1.13 $\mu g \cdot g^{-1}$ for Se in pups), followed by liver (2.04±3.86 $\mu g \cdot g^{-1}$ for Hg and 3.13±3.13 $\mu g \cdot g^{-1}$ for Se in pups). However, two pups (seal no. 29 and no.30) showed high Hg concentrations in the liver with 6.09 $\mu g \cdot g^{-1}$ and 15.6 $\mu g \cdot g^{-1}$, respectively. Correspondingly, high Se concentrations were shown in the liver of these two pups, with 7.25 $\mu g \cdot g^{-1}$ and 13.8 $\mu g \cdot g^{-1}$, respectively. High As and Cd concentrations were also observed in these two liver samples. Hg concentrations were found higher in adult females in muscles, brain, adrenal and thyroid glands and gonads in comparison with those in pups.

The highest concentrations of As were observed in hairs (0.27±0.14 $\mu g \cdot g^{-1}$), followed by kidney (0.15±0.11 $\mu g \cdot g^{-1}$) and thyroid glands (0.11±0.14 $\mu g \cdot g^{-1}$). Significant higher concentrations of As were found in brain, adrenal glands and gonads of adult females than those of pups. Concentrations of Pb were relatively low in all the organs and tissues except hair, which ranged from 0.90 to 24.5 $\mu g \cdot g^{-1}$. However, one gonad sample from pup (seal no. 18) had much higher concentration of Pb (1.33 $\mu g \cdot g^{-1}$) than the other pups (ranged from 0.0029 to 0.058 $\mu g \cdot g^{-1}$).

Cadmium was below the detection limit for more than 50% of the brain samples of pups. Cd concentrations were relatively low compared to other elements, with several adrenal glands, thyroid glands and gonads samples which were under the detection limit. However, two kidney samples revealed extremely high Cd concentrations: 12.9 $\mu g \cdot g^{-1}$ and 20.1 $\mu g \cdot g^{-1}$ of seal no. 21 and no. 32 (adult female), respectively, whereas the other kidney samples ranged from 0.0013 to 0.038 $\mu g \cdot g^{-1}$. Even though this affects considerably the results obtained, no evidence was found to show that such values were biological outliers. Hence these two values were not excluded for further statistical treatment. On the other hand, these outliers were not used in calculations when comparing the Cd concentrations with those of other marine mammals and hence the mean concentration of Cd in kidney of pups became 0.011±0.011 $\mu g \cdot g^{-1}$.

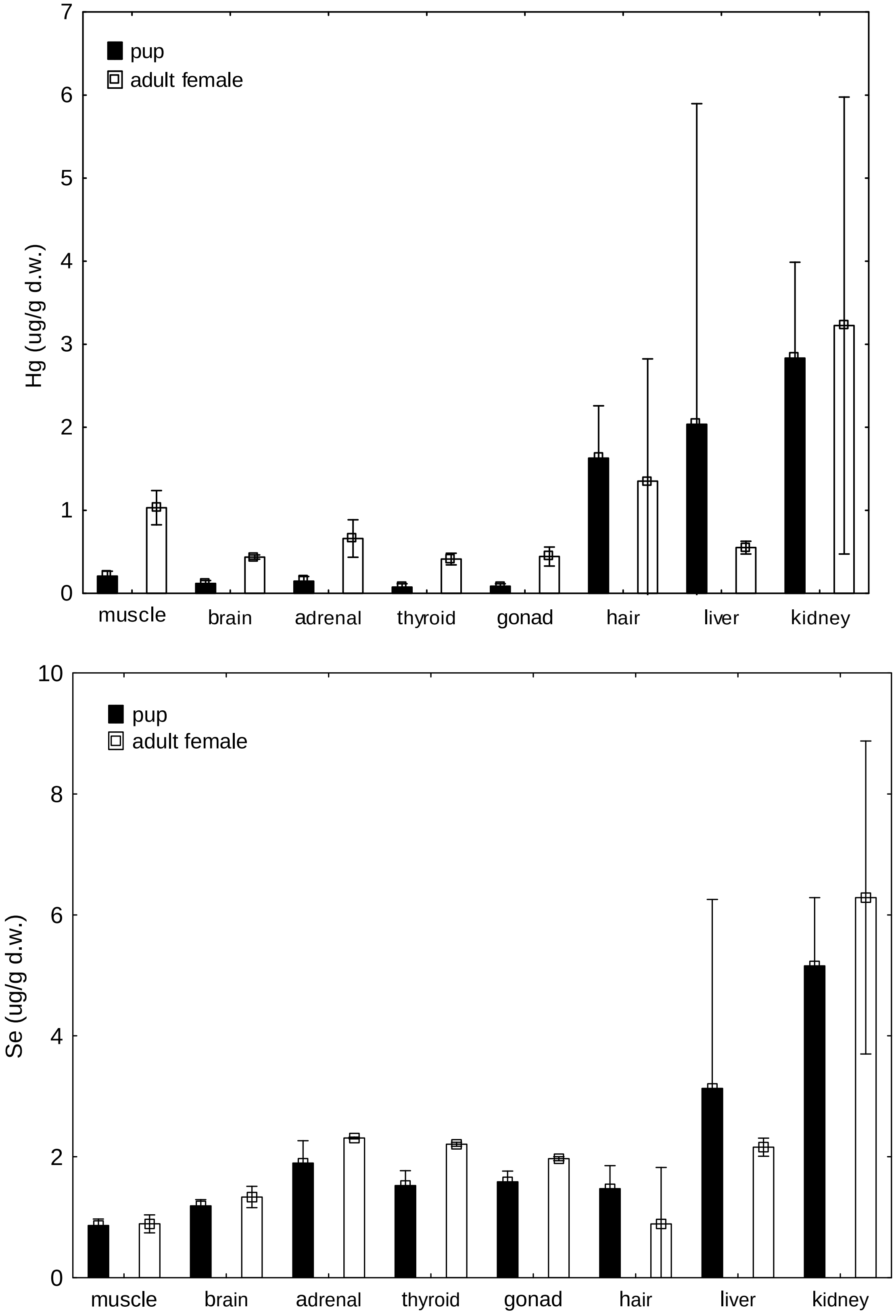
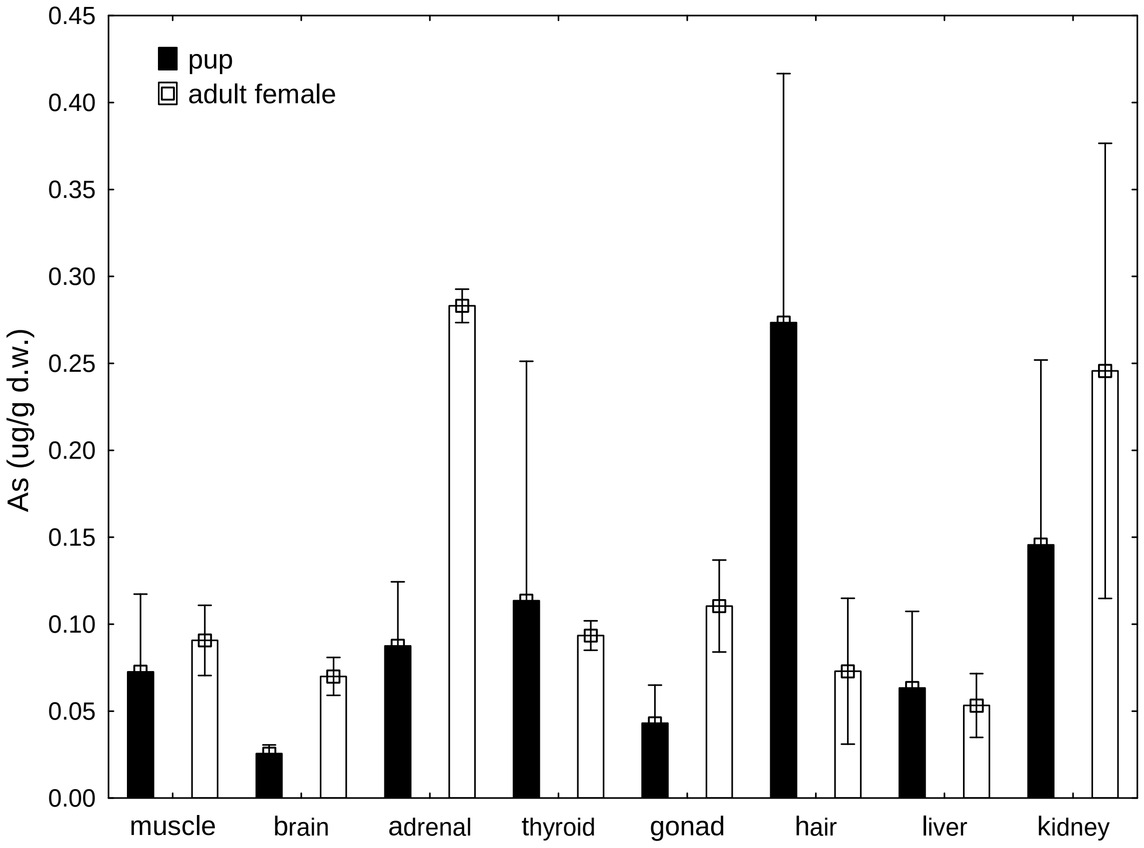


Figure 3.1. Concentrations of Hg and Se (µg/g dry weight) in different organs and tissues in Baikal seals. Bars and whiskers represent mean values and standard deviations, respectively.



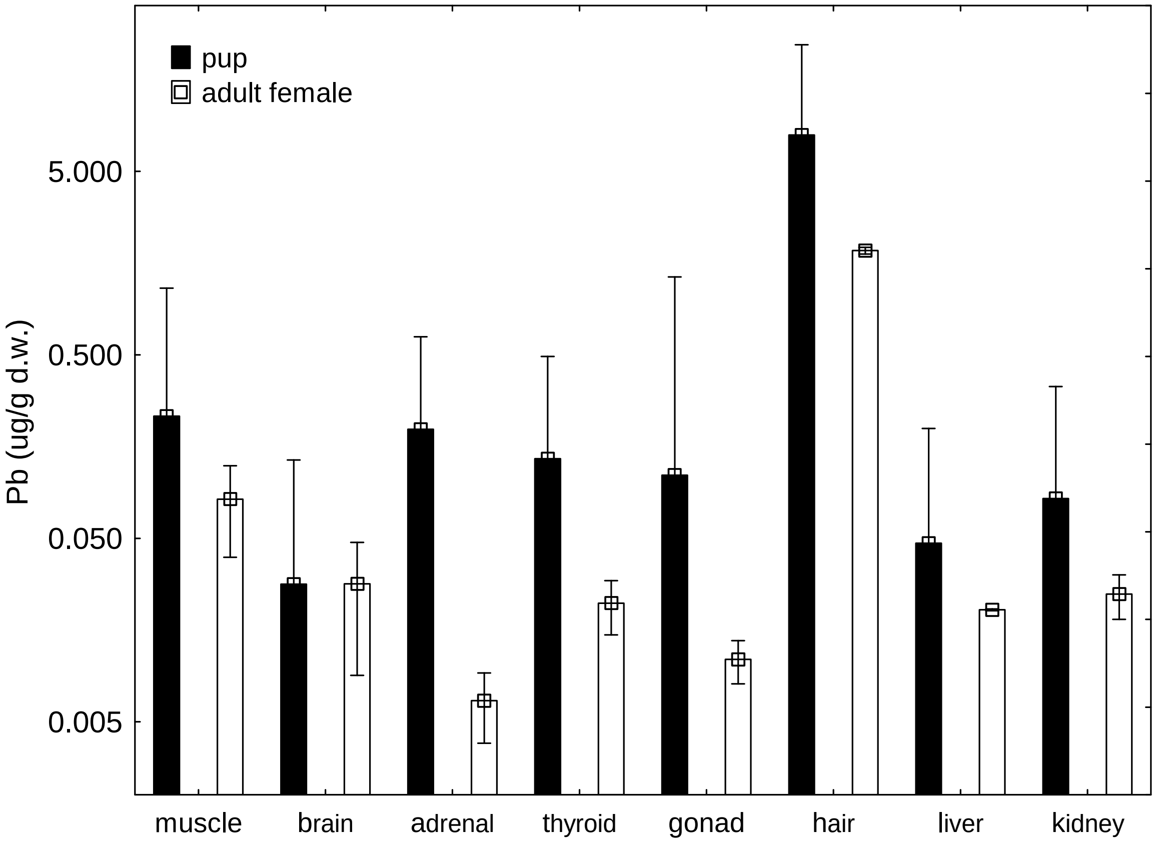


Figure 3.2. Concentrations of As and Pb (μg/g dry weight) in different organs and tissues in Baikal seals. Bars and whiskers represent mean values and standard deviations, respectively.

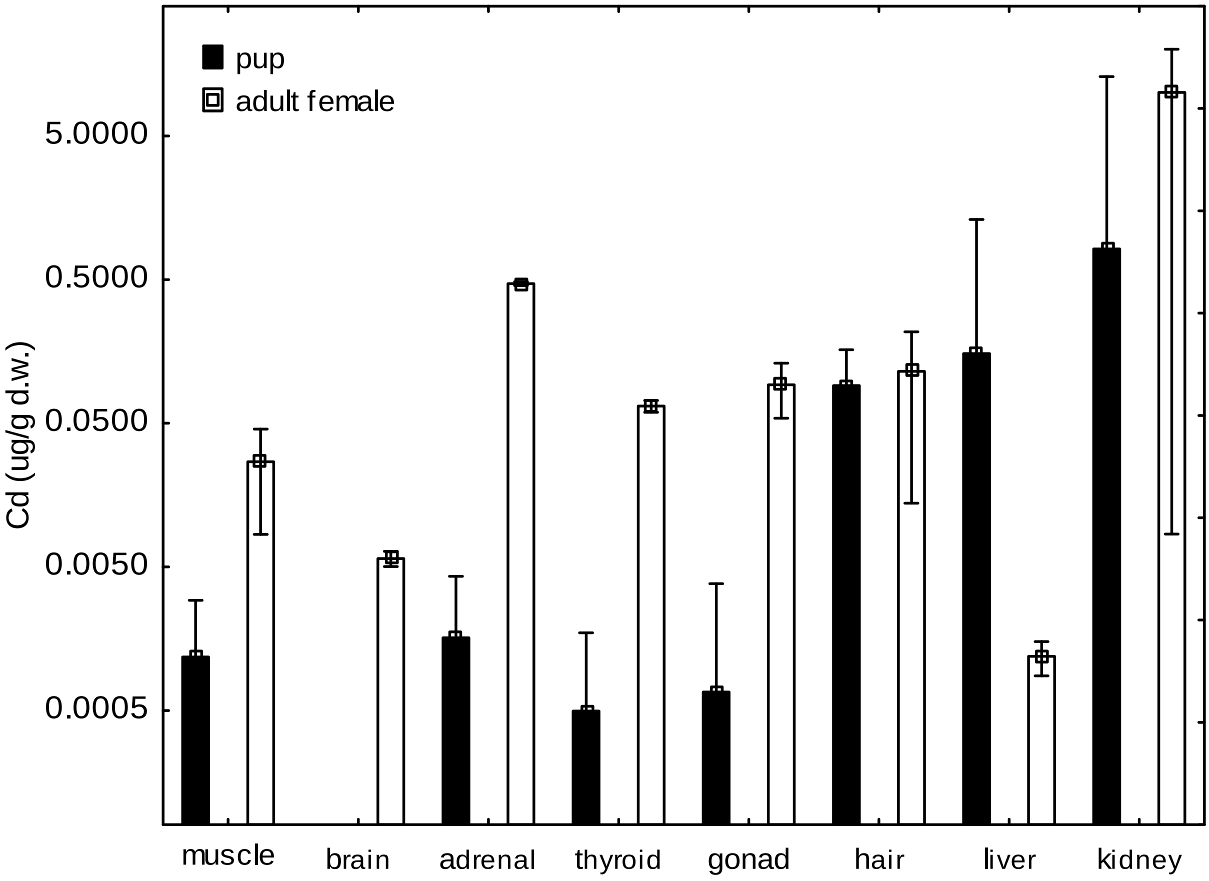


Figure 3.3. Concentrations of Cd (μg/g dry weight) in different organs and tissues in Baikal seals. Bars and whiskers represent mean values and standard deviations, respectively.

3.2 Sex differences in elemental concentrations

No sex-related differences (T-test) was observed between male and female pups, hence this parameter was discarded as an important variable in the further statistical treatment.

3.3 Intra-elemental correlations between organs and tissues

Intra-elemental correlations between different organs and tissues are shown in Table 3.1. Strong correlations were found among muscles, brain, thyroid glands, gonads and adrenal glands of Cd and Hg. However, this is probably due to the much higher concentrations of these elements in adult females than in pups. Concentrations of As in brain, adrenal glands and gonads were also much higher in adult females than in pups. Besides, as mentioned in section 3.1, two pups contained extremely high Hg, Se and Cd concentrations in their livers, one gonad sample from pup contained much higher concentration of Pb than the other samples, and two kidney samples from one pup and one adult female had much higher concentrations of Cd than other seals. These outliers considerably affected the results. Thus, outliers and cadmium, mercury concentrations in brain, muscles, gonads, adrenal and thyroid glands, arsenic concentrations in brain, gonad and adrenal gland of adult females were excluded in the intra-elemental correlation analysis, and new correlations were shown in Table 3.2.

After the outliers were excluded, some correlations of trace element concentrations between organs and tissues were not found, especially for Cd. However, correlations of Hg in muscle - brain, muscle - gonad, muscle – adrenal gland, muscle – thyroid gland, brain - gonad, brain – adrenal gland, brain - thyroid gland and gonad - adrenal gland were still existed but with weaker probabilities, and new correlations of brain - hair, adrenal gland - hair and thyroid gland - hair were found. New correlations of As in muscle - adrenal gland, Pb in gonad - adrenal gland and gonad – hair were also revealed.

Table 3.1. Significant intra-elemental correlations between organs and tissues of Baikal seals..

	Se	**Cd**	**Hg**	**Pb**	**As**
muscle-brain	—	—	0.974***	—	—
muscle-gonad	—	—	0.987***	—	—
muscle-liver	-0.499*	—	—	—	—
muscle-adrenal gland	—	0.812***	0.973***	—	—
muscle-thyroid gland	—	0.850***	0.906***	—	—
muscle-hair	—	0.513*	—	—	—
brain-gonad	0.581*	—	0.961***	—	0.753***
brain-adrenal gland	—	—	0.950***	—	0.881***
brain-thyroid	0.522*	—	0.926***	—	—
gonad-kidney	—	0.921***	—	—	—
gonad-adrenal gland	0.732**	0.900***	0.974***	—	0.608*
gonad-thyroid gland	—	0.868***	0.885***	—	—
liver-hair	0.504*	—	—	—	—
kidney-adrenal gland	—	0.660**	—	—	0.628**
kidney-thyroid gland	—	0.477*	—	—	—
kidney-hair	-0.750**	—	—	—	—
adrenal gland-thyroid gland	—	0.998***	0.852***	—	—
adrenal gland-hair	—	—	—	0.729**	—

*,** and *** indicate *p*<0.05, *p*<0.01 and *p*<0.001, respectively.

Table 3.2. Significant intra-elemental correlations between organs and tissues of Baikal seals (outliers excluded).

	Se	**Cd**	**Hg**	**Pb**	**As**
muscle-brain	—	—	0.775***	—	—
muscle-gonad	—	—	0.739**	—	—
muscle-adrenal gland	—	—	0.720**	—	0.520*
muscle-thyroid gland	—	—	0.510*	—	—
brain-gonad	0.581*	—	0.682**	—	—
brain-adrenal gland	—	—	0.769**	—	—
brain-thyroid gland	0.522*	—	—	—	—
brain-hair	—	—	0.711**	—	—
gonad-adrenal gland	0.732**	0.525*	0.656**	0.538*	—
gonad-thyroid gland	—	0.810***	—	—	—
gonad-hair	—	—	—	0.656**	—
kidney-hair	-0.750**	—	—	—	—
adrenal gland-hair	—	—	0.734**	0.729**	—
thyroid gland-hair	—	—	0.602*	—	—

*,** and *** indicate *p*<0.05, *p*<0.01 and *p*<0.001, respectively.

3.4 Inter-elemental correlations

Inter-elemental correlations of As, Cd, Hg, Pb and Se were tested in all organs and tissues. Results are shown in Table 3.3. Strong correlations between elements were found mostly in liver and kidney. However, as mentioned in section 3.3, this is probably due to the extraordinary high concentrations found in some of the samples (outliers) and data from adult females. Therefore, such data were excluded from the inter-elemental correlation analysis, and then the new results were shown in Table 3.4.

Most of the correlations of elements in liver and kidney were not found after excluded the outliers. However, the correlations of As - Se and Hg - Se in the kidney were not affected by the outliers. New correlations, such as those between Pb and Se in liver were found. The expected significant correlation between Hg and Se in the liver was not found after excluding the outliers (*p*=0.44).

Table 3.3. Correlations between selected elements in different organs and tissues

	muscle	brain	adrenal gland	thyroid gland	gonad	hair	liver	kidney
As-Cd	—	—	0.890***	—	—	—	0.955***	0.828***
As-Hg	—	0.897***	0.784***	—	0.628**	—	0.911***	—
As-Pb	—	—	—	—	0.489*	—	—	—
As-Se	—	—	—	—	—	—	0.941***	0.659**
Cd-Hg	0.659**	—	0.910***	0.960***	0.973***	—	0.928***	0.488*
Cd-Se	—	—	—	0.794**	—	—	0.952***	0.629**
Hg-Se	—	—	—	0.645**	0.529*	—	0.993***	0.588*
Pb-Se	—	—	-0.539*	—	-0.497*	0.457*	—	—

*,** and *** indicate $p<0.05$, $p<0.01$ and $p<0.001$, respectively.

Table 3.4. Correlations between selected elements in different organs and tissues (outliers excluded)

	muscle	brain	adrenal gland	thyroid gland	gonad	hair	liver	kidney
As-Cd	0.550*	—	—	—	0.799**	—	—	—
As-Hg	—	—	—	0.582*	—	—	—	—
As-Se	—	—	—	—	—	—	—	0.659**
Cd-Pb	—	—	0.776**	—	—	—	—	—
Hg-Pb	0.529*	—	—	—	—	—	—	—
Hg-Se	—	—	—	—	—	—	—	0.588*
Pb-Se	—	—	-0.539*	—	—	0.457*	-0.512*	—

*,** and *** indicate $p<0.05$, $p<0.01$ and $p<0.001$, respectively.

3.5 Se:Hg molar ratio

Se:Hg molar ratios were calculated to reveal the detoxification of Hg by Se, and results were shown in Figure 3.4. Differences of Se:Hg molar ratios between adult females and pups were observed. Se:Hg molar ratios were almost at the same level in all the organs and tissues of adult females, which ranged from 1.65-15.58, while variations of Se:Hg molar ratios were observed in organs and tissues of pups, ranged from 2.24-102.17. The highest Se:Hg molar ratio was found in thyroid gland, followed by gonad and adrenal gland. Pups were found to have higher Se:Hg molar ratios than adult females except in liver and kidney.

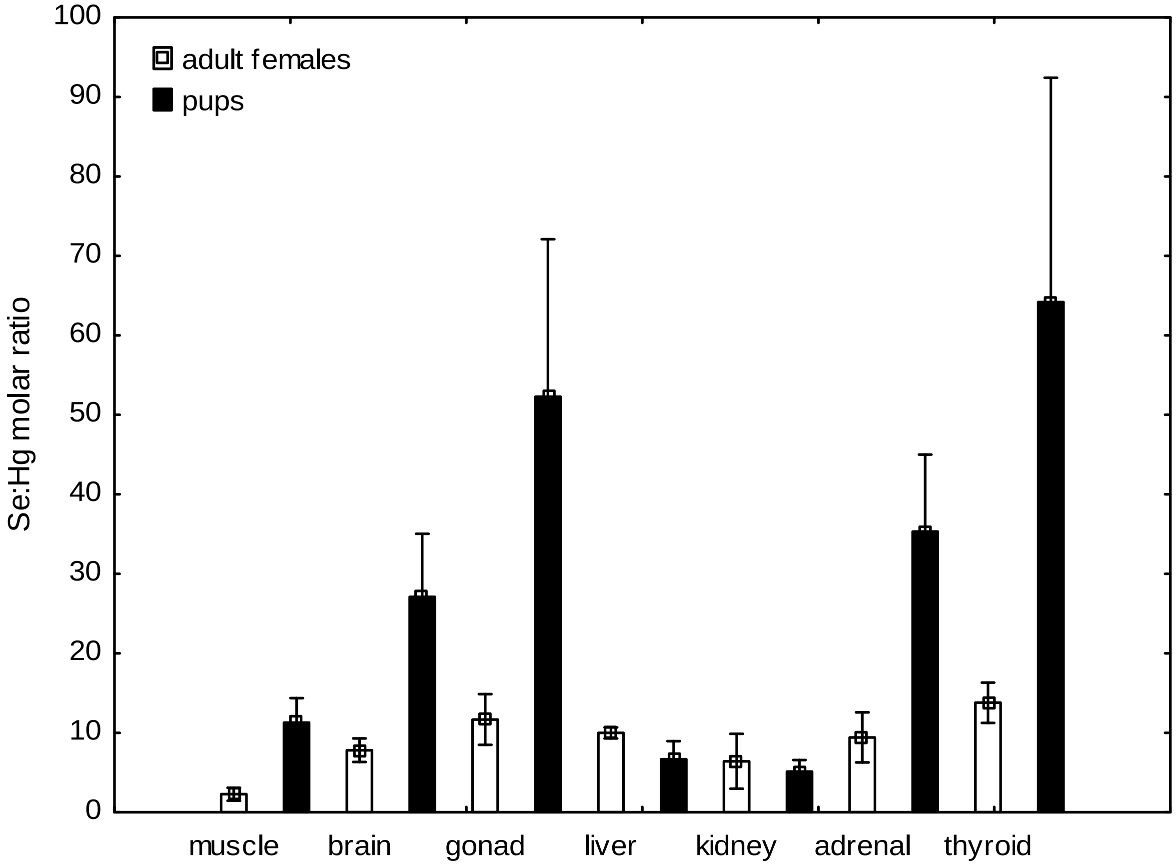


Figure 3.4. The molar relationships between Se and Hg in different organs and tissues of Baikal seals. Bars and whiskers represent mean values and standard deviations, respectively.

3.6 Principal component analysis (PCA)

PCA was performed to investigate the statistical relationships between the trace elements and the distribution of elements in organs and tissues. All of the selected elements were included in the PCA, and samples with concentrations below detection limits were replaced with half of the method detection limit. All the variables were $\log_{10}$-transformed prior to PCA in order to approach normal distribution of the data. Principal component analysis of the trace element concentrations in different organs and tissues resulted in a model ($R^2X=0.835$, $Q^2=0.558$) with 2 significant principal components (PCs), which explain 58.18% and 25.31% of the total variability, respectively.

The PCA revealed that the organ or tissue type was the dominating factor determining the distributions of trace elements. In the loading plot (Fig. 3.5), all the selected trace elements were located to the right along PC1. Pb and Se were located oppositely from each other along PC2 with the highest and lowest PC2 loading, respectively, indicating the significant tissue distribution differences between these two elements. In the score plot (Fig.3.6), points represented hair and kidney samples were located on the right along PC1 and were separated from those represented other organs and tissues. Brain, gonad and thyroid gland seemed to have a similar element composition as they located close to each other. By comparing the score plot with the loading plot, it can be seen that the highest concentration of Se was found in kidney while the highest concentration of Pb was found in hair.

In addition, significant differences of trace elements concentrations between adult females and pups in adrenal gland, brain, gonad, muscle and thyroid gland can be seen on the score plot (Fig 3.6), as the points which represented the adult females were located apart from those represented the pups because of the higher concentrations of As, Cd and Hg in adult females than in pups. Almost all the samples were within the Hotellings T2 range in the score plot except of three hair samples which were characterized by high Pb concentrations. The two liver samples and the two kidney samples (which were mentioned before in section 3.1) were also outside the range and separated from the other liver and kidney samples because of extremely high concentrations of Se, Hg, and Cd in liver and Cd in kidney. Other outliers can also be seen on the score plot, such as a gonad sample and a thyroid gland sample, which had higher scores along PC1 than the other samples because of higher Pb concentrations. One hair sample from an adult female had low scores along PC1 because of the lower Se, Cd and As concentrations in comparison to another adult female and pups.

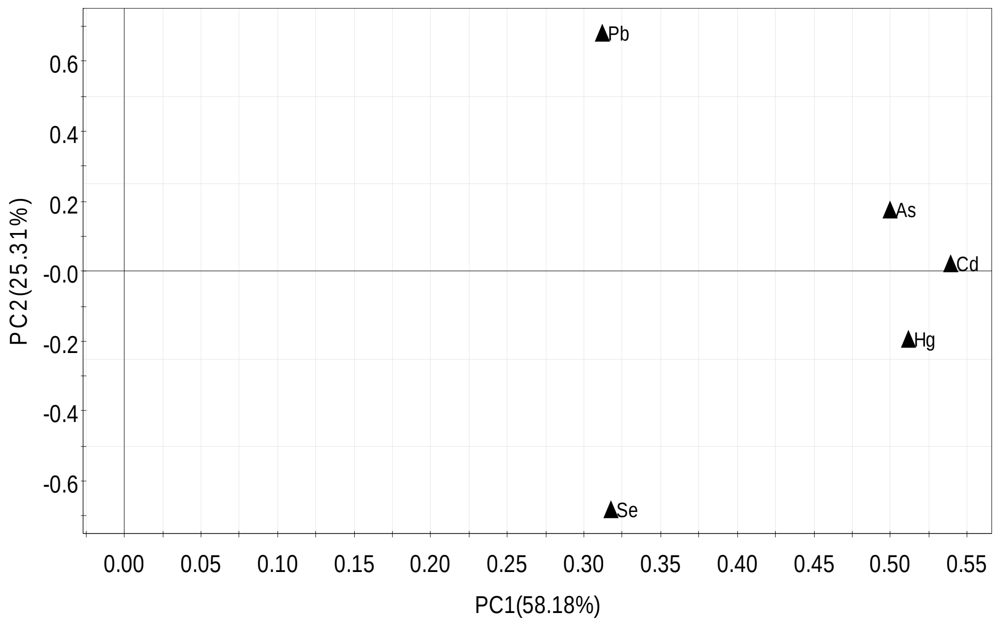


Figure 3.5. PCA loading plot including selected five trace elements analyzed in organs and tissues of Baikal seals (*Phoca sibirica*)

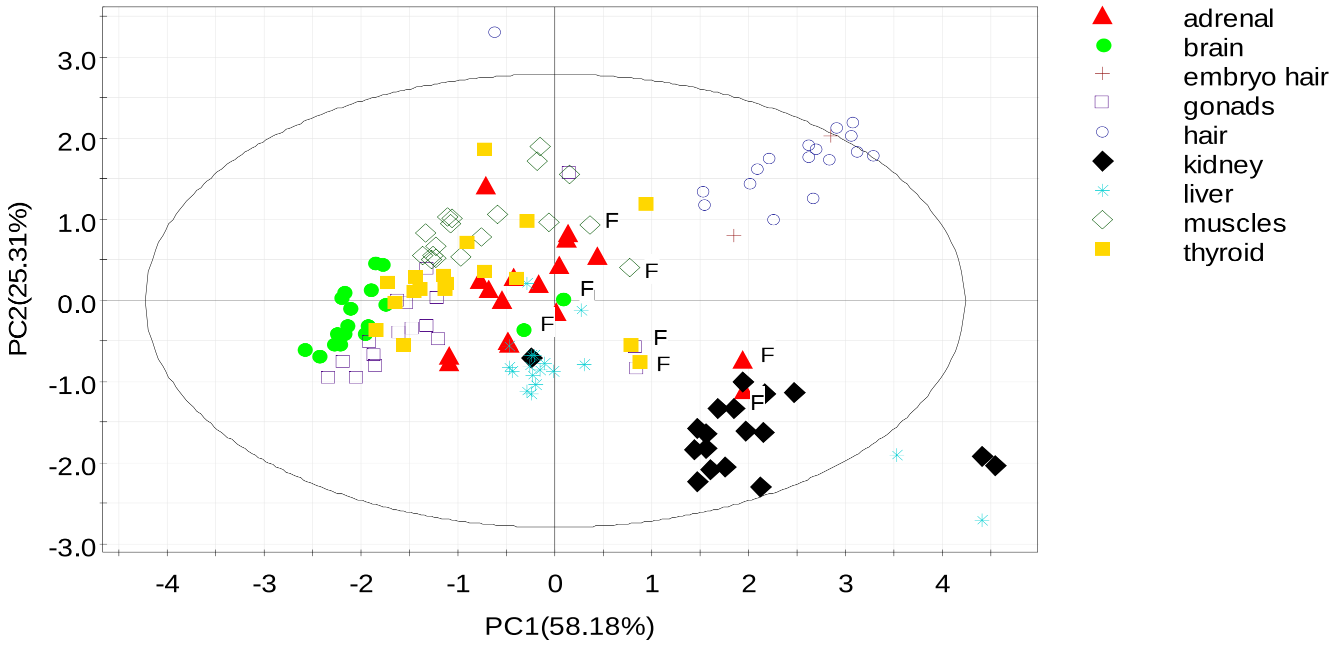


Figure 3.6. PCA score plot of Baikal seals (*Phoca sibirica*). Points marked with F represent data corresponding to adult female.

4. Discussion

4.1 Levels of As, Cd, Hg, Pb and Se in Baikal seals (*Phoca sibirica*)

Hg

Among the five selected trace elements which were analyzed, Hg appears to be of the highest concern due to its toxicity and bioaccumulation potential. Relatively high concentrations of Hg were found in hair, liver and kidney. Higher Hg concentrations were observed in kidney (2.83±1.15 $\mu g \cdot g^{-1}$) than in liver (2.04±3.86 $\mu g \cdot g^{-1}$) of Baikal seal pups, which is in contrary to the studies that reported Hg tend to accumulate in liver (Law 1996, Thompson 1990),. Moreover, Hg concentrations in the liver of two adult females (0.55±0.076 $\mu g \cdot g^{-1}$) were lower than those found in pups. In contrary to this Watanabe et al. (1998) and Ciesielski et al. (2010) reported higher concentrations of Hg in the liver of adult females (3.9±2.9 $\mu g \cdot g^{-1}$ and 5.83± 1.85 $\mu g \cdot g^{-1}$, respectively) than in pups (0.71±0.65 $\mu g \cdot g^{-1}$ and 0.42±0.14 $\mu g \cdot g^{-1}$, respectively). This finding needs to be further explored as there was only limited number of samples from adult females (n=2) in the present study. Besides, significant higher concentrations of Hg in muscles, brain, adrenal and thyroid glands and gonads of adult females than of pups indicate the accumulation of Hg with age.

Mercury concentration in the liver of Baikal seals is lower than that of other seals collected from polluted areas such as the UK coast, with about 10 times higher Hg concentrations in the livers (Miyazaki 2001). Watanabe et al. (1996) also reported that Hg concentration in the liver of Baikal seals is lower in comparison to other seals living in marine environments. A more recent study from Agusa et al. (2011) showed that the concentrations of Hg in the liver, muscles, kidney, gonads and hair samples from harp seals in the Baffin Island, Canada were higher than Baikal seals, especially for the Hg concentrations in the liver. This is probably due to the low background level of Hg in Lake Baikal (Grosheva et al. 2000). In addition, relatively high concentration of Hg in hair indicates an important role of hair in excretion of Hg. Wagemann and Kozlowska (2005) determined that Hg concentrations in epidermal layers were five times greater than dermal layers, and thus Hg in the epidermal layers can be excreted via moulting. Hair functions as an excretory tissue also because considerable amount of Hg can be removed from the blood and retained in hair, and Hg in hair is not considered bioavailable (Brookens et al. 2008).

Hg exists in several interchangeable forms in the biosphere, and the toxicological and biological activity of mercury depends on the chemical form that is taken up, the route when Hg enters the body (skin, inhalation or ingestion), and on the extent to which mercury is absorbed (Kaiser and Tolg 1980). Methylmercury (MeHg) is the most toxic form of mercury (Law 1996). In mammals, Hg might cause damage of the central nervous system including sensory and motor deficits and behavioral impairment (Krishna et al. 2003). Hg might also be hepatotoxic in pinnipeds (Shore and Rattner 2001), and would cause detrimental effect on pinniped testes by altering biosynthesis of steroids in grey seals (Freeman and Sangalang 1977). Ciesielski et al.(2010) reported a high degree of Hg enrichment in the liver of Baikal seals in relation to the surrounding water, indicated a high potential of Hg accumulation though food chain. It is noteworthy that in the present study two liver samples with high Hg concentration (6.09 $\mu g \cdot g^{-1}$ and 15.6 $\mu g \cdot g^{-1}$) were observed in pups. Though the reason in unknown, studies showed Hg is more toxic to developing nervous systems (Goyer and Clarkson 1996). Therefore, Baikal seal pups might be particularly vulnerable to Hg.

Se

Se revealed the highest concentrations compared to other selected elements in all the organs and tissues except in hair. Highest concentration of selenium was found in kidney (5.16±1.13 $\mu g \cdot g^{-1}$), followed by liver (3.13±3.13 $\mu g \cdot g^{-1}$) in pups of Baikal seals. Such distribution of Se indicates the important roles of liver and kidney in Se metabolism, since liver is the main organ for selenoenzyme production (Schomburg, Schweizer and Köhrle 2004), while excretion of Se is mainly in the urine via kidney (Klaassen et al. 2008).

Lower Se concentrations in muscles, liver and kidney were found in harp seal (*Pagophilus groenlandicus*) and hooded seal (*Cystophora cristata*) juveniles from the Greenland Sea (Julshamn and Grahl-Nielsen 2000) than those in Baikal seal pups in this study. Paludan-Müller et al. (1993) also reported lower Se concentrations in the muscles, kidney and liver of pups of harbour propoise (*Phocoena phocoena*) from West Greenland than pups of Baikal seal. In addition, lower Hg concentrations were also reported in these articles, indicates the correlation between Se and Hg.

Many studies have demonstrated the mutual antagonism between Hg and Se (Cuvin-Aralar and Furness 1991, Peterson et al. 2009a, Peterson et al. 2009b, Sørmo et al. 2011). Se is an essential element which is important for antioxidant systems as well as hormone homeostasis.

It is also well-known that Se can form complexes with several metals such as Hg, As, Cu and Cd, and thus Se is of special concern for its detoxification function for these metals (Klaassen et al. 2008). As mentioned in Section 3.1, the two liver samples (seal no. 29 and no. 30) which contained much higher Hg concentrations also contained higher Se concentrations than other pups. This indicates the positive relationship between the concentrations of Hg and Se. High environmental and dietary levels may also lead to increased concentrations and possible toxicity of Se in mammals (Walker 2006). However in a Se deficient environment such as Lake Baikal (Grosheva et al. 2000, Lomonosov et al. 2011), this is not likely to occur. Thus, the high Se concentrations observed in those two liver samples were probably due to the high concentrations of Hg and homeostatical mechanisms that elevate concentrations of Se in order to detoxify Hg and tolerate higher Hg exposure. Besides this, unlike Hg, accumulation of Se was not revealed from the results in the present study. This is probably due to the role of Se as an essential element which is subjected to homeostatic regulation and would maintain at steady concentrations as long as exposure does not overburden the homeostatic mechanisms.

Cd

Cd concentrations were the lowest in all the organs and tissues when compared to the concentrations of other selected elements in Baikal seal pups and adult females. The highest concentration was found in kidney (0.82±3.23 $\mu g \cdot g^{-1}$), followed by liver (0.15±0.41 $\mu g \cdot g^{-1}$), whereas Cd was undetected in brain of pups. However, Ciesielski et al. (2006) reported relatively high concentrations of Cd (0.41±0.17 $\mu g \cdot g^{-1}$) in the liver of Baikal seal pups. Ikemoto et al. (2004) and Watanabe et al. (1998) also reported higher Cd concentrations in liver and kidney of Baikal seals than those in the present study. Cd concentrations measured in the present study were also lower when compared to the juveniles of other marine mammals (Hansen et al. 1990, Holmes et al. 2008, Julshamn and Grahl-Nielsen 2000). Nevertheless Lahaye et al. (2007) found that Cd was undetected in both liver and kidney of the fetus of short-beaked common dolphins (*Delphinus delphis*), and suggested a prevention of Cd to cross the placenta by binding to metallothioneis (MTs).

Higher Cd concentrations in brain, adrenal and thyroid glands, gonads and muscles of adult females than those in pups indicate the accumulation of Cd with age. However, accumulation of Cd was not found in the kidney and liver in the present study though it is well reported in several studies (Dietz, Riget and Johansen 1996, Hansen et al. 1990, Paludan-Müller et al. 1993, Watanabe et al. 1998). Besides this, it should be noted that in the present study two kidney samples (seal no.21 and no.32) exhibited very high Cd concentrations (12.9 $\mu g \cdot g^{-1}$ and 20.1 $\mu g \cdot g^{-1}$), whereas the others ranged from 0.0013 to 0.038 $\mu g \cdot g^{-1}$. The reason for this is unclear, but it may due to the contamination of Cd from the bullet when the seals were shot or an occasionally dietary uptake of Cd contaminated prey.

Studies had found high Cd concentrations in kidney of some ringed seals from Northwest Greenland, which were even higher than the critical concentration of 200 $\mu g \cdot g^{-1}$ wet weight that associated with a kidney damage in mammals including humans (WHO 1992). However, renal damages were not observed in these specimens, suggesting that mammals inhabiting this area have adapted to the high Cd environment (Dietz et al. 1998). Since Cd concentrations in Lake Baikal are low (Grosheva et al. 2000), even small fluctuations in the Cd concentrations may have negative effects on Baikal seals as they have not adapted to high Cd concentrations.

Pb

Pb was detected in all the samples of Baikal seals, and the concentrations were relatively low except the concentrations in hairs, which were considerably higher than those in other organs and tissues. Similar results have also been observed by Ikemoto et al. (2004) that Pb concentrations in hairs of Baikal seals, Caspian seals and Northern fur seals were much higher than those in liver, kidney and muscle, which indicate the accumulation of Pb in hair of these mammals. Since Pb concentrations were similar in all the tissues and organs except hair, it indicates that Baikal seals might be exposed to inorganic Pb because organic Pb would accumulate in kidney, liver and brain (Walsh and Tilson 1984) and would have higher Pb concentrations in these organs. The majority of Pb absorbed from the diet is transferred to bone tissue (Tsuchiya 1979). Pb in bones would have a half-life of several years and would release into the blood in an equilibrium way (Goyer and Clarkson 1996), while soft tissues like liver and kidney have the ability to excrete Pb through urine (Wilson et al. 1998). Therefore, Pb concentrations of soft tissues might only reflect the current and recent exposure (Komarnicki 2000), and might not represent a complete picture of Pb exposure to the Baikal seals.

The Pb concentrations in the present study (0.047±0.051 $\mu g \cdot g^{-1}$ in liver and 0.083±0.11 $\mu g \cdot g^{-1}$ in kidney) were well below the concentrations reported to cause detrimental effects in mammals (5 $\mu g \cdot g^{-1}$ in liver, 15 $\mu g \cdot g^{-1}$ in kidney) (Ma 1996). This is probably due to the low Pb background of Lake Baikal (Grosheva et al. 2000) and indicates toxic effect of Pb is not likely to occur. In addition, in the present study lower Pb concentrations were found in the organs and tissues of adult females than those of pups. The explanation to this might be that Pb concentrations in soft tissues will stabilize, or decrease with age (Tsuchiya 1979). Accumulation of Pb in hair was revealed in comparison to Pb concentrations in other organs and tissues of Baikal seals in the present study. This is consistent with several studies on Ob concentrations on humans (Foo et al. 1993, Nowak and Chmielnicka 2000, Piccinini et al. 1986). Besides this, these authors suggested a correlation of Pb concentrations between hair and blood for humans. However, Wilhelm et al.(2002) found that both endogenous and exogenous factors would contribute to the Pb concentrations in hair, and thus the hair content of Pb cannot be taken as a suitable indicator of the level of Pb in blood and in the whole organism. Since blood samples of Baikal seals were not analyzed in this study, we cannot conclude the correlations of Pb concentrations in hair and blood.

As

Variations of As concentrations among organs and tissues were relatively small compared to Hg, Se, Cd and Pb. Highest concentrations of As in Baikal seal pups in this study were found in hair (0.27±0.14 $\mu g \cdot g^{-1}$), followed by kidney (0.15±0.11 $\mu g \cdot g^{-1}$) and thyroid gland (0.11±0.14 $\mu g \cdot g^{-1}$). Julshamn and Grahl-Nielsen (2000) found higher concentrations of As in the liver of juveniles of harp seals (0.13±0.05 $\mu g \cdot g^{-1}$) and hooded seals (0.27 and 0.64 $\mu g \cdot g^{-1}$) from Greenland sea than those in the liver of Baikal seal pups (0.063±0.044 $\mu g \cdot g^{-1}$) in the present study. Similar results were also reported by Holmes et al. (2008) that higher liver arsenic concentrations were found in Steller sea lion pups (0.18±0.074 $\mu g \cdot g^{-1}$ and 0.16±0.041 $\mu g \cdot g^{-1}$ for Western and Eastern Steller sea lions pups, respectively). Kubota et al. (2001) compared the arsenic concentrations in the liver of Baikal seals with other mammals inhabiting in different environments. The authors found that Baikal and Caspian seals, which inhabiting landlocked waters, contained lower concentrations of As in the liver than those of the marine mammal species. This might due to the differences between marine and freshwater environment. Up till now it has not been established whether As concentrations in marine mammals are higher than those in freshwater mammals, whereas it is generally accepted that As concentrations in marine organisms are much higher than those in terrestrial organisms (Lunde 1977). Kubota et al. (2001) suggested that the low concentrations of As in freshwater mammals might due to the low concentrations of As in the preys in freshwater environment. Kubota et al. (2001) also found no obvious trend with age or body length in As accumulation in the liver of the seals. However, higher As concentrations in brain, adrenal glands and gonads of adult females than those of pups in the present study indicate the potential of arsenic accumulation with age of Baikal seals

Though limited data can be found on abundance and toxicity of As in marine mammals, the detectable concentrations of As in thyroid gland and other organs and tissues may be harmful. For example, it has been reported that exposure to As would cause alterations of the *in vitro* biosynthesis of steroids in the adrenals and testes of grey seals (Freeman and Sangalang 1977). Davey et al.(2007) also reported that *in vitro* exposure to As would cause disruption of some receptor-dependent processes, and thus As is potentially relevant to human developmental problems and disease risk. In addition, although toxic effects of As in marine mammals are poorly understood, ingestion of As-contaminated water could cause skin, liver, lung, kidney and bladder cancer in humans (Smith et al. 1992). Terrestrial mammals are able to methylate and excrete As in urine when exposure does not overburden detoxification mechanisms (Eisler 1988). Kubota et al. (2001) found low concentrations of As in both Baikal seal and its preys and suggested Baikal seals might also have the ability to detoxify and excrete As.

4.2 Relationships of chemical elements between organs and tissues

4.2.1 Intra-elemental correlations

Various correlations of element concentrations between organs and tissues were found in Baikal seals (Table 3.1). Most correlations were observed on Hg concentrations between organs and tissues. Since elements like Hg, Cd and As accumulate with age and only two adult seals were included in the study, data from two adult females were not used for further correlation analysis. Statistical outliers which mentioned in Section 3.3 were also excluded in order to show the precise correlations between organs and tissues.

After excluding data from adult females and outliers (Table 3.2), positive correlations of Hg concentration between muscles, brain, gonads, adrenal and thyroid glands were found and this is might due to the age-dependant accumulation of Hg in these organs and tissues. Several correlations of Se (brain-gonads, brain-thyroid glands, gonads-adrenal glands, kidney-hair) were also revealed, however this relationship were statistically less significant. This might be the consequence of the homeostatic control of the concentrations of Se, since its important role as an essential element.

It is well known that a correlation of Hg concentrations between liver and kidney exist in marine mammals, and this was reported by many studies (Agusa et al. 2011, Hansen et al. 1990, Ikemoto et al. 2004, Medvedev, Panichev and Hyvärinen 1997, Paludan-Müller et al. 1993, Watanabe et al. 1996, Watanabe et al. 2002). These authors suggested Hg accumulates with age in both liver and kidney. However, such a correlation was not revealed in the present study. It is known that Hg accumulates in liver with age (Klaassen et al. 2008, Krishna et al. 2003). In the present study, such age-dependant accumulation of Hg was not found in the liver of Baikal seals. This might be explained that hepatic Hg concentrations were reported to be elevated up to about 10 years of age and afterwards the concentrations become variable in mature females (Watanabe et al. 1998), and thus indicate that the concentrations of Hg in the liver of adult females are not necessarily higher than those of pups. Besides this, in the present study concentrations of Hg in kidney were almost 3 times higher than the concentrations of Hg in liver of pups. Such results were inconsistent with the previous studies on juveniles of marine mammals where hepatic Hg concentrations were found to be higher than or comparable to the Hg concentrations in kidney (Julshamn et al. 1987, Julshamn and Grahl-Nielsen 2000, Watanabe et al. 1998, Holmes et al. 2008). The reason for the high renal Hg concentrations observed in pups in the present study is unclear but it might indicate that the main chemical form of Hg in Baikal seals is inorganic Hg since kidney is the major target of distribution of inorganic Hg (Klaassen et al. 2008).

Hair has been used for time trend monitoring of Hg in humans (Gibson 1989, Houtman et al. 1982). It has been also suggested that hair can be used to evaluate the level of trace elements in pinnipeds (Fossi and Marsili 1997). In fact, Ikemoto et al. (2004) and Watanabe et al. (1996) have reported the correlations of Hg concentrations of hair to kidney and liver of Baikal seals. Agusa et al. (2011) also found the Hg correlations of hair to gonad, liver and kidney of harp seals from Canada. In the present study, correlations of Hg concentrations were found between hair and brain, thyroid and adrenal glands, but correlations between hair and liver and kidney were not significant. Watanabe et al. (1996) concluded that the significant higher concentrations of trace elements in hair than those in other organs and tissues was one of the requirements for using hair as monitoring factor of chemical elements in animals. Therefore, the reason for the absence of correlations of Hg between hair and kidney and hair and liver in the present study might be the relatively low concentrations of Hg in hair from pups compared to adult Baikal seals. Besides this, close to significant correlations was found between hair and muscle (p=0.054). Therefore using hair for monitoring Hg level in the organs and tissues of marine mammals might be inappropriate. Care should be taken when analyzing hairs collected from young animals and drawing conclusion about the entire body burden of specimen.

4.2.2 Inter-elemental correlations

Multitude inter-elemental correlations were found in organs and tissues of Baikal seals. However, due to the small number of samples, two adult females were excluded from the correlation analysis (see section 4.2.1) in order to show the precise correlations between elements.

After excluding data corresponding to adult females, some of the correlations between elements remained unchanged. Among the observed relationships, correlations between Hg and Se are of special concern. It is known that Hg can accumulate with age, which increase is accompanied with an increase of Se levels. This phenomenon was explained via formation of Hg-Se complexes in marine mammals (Ikemoto et al. 2004, Itano et al. 1984, Kunito et al. 2004). Thus, correlations between Hg and Se have also been reported in many studies. For example, Hansen et al.(1990) found the correlations between Hg and Se in livers of minke whale ($0.001 \leq p \leq 0.01$), belugas ($p<0.001$) and narwhals ($p<0.001$) from West Greenland.

Agusa et al. (2011) and Paludan-Müller et al. (1993) observed the correlations between Hg and Se in the liver of harp seals from Canada ($p<0.001$) and harbour propoise from West Greenland ($p<0.001$), respectively. However, in the present study, significant correlations between Hg and Se were not found in organs and tissues except kidney. This is probably due to the fact that seals analyzed were pups, not adults. This result is also consistent with the results from Lahaye et al. (2007) and Wagemann et al. (1988). In their study they observed strong correlation between Hg and Se in the liver of the mothers of the short-beaked common dolphins ($p<0.001$) and harp seals ($p<0.001$), respectively. In contrast, there was no correlation between Hg and Se observed in the liver of fetus. Paludan-Müller et al. (1993) concluded that the strong correlation between Hg and Se would only be found when the Se:Hg molar ratio approach 1:1. Study also elucidated that the Hg-Se complex is the last stage of Hg detoxification, and thus in the liver of young animals, low level of Hg would be detoxified by other processes (Palmisano, Cardellicchio and Zambonin 1995). This might be the reason for the absence of this correlation in organs and tissues of Baikal seal pups in the present study. A weak correlation between Hg and Se ($p<0.05$) was found in kidney, and the two liver samples (seal no.29 and no.30) with the highest Se and Hg concentrations were found to have much lower Se:Hg molar ratio (3.03 and 2.24, respectively) compared to the other liver samples (ranging from 3.93 to 9.37), indicating the formation of Hg-Se complexes and thus revealing the detoxification of Hg by Se.

In the present study, positive correlations between Cd and Hg were found in muscle, adrenal and thyroid glands, gonads, liver and kidney if adult females were also included in the analysis (Table 3.3). Studies also reported positive correlations between Cd and Hg in different organs and tissues, mainly kidney and liver, in marine mammals (Hansen et al. 1990, Noda et al. 1995, Paludan-Müller et al. 1993, Watanabe et al. 1996, Watanabe et al. 2002), and the authors believed that the correlation between Hg and Cd was due to the accumulation of both elements in the organs and tissues independently with age. In the present study, correlations between Hg and Cd found in muscle, adrenal and thyroid glands and gonads were due to significant higher concentrations in the two adult females than those in pups, whereas the correlations observed in liver and kidney were due to the high concentrations of Hg and Cd in livers of seal no.29 and no.30, high concentrations of Cd in seal no.21 and no.32, respectively. Therefore after excluded those data, no correlations was found between Hg and Cd in organs and tissues (Table 3.4). It should be noted that those two liver samples with higher Hg and Se concentrations had also much higher Cd concentrations than other liver samples from pups, indicating the correlation between Hg and Cd in the liver since Cd concentrations elevated with the higher Hg concentration. The absence of correlation between Hg and Cd in the present study might due to the concentrations of Cd in pups were too low to observe this correlation.

4.3 Se:Hg molar ratios

The antagonistic effect of Se on Hg induced toxicity has been recognized for 50 years (Pařízek and Ošťádalová 1967). It has been also demonstrated in a variety of animals (Cuvin-Aralar and Furness 1991, Peterson et al. 2009a, Peterson et al. 2009b, Sørmo et al. 2011). The antagonistic effect between Se and Hg has become one of the strongest and most general examples of interactions between chemical elements. Several mechanisms of the detoxification of Hg by Se were proposed (Cuvin-Aralar and Furness 1991). One of them comprises that Se can sequester Hg and thus reduce the bioavailability of Hg in the organism. Conversely, Hg has strong affinity for Se and thus inhibits the activity of Se-dependent functions by forming the insoluble Hg selenide species (Se-Hg complexes). Therefore, measuring only concentration and bioaccumulation of Hg might be insufficient and might provide an inadequate conclusion about the potential toxicities and risks of Hg exposure, especially in oligotrophic lakes such as Lake Baikal. Studies showed that if Se is in a molar excess of Hg in the cell, the inhibition of Se-dependent functions is not likely to occur. It has been also suggested that a tissue Se:Hg molar ratio greater than one is a threshold for the protecting action of Se against Hg toxicity (Peterson et al. 2009b, Ralston, Blackwell and Raymond 2007).

In the present study and some previous studies (Grosheva et al. 2000, Ikemoto et al. 2004, Watanabe et al. 1996), Hg concentrations in the Lake Baikal and its biota were found to be low compared to other freshwater or marine environments. However, since Lake Baikal is a Se deficient environment, it is more likely that Hg would cause toxic effects on the wildlife even though Hg is at a relatively moderate level. In the present study, Se:Hg molar ratios were well above the suggested protective ratio of 1 (ranged from 2.24 to 102). These molar ratios are much higher than previously reported values for marine mammals (Dietz, Riget and Born 2000, Nigro and Leonzio 1996). In addition, lower Se:Hg molar ratios in adult females than in pups were also observed. This is due to the accumulation of Hg with age and thus adult females have higher concentrations of Hg than pups. But Se:Hg molar ratios in liver and kidney from adult females are at the same level as pups. The reason might be that liver and kidney have different accumulation pattern of Hg in relation to age (Watanabe et al. 1998).

In this study, the great molar excess of Se over Hg in the organs and tissues of the endemic Baikal seals indicates that though Lake Baikal is a Se deficient environment, the Se concentrations in Baikal seals, especially in pups, are high enough to prohibit Hg induced toxicity. This is probably due to the low Hg concentrations in Lake Baikal and its biota. Therefore, there is no potential risk of Hg induced toxicity in Baikal seals at present.

4.4 Maternal transfer of As, Cd, Hg, Pb and Se

It is well known that fetus can receive toxicants from mother during gestation and lactation (Klaassen et al. 2008). Such toxicants are mainly lipophilic organic contaminants, and previous studies have reported the maternal transfer of such organic contaminants in Baikal seals (Iwata et al. 2004, Ishibashi et al. 2008). Chemical elements such as Hg, Co, As and Pb have also the ability to be transferred from mother to fetus (Brodner et al. 2004, Kubota et al. 2005, Wagemann et al. 1988). In a relatively less metal contaminated environment such as Lake Baikal, toxic elements in pups might mainly come from mothers during gestation and lactation, and maternal transfer might be an important process related to the toxicants exposure in the early life stage of Baikal seals. Thus it is of interest to investigate the maternal transfer level of As, Cd, Hg, Pb and Se in Baikal seals.

Among these five selected elements, maternal transfer of Hg is probably of most concern. Wagemann et al. (1988) suggested that Hg would be transferred from mother to fetus of harp seals by crossing the placenta, and this is the main route of maternal transfer of Hg from mother to fetus. Adult female Baikal seals can excrete Hg via molting, parturition and lactation (Ciesielski et al. 2010), After parturition, females eliminate pollutants by transferring them to their pups, a phenomenon which may jeopardize the offspring (Medvedev et al. 1997). Hg exists in several forms in the biosphere, and the most toxic form is methylmecury (MeHg). Almost all the Hg present in the tissue of fish is methylated. The MeHg in the fish is almost completely absorbed by the gastrointestinal tract when fish is ingested, and subsequently distributed throughout the body. MeHg can easily penetrates the blood-placenta barrier in humans and other mammals (Drasch, Horvat and Stoeppler 2004). Wolfe et al. (1998) reported that MeHg will concentrate selectively in the fetal brain, and the concentrations of MeHg in the fetus can be twice as high as in the maternal brain in rodents fed by MeHg. In our study we measured exclusively total Hg concentration, but comparable level of Hg in kidney and liver between adult females and pups were observed, which indicate that adult females might excrete some Hg via parturition and lactation.

A few studies have reported Hg in the natal hair of pinnipeds (Beckmen et al. 2002, Hyvärinen and Sipilä 1984, Hyvärinen et al. 1998). Hyvärinen and Sipilä (1984) have studied the Hg, Cd and Pb concentrations in the natal hair, pup hair and adult hair of the Saimaa ringed seals and found that the Hg concentrations were higher in natal hair than those in pup hair and adult hair, while the concentrations of Cd and Pb showed no difference between all the hair samples. A more recent study from Castellini et al. (2012) also found significantly higher Hg concentration in hair of young pups (≤ 3 months) than old pups (ca. 5 months) and yearlings of Steller sea lion. This is consistent with the results in the present study, since Hg concentrations in natal hair of Baikal seals (3.06±0.95 $\mu g \cdot g^{-1}$) were higher than those of pups (1.63±0.63 $\mu g \cdot g^{-1}$). Therefore, it indicates that Baikal seal fetus receive Hg from mother and then excrete it into natal hair through blood (Brookens et al. 2008). Since Hg is not considered bioavailable in hair (Wenzel et al. 1993), excretion of Hg by concentrating it into natal hair can protect fetus from the toxicity of MeHg, which is the main form of Hg transferred through placenta.

Baikal seals are born with a white woolly coat which will be molted after 6-8 weeks and replaced by a silvery grey coat. All of the Baikal seal pups at the present study were in such silvery grey hair, and Castellini et al. (2012) suggested the Hg concentration in such hair can represent the dietary exposure to Hg through milk since pups get the hair before they begin to feed independently. That means the hair has grown when milk from the mother has been the only food. This also indicates that placenta plays a greater role in Hg transfer to offspring than does milk, since the Hg concentrations is higher in the natal hair than those in the hair of pups (Habran et al. 2011). Thus, it can be concluded that Baikal seals might encounter significant exposure to Hg in utero, which is a critical development period, and lactation is less important route of Hg exposure for pups. According to previous report (Thompson 1996) that the toxic threshold for total Hg concentration in hair in terrestrial mammals was 20 $\mu g \cdot g^{-1}$, and human maternal hair Hg concentrations of 10-20 $\mu g \cdot g^{-1}$ may indicate exposure great enough to impact fetal neurodevelopment (WHO 1990), in the present study the concentrations of Hg in hair of pups and adult females are well under this threshold. Regarding the relatively low Hg concentrations in the organs and tissues of adult females and pups in this study, maternal transfer of Hg through placenta and milk is unlikely to cause severe toxic effects to pups in Lake Baikal.

Unlike Hg, Habran et al. (2011) reported that the Se uptake from milk appears to be higher than from the placenta since these authors found substantial quantities of Se in the milk of northern elephant seals. In the same study it was also found that no correlation exist between Hg and Se in blood and milk and thus it was concluded that different maternal transfer processes are crucial for Hg and Se. During lactation, the mammary-gland regulation mechanism controls the synthesis and secretion of Se compounds, with the highest amount of Se being excreted at the beginning of lactation (Dumont, Vanhaecke and Cornelis 2006).

Information on Se in marine mammals is limited, and studies about maternal transfer of Se focus on Se concentrations in milk and plasma, which are not included in the present study. Se concentrations in organs and tissues in Baikal seal pups were found to be fairly similar in both mothers and pups (Fig. 3.1), thus it is difficult to evaluate the rate of maternal transfer of Se in Baikal seals. Besides this, maternal transfer of Se seems to be related to the chemical form of Se (Anan et al. 2009, Awadeh, Kincaid and Johnson 1998, Kim and Mahan 2001). For example, Kim and Mahan (2001) observed different toxicity when pigs were fed either with inorganic or organic Se source. Results have shown that organic Se seemed to exert its toxicity more on reproductive performance during gestation, whereas inorganic Se was more detrimental to the nursing pig during lactation. Therefore, further study on the Se and its different chemical forms concentrations in plasma and milk is required for detail explanation of maternal transfer of Se in Baikal seals.

It has been reported that Cd has a limited placenta transfer compared to Hg (Wagemann et al. 1988). This is because Cd can bind to metallothioneins (MTs). MTs play an important role in metal homeostasis and detoxification. For example, MTs may detoxify Cd in animals which are able to accumulate Cd in their tissues (Webb 1979). Pregnant and lactating females have in general an increased induction of MTs in order to accommodate increasing demand and storage of essential elements (Solaiman et al. 2001). Furthermore, Hanlon et al. (1982) reported that Cd can bind to MTs and thus prevent the transfer of Cd across the placenta. Moreover, placenta itself contains MTs and Cd will bind to the placenta's MTs and results in high concentrations in this organ (Kuriwaki et al. 2005). Several studies have observed such a phenomenon as they found Cd concentrations in the organs and tissues of mothers are higher than those of pups (Ciesielski et al. 2006, Lahaye et al. 2007, Lau, Joseph and Cherian 1998). In the present study, Cd concentrations were very low in organs and tissues of pups, and higher Cd concentrations shown in musclea, brain, adrenal and thyroid glands and gonads of adult females than those of pups. This concentration difference might both due to the Cd age-dependent accumulation in adult females and the role of placenta in adult females to prevent Cd transfer to fetus. In addition, concentrations of Cd in natal hair (0.051±0.020 $\mu g \cdot g^{-1}$) were found no higher than those in hair of pups (0.091±0.044 $\mu g \cdot g^{-1}$) and adult females (0.11±0.14 $\mu g \cdot g^{-1}$) in the present study. This is consistent with the result in the study from Hyvärinen and Sipilä (1984) where Cd concentrations in natal hair was similar to or lower than the hair samples of all the age groups of the Saimaa ringed seals. This indicates that the prevention of maternal transfer of Cd during gestation also exist in Baikal seals.

Placenta can protect the fetus against the toxic effect of Cd, but it is still possible that Cd penetrates the epithelia of the mammary glands and enters the milk of female marine mammals (Frodello, Viale and Marchand 2002). Thus Cd from mother can be transferred to offspring via lactation. This might be also true for Baikal seals since we observed comparable Cd levels in the liver and kidney of adult females and pups.

It has been reported that there was a limitation of the placenta transfer of Pb, since Pb concentrations in the natal hair and the hair from various age groups of Saimaa ringed seals showed no differences (Hyvärinen and Sipilä 1984). This is consistent with the results in the present study. Besides this, lead that is stored in the bone of mothers might be released during lactation due to an altered metabolism (Silbergeld 1991). This remobilization may represent an important contamination factor for suckling young pups and indicates that lactation might be the main route of Pb maternal transfer. However, Gulson et al. (1997) reported that Pb can also easily cross the placenta and Pb can be mobilized from bones at an accelerated rate during gestation and subsequently transferred to fetus. From our results lactation seems to be the main route of maternal transfer of Pb. Since Pb concentrations in all the organs and tissues of both adult females and pups are relatively low in this study, toxic effects of Pb exposure are not likely to occur. According to the studies on maternal transfer of Pb, its concentrations in blood and bone might provide a better insight of the maternal transfer in Baikal seals.

Few studies are published on the maternal transfer of As in marine mammals. Kubota et al. (2005) have measured the As concentrations in organs of a mother-fetus pair of Dall's porpoise and found that the concentrations of As in fetus were lower than those in mother. It was suggested that placental transfer of As, like other toxic elements, seems to be limited to some extent by physiological system such as placental barrier in marine mammals. Another study on placenta transfer of toxic elements in humans found that placenta partially blocks the passage of As (Iyengar and Rapp 2001). However, due to lack of data from maternal and fetal blood, the authors stated that further information is needed for conclusion. In general, As concentrations in the organs and tissues of both adult females and pups were quite low in this study. Concentrations of As in adult females were in the same order of magnitude as in pups, except for brain, adrenal glands and gonad. In these organs As concentrations were a bit higher than in adult females (0.070±0.011 $\mu g \cdot g^{-1}$, 0.28±0.010 $\mu g \cdot g^{-1}$ and 0.11±0.026 $\mu g \cdot g^{-1}$, respectively) than in pups (0.026±0.0050 $\mu g \cdot g^{-1}$, 0.088±0.037 $\mu g \cdot g^{-1}$ and 0.043±0.022 $\mu g \cdot g^{-1}$, respectively). Therefore, a limited placental transfer seems also occur in Baikal seals.

5. Conclusion

In the present study, concentrations of As (arsenic), Cd (cadmium), Hg (mercury), Pb (lead) and Se (selenium) were measured in muscles, brain, adrenal and thyroid glands, gonads, liver, kidney and hairs of Baikal seal pups and adult females. In general, concentrations of these five selected elements were lower in Baikal seals than those published for other marine mammals. This finding confirms the low natural background of As, Cd, Hg and Pb in Lake Baikal. The high Se:Hg molar ratios observed in all the organs and tissues indicates that even in a Se deficiency environment as Lake Baikal, Se concentrations were high enough to prohibit Hg induced toxicity, and thus indicates there is no risk of the Hg toxicity in Baikal seals at present.

In Baikal seal pups, correlation between concentrations of Se and Hg was found in kidney. This indicates the important role of Se in the detoxification of Hg. The absence of correlations between Se and Hg in liver and other tissues and organs might be due to the high Se:Hg molar ratios. When molar Se is in great excess of molar Hg, other mechanism may be important for detoxification of Hg. Unlike in other marine mammals, significant correlations of Hg concentrations between hair and kidney and between hair and liver were not observed in the present study. This indicates that use of hair for monitoring Hg levels in organs and tissues of marine mammals might be inappropriate in pups.

Results in this study showed that the main route of maternal transfer of Hg might be via parturition, while the main route of maternal transfer of Cd and Pb might be via lactation, since the placenta seems to have a limited transport of Cd and Pb. The placenta also seems to partially block the passage of As. Maternal transfer of the selected chemical elements was found to constitute low potential risk for pups due to the low concentrations of the elements in both adult females and pups. However, the entire process of maternal transfer of these five elements in Baikal seals is unclear. Further study is required such as elements concentrations in plasma and milk, which might provide a better understanding of maternal transfer in Baikal seals.

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Appendix

Appendix 1

Biometric data

Table a1: Biometric data of sampled Baikal seals from the Lake Baikal

ID	Sampling Date	Sex	[a]BW(kg)	[b]NT(cm)	[c]TL(cm)	Girth(cm)	Note
Ph-18	16.04.2011	M	19.1	85	101	80	pup
Ph-19	16.04.2011	M	24.5	86.7	105	123	pup
Ph-20	16.04.2011	M	17	83	95.5	124	pup
Ph-21	16.04.2011	M	18.5	84	101.5	76.5	pup
Ph-22	16.04.2011	F	18.3	84.5	100	76	pup
Ph-23	16.04.2011	F	20	81	97.5		pup
Ph-24	17.04.2011	F	17	72	96	73	pup
Ph-25	17.04.2011	F	12.6	70	88	70.5	pup
Ph-26	17.04.2011	F	18	77	90	79	pup
Ph-27	17.04.2011	M	22.1	91	106	79	pup
Ph-28	17.04.2011	M	20.25	84	101	80	pup
Ph-29	17.04.2011	M	22	89	103.5	79	pup
Ph-30	18.04.2011	F	21.4	84.5	100	78	pup
Ph-31	18.04.2011	F	20.4	84	99	80	pup
Ph-32	18.04.2011	F	54.4	129	148	115	Adult female
Ph-33	18.04.2011	F	42.5	114	136	85	Adult female
Ph-34	18.04.2011	M	14.9	78	91	63	From Ph-32
Ph-35	18.04.2011	F	15.9	94	77	74	From Ph-33

[a] BW: body weight

[b] NT: length from nose to tale

[c] TL: total length

Appendix 2

Elemental recoveries

Table a2: Quantified and certified element concentrations in reference materials, Chicken GBW10018 (chicken muscle) and DOLT-3 (dogfish liver), used for assessment of accuracy of ICP-MS instrument.

Element	**Isotope**	**Chicken GBW10018 (chicken muscle)**				**DOLT-3 (dogfish liver)**			
		Conc. (µg/g)	**SD**	**Certified value (µg/g)**	**Recovery (%)**	**Conc. (µg/g)**	**SD**	**Certified value (µg/g)**	**Recovery (%)**
As	75	0.12264	3.425	0.109±0.013	112.51	9.86749	3.2	10.2±0.5	96.74
Cd	114	0.00334	10.4	0.005	66.78	19.7368	3.77	19.4±0.6	101.74
Hg	202	0.00694	6.05	0.0036±0.0015	192.64	3.31149	2	3.37±0.14	98.26
Pb	208	0.06694	3.575	0.11±0.02	60.86	0.40345	1.2	0.32±0.05	126.08
Se	78	0.56880	3.95	0.49±0.06	116.08	7.27010	2.1	7.06±0.48	102.98

Appendix 3

Table a3: Method detection limit[a]

Element	MDL1	MDL2	MDL3	MDL4	MDL5	MDL6	MDL7	MDL8	MDL9
	hair (0.01g)	**hair (0.03g)**	**hair (0.04g)**	**hair (0.06g)**	**hair (0.10g)**	**hair (0.15g)**	**adrenal (0.20g)**	**thyroid, hair (0.30g)**	**muscle, brain, gonad, liver, kidney (0.40g)**
As	0.15	0.050	0.038	0.025	0.015	0.010	0.0075	0.0050	0.0038
Cd	0.012	0.0040	0.0030	0.0020	0.0012	0.00080	0.00060	0.00040	0.00030
Hg	0.0060	0.0036	0.0036	0.0036	0.0036	0.0036	0.0036	0.0036	0.0036
Pb	0.012	0.0040	0.0030	0.0020	0.0012	0.00080	0.00060	0.00048	0.00048
Se	0.90	0.30	0.23	0.15	0.090	0.060	0.045	0.030	0.023

[a]: Method detection limits were determined by comparing instrument detection limit with detection limits calculated as 3 times of the standard deviation of blank samples, and then selecting the value of the two. In order to obtain accurate MDLs, the average weight for different organs and tissues were calculated, and 9 different MDLs (µg/g) were calculated based on these weight groups.

Appendix 4

Chemical element concentrations

Table a4: Mean concentrations and standard deviations, median, minimum and maximum values of As, Cd, Hg, Pb and Se (µg/g dry wt.) in different organs and tissues of Baikal seals.

Element	Organ	Age	n	Mean±SD (µg/g)	Median (µg/g)	Min (µg/g)	Max (µg/g)
As	muscle	pups	16	0.073±0.045	0.060	0.029	0.20
		mothers	2	0.091±0.020	—	0.076	0.11
	brain	pups	16	0.026±0.0050	0.024	0.019	0.036
		mothers	2	0.070±0.011	—	0.062	0.078
	adrenal gland	pups	15	0.088±0.037	0.082	0.040	0.20
		mothers	2	0.28±0.010	—	0.28	0.29
	thyroid gland	pups	16	0.11±0.14	0.077	0.034	0.61
		mothers	2	0.094±0.0085	—	0.088	0.10
	gonad	pups	15	0.043±0.022	0.033	0.023	0.11
		mothers	2	0.11±0.026	—	0.092	0.13
	liver	pups	16	0.063±0.044	0.049	0.035	0.19
		mothers	2	0.053±0.018	—	0.040	0.066
	kidney	pups	16	0.15±0.11	0.12	0.038	0.53
		mothers	2	0.25±0.13	—	0.15	0.34
	hair	pups	15	0.27±0.14	0.25	0.10	0.63
		mothers	2	0.073±0.042	—	0.043	0.10
	natal hair	—	2	0.20±0.17	—	0.083	0.33
Cd	muscle	pups	16	0.0012±0.00074	0.0010	0.00031	0.0029
		mothers	2	0.027±0.026	—	0.0084	0.046
	brain	pups	16	—	<0.0003	<0.0003	0.00046
		mothers	2	0.0057±0.0010	—	0.0050	0.0064
	adrenal gland	pups	15	0.0016±0.0014	0.0010	<0.0006	0.0043
		mothers	2	0.47±0.021	—	0.45	0.48
	thyroid gland	pups	16	0.00049±0.00041	0.00043	<0.0004	0.0017
		mothers	2	0.066±0.0088	—	0.060	0.072
	gonad	pups	15	0.00067±0.00092	0.00047	<0.0003	0.0038
		mothers	2	0.092±0.054	—	0.054	0.13
	liver	pups	16	0.15±0.41	0.0017	0.00077	1.31
		mothers	2	0.0012±0.00045	—	0.00087	0.0015
	kidney	pups	16	0.82±3.23	0.0082	0.0013	12.93
		mothers	2	10.06±14.21	—	0.0084	20.11
	hair	pups	15	0.091±0.044	0.086	0.020	0.16
		mothers	2	0.11±0.14	—	0.014	0.22
	natal hair	—	2	0.051±0.020	—	0.036	0.065
Hg	muscle	pups	16	0.21±0.059	0.21	0.11	0.35
		mothers	2	1.03±0.21	—	0.89	1.18
	brain	pups	16	0.12±0.034	0.11	0.082	0.20
		mothers	2	0.44±0.026	—	0.42	0.46
	adrenal gland	pups	15	0.15±0.055	0.13	0.069	0.28
		mothers	2	0.66±0.23	—	0.50	0.82
	thyroid gland	pups	16	0.08±0.041	0.056	0.030	0.16
		mothers	2	0.41±0.070	—	0.36	0.46
	gonads	pups	15	0.087±0.030	0.081	0.050	0.16
		mothers	2	0.44±0.114	—	0.36	0.53

Element	Organ	Age	n	Mean±SD (μg/g)	Median (μg/g)	Min (μg/g)	Max (μg/g)
Hg	liver	pups	16	2.04±3.86	0.69	0.55	15.61
		mothers	2	0.55±0.076	—	0.50	0.61
	kidney	pups	16	2.83±1.15	2.74	0.73	5.69
		mothers	2	3.22±2.75	—	1.28	5.17
	hair	pups	15	1.63±0.63	1.52	0.91	3.11
		mothers	2	1.35±1.47	—	0.31	2.39
	natal hair	—	2	3.06±0.95	—	2.39	3.74
Pb	muscle	pups	16	0.23±0.34	0.085	0.020	1.16
		mothers	2	0.082±0.060	—	0.039	0.12
	brain	pups	16	0.028±0.037	0.014	0.0044	0.13
		mothers	2	0.028±0.027	—	0.0090	0.048
	adrenal gland	pups	15	0.20±0.19	0.13	0.0077	0.63
		mothers	2	0.0065±0.0038	—	0.0038	0.0092
	thyroid gland	pups	16	0.14±0.16	0.063	0.0071	0.49
		mothers	2	0.022±0.010	—	0.015	0.029
	gonad	pups	15	0.11±0.34	0.018	0.0029	1.33
		mothers	2	0.011±0.0041	—	0.0081	0.014
	liver	pups	16	0.047±0.051	0.011	0.0081	0.014
		mothers	2	0.020±0.00033	—	0.020	0.021
	kidney	pups	16	0.083±0.11	0.035	0.0056	0.34
		mothers	2	0.025±0.010	—	0.018	0.032
	hair	pups	15	7.89±6.15	7.49	0.90	24.49
		mothers	2	1.85±0.11	—	1.77	1.93
	natal hair	—	2	4.95±5.15	—	1.31	8.60
Se	muscle	pups	16	0.87±0.11	0.86	0.68	1.07
		mothers	2	0.89±0.15	—	0.79	1.00
	brain	pups	16	1.19±0.10	1.19	1.00	1.37
		mothers	2	1.33±0.18	—	1.21	1.46
	adrenal gland	pups	15	1.90±0.37	1.85	0.96	2.59
		mothers	2	2.31±0.014	—	2.30	2.32
	thyroid gland	pups	16	1.52±0.25	1.55	0.91	1.91
		mothers	2	2.21±0.032	—	2.19	2.23
	gonad	pups	15	1.59±0.18	1.63	1.25	1.94
		mothers	2	1.97±0.033	—	1.94	1.99
	liver	pups	16	3.13±3.13	2.10	1.54	13.77
		mothers	2	2.16±0.15	—	2.05	2.26
	kidney	pups	16	5.16±1.13	5.19	2.18	6.80
		mothers	2	6.29±2.59	—	4.46	8.12
	hair	pups	15	1.47±0.38	1.34	1.03	2.47
		mothers	2	0.89±0.93	—	0.23	1.55
	natal hair	—	2	1.40±0.32	—	1.17	1.62