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Determination of Selected Environmental Pollutants in Tissue Samples of Livestock

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Statement

I declare, that I have elaborated with diploma thesis “Determination of Selected Environmental Pollutants in Tissue Samples of Livestock” independently and I have used only quotations listed in References.

In Prague: 18. 4. 2012

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Ondřej Šimoník

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Abstract

Pilot survey which was realized in autumn term 2011 in selected localities of Mongolia with higher levels of burden caused by anthropogenic influences. The contamination of cadmium, lead, mercury and arsenic was monitored in animal tissues of cattle, sheep and horses. Animals are bred on extensive pasture year-round. Determination of selected heavy metal concentration was performed particularly for assessment of potential risk for the human population in relation to the possible contamination of the food chain with pollutants.

In total 415 samples of livestock tissues (bovine, ovine, equine) were collected in field conditions in Mongolia, from this quantity 24 mixed samples were created, which represented different districts and regions. Analyses focused on the presence of pollutants was performed in accredited laboratory from mixed samples of liver parenchym and skeletal muscle from sheep, cattle and horses from selected regions. Included Coupled Plasma Spectrometry (ICP-MS) was used for determination of cadmium, lead and arsenic. Mercury content was quantified in tissues with Atomic Absorb Spectrometry (AAS)

Presumption of higher concentration level of pollutants in northern part of Mongolia (in locality with the highest number of illegal and legal mining activities) was not statistical proved. However in comparison with this area there was statistically confirmed a higher concentration of cadmium ($p < 0,05$) and arsenic ($p < 0,01$) in animal tissues originating from adjacent areas to capital Ulaanbaatar, which is situated in central region Tuv.

In relation to traditional Mongolian cuisine based on mutton meat, exceeding of maximum limits according to European Commission Regulation (ES) 1881/2006 was not showed in 95% of analyzed samples of mutton muscle. In case of beef muscle no analyzed sample was above maximum limit. Detailed targeted analysis carried out in regular time intervals will be henceforth implemented in cooperation with scientific-research partners from Mongolia.

keywords: Mongolia, animal tissues, livestock, contamination, heavy metals, cadmium, lead, arsenic, mercury, monitoring,

Abstrakt

V pilotním výzkumu, který byl realizován v podzimních měsících roku 2011 ve vybraných lokalitách Mongolska s vyšším stupněm zatížení antropogenními vlivy, byla sledována kontaminace kadmiem, olovem, rtutí a arsenem u živočišných tkáních skotu, ovcí a koní. Zvířata jsou celoročně chována extenzivním pasteveckým způsobem. Zjišťování koncentrací vybraných těžkých kovů bylo prováděno především za účelem posouzení potenciálního zdravotního rizika pro humánní populaci v souvislosti s možnou kontaminací potravinového řetězce těmito polutanty.

Celkem bylo v terénních podmínkách Mongolska odebráno 415 vzorků živočišných tkání pasoucího se dobytka (skot, ovce, koně), z nichž bylo cíleně vytvořeno 24 směsných vzorků, reprezentujících různé regiony. Analýzy přítomnosti polutantů byly provedeny v akreditované laboratoři ze směsných vzorků jaterního parenchymu a kosterní svaloviny ovcí, skotu a koní, původem z chovu zvířat sledovaných oblastí. V případě kadmia, olova a arsenu byla ke stanovení obsahu těchto prvků použita metoda Hmotnostní Spektrometrie s Indukčně Vázaným Plazmatem (ICP-MS). Obsah rtuti byl ve tkáních stanoven pomocí Atomové Absorpční Spektrometrie (AAS).

Předpoklad zvýšené hladiny koncentrace polutantů v severní části Mongolska (v oblasti, kde probíhá nejvíce legální, ale i ilegální těžební činnosti), nebyl statisticky prokázán.

Ve srovnání s touto průmyslovou oblastí severní části Mongolska byla statisticky potvrzena vyšší koncentrace kadmia ($p < 0,05$) a arsenu ($p < 0,01$) v živočišných tkáních monitorovaných zvířat pocházejících z přilehlých okresů hlavního města Ulánbátaru, ležícího v centrálním kraji Tuv.

V souvislosti s tradiční mongolskou kuchyní, založenou převážně na konzumaci skopového masa, nebylo prokázáno překročení maximálních limitů posuzovaných podle Evropského Nařízení (ES) 1881/2006 a to u 95% analyzovaných vzorků skopové svaloviny. V případě hovězí svaloviny žádný z analyzovaných vzorků stanovené limity sledovaných polutantů nepřesáhl. Podrobnější cílené analýzy, prováděné v pravidelných časových intervalech budou i nadále realizovány ve spolupráci s vědecko-výzkumnými partnery z Mongolska.

klíčová slova: Mongolsko, živočišné tkáně, hospodářská zvířata, kontaminace, těžké kovy, kadmium, olovo, arsen, rtuť, monitoring,

List of abbreviations

ICP-MS - Inductively Coupled Plasma Mass Spectrometer

AMA – Atomic Mercury Analyzer

NIST – National Institute of Standards and Technology

SRM – Standard Reference Material

GDP – Gross domestic product

WHO – World Health Organization

Cd-Mt – Cadmium Metallothionein

SVI – State Veterinary Institute

ASTM - American Standard Test Method

EPA – Environmental protection agency

ATSDR – Agency for Toxic Substances and Disease Registry

ppm – parts per million

ppb – parts per billion

IFCS - Intergovernmental Forum on Chemical Safety

MECA – Manufacturers of Emission Controls Association

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1 Introduction

Toxic metals represent significant factor for environment, which can have negative influence to metabolic processes in living organisms. The increasing of metal concentration in the environment, in atmosphere, water, soil and in foodstuffs is serious hygienic problem of which extension rises constantly (Watson, 2001). By Jakimska et al. (2011) group of heavy metals include metals and metalloids and also according to (Beneš, 1994) their compounds, which can be potentially hazardous due to their toxicity and consequently by harmful effects to human being. As the most dangerous are assumed cadmium and other are lead, mercury, chromium and arsenic. Metals in nature as Brimer (2011) additionally presents, are not dissociated by water or soil microorganisms in contrast to organic substances, but the both may have negative influence to the environment. Increasing metal concentration in soil and water often causes higher level of metals in alimentary products with animal and plant origin as well (Bencko et al., 1995). As Bradl (2005) states heavy metals can occur in from different sources and in various forms, as gaseous, particulate and aqueous.

The main natural source are parent rocks and metallic minerals, anthropogenic sources involve mining, smelting, metal finishing and also agricultural activities are include to this group. According to Stoepler (1992) regional and global increase of heavy metals concentration in plough layer, plants, animals, lakes, rivers, oceanic regions, foodstuffs is created mainly by intensified industry and mining. The atmosphere is affected by coal combustion in power plants or in households also by waste combustion in special incineration plant or in waste burning in households. Self–evidently other source of air pollution is usage of leaded fuel. Water from mining places, water polluted by tailings, waste waters etc. have huge negative effect to surface flows (Bencko et al., 1995). In the past, there was small attention to prevention introduction these hazardous agents to the environment but fortunately this have been changed in many countries and due to enhanced legislation about capture and treatment of pollutants load is decreased (Bradl, 2005). But according to Liu (2003) in some countries heavy metals have still increasing tendency of their content in the air, water and soil in cities and also in suburban areas.

2 Aims of thesis

The pollutants which have been selected are heavy metals, namely cadmium, lead, mercury and arsenic have been selected because of their no naturalness biological function, ability to accumulation in food chain and possible unfavourable effect to humans and animals as well (Čelechovská, 2008). Therefore the knowledge about concentration in livestock are consequential to evaluation of risk from view point of intake by human (Liu, 2003).

This thesis is focused on the two main aims:

1. To analyse and to evaluate heavy metal pollutants in animal tissues, mainly in liver and muscles which originate from different parts of Mongolia.

2. Assess hazards for consumers from the point of view to area of livestock origin.

Two hypothesis have been established:

1. Mutton is main source of Mongolian nutrition and exists risk of heavy metals with the biggest intake of one.

2. The content of pollutant in mining areas and around cities will be significantly higher than outside this localities.

3 Literature review

3.1 Sources of heavy metals

Metals are released into the environment from natural or anthropogenic sources and as Sharma et al. (2009) say, heavy metals are ranked among persistent pollutants because of they are not biologically degradable. Transport of heavy metals in the environment is illustrated in Appendix 1. Natural resources are volcanic activity, wind erosion, forest fires, microorganisms and plant products others are as Brimer (2010) additionally presents soil are rock erosion and other soil processes quantity, is particularly influenced by content and rock release ability. Heavy metals are removed by dry or wet deposition, from the atmosphere to the soil, surface water bodies or to plants. It is absorbed particularly on biochemically elements with 0,1 – 10 μm proportions. The toxicity of heavy metals is dependent on their concentration and exposition length (Beneš, 1994).

Anthropogenic sources involve emission from power plants, industrial activities including mining, traffic and agriculture. Soil contamination includes industrial activities, application of organic and inorganic fertilizers, sludge and industrial made composts, then as Bradl (2005) presents the contaminants can be released at beginning of production chain during mining ores as well as during products use and also at the end of this chain as a waste deposition.

Influencing on surface water can be direct – effluents, direct fallout or indirectly entrance by dry and wet atmospheric deposition, metal leaching from soil by water or indirect by wet or dry deposition. The well leachable group of metals involves cadmium, thallium, zinc, copper, chromium, nickel and as Liu (2010) quotes those elements can introduce risk of secondary contamination (e.g. from landfills of urban solid wastes). Composition of water is also influenced by chemical reactions during contact water with rocks (Beneš, 1994).

3.2 Anthropogenic sources

3.2.1 Mining generally and in Mongolia

Especially within storing the waste rock which is produced by extraction process in mine tailings or rock spoils heavy metals can leak out. This process is encouraged mainly by acid conditions and heavy metals are mobilized from stored waste rock, what can cause environmental problem representes health problems through respiration, drinking and eating food grown on influenced area.

Example of serious result is gold extraction from placer where the mercury is used to extraction gold by amalgam method and mercury is released by flushing or burning (Bradl, 2005). Appel (2005) quoted that about 650 tons of mercury were released annually through small scale mining but, Intergovernmental Forum on Chemical Safety (IFCS) (2006) stated that more than 800 tons per year were used up by small scale miners. Other dangerous aerosols which are produced during driving of lead, zinc, copper and cadmium ores (Beneš, 1994). Major operating mines and deposits in the Mongolia is showed in Appendix 2.

Mining in Mongolia

The mining sector is considered as a main part of Mongolian economy, it contributes about 17 % to GDP and 58 % to export proceeds. Before change to market economy which has started in 1992 mining industry was owned by state and it was jointed with Russian, Bulgarian, Czechoslovakian, Hungarian enterprises. Since 1992 Mongolia have mostly cooperated with Canada, Australia, Russia, China companies and exploration and mining is on high level and more than 12 000 of people are employed by mining section, but artisanal mining as a informal part, increases this number many times (World Bank, 2006).

Artisanal small-scale miners are known as a people with nickname “ninja” because of plastic bowl is carried fastened to their back. This artisanal small scale mining is not traditional part of Mongolian economy, nevertheless there are 30 000 – 100 000 informal gold miners. In other numbers there are 784 mining companies and 204 from these are small scale type, and those cover 60652,98 hectares of land (WHO,2005). The term artisanal small scale mining is denoted as a exploitation various products by individuals or groups whose knowledge of safety methods are very poor. In instance of gold mining, tunnel caves which are made usually with shovels, hammers and mattocks, represent mortal trap for labours this means that this mining methods is unsafe only for environment but also for workers because of usage of high toxic mercury for extraction. Mercury is used during method of extraction gold which is called amalgamation, is added into mineral concentrate and gold is dissolved in mercury, result is developed amalgam. Process is showed in Appendix 3. Amalgam is replaced into iron cup and heated up, to scalding of mercury. Method is very efficient, but during it the large amount of mercury may release to the environment (Appel, 2005). As Šourek (2008) presents separation during placer mining, no technological complicated equipment requirements, and high abundance in rivers is main reason to that gold is the most frequent exploited commodity by middle and small miners in Mongolia.

The base of Mongolian mining as World bank (2006) present, is in sourcing of copper, gold, coal, molybdenum and fluorspar as well as their export (World bank, 2006). As Žáček et al. (2005) showed mainly copper and molybdenum deposit is situated in vicinity of Erdenet city in the north part of Mongolia and belongs in world largest deposits in terms of volume of mining as well as number of geological reserves. This bearing contributes 15 – 25 % to GDP and copper – molybdenum concentrate represent crucial export item which create 30 – 40 % of total Mongolian exports.

Additionally growth of artisanal small scale mining activities (particularly gold extraction) in Mongolia is manifestation of poverty which is partially caused by transition to market economy, then job loss and lack of social safety. Poverty is also interfaced with environment especially with natural disasters called-dzud which is related to conditions in step pastures (World bank, 2006). The winter in 2009/2010 was extremely hard and has caused that 8,711 families or approximately 43,555 Mongolians lost all their livestock during dzud and 163,780 herders lost half of their wealth, therefore they had no money to buy foodstuff or medicines, and they were unable to send their children to school (UNDP, 2010) Dzuds can be in form called "white dzud" high snow accumulation and ice crust on surface or also "black dzud" is known in conditions with low precipitations when absence of snow cause no source of water. This disasters do not effect only during winter but also forage growth in summer is negatively influenced by the low precipitation.

There is different in composition of ninjas in various regions determined by World Bank (2006), in north part artisanal small-scale mining society includes ex-herders or ex-farm workers but also students and poor urban people. In Gobi region in the south, majority is formed by herders who lost their livestock during dzud. Other reason to desist from herders life is according to Horlemann and Dombrowsky (2011) vision of higher incomes along with better life, herders leaved their livestock which provides them stable livelihood, and start to work as an artisanal miners. Their vision or wishes are not always fulfilled and this is connected with social problems and with risk of decline of traditional nomadic-pasture life, as well.

3.2.2 Coal and petroleum combustion

The environment is additionally influenced by combustion of fossil fuels which is used to generate power in plants (Bradl, 2005). The most dangerous is combustion of coal with content of Beryllium, Lead and Silver (Beneš, 1994) and according to Tian (2010) coal combustion is main anthropogenic source for mercury and arsenic. By Bradl (2005) other heavy metals which are released during combustion process are cadmium, molybdenum, selenium and zinc. Their concentration is depends on conditions during combustion, control mechanisms and disposing of. The disposal of fly ash from this combustion leads to mobilization of these metals into waters, soils and ground water. It is not only combustion in plants but also fossil fuels and wood which used in households have influence. Due to energy requirements increase in relation with ascending human population, also higher level of heavy metals is expected.

In case of Mongolia (WHO, 2005) showed that 60% of inhabitants use solid fuels, 14% of means of transport use diesel and share of usage of unleaded gasoline is zero, despite that Manufacturers of Emission Controls Association (MECA) (2003) showed the Mongolia as a leaded fuel free. Especially in the capital Ulaanbaatar, there are power plants which burn large quantities of coal to generate electricity and hot water. As WHO (2005) presents power stations use about 5 million ton of coal annually and households demand of coal to produce heat also contribute, with annually consumption about 200 000 tons of coal and 160 000 m3 of wood, to pollution.

3.2.3 Solid waste disposal

The solid waste is produced in thousands of million of tons every year in relation with growing of large urban areas and mega cities. When the waste products are disposed of without proper treatment and deposited without appropriate requirements that mean in places with high permeability, in vicinity of water bodies, with no barrier systems against dissemination or leaching of, it represents high risk of pollution (Bradl, 2005).

In Mongolia this thing is also very important issue, there are about 450 waste collection points (means open space) with total area over 3000 hectares. The 220 of these points are situated only in Ulaanbaatar, but the collection of waste is not well organized (WHO, 2005). The main source of waste production are Ger areas (ger is traditional dwelling of Mongolian people) which contribute with 48%, next are apartments with 32% and last are offices, restaurants. Fortunately as WHO and MOH (2002) present the current situation is much more better than in early 1990’s when the waste were open burned, this was stopped because of the resulting air pollution and also due to regulations. Health Care Waste Management Improvement Regulation was agreed in October 2002 and the law on municipal and industrial waste was ratified in 2003.

3.3 Mongolia and characteristics of selected area

Size of Mongolia is three times bigger than France and of half smaller than India. Country have very low population density, countrywide average is 1,6 per km2, but in area except capital Ulaanbaatar and centrals of 21 regions, the number is 0,6 per km2 (Juřička et al., 2010). Additionally as WHO (2005) presents Mongolia belongs in group of 60 countries with limited fresh water sources, with 0,00004 % portion of whole water resource in the world. Mongolian rivers have been exposed to very serious pollution in last years. Contamination of streams by cyanides and mercury, as a result of legal and more frequently of illegal mining, does not represent only threat to rivers. But also to large areas of underground water and river basins. Consequences are depreciation water in wells with potable water and also in watering places for livestock.

The major reasons of pollution in the present age are not untreated waste water from industry, factories, tanneries or households particularly in the cities but illegal and legal extraction of precious metals, especially gold from river placers, are the most important contributor to contamination of environment (Spiegel and Veiga, 2009).

Selenge river basin

This area is huge, presents 20% of Mongolian territory and involve 9 regions (Arkhangai, Bulgan, Ovorkhangai, Khuvsgol, Orkhon, Selenge, Tuv, Darkhan-Uul and Ulaanbaatar) which include 129 districts. In 2008, this basin was home for 1 818 900 inhabitants from total number 2 594 800. Additionally 55% of river basin area is used for agricultural purposes (i.e. livestock husbandry and plant production) which mean that this region is very important form viewpoint of ecology and economy (Mun et al., 2008).

One of the test case and the most important is particularly this river basin, which is situated in same named region. Selenge is the biggest Mongolian river, which estuary to Russian lake Baikal. And as Pavlov et al. (2008) further quote, from year 1990 Selenge basin have been strongly affected particularly by extension of gold mining. Catchment area is the most affected with both types of mining activities, main contribution are huge gold placers, 3400 official mining companies and 7000 illegal groups have worked here for 15 years. Illegal mining is realized particularly with artisanal, small and middle scale miners. This mining is based on processing of generated wastes, heaps after exploration or during it, but also on small deposits which are not attractive for big companies (Juřička et al., 2010). Majority of informal activities are realized with poor subjects with very low level of equipment which represent hazard of discharge pollutants, mercury and cyanide into the environment during alteration of mined metals concentrates and separation of gold, which requires usage of these dangerous chemicals. Moreover the knowledge of miners and general awareness of pollution hazards are very low rather zero, and consequences for ecosystem and public health are grave (Spiegel and Veiga, 2009). Accumulation of mercury in the environment and devastation of localities during or after finishing of mining, have fatal and destructive after-effects to whole ecosystem of river landscape (Juřička et al., 2010). Nevertheless as (Šourek, 2008) quotes, contaminated area is larger than river vicinity and the most important source of potable water, underground waters and watering places for livestock can be affected. This fact has also been confirmed by Mun et al. (2008) who presents that extension of heavy metals can be larger, because of fact that the Selenge river is one of tributaries of the deepest fresh water lake in the world (i.e. Baikal) which is consider as the biggest source of surface potable water worldwide. Unfortunately fast development of illegal mining is additionally maintained by inadequate governmental control, monitoring. (Juřička et al., 2010).

Additionally as Mun et al. (2008) present, Selenge basin includes the three biggest cities of Mongolia (i.e. Ulaanbaatar, Darkhan, Erdenet) and 10-70% of waste water (treated and untreated) is released into Selenge river which represent major water supply. Also big proportion of agriculture belongs to this region and according to Dolgormaa (2004), task of Law Protection from Toxic Chemicals which have been established in 1995, does not always fill the role. And particularly in countryside people use banned DDT bought in black markets, which fall into organochlorine pesticides with harmful and persistent effect to environment and individuals.

3.4 General accumulation and toxicity of heavy metals

Heavy metals are present in all foodstuffs due to their ubiquity. Some of them have nutritional importance as an essential effect but only in given concentrations, ranges or doses. Other elements or compounds as a lead, cadmium, mercury, arsenic and thallium have opposite toxic effect and health problems can be caused by them (Merian, 1991). Burden of food chain by heavy metals can comes from water, soil, plants and animals as well. Then as Brimer (2011) presents individual elements and their compounds can show different effect and behavior, this differences and their causes is little known but it can be possible that ionized metals is bound to storage structures with dependence on their reactivity. Soaking and distribution are unfolded by solubility and ability to infiltrate lipid membranes. Oxidation state, forms (i.e. organic, inorganic) according to Sharma et al. (2009), must be distinguished in association with toxicity of metals. Generally, in case of organic compounds likelihood entry and accumulation in organism is higher in comparison with inorganic.

Disposable dosage does not cause identifiable impact to the person but extended time of exposure over longer period can induce various toxic effects which can bring damages of characteristic organs, cancer or allergic reactions (Merian, 1991). As Adriano (2001) quotes the toxic action usually extend to multiple target organs and systems, never only single biochemical, biophysical or enzyme are affected. There are two groups of action of influence: local and systemic effects. In connection of first organism exposition to toxic factor, local effect is occurs. Systemic effect require absorption and distribution from area of entry. Adverse effect of metal compounds can proceed in different parts of organism, usually with cardinal impact to one or two organs which is called the target organs of toxicity. Those organs always have the highest concentration of metal and can be affect adversely by reversible or irreversible changes.

The most important is low-level and long-term effectiveness, so feasible chronic effect, because those are the most widespread way of environmental as well as occupational exposure (Sharma et al., 2009). By Vernet (1991) long-term exposure with low doses means, that signs of toxicity are appear after certain time. In general chronic effects set in, when factor are accumulated in organism, or when agent produces non-eversible toxic effect. Not always the accumulation in tissues induce toxic effect as Pichery et al. (2011) present, some metals make complexes and those are stored e.g. in bones, teeth exactly in case of lead due to this fact illness often does not recognized during clinical investigation and many cases of metal intoxications stay undiagnosed.

3.5 Pathways of heavy metals access

The main ways which lead to contamination of organism are through atmospheric disposal in water and soil, through contaminated water and food (Bradl, 2005). Predominant part of metal compounds is transferred through food and drinking water. In case of work environment, e.g. exposure to mercury in chloralkali plants, exposure to dust with content of arsenic, cadmium, lead, manganese, nickel in metal smelters, main pathway is inhalation (Merian, 1991). Diagram of the exposure pathways is prefaced by Appendix 4.

The most important entrance pathways of metals to organism involve as Bencko et al. (1995) presents, lungs and digestion tract next is absorption through skin which can proceeded only in certain circumstances and intraplacentar transport relevant for fetus development.

3.5.1 Ingestion

Orally intake of pollutants can be influenced with food, beverages and by ingestion of contaminated soil or dust, as well (Merian, 1991). In the case of brew intake contaminants are received directly by drinking contaminated water or indirectly by utilization this water for cooking or using it for field or pastures irrigation. Also in these eventualities the contamination can be natural or anthropogenic origin (Bradl, 2005).
Alarming situation according to Ahmad et al. (2010), is in West India and Bangladesh where twenty millions people are affected by arsenicosis due to drinking contaminated water., and by higher levels of cadmium due to contaminated fruit and vegetable which are irrigated with contaminated industrial and municipal waste water with significant contribution to higher levels of cadmium and other metals.

Generally the terrestrial human food chain is the most significant pathway to metals exposure to population. Main routes are:
polluted soil – plant – human

polluted soil – plant – home gardener

polluted soil – plant – animal – human

polluted soil – animal – human

polluted water/sediment – plant – human

polluted water – plant – aquatic animal – human

polluted water/sediment – plant/animal – waterfowl – human

polluted water/sediment – animal – human (Adriano, 2001).

Food with bioacumulated or natural content of heavy metals can represent risk for consumer (Bradl, 2005). Absorption from intestines is the most important in comparison with soaking up in mouth or stomach (Merian, 1991). The most rapid soaking is in central part of small intestine (jejunum, ileum). Parts with low absorption are final part of ileum and colon, but passage through these sections is slow, therefore absorption can be significant (Bencko et al., 1995). The absorption is done by passive diffusion in dependence on concentration gradient between blood and intestinal lumen, this transfer is enabled by high concentration gradient which is maintained by strong blood circulation in intestinal (Merian, 1991).

Other ways to transport through enterocytes as Bencko et al. (1995) say, are active transport against concentration gradient, facilitation diffusion with assistance of protein carrier and also by pinocytosis in case of protein absorption in newborns.

The amounts of absorption is dependent on kind of metal on its chemical form and animal species (Merian, 1991), e.g. inorganic As(III) and As(V) compounds by Gunduz et al. (2010) can be absorbed more than 90%, elemental Hg 0,5% in other hand organic methyl Hg compounds more than 80% and water soluble lead salts about can be absorbed from10%. As Bencko et al. (1995) quotes absorbability and speed of absorption can be also affected thanks to capability of pH in digestive tract to change valence of metals.

3.5.2 Respiration

Metals can enter into organism as a gases, vapours or particles (Bencko et al., 1995). The particles with diameter 2,5 μm and less, introduce by Zhang (2009) the most probable reason of negative effect to human health in comparison with particles of 10-100 μm in diameter. Number of released substances into atmospheric environment is increased accordingly to higher population and developing industrial activities. These numbers are at level thousands of tons every year(Bradl, 2005).

Deposition of aerosols is given by physical mechanisms: impaction, gravitational sedimentation and diffusion in different parts of respiratory system. The impaction is main deposition mechanism for particles with size of micrometers particularly in nasopharyngeal cavity. Elementary particles with tenth of to several micrometers are deposited in smoother ways by process of gravitational sedimentation. And in the smoothest air ways are stored particles, via diffusion (Vernet,1991).

According to Bencko et al. (1995) aerosol particles soluble in body fluids are absorbed from lungs to circulatory system mainly in places with tight contact among capillary surface and particle. Less soluble particles can be soluble in lung surfactant in dependence to their water solubility, insoluble particles can reach blood circulation by penetration of capillary membrane.

By Brimer (2011) in case of metals in gaseous and vapour form with good water solubility they are dissolved in mucous layer on surface of nasopharyngeal and tracheobronchial system, therefore they never reach to the alveolar level. On the other hand insoluble metals are soaked in alveols. Additionally presents that covering of alveolar cells with phospholipids coat is main factor for gas and vapour penetration.

3.5.3 Resorption through skin

Mainly structure, regenerative attribute, repulsive properties and integrity have positive influence to avoid soaking of water soluble toxic substances, particularly of inorganic electrolytes, high molecular compounds and elementary particle (Bencko et al., 1995). On the other hand Filon et al. (2009) say, that organic and inorganic metal compounds, which are soluble or distributable in fats (i.e. mercury, zinc compounds, copper, arsenic, cobalt, nickel and for tetraethyl lead) are well assimilated with skin as well.

Additionally skin does not protect organism against fat soluble matters. In case of animals with high layer of fat, those substances are lodged in this part of skin. Transport can pass through follicular glands (Bencko et al., 1995).

3.5.4 Intraplacentar transmission

Penetration and interception are influenced by structure differences in placenta which is connected with animal species. Also gravidity stadium and chemical form of metal contribute to various bounding and penetration (Bencko, 1995).

In case of human, cadmium is accumulated mainly in placenta but after parturition higher levels in newborn hair has been found. Cadmium can cause early birth, low birth weight and also changes in genes. In the Iran has been determined by Savabiesfahani et al. (2012) that lead level in fetus blood has been same as in maternal blood because there is no placental-fetal obstacle. And fetal blood lead content can be already found between 12th to 14th week (Bencko et al., 1995). Ask et al. (2002) present that organic form of mercury (i.e. methyl mercury) and inorganic mercury are highly suitable to be transported through placenta as well.

3.6 Health risks

Present of trace elements are divided to 4 groups, essential, probably essential, not essential, toxic. Fang et al. (2006) characterize those groups in this way, the first group involves iron, iodine, copper, zinc, manganese, cobalt, molybdenum, chromium, which are indispensable from viewpoint of maintenance of basic functions. Into the second group of probably essential elements belong – nickel, bromine, arsenic, vanadium, barium, strontium, their essentiality does not have been fully proved. In third group there are elements which are constantly present in various concentration as a part of tissues, but with low knowledge of their task in metabolic processes e.g. aluminum, silver, gold, lead, titanium.

Arsenic, cadmium, lead and mercury represent the group of toxic elements, and by Bencko et. al. (1995) these trace elements are ordinarily analyzed in biological as well as in environmental materials with concentration in range ng/kg to ng/l that means in ultratracelevel. But can be increased by anthropogenic pollution to level mg/kg, therefore clear analytical methods are required for food control. Biological risk is represented by function of the heavy metal burst, their solubility and mobility, these characteristics specify different bioavailability and bioaccumulation. Absorbed element may develop adverse effects especially in case of toxic cadmium, arsenic, mercury, lead. (Bradl, 2005).

The keystone of toxic effects connected with above mentioned heavy metals in biological systems as Brimer (2011) states, is reaction with ligands which are essential to their standard functions. High affinity of heavy metals to SH- (thiol) groups is well know, others are –OH, COO-, PO_4H_2-, NH_2- groups (Bencko et al., 1995). Therefore mechanism of toxic effect, is explained as an inactivation of some enzymatic systems. Biotoxicity is developed directly to organisms (e.g. inhalation or dermal contact) or indirectly through food chain transmission and final ingestion by which larger part of population can be exposed (Adriano, 2001).

3.7 Selected heavy metals Cd, Pb, Hg, As

Cadmium

Not essential but highly toxic element for plants and animals with concentration in air, natural waters, sediments, food and animal tissues in range from ng per kg to mg per kg (Stoeppler, 1992). Occurrence can be in various forms, and as Abdel-Sabour (2001) presents it can ranges between compartments of environment with different mobility, this lead to various accumulation in soil and living organisms. As additionally says, cadmium cannot be destroyed by any natural process. Higher aggregation is expected with increasing of discharging but also by Adriano (2001) attention must be kept on natural sources.

Sources and contents of cadmium in animal tissues

Main source of cadmium for humans by Beneš (1994) is food, after deposition from environment and smoking. Food can be contaminated particularly from sewage sludge and by phosphate fertilizers, pesticides, industrial pollution, other important source of pollution is fuel combustion (Bencko et al., 1995). According to Abdel-Sabour (2001) it is not only fuel combustion but combustion of all fossil fuels represents sources of cadmium and others are burning of iron, steel and also incineration of solid waste. Nevertheless not only combustion is included to sources as Agrawal et al. (2010) say, but also fate of rest after it can present risk, thanks to disposal on heaps. Further the cadmium has close relation with zinc mainly with zinc, lead-zinc, lead-copper-zinc ores and therefore source of cadmium are also mining operations (WHO, 2004).

In general consumption of agronomic food crops is accepted as the most critical pathway for population (Adriano, 2001). Exposure via air is occurred mainly in occupational environment and the intake through the human digestive tract is approximately 5% of an ingested amount of cadmium, depending on the dose and diet composition (Jin et al., 2002).

Element is mostly discovered in plant tissues as a potatoes, leafy vegetable and products with grain crop origin but animal origin is also important. According to Ragaini et al. (1977) average levels (ppm) in animal tissues were: cattle liver 0,34 and 0,06; cattle kidney 1,67 and 0,22; swine liver 0,24 and 0,14; swine kidney 0,99 and 0,39 ppm from contaminated and non-contaminated areas. Adriano (2001) quotes that generally average concentration in kidney from no-loaded areas is about 0,05 ppm which means generally higher than in other tissues as a meat or than in dairy products.

Jorhem et al. (1991) state same claim but in connection with liver and with different content, around 1 ppm with sporadic occurrence above this value. By research of cadmium contain in animal tissues which have been done by Jorhem (1999) in Sweden determined, that levels in horse meat are usually higher than in case of other mammals, frequently meat contamination exceeds generally 0,01 ppm up to amounts of 50 ppm. As survey shows, average concentrations of muscle, kidney and liver from 8 years old horses were 0,042; 2,5 and 18 ppm, Hecht and Kumpulainen (1995) provide similar values 0,230 ppm in muscle tissues from Germany. Further, research Jorhem et al. (1999) suggests higher concentration in reindeer muscles in comparison with sheep, what point that their main feed - lichens is characterized by feature to accumulate metals. Mean concentration was 0,6 ppm in liver and 2,7 ppm in kidney, but there were no information about age of animals. In comparison with details about tissues from Liu (2003), levels of samples from smelter area are higher, exactly 8,3 ppm (liver) and 27,2 ppm (kidney), samples from sheep assign mean levels (in same order) 7,92 and 25,39 ppm. Interesting are the similar contents in liver of reindeer (0,6 ppm) and muscle from sheep from smelter are 0,62 ppm.

According to Rudy (2009) who found out contamination of liver and meat from cattle in different stages of age from 1,5 to 12 years, there were changes in concentration in relation with this factor (cattle was bred in non-contaminated area in Poland). In group of 1,5 to 2 years, mean concentration in muscle was 0,009 and increased in relation with age up to 0,021 ppm in 12 years old cattle. Same tendency was showed also in case of liver, in same groups it increased from 0,123 ppm to 0,278 ppm. In the making of this survey, correlation between cadmium and lead was also found namely in liver and muscle. Čelechovská et al. (2008) quote, on base of survey from northern Moravia, that mean levels in muscles from cattle of 3 to 4 years age were 0,004 ppm which entail lower concentration in comparison with details of 1,5 to 2 years old cattle from Poland and in case of liver situation is same, as well.

Further according to Jorhem (1999), in samples of sheep origin there were mean concentration in muscles and kidney 0,0034; 1,000 ppm. By comparison with lamb, levels were lower 0,019 and 0,12 ppm, which shows relation between concentration and age.

Metal accumulation in food chain

Animals more than 10 years old have kidney concentration up to 40 mg per kg and on other hand young animals around 3 year old showed very low content in kidneys around 0,5 mg per kg or around 1 mg per kg and more by consumption fodder with cadmium content 0,5 mg per kg of dry matter. Other organ with predisposition to accumulate cadmium is the liver with levels 0,08 mg per kg in young animals and from 0,3 to 1,0 mg per kg in older individuals (Johnson, 1981). Burden of sheep kidneys is influenced by concentration of 6 mg per kg which may lead to 10 mg per kg of tissue (Merian, 1991).

Hazards to human health

Cadmium enters food chain mainly from soil in according to soil type and pH. Level is influenced mainly by agricultural fertilizers and sludge, also can be increased with dust from industry by leave resorption. The contents can be from 0,005 to 0,1 mg per kg and by influence of atmospheric contamination increased to level up to 50 mg per kg. Due to contaminated fodder, cadmium is omnipresent in food. One-third of cadmium load to human being derives from animal products and the rest from plant products (Merian, 1991). Result, accumulation in some tissues is induced by oral uptake which outweighs excretion (Brimer, 2010).

Retention of cadmium for human is up to 25 % in comparison with poultry where the value is 7 %. Generally has different hurtful influences, which are caused with acute or chronic exposure, male organism is usually more sensitive than female organism (Beneš, 1994).

Health problems mainly disorders are connected with calcium and vitamin D – osteomalacia, osteoporosis, those are caused by interference of metabolism of these important substances of human being (Adriano, 2001). Also in case of animals, changes in vitamin D level can lead to higher absorption of cadmium, especially in case of low doses of this vitamin which has positive influence to absorption of cadmium (Bencko et al., 1995).

Main problem for patients, whose were affected with chronically exposition, is kidney damage. Those are reached in form of cadmium-metallothionein (Cd-Mt) which are filtrated into glomerulus and tubulus and after swallowed up to the proximal tubulus. The remaining Cd-Mt in tubulus cells generates major part of cadmium burden to human body (Barbier, 2005). This creates excretion of low weight proteins α2-, β2-, γ globulins, what is caused proteinuria, due to strong affinity to sulphur protein which plays main role in bioaccumulation in kidney and liver. Proteinuria is the first sign of toxicity and formation of kidney stone are results of long-term chronic oral exposition (Adriano, 1991). Handling of cadmium in human body and effects to several organs are showed in Appendix 5. Other harmful effect of cadmium is to reproduction system, exactly secretion of steroid hormones – testosterone and progesterone (Piasek, 1999). When high dosages inhibit progesterone biosynthesis and low doses have opposite effect (Henson et al., 2004). The exposure of pregnant women increases low birth weight and spontaneous abortion. Interaction between cadmium and bone mineralization has been determined as a influence on function of osteoblast and osteoclast through dysfunction of kidney (Fréry, 1993).

Low bone mineralization, osteoporosis and pain associated with bone are symptoms of the disease called Itai-Itai which is connected with this heavy metal. Usually patients with these symptoms have high level of cadmium in diet particularly due eating rice irrigated with cadmium polluted water originating from mining (Nogawa et al., 2004). In the Jinzu and Kakehashi river basin the daily intake was 3,4 mg/kg for comparison, rural daily intake in the Netherland has been determined by Adriano (2001) in range from 20 – 32 μg/kg.

Cancerous effect have been proved only partially by (Walkees et al., 1989) whose has found that, injection of cadmium chloride can induce prostate cancer in Wistar rats, mainly testicular necrosis followed by testicular interstitial tumors.

In 2005 it has been classified as a human renal carcinogen in group one (Godt et al., 2006).

Lead

Lead is characterized by Watson (2001) as a omnipresent and can be found innately as a metallic lead, inorganic ions and salts in different foods. The mean content in earth cortex is 13 to 16 ppm. In arable soil in level 10 – 30 ppm and higher values is linked with geochemical reservoir, densely inhabited places and around industrial zones (Adriano, 2001). Additionally by Watson (2001) with dispensable function for humans, represents major environmental and public risk particularly to young children and fetuses in growth, where the toxic effect is grave . Also for livestock, fish and wildlife according to Bradl (2005) is nonessential and introduces non-specific toxin with adverse effect to their enzymatic activities with poisonous influence to blood, central nervous system and for reproduction function.

Sources of Lead

The lead can be released from natural and as well as from anthropogenic sources (Beneš, 1994). But second named source is the most responsible for higher levels of these element in the environment (ATSDR, 2007). Volcanic activity, erosion and windblown dust are included to native mines. The considerable amount of lead is originated as a product of natural radioactivity decay (Beneš, 1994).

In the past centuries levels has been increased 1000 times as a outcome of human activities (ATSDR, 2007). To the main anthropogenic sources are listed by Beneš (1994) mining, lead ore smelting, coal combustion, emissions from lead fuel using in motor engines, agrochemicals (e.g. phosphates, insecticides) and munitions.

When lead is released to the air, rain and dust particles are source to the water and land. Other threat is fact that small lead articles can travel long distances, average time of lead presence in the air is 10 days during which can cover long way up to in orders thousands of kilometers (ATSDR, 2007).

According to Flegal and Smith (1995) lead in rivers, lakes and streams can remain bounded to particles or sediment for many years. This sources of lead, which can further to influence plants and animals originates from atmosphere deposition, sewage water from industries, waste water from urban sites and from slag heaps. The dumps of ammunition industry or battery industry and town landfills with disposal of lead containing material, are also important.

Usage of leaded fuel lead to emitting particularly lead-bromide and lead-chloride compounds which are transformed in atmosphere or in soil to less soluble Lead (II) – sulfide. The restriction of using leaded fuel has contributed to reduction of emission (e.g. in 1979 in United States were released 94,6 million kilograms of lead to the air) after banning the quantity was 2,2 million kg (ATSDR, 2007). Unfortunately, in the present time as MECA (2003) showed there are some countries in Africa, Asia, Oceania, where the usage of leaded oil does not prohibited. In case of Mongolia it should be used only unleaded fuel according to MECA but by the Environmental Health Country Profile, which has been prepared by WHO (2005), shows zero percentage proportion of using unleaded fuel.

Metal accumulation in food chain

Generally plant foodstuffs have higher content than alimentary products with animal origin. Human mean weekly intake is from 0,3 – 1,0 mg and concentrations around 3 mg in food is determined by Merian (1991) as toxicologically unimportant. Main pathway of animal tissues contamination is through ingestion of contaminated feed other route is by respiratory system as (ATSDR, 2007) and according to Merian (1991) creates 10 % of total load, due to higher resorption rate. Also he wrote that accumulation is changed by type of tissue and life length and Rudy (2009) follow up with dependency on species and age of animals.

If the organism has been intoxicated lead does not assign acute hazard, nevertheless content particularly in liver and kidneys from animals which are kept near emission sources (i.e. highly polluted areas) can be in range from 3 to 10 ppm as Merian (1991) quotes, and this means impropriety for human consumption. Liu (2003) confirmed those contents, namely by analyses of kidney and liver tissues which are taken from horses and sheep around smelter in Gansu region in China. Also he showed that levels can be higher than 10 ppm, above mentioned by Merian (1991). In instance of horses average concentrations (ppm) in liver were 23,72 and in kidney 68,83 whereas blood level was 0,28 ppm, sheep were examined by blood (0,34 ppm) and fur samples (3,64 ppm). All animals assigned clinical symptoms of poisoning.

Out of polluted areas very low content, no more than 0,1 ppm of muscle is usual but in rare cases more than 0,1 ppm of liver can occurs, according to Jorhem et al. (1991) this rarely incidence is connected with 0,5 ppm. As Jorhem (1999) present on basis of survey from Sweden, which was focused also to cadmium detection in different tissues of animal origin, mean grades of lead in selected sheep tissues (i.e. muscle and kidney) were 0,002 and 0,046 ppm. The likeness among concentrations of lamb (same standing of tissues) 0,002 and 0,053 ppm, shows that there are no significant changes between animals slaughtered in 6 month and in 2 to 3 years. Analyses of horse samples displayed same results as in case of other animals produce meat, what is interesting in comparison with cadmium. Mean level were 0,002 ppm of muscles, 013 ppm of liver and 0,047ppm in kidney. Additionally Rudy (2009) presents degree of lead contamination in muscles and liver of cattle which were bred by semi-extensive farming in Poland regions, Podkarpackie and Lubelskie without industrial load. Concentrations in first named tissues was in range from 0,051 to 0,075 ppm and in liver from 0,125 to 0,179 ppm, those values were measured in selected groups with span of age from 1,5 to 12 years. In comparison with survey focused on the northern Moravia (cattle in age 3 to 4 years), which was done by Čelechovská et al. (2008), quoted ranges by Rudy (2009) of 1,5 to 2 years old cattle with origin in Poland are higher. Namely concentration in muscle (0,041 ppm), in liver (0,059 ppm) compared to (in the same order) 0,051ppm and 0,125 ppm from Poland.

Effects on human health

The main forms of lead which can have adverse effect to living organisms and which are predominantly present in environment are inorganic lead compounds (i.e. salts, oxides and sulfides). Also organic form (e.g. alkyl-lead) compounds can have toxic effect, but through metabolism of inorganic lead (ATSDR, 2007). Distribution is mainly among blood, soft and hard tissue, as Adriano (2001) presents mineralizing tissue of adults contains 95 % of total body load with unstable component which can be moved to blood. And according to authors Faikŏglu et al. (2006) this inert pool can be responsible for increasing lead level in blood even after removal from place with source of contamination. This mobilizing is depends on physiological stress, pregnancy, presence of chronic disease, lactation.

From viewpoint of bioavailability, particularly chemical, physical form of lead and physiological state of person are crucial for the rate of absorption. Lead which was inhaled to respiratory system is fully absorbed. While less quantity is absorbed in gastrointestinal tract, in case of adult person 10 to 15 % are ingested. For pregnant women and children level of ingestion is higher, more than 50%, additionally higher degree of absorption is also connected with lack of calcium and iron. Exposition of humans to higher levels then basic is common, baseline levels in soil or dust is not connected with human influence (ATSDR, 2007).

Effect to children health

Mainly infants can be afflicted by lead poisoning by Winneke et al. (1996) ever since low doses. This fact represents lead intoxication as an environmental and public risk in global scale (Adriano, 2001). From view of distribution, the lead poisoning of children is the most expanded environmental problem of this element. According Pichery et al. (2011) especially children under 6 years old are exposed to higher doses of lead. Higher exposition has been found because of some factors, such as higher hand dust contamination, more common hand to mouth intermission, higher absorption and high susceptibility to lead effectiveness. By Othman (2001) others fact which is responsible to higher exposition, is larger quantity of food with relation to body weight particularly in group of infants under two years. Lanphear et al. (2000) wrote that dose, already less than 0,05 mg per liter has the adverse effect to cognitive and abilities to learn of 6-16 years old children.

Additionally infants can be influenced already before birth, by burdened mother body, due to lead ability to be transport through the placenta (Pichery et al., 2011). For this reality socioeconomic factor also has the importance. Particularly in the case of developing countries, higher numbers of afflicted infants is caused by low and insufficient pollution control or using folk medicines with content of lead but according to Adriano (2001) this problem does not connect only with these countries also e.g. with Mexico where people use lead to cure of ordinary illnesses as a constipation or to force children to eat meals which they do not like. Factor which plays role at measurement of encumbrance by lead is distance from places with industry or municipal areas, where lead levels in soil, air and with this connected amount lead in human blood are higher than in countryside. Using of unleaded gasoline was considered as source with the biggest importance (Adriano, 2001). Widening the fuel with lead has been banned in USA, Canada and Europe since 1970's. But in China, Russia, India and other mainly developing countries leaded fuel usage is continuing (Bradl, 2005).

Effect to adults

The most often form of acute poisoning is digestive disorder with anorexia, dyspepsia, constipation and colic convulsions. The acute encephalopathy may rarely occurs, symptoms are anemia, vomiting, ataxia, stupor, in more serious coma with heart failure. The concentration in case of this type of poisoning is in range 0,080 to 0.300 mg per 100 ml (Bencko et al., 1995). The encephalopathy is seldom seen in adults but more easily is induced, as a long term exposure, in small children, firstly during early postnatal period damages are the largest. Those damages is connected with effects of mental deficiency which can be created with passage of burdened mother blood to fetal brain.

As Brimer (2011) presents, other organs which can be affected by unfavorable effect are kidneys, as a result of high, medium or low period of exposition. Lead is bounded into tubule cells to proteins with sulfhydryl groups (-SH) which are specific by strong affinity. According to Navaro-Moreno et al. (2009) originating of these compounds may lead to synthesis of acidic protein and long accumulation lead to morphological alterations in proximal tubule epithelial cells and in other structural components. This changes may cause glucosuria, proteinuria nad hematuria (Brimer, 2011).

Further place, when lead can to cause by Lee et al. (2001) are liver where is the place of vitamin D conversion (i.e. hydroxylation) to hormonal form - dihydroxyvitamin D, there interference proceeds. Again children are more susceptible, ranges from 0,033 – 0,120 μg per deciliter of blood showed decreased in serum level of above-mentioned hormonal form of vitamin D. Immunological characteristics can be also influenced as a effect on the cellular compound of immune system, apart from humoral component, but it has not been determined, exactly.

Occupational and environmental exposure can leads to premature childbirth or miscarriage (ATSDR, 2007). In case of occupational problem (e.g. smelter in Sweden), female workers had higher frequency of spontaneous abortion in number of 294 gravidities (Borja-Aburto et al., 1999). Also women who worked or lived in highly contaminated areas had greater likelihood of abortions (ATSDR, 2007). Faikŏglu et al. (2006) confirm this fact, of influence of contaminated area and present that lead can be accumulated in bones which can cause abortion after removal from this area. Because of mobilizing lead accordingly with calcium from skeleton during pregnancy, which equalize higher requirement of this element. Lead and also calcium are transferred through placenta to fetus. In case of male, exposition can means decrease of fertility caused by lower quality of sperm cells, especially in instance of occupational exposure. This unfavorable effect have been indicated by Sállmen et al. (2000) in connection with sperm chromatin and existence of sperm abnormalities, there is no effect to releasing of androgens from testes.

Mercury

Main portion of mercury is in atmosphere as a ubiquitous in vapor form, volatility is increased by higher temperature (Bradl, 2005) because of this attribute according to Agamuthu and Mahlingan (2005) mercury has been accepted as global problem. Additionally non-essentiality for fauna and flora which has been described by Jitaru and Adams (2004) is very important and many researches deal with mercury, attention is particularly set to movements and transformation in milieu. Cycle of mercury is showed in appendix 6. Agency for Toxic Substances and Disease Registry (2009) quoted that the emission peak of mercury was around year 1970 world production was 20 000 tons per year from this number 5000 – 10 000 tons were release to the environment by combustion of fossil fuels. In comparison with anthropogenic activity volcanic activity is annually responsible for 1000 t of mercury. IFCS (2006) presented that world and EU demand decreasing, in case of EU this situation represents the plan which suggests closure of 50 chlor-alkali units up to the end of year 2020.

Forms of mercury

It can be found in elemental form Hg^0 which is characteristic with easily forming alloys, called amalgams, with other metals (e.g. gold, silver, zinc, cadmium), mainly used to gold extraction from ores. Other versions are inorganic ions Hg^{+1} and Hg^{+2} (Stoeppler, 1992) as Jing et al. (2007) wrote, effect of second named form is dependent on adsorption and desorption. Next forms are mercurous and mercuric salts and those are created by these named ions. Organic compounds are methylmercury $CH3Hg^{+}X$(monomethylmercuriccation) and dimethylmercury (CH3)2Hg (Brimer, 2010), process of methylation as Boening (2000) has described, leads to higher availability, mobility and also toxicity. Main problem from viewpoint of toxicity according to Melamed et al. (1998) is the feature of mercury to be transformed from any inorganic version (i.e. less toxic) to particularly this organic form. Generally products of this process are lipophilic and highly toxic for human and animals (King et al., 2002) and can be found in soil, water, sediments and living organisms (Stoeppler, 1992). Those forms of mercury can be decomposed to elemental size after converted to HgS and be evaporated to atmosphere. Mercury in atmosphere can be transported for thousands of kilometers and hence can be spread with large distance from source (Bradl, 2005).

Anthropogenic and natural sources

Naturally it is occurred in soils in concentrations from a few ppb to hundred ppb, normal content is less than 100 ppb, contrary near gold, molybdenum mines burden is higher in range from 50 to 250 ppb sometimes more than 2000 ppb. In vicinity of mercury mines there is concentration in dozens of thousands ppb (Adriano, 2001). Bradl (2005) presented that the main anthropogenic sources are ores mining and smelting, industrial processes utilize mercury and as López et al. (2003) quote, brown coal and wood combustion, combustion of municipal wastes, manufacturing of the cement and sludge. Other human utilization is common in electrical, electrolysis, dental and paper industry also in agriculture is used particularly for seed treatment or as a spray against plant diseases (ATSDR,1999)

Artisanal mining as illegal activities were represented, by recent study performed by Melamed and Villas Boas (1998), as a huge problem in Amazon ecosystem, but impact of used elemental mercury during process called amalgamation is same in other regions. As Tumenbayar et al. (2000) presented, also in Mongolia this problem exists as illegal mercury usage to recover gold from waste which originate by also illegal or legal hard-rock mining. This can have negative influence to streams, in the case of Tumenbayar et al. survey mercury using alongside Boroo river can affect other water bodies in the north - Selenge, Orkhon which flow into Baikal lake.

Terrestrial and aquatic occurrence

The process biotransformation of inorganic mercury to methylated organic mercury, which is crucial to transfer into the food chain, can be influenced by water bodies (Adriano, 2001). This course is called methylation and it has been described by Boening (2000) as a reason to modify mobility, availability and also toxicity. Other influence has been determined by Macalady et al. (2000) as effect of sulphur reducing bacteria and iron reducing bacteria but according to Coelho-Souza et al. (2006) also another types of bacteria can have effect. Next relation is with pH, organic carbon. There is a relationship among fish and water pH when content of methyl mercury is inflicted on acidic pH. In general, speed of uptake and bioavailability is impacted with low pH and water hardness, bioaccumulativeness is higher in relation with solubility in fat. Not only methyl mercury can be accumulated, inorganic mercury may be also absorbed but in slower rate and with low effect (Adriano, 2001). Speed of bounding has been determined by Downs (1998) with result that methyl mercury is bounded up to 100 times more faster than inorganic form and its concentrations in aquatic organisms is higher than in water. Other comparison is that organic version is eliminated slowly from organism, in range of months and years. Therefore accumulation of inorganic mercury is reduced (Stoeppler, 1992). Nevertheless both, inorganic salts and organic form present threat during usage of mercury to recovery of gold form heaps after hard-rock mining procedures (Tumenbayar et al., 2000).

Bioaccumulation and toxicity of mercury in terrestrial ecosystem are lower due to strong isolation in soil and it can influence water bodies. Thanks to deposition of air mercury, it can serves as long-range to water. Transfer from soil to roots and then to leaves is very slow and subordinated with soil properties, plant species and form of mercury, therefore this pathway does not important to intoxication of animals (Adriano, 2011). For example in plants, which were collected during survey of Molina et al. (2006) in vicinity of Almaden mercury mine in Spain, there were determined levels only one to three time higher in comparison with vegetation out of this area although level of mercury in soil was elevated more than 100 times (Adriano, 2001). In result the burden of terrestrial herbivores is lower by comparison with aquatic organism (ATSDR, 2007). Typical content in deer, moose, caribou liver tissue is less than 0,15 ppm and in muscle tissue less than 0,10 ppm, muscle concentration in carnivores is usually about 0,20 ppm (Adriano, 2001). Rudy (2009) states levels in cattle bred partially in pastures in Poland, exactly in Podkarpackie and Lubelskie provinces. Mean load is detected under 0,001 ppm in meat and liver samples in all groups of cattle (i.e. from 1,5 to 12 age). As Čelechovská et al. (2008) present, that mean concentration in muscles from cattle was same (0,0011 ppm) but during analyses of liver and kidney higher levels were found, videlicet 0,0033 ppm and 0,018 ppm.

Effects on human health

Mercury in the environment represents by Bridges et al. (2010) risk to human especially to internal organs as a brain, intestine, kidneys, liver. Negative impact to human is depends on mercury form, the most significant role from point of view of human health plays methyl mercury (Stoeppler, 1992) so as Rooney (2007) specified, organic mercury originating by binding of covalent binds with carbon from function group (i.e. methyl). In comparison Bradl (2005) quoted that primary mercury is well absorbed in respiratory tract but less in gastrointestinal tract and Brimer (2010) represent that majority of mercurous salts are insoluble, therefore mercurous ions are not toxic. And further that once absorbed it can be distributed through the body, also placental and brain barriers can be passed. When it is already present in the body, it is oxidized in the red corpuscles to the well soluble mercuric ions Hg^{2+}. In the opposite site many salts of above mentioned mercuric ($Hg2^{+}$) are easily absorbed in duodenum. In the result inorganic mercury and methyl mercury are particularly assimilated in the gastrointestinal tract, first kind in amount of 7 - 15% and second 90 – 95% (Adriano, 2001). Organic forms are generally well absorbed, methyl-mercury is formed in the environment partially by microbial methylation, particularly by sulfatereducing bacteria (Brimer, 2010), accurately according to Rooney (2007) inorganic ions are methylated namely in soil and water.

Toxic features are tribute to affinity for thiol group –SH, in common with cadmium and lead (Brimer, 2010). Bridges et al. (2010) have specified that those groups are part of bio-molecules, accurately glutathionein, cysteine, homocysteine. The toxicity varies in relation with mercury species, due to it has a large extent. This variability also influences distribution through the body after absorption. Non-liphophilic and medium lipophilic forms of compounds are known with their toxicity to intestine surface and ability to be absorbed to proximal tubules in kidneys where induce formation of metallothioneins and those are bound to the kidney cells (Brimer, 2010). This author also described, that the absorption in tubules is positively influenced with concentration of glutathionein in tubular cells. According to experiment with rats which have been done by Massany et al. (2007) this action leads to changes in renal structure – reduction of glomeruli diameter and quality of cells, which means kidney damages. Other organ which can be influenced is neural system because methyl mercury is the neurotoxin which creates non-reversible changes, damages in nervous system of fetuses and in the case of adults neurological deterioration can be caused by this form of mercury (Collen et al., 2003) and this toxicity is connected with elemental mercury, too.

As Adriano (2001) presented the most important exposure way for humans is fish and fish products intake. In addition the content of lipophilic methyl mercury and polychlorinated biphenyls caused impossibility of reduction during cooking (Colllen et al., 2003) . Seaweed and marine algae is very important potential source of methyl-mercury as Brimer (2010) adduced, due the higher content of methyl iodide in seawater which caused chemical methylation. Other pathways are drinking contaminated water, food or cutaneous entering from water (Adriano, 2001). Pregnant women and less than 14 age infants are in higher risk from methyl mercury because its ability to cross placental barriers and in the case of young children their lower bodyweight (Bradl, 2005). Exact impacts are described as a neurobehavioral alterations (i.e. mental retardation) and blindness or ataxia. (Zahir et al., 2005) presented that mothers who consume food intoxicated by mercury can influence their babies through breast milk, and motor skills or memory performance of infants are decreased already in supposedly safe doses. Likewise the adults can be influenced also by low doses with same changes in memory and in attention. Other effects is various from impaired movement and sensory defects to tremors, spasm and death (Stoeppler, 1992).

Metal accumulation in food chain

Mercury is well known for its bioaccumulation in aquatic food chain in comparison with terrestrial food chain and finally ATSDR (1999) described aquatic ecosystems as more important. The normal values 0,03 to 0,06 mg per kg can be increase with sewage sludge up to 3 or 4 mg per kg. Weekly man intake of mercury is between 0,03 and 0,05 mg and higher numbers is not expected in future (Merian, 1991). Bencko et al. (1995) presented, that in countries where the fish meat is major part of diet, average intake is 0,001-0,002 $mg.kg^{-1}$ of methyl-mercury per person. Agah et al. (2009) further found out during research in Persian gulf, that fishermen which consumed several fish meals per week, fish muscles contain mercury in range from 0,02 – 1,30 mg per kg, had scalp hair mercury concentration around 2,9 mg per kg with 60% of methyl-mercury.

The most important environment in view of accumulation is water, the most common is methyl- mercury, which is rapidly received to aquatic food chain and has larger range in living tissue than inorganic forms. The top of freshwater and seawater food chain carnivorous fish (e.g. pike, shark, swordfish etc.) their tissues contain much more mercury than surrounding water and smaller fish, thanks to biomagnification (ATSDR, 1999). Bioaccumulation is influenced by pH of water, in the case of lower pH together with low dissolved oxygen content, this process is greater (Ponce and Bloom, 1991). Accumulation level from 100 to 1000 in comparison this level in terrestrial food chain is 2 to 5.

Also terrestrial environment is important and according to Gabriel and Williamson (2003) have high influence and mercury contributing to water bodies. As Bishop et al. (1991) have presented huge amount of methyl mercury originating from basins. Plants grown on contaminated soils do not uptake high amounts of mercury but according hypothesis of Grigal (2002) 60% of atmospheric mercury which fall down to soil, is removed to the lakes, rivers etc. Mercury content in soil is influenced by mineral dissolution and regionally by industry and agriculture (Merian, 1991), base mercury levels in grasses are usually around 0,1 mg per kg of dry weight. Biotransfer factors for mercury from soil to plant to animals has not been fully determined (ATSDR, 1999) however as Gabriel and Williamson (2004) have presented, mercury levels in animals are also inferred from locality, air modeling, consumption rates and plant contents.

Arsenic

Sources of arsenic

Natural sources as ATSDR (2005) described, are active volcanoes as well as dormant volcanic activity, very interesting fount is cotton processing, exactly phase of removing seeds from the raw cotton in roller gin.

The main anthropogenic sources due to which arsenic is released to the environment are smelting operations, agriculture, forestry and manufacturing (Stoeppler, 1992) further according to Ötles et al. (2010) also mining, rendering operations, coal and wood burning and also municipal waste incineration. (ATSDR, 2005) determined that smelting of cooper and coal combustion represent 65% of all anthropogenic emissions. Furthermore thanks to high toxicity, it is used in agriculture as a part of pesticides, herbicides and silvicides and also as a protective compound of wood. (Bradl, 2005). Haffert and Craw (2007) wrote down that occurrence of arsenic is as component of lead, zinc, gold and copper ores and can be discharged during smelting process and incurred combustion gases and particulates can affect ecosystem around. Also from refining of these ores, arsenic compounds can be released in this case especially arsenic oxide. Main problem by Roussel et al. (1998) is possibility to pollution of water which can be caused by disposal of slag in heaps, particularly arsenic-abundant wastes lead to natural process of arsenic mobilization.

Different amounts in ppm of arsenic are exuded by coal and oil combustion. Average concentrations can be from 100 ppm to 1000 ppm. It is not only industrial combustion but also domestic combustion of coal with general level of arsenic ranging from 90 to 1200 mg . kg^{-1} (Adriano, 2001)

WHO (2005) during survey in Ulaanbaatar found out that there is average demand of coal 5 million tons only to power generation in plants and 200 thousand of coal are burned in households. Further arsenic content in coal from the 10 biggest coal pits is in range from 121 to 183 ppm. Also in southern China, people are exposed to high doses of arsenic due to coal burning inside houses in open pits for cooking and also for drying corn. The air As level is from 0,04 to 0,13 mg . m-3 and content in coal is in range from 1,5 to 11 mg . kg^{-1} (Adriano, 2001).

In normal vegetation content is about 0,1 to 1 mg per kg of dry matter and plants in area which have been polluted by industry or agriculture the concentration is increased to 1,0 mg per kg up to 20 mg per kg. The risk of arsenic in vegetation is limited, higher accumulations are allocated in marine organisms and from those is transformed to fish meals as a fodder for poultry and swine feeding, in mean contents about 0,4 ppm (Azizur, 2008). And thus higher concentration in animal tissues can be expected in pork about 0,1 ppm, in pork kidney 0,5 ppm and in pork liver from 0,05 to 5 ppm. In kidneys and liver from cattle which was bred in unpolluted areas the concentrations were less than 0,1 mg or up to 5 ppm (Merian, 1991). Muscle content was found during ecological audit which has been performed by Žáček et al. in period from 2003-2005 around the one of biggest Cu-Mo mine, which is located in vicinity of the third biggest city of Mongolia – Erdenet. Concentrations were determined in levels less than 0,005 ppm in three animals (sheep and goat) from total four examined. Other study by López et al. (2000) from Galicia (i.e. northwest Spain) showed mean concentrations (ppm) in muscle, liver and also from kidneys: 0,004; 0,01; 0,02 respectively. Other authors Nriagu et al. (2009) presented details of arsenic levels from Jamaica (through whole country), arithmetic mean level in liver was 0,016 ppm and maximum was 0,358 ppm.

Toxicity

This element is omnipresent and can be found in all soils and other components of environment (Adriano, 2001). Its toxicity, how Ötles et al. (2010) wrote, is connected already with low (ppb) concentrations in environment. Additionally it is assumed as a toxic metalloid which deals with the whole world and contamination of foodstuffs and that arsenic is expanded global problem. The main species (i.e. oxidative states) of arsenic in nature particularly in soils and water are arsenate (V), arsenite (III). In addition the most important compounds are white arsenic, sulfid, copper acetarsenite, calcium arsenate and lead arsenate.

Thanks to the highest mobility and solubility, the arsenite species are the most toxic, also pentavalent arsenic are poisonous substance but trivalent form is more toxic due to its higher mobility in sediments and groundwater (Bradl, 2005) and also as Ötles et al. (2010) wrote, for its higher stability. The both exhibit feature to easily accumulation in living tissues because of their high affinity for proteins, lipids and cell components (Adriano, 2001). For humans arsines, inorganic arsenites, organic trivalent compounds arsenoxides are more toxic in comparison with lower toxicity of elemental and organic pentavalent compounds. The most common exposure ways for human are ingestion and inahalation.. The drinking of contaminated water is considered as a main risk (Bradl, 2005), drinking limit has been established by WHO (2001) to 0,01 mg per liter. Others ways to exposition are through foodstuffs as a rice, fish or milk (Ötles et al., 2010). Situation in Mongolia exactly in Ulaanbaatar is described in health survey which have been set up by WHO (2005) and it shows that up 82,4% of the exanimate people had signs of arsenic poisoning, 16, 5% had light and 1% moderate indications.

Health effects

Arsenic as inorganic or organic in food is also identifiable. The process of inorganic form which lead to methylated arsenic is controlled by metabolism, from arsenate to arsenite and finally due to self methylation. And how Ötles et al. (2010) presented this transformed inorganic compounds are less hurtful.

Problem of chronic intoxication as Kapaj et al. (2006) note, has been reported from many areas on the world, and mainly is inflicted with inorganic arsenic which is highly toxic, this form is innately (i.e. by disintegration, erosion) cumulative in soil or by mentioned anthropogenic sources. Organic arsenic which has been considered as less hurtful is mainly present in food. Huge extension is caused due to possible presence in water which can be used as potable water, irrigation water or in industry (Smith et al. 2000). With water the term arsenicosis is connected and has been described by Kapaj et al. (2006) as a disease which are brought on by long-term using of water with high amounts of arsenic but ingestion is not single pathway according to Smith et al. (2000) and also through skin and respiratory system body can be influenced.

Organs which can be usually affected are liver, kidney, skin, circulatory system and whole gastrointestinal tract. Dermal changes are in form of lesions (e.g. hyper pigmentation, excessive hair falls out) others are skin blackening especially on the palms, soles, chest and limbs, these changes can be connected with cancer. Arsenic has been put by International Agency for Research on Cancer (1980) into group of human carcinogens. Occurrence of these sings has been connected with 5–10 years length of exposure mainly by ingestion and as Rossman et al., (2004) showed, particularly reported from Taiwan, Bangladesh, India, Argentina. Next stage is characterized with changes on internal organs – maximization of liver, kidney, spleen and further with conjunctivitis, bronchitis. The last stage are mainly skin tumors and others organs and after gangrene may occurs (Ötles et al., 2010).

Other effects can be anemia (ATSDR, 2007) in form of diseases – unfavourable impact to immunity causes higher susceptibility to viral or bacterial diseases, due to it can be equated with other disease which is not connected with arsenic (Miller, 1997). Stone (2008) alerted to high contaminated food mainly with inorganic form, particularly in connection with paddy rice because half of humankind is dependent on it. Diet established on these contaminated rice, may lead to cancer. But on the other hand diet with sufficient doses of vitamin C, high-proteins, high-carbohydrates, high-fats, can influences negatively to arsenic toxicity (Roychodhury et al. 2003).

4 Materials and methods

As follow from aims, collection of samples and their analysis were focused on the heavy metals. Sampling was proceed exclusively in territory of the Mongolia, accurately in selected counties and districts. Period of collection was from the end of August to the end of November 2011. Analyses were taken in length of time from the January to the April 2012, and were carried out in the Chemistry Department of State Veterinary Institute in Prague. Samples of liver were examined due to high accumulation then, as well, muscles were analyzed particularly thanks to their importance for human nutrition. Those were obtained, handled and processed in way with the aim of a maximum chance of detecting the substance. Sample handling procedures were prevented the possibility of contamination or loss of analytes.

4.1 Field methods

Samples were collected or purchased in farmers' markets, central purchasing places and in local slaughterhouses. Location of sampling points were in the capital of Mongolia – Ulaanbaatar, in cities Darkhan, Bulgan and Sainshand which are situated in central and southern region. To determination of the heavy metal kontent in livestock tissues were wi thdrawn their skeletal muscle and liver parenchym. Total of 415 tissue samples were taken from sheep and cattle age 2-4 years, in sporadic cases tissue samples came from horses 3-6 years old and goats with same span of age as a sheep. Information about slaughter age were obtained on basis of information from book of author Төмөржав (1989) and from herders. Overview of total samples is listed in Appendix 13.

From all collected 415 samples, 119 were analyzed from which were created 24 mixed samples. One mixed sample included averagely 5 subtotals, overview of number of mixed samples with included partials is listed in Table 1. Exactly 19 mixed samples originated from the area, where higher burden was expected and last 5 mixed samples result from territories with lower expected encumbrance. The map with locations of mixed samples collection is listed in Appendix 14. Number of possible analyzed samples was constrained by financial limit.

Animal tissue samples were placed in micro sampling tubes with screw locks and fixed with 96% ethanol. After preservation, samples were stored and adjusted for transport to subsequent processing in the accredited laboratory of Chemistry department of the State Veterinary Institute (SVI) in Prague-Lysolaje. (Certificate of accreditation is given in Appendix 7)

Table 1: Overview of number of analyzed sample (mixed, subtotal) and their origin

No. of mixed sample	Tissue	Quantity of partial samples
	County	
	Mutton muscle	
1	Eroo	5
2	Orkhon	5
3	Orkhon Tuul	5
4	Baruun Buren	5
5	Javkhlant	5
6	Khuskhaat	5
7	Darkhan	5
8	Zaamar	5
9	Erdene	5
10	Sainshand	5
	Mutton liver	
11	Baruun Buren	5
12	Darkhan	5
13	Khongor	5
	Beef muscle	
14	Baruun Buren	4
15	Eroo	3
16	Darkhan	5
17	Sainshand	5
	Horse muscle	
18	Sant	4
19	Sainshand	5
	Mutton liver	
20	Uliastai	5
21	Uvs	8
22	Terlj	5
	Goat liver	
23	Terlj	5
	Horse liver	
24	Terlj	5

4.1.1 Markets

To obtaining samples were selected market halls in the capital Ulaanbaatar: Naraan Tuul, Bayan Dzurkh, Mercury, from these places samples of unknown provenance originated. Overview of places, animal species and numbers of tissue samples is listed in Appendix 8. In the cutting part of Merkuri market were collected 14 muscle samples and 15 samples of liver. In the meat section of market Naraan Tuul were collected 17 muscle samples. In the Bayan Dzurkh market 24 muscle samples came from.

In the district town Bulgan 16 muscle samples were collected, exactly from 3 regions (Bulgan, Arkhangai, Selenge) and one sample of liver from Selenge region. Above mentioned samples came from districts, Orkhon, Bayan Agt, Bulgan, Buregkhangai, Erdenemandal.

Similarly was performed collection in farmer's market in town Sainshand in south part of Mongolia, where total of 17 muscle samples were obtained from adjacent area of district city. Summary of samples which were acquired in these markets is listed in Appendix 9.

4.1.2 Purchasin places

In Mongolian conditions carcasses are sold by herdsmen to local merchants with meat and slaughter products.

In northern part of Mongolia, in Darkhan were collected 97 muscle samples with origin in the following districts: Erro, Orkhon, Orkhon Tuul, Baruun Buren, Khongor, Darkhan, Javkhlant, Khuskhaat, Navcal, Mandal, Sant. Additionally 38 liver tissue samples were collected with origin from districts: Baruun Buren, Darkhan, Tsagan Tolgot, Mandal Gobi. In the capital of Mongolia, Ulaanbaatar purchasing place is situated in farmer's market Naaran Tuul II, where were obtained 103 samples with origin from regions: Zavkhan, Khentii, Uvurkhangai, Arkhangai, Uvs and 13 samples from districts Erdene and Tsagan Ovoo. Overview of obtained samples is listed in Appendix 10.

4.1.3 Slaughterhouses

After the carcass processing, samples were taken particularly with origion from the central region Tuv, which is main attraction zone. A total of 39 liver samples were purchased from districts Zaamar, Jargalant, Bornuur, Erdene, Cengel Toson Uul, Uliastai, Bayan Tsogt and 19 liver samples from the National Park Terlj. Overview of samples is listed in Appendix 11.

4.1.4 Preservation and storage

Tissue sample from farm animals (muscle, liver) with weight approximately 1-3 g were placed into micro-sampling tube of 2 ml volume with screw caps and then fixed with 96% ethanol. Accurate volume of ethanol 1 ml was ensured by using a calibrated pipette. After closing, tubes are marked with label with registered serial number and information about animal species, tissues, and date of collection locality.

In this way adjusted samples were stored in temperature around 20 °C. Transport to the State Veterinary Institute in Czech Republic in Prague-Lysolaje proceeded in agreement with local authorities, Mongolian State Veterinary Service in Ulaanbaatar. (Copies of laboratory examination of hazard agents are listed in Appendix12)

4.2 Laboratory methods

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

For sample treatment was used concentrated nitric acid (68,4% HNO3), suprapure, hydrogen peroxide (H2O2), demineralized water, without all of ion soluble matters and silicon.

Microwave digestion of examined tissues was proceed in equipment ETHOS PLUS from MILESTONE company. For determination of heavy metal presence (Pb, Cd, As) was used included 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 SPECTR 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 all samples were put into microwave device for process of sample mineralization.

4.2.1 Mineralization by microwave device

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

Methodic procedure

Mixed sample composed from 5 partial samples with origin in same county (every time separately liver and muscle) in weight from 1 to 3 g was placed into teflon vessel. Afterwards 4 ml of concentrated nitric acid was added. After 30 minutes was added 2 ml of concentrated hydrogen peroxide (30%). Mixture was covered by cap, left for at least 1 hour to react, applicated to plastic segment and closed by moment key. This way adjusted sample was put in to 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.

4.2.2 Measurement of heavy metals

For content identification of lead, cadmium and arsenic was used method which combine inducted coupled plasma and mass spectrometry (ICP-MS), using isotopes Pb207, Cd111, As75.

Before measurement content of nitric acid was reduced 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 SPECTR AA 220Z, due to the elimination of error.

Before determination of mercury concentration, the removal of nitric acid overspill was not recommended because of potential losses of quantified elements, hence dilution of mineralizate was done. The measurement of mercury was proceed by atomic absorb spectrometer (AMA 254). Quantity of 200 μl of sample 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.

4.3 Statistics analysis

Results were subjected to statistical evaluation in software STATISTICA Cz version 10.0, (Statsoft CR Inc.)

For statistical purposes samples below the level of detection of selected elements, were assigned a values of these detection limits, as was used in papers (López et al., 2003; Waegeneers et al., 2009; Noël et al., 2011). Afterwards data were prepared to table in the MS EXCEL 2007.

Before analysis data were tested for normality using the Kolmogorov-Smirnov and Lillieforsov test. Because of irregular data distribution Kruskal-Wallis ANOVA non-parametric statistical test was used and variances between groups were tested by multiple comparison test.

Mixed samples of horse liver were not included to statistical analysis by reason of possible influence to results. Since as (Baldini et al.,2000) and (Beldoménico et al., 2001) presented this tissue together with kidney and spleen achieve substantially higher levels in comparison with other domestic animal.

5 Results

Given results was obtained by analysis of 119 animal tissue samples (29 partial samples). Investigation was done in skeletal muscle and liver parenchym, which were taken from markets, slaughterhouses and purchasing places in different counties listed in field methods, and originating from livestock – cattle, sheep, horse. In case of bovine tissues only analysis of skeletal muscles were performed because of low availability of liver parenchym with known origin.

Concentration of lead and cadmium was evaluated according to maximum limits established by Commission Regulation (EC) No. 1881/2006 which sets maximum levels for certain contaminants in foodstuffs (European Environmental Agency, 2006). Limits for arsenic and mercury are established by SVI for monitoring of contaminants, those limits were been found out on the basis of personal communication with laboratory worker of SVI Ing. Buhrová (2012).

Exceeding of these maximum limits was found out in case of cadmium in mutton muscle which came from counties Erdene and Zaamar. In other case it was horse liver from National park Terlj. Increased concentration in comparison with other samples from collection were additionally determined in mutton liver also from area of Nation Park Terlj (in vicinity of capital Ulaanbaatar) exactly in case of lead, cadmium. Higher level of mercury was in mutton muscle from Zaamar district.

5.1 Evaluation of heavy metals exceeding

Bovine skeletal muscle

After executed analysis of bovine muscle result from Table 2 that concentration of selected heavy metals – lead, cadmium and arsenic – established by method ICP-MS were not exceeded maximum limits. As well as in instance of mercury which was quantified by method AAS-AMA.

Table 2: Concentration of selected heavy metals Hg, Pb, Cd, As in bovine skeletal muscle originating from different counties and districts of Mongolia (comparison with maximum limits)

County	District	Hg (ppm)	Pb (ppm)	Cd (ppm)	As (ppm)
Selenge	Baruun Buren	<0,002	<0,05	0,023	0,02
	Eroo	<0,002	<0,05	<0,005	<0,02
Dornogobi	Sainshand	<0,002	<0,05	<0,005	0,03
Darkhan Uul	Darkhan	<0,002	<0,05	<0,005	0,03
Maximum limits		**0,05	*0,1	*0,05	**0,1

Note: * maximum limits – according to Commission Regulation (EC) No. 1881/2006

** maximum limits – according to SVI rules for monitoring of contaminants

Ovine skeletal muscle

Fromanalyzed samples of ovine muscle was ascertained concentration of cadmium excess of maximum limits established by Commission Regulation (EC) No. 1881/2006 (see Table 3). Samples with origin from districts Zaamar and Erdene which are situated in county Tuv have 15x and more than 1,5x higher concentration, respectively.

Table 3: Concentration of selected heavy metals Hg, Pb, Cd, As in ovine skeletal muscle with origin from different counties and districts of Mongolia (comparison with maximum limits)

County	District	Hg (ppm)	Pb (ppm)	Cd (ppm)	As (ppm)
Selenge	Eroo	<0.002	<0.05	<0.005	<0.02
Selenge	Orkhon	<0.002	<0.05	<0.005	<0.02
Selenge	Orkhon Tuul	<0.002	<0.05	<0.005	<0.02
Selenge	Baruun Buren	<0.002	<0.05	<0.005	<0.02
Selenge	Javkhlant	<0.002	0.05	<0.005	<0.02
Selenge	Khuskhaat	<0.002	<0.05	<0.005	<0.02
Darkhan Uul	Darkhan	<0.002	0.07	<0.005	0.03
Tuv	Zaamar	0.005	0.05	0.75	0.05
Tuv	Erdene	<0.002	0.08	0.086	0.05
Dornogobi	Sainshand	<0.002	<0.05	<0.005	0.03
Maximum limits		**0.05	*0.1	*0.05	**0.1

Note: * maximum limits – according to Commission Regulation (EC) No. 1881/2006

** maximum limits – according to SVI rules for monitoring of contaminants

green colour – exceeding of maximum limit

red colour – increased concentration

Ovine liver parenchym

Analysis of mixed sample from National Park Terlj, which is situated in central county Tuv showed in case of cadmium increased concentration in comparison with other samples in collection (see Table 4). Lead concentration reached to the highest level 0,25 ppm from county Tuv (National Park Terlj) in rest of samples concentration was lower than given limit i.e. 0.05 ppm. In instance of cadmium the highest concentration 0,109 ppm was from county Tuv (National Park Terlj).

Table 4: Concentration of selected heavy metals Hg, Pb, Cd, As in ovine liver parenchym from different counties and districts of Mongolia (comparison with maximum limits)

County	District	Hg (ppm)	Pb (ppm)	Cd (ppm)	As (ppm)
Selenge	Baruun Buren	<0,002	<0,05	0,038	0,02
Darkhan Uul	Darkhan	<0,002	<0,05	0,026	0,03
Darkhan Uul	Khongor	<0,002	<0,05	0,045	0,05
Tuv	Terlj	0,002	**0,25**	**0,109**	0,07
Uvs	Davst	<0,002	<0,05	<0,005	0,02
Zavkhan	Uliastai	<0,002	<0,05	0,061	0,05
Maximum limits		**0,05	*0,5	*0,5	**0,1

Note: * maximum limits – according to Commission Regulation (EC) No. 1881/2006

** maximum limits – according to SVI rules for monitoring of contaminants

red colour – increased concentration

Equine skeletal muscle

Examination of equine skeletal muscle to presence of heavy metals Hg, Pb, Cd, As showed (see Table 5) that all concentration were below value of maximum limits given by Commission Regulation (EC) No. 1881/2006 and by rules of SVI Prague for monitoring of contaminants.

Table 5: Concentration of selected heavy metals Hg, Pb, Cd, As in equine skeletal muscle from different counties and districts of Mongolia (comparison with maximum limits)

County	District	Hg (ppm)	Pb (ppm)	Cd (ppm)	As (ppm)
Selenge	Sant	<0,002	<0,05	0,027	0,03
Dornogobi	Sainshand	<0,002	<0,05	0,015	0,04
Maximum limits		**0,05	*0,1	*0,2	**0,1

Note: * maximum limits – according to Commission Regulation (EC) No. 1881/2006

** maximum limits – according to SVI Prague rules for monitoring of contaminants

Equine liver parenchym

In samples of equine liver concentration of cadmium were exceeded maximum limit given by Commission Regulation (EC) No. 1881/2006 videlicet 5,5-fold.

Table 6: Concentration of selected heavy metals Hg, Pb, Cd, As in equine liver parenchym from Tuv county of Mongolia (comparison with maximum limits)

County	District	Hg (ppm)	Pb (ppm)	Cd (ppm)	As (ppm)
Tuv	Terlj	**0,021**	0,13	**2,77**	0,06
Maximum limits		**0,05	*0,5	*0,5	**0,1

Note: * maximum limits – according to Commission Regulation (EC) No. 1881/2006

** maximum limits – according to SVI Prague rules for monitoring of contaminants

green colour - exceeding of maximum limit

5.2 Differences of Hg, Pb, Cd, As concentrations in animal tissues in selected regions of Mongolia

As result from Figure 1, in liver parenchym and skeletal muscle of tested animal, there was no significant differences ($p > 0,05$) in mercury concentration among selected counties of Mongolia [Kruskal-Wallis ANOVA: $H(5;23) = 4.75$; $p = 0.4471$].

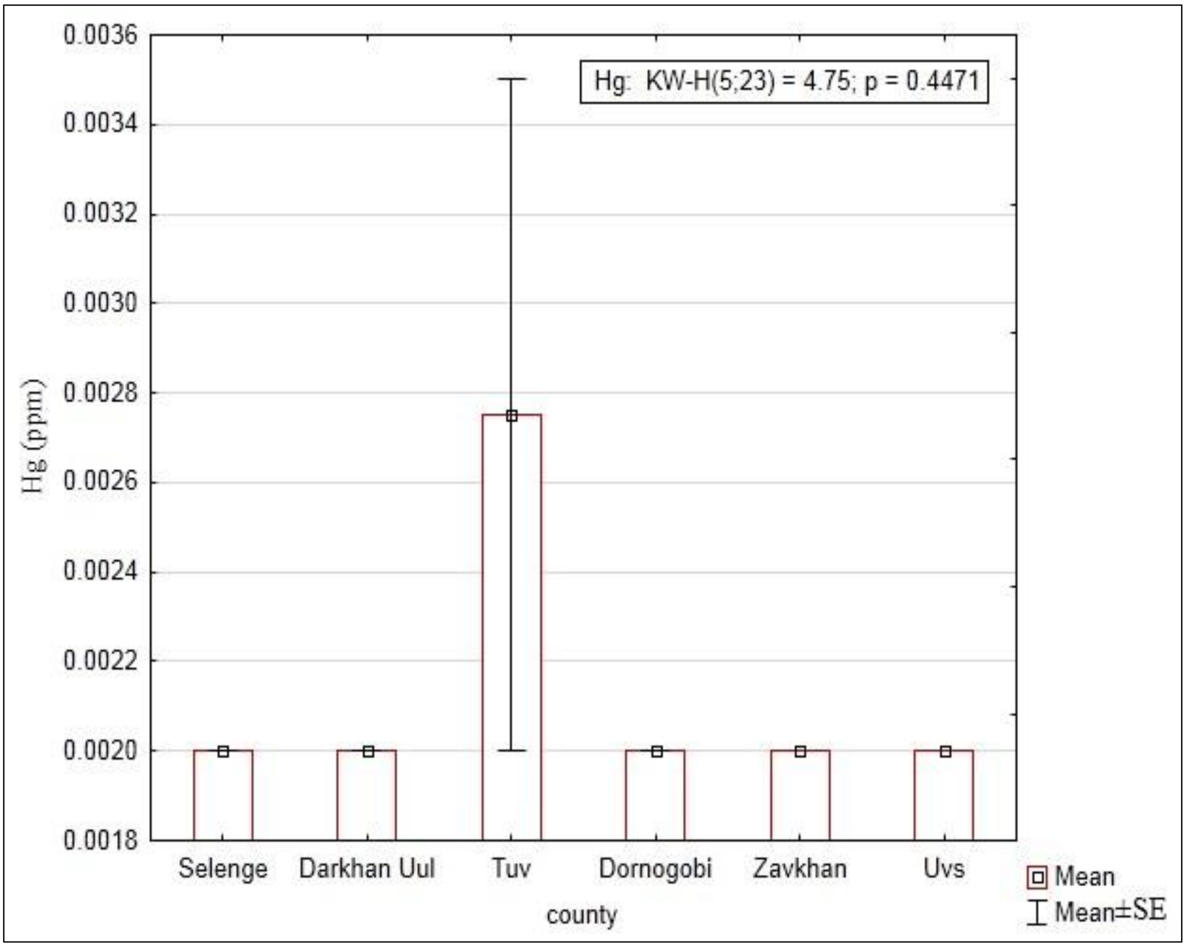

Figure 1: Statistically no significant difference of mercury concentration in realtion to selected counties

In concentration of cadmium and lead (Figure 2; Figure 3) in skeletal muscle, statistical significant differece was showed ($p < 0,05$) [Cd: Kruskal-Wallis ANOVA: $H(5;23) = 13.9293$; $p = 0.0161$) and Pb: Kruskal-Wallis ANOVA: $H(5;23) = 12.7919$; $p = 0.0254$] And multiple comparison test affirmed that county Tuv has significantly higher Selenge ($p < 0,05$) concentration of cadmium than Selenge.

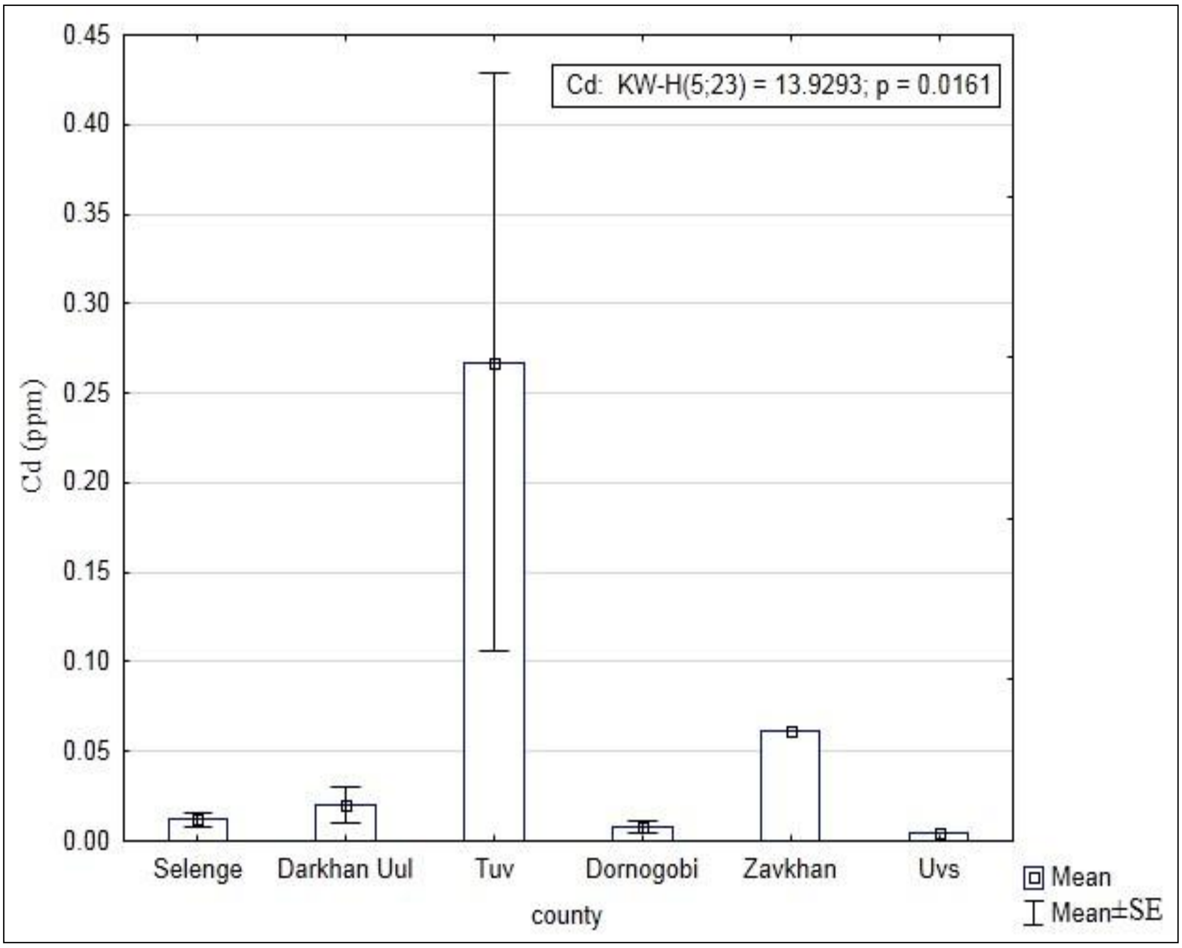

Figure 2: Statistically significant different of cadmium concentration in relation to the selected counties

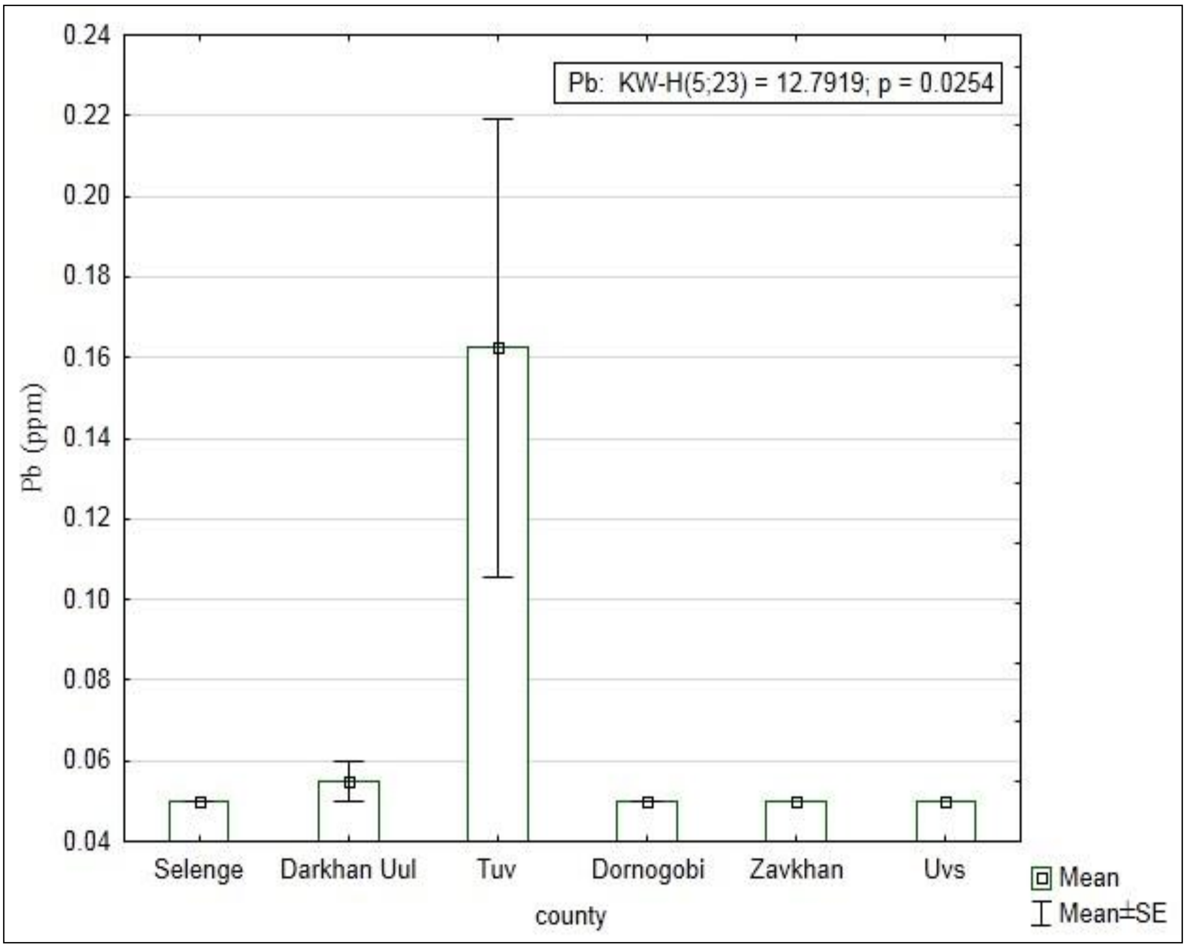

Figure 3: Statistically significant diference of lead concentration in relation to the selected counties

Differences in arsenic concentrations in animal tissues (liver parenchym, skeletal muscle) in selected counties were statistically relevant ($p < 0,01$), [As: Kruskal-Wallis ANOVA: $H(5;23) = 19,5194$; $p = 0.0015$] (see Figure 4). And on the base of multiple comparison test was additionally confirmed significant difference of Tuv county ($p < 0,01$) by comparison with Selenge county, in arsenic concentration in liver parenchym and skeletal muscle.

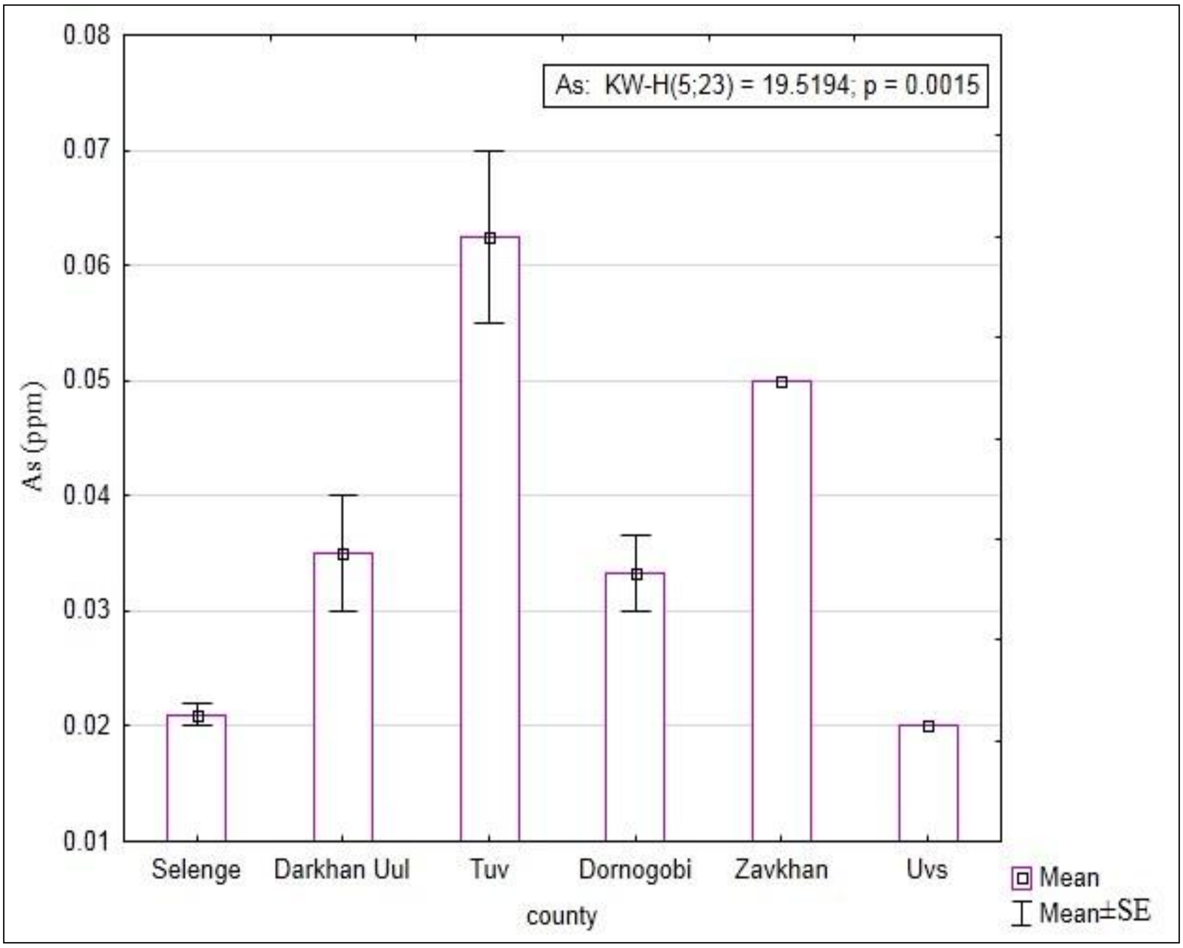

Figure 4: Statistically significant diference in case of arsenic concentration in relation to the selected regions

6 Discussions

The survey aimed only to determination of heavy metals in animal (livestock) tissues was pilot in territory of the Mongolia. Moreover, the main aim carried was to assess and to evaluate concentration of heavy metals in animal tissues originating from different parts of Mongolia and as well as, to assess the health risk for consumers in view of intake of these elements through food, particularly meat and liver. The livestock in total number were approximately 42-50 million heads which is bred exclusively with extensive type of farming on unspoiled land but also around cities and in areas burdened by mining. In addition, this type of farming with nomadic lifestyle provides general situation of the environment on larger area as presented in (Snively and Flaspohler, 2006) a case study connected with waterfowl migration and mining regions.

Samples were originated from counties Selenge, Tuv, Darkhan Uul with assumed higher burden and from regions with probable lower load Uvs, National Park Terlj, Zavkhan, Dornogobi. The burden of the affected counties is connected to combustion of fossil fuels around cities and to illegal or legal mining activities. In European Union conditions monitoring of contaminants and residues is conducted in accordance with Commission Directive (EC) 96/23, (EC) 96/22 and Commission Decision (EC) 97/74 and 98/179 which have been transported to legislation in member states of EU.

During the study, samples of animal tissues were collected from counties of Selenge, Darkhan Uul, Tuv, Uvs, Zavkhan and Dornogobi. Whereas the total number of taken samples from Mongolia was 415 afterwards 24 mixed samples (including 5 partial samples) were created which represented appropriate region (county), those were processed in laboratory of State Veterinary Institute (SVI) in Prague. Analyses were performed in accordance with Standard Operational Technique appointed by SVI in case of cadmium, lead and arsenic, ICP-MS was used, and in case of mercury AAS analytical method was used. Similarly, analysis proceed in studies performed by (López et al., 2003; Chibunda & Jansen, 2009) where AAS analytic method was used for the determination of mercury. However, in case of the determination of other heavy metal; lead, cadmium and arsenic, AAS was used as well (López et al., 2000) which is the opposite of the determination of this elements in my survey. The other authors who used AAS analytic method were (Baldini et al., 2000; Sedki et al., 2003) to establish cadmium levels, (Jorhem, 1999; Farmer and Farmer, 2000) in case of cadmium and lead.

Statistically significant difference in concentration of elements Pb, Cd, As was ascertained in county Tuv (see. in Figures 2,3,4 and also in Appendix 15). Exactly statistically significant difference was determined among the above mentioned county and Selenge region. Therefore the part of hypothesis number 1, which supposed higher levels of selected pollutants in vicinity of cities was confirmed. On the other hand, the significant difference partially invalidates this hypothesis, when concentration of heavy metals in tissues originating from counties affected by mining will be significantly higher than out of these areas. This hypothesis was established on basis of previous studies of (Pavlov et al., 2008; Šourek, 2008; Spiegel and Veiga, 2009), who presented that Selenge river basin which flows through same-named region is affected by illegal gold mining particularly by the release of mercury during the process of amalgamation (extraction of gold from wastes) originating from hard-rock mining. Tumenbayar et al. (2000) additionally quote that contamination can be caused also by improper storage and handling with mercury. Authors Spiegel and Veiga (2009) specified that in this area 7000 mining groups are situated and Šourek (2008) phrased that contaminated area can be higher than only along of the river Selenge.

Not only mercury but others selected heavy metals such as lead, cadmium, arsenic was expected in higher concentration due to more populous places which is connected with increased degree of combustion of fossil fuels and waste.

Within Tuv county, samples were analyzed from districts Zaamar, Erdene and Terlj, those are situated in vicinity of capital Ulaanbaatar. This is the coldest capital throughout the world where coal heating plants have to generate heat for 9 months in a year as World Bank (2004) present that there were combusted huge amounts of coal. Three coal heating plants, in 2005 burned 5 million tons of coal, whereas consumption of households was estimated to be 200 000 – 350 000 tons of coal and 160 000 m3 of wood, but this figures could be higher, thanks to large area of urban slums (Ger areas) in periphery of the city. Davy et al. (2011) on the basis of study which investigated air pollution in the vicinity of capital, represented that tendency of this influence is increasing and particularly connected with local population growth and sequential higher coal combustion in plants and households. This activity is the main anthropogenic source of mercury and arsenic as Tian et al. (2010) presented, and additionally cadmium is also another source (Bradl, 2005). The elevated concentration of lead contributes to a fact that in the city and its surroundings there is higher traffic density and as WHO (2005) quoted leaded fuel is ordinarily used to drive as the means of transport in Mongolia.

In mixed samples of ovine muscle it was detected that cadmium concentration was above limit, 0, 75 ppm, which means that fifteen fold exceeding of maximum limit 0, 05 ppm established by Commission Regulation (EC) No. 1881/2006. To evaluation of concentration, this regulation was used because Mongolian veterinary and health service attempts to harmonize and adopt EU legislative conditions. In muscle, it was additionally found that increased level of mercury in concentration 0,005 ppm, was increasing in comparison with others samples collected when mercury concentration was below detection limit of 0,002 ppm.

The above mentioned data originates from the analysis of animal tissues from Zaamar district which belongs to the northern part of Tuv region as shown in Table 3 in chapter Results.

Given concentrations are likely caused by the fact that in Zaamar district the huge gold placers are situated there and their number is increasing. The location of these places is along the river which is about 50 km in direction to the river Selenge and Orkhon. As Farrington (2000) quoted the area is negatively affected by extraction of sand deposits and mud, storing of barren rock and increased traffic. In the case of cadmium and mercury, important influence is the increasing of population density in association with higher combustion of fossil fuels (coal and wood) or also burning of waste from households. Those as Abdel-Sabour (2001) presented are one of the anthropogenic sources for cadmium and also for mercury according to López et al. (2003).

Increased concentration of mercury can be connected with illegal usage of extraction of gold from wastes after hard-rock mining, because as Farrington (2000) states that at mining of gold by panning toxic matters are not produced. However, in case that some illegal mining groups use mercury to processing of mining wastes (also it is connected with improper storage) there is a probability that inorganic salts leak out to water flow. Moreover, Tumenbayar et al. (2000) described those droplets with diameter bigger than 5 mm represent the highest risk of distribution elements to river and contamination of other rivers in the basin. These facts are in addition connected by low level of education and awareness about hazards of mercury (World Bank, 2006).

The other collection of samples with above limit concentration of cadmium 0,086 ppm was mixed sample of ovine skeletal muscle originating from district Erdene, county Tuv, in vicinity of capital Ulaanbaatar. Established concentration was slightly higher in comparison with mean value 0,06 ppm determined by Farmer and Farmer (2000) for ovine muscle from surroundings of region Oskemen characterized with smelter industry in eastern part of Kazakhstan. By comparison with mean values 0,0034 ppm determined in muscle of sheep through whole Sweden (Jorhem, 1999) concentration from district Erdene is again higher.

Maximum limit of cadmium appointed by Commission Regulation (EC) No. 1881/2006 for ovine muscle is 0,05 ppm. This exceeding could be caused with surroundings of capital, where high degree of fossil fuel and waste combustion are (World Bank, 2004; WHO, 2005).

Similar situation occurred in collection of equine liver parenchym samples from Terlj National Park (eastern course, from the Ulaanbaatar), maximum limit for cadmium 0,5 ppm appointed by Commission Regulation (EC) No. 1881/2006 was five-fold exceeded. Concentration in mixed sample of liver parenchym was 2,77 ppm. Similar result was presented by Farmer and Farmer (2000) at survey in eastern Kazakhstan. In addition this measured value was very close to concentration from analysis of equine liver originating from Sweden (Jorhem, 1999) where mean value of cadmium in liver was 2,5 ppm. Parallel concentration was determined by Baldini et al. (2000) through analysis of equine liver samples from horses originating from different countries of European Union. Values ware in the range of 0.07-18.12 ppm and mean concentration was 2.46 so again very similar to level of cadmium concentration in examined livers from Mongolian horses. As Falandysz (1993) presented high concentration of this element in comparison with other domestic animals can be explained by higher ability of horses to accumulate cadmium in liver tissue.

In the bovine muscle there was no case exceeding of lead, cadmium (see Table 2 in chapter Results) according to maximum limits appointed by Commission Regulation (EC) No. 1881/2006 nor in case of mercury and arsenic by maximum limits fixed by State Veterinary Institute (SVI) in Prague for monitoring of contaminants. Nor in the instance of skeletal muscle of cattle from Selenge region where increased content was expected. In comparison with survey of Chibunda and Janssen (2009) who found out residues of mercury in Tanzania in Geita district under huge influence of illegal artisanal gold mining, concentration of mercury established from bovine muscle from Selenge was lower, all were below detection limit 0,002 ppm. Whereas the range of concentration in muscle from cattle originating from affected area in Tanzania was 0.022 – 0.081 ppm. There is possibility of comparison with analysis of bovine muscle done by López et al. (2003) originating from northwest part of Spain – Galicia, Asturias. Secondly, is characterized by industrial activity, nevertheless, maximum concentration was 0,003 ppm then similar content of mercury as in samples from Selenge region.

In the case of equine liver, have to be respect probable feature for higher accumulation of heavy metals particularly cadmium due to their higher age of slaughter. The above mentioned facts can present risk for end user mainly in case of inordinate consumption what is very important for individuals (families) who live on or below poverty line. Meat, for those people is not financially available than offal and purchase of kidneys and liver presents hazardous commodity not only for adults but for children as well. It will be necessary to perform veterinary and medical monitoring in regular periods and thus serve as a basis for local authorities to protect the environment not only in vicinity of capital Ulaanbaatar but as well as independent international organization which are act in Mongolia should point out this fact and to negotiate with governments performers about decreasing of health risks for this social group.

The levels regarding to arsenic were all below limits according to SVI Prague standards for monitoring of contaminants (Commission Regulation (EC) No. 1881/2006 although it does not include maximum limits for arsenic). Wherefore, the concentration in ovine, bovine and equine tissues were in range of detectable limit up to 0,07 ppm. By comparison with results from ecological audit which was performed by Žáček et al. (2005) in vicinity of the city Erdenet where the biggest porphyry deposit in the world is situated, established concentration of arsenic was lower, but only low number 4 samples was examined because this analysis represented only partial result. The concentration in tissues from selected livestock from Mongolia was from under detectable level up to 0,07 ppm and in the case of ovine tissues originating from Erdenet content of arsenic; it was below detectable level of 0,005 in 3 of 4 analyzed samples.

The levels of heavy metals concentration in majority of analyzed samples were below maximum limits of cadmium and lead appointed by Commission Regulation (EC) No. 1881/2006 and below arsenic, mercury limits according to standards of SVI Prague for contaminants monitoring. Those limits are set down in interest of public health and therefore hypothesis number 2, that mutton as main type of meat consumed by Mongolians represents health risk from point of view of heavy metals intake by organism of consumers i.e. Mongolian inhabitants, was invalidated.

Appointed concentration of heavy metals in animal tissues from Mongolia which were analyzed during this research in comparison with occurrence of these trace elements in other countries, have screening testified importance of pollution in Mongolian territory in the year 2011. Therefore, with respect to that, it was pilot research project, when evaluation was done according to European Regulation (EC) No. 1881/2006 and Standards prescribed by SVI Prague to monitoring of contaminants. With that been said, results could be claimed to be attained as a basis for sequential monitoring of burdened regions by veterinary services.

Results can be practically applicable in veterinary precautions leading to standardization of monitoring of pollutants in animal tissues in the environment (water and feed) and also in human population.

7 Conclusions

Survey focused on determination of heavy metals in animal tissues originating from Mongolia was pilot project designed in selected Mongolian regions. Generally it can be said that heavy metals in tissues from sheep and cattle (2-4 years age) occurred in low concentrations, which can be comparable with values from monitoring in European Union (e.g. Spain). In collection of equine liver level of concentration was multiple higher than maximum limit (ES) 1881/2006 allows, however similar concentration was proved in EU studies, in Sweden and Italy. The fact that most of determined concentrations of heavy metals were below detectable limits, selected animal tissues do not represent significant health risk for consumers.

In case of sample collection from districts of Erdene and Zaamar in vicinity of the capital Ulaanbaatar, excessive content of cadmium it was discovered. Possible to conclude that this situation is probably caused by higher air pollution due to increasing of coal combustion in three coal heating plants which are situated in the capital. Additionally huge contribution represents coal and waste combustion in Ger areas on periphery.

In collection of samples from Erdene district influence of coal combustion is likely to obvious, thanks to expansive local deposits of fossil fuel (coal) which is the only energy source for the inhabitants. For the prevention of unfavorable effect of exhalations, further education for target groups of dwellers in Ger areas could be performed, mainly focused on burning of municipal waste.

Another solution can be government price support of stoves with better coal combustion properties apart from traditional. This has been partially solved by “Millennium Challenge Corporation” which cooperates with Mongolian government. Modern type of stove shows of 80% lower smoke exhalation in comparison with traditional type.

Motor and bus traffic in surroundings of the capital represent contribution as well, particularly since there is zero usage of unleaded fuel. Therefore, the complete elimination or partial restriction could have significantly influence to reduction of pollutants.

With respect to increased content of mercury in sample collection from district Zaamar, this fact can be explained with potential release of mercury during illegal gold mining, which are extended in this area. Determined concentration of mercury can present base for consequent monitoring because of mining sites are situated in the Tuul river basin and cover area of several dozens of square kilometers. Achieved results can be a starting-point for other survey focused on potentially threatened areas e.g., regions along the Selenge river where expected higher contamination of animal tissues was not affirmed. Thanks to high burden by illegal gold mining that could need to perform larger scale studies with higher analyzed samples, monitoring of illegal mining groups and currently to carry out further education in local communities. To prevention of heavy metals access to the food chain and occurrence of excessive values of pollutants in livestock tissues and in inhabitants as well.

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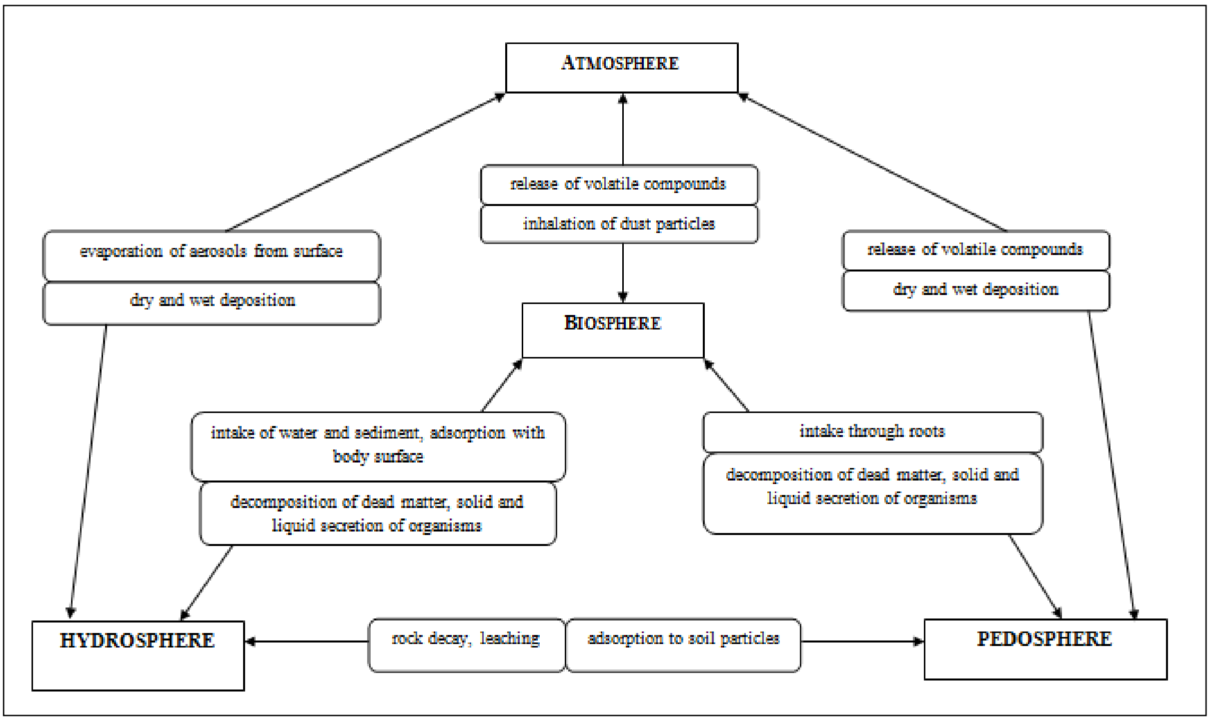
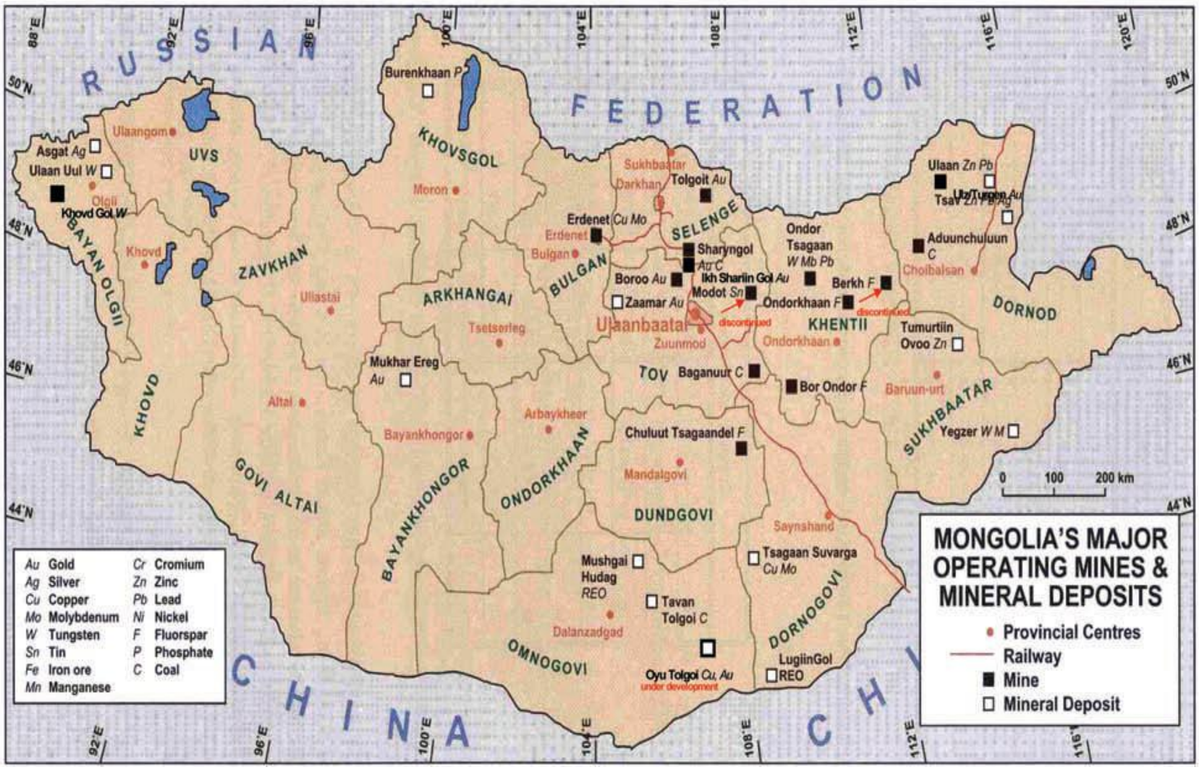
List of appendices

Appendix 1: Transport of heavy metals in the environment (Beneš, 1994)

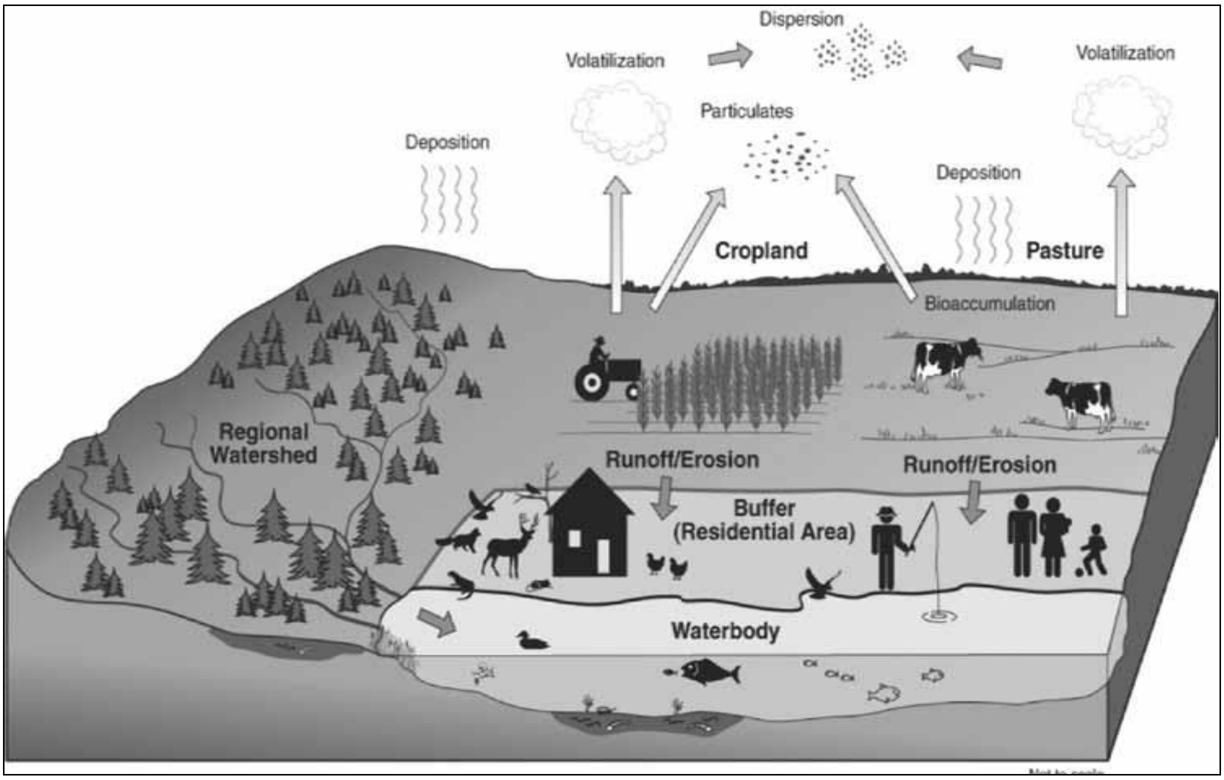


Appendix 2: Major operating mines and mineral deposits in the Monoglia (World Bank, 2006)

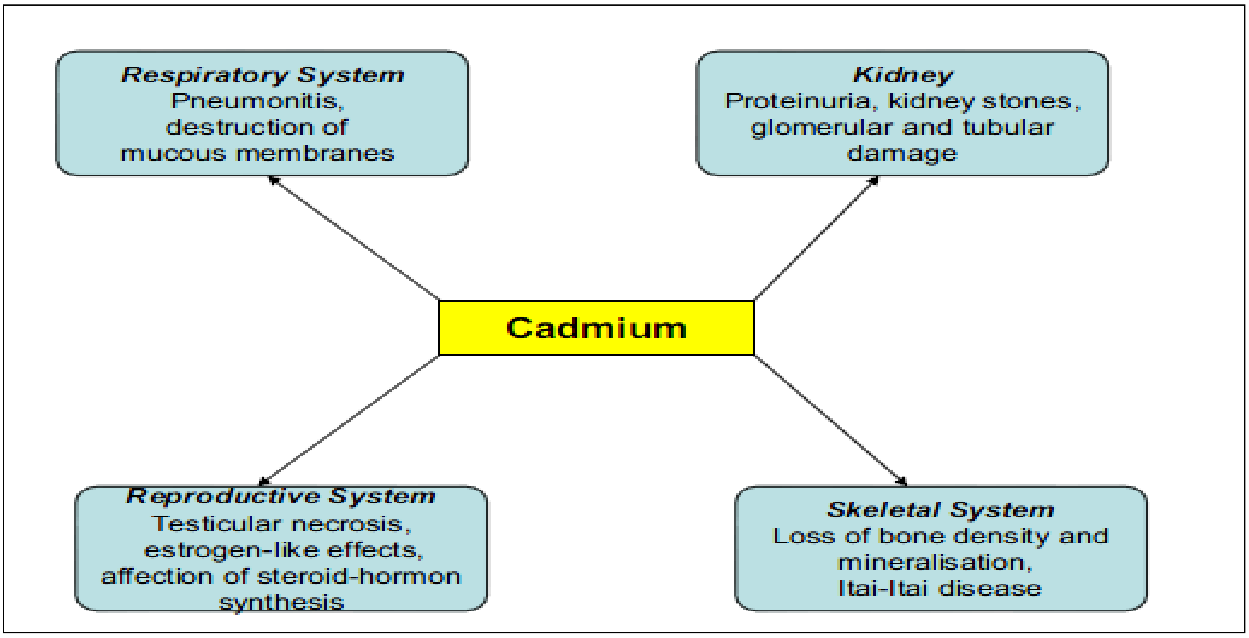


Appendix 3: Children panning powdered rock for gold, after treatment with mercury in vicinity to village well (Tumenbayar, 2000)

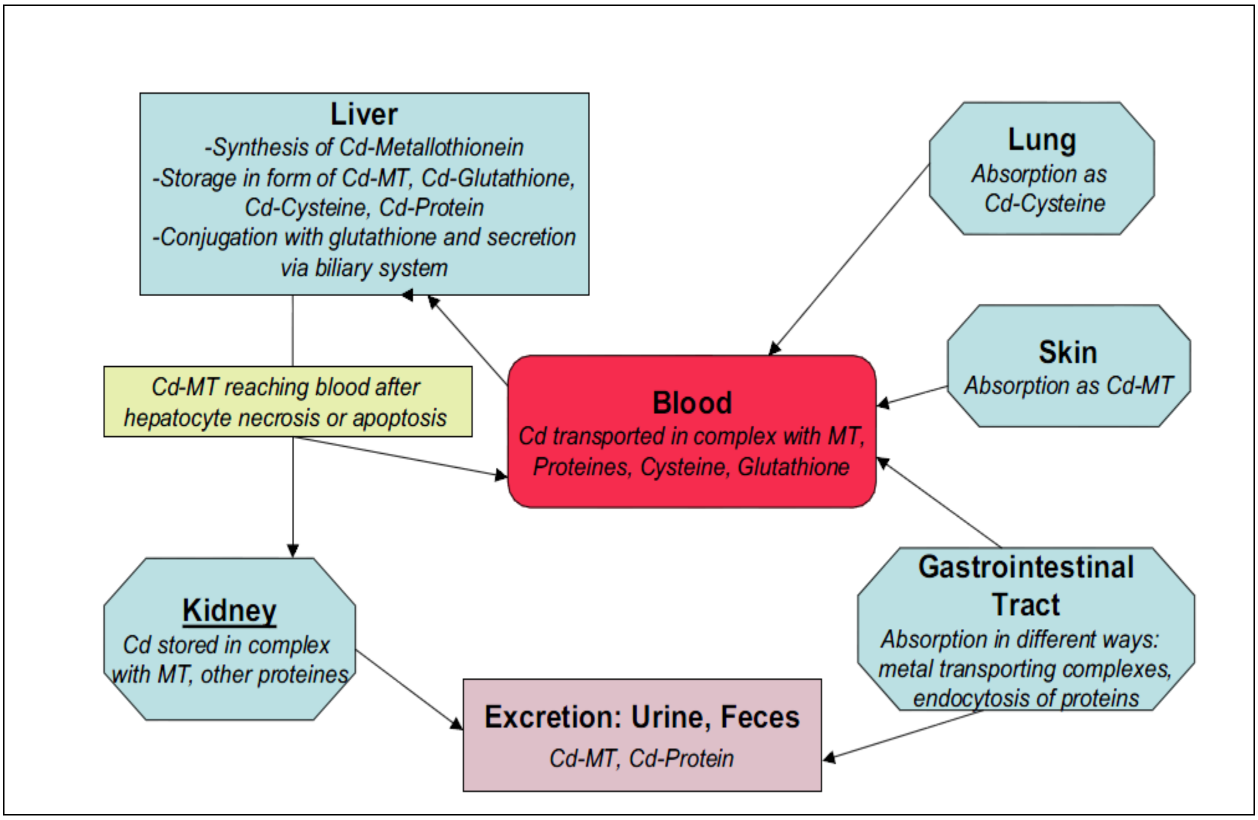
Appendix 4: Diagram of the exposure pathways (Water Environment Research Foundation, 2010)



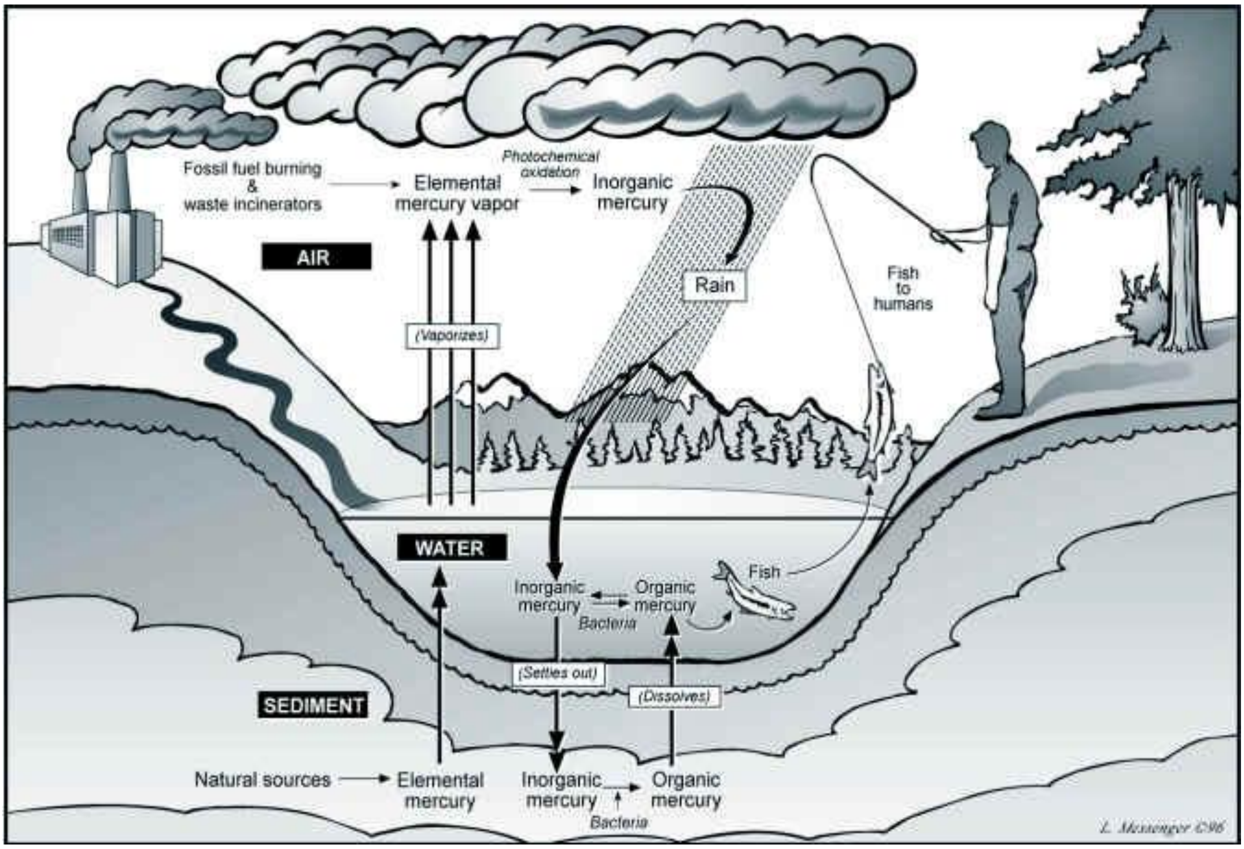
Appendix 5: Effects of cadmium to several organs (Godt et al., 2006)



Handling of cadmium in human body (Godt et al., 2006)



Appendix 6: Cycle of the mercury (Noë, 2011)



Appendix 7: Certificate of accreditation of Chemistry Department State Veterinary Institute Prague – Lysolaje

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.

Appendix 8: Table of samples with unknown origin taken at farmers' markets in capital Ulaanbaatar

	Number of samples Total Number	Animal Species	Tissue	
			Muscle	Liver
Merkuri	8	Ov	x	
	6	Bo	x	
	14			
	7	Ov		x
	8	Bo		x
	15			
Naaran Tuul	10	Ov	x	
	7	Bo	x	
	17			
Bayan Dzurkh	11	Ov	x	
	13	Bo	x	
	24			

Appendix 9: Table of samples with known origin collected at farmers' markets in cities Bulan and Sainshand

Origin		Number	Animal Species	Tissue	
region	**district**			**muscle**	**liver**
Market in Bulgan					
Selenge	Orchon	1	Cp	x	
Arkhangai	Erdenemandal	1	Cp	x	
Bulgan	Bayan Agt	1	Eq	x	
Selenge	Orchon	2	Eq	x	
Bulgan	Bulgan	1	Bo	x	
Arkhangai	Erdenemandal	2	Bo	x	
Arkhangai	Erdenemandal	1	Bo	x	
Bulgan	Buregkhangai	1	Bo	x	
Bulgan	Bulgan	1	Bo	x	
Bulgan	Bulgan	2	Bo	x	
Selenge	Orkhon	2	Ov	x	
		16			
Selenge	Orkhon	1	Eq		x
		1			
Market in Sainshand					
Dornogobi	Sainshand	7	Ov	x	
Dornogobi	Sainshand	5	Eq	x	
Dornogobi	Sainshand	5	Bo	x	
		17			

Appendix 10: Table of samples from purchasing places in capital Ulaanbaatar and from Darkhan

Origin		**Number**	**Animal Species**	**Tissue**	
Region	**District**			**Muscle**	**Liver**
Purchasing place in Ulaanbaatar					
Zaavkhan		10	Ov	x	
Khentii		28	Ov	x	
Uvurkhangai		24	Ov	x	
Dornod	Tsagan Ovoo	7	Ov	x	
Arkhangai		25	Ov	x	
Uvs		16	Ov	x	
Sukhbaatar	Erdene	6	Ov	x	
		116			
Purchasing place in Darkhan					
Selenge	Eroo	16	Ov	x	
Selenge	Baruun Buren	3	Ov	x	
Selenge	Javkhlant	2	Ov	x	
Darchan	Chongor	2	Ov	x	
Selenge	Orchon	7	Ov	x	
Selenge	Javkhlant	6	Ov	x	
Darkhan Uul	Darkhan	9	Ov	x	
Selenge	Eroo	6	Ov	x	
Selenge	Orchon Tuul	8	Ov	x	
Selenge	Baruun Buren	5	Ov	x	
Selenge	Khuskhaat	10	Ov	x	
Selenge	Navcal	3	Bo	x	
Selenge	Baruun Buren	4	Bo	x	
Selenge	Mandal	1	Bo	x	
Selenge	Eroo	3	Bo	x	
Selenge	Darkhan	8	Bo	x	
Selenge	Sant	4	Eq	x	
		97			
Selenge	Baruun Buren	5	Ov		x
Darkhan Uul	Darkhan	10	Ov		x
Darkhan Uul	Hongor	13	Ov		x
Darkhan Uul	Tsagaan Tolgot	6	Ov		x
Dundgovi	Mandal Govi	4	Bo		x
		38			

Appendix 11: Table of samples with known origin from slaughter in the Ulaanbaatar

Origin			Animal	Tissue
Region	District	Number	Species	Liver
Tuv	Zaamar	5	Ov	x
	Jargalant	5	Ov	x
Tuv	Bornuur	5	Ov	x
Tuv	Erdene	5	Ov	x
Khuvsgul	Toson Cengel Ull	3	Ov	x
Uliastai	Zavkhan	9	Ov	x
Tuv	Terlj	7	Ov	x
Tuv	Terlj	7	Cp	x
Tuv	Bayan Tsogt	7	Cp	x
Tuv	Terlj	5	Eq	x

Appendix 12: Copy of laboratory examination of hazard agents from the capital of Mongolia Ulaanbaatar

НИЙСЛЭЛИЙН МАЛ ЭМНЭЛЭГ АРИУН ЦЭВРИЙН ЛАБОРАТОРИ

Баянгол дүүрэг, 2-р хороо, Чингүүнжавын гудамж
Улаанбаатар, Монгол шуудан компани-35
Шуудангийн хайрцаг 29
Факс: (976) 70144009
Утас: 70144003

ИТГЭМЖЛЭГДСЭН ЛАБОРАТОРИЙН СОРИЛТЫН ДҮН

Дугаар *1021* Олгосон: *2011.11.22*

Үйлчлүүлэгчийн нэр: *Чехийн ХААИС-ийн оюутан Simonik Ondrej*
Шинжилгээний зорилго:
Дээжийн нэр: *МЭАЦМШ , Нян судлал*
Дээжийн хэмжээ: *Элэг - 1 дээж*
Дээж хүлээж авсан огноо: *0.2 кг*
Шинжилсэн хугацаа: *2011.11.21*
2011.11.21 - 11.22 *Протокол № 177*

№	Шинжилгээний үзүүлэлтүүд	Шинжилгээний стандартын дугаар	Байвал зохих үзүүлэлт	Байгаа үзүүлэлт	Тайлбар
1	*Өнгө:*	*MNS 1023:2007*	*Тухайн нэр төрөлдөө тохирсон өнгө, үнэртэй*	*Тухайн нэр төрөлдөө тохирсон өнгө ,үнэртэй*	
2	*Амт, үнэр:*	*MNS 1023:2007*			
3	*Биелэг байдал:*	*MNS 1023:2007*	*Чийглэг хэвийн*	*Чийглэг хэвийн*	
4	*Staph aureus-0,1g*	*MNS 4378:96*	*Илрэхгүй*	*Илрээгүй*	
5	*E.Coli-0,01g*	*MNS 4378:96*	*Илрэхгүй*	*Илрээгүй*	
6	*Sal.spp- 25 g*	*MNS 4378:96*	*Илрэхгүй*	*Илрээгүй*	

Тайлбар 1: MNS стандартад (+) тохирно. (-) тохирохгүй
Тайлбар 2: Сорилтын дүн тухайн дээжинд хүчинтэй

Тэмдэг

Стандартчилал хэмжилзүйн газраас итгэмжлэгдсэн лаборатори С № 078

Шинжилгээ гүйцэтгэсэн: *Д.Уранчимэг*
Шинжлэгч эмч ..../ *Б.Баттунгалаг* /
Хянасан: *Д.Лхамноржим*
Лабораторийн эрхлэгч: ..../ /

Сорилтын лабораторийн зөвшөөрөлгүйгээр хуулбарлахыг хориглоно.

Appendix 13: Overview of collected samples

Table of samples with known origin

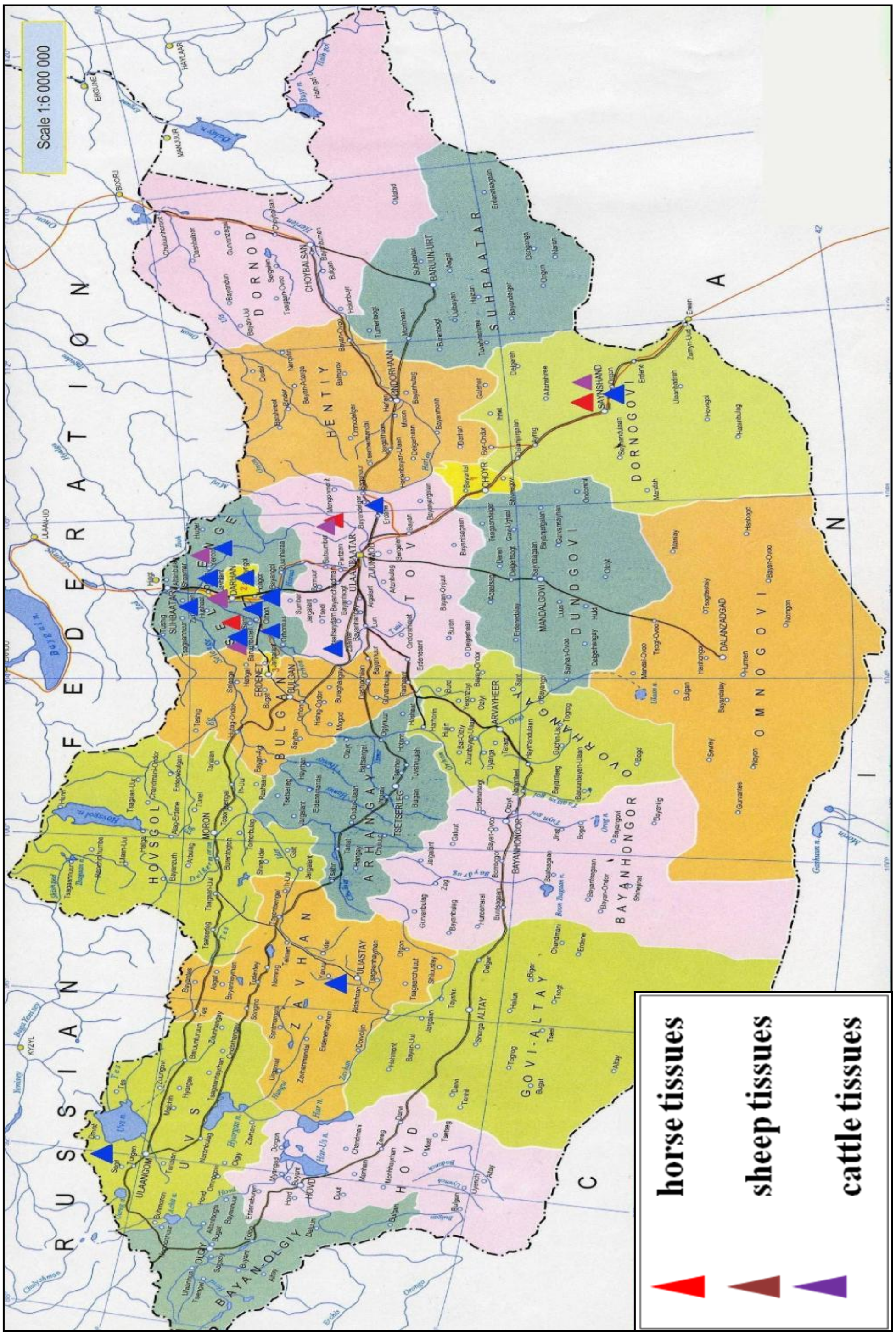
county	district	species	muscle	liver	Total from county
Selenge	Eroo	sheep	22		**78**
		cattle	3		
	Orkhon	sheep	7		
	Orkhon Tuul	sheep	8		
	Baruun Buren	sheep	8	5	
		cattle	4		
	Mandal	cattle	1		
	Sant	horse	4		
	Khushaat	sheep	10		
	Tsagan Tolgot	sheep		6	
Bulgan	Orkhon	sheep	2		**12**
		goat	1		
		horse	2	1	
	Bayan-Agt	horse	1		
	Bulgan	cattle	4		
	Buregkhangai	cattle	1		
Darkhan Uul	Darkhan	sheep	9		**42**
		cattle	8		
	Khongor	sheep	2	13	
	bez sumu	sheep		10	
Khuvsgul	Toson Cengel Uul	sheep		3	**9**
		sheep	6		

Uvs		sheep	16		**16**
Zavkhan	Uliastai	sheep	10		**19**
		sheep		9	
Arkhangai	Erdenemandal	cattle	3		**29**
		sheep	25		
		goat	1		
Uvurkhangai		sheep	24		**24**
Dundgovi	Mandal Govi	cattle		4	**4**
Tuv	Javkhlant	sheep	8		**54**
	Zaamar	sheep		5	
	Jargalant	sheep		5	
	Bornuur	sheep		5	
	Erdene	sheep		12	
		goat		7	
		horse		5	
	Bayan Tsogt	sheep		7	
Dornogobi	Sainshand	sheep	7		**17**
		horse	5		
		cattle	5		
Khentii		sheep	28		**28**
Dornod	Tsagan Ovoo	sheep	7		**7**
Sukhbaatar	Erdenetsagan	sheep	6		**6**
	Total numbers of samples		**238**	**97**	**345**

Table of samples with unknown origin only from markets in Ulaanbaatar

market	species	muscle	liver
Merkuri	sheep	8	7
	cattle	6	8
Naaran Tuul	sheep	10	
	cattle	7	
Bayan Dzurkh	sheep	11	
	cattle	13	
total / tissue		55	15
total number of samples		**70**	

Appendix 14: Sampling places on the map (mixed samples)



Appendix 15: Figure of statisticaly determined differences in concentrations of selected heavy metals in relation to regions.