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Analysis of Selected Heavy Metals in Tissue of Fish

M. Sc. DIPLOMA THESIS

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Statement

I declare that I worked out this M.Sc. diploma thesis titled „Analysis of Selected Heavy Metals in Tissue of Fish“ individually and I used only literature that is cited and mentioned in references. I agree with storing this thesis in the library of CULS Prague and enabling it for study use.

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Bc. Kateřina Jacklová

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Author's Abstract

Analysis of Selected Heavy Metals in Tissue of Fish

Heavy metals are chemical components belonging to transitional metals, metalloids, lanthanides and actinides. The occurrence of toxic heavy metals in higher non-permitted values can cause health problems in human and also in animals. The main goal of this thesis was determination of levels of selected heavy metals (Cd, Pb, Hg, As) in tissues of fish (muscle or liver). One set of samples of tissues was collected in Mongolia (Arkhangai and Khuvsgul area from August till October 2011) and analysed in accredited laboratory of State Veterinary Institute in Prague – Lysolaje. For comparison was used second set of samples from databases of State Veterinary Administration of the Czech Republic for last 10 years (2000-2010). The samples of databases were divided into three categories- common carp, trout and other freshwater fish. Total sum of analyzed Czech fish tissues were 4101 samples and Mongolian 20 representative samples. Methods for determination of selected heavy metals were Atomic Absorption Spectrometry and Included Coupled Plasma- Mass Spectrometry, which were chosen by laboratory of State Veterinary Institute in Prague- Lysolaje (all analyses were done there).

The concentration of different metals in fish tissues followed the this order :

AS>Hg>Cd>Pb

The maximum value of arsenic was 3,6 mg/kg (HL is not determined by EU, due to the CR is 1,000 mg/kg). The highest value of cadmium was0,083 mg/kg (HL for Cd= 0,050 mg/kg). The maximum value in lead was 4,00mg/kg (HL for Pb=0,500 mg/kg) and the highest value of mercury was 0,880 mg/kg (HL for Hg=0,300 mg/kg).

Regular control of food chain, water and soils for heavy metals are necessary.

Key words: fish tissue, cadmium, lead, mercury, arsenic, toxicity,atomic absorption spectrometry

Autorský referát

Analýzy vybraných těžkých kovů v tkáních ryb

Těžké kovy jsou chemické prvky, které se řadí mezi přechodné kovy, polokovy, lanthanoidy a aktinoidy. Výskyt toxických těžkých kovů ve vyšších nepřípustných hodnotách může způsobit zdravotní problémy jak u lidí tak u zvířat. Hlavním cílem této práce bylo stanovení hodnot vybraných těžkých kovů (Cd, Pb, Hg, As) ve svalové a jaterní tkáni ryb. Jedna sada vzorků tkání byla odebírána v Mongolsku (oblast Arkhangai a Khuvsgul v termínu od srpna do října 2011). Tyto vzorky byly analyzovány v akreditované laboratoři Státního Veterinárního Ústavu v Praze – Lysolajích. Pro porovnání byla použita druhá sada vzorků z databáze Státní Veterinární Správy České republiky za posledních 10 let (2000-2010). Vzorky z databáze byly rozděleny do tří kategorií – kapr obecný, pstruzi a ostatní sladkovodní ryby. Celkový počet analyzovaných vzorků tkání českých ryb byl 4101 a z mongolských ryb 20. Pro stanovení hodnot těžkých kovů byla použita atomová absorpční spektrofotometrie hmotnostní spektrometrie s vázaným plazmatem. Tyto metody byly zvoleny akreditovanou laboratoří Státního Veterinárního ústavu v Praze – Lysolajích (všechny analýzy byly prováděny v této laboratoři).

Koncentrace těžkých kovů v tkáních ryb byla následná:

AS>Hg>Cd>Pb

Maximální hodnota arsenu byla 3,6 mg/kg (hygienický limit není stanoven Evropskou Unií, ale limit pro ČR je 1,000mg/kg. Nejvyšší hodnota kadmia byla 0,083 mg/kg(hygienický limit je 0,050 mg/kg) Maximální hodnota olova byla 4,00 mg/kg(hygienický limit je 0,500 mg/kg) a nejvyšší hodnota pro rtuť byla 0,880 mg/kg(hygienický limitje 0,300 mg/kg).Pravidelné kontroly těžkých kovů ve vodě ,půdě a potravním řetězci jsou potřebné.

Klíčová slova: tkáně ryb, kadmium, olovo, měď, arsen, toxicita, atomová absorpční spektrometrie

The Contents

1. Introduction

Heavy metals are chemical elements, which are related to mainly transitional metals, metalloids, lanthanides and actinides. A lot of different expressions could be found. Some of them are divided metals according to their density, an atomic number, an atomic weight or toxicity (ADRIANO, 2001). The main natural sources are parent rocks and metallic minerals, anthropogenic sources involve mining, smelting, metal finishing and also agricultural activities are include to this group (BENCKO et. al., 1995).

The well-known chemical contaminants (heavy metals and persistent organic pollutants) may represent a potential human health hazard (HUSS, 2004). These elements have negative influence also on animals. However they could be toxic, but on the other hand they are essential for human. Increasing potential of toxicity of heavy metals can cause serious ecological problem. Metals are non-degradable and they are accumulated in soils, water and living organisms (CIBULKA, 1991).

Concentration of metals in natural areas, namely in water, atmosphere, foodstuff and in soil is the reason of increasing serious problem with hygiene (WATSON, 2001). Most of the heavy metals have ability to accumulate from water to sediments and later to living organisms. The highest coefficient of accumulation has cadmium, lead and mercury (ADÁMEK, 2010). Some amount of harmful substances may occur in fish and water animals. The lowest concentrations of these elements are detected in fish from aquaculture (INGR, 2010).

Concerning for these contaminants are mainly related to fish harvested in estuaries, fresh water, and coastal waters. In these areas are located shore-side industries or intensive agriculture. Thus there is a possibility of higher usage of large amounts of pesticides or other agro-chemicals (HUSS, 2004)

INGR in (2010) noticed, that fish are important part of healthy nutrition of human. Contain omega 3- fatty acids, which are necessary for organisms.

It is necessary to know the factors, which influence the uptake of the metals in the body and consequently their localization in the tissues (SCHEGEL et. al., 2008)

Atomic absorption spectrometry (AAS) is one of the most widely used analytical methods for trace element analysis. The determination of about 68 elements (metals and metalloids) is allowed (KOMÁREK, 2000).

2. Aims of the Thesis

This thesis deals with the analyses of heavy metals. It focuses on cadmium, lead, mercury and arsenic in fish tissues (muscle and liver tissue) in the Czech Republic and Mongolia. The aim therefore is to assess concentration of selected heavy metals, which would be compared with hygienic limits recommended by European Union. There are also some health risks, which should be evaluated. All would be mentioned in the tables. I assume that mercury would be the element often with values over the hygienic limit in Mongolia, due to the exploitation of individual areas in Mongolia (gold mining, copper mining) can be assumed illegal use of mercury from obtaining of gold. Although the topic is quite important because of really high consumption of fish by human, we need to know some health risks of the heavy metals concentration in fish tissues.

2.1. Hypothesis:

H1: Due to the exploitation of individual areas in Mongolia (gold mining, copper mining) can be assumed illegal use of mercury from obtaining of gold.

H2: In freshwater fish will be higher concentrations of selected heavy metals than in Common carp or trout.

3. Bibliographic Research

3.1. Fish in general

Fish are an ancient group of vertebrates, which appeared on Earth in older Palaeozoic era in period called the Silurian more than 400 million years ago. However more significant development occurred in the following section Devonian.

Next development was interesting in terms of quantity and appearance of various forms, which we know nowadays. They inhabit our planet for a very long time and still represent the top of adaptation of organisms in aquatic systems (BALONEK, 2004)

Fish body is usually spindle and hydrodynamically shaped. Three main parts are head, trunk and tail. Some fish are adapted to specific conditions, so the shape of their body is unique (flat fish, seahorses or deep fish). Conditions in which fish live have a major influence on appearance and colour. Mouth of fish is normally full of rows with teeth (BALONEK, 2004)

The head has probably the most complicated structure of whole body. The Brain and gills are hidden in head and there is the place for variety of sensory organs. Important parts for moving in the water are fins. They are located in different parts of body and play role in several functions- drive the fish, stabilize the body and allows doing other things during the move and on the spot. Skin is covered with scales. They can have unusual size or they are not developed, so the skin is bare (BALONEK, 2004).

3.2. Presence of most common fish in the Czech Republic and Mongolia

The Czech Republic is right at the barrier of 3 different drainage areas- Baltic Sea, North Sea, Black Sea. The richest for different species is the Black Sea. Total sum of species of fish in the Czech Republic is variable due to the natural migration and random settings by human (DUS, 2010).

The most common species of fish in rivers, lakes, ponds, steams or dams from Czech Republic are from family *Acipenseridae, Anguillidae, Clupeidae, Esocidae,*

Salmonidae, Siluridae, Percidae. Surely the most widespread fish is from family *Cyprinidae*-Common carp (*Cyprinus carpio)* (MZ, 2010)

Kingdom	Animalia	animals
Phylum	Chordata	chordates
Subphylum	Vertebrata	vertebrates
Superclass	Osteichthyes	bony fishes
Class	Actinopterygii	spiny rayed fishes
Subclass	Neopterygii	neopterygians
Order	Cypriniformes	
Family	Cyprinidae	
Genus	Cyprinus (Linnaeus, 1758)	
Species	Cyprinus carpio(Linnaeus, 1758)	common carp

3.2.1. Common Carp (Cyprinus carpio)

Fig.1: Taxonomy of Common carp *(Cyprinus carpio)*, (ITIS[a], 2012)

Common carp *(Cyprinus carpio*) is a freshwater fish from family *Cyprinidae* (see Fig.1). Probably carps were the first domesticated fish and they are the most important genus of fish used in aquaculture. In the Czech Republic is the traditional fish with huge economic importance (DUS, 2010).

Priceless advantages are that the growth is really fast and quality of meat is high. Family Cyprinid*ae* is widespread all over the world, breeding of this family is on high level and all coloured forms are known especially in Japan. However some species were introduced to Australia, where are considered as an extremely dangerous species with bad influence on freshwater ecosystems (ANDREJI et. al., 2006), (VANDEPUTTE, 2003).

Fig.2- Common carp *(Cyprinus carpio)*

Weight of Common Carp could be in good conditions around 20 kilograms and length is around 1 meter (see Fig.2). The shape of the body is prolong and covered with scales. Muzzle is movable with 4 hairs on its circuit. Carp mouth is without teeth, but food is processed by special bones with 3 rows of teeth. Eyes are moving with gold, deep green colour. Carps have got dorsal, pectoral, abdominal, anal and caudal fins. They are omnivores (DUS, 2010).

3.3. Fish in Mongolia

The Mongolia is also at the barrier of 3 different drainage areas- Arctic Ocean, Pacific Ocean and Central Asian Internal Drainage. The last named is the largest on and includes the Great Lakes depression basin including Uvs Lake, Khar- Us Lake, Khar Lake and Khyargas Lake. Khuvsgul Lake is the second biggest freshwater lake in Asia. Its water contributes to Baikal Lake. This lake is rich for different species of fish and famous for game fishing (BATNASAN, 2003)

In Mongolia you can find a lot of fish from family *Esocidae, Salmonidae, Percidae* and *Cyprinidae*. The most well-known fish from family *Salmonidae* is Taimen *(Hucho taimen)* (BATNASAN, 2003)

3.3.1. Taimen (Hucho taimen)

The taimen, which is also known as Mongolian terror trout or Siberian taimen, is species of fish from family *Salmonidae* (see Fig. 3). Distribution of these fish is mainly, on east part of the Asia, in Mongolia and Russia. Taimens live in flowing water and sometimes are found in lakes (HOLČÍK, 1988)

Kingdom	Animalia	animals
Phylum	Chordata	chordates
Subphylum	Vertebrata	vertebrates
Superclass	Osteichthyes	bony fishes
Class	Actinopterygii	spiny rayed fishes
Subclass	Neopterygii	neopterygians
Order	Salmoniformes	salmons
Family	Salmonidae	
Genus	Hucho (Günther, 1866)	
Species	Hucho taimen(Pallas, 1773)	taimen

Fig.3: Taxonomy of taimen *(Hucho taimen)*, (ITIS[b], 2012)

The taimen is rich in colours, olive green on the head and reddish brown in the tail. Fins are dark red and belly is from white to dark grey (see Fig.4). Taimens are mainly piscivores, but they eat small rodents and birds (HOLČÍK, 1988).

Fig.4-The Taimen (*Hucho taimen)*

Largest salmonid in the world is taimen. The maximum size is not well-known, but a fish caught in Russian Kotui River was 210 cm long and weight was 105kg. This is the largest reliable record. It could be for this reason they became a valued game trophy, especially for fly fishermen (HOLČÍK, 1988).

3.4. Fish as a source for human nutrition

The important role in world's food system plays fish. It is increasingly seen in the rich world as a healthy luxury food (EMERSON and HJORLEIFUL, 2009). Fish are major part of healthy nutrition. People are searching for all kinds of fish. The most consumed fish are from the sea (MZ, 2010).

3.4.1. Nutrition value of fish meat

The content of omega-3- fatty acids is high and really necessary from human. The reason is that human organism cannot create them. It also contains a high valuable biological proteins and other substances needed as vitamins and microelements. Balanced diet with the presence of fish helps to prevent cardiovascular diseases, in children promotes healthy growth and development of body tissues (MZ, 2010). According to INGR (2010) has from nutritional point of view it is advantageous the presence of potassium and sodium in fish meat. We get really high amount of potassium from fish, but on the other hand a relatively small amount of sodium.

The nutritional value of fish meat is derived from its chemical composition and is highlighted by relatively huge utilization of nutrients by human body. Overall, the nutritional value of seafood is much higher than the freshwater fish. Fish proteins have high biological value. They are well-digestible and usable, especially for the absence of collagenous proteins of fibrous tissues. Advantage of biological value of fish meat is also for positive content of essential amino acids. The nutritional value is excellent due to the lipids in liver and muscle tissues, especially seafood. The lipophilic vitamin A, D and essential fatty acids are also present in seafood in high amount. The most important minerals obtained from fish meat are iodine, calcium and phosphorus (INGR, 2010).

The concentrations of heavy metals in the muscle tissues are the most important, because they are the most common consumed part of fish. This valid for seafood and also for freshwater fish (CANLI et.al., 1998)

3.4.2. Influence of environment on fish population and quality of their production

However in fish and aquatic animals can occur certain amount of harmful substances. The lowest concentrations of these harmful substances are found in fish from aquaculture. Usually these fish are the market ones.

For evaluation of the health risks arising from fish consumption are monitored these toxic substances:

- Toxic metals (Mercury, Methylmercury = toxic form of mercury, lead, cadmium)
- Hazardous organic compounds (DDT, PCBs)

Generally it is expected, that older fish leads to higher accumulation of toxic substances (MZ, 2010).

3.5. Contamination of environment by toxic metals

The heavy metals constitute the largest problem in the area of harmful contaminants of inorganic origin. Heavy metals are defined as a group of elements between copper and lead in the periodic table of elements with a density greater than 5 $g.cm^{-3}$. The most dangerous is cadmium, as well as lead, mercury and chromium. However, problems can cause also copper, zinc, molybdenum, nickel, etc. Some sources say this descending order of contaminants with harmful effects: for crop production: Hg, Pb, Cd, etc.; _ for animal production: Cd, Hg, Pb,

The pollution by heavy metals is related to their accumulation in soil, plant and animal material (GREENWOOD and EARNSHAW, 1993), (BRANDL, 2005).

The occurrence of chemical contaminants in various food stuffs is a serious problem at the global level. In fish it is related with pollution of flowing water, dams, seas and oceans. The most serious contamination in fish and fish products is by mercury, cadmium and polychlorinated biphenyls (INGR, 2010). WHO set for contaminants the

detection limits and additive value ADI (Acceptable Daily Intake). ADI is for substances expressed in mg per 1 kg of body weight and means the dose that can be received during whole life. It is determined with usage of a safety factor of 100, but for the health status is better less drawing from the ADI limit (INGR, 2010).

Accumulation of selected heavy metals in tissue of fish, is influenced by concentrations of metals in food organisms and water, exposure duration and factors on physiochemical bases (JEZIERSKA and WITESKA, 2001)

3.5.1. Source of Pb, Hg, Cd, As in atmosphere

Gaseous, liquid and solid proportions of trace substances in different composition and origin are raised into the air and can affect many atmospheric processes as decreasing of visibility, causing various thermal changes and formation of clouds.

Transport of substances in the atmosphere (MILLER and ROBINSON, 1988).
a) Local Transport
b) Regional transport
c) Global transport

Exception in this is mercury, which can be transported for a long distances without chemical changes (PETERSEN, 1989)

Trace elements are mainly distributed in the atmosphere from combustion processes, this become a global problem (CIBULKA, 1991).

3.5.2. Source of Pb, Hg, Cd, As in water environment

At least in small amount are contains of almost all metals in waters. The enrichment of water by heavy metals occurs by contact of water with rocks and soil, volcanic activity and anthropogenic pollution from industrial agglomerations (PITTER, 1999).
Currently is increasing the water contamination by heavy metals and if their income

exceeds the body's ability to excrete, these substances can cause toxicity. Some substances are not capable of secreting by organisms. These elements accumulate in tissues and can be particularly dangerous (POPL and FÄHNRICH, 1999).

3.5.3. Source of Pb, Hg, Cd ,As in soils

Contamination of soil is sorted with water and wind erosion, land degradation by declining of organic matter and disruption of water processes, which could affect the soil functions. The contamination of soils involved many inorganic and organic substances, the source in the soil can be of natural processes and anthropogenic activity. The potential hazards of these substances is assessed in terms of ecological toxicology (effects on other components of the ecosystem), in terms of human toxicology (acting on the human organisms) and in economic term (reduction of profitability of crop production) (BENEŠ, 1993).

According to BENEŠ (1993), who described the three possibilities of entrance of metals into the soil:

1) natural - primary (formed minerals, rocks, bearings)

2) natural - secondary (consisting of various products of natural processes such as dust storms, often with a high content of organic matter, or volcanic activity, atmospheric fallout and precipitation)

3) anthropogenic (due to wide range of human activities, the source of elements is in the application of various raw materials, fertilizers, pesticides, fly ash, sludge, irrigation water, etc.)

Under the normal conditions are metals found in soil in rather small quantities, but on the other hand due to the anthropogenic influences their content in the soil increases. This is related to the toxicity of these metals, which depends on their in the soil. Hence is necessary to monitor the contents of elements in soils, plants, food chain and also in human population (ADRIANO, 2010).

Increasing potential of gold mining was observed in Mongolia since 2003. About 3000 tonnes of gold are the reserves of Mongolia. The gold mining in 2002 yielded 12.1 tonnes in Mongolia. From hard rock the gold is isolated using mercury by untrained

employees. This situation is very reduced. But informal gold mining rapidly increases in Mongolia. And this process will persist for several decades (MURRAY, 2003).

Soils polluted with arsenic can be considered as agents of health hazards of plants, animals and also human (SMITH et.al., 1998).

3.5.4. Metals in living organisms

CIBULKA (1991) mentioned that heavy metals, namely Hg, Pb, Cd, are in the organs and tissues of fish found in inorganic form, but mainly in organic compounds. For example all the mercury in muscle tissues of fish is in the form of toxic organic compounds, methylmercury.

Specific pollution represents substances, which can cause damages, death of fish or accumulation in tissues. In extremely high consumption of contaminated fish can damage human health. They are mainly different metals and their salts, which get into the water from production processes or at their usage in nature. Among the most harmful substances belongs the mercury, lead, copper, cadmium, aluminium, chromium, manganese or iron. A special subset of elements is consist of compounds and elements with the effect of bioaccumulation. These substances are able to be stored in tissues of fish (muscle, liver, spleen, kidneys, etc.) even if they not cause their death directly. This group includes mainly heavy metals as mercury, lead, copper, radioactive elements and polychlorinated biphenyls (DUS, 2010)

The bioaccumulation of metals in an animal depends on a multitude of factors: biotic ones, like its body dimensions and mass, age, sex diet and metabolism. An abiotic factors are known as the distribution of metals in its environment, salinity, temperature, pH of the water, habitat type, and interactions with other metals.

> The complex process named bioaccumulation is requiring the simultaneous examination of metal levels in the tissue of animals from at least two adjacent trophic levels. Most commonly, metals concentrations are higher in larger animals. The reason is that they are the end members of the chain, so the source of food are smaller organisms (BRANDL,2005),(O´NEILL, 1995).

The levels of Pb and Cd detected in muscle tissue of livestock animals were constantly low, commonly equal to limits or below. The concentrations of both metals

occurred with higher frequency in liver and kidney than in muscle tissue, but rarely exceed O,5g/kg for Pb,or 1g/kg for Cd (LARS, 1999).

3.6. Absorption of metals

BRANDL (2005) published the main ways contamination of organism lead through the atmospheric disposal in soil and water, over the contaminated food and water.

Most of the compounds of metal are moved through food and drinking water. In case of exposure to dust with content of arsenic, cadmium, lead in metal smelters is the major pathway in inhalation (MERIAN, 1991).

Gates of selected metals to organism are lungs and digestion tract. Possibility could be also the absorption through skin (BENCKO et al., 1995).

3.6.1. Cadmium

Cadmium is an element belonging to the group of heavy metals. Cd is one of the most dangerous toxic substances, which can easily enter the food chain. From the soil is easily transferred into plants (70%), in smaller rate is taken by plants from the atmosphere (from 20 -40%) (HERČÍK et. al., 1995)

The cadmium accumulation is firstly in tissues of major organs than in muscles (MOORE and RAMAMURTHY, 1984).

Cadmium (Cd) is one of the most dangerous elements in the environment, which penetrates from communal waste in the processing of ores, metals and in the form of superphosphates that are used for fertilization, from sewage sludge and from the combustion of oil and gas. The source may be also the combustion of plastics, coal and fuel (BRANDL, 2005), (BRIMMER, 2011).

Toxicity of cadmium in fish usually includes damage of the kidney or injury of the gills. Often is connected with failure of respiratory functions or asphyxiation. But the worst case could be death (SORENSEN, 1991).

In the human organism the cadmium accumulates in the liver, kidneys, pancreas and genital organs, where damages the epithelium of the testis. From placenta can cadmium penetrate to the fetus and damage it. In women, Cd can cause the infertility (SOVA,1995).

Cadmium causes itai-itai, anemia, cardiac enlargement, gonadal atrophy, failure of kidney, pulmonary emphysema and different bone diseases (KHALLAF et.al., 1998).

3.6.2. Lead

Lead can be found in all parts of nature. It is assumed that in regions, which are not contaminated by human activities yet, the lead concentrations should not exceed 1 ng.m-3 in the atmosphere. Some measurements, which took place in Greenland and in the New Earth, this assumption confirmed (BENCKO et. al., 1995).

The simple ion Pb^{2+} dominates in natural waters from dissolved forms of occurrence, it also depends on the value of pH and concentration of all carbon dioxide. Lead has a high storage rate and therefore accumulates significantly not only in sediments and sludge, but also in biomass of microorganisms and plants. Pb is also considered a potential carcinogen, especially harmful to children's body. Lead is stored mainly in the bones, partly in the blood (BENCKO et al. in 1995).

Lead (Pb), according to earlier data, was found in the biosphere in amount around 440 thousand tons. From this amount was 60% of gasoline, 25% from industries, 8% of mining and 5% from fossil fuels. For further penetration of lead occurs from heating plants, industries of aluminium, mines of magnesite, eventually of battery plants. Gate into the food chain is consists of air, water and soil where lead penetrates into the plants, feed, fish, wildlife and livestock animals and eventually, to human on the top of the food chain.

30-50% of lead intake to the body penetrates by inhalation, in the lungs the lead is bound on the red blood cells and then they disperse it to the whole organism, especially liver, kidney and in mostly to the bones.

In organism has lead a negative effect on antibody production, it can penetrate through the placenta to the fetus and damage it (SOVA, 1995).

Poisoning by Pb can caused vomiting, weight losses, losses of coordination, encephalopathy and insomnia etc. (KHALLAF et.al., 1998).

Lead is associated with adverse outcomes in children. Most common are learning difficulties of impaired neurobehavioral development (MONTORO and VELEZ, 2004).

3.6.3. Mercury

The occurrence of mercury in nature is low, the source is in the mercuric sulfide HgS. It is also part of other sulphide ores, when during the roasting enters the atmosphere. An important source of mercury are atmospheric water, which are contaminated in combustion of fossil fuels, as well as in industrial waste water processing, agriculture, from electrochemical processes and chemical laboratory (PITTER, 1999).
In water is present as metal Hg^0 in the form of inorganic ion Hg_2^{2+},Hg^{2+} and in form of methylmercury CH_3HG^+ and dimethylmercury $(CH_3)_2Hg$).

Toxic substance is methylmercury and ethylmercury (INGR, 2010). They are highly volatile and therefore can easily by aeration move from the water into the atmosphere. A significant problem is the high toxicity of mercury and strong ability to accumulate in the biomass (especially in animal tissues as kidneys, liver and muscle (POPL and FAHNRICH, 1999).

Mercury (Hg) enters the food chain from the air, soil and water to which contamination occurs from factories, where the mercury is processed. The use of mercury in agriculture and discharge waste of mercury into waterways threatens fish, birds and wild animals (ADRIANO, 2001).
Mercury is the most toxic heavy metals, which can damage the nervous system, kidneys, liver, and can penetrate also into the fetus. In adults, mercury poisoning people with signs of disorders of perception, may be a partial paralyzed of hands, tongue and around the mouth. Damages could be a failure of memory, hearing and hallucinations may occur (SOVA, 1995).

Average content of mercury in seafood is usually below 0,1 and almost always below 0,15 mg/kg, in freshwater it is below 0,2 mg/kg (INGR, 2010)

3.6.4. Arsenic

STOEPPLER (1992) mentioned that major anthropogenic sources of arsenic are related to the agriculture, forestry and manufacturing. Another are mining, rendering operations, coal and wood burning (STOEPPLER, 1992).

The arsenic in different form can penetrate the soils in the solid phase (tailings). The process of chemical oxidation of the tailings is the reason of soil contamination. The arsenic can be accumulated in polluted soils because it is only partially removed by leaching, methylation, and erosion or it is slightly taken up and accumulated by plants. Different forms of arsenic observed in soils can be classified into two groups : organic and inorganic compounds. Inorganic compounds have different water solubility than organic compounds thus they appear in soil with higher frequency (AQUILAR et.al., 2007).

The level of arsenic, appeared in soils used in agriculture, does not cause a serious environmental problem. However there could be individual areas associated with deposits of silver and gold and connected with elevated concentrations (KRÁLOVÁ et.al., 2010)

Pollution of water with arsenic is problematic. The two main form of arsenic can accumulate in organisms in case of high affinity of proteins, cell compounds and lipids (ADRIANO, 2001).

For human is the major hazard drinking of contaminated water, inhalation and ingestion Organs with the high accumulation effect are gastrointestinal tract, liver, kidney,skin and circulatory system (BRANDL, 2005).

3.7. Health prevention

After all findings and analyses, everything should be compared with hygienic limits of selected heavy metals and there have to be the requirement for safe consumption of food. Following the results and limits, there should not be found a value above the hygienic limit.
Mercury is a contaminant, which limits the consumption of fish meat at the level of food safety (MZ, 2010).

Hygienic limit allows the daily intake of heavy metals in all specific natural areas (ANDREJI et.al., 2006), (CIBULKA,1991)

In 1998 HAN et.al. highlighted that extremely high values of metals can lead to serious problems with health or even to death.

3.8. Methods suitable for analysis in biological material

Monitored heavy metals had values, which were introduced on a wet weight basis in mg/kg and compared with Legislation of EU, especially EC Directive 2001/22 (ANDREJI et.al., 2006).

The maximum of residue levels in food is regulated by Regulation of European Commission No1881/2006.

Regular and systematic monitoring is recommendable, in case of environmental and public health aspects (DJEDJIBEGOVIC et. al., 2012).

Atomic absorption spectrometry (AAS) is one of the most widely used analytical methods for trace element analysis. Allows to determine up to 68 elements, all metal and metalloids at low concentration (KOMÁREK, 2000), (ČERNOHORSKÝ and JANDERA, 1997).

Flame atomic absortion spectrometry (FAAS)

The oldest type of atomization is the atomization in the flame. The principle of this method is to convert the solution in the aerosol mist and the introduction of aerosol into the mixture of flame. The FAAS is most commonly used the flame of acetylene - air.

Due to this is possible to determine more than 30 elements (ČERNOHORSKÝ and JANDERA, 1997).

Because the sensitivity of flame AAS is less than that would be sufficient to tackle range of analytical problems (environmental analysis, analysis of raw chemicals, etc.) were searched new atomization technologies. One option is to atomize the sample in Electrothermal atomizer (ETA) (ČERNOHORSKÝ and JANDERA, 1997). Currently in electrothermal atomizers are analyzed liquid samples, or well homogenized suspension, which can be dose in the same form as in liquid one. The volumes range from 5 to 100 μl, due to the dependence on the type of atomizer and dosed liquid. The main advantage of flameless atomizer is the fact that the entire quantity involved in the absorption of primary radiation. It is attained much higher concentration of free atoms in the gas phase at very low volume of atomizer (KOMÁREK, 2000), (ČERNOHORSKÝ and JANDERA, 1997).

Atomic emission spectrometry is based on registration of photons, which were developed by transition of valence electrons from huger energy status to lower.

During the AES is measured radiation of emitted atoms or ions in the excited status, which is created by the deexcitation (ČERNOHORSKÝ and JANDERA, 1997).

Inductively coupled plasma mass spectrometry is a sensitive method for measurement of environmental pollution. This method is usually used for quantification of trace heavy metals such as Pb, As, Cd etc. (HOLÁ et. al., 2009).

This method is characterised low detection limits, wider linear dynamic range, simple spectral interpretation, less matrix, capability of simultaneous multielemental measurement or spectral interferences (DJEDJIBEGOVIC J. et. al., 2012).

4. Materials and Methods

Monitoring of contaminants in food chain is really necessary to do every year. Controlling of contamination of food, feed and other materials for production and biomonitoring of animals have to be done regularly. In fish have to be done analyses for detection of heavy metals and other pollutants, because they may caused serious diseases and there is a risk of health problems for human. The European Commission makes the protection of public health by determination of hygienic limits a lot of elements. Also methods for samplings, preparation of samples and analyses of heavy metals in fish tissues are describes in Directive 2001/22 of EC.

Fish tissue samplings were used for detection of presence of heavy metals in tissue of fish, all samples were examinated with cooperation with State Veterinary Administration of the Czech Republic (SVA CR), when were analyzed 4101 samples (Common carp (*Cyprinus carpio)*, Trout and other freshwater fish) during last 10 years. All samples were collected in rivers and ponds from the Czech Republic. Further was taken the opportunity to compared selected elements in Czech fish samples with Mongolian fish samples in sum of 20 samplings (*Esox lucius, Silurus glanis, Rutilus rutilus, Perca fluviatilis etc.*). The quantitative content of individual heavy metals was collated in two groups of analyzed samples, from the Czech Republic and Mongolia.

4.1. Monitoring in the Czech Republic

Total sum of samples of tissue of fish (originally from Czech rivers and ponds), which were used for analyses, was 4101 samplings. Samples were divided into three main groups: Common carp, trout and other freshwater fish. For detection of presence of selected pollutants (lead, mercury, cadmium, arsenic) was used the database of State Veterinary Administration of the Czech Republic, which monitored and analyzed representative samples from the whole Czech Republic every year, follow the legislation of European Union (Regulation of European Commission No.1881/2006). Relevant data were extracted from annual report of last 10 years (2000- 2010) and their content was mainly focused on contamination of foreign substances in the food chain and animal production.

4.2. Monitoring in Mongolia

Collection of samples was performed in Mongolian filed conditions from August to October 2011 by my colleague Bc.Ondřej Šimoník. Samples were obtained at fish markets or directly harvested in Mongolian rivers and lakes. Origin of samples was from Khuvsgul area and Arkhangai (see Tab.1 and Fig.5). The most frequent occurrence of heavy metals, which are suitable for analyses are mainly in liver or muscle tissue of fish (BRANDL, 2005). Fish tissue samples were stored in sample tubes with screw caps and fixed with 96% of ethanol.

Afterwards the samples were prepared for transport in cooperation with the Mongolian Veterinary Services and delivered to laboratory of State Veterinary Institute in Prague- Lysolaje (SVI).

Tab.1 –Origin of Mongolian freshwater fish samples

Mongolian freshwater fish samples		
sample	**Organ**	**Region**
1	Muscle	Khuvsgul
2	Muscle	Arkhangai
3	Liver	Arkhangai
4	Liver	Arkhangai
5	Muscle	Arkhangai

Fig.5 – The map of Mongolia with areas of the samples

4.3. Preservation and storage of samples

Fish tissue samples (liver and muscle tissues) with weight approximately 1-2 g were placed into micro sampling tubes of 2 ml volume and fixed with 96% ethanol (see Fig.6) The volume of ethanol was ensured by using calibrated pipette in volume of 1 ml. Closed and described micro sampling tubes were sorted into sets and stored at room temperature ±20 °C. Their transport was approved by the Mongolian Veterinary Services in cooperation with the State Veterinary Institute in Prague-Lysolaje in the Czech Republic.

Fig.6- micro sampling tube with sample

Fig.7- State Veterinary Institute in Prague

State Veterinary Institute is an organization established by the Ministry of Agriculture of the Czech Republic (see Fig.7). A lot of activities are done in SVI. It performs health tests for animals (needed for export or import), diagnosis of infectious animal diseases (Avian Influenza, Bovine Spongiform Encephalopathy, Rabies and Trichinella), analyses for determination of non-infectious diseases, hematological and biological tests, analyses of death animals (toxicological tests), examination and monitoring of food, food safety (for import and export), complex chemical analyses of food; water and feed, bacteriological and chemical analyses of water and feed. Finally, professionals provide training and educational activities.

With both organizations in the Czech Republic and in Mongolia works the Czech University of Life sciences Prague, especially Institute of Tropics and Subtropics, based on long tradition (2007-2009 and following 2010-2012 Mongolian projects). All analyses were done under the professionals from department of chemistry, namely by Ing. Jan Rosmus and technician Ing. Ivana Buhrová

4.4. Frequency of analyses

All analyses were performed with the assistance of named specialists from SVI Prague during the normal laboratory conditions in period of one month.

Due to the different determination of heavy metals, methods were non similar in preparation and detection of elements. Some analyses were done together (determination of lead, cadmium and arsenic) or individually (determination of mercury). Results from analyses were reported in laboratory protocols and tables, then were compared with standards according to Regulation of European Commission No. 1881/2006, to made a decision whether are suitable or non suitable with strict hygienic limits.

For detection of cadmium, lead and mercury is most common used method of Atomic Absorption Spectrometry (AAS), but also method of Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), which were done in cooperation with technician Ing. Ivana Buhrová from laboratory of chemistry in SVI Prague. All instruments and methods are certified to ensure the quality and accuracy of results. They also have valid accreditation (see App.1) and all outputs were on high level of diagnosis and treatment also required by legislation of EU.

4.5. Laboratory methods

Detection of concentration of selected elements was done according to standard operation technique, which has been established by State Veterinary Institute (SVI) in Prague- Lysolaje.

For sample treatment was used concentrated nitric acid (68,4% HNO_3), suprapure, hydrogen peroxide ($H_2O_{2)}$, demineralized water (without all ion soluble matters and silicon).

Microwave digestion of examined tissue was proceed in equipment ETHOS PLUS from MILESTONE company. For determination of heavy metal presence (Pb, Cd, Hg, As) was used Inductively Coupled Plasma- Mass Spectrometry (ICP-MS) method, which was performed on ICP-MS device from Australian company VARIAN. Control measurement was done by atomic absorb spectrometer SPECTRA AA 220Z also from VARIAN company. Determination of mercury took place in equipment atomic mercury analyzer (AMA 254), ALTEC producer.

As standard reference materials (SRM) for our analysis were used products of National Institute of Standards and Technology, Oyster tissue – SRM NIST 1566b and Bovine liver – SRM NIST 1577c.

Due to fixation of samples with 96% ethanol, before microwave digestion all preservative matter has to be evaporated. Mixed sample was inserted to mineralization vessel and left in laboratory temperature at least 2 days. After it our samples were put in to microwave device for process of sample mineralization (see Fig.8)

4.5.1. Mineralization by microwave device

This procedure is used to elimination of organic materials from sample by oxidation and then transformation of analyte to form of inorganic solution. Microwave digestion performed in high pressure teflon vessels, which were properly washed and treated with diluted nitric acid.

Fig.8- Microwave device in laboratory

Fig.9- Samples ready for analyses

4.5.2. Methodological procedure

Mixed sample composed from 2-3 partial samples (every time separately liver and muscle), in weight from 1-3 g was placed into teflon vessels. Afterwards 4 ml of concentrated nitric acid were added. After 30 minutes were added 2 ml of concentrated hydrogen peroxide (30%). Mixture was covered by cap, left for at least 1 hour to react, applicated into the plastic segment and closed by moment key. This way adjusted sample was put in to the microwave device for 20 minutes. Consequently cooled down vessels mineralizate was quantitatively transformed by demineralized water to volumetric flask. Adjusted sample was prepared to sequential analysis (See Fig.9)

4.5.3. Measurement of heavy metals

For content identification lead, cadmium, arsenic was used method which combined inductively coupled plasma and mass spectrometry (ICP-MS), using by isotopes Pb^{207}, Cd^{111}, As^{75}.

Before measurement was reduced content of nitric acid by dilution with demineralized water, in case of possibility of damage to ICP-MS device. Samples were applied to small vials, which were put in to carousel of autosampler and then automatically evaluated. Control measurement was performed by device SPETRAA 220Z, due to the elimination of errors.

Before determination of mercury concentration, the removal of nitric acid overspill was not recommended because of potential losses of quantified element, hence dilution of mineralizate was done. The measurement of mercury was proceed by atomic absorb spectrometer (AMA 254). Quantity of 200 µl of sample was put on weighing boat, which was inserted to AMA 254 and there was sample dried, thermal decomposition in oxygen flow and consequently detection and measuring of mercury concentration. (see Fig 10 +Fig. 11)

Fig.10- the weighing boat

Fig.11-The ICP-MS device

5. Results

All results were compared with Regulation of European Commission No. 1881/2006, which have to follow all member states of European Union. For all hygienic limits was used this Regulation (Cd, Pb, Hg). Just the hygienic limit for arsenic is not detected by this Regulation, so in this case was used hygienic limit of Czech State Veterinary Administration.

Fig.12: The map of contaminated areas in the Czech Republic

5.1. Common carp in Czech Republic (2000-2010)

Analyzed samples of Common carp *(Cyprinus carpio),* from muscle tissue origin, contained the selected elements (cadmium, lead, mercury, arsenic), which were almost in all cases at low hygienic limits. During the last ten years was total sum of samples 2538

samplings. Just 9 samples were over the hygienic limit established by European Union (see tab.2). For example finding of lead in two samples was thirteen times higher than the hygienic limit of EU, detection limit of lead is 0, 300 mg/kg. Similarly in 2004 the finding of mercury in muscle tissue of carp even three times higher than hygienic limit of EU for this element. The Arsenic detection was two times above limit, which was set by State Veterinary Administration of the Czech Republic, because EU still did not set the hygienic limit for the finding of this element in muscle tissue of fish (see Tab.4, Fig.13,14,15,16)

Tab.2- Values of As and Cd in tissues of Common carp *(Cyprinus carpio)* in 2000-2010

ARSENIC in Common Carp					
YEAR	HL	N of samples	Increased value	Over the HL	MAX. value
2000	1,000 mg/kg	66	0	0	
2001	1,000 mg/kg	102	1	0	
2002	1,000 mg/kg	94	0	0	
2003	1,000 mg/kg	96	0	0	
2004	1,000 mg/kg	95	0	0	
2005	1,000 mg/kg	83	0	1	1,3 mg/kg
2006	1,000 mg/kg	17	0	0	
2007	1,000 mg/kg	19	0	0	
2008	1,000 mg/kg	17	0	2	2,31-2,4 mg/kg
2009	1,000 mg/kg	18	0	0	
2010	1,000 mg/kg	13	0	0	
Σ		620	1	3	
CADMIUM in Common Carp					
YEAR	HL	N of samples	Increased value	Over the HL	MAX. value
2000	0,100 mg/kg	60	0	0	
2001	0,100 mg/kg	102	3	0	
2002	0,100 mg/kg	94	0	0	
2003	0,050 mg/kg	96	1	0	
2004	0,050 mg/kg	95	4	1	0,083 mg/kg
2005	0,050 mg/kg	87	7	1	0,078 mg/kg
2006	0,050 mg/kg	17	0	0	
2007	0,050 mg/kg	19	0	0	
2008	0,050 mg/kg	18	0	0	
2009	0,050 mg/kg	20	0	0	
2010	0,050 mg/kg	15	0	0	
Σ		623	15	2	

Tab.3- Values of Hg and Pb in tissues of Common carp *(Cyprinus carpio)* in 2000-2010

MERCURY in Common Carp					
YEAR	HL	N of samples	Increased value	Over the HL	MAX. value
2000	0,500 mg/kg	60	0	0	
2001	0,500 mg/kg	115	4	0	
2002	0,100 mk/kg	94	0	1	0,113 mg/kg
2003	0,100 mg/kg	96	18	0	
2004	0,100 mg/kg	95	14	1	0,449 mg/kg
2005	0,500 mg/kg	87	0	0	
2006	0,500 mg/kg	17	0	0	
2007	0,500 mg/kg	19	0	0	
2008	0,500 mg/kg	29	0	0	
2009	0,800 mg/kg	33	0	0	
2010	0,500 mg/kg	27	0	0	
Σ		672	36	2	
LEAD in Common Carp					
YEAR	HL	N of samples	Increased value	Over the HL	MAX. value
2000	0,500 mg/kg	60	0	0	
2001	0,500 mg/kg	102	0	0	
2002	0,500 mg/kg	94	0	0	
2003	0,200 mg/kg	96	9	0	
2004	0,200 mg/kg	95	5	0	
2005	0,200 mg/kg	87	2	0	
2006	0,200 mg/kg	17	0	0	
2007	0,200 mg/kg	19	0	0	
2008	0,300 mg/kg	18	0	2	3,8-4,01 mg/kg
2009	0,300 mg/kg	20	0	0	
2010	0,300 mg/kg	15	0	0	
Σ		623	16	2	

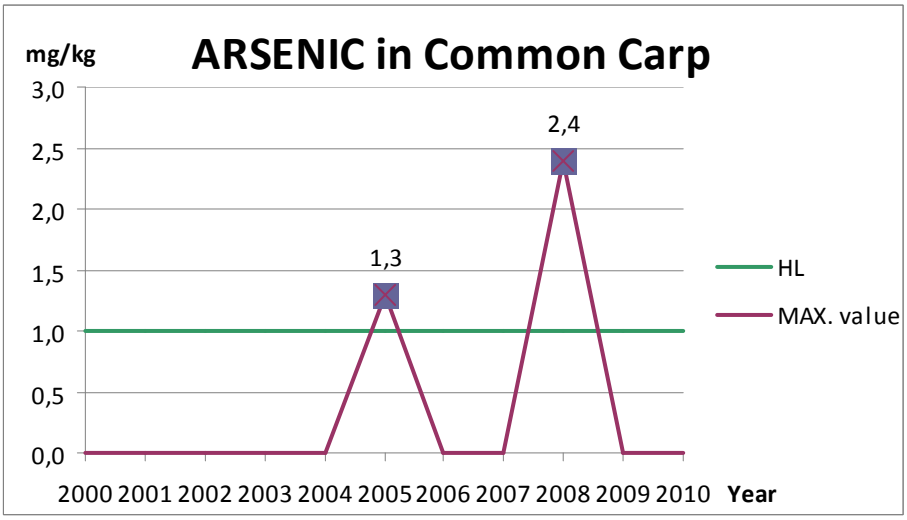

Fig.13- Maximum value of Arsenic in Common carp in CR

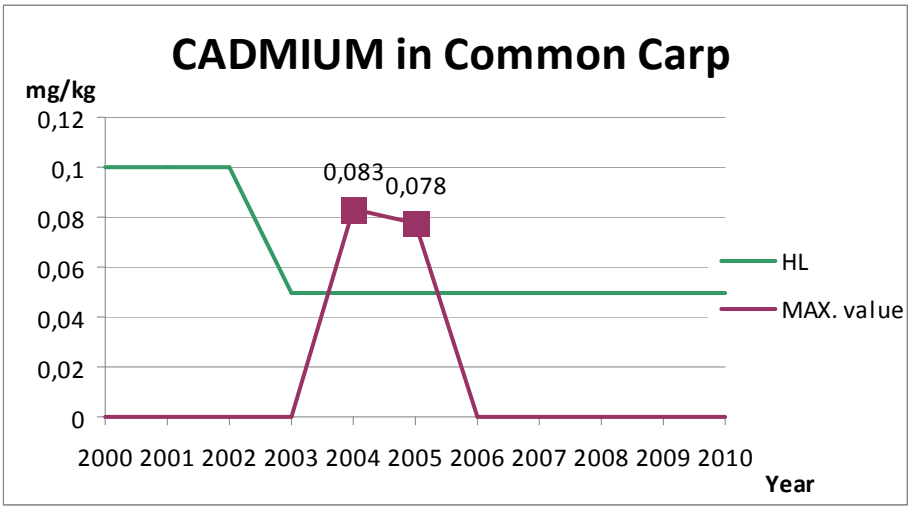

Fig.14- Maximum value of cadmium in Common carp in CR

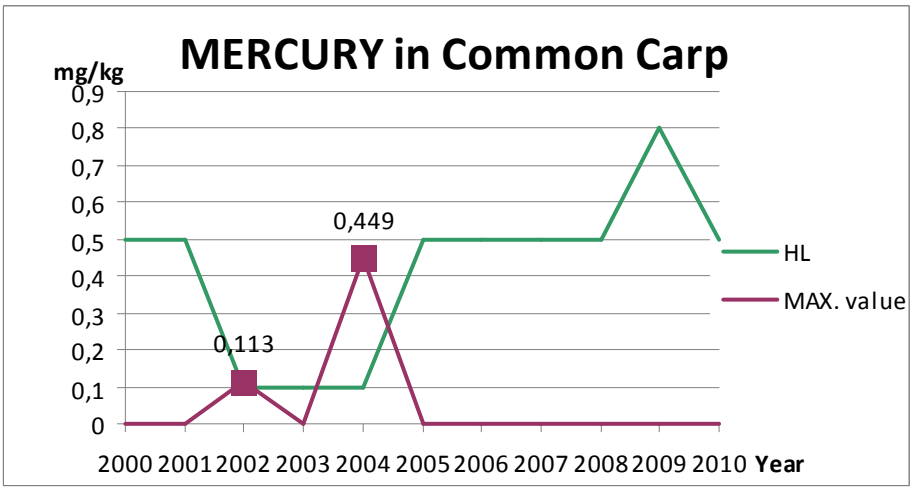

Fig.15- Maximum value of mercury in Common carp in CR

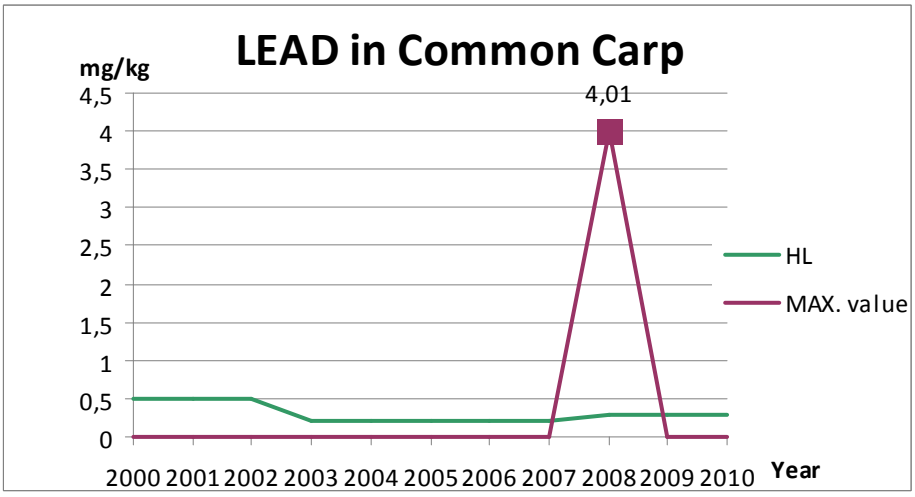

Fig.16- Maximum value of lead in Common carp in CR

5.2. Trout in Czech Republic (2000-2010)

The situation in species of trout during the monitoring of heavy metals in conditions of the Czech Republic was much favourable. Between the years of 2000 to 2010 were all selected chemical elements (Cd, Hg, Pb, As) under or right at the barrier of hygienic limits. Excess finding were detected in muscle tissue samples of trout. The presence of 4 selected elements, from all 485 samples of trout muscle tissues, was with

positively detected in 13 samples during last ten years and were over set parameters (see tab.4+5).The highest value of arsenic was 3,6 mg/kg, however the limit in the Czech Republic is set for 1,0 mg/kg. This means, that the hygienic limit was exceed approximately about 300%.(See Fig.17)

Tab. 4- Values of As and Cd in tissues of Trout in 2000-2010

ARSENIC in Trout					
YEAR	HL	N of samples	Increased value	Over the HL	MAX. value
2000	1,000 mg/kg	18	4	0	
2001	1,000 mg/kg	31	11	1	1,157 mg/kg
2002	1,000 mg/kg	20	7	0	
2003	1,000 mg/kg	15	2	3	1,110-1,180 mg/kg
2004	1,000 mg/kg	16	7	5	1,270-3,600 mg/kg
2005	1,000 mg/kg	13	4	4	1,09-2,35 mg/kg
2006	1,000 mg/kg	3	3	0	
2007	1,000 mg/kg	3	0	0	
2008	1,000 mg/kg	4	2	0	
2009	1,000 mg/kg	2	0	0	
2010	1,000 mg/kg	1	1	0	
Σ		126	41	13	
CADMIUM in Trout					
YEAR	HL	N of samples	Increased value	Over the HL	MAX. value
2000	0,100 mg/kg	18	0	0	
2001	0,100 mg/kg	16	0	0	
2002	0,100 mg/kg	20	0	0	
2003	0,050 mg/kg	15	1	0	
2004	0,050 mg/kg	16	0	0	
2005	0,050 mg/kg	15	0	0	
2006	0,050 mg/kg	3	0	0	
2007	0,050 mg/kg	4	0	0	
2008	0,050 mg/kg	4	0	0	
2009	0,050 mg/kg	2	0	0	
2010	0,050 mg/kg	1	0	0	
Σ		114	1	0	

Tab.5- Values of Hg and Pb in tissues of Trout in 2000-2010

MERCURY in Trout					
YEAR	HL	N of samples	Increased value	Over the HL	MAX. value
2000	0,500 mg/kg	18	0	0	
2001	0,500 mg/kg	31	0	0	
2002	0,500 mg/kg	20	0	0	
2003	0,500 mg/kg	15	0	0	
2004	0,100 mg/kg	16	0	0	
2005	0,500 mg/kg	15	0	0	
2006	0,500 mg/kg	3	0	0	
2007	0,500 mg/kg	4	0	0	
2008	0,500 mg/kg	8	0	0	
2009	0,800 mg/kg	6	0	0	
2010	0,500 mg/kg	6	0	0	
Σ		142	0	0	
LEAD in Trout					
YEAR	HL	N of samples	Increased value	Over the HL	MAX. value
2000	0,500 mg/kg	18	0	0	
2001	0,500 mg/kg	5	0	0	
2002	0,500 mg/kg	20	0	0	
2003	0,200 mg/kg	15	0	0	
2004	0,200 mg/kg	16	0	0	
2005	0,200 mg/kg	15	1	0	
2006	0,200 mg/kg	3	0	0	
2007	0,200 mg/kg	4	0	0	
2008	0,300 mg/kg	4	0	0	
2009	0,300 mg/kg	2	0	0	
2010	0,300 mg/kg	1	0	0	
Σ		103	1	0	

5.3. Freshwater fish in Czech Republic (2000- 2010)

Monitoring of heavy metals was settled also in other freshwater fish. Also the main detected elements in animal tissues were cadmium, arsenic, lead and mercury. Total sum of analyzed samples was 1078 during last ten years. Positive cases were recorded in arsenic and mercury, when in both elements was find out the exceed limit in 13 samples. Values of arsenic were slightly over the hygienic limit (see Tab8+9.) On the other hand the values of mercury were extremely over the hygienic limit, in 2003 was the maximum value of samples 0,585 mg/kg (hygienic limit for this year was 0,100 mg/kg in CR).

5.4. Freshwater fish in Mongolia (August 2011- October 2011)

Mongolian fish tissue samples (*Esox lucius, Silurus glanis, Rutilus rutilus, Perca fluviatilis etc.*) were obtained from liver and muscle. Increased values over the hygienic limit were detected in cadmium and mercury (see tab 8). In samples from Arkhangai area was the value of cadmium from 0,12 mg/kg to 0,19 mg/kg in muscle and liver tissue. Positive finding were in 3 samples and the limit for cadmium was 0,050 mg/kg. Similarly the content of mercury was 0,880 mg/kg, however the hygienic limit due to the Regulation of EU was set as 0,500 mg/kg. This sample had the origin also in Arkhangai, when the analyzed part of fish was from liver tissue (see tab.9). Analyses of heavy metals in fish tissues were done according to EU methodology and in certified laboratories on department of the State Veterinary Institute in Prague- Lysolaje. In other elements (As and Pb) the limits were not exceeded.(see Tab.6,7,8,9 and Fig. 18,19,20).

Tab. 6- Detail of analyses of all Mongolian samples

ARSENIC in Mongolia Fish		
	HL	Value
1		0,11 mg/kg
2		0,08 mg/kg
3		0,08 mg/kg
4		0,05 mg/kg
5		0,19 mg/kg

CADMIUM in Mongolia Fish		
	HL	Value
1	0,050 mg/kg	0,005 mg/kg
2	0,050 mg/kg	<0,005 mg/kg
3	0,050 mg/kg	0,12 mg/kg
4	0,050 mg/kg	0,19 mg/kg
5	0,050 mg/kg	0,150 mg/kg

MERCURY in Mongolia Fish		
	HL	Value
1	0,500 mg/kg	0,043 mg/kg
2	0,500 mg/kg	0,261 mg/kg
3	0,500 mg/kg	0,030 mg/kg
4	0,500 mg/kg	0,880 mg/kg
5	0,500 mg/kg	0,247 mg/kg

LEAD in Mongolia Fish		
	HL	Value
1	0,300 mg/kg	0,09 mg/kg
2	0,300 mg/kg	<0,05 mg/kg
3	0,300 mg/kg	0,13 mg/kg
4	0,300 mg/kg	<0,05 mg/kg
5	0,300 mg/kg	0,06 mg/kg

Tab.7- Hygienic limits by EU

Element	HL by EU
Cadmium	0,050 mg/kg
Mercury	0,500 mg/kg
Lead	0,300 mg/kg

Tab. 8- Values of As and Cd in tissues of other freshwater fish in CR (2000-2010) and MNG (2011)

ARSENIC in other freshwater fish (CR)					
YEAR	HL	N of samples	Increased value	Over the HL	MAX. value
2000	1,000 mg/kg	106	4	1	1,280 mg/kg
2001	1,000 mg/kg	23	0	0	
2002	1,000 mg/kg	68	0	1	1,440 mg/kg
2003	1,000 mg/kg	32	9	1	1,270 mg/kg
2004	1,000 mg/kg	21	0	1	1,540 mg/kg
2005	1,000 mg/kg	5	0	0	
2006	1,000 mg/kg	2	1	0	
2007	1,000 mg/kg	2	0	0	
2008	1,000 mg/kg	1	0	0	
2009	1,000 mg/kg	1	0	0	
2010	1,000 mg/kg	1	1	0	
Σ		262	15	4	
ARSENIC in other freshwater fish (MNG)					
2011		5	0	0	
Σ		5	0	0	
CADMIUM in other freshwater fish (CR)					
YEAR	HL	N of samples	Increased value	Over the HL	MAX. value
2000	0,100 mg/kg	106	0	0	
2001	0,100 mg/kg	25	1	0	
2002	0,100 mg/kg	68	0	0	
2003	0,050 mg/kg	32	1	0	
2004	0,050 mg/kg	21	1	0	
2005	0,050 mg/kg	6	0	0	
2006	0,050 mg/kg	5	0	0	
2007	0,050 mg/kg	2	0	0	
2008	0,050 mg/kg	1	0	0	
2009	0,050 mg/kg	1	0	0	
2010	0,050 mg/kg	1	0	0	
Σ		268	3	0	
CADMIUM in other freshwater fish (MNG)					
2011	0,050 mg/kg	5	0	3	0,12-0,19 mg/kg
Σ		5	0	3	

Tab.9- Values of Hg and Pb in tissues of other freshwater fish in CR (2000-2010) and MNG (2011)

MERCURY in other freshwater fish (CR)					
YEAR	HL	N of samples	Increased value	Over the HL	MAX. value
2000	0,500 mg/kg	106	0	0	
2001	0,500 mg/kg	31	1	1	0,569 mg/kg
2002	0,100 mg/kg	73	0	4	0,163- 0,266 mg/kg
2003	0,100 mg/kg	32	0	2	0,1265-0,585 mg/kg
2004	0,100 mg/kg	21	7	2	0,208-0,361 mg/kg
2005	0,500 mg/kg	6	0	0	
2006	0,500 mg/kg	5	1	0	
2007	0,500 mg/kg	2	0	0	
2008	0,500 mg/kg	1	0	0	
2009	0,800 mg/kg	2	0	0	
2010	0,500 mg/kg	1	0	0	
∑		280	9	9	
MERCURY in other freshwater fish (MNG)					
2011	0,500 mg/kg	5	0	1	0,880 mg/kg
∑		5	0	1	
LEAD in other freshwater fish (CR)					
YEAR	HL	N of samples	Increased value	Over the HL	MAX. value
2000	0,500 mg/kg	106	0	0	
2001	0,500 mg/kg	25	0	0	
2002	0,500 mg/kg	68	0	0	
2003	0,200 mg/kg	32	0	0	
2004	0,400 mg/kg	21	0	0	
2005	0,200 mg/kg	6	1	0	
2006	0,200 mg/kg	5	0	0	
2007	0,200 mg/kg	2	0	0	
2008	0,300 mg/kg	1	0	0	
2009	0,300 mg/kg	1	0	0	
2010	0,300 mg/kg	1	0	0	
∑		268	1	0	
LEAD in other freshwater fish (MNG)					
2011	0,300 mg/kg	5	0	0	
∑		5	0	0	

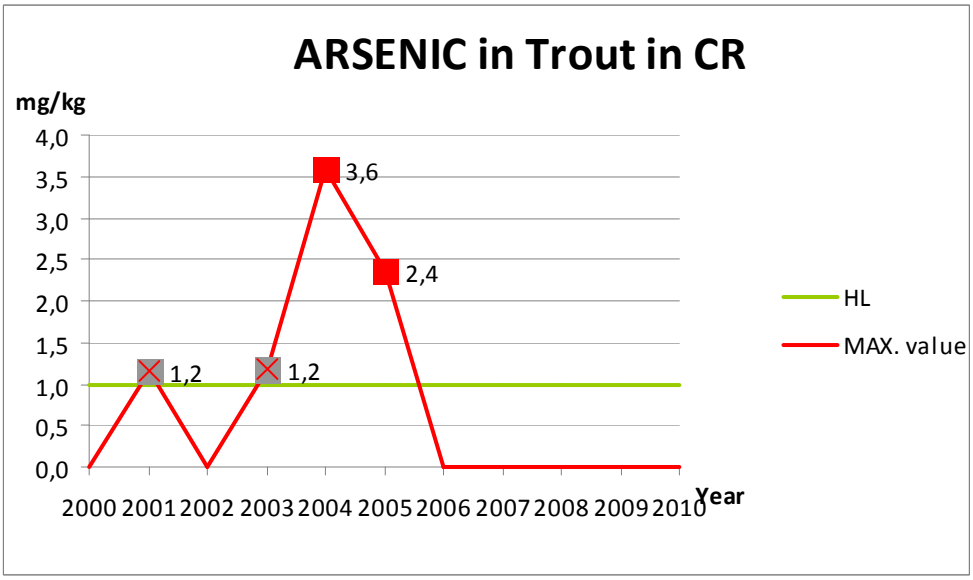

Fig.17- Maximum value of Arsenic in Trout in CR

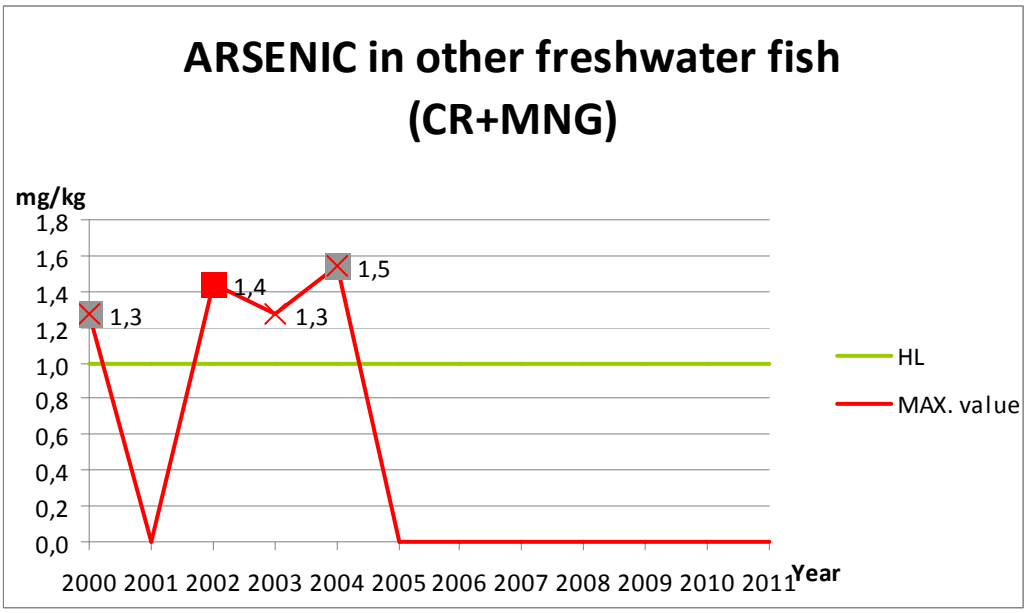

Fig.18- Maximum value of Arsenic in other freshwater fish (CR+MNG)

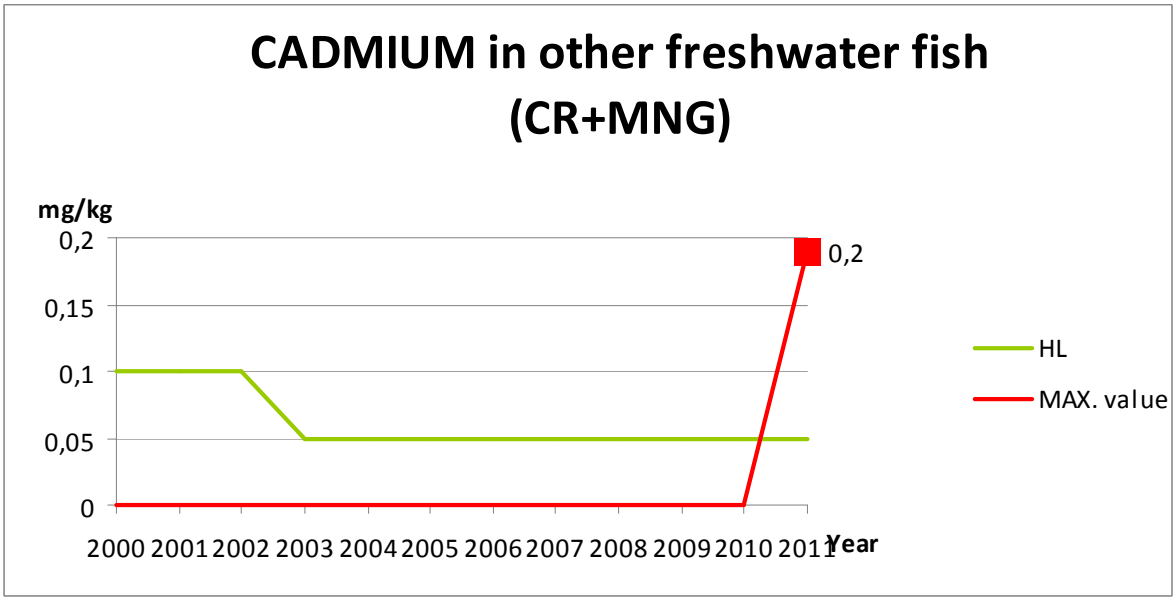

Fig.19- Maximum value of Cadmium in other freshwater fish (CR+MNG)

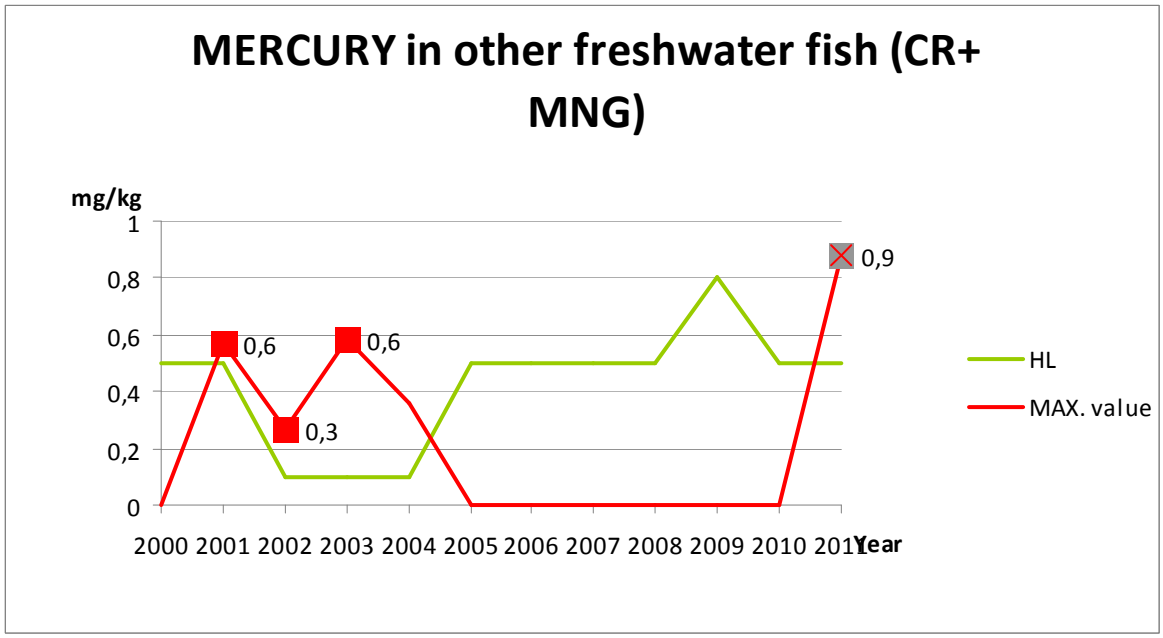

Fig.20- Maximum value of Mercury in other freshwater fish (CR+MNG)

6. Discussions

Fish and fish products reach higher popularity, due to their good nutritional value. LAURETTI (1998) and INGR (2010) agree with the importance of good quality and safety of fish and fish products. Therefore the monitoring of contaminants should be not underestimated. This was confirmed in our analysis, where the periodical monitoring in the Czech Republic showed decreasing values of heavy metals and excess findings were rare. The control of contaminants should be realized mainly in areas, where the possibility of pollution of watercourses and pastures is increased by industrial development, coal and gold mining and mining of other metals. The limits are determined by regulation of European Commission No. 1881/ 2006. Although the limits are not defined for arsenic but it could be possible to work with the standard of State Veterinary Institute in Prague – Lysolaje, which is valid from an earlier period for the Czech Republic.

CIBULKA (1991) noticed that the most dangerous compound of mercury is methylmercury. Found mercury levels, with the base of methylmercury, are several times higher than the permitted limit, which is 0.500 mg/kg, according to EU. In 2003 and 2004 were in the Czech Republic high findings of mercury in fish, especially in trout and other freshwater fish. Decreased amount above permitted limit could be caused by extensive flooding in the Czech Republic in 2002 (annual report of Ministry of Agriculture, 2002, and 2003).

BRANDL (2005) mentioned that occurrence of heavy metals in higher concentrations is more visible in organs, such as liver and kidneys, than in muscle tissue of live organisms. It was also confirmed in our research where the mercury concentration in fish liver tissue, form Arkhangai area, achieved the value of 0.880 mg / kg. On the other muscle tissue reached the maximum value of 0.261 mg / kg. Finding of mercury, in levels above limits, came from the Mongolian area, where gold mining is placed. This mining is usually illegal and so the big amount of mercury penetrates to environment due to this illegal process. Thus the suggested hypothesis was confirmed according to analysis of mercury determination using Atomic Absorption Spectrometry.

For arsenic none hygienic limits are determined in regulation of European Union. But in databases of State Veterinary Administration in the Czech Republic is notice the

value for arsenic, which is 1.000mg/kg. The levels of arsenic of some areas in the Czech Republic exceed the values determined by hygienic limits. The reason may be that arsenic it he compound of sediment and so it is dislodged during leaching into water. Consequently the water is contaminated by this process and also presented organisms. The similar situation was monitored in Nepal (Bangladesh), where the arsenic is the compound of earth ground and it is dislodged into groundwater during the well drilling. The consequent circulating of arsenic is caused by water cycle, which should be connected with huge floods in the Czech Republic in 2002. So the high contents of arsenic and mercury were extremely over the hygienic limits. It could be the reason of high values. Arsenic and mercury are naturally in soils and in the water, however the movement during the floods were huge. Afterwards fish and other animals ,which are living in the water or olive in the pasture near it. In these animals was high content of selected animals. (Annual reports of SVA of CR from 2003 and 2004)

Results of our study correspond with mean content of Cd in muscle tissue of examine d fish was below the maximum available leve l (EC,2006).

Also results of mercury was similar to other studies (JEZIESKA and WITESKA, 2001), (EC, 2006).

Positive findings of arsenic in the Czech Republic come from area around Hodonín, where the wells for extraction of natural gas are widespread.

The occurrence of high concentration of lead in animal tissue usually dos not exceed the hygienic limits. But when it will happen the contents are very high. Justification based on a study of (CIBULKA, 1991) (ADRIANO, 2001), with which I fully agree, determined the high concentrations of lead due to its usage in motor vehicles. In the European Union the usage of leaded petrol is prohibited. The positive samples available from the Czech Republic came from areas where the wastewater treatment plants were located near rivers and pounds.

The higher concentrations of selected heavy metals were confirmed in freshwater fish from rivers. FIRAT et.al.,(2011) mentioned that smaller fish contain lower concentrations of all elements than the bigger predatory fish. The higher accumulation of components in liver, kidneys and muscle of bigger predatory fish is caused by longer life. The cycle continues in bigger mammals, because the fish are part of food chain.

7. Conclusions

The attention of specialist and laic community is focused on evaluation of nuisance effect of heavy metals on environment. The heavy metals are natural compounds of soils and minerals and they are represented in low concentrations. Thus they don´t have harmful effect on health of fauna and flora. But on the other hand contamination of soils by heavy metals increases due to growing human activity in mining, refining of ores, wastewater treatment, establishment of landfills especially with hazardous wastes, combustion of leaded fuel and in many other industrial procedures. All of these processes can cause higher occurrence of heavy metals incoming to environment. Therefore these activities should be realized in reasonable level, because of observance of hygienic limits in given elements. Together with this fact is necessary to increase the cognizance about health risks and impacts of heavy metals on animals (on pasture and in wildlife), plants and water resources.

The first hypothesis was confirmed, due to the results of mercury (Arkhangai area: Pb=0,880mg/kg), which was above the hygienic limit for this element. However the Arkhangai area in not the major one of illegal mining of gold in Mongolia, the positive sample was found in liver tissue of freshwater fish.

Secondly, this hypothesis was also confirmed, because the most positive samples(17 samplings from whole analyses) for all selected heavy metals, was detected in group of freshwater fish from the Czech Republic and Mongolia. The group of trout in the Czech Republic was almost whole negative, just few positive samples on arsenic were found.

Among the biggest hazards belong determination of heavy metals in live organisms and their tissues, which are used for consumption (game animals, livestock, fish) and the last consumer is human. The majority of components is toxic in high concentration and can cause serious diseases.

The monitoring of extraneous substances and heavy metals in periodic intervals is realized due to legislative regulation determined by the European Union and thereby the Czech Republic. The determined limits should be satisfied by member states in reason of globalization of food trade. Due to periodic observation could be realized the goal-directed

monitoring of regions in the Czech Republic, where are the limits exceeded repetitively. The publishing of critical areas is an effective protection and prevention of animal and human health. The administration procedure can be propounded in case of repeating pollution and infringement of laws and regulations determined by the European Union.

In the Czech Republic concentrations of selected heavy metals (Cd, As, Hg) in freshwater fish were above hygienic limits. The values of Pb did not pass the permitted regulation determined by EU.

There is a consideration of implementation the legislative regulations and harmonization on requirements of EU in Mongolian. Due to cooperation with the Czech Republic and member states the laboratory analyses and determining criteria are implemented.

In Mongolian conditions, they are thinking of introduction of legislative measures and the harmonization of the conditions of the EU and through mutual cooperation with the CR and EU member States are gradually introducing laboratory analysis and some criteria are established. The reason is that in Mongolia live about 42-50 million of cattle and huge amount of fish are in their rivers. So the Mongolian farmers and producers want to export they product to EU countries.

Exceeding the hygienic limits of elements (Pb and Hg) in fish production was significant. This is the fact, which should make the trade with Mongolian products impossible.

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9. List of the Contractions Used in this Thesis

AAS = Atomic absorption spectrometry

ADI= Acceptable daily intake

AES = Atomic emission spectrometry

AMA =Atomic mercury analyzer

As=Arsenic

Bc= bachelor

Cd= Cadmium

CR= Czech Republic

CULS = Czech University of Life Science

DDT= dichlorodiphenyltrichloroethane

EC= European Commission

ETA AAS = Electrothermal atomization atomic absorption spectrometry

ETA= Electrothermal atomizer

Etc.=Etcetera

EU=European Union

FAAS = Flame atomic absorption spectrometry

Fig. = figure

Hg= Mercury

HL= Hygienic limit

ICP-MS = Inductively coupled plasma mass spectrometry

ITIS= Integrated taxonomic information system

MAX= Maximum

MNG = Mongolia

N= number

NIST= National Institute of Standards and Technology

Pb=Lead

PCBs= polychlorinated biphenyls

pp. = pages

SRM= Standard reference material

SVA= State Veterinary Administration

SVI = State Veterinary Institute

Tab. = table

WHO = World Health Organisation

APPENDICES

List of the Appendices:

APPENDIX 1

Accreditation of laboratory in State Veterinary Institute in Prague

NÁRODNÍ AKREDITAČNÍ ORGÁN

Czech Accreditation Institute
Public Service Company
110 00 Praha 1 - Nové Město, Opletalova 41

issues this

CERTIFICATE OF ACCREDITATION

No. **186 / 2004**

to

Testing Laboratory No. **1176.1**

Státní veterinární ústav Praha
Oddělení chemie
Sídlištní 136/24, 165 03 Praha 6

Scope of accreditation:

Chemical and physical tests of consumables, raw materials, biological material, animal feeding stuffs and water to the extent as specified in the appendix to this Certificate.

Ing. Jan Rosmus shall act on behalf of the accredited testing laboratory, and Ing. Jan Rosmus, Ing. Jaroslav Šebesta and Ing. Hana Rolencová shall be responsible for the correctness of relevant test reports.

This Certificate of Accreditation has been issued by Czech Accreditation Institute, Public Service Company, on the basis of assessment of fulfilment of the accreditation criteria in accordance with

ČSN EN ISO/IEC 17025

and after having found that the testing laboratory has been qualified for objective and independent testing to the extent of the scope of accreditation.

In its activities, performed within the scope and for the period of validity of this Certificate, the holder of this Certificate is entitled to use the identification "Accredited Testing Laboratory No. 1176.1" next to its name (including official stamp) provided it observes all relevant regulations relating to the activity of accredited testing laboratory, including regulations issued by Czech Accreditation Institute, Public Service Company.

Should it be proved that the holder of this Certificate fails to meet the accreditation criteria decisive for the issue hereof and the obligations conditioning accreditation, Czech Accreditation Institute, Public Service Company, may either suspend the validity of or withdraw or change this Certificate.

This Certificate is valid until: **28 February 2006**
and replaces completely the CAI's Certificate of Accreditation No. 227/2003 of 4 June 2003

Prague: 16 April 2004

Český institut pro akreditaci, o.p.s. # 1 #

Jiří Růžička
Director
Czech Accreditation Institute
Public Service Company

Instruction:
The holder can enter a written caveat against this Certificate, provided it concerns the scope of accreditation, in 10 days from the receipt hereof. Timely submitted caveat has no dilatory effect.