UNIVERSITY OF NOVA GORICA
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EVALUATION OF THE IMPACT OF LEAD POLLUTION ON PEOPLE´S HEALTH IN THE VICINITY OF A LEAD-GLASS FACTORY

MASTER´S THESIS

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ABSTRACT

Environmental lead toxicity is an old but persistant public health problem throughout the world, and children are more susceptible to lead impact than adults because of their behavior. Over the last decades children´s blood lead level (B-Pb) have fallen significantly in a number of countries. Despite this reduction, childhood lead toxicity continues to be a major public health problem for a certain at-risk group of children, and concern remains over the effects of lead on intellectual development. Lead is dangerous at all levels in children.

The sources of lead exposure vary among and within countries depending on past and current uses.

Lead-glass manufacturing is a known source of pollution from lead, so we studied the lead burden among children who live near a lead-glass factory. Lead-glass production in Rogaška Slatina has been well known since 1665. There has been no previous study under taken for this area.

Human biomonitoring was made from blood and hair samples. We tested 49 children in Rogaška Slatina (mean age 11.4), from 10 to 15 years of age. The children in Rogaška Slatina had B-Pb in range from 4 to 48 μg/L, the median was 20 μg/L and the average was 19.81 μg/L. The control group (children from Rakitna) had B-Pb from 10 to 18 μg/L, the median was 10 μg/L and the average 11.7 μg/L. There were statistically significant differences between both groups ($p<0.001$). The hair Pb ranges from 0.07 to 6.26 μg/g, the median 0.91 μg/g and the average 1.36 μg/g in children from Rogaška Slatina, and from 0.13 to 0.82 μg/g in control group (median 0.39 μg/g and average 0.40 μg/g). The differences in median values between both groups was statistically significant ($p<0.003$).

Between B-Pb and H-Pb on both locations is statistically significant diference ($p<0.001$ and $p=0.03$).

The environmental burden with lead was measured by soil-Pb, air-Pb and vegetable Pb.

Measurements of soil were performed in an area around Rogaška Slatina, for control in Rakitna. Contrary to the expectations, measurement of lead in soils near the glass factory demonstrated only minor increase, insignificant to cause deleterious health effects. Only at one sampling site, above the factory, relatively high Pb concentrations were found (293 mg/kg). We assumed that Pb containing particles, emmited from the factory were transported across larger distances. The direction of prevailing wind in this region is toward that direction. We repeated sampling above the factory at different heights. The highest Pb content was at meters above sea level (MSL) which was nearest the lead-glass factory chimney (1192 mg/kg; average at that MSL 912 mg/kg) and it was above the critical value. The soil-Pb near the chimney was a little smaller to those, measured in the Mežiška Valley area in 2002. Comparison with soil-Pb in Rakitna showed statistically significant differences between locations ($p=0.006$). We concluded that soil across center of Rogaška Slatina was not lead polluted but above lead-glass factory it was. In that direction there is a village and people have cultivated fields. In comparison with data from the survey of soil pollution in Slovenia (ROTS) report for period 1989 – 2007, where the average soil-Pb content in Slovenia was 36 mg/kg, in Rogaška Slatina was higher eg. 59 mg/kg in the center and 301 mg/kg above

the factory chimney, which is comparable to the average content in the Upper Mežiška Valley (320 mg/kg), which is area known to be highly contaminated with lead in Slovenia.

Emission of lead in Slovenia in dust precipitates have been decreased since 1994, when the use of catalytic converters in new cars with petrol engines became mandatory, and leaded gasoline cannot be consumed. In July 2001 Slovenian legislation prohibited the use and maketing of leaded gasoline. Thus there disappeared the largest source of lead in Slovenia.

Samples of vegetables were taken in 2006 at five different sites in Rogaška Slatina, and all were near the lead-glass factory. Eighteen samples of string bean, chicory, carrot, parsley, pepper, cabage, kale and red beet were obtained. One of the samples exceeded the acceptable level (parsley leaves).

We can conclude that in the vicinity of the lead glass factory there are influences on the lead burden, but in limited volume. Children in Rogaška Slatina have B-Pb bellow limit of 100 μg/L, therefore, they are not threatened according to the CDC recommendation, despite the fact, that they have a significantly higher amount of blood lead level than the control group. It is necessary, therefore, to determine which level of lead in blood is the one usual for local residents and further investigation of children, which are above the 95th percentile requires further research. That is especially important because studies demonstrate a negative correlation between BLL, which is bellow 100 μg/L and cognitive function in children.

KEY WORDS: lead-glass factory, lead poisoning, blood lead, hair lead, soil lead, lead in dust particulates

POVZETEK

Toksičnost svinca v okolju je star, vendar perzistenten problem javnega zdravja po vsem svetu. Najbolj dovzetni so otroci zaradi svojega vedenja. V zadnjih desetletjih je nivo svinca v krvi otrok pomembno upadel v številnih državah. Kljub temu zmanjšanju zastrupitev otrok s svincem ostaja velik problem javnega zdravja, pri nekaterih ogroženih skupinah, zaradi vpliva na intelektualni razvoj. Svinec je nevaren za otroke v vseh dozah.

Viri svinca se razlikujejo med državami in znotraj njih glede na pretekle in sedanjo rabo.

Pridelava svinčenega stekla je znan vir onesnaževanja s svincem, zato smo proučili obremenjenost otrok, ki živijo v neposredni bližini steklarne. Proizvodnja svinčenega stekla v Rogaški Slatini je znana od leta 1665. Do sedaj ni bilo opravljene študije obremenjenosti s svincem na tem območju.

Humani biomonitoring je bil opravljen z analizo krvi in las. Testirali smo 49 otrok v Rogaški Slatini (povprečna starost 11.4), starih od 10 do 15 let. Otroci v Rogaški Slatini so imeli vrednost svinca v krvi v območju med 4 in 48 μg/L, mediana je bila 20 μg/L, povprečno 19.81 μg/L. Kontrolna skupina (otroci iz Rakitne) je imela v krvi med 10 in 18 μg/L svinca (mediana 10 μg/L, povprečno 11.7 μg/L). Med obema skupinama so bile

statistično pomembne razlike ($p<0.001$). Nivo svinca v laseh se je gibal od 0.07 do 6.26 µg/g (mediana 0.91 µg/g in povprečje 1.36 µg/g) pri otrocih iz Rogaške Slatine in med 0.13 in 0.82 µg/g (mediana 0.39 µg/g, povprečje 0.40 µg/g) pri kontrolni skupini. Razlika v srednji vrednosti med obema skupinama je statistično značilna ($p<0.003$).

Med svincem v krvi in svincem v laseh na obeh lokacijah je statistično značilna razlika ($p<0.001$ in $p=0.03$).

Obremenitev okolja s svincem smo določili iz vzorcev tal, zraka in zelenjave.

Meritve tal so bile izvedene v okolici steklarne Rogaška Slatina, za primerjavo smo vzorčili zemljo na Rakitni. V nasprotju s pričakovanji, je bila količina svinca v tleh v bližini tovarne stekla le malenkost povečana, v koncentracijah, ki niso škodljive za zdravje. Samo v enem vzorcu tal nad tovarno je bila ugotovljena relativno visoka koncentracija svinca (293 mg/kg). Predvidevali smo, da svinec potuje z zračnimi delci, ki uhajajo iz tovarne na večje razdalje. Tudi smer vetrov, ki prevladujejo v tej regiji, je v tej smeri. Ponovili smo vzorčenje nad tovarno na različnih nadmorskih višinah. Najvišja vsebnost svinca v zemlji je na višini, ki je najbližja steklarni (1192 mg/kg, povprečna za to nadmorsko višino 912 mg/kg) in je nad kritično vrednostjo, ki jo določa predpis. Nivo svinca na tem delu je nekoliko manjši od tistega, ki je bil izmerjen v območju Mežiške doline leta 2002. Primerjava s svincem v zemlji na Rakitni je pokazala statistično pomembne razlike med lokacijama ($p=0.006$). Zaključili smo, da tla v centru Rogaške Slatine niso prekomerno onesnažena s svincem, medtem ko je vrednost svinca nad steklarno močno povečana. V tej smeri je tudi naselje, kjer ljudje obdelujejo polja. V primerjavi s poročilom Raziskave onesnaženosti tal Slovenije (ROTS) za obdobje 1989 - 2007, kjer je povprečna vsebnost svinca v zemlji v Sloveniji 36 mg/kg, je vsebnost v Rogaški Slatini višja tj. 59 mg/kg v centru in petkrat višja (301 mg/kg) nad steklarno, kar je primerljivo s povprečno vsebnostjo svinca v zemlji v Zgornji Mežiški dolini (320 mg/kg), ki velja za najbolj onesnažen del v Sloveniji.

Emisije svinca v Sloveniji s prašnimi delci so se zmanjševale od leta 1994, ko je postala obvezna uporaba katalizatorjev v novih avtomoblih z bencinskim motorjem, ki ne morejo uporabljati osvinčenega bencina. Z julijem 2001 je v Sloveniji stopila v veljavo prepoved uporabe osvinčenega bencina za promet. Tako je izginil največji vir svinca v Sloveniji.

Vzorce zelenjave smo odvzeli v letu 2006 na pet različnih mestih v Rogaški Slatini. Vsa vzorčna mesta so bila v okolici steklarne. Odvzeli smo 18 vzorcev stročjega fižola, radiča, korenja, peteršilja, paprike, zelja, ohrovta in rdeče pese. Količina svinca nad sprejemljivo mejo je bila samo v enem vzorcu, v listih peteršilja.

Zaključimo lahko, da bližina steklarne vpliva na obrmenjenost s svincem, vendar v mejnem obsegu. Otroci v Rogaški Slatini imajo nivo svinca v krvi pod mejo 100 µg/L, zato v skladu s priporočili CDC niso »neposredno ogroženi«, kljub temu, da imajo pomembno višjo vsebnost svinca kot kontrolna skupina. Potrebno je ugotoviti, katera vrednost svinca v krvi je tista, ki je običajna za lokalno prebivalstvo in podrobneje preiskati otroke, ki so nad 95-percentilo. To je še posebej pomembno, saj študije dokazujejo negativno korelacijo med nivojem svinca v krvi, ki je <100 µg/L in kognitivno funkcijo otrok.

KLJUČNE BESEDE: steklarna, zastrupitev s svincem, svinec v krvi, svinec v laseh, svinec v zemlji, svinec v zračnih sedimentih

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ABBREVATIONS AND SYMBOLS

AAS: atomic absorption spectroscopy

ALA: δ-aminolevulanic acid

ALAD: δ-aminolevulanic acid dehydratase

ATSDR: Agency for Toxic Substances and Disease Registry

B-Pb: blood Pb

BSID: Bayley Scales of Infant

BW: body weight

CDC: Center for Disease Control and Prevention

CRM: certified reference material

DM: dry matter

DTIE: Division of Technology, Industry and Economics

EBLL: elevated blood lead level

EDTA: ethylenediaminetetraacetic acid

EPA: United States Environmental Protection Agency

FAAS: flame atomic absorption spectroscopy

FT4: free thyroxine

H-Pb: hair Pb

IARC: International Agency for Research on Cancer

ICP-MS: inductively coupled plasma mass specrtoscopy

IQ: intelligence quotient

LOD: limit of detection

LOQ: limit of quantitation

MSL: meter height above sea level

NHANES: US National Health and Nutrition Examination

PTWI: provisional tolerable weekly intake

ROTS: raziskave onesnaženosti tal Slovenije

SD: standard deviation

WHO: World Health Organization

WW: wet weight

XRF: X-rays fluorescence

1 INTRODUCTION

Humans are exposed to many different harmful substances, which may affect people´s health in many ways. Consequences of exsposure to those substances are usually known as accidents or high levels professional exposure. Input of some harmful substances present health risks even when concentrations are low, but exposure is long (Ernhart, 1989; Rothenberg, 1989; Pocock, 1994; Coony, 1991, Committee on Environmental Health, 1993; Mendelsohn, 1998). This is especially true with substances, which accumulate in the human body, such as mercury, cadmium or lead.

Lead is a common environmental contaminant, and an exposure to lead is a preventable risk. Lead is widely used in industry for building construction, lead-acid batteries, bullets and shot. It is also found in pipes, weights, cable covers and as sheets used to shields from radiation. Lead is commonly found in soil especially near roadways, older houses, old orchards, mining areas, industrial sites, near power plants, incinerators, landfills, and hazardous waste sites (CDC, 2007).

Sources of lead exposure vary among countries. Leaded gasoline accounted for 80 to 90% of airborne lead pollution in large cities where it was previously used worldwide. Countries that have phased lead out of gasoline have reported corresponding decreases in lead concentrations in air and human blood. Metal smelters are another potential source of contamination for air and soil and can contribute to lead posioning. After smelter emissions cease, historic soil contamination can pose an ongoing threat. Informal lead-smelting operations often operated at or near the home, can be a source of lead exposure for nearby residents. The most common source of lead posioning for the majority of young children in the United States is deteriorated, eladed residential paint and lead-contaminated house dust and soil (United Nations Environment Programme, 2010).
People, who are not occupationaly exposed to lead, intake lead especially from food, water and air.

Lead poisoning is an important environmental pollutant that can have life-long adverse health effects. Most susceptible are children. Lead affects virtually every organ or system in the body. The adverse health effects range from death to impaired cognitive and behavioral development that can have lifelong effects for children.
Lead toxicity can be examined at three levels: biochemical, subclinical and clinical. Lead is distributed into all cells. Toxicity is mediated through several mechanisms. At a subclinical level, most children with increased lead exposure and absorption are asymptomatic. The decrements in cognitive abilities and the behavioral disturbances attributable to lead toxicity may lag behind the period of lead ingestion and become most apparent when the children begin attend school, long after blood lead levels have declined for most of them. At extremely high levels, generally greater than 1000 μg/L, children may exhibit signs of increased intracranial pressure and have seizures or lapse into coma (encephalopathy).

Lead is associated with negative outcomes in children, including impaired cognitive, motor, behavioral, and physical abilities (CDC, 2005b; CDC, 2002). The CDC defined blood lead level that should prompt public health actions are 100 μg/L. Concurrently, CDC also recognized that a blood lead level of 100 μg/L did not define a threshold for the harmful effects of lead (CDC, 1991a). Recent research demonstrates that cognitive impairment is associated with blood lead levels bellow 100 μg/L (Lanphear, 2000).

The largest and highly contaminated lead area in Slovenia is the Upper Mežiška Valley. About 19 million tons of the lead and zinc ores were excavated in total. First data on health risks and workers health protection date from the second half of the 19th century. Health studies of the inhabitants in the Meža Valley stretch over 50 years of research (Ivartnik, 2009).

Lead-glass manufacturing is also known as potential source of lead to environment (Baxter, 1985). Few studies have been undertaken into lead exposure among stained glass workers. Landrigan concluded that family members of stained-glass workers also had blood lead levels > 100 μg/L (Landrigan, 1980).

In Slovenia, located in Rogaška Slatina, there is lead-glass factory. Lead-glass manufacturing, and especially grinding and cutting the glass, is reknowned source for pollution with lead to environment. In Slovenia there was no prior study, which could give an assesement of the environmental burden of lead to people who live near the lead glass manufacturing installation.

Because the lead-glass factory lies in the middle of the town, our main objective was to find out whether the proximity of lead-glass manufacturing had any influence on the lead burden to people who live there.

Our hypothesis was: »The vicinity of lead-glass factory influences on the lead bioburden to people, who live in the vicinity of the lead-glass factory.«

Our specific goals were:

- determine blood and hair lead levels of children in Rogaška Slatina;

- determine if children in Rogaška Slatina had higher blood and hair lead levels than the control group;

- determine the extent of pollution in soil around lead-glass factory;

- determine the air quality in that area.

1.1 History of lead

»Lead« is an Anglo-Saxon word for the element initially known by the Latin word *plumbum*, which serves as the root of plumbism, meaning »lead posioning« (Lide, 1994).
The toxic nature of lead has been well known since 2000 BC (Needleman, 1990). Intoxication with lead is probably the most important toxic incidents in the evolution of our civilization. Alchemics belived, that heavy grey is the oldest metal and they connected it with pale and slow Saturn, the planet, described with leaden pither from which the cold water runs. Lead is one of the first metals known by Egyptian, Jews and the Phoenicians. Use of lead in pottery was known in ancient Egypt between 7000 and 5000 B. C. Lead colors were used for pottery glaze. No doubt, the toxic effect was also known to the Greeks, Romans and Arabians. The abdomen colics were the first manifestation of saturnism (Paloucek, 1993; Castiglia, 1995; Philip, 1994). Lead poisoning, from boiling grape juice in lead pots, or for storing ang curing beverages in

lead-lined containers, may have contributed to the fall of Roman Empire. Lead pipes bearing the insignia of Roman Emperors are still in service today (Trotter, 1990; Nriagu 1983a b; Gilfillan, 1965). There are few mentions of saturnism in Middle Ages. In the first half of the 19th. century a few publications on lead poisoning appeared (Stanković, 1986). Despite this, lead has become widely dispersed throughout the environment because of the number of human uses for it (Figure 1).

Figure 1: *Lead coin*

1.2 Lead in the environment

Lead may enter the environment through various sources, such as leaded petrol (in countries where this type of fuel is still available), glazed ceramics, leaded pipes, recycling of car batteries, smelters, certain toys and trinkets and leaded paint, among numerous other sources (Figure 2). Tetraethyl and tetramethyl lead are important because of their extensive use as antiknock compounds in petrol (WHO, 1996.)

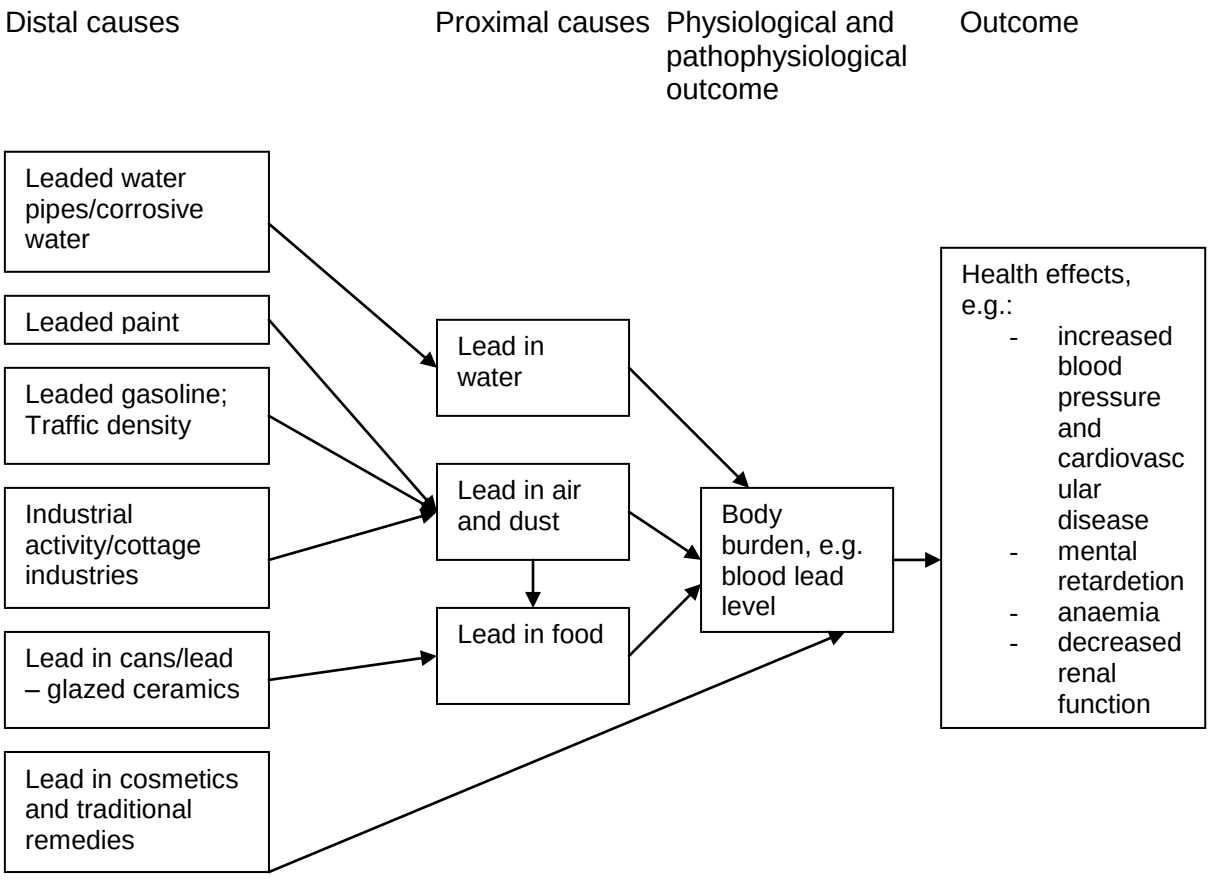


Figure 2: *Framework for lead exposure (modified from Fewtrell, 2004.)*

Lead has a long half-life in the human body. Half - life for lead is 20 years (Rabinowitz, 1976).

1.2.1 Lead in the atmosphere

Concentration of lead in atmosphere depends on numerous factors, including proximity to roads and point sources. Transport and distribution of lead from sources into environment mainly transfer through the atmosphere. Rain removes the majority of lead from the air. When water runs through the ground, lead eliminates quickly, because of the high possibility of organic particles that bind to lead. Because of this mechanism, water usually has low concentrations of lead. The majority of lead in the air is in dust particles, which are smaller than 1 μm, therefore, they can travel with air over very long distances. They usually stay in the low troposphere from 3 to 6 weeks, in the higher part of the troposphere from 3 to 4 weeks (Evans, 1985).

In the year 2007 in Slovenia there were measurements of heavy metals taken in PM10 particles at three different mesurement points. The ammount of Pb was lower than yearly aim value (Bolte, 2008).

1.2.2 Lead in dust and soil

Sources of lead in dust and soil include lead that falls to the ground from the air, and weathering and chipping of lead – based paint from buildings, bridges, and other structures. Thus lead content in soil represents an integrated value of lead pollution within the region.

Once lead falls onto the soil, it adheres strongly to soil particles and remains in the upper layer of soil. That is why past uses of lead such as lead in gasoline, house paint, and pesticides are important constituents in the amount of lead found in soil.

Dust and soil represent important sources of lead, especially for children. Lead remains in soil many years after it enters the ground. Factors affecting the mobility and availability of lead are: soil texture and clay minerology, pH, organic matter and cation exchange capacity and plant factors.

One of the most important factor which influenzes the solubility, and consequently the accsessibilty of inorganic matter, is soil reaction, which is expressed by pH. Generally, the accsessibilty of metal is higher in lower pH, when there are less negative charged parts avaliable for adsorption. The issue is not just measurable lead but the accesibility and bioavialability of the lead. Large particles containing lead are less easily absorbed and, therefore less bioavaliable (Mielke, 1998).

The regulative, boundary level of Pb in PM_{10} till 2008 was 100 $\mu g/m^2$ per day, and 500 ng/m^3 per year.

The first comprehensive study of soil-Pb pollution in the Ljubljana region was performed in the year 1995 (Šajn, 1995). In the suburban area a median lead content in the soil of 41 μg/g was reported. However, significant differences were found between field, meadow (36 μg/g) and forest soils (69 μg/g). In the urban area of

Ljubljana a higher median Pb in the soil was recorded (60 µg/g). Anomously high values were found in the whole area of city centre, in the northern part of the city (317 to 712 µg/g), near the main roads and road crossings and in the car parks (322 to 1196 µg/g) (Štupar, 2007b).

Šajn made the first measurements of Pb distribution in the soil profile from 0 to 5 cm, 5 to 20 cm and 20 to 30 cm across the Ljubljana region. He observed that lead has the tendency of decerasing with depth in all types of unperturbed grounds. The contribution of anthropogenically derived lead in soil was thus assumed to be susbstantial in the upper 20 cm layer and approaches the natural level beyond 30 to 40 cm depth (Šajn, 1995).

In a research report on ground pollution in Slovenia, 2007, was reported that Pb amounted from 10 to 2000 mg/kg of soil. Eleven locations had exceeded boundary level of 85 mg/kg (10 of those reached the warning level of 100 mg/kg). In one location the critical value of 530 mg/kg was excedeed. Average value in the upper layer was 114 mg/kg (Figure 3), which was more than the warning level, and the mediana was 51.5 mg/kg. The mediana in the lower layer was 36.0 mg/kg. In the report on environment in Slovenia for 2009 the excessive warning level for Pb was found in different parts of Slovenia (Muta, Duplek, Žerjav, Bovec, Kranjska Gora, Celje, Ormož, Litija, Ilirska Bistrica) (Urbančič, 2009).

Study in the Mežica Valley, made in 2008, reported the ammount of Pb from 26 to 4483 mg/kg, mediana 320.5 mg/kg. Boundary value was exceeded in 40 samples, they all were above the warning value, eighteen were above critical emmision value for Pb. The study established that of first concern were the high values of Pb in samples of soil from kindergarten, public playgrounds and arrivals, which were settled with material from Žerjav (Zupan, 2008).

Warning level (100 – 350 mg/kg)

Critical value (> 530 mg/kg)

Figure 3: *Pb in upper layer of soil in Slovenia in 2007 (Modified from Zupan, 2008)*

1.2.3 Lead in food

Prepared food contains small but significant amounts of lead. Lead content is increasing when the water used for cooking or the cooking utensils contains lead, or the food, especially if acidic, has been stored in lead – ceramic pottery ware or lead – soldered cans. The intake of lead from lead – soldered cans is declining as the use of lead – free solders become more widespread in the food processing industry (WHO, 1996).

Passage of lead from air into organisms is direct or indirect. Mode and level of lead accumulation in plants depends on growth mode. Lead primarily concentrates in the roots and is generally poorly translocated to the vegetative parts and particularly to the reproductive organs. The concentration of lead does not increase when concentration of lead in soil increases. That is in accordance with findings that lead in soil is poorly mobile and that vegetables adopt – especially those with big leafs – lead directly from the atmosphere.

For Pb in food, drinking water and wines, the reduction in the number of sources contaminated has been a major factor in reducing risk. For, although adventagious contamination of wine and foodstuffs by Pb has been drastically reduced, the ubiquitiouse nature of this element means there will always be the posibility of low levels in some foods. Information on the speciation and bioavaliability will inform the assessment of risk posed by the ingestion of Pb from the sources.

Nutritional factors (Ahamed, Siddiqui, 2007) known to influence susceptibility to lead effects is shown in Figure 4:

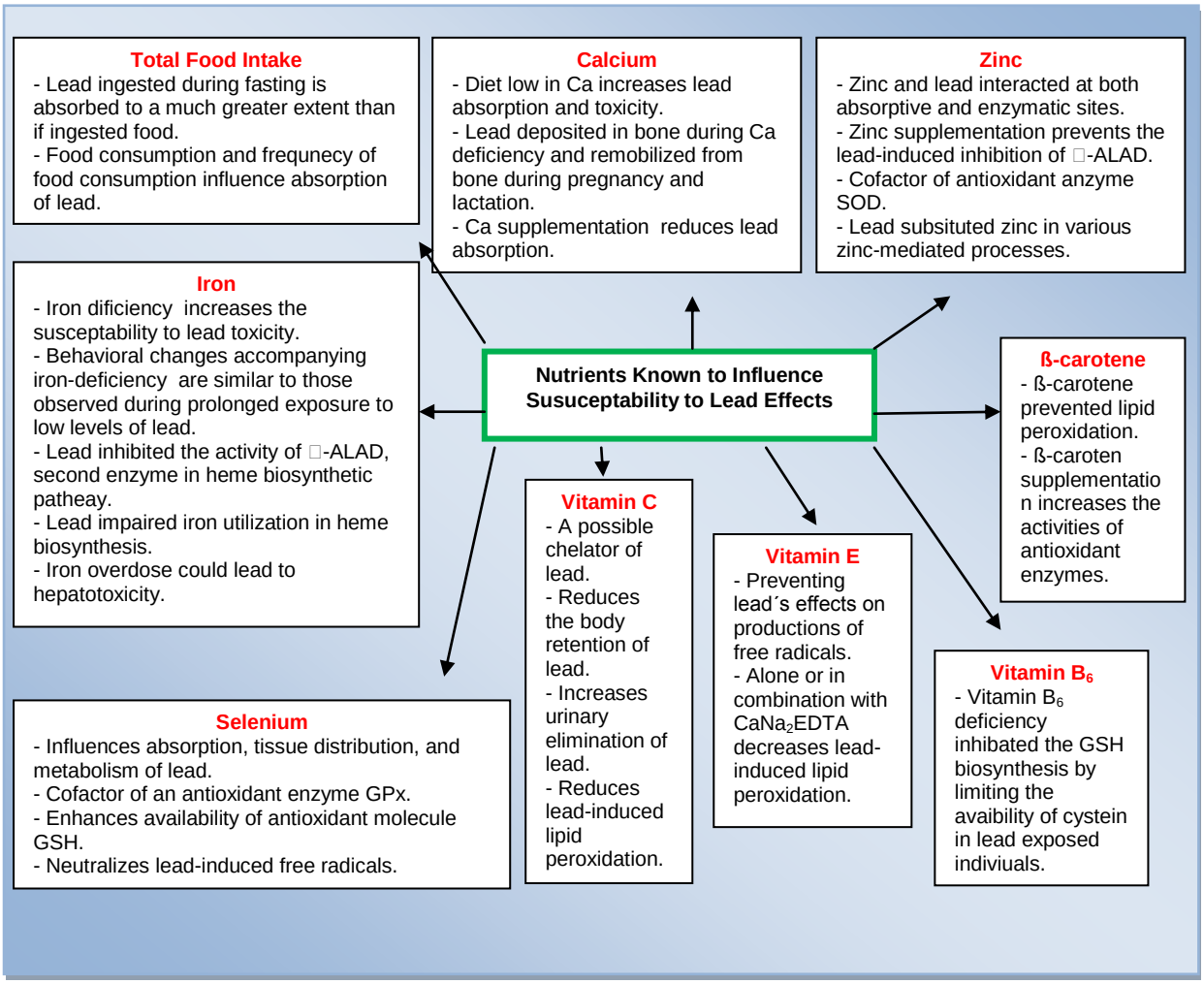


Figure 4: *Nutritional factors known to influence susceptibilty to lead effects (taken from Ahamed, Siddiqui, 2007).*

A child absorbs less lead just after eating than during the period between meals (Chaney, 1989).

Reports show great variability of lead contained in different food types. The most lead contaminated food was: fish, entrails, cereals, vegetable and fruit (Galal Gorchev, Jelinek, 1985).

The concentration of lead in entrails was usually between 50 to 200 µg/kg and is mainly higher that in meat of the same animals. The concentration in meat is nearly higher then 100 µg/kg. Mean concentration of lead in meat is about 50 µg/kg and lower (WHO, 1996). Relatively high concentration of lead is also found in leaf vegetables, especeally in spinach and lettuce. Contamination of lead is between 50 and 100 µg/kg.

Report for Slovenia showed a low concentration of lead in onion, apple, turnip, cabbage, gherkin, tomato, pumpkin and paper. Low concentrations of lead were also

found in meat specimens. The highest median was in samples of preserved meat products (Table 1). Proportionally, a high concetration of lead was found in entrails, fish and eggs. Lead concentrations were above legal level for the EU in: red beet, carrot, potato and flour (Eržen, 2004).

Table 1: *Pb amount in certain foodstuffs and their contribution to weakly consumption in Slovenia (Modified from Eržen, 2004).*

Foodstuff	Average c of Pb in µg/kg	Estimated weekly consumption amount of Pb in µg
1.MILK AND DIARY		**43.1**
2. MEAT AND MEAT PRODUCTS		**49.1**
3. FISH, CRAYFISH, SEA SHELL		**6.3**
4. CEREALS AND PRODUCTS		**183.5**
5. FRUIT AND PRODUCTS		**70.7**
6. VEGETABLE AND PRODUCTS		**78.4**
Potato	30	29.5
Carrot	50	3.5
Onion	20	2.5
Salad	40	12.4
Cabbage, turnipe	20	2.5
Spinach, mangold	70	1.2
Been	40	6.4
String beans	10	0.5
Tomato	20	3.5
Pepper	20	1.6
Cucumba, pumpkin	20	0.4
Red beet	50	4.8
7. GREASE, OILS		**20.0**
8. EGGS		**13.8**
9. SUGAR, SALT, SPICES		**25.9**
10. DRINKS		**63.1**

In consideration of data regarding consumed food, adults, weighing 70 kg, consume 554 µg Pb / week (Table 2), which is 79.1 µg Pb/day, respectively 1.1 µg/kg BW/day. With consideration of adaptation factor for joint amount consumed foods, the ammount of consumed Pb is 663 µg/week, respectively 1.4 µg/kg of BW/day.

Table 2: *Esitimated contribution of individual group of foodstuffs to total consumed amount of Pb with food per habitant in Slovenia and to part of provisional tolerable weekly intake (PTWI) (Eržen, 2004).*

Foodstuffs	Amount of food/person/day in g	Amount of consumed Pb/person/week in µg	Part of PTWI* per person with BW 70 kg in %
Milk and diary	360.5	43.1	2.5
Meat and products	123.6	49.1	2.8
Fish, crayfish, sea shell	14.5	6.3	1.3
Cereals and products	298.6	183.5	10.5
Fruit and products	192.9	70.7	4.0
Vegetables and products	334.2	78.4	4.5
Greas, oils	44.4	20.0	1.1
Eggs	26.3	13.8	0.8
Suggar, salt, spices	48.8	25.9	1.5
Drinks	149.3	63.1	3.6
TOGETHER	**1593.2**	**554.0**	**326**

*PTWI for Pb is 25 µg/kg for all age groups, e.g. 1750 µg for person with 70 kg of BW (WHO, 1999)

On basis of all data it was estimated that PTWI for Pb in adults was almost 40% within food.

For dietary intake of lead the cereals are most important, as well as some species of vegetables, especially rhizome varieties. The average concentrations in this food are up to 50 µg/kg, but they represent almost half of the dietary intake (Galal Gorchev, 1993). Most human food contains < 1 ppm (< 1000 µg/kg) Pb when uncontaminated.

The average concentration of Pb in the United States diet is roughly 0.2 ppm (Mahaffey, 2000). The average daily dietary lead intake is 1.4 µg/kg body weight (BW)/day in Slovenia e.g. 89 µg/day in 70 kg weighting adult (Eržen, 2004), 27 µg/day in Sweden, 66 µg/day in Finland, > 20 µg/day in Germany (Anke, 2004), 90 µg/day in Belgium and 177 µg/day in Mexico. In some countries, dietary intakes as high as 500 µg/day have been reported (WHO, 1996).

1.2.4 Lead in water

Lead in water originates from a variety of sources: atmosphere, geological formation, and in the case of drinking water – plumbing fixtures and lead pipes.
The WHO has established a guideline value for maximal lead levels in drinking water of 0.010 mg/L (CAC/RCP, 2004).

Very high Pb concentrations can be expected in householdes with Pb plumbing. An amount of lead in the water depends on physical and chemical properties of the drinking water (pH, containing of minerals, water temperature, the presence of chloride and dissolved oxygene) and standing time within the waterworks.
Adults absorb 35 – 50 % of lead from drinking water and in children this can exceed 60 %. Lead levels in drinking water in the USA found the geometric mean to be 2.8 µg/L.

The median level of lead in drinking – water samples from five Canadian cities was 2.0 µg/L.
If a concentration of 5 µg/L in drinking – water is assumed, the total intake of lead from this source can be calculated to range from 3.8 µg/day for an infant to 10 µg/day for an adult (WHO, 1996).

1.3 Kinetics and metabolism of lead in humans

The kinetics in the uptake, distribution, and equilibration of lead in blood, bone, and soft tissue are complexed. With short – term exposure to a high dose, the B-Pb may rise and fall relatively quickly (e.g., within days), but some of this decline may be due to redistribution rather than excrection. Once a significant burden has been stored in bone, lead has remarkably long half – life, as long as 10 years in some studies or even 20 years (Dart, 2004).

The amount of lead is increasing with years. In the process of decalcination of bone tissue, which appears in pregnancy, in eldery people or in some diseases, the amount in bones is decreasing, and the B-Pb is increasing. The lead is an important source of internal exposure. Mobilization of lead from bones during pregnancy presents an important risk for over burden of mother and infant. Lead passes over from mother to fetus during all gestation stages, but it is more expressive in the last stage (third tri-mester).

Lead is absorbed readily through the lungs. Like other compounds, particulates less then 5 µm in diameter can reach the alveoli for absorption. Larger particles are trapped by air passage mucus and produce gastrointestinal exposure after swalloving. Orally, adults absorb a small amnount (20 to 30%), but children absorb up to 50% of intake lead. Several soil ingestion studies have indicated that some children ingest susbtantial ammounts of soil on given day. Although the EPA has assumed that 95% of children ingest 200 mg soil/day or less for exposure assessment purposes, some children have been observed to ingest up to 25 – 60 g soil during a single day (Calabrese, 1997). Bioavaliability may be increased by iron deficiency and other factors. In contrast to inorganic lead, organic lead compounds can be absorbed dermally. By measuring lead in tissues such as skin and muscle, rather than relying exclusevely on blood, Stauber et al and Florence et al demonstrated that exposure to lead nitrate appeared to penetrate the skin barrier of mice and humans (Stauber, 1994; Florence, 1998).

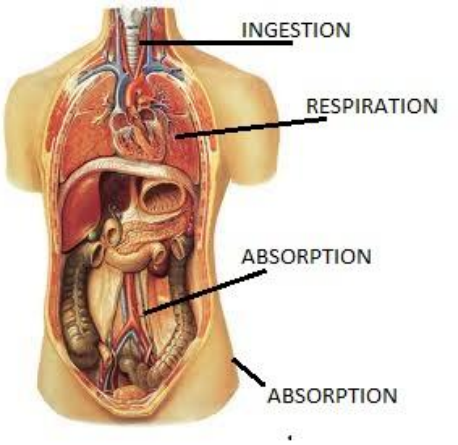


Figure 5: *Routes of lead entry into the human body and its absorption within the body (modified from www.holyiokesbiology111.wikispaces.com)*

In adults, lead is excreted by the kidney at a rate of approximately 30 µg/day. With increasing body stores, this amount may reach 200 µg/day. Excretion may be due both to glomerular filtration and shedding of tubular epithelil cells, within which lead is concentrated. The extent of fecal excretion in humans is uncertain. Lead balance studies indicated that fecal lead nearly matched an oral intake of lead (CDC, 1991a).

Lead crosses the placenta and enters human breast milk (2 to 30 µg/L). Blood levels are generally similar in infants and mothers, although a study in the Mežiška Valley in Slovenia showed that the burden of infants with lead dependes on lead concentrations in their mother's milk. Concentration of lead in milk from 33 mothers in a polluted area was much higher than in milk from mothers in a non polluted area (Primerjalna študija onesnaženosti okolja v Zgornji Mežiški dolini med stanji v letih 1989 in 2001, 2002). Because pregnancy is a period during which maternal calcium soters are mobilized, a significant amount of lead may be transferred simultaniously to the devloping fetus.
In an epidemiological study of women environmentally exposed to lead in Mexico City, maternal bone lead levels were related to a decline in infant birth weight (Gonzales-Cossio, 1997). In a companion study, maternal bone lead levels were related to smaller head circumference and shorter lenght at birth (Chuang, 2001).

1.4 Pathophysiology

Absorption of ingeasted lead is influenced by it´s form and particle size, as well as concurrent iron and calcium absorption. Almost all lead is bound to the red cell after absorption. Research shows that after a dose exceeds 500 µg (even of small particles) there is a dramatic fattening of the absorption capability of lead in food, soil, dust, drinking water, and paint (Brunekreef, 1981; Angle, 1982; Bornschein, 1986). Lead is distributed extensively throughout tissues – all cells, with highest concentrations in bone, teeth, liver, lung, kidney, brain and spleen. Most absorbed lead is deposited in the bones, where it is substituted for calcium in the matrix. It does not seem to damage

the bone itself and may act as a sink, protecting other organs. This, however, creates a long – term storage depot, allowing the accumulation of lead in the body.
Lead impairs a variety of enzyme systems. Lead has affinity to sulphhydryl groups and is toxic to zinc- and calcium- dependent enzyme systems. Two enzymes in heme synthesis are affected (i.e., inhibited) by lead – the cytoplasmic enzyme ALA-D and ferrochelatase, a mitochondrial enzyme. Interference with ALA-D is dose – related, occurs at a B-Pb between 100 and 200 µg/L, and is completed at a B-Pb of 70 and 90 µg/dL (Yaping, 2006).
Ferrochatalase catalyzes the transfer of iron from ferritin to protoporphiryn and forms heme. Ferrochatalase inhibition by lead results in an increase of coproporphiryne excretion in urine and in increase of protoporphiryne in red blood cells.

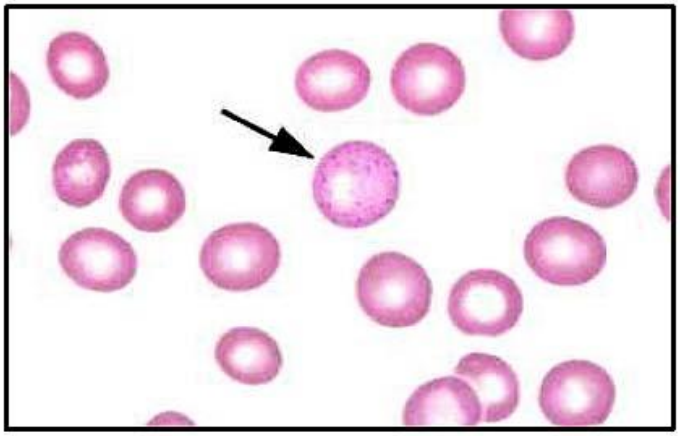


Figure 6: *Peripheral blood smear in a patient with lead poisoning (modified from http://www.wadsworth.org/chemheme/microscope/basostip.htm, 2010).*

Lead interferes with deoxyribonucleic acid transcriptation factors through binding to cystein sites. It binds and interferes with the ability of calcium to trigger exocytosis of neutrotransmitters. It interferse with calcium – dependent protein kinase C, which regulates many cellular events, such as the regulation of cell – growth, learning, and memory.
Lead also interferes with enzymes important in maintaining the integrity of membranes and affetcting steroid metabolism. Vitamin D synthesis in renal tubular cells is affected by lead, owing to an interfernce with a heme – containing hydroxylase enzyme that converts 25 – hydroxyvitamin D do 1,25 – hydroxyvitamin D.
Lead targets motor axons and produces axonal degeneration and segmental demyelination (CDC, 1991a).

1.5 Effects on humans

Lead has many health effects on humans. Overt signs of acute intoxication include dullness, restlessnes, irritability, poor attention span, headaches, muscle tremor, abdominal cramps, kidney damage, hallutinations, and loss of memory, encephalopathy occuring at B-Pb of 100 – 120 µg/dL, in adults and 80 – 100 µg/dL in children. Signs of chronic lead toxicity, including tiredness, sleeplessness, irritability, headaches, joint pain, and gastrointestinal sysptoms, lower scores on psycometric tests, disturbances in mood, and symptoms of peripheral neuropathy were observed in occupationally exposed populations at B-Pb of 40 – 60 µg/dL (WHO, 1996).

In analysis, Schwartz in 1994, showed that an increase in B-Pb from 10 to 20 µg/dL was associated with a mean decrease of 2.6 IQ points (Schwartz, 1994). The results suggested that the effect was likely to continue below 10 µg/dL. There is some evidence that cognitive effects of lead occur at BLL below 5 µg/dL (Lanphear, 2000). For Slovenia, research from 1989 to 2001 showed no influence of higher B-Pb to developmental, cognitive and behaviour characteristics of children in the Mežiška Valley (Primerjalna študija onesnaženosti okolja v Zgornji Mežiški dolini med stanji v letih 1989 in 2001, 2002).

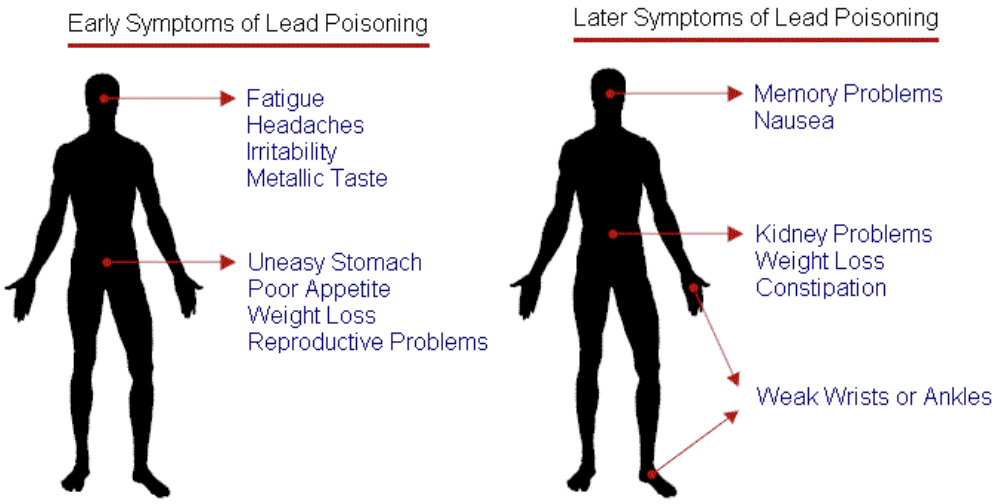


Figure 7: *Early and later symptoms of lead poisoning (modified from http://www.kdheks.gov/ables/faq.htm, 2009).*

1.5.1 Reproductive effects

Gonadal dysfunction in men, including depressed sperm counts, has been associated with B-Pb of 40 to 50 µg/dL. Reproductive dysfunction may also occur in females occupationally exposed to lead (IARC, 1980).
Low-to-moderate lead exposures may increase the risk for spontaneous abortion. In the prospecitive Mexico City Study, a striking dose-response relation between B-Pb and risk for spontaneous abortion was found (odds ratio 1.8 for every 5 µg/dL increase in blood lead) (Hertz-Picciotto, 2000). Two studies reported that women with elevated lead exposure from occupational settings are at increased risk of developing infertility compared with women with no such exposure (Rom, 1976; Landrigan, 2000). Studies in Taiwan showed that women with B-Pb greater than 250 µg/L were associated with a threefold increased risk for infertility. Women´s B-Pb was a significant predictor of the serum estradiol concentration (Shu-Hao, 2006).
The reproductive effects in men were observed in a study in Croatia in 2006 (increase in immature sperm concentration, in percentage of pathological sperm, wide sperm, round sperm, and short sperm, in serum levels of testosteron and estradiol, and decrease in seminal plasma zinc and in serum prolactin. These reproductive effects were observed at low-level lead exposure (B-Pb median 49 µg/L) common for the general population worldwide (Telišman, 2007).

1.5.2 Neurological effects in infants and children

Acute lead poisoning may produce encephalopathy. Ataxia, altered consciousness, and seizures have been reported in children with a B-Pb higher than 100 µg/dL (WHO, 1996).
The effects of chronic low – level lead poisoning had been addressed through large, complex epidemiologic studies. The evidence indicated that young children have subtle impairment of neuropsychiatric development when B-Pb is elevated. Few studies have examined the long-term effects of childhood lead poisoning. A study by White et al. among 34 Boston subjects and 20 matched controls, 50 years after diagnosis of symptomatic lead posisoning, suggested that a permanent pattern of cognitive dysfunction may result from childhood lead poisoning. The authors suggested that cognitive deficits among previously lead-posioned adults may explain lower occupational achivement in this group (White, 1993).

Risk factors for childhood lead exposure are:

- second year of life;
- history of lead poisoning in a sibling or playmate;
- history of pica;
- living in house built before 1960;
- parents working in a lead industry;
- reside near an active lead industry.

From autoposy studies in children exposed to lead, it was found that there are two types of morphological effects on the central nervous system. First, there is a cerebral edema, which may be reversible. Second, there is a direct loss of neurons in certain areas of the brain (such as gray matter, hypothalamus, and basal ganglia) where neurons are most concentrated (Que Hee, 1993).

Lanphear et al in 2000 showed that the magnitude of the association between concurent B-Pb and academic achivement in 6- and 16-years-olds was more steeply inverse when analyses were restricted to children with B-Pb < 25 µg/L rather than when analyses included all children with B-Pb < 100µg/L (Lanphear, 2000). Assesements that have conducted at older ages often have revealed inverse associations between children´s B-Pb and their scores, at later ages, on tests of motor development or visual-motor skills.
Study in the U.S., 1994 and 1995, concluded that B-Pb below 100µg/L, were inversely associated with children´s IQ scores at 3 and 5 years of age, and associated declines in IQ were greater at these concentrations than at higher concentrations (Canfield, 2003).
The meta-analysis of Schwartz (Schwartz, 1994) showed a reduction of 2.6 in IQ points for an increase in blood lead of 100 to 200 µg/L. This analysis included eight cross-sectional and longitudinal studies, the largest longitudinal study being the Port Pirie cohort study in Australia, with about 500 participants and a follow-up after several years (Baghurst, 1992). The meta-analysis also reported that the effect was likely to continue between 100 and 50 µg/L, with an even steeper curve. Above a blood lead level of 200 µg/L, a loss of 3.5 IQ points has been assumed (Fewtrell, 2004).

1.5.3 Elevated blood pressure

Elevated blood presure is another effect seen at low B-Pb. Although in a strict sense it is not a health outcome, elevated blood pressure has been associated with an increased risk of cardiovascular and cerebrovascular disease. The association between the B-Pb and blood pressure is strongest for increases in systolic blood pressure in adult males. Systolic and diastolic blood pressures have been shown to be higher in lead exposed workers than in the control group (Verschoor, 1987), and systolic blood pressure was positively correlated with blood lead levels in a number of studies (Maheswaran, 1993; Schuhmacher, 1994). Schwartz showed that decreases in B-Pb from 10 µg/dL to 5 µg/dL were associated with a decrease of 1,25 mmHg in systolic blood pressure; other studies found decreases of 2 mm Hg for reductions in blood lead from 20 µg/dL to 15 µg/dL, and from 15 µg/dL to 10 µg/dL. In women, the association between systolic blood pressure and blood lead is weaker and less – well documented (Schwartz, 1994).
Large-scale mortality studies of individuals in the lead-smelting and battery industries have supported the conection between lead and hypertension. In an American population between 1946 and 1970, most workers had mean B-Pb of 400 – 700 µg/L. Little is known about the natural history of the development of hypertension in lead posioning, its pathophysiology and relation to renal effects, and the effects of intervention (Dart, 2004). In systematic review of lead exposure and cardiovascular diseases in 2007, Navas-Ancien et al found sufficient evidence to infer a causal relationship between hypertension and lead exposure (Navas-Ancien, 2007).

1.5.4 Gastrointestinal effects

One of the earliest clinical signs of lead posisoning can be nonspecific, gastrointestinal effects. Symptoms include abdominal pain, constipation, cramps, nausea, vomiting, anorexia and weight loss. Lead may exert a direct effect on visceral smooth muscle tone and vascular supply in the gastrointestinal tract. At higher blood lead levels (> 800 µg/L), severe abdominal cramping and constipation may occur (Fischbein, 1998).

1.5.5 Anaemia

The haemopoeitic system is very sensitive to the effects of lead, and subclinical effects on a number of enzymes involved with haem biosynthesis have been seen at very low lead levels.
A study from 1968 to 1969 in Žerjav, Slovenia, showed an increased number of reticulosits 18.1 % with adult male and 34 % in adult females (Primerjalna študija onesnaženosti okolja v Zgornji Mežiški dolini med stanji v letih 1989 in 2001, 2002).

1.5.6 Renal effects

Acute effects are generally seen on children, when exposure is primarily through the oral route, while chronic effects are more common in adults, occupationally exposed to lead, where the principal route is inhalation.
Lead accumulates in the proximal tubular cells, a process that explains the marked effect on urate excretion. In addition, Fanconi´s syndrome (proteinuria, aminoaciduria, and phosphaturia) have been described as the result of lead accumulation. Inclusion

bodies have been found in renal tubular cells. These inclusions may represent the binding of lead by a renal binding protein that mitigates the effects of lead. As toxicity progresses, chronic interstitial nephritis may develop, in some cases progressing to end-stage renal failure (WHO, 1996).
Few studies have examined the renal effects of lead in children. A study in Romanian children ages 3 to 6 years with an average B-Pb of 340 µg/L, showed a significant relationship between the BLL and N-acetyl-ß-D-glucosaminidase activity in urine (Dart, 2004).

Blood lead levels, ranging from 23 – 725 µg/L, were shown to correlate with measures of glomerular dysfunction in a study of a large European population (Staessen, 1992). Low-level exposure to lead has been shown to impair renal function and accelerate age-related loss of renal function in asymptomatic middle-aged and older men, among whom increased serum creatinine levels were positively correlated with blood lead levels (Kim, 1996).

1.5.7 Pulmonary effects

Although a recent report identified lead pneumoconiosis in lead miners, no reports have cited pulmonary dysfunction among other intoxiacated populations (Dart, 2004).

1.5.8 Endocrine effects

Lead causes decreased serum thyroxine levels, effects on adrenal hormones, and changes in levels of vitamin D (Dart, 2004). The effects of lead on thyroid function have been known for 50 years. The study of Slingerland in 1955 (Slingerland, 1955) was the first to show diminished iodine uptake by the thyroide gland and Sandstead in 1969 conformed that results in rats in an in vivo study (Sandstead, 1969). A study by Dundar et al in 2005 showed that long-term low-level lead exposure may lead to reduced FT4 (free thyroxine) level without significant changes in Thyrotrophin and free triodothyronine levels in adolescents even at low B-Pb levels (mean 73 µg/L) (Dundar, 2006).

1.5.9 Cancer

In 1987, the IARC classified lead and inorganic lead compounds as „possibly carcinogenic to humans (group 2B)“ on the basis of sufficiant animal data and insufficiant human data. On the basis of inadequate evidence from two epidemiological human studies as well as animal studies, organolead compounds were considered as not classifiable as to their carcingenicty to humans (group 3). In 2004, the IARC reevaluated the potential carcingenic hazards to humans from exposure to inorganic lead compounds and reached the following overall evaluations: inorganic lead compounds are probably carcinogenic to humans (group 2A), organic lead compounds are not calsiffiable as to their carcinogenicity to humans (group 3) (IARC, 1980).
Lead is weakly mutagenic but exerts pronounced indirect genotoxic effects and increases the mutagenicity of other mutagens. These indirect genotoxic effects are observed at low, nontoxic concentrations, possibly via interference with DNA repair processes (Cornelis, 2005).

1.5.10 Mutagenicity

Though some research shows lead compounds are capable of inducing gene and chromosome mutations, lead is clearly not a powerful mutagen nor even a consistent mutagen among various test systems. Lead´s ability to interfere with the fidelity of DNA synthesis surely provides a mechanistically plausible way by which it may be capable of causing both mutation and cancer (Johnson, 1998). However, the *in vitro* demonstration of this infidelity may not apply to intact cells or to the complete organism at environmentally relevant concentrations (Hartwig, 1994).

1.6 Excretion of lead from human body

Non absorbed lead excretes from body with feces; inhaled lead that has been swallowed after clearance by the mucociliary process and not absorbed in the gastrointestinal tract is also eliminated in the feces. Excretion of lead with urine goes through glomerulofiltration, where it comes to partial tubular reabsorption. Aborted lead that is not retained is mainly excreted through urine (about 75%) and feces via biliary excretion (about 10 – 20%). It is also excreted in sweat, nails, and hair (WHO, 1996).

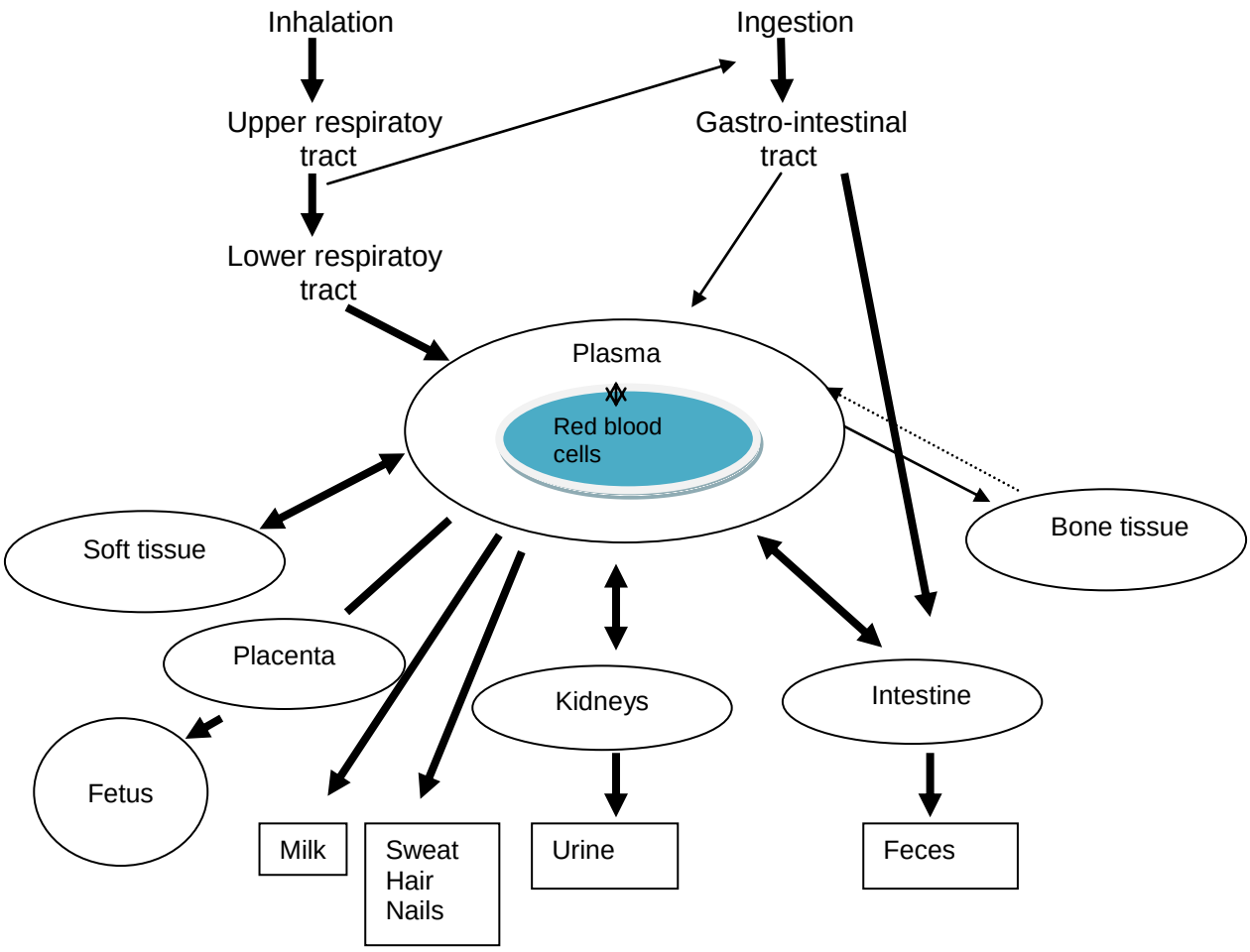


Figure 8: *Main Aspects of lead toxicokinetiks.*

1.7 Clinical trails, epidemiological investigation and estimation of risk for human health

Higher levels of lead in humans cause hem synthesis disturbance. This leads to aenemia. Clinical signs depend on aenemia degree, but it usually occurs in conjunction with nausea, paleness and tiredness. In concentrations of lead above 200 µg/L an inhibition of dehydratasa delta-aminolevulenic acid occurs. Concentrations of lead above 400 µg/L causes 70% inhibition of that enzyme in 20% of people.
Because the central nervouse system is particulary sensitive to oxygene deficiency, the consequences occur even before aenemia. The effect of lead on the central nervous system dependes on the duration and extent of exposure.

So far it is not known, what value of Pb in humans is harmful. Research conducted since 1991 has strengthened the evidence that children´s physical and mental development can be affected at B-Pb < 100 µg/L.
During period from the year of 2000 to 2004, a workgroup of the CDC´s Advisory Committee on Childhood Lead Poisoning Prevention reviewed the scientific literature regarding adverse health effects associated with B-Pb <100 µg/L, including 23 published reports that analazed 16 separated populations with IQ or general cognitive index outcomes and 12 publications related on other health outcomes. The workgroup concluded that an inverse association exists between B-Pb and cognitive functions with no evidence of a weaker association in populations with lower B-Pb.

1.7.1 Children´s B-Pb patterns

B-Pb increase quickly after an acute exposure, then gradually (over weeks) reach equilibrium with body stores of lead. Lead is distributed unevenly within the human body; in children approximately 70 % is stored in the bone tissue. The residence time of lead in bone can be decades. Thus, an elevated B-Pb will decline within a few weeks to months after an acute exposure. However, for those chldren with chronic lead exposure and, presumably higher bone lead stores, the decline in B-Pb can take much longer (CDC, 2005b).

A newborn infant´s B-Pb closely reflects that of the mother. During the period from the year 1999 to 2002, the geometric mean B-Pb for US women aged 20 – 59 years was 12 µg/L, with 0,3 % having a B-Pb ≥ 100 µg/L (CDC, 2005a).

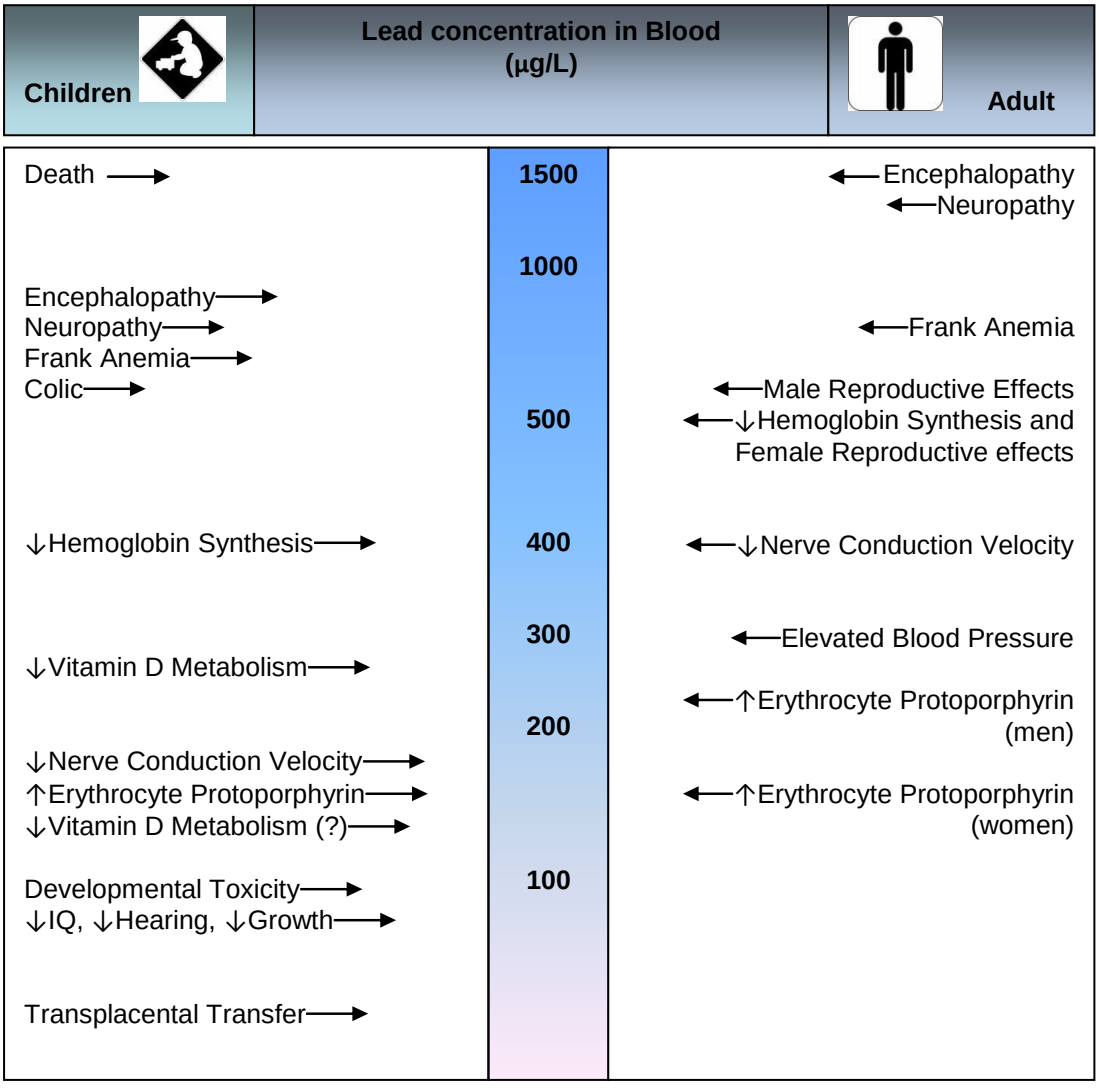
During period from the year 1989 to 2001 a group of researchers investigated 3-year and 12-year old children in the Mežiška valey, and found no influence of higher B-Pb on development, cognitive and behavorial characteristics in children. For the period from 1997 to 2000 researches could not concluded the influence of lead from environment on fertility disturbance, because there were insufficent data (premature born before 37 weeks of gestation, low birth weight infants) (Primerjalna študija onesnaženosti okolja v Zgornji Mežiški dolini med stanji v letih 1989 in 2001, 2002).

1.7.2 Adults

Because of the sensitivity of the fetus nervous system toward lead, mothers (in time of gestation) are the most vulnerable population group. In adults, the changes on the central nervous system are showed in B-Pb at around 300 µg/L. Increased amounnts

of protoporphiryn in red blood cells and frequent high blood pressure were also found in that group (Korick, 1999; Nain Feng, 1999).

When humans were exposed to Pb at even higher values – between 500 to 1000 µg/L B-Pb, signs of central nervous system damage can occur.
The adverse health effects range from death to impaired cognitive and behavioral development that can have lifelong consequences. They are presented in Figure 9 (Meyer, 2008).



Note: ↑ = increased function and ↓ decreased function.

Figure 9: *Blood – lead levels associated with adverse health effects (modified from Meyer, 2008).*

Lead is ranged at the 2B category of carcinogenicity. There is insufficient evidence, that it causes cancer in humans, but researches in animals confirm that effect.

Analisys of deaths in the Mežiška Valley area from 1991 to 2000 showed that population is more jeopardized in comparison with most of other Slovenian regions (Primerjalna študija onesnaženosti okolja v Zgornji Mežiški dolini med stanji v letih 1989 in 2001, 2002).

1.7.3 Nonoccupational exposure and burden of humans with lead

Burden degree of humans with lead is various and it depends on lifestyle, nutritional status and environment. There are differences between particular biological groups of the population with the same exposure levels.
The most vanurable group is children in age younger than 6 years, because:

- absorption of lead from gastrointestinal organ is considerable (about 50 %, 10 % in adults);
- apperance of deficitary consumption of Fe, Ca and vitamin D, which acclerate absorption of lead from the intestines;
- children do not have a developed haematoencephalic barrier;
- haemoatopoetic and nervous lead effects occur earlier in children than in adults;
- children eat more food on BW unit than adults, so intake of lead is bigger;
- some child characteristics allow bigger intake of lead versus adults (poor hygiene, they spend lot of time outside, they put hands in mouth).

Many nutritional and behavioral factors associated with iron deficiency may also be found in children with elevated B-Pb.
Because of the frequency of inadequate calcium intake among all children, it is important to verify that a child with an elevated B-Pb is receiving enough calcium (Birt, 2002).

1.8 The assessment of Pb burden

1.8.1 Biomarkers of exposure

The measurement of Pb in whole blood is the most widely used biomarker to assess the absorbed dose of inorganic lead. However organic lead exposure can affect blood lead levels, probably after dealkylation to inorganic lead.
Since half-life of lead in blood is about 35 days, B-Pb mainly reflects relatively recent exposure. In view of the cycling of lead between blood, bone, and soft tissue, lead in the blood is also influenced by lead stored in tissue that reenters the blood during tissue mobilization. When bone mobilization is accelerated, or when a lead worker is removed from exposure to lead, a greater fraction of B-Pb will be derived from tissue stores. It is important to keep in mind that the relationship between lead exposure and B-Pb concentrations is curvilinear; the increase in the B-Pb concentration is less at high exposure than at low exposure (Sakai, 2000).

Plasma lead concentrations may better reflect the “metabolic acite” fraction of lead in blood and define the relationship between B-Pb and tissue or organ accumulation (and effect). However, in view of the difficulty of accurately measuring very low concentrations of lead, the determination of lead in plasma has not been recommended for the routine monitoring for lead exposure. This could change with inductively coupled

plasma (ICP-MS), which has a high analytical sensitivity and can achieve a much lower limit of detection than AAS methods. It is suggested that the considerable variation reported between symptoms and signs and B-Pb might be caused by differences between people in the distribution of lead between erythrocytes and plasma (Shutz, 1996).

In many countries, the progressive removal of organolead from gasoline and other preventive measures has been associated with a progressive decline in B-Pb for the general population. In nonoccupationally exposed adults, B-Pb rarely exceeds 100 – 150 µg/L. The US National Health and Nutrition Examination surveys (NHANES) concluded that the mean B-Pb level of persons aged 1 to 74 years dropped 78% (from 128 to 28 µg/L). According to Center for Disease Control and Prevention, the goal of all activities preventing lead poisoning should be reducing children´s B-Pb below 100 µg/L.
Because of the large number of studies that have assessed cognitive function as an outcome, the review and conclusions by the CDC work group primarily focus on this health domain. The consensus is that the overall weight of available evidence supports an inverse association between B-Pb <100 µg/L and the cognitive function of children. The evidence for such association is bolstered by the consistency across cross-sectional and longitudinal studies in varied settings with blood lead distributions overlapping 100 µg/L and by the lack of any trend towards a weaker association in studies with lower population mean B-Pb. More recent studies and analyses best suited to examining this association (Canfield, 2003; Bellinger, 2004) have added to, rather than refuted, evidence for such an association noted in prior CDC guidance (1991). Relatively few studies have directly examined the association between B-Pb <100 µg/L and health status among children and many of those that have are cross sectional studies in which data are unavailable on B-Pb earlier in life and key covariates. Nonetheless, the available data from these studies are consistent with associations between higher B-Pb and poorer health indicators for values <100 µg/L (CDC, 2005b).

The measurement of the heme precursor, protoporphyrin, is used as a marker for lead toxicity. Protoporphyrin levels ≥ 35 µg/dL in whole blood are associated with B-Pb levels greater than 250 to 500 µg/L (Piomelli, 1977). Protoporphyrin levels increase several weeks after B-Pb concentrations have risen. After cessation of further ingestion, the decline in protoporphyrin follows the fall in B-Pb in a similar manner. The measurement of protoporphyrin was recommended by CDC until 1991, when the allowable limit for B-Pb levels in children was lowered to 100 µg/L. At this quantity of lead, the protoporphyrin is an insensitive tool for screening purposes (CDC, 1991a).

Although urinary lead level is used occasionally as a screening test to measure current exposure, its use as a biomarker of environmental lead exposure appears to be of limited value in view of the relatively low and variable level of lead excreted in the urine (Cornelis, 2005).
The determination of lead in urine (Urine-Pb) is considered to reflect Pb that was diffused from plasma and is excreted through the kidneys. Collection of urine for Pb measurements is non-invasive and is favored for long-term biomonitoring, especially for occupational exposures.

Teeth can be useful tissues for assessing long-term lead accumulation from prenatal exposures to the time of shedding the tooth. The accumulation of lead in teeth has been used as a measure of environmental exposure of children to lead in several epidemiological studies.

The human skeleton begins to accumulate lead during fetal development and continues to about age 60 (Pounds, 1991). Skeletal lead (finger, tibia, patella, calcaneus) can be measured by bone bipsy and more interestingly by noninvasive X-rays fluorescence (L-shell XRF (L-XRF) and K-shell XRF (K-XRF)). This technique is not applicable routinely at present but constitutes a powerfull analytical methodology for evaluating bone lead levels as a measure of time-integrated (i.e. cumulative) lead dosages in epidemiological studies of the effects of chronic lead exposure.

Hair lead (H-Pb) has been proposed as a noninvasive indicator of exposure to lead. However, hair color, texture, treatment, location on the body and growth phase can influence the metal analysis of hair. Rodrigueas et al in 2008 reported a weak correlation between B-Pb and H-Pb levels. This was also the conclusion in a study by Štupar et al (Rodrigues, 2008; Štupar, 2007a).

Correlation between H-Pb and B-Pb varies tremendously ($r = 0.03 – 0.76$). It appears that the level of environmental or occupational lead pollution considerably affects the strength of this correlation. Two studies in 1993 found the strongest correlation for heavily exposed lead-battery workers, while the correlations were much lower in the control population.

Hair structure containes a high amount of sulfur because the amino acid cystein is a key component of the keratin proteins in hair fiber. The sulfur in cystein molecules adjacents keratin protein links together in disulfide chemical bonds. Some heavy metals (such as mercury and lead) have a high affinity to sulfur.
There is approximately a 20-day lag between the concentration of trace elements in the first centimeter next to the scalp and the corresponding average monthly blood lead level.

The ability to distinguish between Pb that is endogenous, i.e., absorbed into the blood and incorporated into the hair matrix, and Pb that is exogenous, i.e., derived from external contamination, is a major problem. During the washing step, it is assumed that exogenous Pb is completely removed whereas endogenous Pb is not (Barbosa, 2006).

Saliva has been proposed as a diagnostic specimen for various purposes, as it is easily collected (Silbergeld, 1993). However, in the absence of consistent and dependable saliva Pb measurements, it is not generally accepted as a reliable biomarker of Pb exposure. Saliva shows large variations in its ion content throughout the day, coupled with changes in salivary flow rates before, during, and after meals. Variations also arise depending on the manner in which saliva collection is stimulated (or not), and on the nutritional and hormonal status of the individual.
There are some data to suggest an association between Pb levels in saliva and those in either plasma or blood (Pan, 1981; Omokhodion and Crockford, 1991). Moreover, it has been argued that Pb in saliva is the direct excretion of the Pb fraction in diffusible plasma, i.e., the fraction not bound to proteins (Omokhodion and Crockford, 1991). Despite the associations reported in the literature, the older saliva Pb concentrations were quite high, and the values vary from study to study. On the other hand, recent data suggest much lower saliva Pb levels, in both exposed and unexposed subjects (Koh, 2003; Wilhelm, 2002). According to Wilhelm et al, Pb content in the saliva of unexposed children was usually below 0.15 µg/dL.
Uncontrolled variation in salivary flow rates, lack of standard or certified reference materials, and absence of reliable reference values for human populations are major factors that limit the utility of saliva Pb measurements. In addition, the very low levels of

Pb present in saliva serve to limit the range of suitable analytical techniques thereby further diminishing the utility and reliability of this biomarker for prediction of Pb exposure.

1.9 Estimation of lead burden and treatment

1.9.1 Estimation of lead burden in children

High risk groups that may be targeted include low-income, urban, those with pervasive developmental disorders and those, living in geographic regions associated with older houses or associated with a source of pollution.

The whole B-Pb is the standard for diagnosis of childhood lead poisoning. A classification system based on blood lead level has been established by the CDC (Table 3).

Table 3: *Summary of recommendations for children with confirmed (venous) elevated blood lead levels (Modified from CDC, 2002).*

Class	Blood lead µg/L	Suggested interpretaion and suggested treatment
I	≤90	Normal; rescreen as indicated
IIA	100 - 140	Educate parents, rescreen in 3 months, report
IIB	150 - 190	Educate parents, test for and correct iron difficiency, rescreen in 3 months; if level persist, proceed as class III
III	200 - 440	Retest within 1 month, complete medical evaluation; begin environmental assessment, consider chelation therapy
IV	450 - 690	Retest within 48 hours; complete medical evaluation - complete history and physical exam, complete neurological exam ; begin environmental assessment and medical treatment, including chelation, within 48
V	≥700	Medical emergency; retest immediately; hospitalize, complete history and physical exam, complete neurological exam and begin treatment immidiately, identify and remove source of lead

B-Pb below 100 µg/L is also important because many studies report on decreased IQ potential in low level lead exposure.
There is strong link between low-dose lead exposure and intelectual deficit in children (Needleman, 1990). Study by Jusko et al showed that children with B-Pb in the 50 – 99 µg/L range had significantly lower IQ scores than children who had B-Pb <50 µg/L. Further, additional nonlinear analysis of peak exposure throughout early childhood indicated that B-Pb as low as about 20 µg/L may be associated with declines in Full-Scale IQ. The effect of blood lead on child intellectual development is larger for equal increments of lead <100 µg/L than it is at higher levels. The performance IQ was more strongly associated with B-Pb than was verbal IQ (Jusko, 2008).
Lanphear et al showed that the magnitude of the estimated association between concurrent B-Pb and academic achievement in 6- and 16-years-olds was more steeply increased when analyses were restricted to children with a B-Pb <25 µg/L than when

the analyses included all children with B-Pb <100 µg/L (Lanphear, 2000). In the study by Tellez-Rojo et al, it was found that among infants whose B-Pb did not exceed 100 µg/L at 12 or 24 months of age, scores both on a Mental Development Index and the Psychomotor Development Index of the Bayley Scales of Infant (BSID II) were inversely related to B-Pb at 24 months, adjusting for such covariates as maternal IQ, birth weight, gender, age, and umbilical cord B-Pb (Tellez-Rojo, 2006).
Canfield et al in 2003 found that an increase in the lifetime average B-Pb of 10 µg/L was associated with decrease of 0.87 IQ points (Canfield, 2003).

1.9.2 Estimation of lead burden in adults

Any worker whose B-Pb is more than 500 µg/L must be removed from work immediately; a level more than 400 µg/L must undergo medical evaluation. Routine laboratory work may reveal decreased hemoglobin and hematocrit, but severe aenemia is not common. The classic sign of basophilic stippling of erythrocytes rarely is seen.

The first step in treatment is to identify the source of exposure, to identify any other individuals (family member or coworkers) who also may have been exposed, and to terminate the exposure. The affected individuals should not return to the work or home activity that caused poisoning until all risk of further exposure has been eliminated. Treatment of lead poisoning may involve source abatement, behavioral modification programs, dietary manipulation, and chelation.

Management for lead-exposed adults is in Table 4.

Table 4: *Health-based management recommendations for lead-exposed adults. (Modified from Kosnett, 2007).*

Blood lead level µg/L	Short term risks (lead exposure < 1 year)	Long term risks (lead exposure ≥ 1 year)	Management
<50	None documented	None documented	None indicated
50 - 90	Possible spontaneous abortion	Possible spontaneous abortion	Discuss health risk
100 - 190	Possible spontaneous abortion Possible postnatal developmental delay Reduced birth weight	Possible spontaneous abortion Reduced birth weight Possible postanatal developmental delay Hypertension and kidney disfunction Possible subclinical neurocognitive deficits	As above for B-Pb 50 – 90 µg/L, plus: Decrease lead exposure Increase biological monitoring Consider removal from lead exposure to avoid long-term risks if exposure control over an extended period does not decrease B-Pb <100 µg/L, or if medical condition present that increases risk with continued exposure

200 - 290	Possible spontaneous abortion Possible postnatal developmental delay Reduced birth weight	Possible spontaneous abortion Possible postanatal developmental delay Reduced birth weight Hypertension and kidney disfunction Possible subclinical neurocognitive deficits	Remove from lead exposure if repeat B-Pb measured in 4 weeks remains ≥200 μg/L
300 - 390	Spontaneous abortion Possible postnatal developmental delay Reduced birth weight	Spontaneous abortion Reduced birth weight Possible postanatal developmental delay Hypertension and kidney disfunction Possible neurocognitive deficits Possible nonspecific symptoms	Remove from lead exposure
400 - 790	Spontaneous abortion Reduced birth weight Possible postnatal developmental delay Nonspecific symptoms Neurocognitive deficits Sperm abnormalities	Spontaneous abortion Reduced birth weight Possible postanatal developmental delay Nonspecific symptoms[b] Hypertension Kidney disfunction/neuropathy Subclinical peripheral neuropathy Neurocognitive deficits Sperm abnormalities Anemia Coli Possible gout	Remove from lead exposure Refer the prompt medical evaluation Consider chelation therapy for B-Pb >500μg/L with significant symptoms or signs of lead toxicity
≥800	Spontaneous abortion Reduced birth weight Possible postnatal developmental delay Nonspecific symptoms Neurocognitive deficits	Spontaneous abortion Reduced birth weight Possible postanatal developmental delay Nonspecific symptoms Hypertension Nephropathy Peripheral neuropathy	Remove from lead exposure Refer the immediate/urgent medical evaluation Probable chelation therapy

	Encephalopathy Sperm abnormalities Anemia Colic	Neurocognitive deficits Sperm abnormalities Anemia Coli Gout	

2 PURPOSE OF THE RESEARCH

Purpose of the research was to study if there is any influence on lead levels in blood and hair (biological material) of children included in research, as also on environment, because of the vicinity of lead-glass factory.

That datas allow us to:

- estimate burden of children with lead;

- determinate the correlation between pollution rate and B-Pb and H-Pb of subjects;

- demonstrate the important difference between subjects in polluted area and control group;

- evaluate the extent of contamination with lead in investigated area;

- emphasize a holistic approach to the analisys of exposure.

3 MATERIAL AND METHODS

3.1 Selection of participants in the study

Forty eight healthy children, 33 males (69%) and 15 females (31%) participated in the study. The control group were children consisting of 11 males and 8 females from Rakitna. Parents of every child agreed with the research Annex F).

3.2 Sort of research

For checking assumption, that the vicinity of lead-glass factory influences on people's health, more than people who live in nonpolluted areas, the analytical longitudinal ecological epidemiological method was used.
The examined units were groups of children from age 6 to 14 from Rogaška Slatina and Rakitna.
Level of burden with lead in examined subjects was checked by analysis of full blood and hair. We also examined the correlation between them.

3.3 Protocol and instrumentation

3.3.1 Human Biomonitoring

Lead in hair and blood samples was measured with graphite electrothermal atomic absorption spectrometry.

In both groups blood and hair lead samples were taken in the period from 2007 and 2008, blood samples were collected at locations and then analyzed in the laboratory of the Regional Public Health Institute, Celje, and the Institute of Clinical Chemistry and Biochemistry at the University Medical Centre, Ljubljana.

Blood samples

A trained nurse collected 2 mL blood samples from participants. Blood samples were collected in trace-metal-free evacuated tubes (BD 3E Vacuatiner®) containing K-EDTA to prevent coagulation. Determination of lead was performed after dilutions with Triton X-100 1:5 with graphite electrothermal AAS at wavelenght 283.3 nm. Each blood lead sample was measured in two parallels and the result was presented as an average of both measurements.
The method has the following characteristics: the limit of detection (LOD) was 5 μg/L for group from Rakitna and LOD for group from Rogaška Slatina was 2.2 μg/L, because it was done in two different laboratories. To assure the accuracy of measurement, certified reference material BCR CRM 194 (bovine blood) with declared value of 126±4 μg/L Pb was used. It was prepared in the same way as the blood samples. Limit of quantitation (LOQ) for B-Pb from control group was 10 μg/L, and for group in Rogaška Slatina was 3 μg/L.

The measurement results for the reference material showed a satisfactory agreement with declared value (Table 5).

Table 5: *Measurements of the certified reference material BCR CRM 194*

Measured values (mg/L)			
1.	*130*	*5.*	*130*
2.	*128*	*6.*	*145*
3.	*140*	*7.*	*103*
4.	*111*	*8.*	*118*
Average± SD		*125.6 ± 14.2 μg/L*	
Declare value		*126 ± 4 μg/L*	

Amount of Pb in blood sample was calculated by the equation

$$B\text{-}Pb = \frac{(CvxF - Csl)xVk}{Mv}\,(\mu g/L) \qquad \textit{(Equation 1)}$$

Where: Cv = measured concentration of Pb in sample in μg/L;
Csl = concentration of lead in blind in μg/L;
Vk = final volume of sample;
Mv = initial volume of sample;
F = dilution factor, if sample exceed moderation curve.

Hair samples

On the same day as the blood collection, hair samples were taken from the occipital area of the head, close to the scalp. Hair was cut on the right and left side (30 – 50 hair in one mop).

Hair was washed in dilution of n-heksan and ethanol (1:1) for 30 minutes twice, then in ethanol for 5 minutes and rinsed with de-ionized water twice (5 minutes). After washing, samples were dried at 80ºC for 6 hours.
100 mg from each sample was irrigate with 5 mL 65% HNO_3, stand in laminar-flow hood over night (at least 12 hours), slowly warmed to 60 – 80ºC. Small amount of 30% H_2O_2 was added. Samples were then warmed almost up to the end (0.5 mL left). Samples were rinsed and diluted with de-ionized water in relation 1:5. Hair lead samples were measured with flame atomic absorption spectrometry (FAAS. Each hair lead sample was measured in three parallels and the result was presented as an average of all three measurements.
Certified reference material was Chinese hair CRM GBW 09191 (declare value 7.2 ± 0.7 μg/g, measured value 6.75 μg/g).

Amount of Pb in hair sample was calculated by equation

$$H\text{-}Pb = \frac{(Cv \times F - Csl) \times Vk}{Mz} (\mu g/g) \qquad \textit{(Equation 2)}$$

Where: Cv = measured concentration of Pb in sample in μg/L;
Csl = concentration of lead in blind in μg/L;
Vk = final volume of sample;
Mz = initial volume of sample;
F = dilution factor, if sample exceed moderation curve.

3.3.2 Environmental measurements

Lead in samples of soil and vegetables were measured with ICP spectrometer Varian 820 MS. Samples were analyzed in the laboratory of the Regional Public Health Institute, Celje.

Soil samples

Samples of soil were taken in depth of 0 – 25 cm from cultivated land and 0 – 5 cm from another type (Figure 12). Soil samples were collected using a stainless steel screw auger.

Figure 10: *Sampling site at the top of the hill, lead-glass factory in Rogaška Slatina.*

At first sampling (Figure 13) soil around the lead-glass factory was sampled.

Lead-glass factory

Figure 11: *First sampling around the lead-glass factory.*

At second sampling we examined in detail the hill above factory chimney and took samples at different MSL above the chimney (Figure 14).

Figure 12: *Second sampling direction above lead-glass factory in Rogaška Slatina.*

Samples were homogenizied, dried in air chamber until they reached constant weight, crushed, sieved through 0.25 mm sieve. 0.5 g of each sample was irrigate with 2 mL of 65% HNO_3 and 5 ml of 37% HCl. Disconnection of samples was obtained in a microwave oven Milestone 1200 MEGA. Samples were rinsed, diluted with de-ionized water to a final volume of 50 ml. Determination of soli lead in extract solution was performed by ICP-MS and calibration with standard solutions containing the same concentration of acids as sample. Montana II NIST-SRM 2711 soil sample (certified value 1162 ± 30 μg/g of Pb) was employed to validate the results of the soil sample analysis. Each soil lead sample was measured in two parallels and the result was presented as an average of both measurements.

Amount of Pb in sample was calculated by equation

$$Soil\text{-}Pb = \frac{(Cv \times F - Csl) \times Vk}{Mz \times 1000}\ (mg/kg) \qquad \textit{(Equation 3)}$$

Where: Cv = measured concentration of Pb in sample in mg/L;
Csl = concentration of lead in blind in mg/L;
Vk = final volume of sample ml;
Mz = initial mass of sample in g;
F = dilution factor, if sample exceed moderation curve.

Samples of vegetables

Samples were cleaned, mixed and frozen. Before taking measurements, the sample was defrosted and dried at 60ºC for 1 – 2 days. After drying, the sample was crushed in a mill. Approximatly 0.5 to 1 g from each sample was weighed, then 5 ml of 65% HNO_3 and 1 ml of 30% H_2O_2 was added. Moderate curve was made with a defined amount of Pb (5, 10, 20 and 30 μg/L). LOQ was 0.1 mg/kg. Each vegetable lead sample was measured in two parallels and the result was presented as an average of both measurements.

Amount of Pb in sample was calculated by equation

$$Vegetable\ Pb = \frac{(Cv \times F - Csl) \times Vk}{Mz} / 1000\ (mg/kg) \qquad \textit{(Equation 4)}$$

Where: Cv = measured concentration of Pb in sample in μg/L;
Csl = concentration of lead in blind in μg/L;
Vk = final volume of sample in ml;
Mz = initial mass of sample in g;
F = dilution factor, if sample exceed moderation curve.

Pb in air

For the purpose of monitoring lead, three sampling sites were selected within the residential area. Air precipitates were sampling monthly, beginning in June 2005 untill April 2007, employing NILU samplers (Norwegian Insititute for Air Research). Sampling sites are presented in figure 15.

Lead-glass factory

Figure 13: *Sampling points for vegetable.*

Volume of the collected sample was measured and the content filtered through the polycarbonate membrane filter (pore size 0.3 μm) to separate the particular matter. The mass of the latter was determined by weighing prior to dissolution in the mixture of H_2O_2 and HNO_3. Lead content in the solution was measured directly by Electrothermal AAS at the 283.3 nm resonance line employing graphite furnace atomization. Each air lead sample was measured in two parallels and the result was presented as an average of both measurements.

Pb atmospheric fall-out in sample was calculated by equation

$$Air\ Pb = \frac{(Cv \times F) \times Vk}{} \times 10000/s/Nd \qquad \textit{(Equation 5)}$$

Where: Cv = measured concentration of Pb in sample in mg/L;
Vk = final volume of sample in ml;
F = dilution factor, if sample exceed moderation curve;
s = surface of hole in cm^2;
N d = number of days.

3.3.3 Reagents

All reagents were of analytical-reagent grade. Acids used for decomposition of samples were suprapure quality. A clean laboratory and laminar-flow hood were used for preparing solutions. High purity de-ionized water (resistivity 18.2 MΩ cm), obtained from a Milli-Q water.

3.4 Description of the site

Rogaška Slatina is a small town with 11,284 inhabitants, situated on the Slovenian – Croatian border.

Figure 14: *Map of Rogaška Slatina and the lead-glass factory in Rogaška Slatina (taken from point of sampling).*

It is well known health resort, famous for its magnesium rich mineral water. The major occupation of local people is farming. The only industrial plant in the town is a glass factory, established in 1927. Since then the size of the plant and production of lead containing glass has increasing rapidly, reaching a culmination in 1985. At the end of 2008, the production of glass was decreasing.

Lead and hydrofluoric acid are the main pollutants resulting from the production of crystal glass. Crystal glass is one of the most attractive forms in which lead is used. Lead oxide (PbO) is used in producing fine “crystal glass” and “flint glass” of a high index of refraction for achromatic lenses. Lead glass forms part of the silica-potassium-lead system, where lead replaces the calcium content of typical potash glass. Lead glass contains typically 18 – 35 mol% PbO, while modern lead crystal, historically also known as *flint glass* due to the original silica source, contains a minimum of 24% lead oxide. The very finest quality crystal has up to 36 – 70% lead content. It adds clarity, desity and brilliance to glass products.

3.5 Statistical analysis

Our hypothesis was that the vicinity of the lead-glass factory influenced the lead burden in humans and the environment.

To test if there were important differences in anthropogenic measurements and environmental data between locations, the statistical analysis was performed with the PAST program (Hammer, 2001).
The differences were considered statistically significant if the p value was less than 0.05.

4 RESULTS AND DISCUSSION

4.1 Determination of lead levels in blood and hair samples

Subject´s mean age was 11.4 years, number of participants was 48. Minimum age was 10 and maximum 15, mediana 10. Study included 13 (27%) females and 35 males (73%).
In the control group there were 19 subjects (8 females and 11 males), ranging from 9 to 17 years of age, mediana 13.

4.1.1 Blood Pb (B-Pb)

The subjects had B-Pb values from 4 – 48 μg/L (SD 8.9), the mediana was 20 μg/L, average 19.8 μg/L.
The control group had B-Pb values from 10 to 18 μg/L (SD 2.5), the mediana was 10 μg/L, average 11.7 μg/L.
The average B-Pb value for males in Rogaška Slatina was 20.7 μg/L (in control group 11.2 μg/L) and for females 17.6 μg/L (in control group 11.6 μg/L).

Table 6: *Mean (SD) B-Pb of children from Rogaška Slatina and control group.*

	N	Median	B-Pb in μg/L (SD)
Group from Rog.Sl.	48	20	19.8 (8.9)
Control group	19	10	11.7 (2.5)

Majority of subjects had BLL between 10 and 30 μg/L (Figure 17).

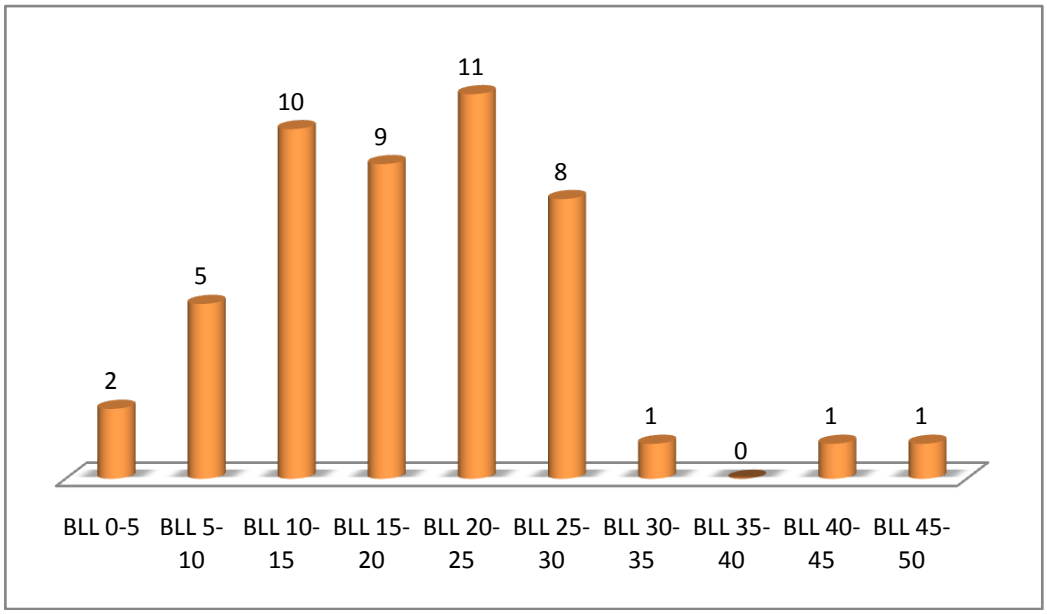


Figure 15: *The frequency distribution for B-Pb values in children from Rogaška Slatina (N=48).*

Differences between boys and girls are shown in the following Figure 16 (median and average).

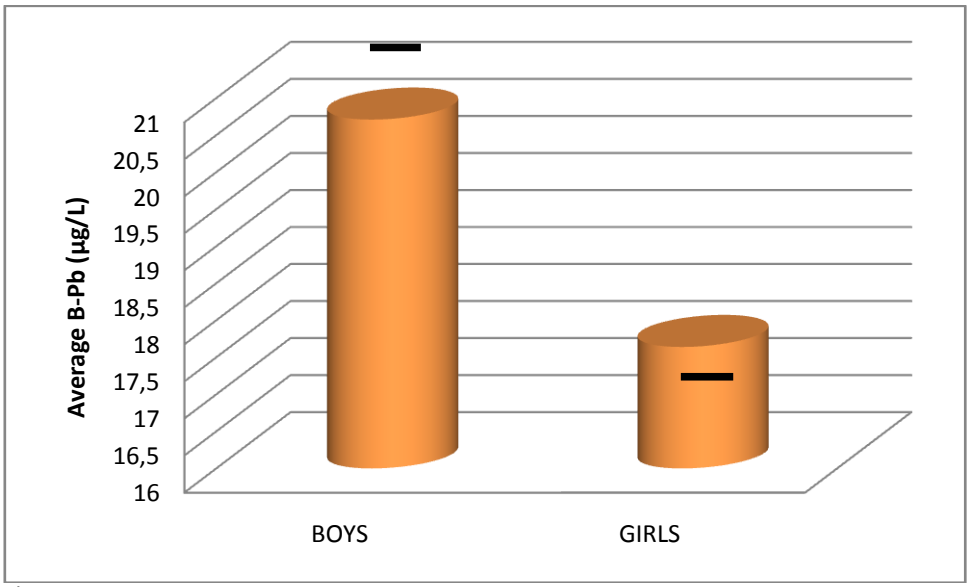
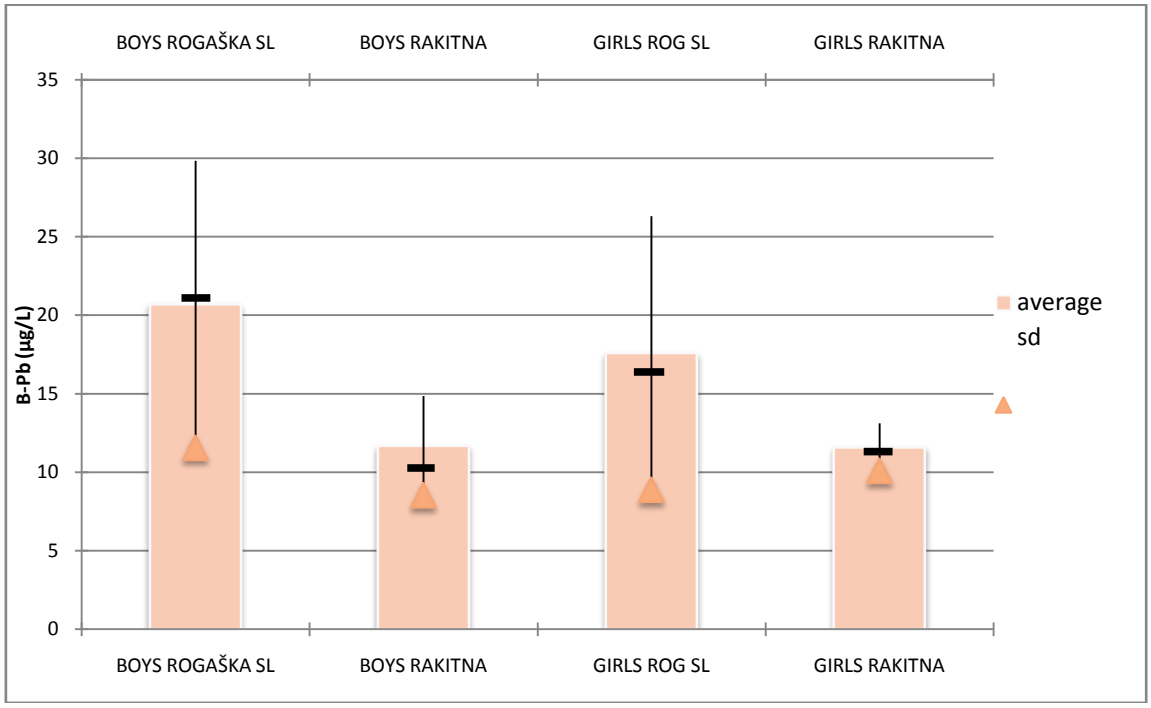


Figure 16: *Differences between median and average B-PbL in boys and girls in Rogaška Slatina (N=48).*

Boys in Rogaška Slatina had a higher median B-Pb than girls.

The median and average B-Pb between boys and girls in both locations is presented in Figure 17.



Median value ——

Figure 17: *Median and average B-Pb in µg/L between sexes in both locations with SD (N=68).*

Distribution of B-Pb in the group of children from Rogaška Slatina and from the control group from Rakitna is shown in the following figure. The outliners indicate the smallest and the largest values and the boxes indicate the 25th and 75th quartiles. The lines in the boxes are median values.

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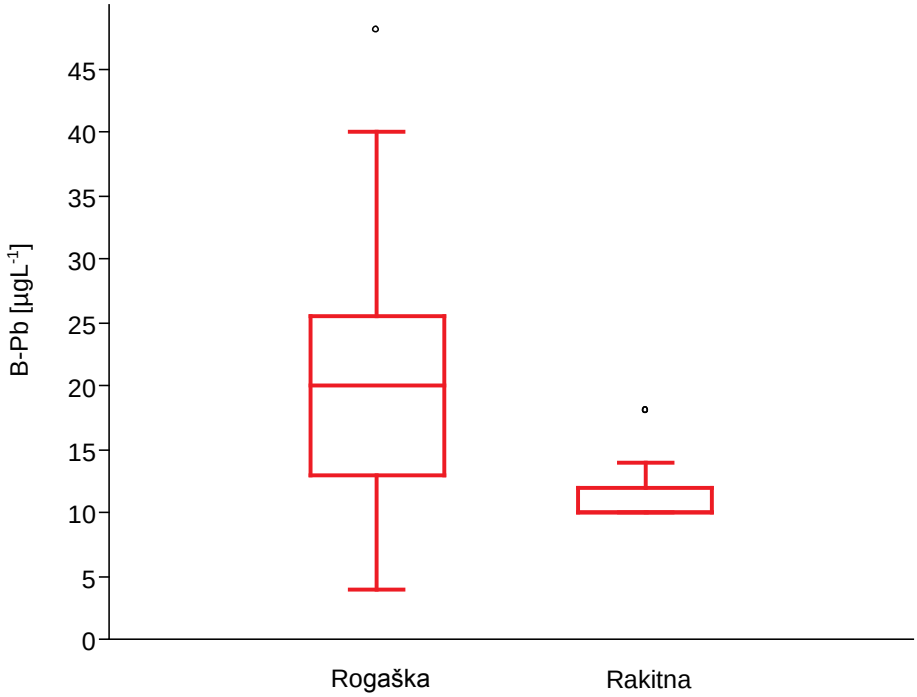


Figure 18: *Distribution of blood lead levels in the reference group and group from Rogaška Slatina.*

Measured blood lead levels were between 4 and 48 µg/L. The analysis revealed that none of the examined children had blood lead level equal or greater than 100 µg/L, furthermore, none had a blood lead level equal or greater than 50 µg/L. An average blood lead level for children from Rogaška Slatina was 19.8 µg/L, median was 20 µg/L, mode 13 µg/L.

In the control group an average blood lead level was 11.7 µg/L, median 10 µg/L and mode 10 µg/L.

Median blood lead level for the boys in Rogaška Slatina was higher than for the girls. There are many factors which may influence the blood lead burden, and behavior is one of them. It is believed that boys spend more time playing outside on the ground and that they are not as clean as girls.

To confirm that the values for blood lead levels were significantly higher in children from Rogaška Slatina than in children from Rakitna, we used the Mann-Whitney test ($p<0.001$). It must be pointed out that the actual B-Pb for Rakitna group is most probably even lower than the average value of 11.7 µg/L, since several results were below the LOD value of 10 µg/L, which were all included in the calculation of the average and counted as 10 µg/L.

Comparison of blood lead levels revealed that children in Rogaška Slatina had 6-times lower values than children form the Upper Meža Valley (geometric mean was 95.8 µg/L) (Ivartnik, 2009), and lower median B-Pb values than it was found in the Slovenian male population of 35 µg/L by Štupar, and Eržen (Štupar, 2007b, Eržen, 2004).

Children with concentrations less than 100 μg/L are not currently considered to have excess lead exposure.
A threshold value below which lead has no aparent adverse developmental effect has not been identified. The 1991 CDC statement on childhood lead poisoning (CDC, 1991a) set 100 μg/L as the screening action guideline.

In Figure 19 are blood lead levels, proposed by CDC through the years.

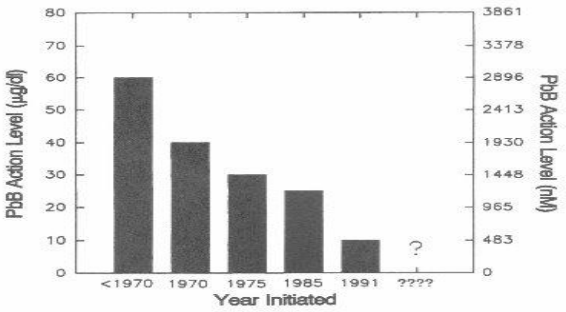


***Figure 19**: Blood lead action levels proposed by the CDC and the Public Health Service (Copied from CDC, 1991a).*

Although this blood lead level was intended to serve as a risk guidance and management tool at the community level, it has been widely imbued with biological significance for the individual child. Indeed, it often seems to be interpreted as a threshold, such that a level <100 μg/L is viewed as »safe« and a higher level is »toxic«. No single number can be cited as a threshold, divorced from a context that specifies factors such as the endpoint of interest, the age at exposure and at assessment, the duration of blood lead elevation, and characteristics of the child´s rearing environment. Even after 2 decades of steady decline in population blood lead levels (Pirkle, 1994), the mean still lies between 1 (Mushak, 1993) and 2 (Flegal, Smith, 1992) orders of magnitude greater than estimates of natural background levels in humans. Among children in the Boston prospective study, for whom the mean blood lead level at age 2 years was 70 μg/L, a significant inverse association was found between blood lead levels and both IQ and academic achievement at 10 years of age (Bellinger, 1992).

It may be usefull to explore intellectual impairment in children in Rogaška Slatina. In the Third National Health and Nutrition Examination Survey in USA (Lanphear, 2000) a sample, among 4853 6- to 16-year-old children, current blood lead was inversely associated with 4 measures of cognitive function, even when the sample was restricted to children with blood lead levels <50 μg/L. In the study of Canfield in 2003 in the subgroup of 101 children whose blood lead levels were <100 μg/L at 6, 12, 18, 24, 36, 48, and 60 months of age, significant covariate-adjusted associations were observed between blood lead lelvel and IQ at ages 3 and 5 (Canfield, 2003). In the linear model involving the full range of lead values in this sample, the estimated IQ loss was 4.6 points for each increase in the blood lead concentration 100 μg/L. In contrast, for children whose blood lead concentrations remain bellow 100 μg/L, the estimated loss in

IQ was considerably greater. (Lanphear, 2000; Lanphear, 2005; Tellez-Rojo, 2006; Hu, 2006; Schnaas, 2006). A second cross-sectional study that used data from the third NHANES indicated greater possible effects on reading and math scores among children with blood lead concentration bellow 50 μg/L than among those with higher concentrations. Similarity was found in some cohorts with a mean as low as 10 – 20 μg/L (Emory, 2003; Jedrychowski, 2007).
Some have recommended that the screening guideline shoud be reduced to a level as low as 20 μg/L (Gilbert, 2006).

In a pooled analysis of seven major prospective studies involving 1333 children (Lanphear, 2005), a log-linear model, the functional form that best described the relationship, predicted a 9.2-point decline in IQ over the range less than 10 – 300 μg/L. Two-thirds of this decline (6.2 points) was predicted to occur in the range of less than 10 - 99 μg/L, with an additional 1.9-point decline between 100 and 199 μg/L, and a 1.1-point decline between 200 an 300 μg/L. The mechanism that would generate such a supralinear relationship is unknown, presumably it involves a lead-sensitive pathway that is rapidly saturated at blood lead levels below 100 μg/L and others, less rapidly saturated pathways, at blood lead levels above 100 μg/L. Nonlinear relationships are, however, common in toxicology (Calabrese, 2003) and have been observed in a neurodevelopmental study of methylmercury exposure (Budtz-Jorgensen, 2000).
As lead is a bioaccumulative toxicant with complex kinetics, however, concurent blood lead level at school age is likely to be a reasonable proxy for lifetime exposure.

Based on the results we have got, it can be said that the group with higher risk for elevated blood lead levels are boys living in the community of Rogaška Slatina.

4.1.2 Hair Pb

The subjects has H-Pb values from 0.07 – 6.26 μg/g (1.5), the mediana 0.91 μg/g, average was 1.36 μg/g.
The control group has H-Pb values from 0.13 – 0.82 μ/g (0.2), the mediana 0.39 μg/g, average was 0.40 μg/g.

Table 7: *Mean (SD) H-Pb of children from Rogaška Slatina and control group.*

	N	Median	H-Pb in μg/g
Group from Rog.Sl.	36	0.91	1.36 (1.5)
Control group	16	0.39	0.40 (0.2)

The difference in the median values between the two groups was greater than would be expected by chance; thus, there is a statistically significant difference ($p < 0.003$). For confirmation, we used the Mann-Whitney test.

Distribution of H-Pb in the group of children from Rogaška Slatina and the control group from Rakitna is shown in the following Figure 20. The outliners indicate the smallest and the largest values and the boxes indicate the 25^{th} and 75^{th} quartiles. The lines in the boxes are median values.

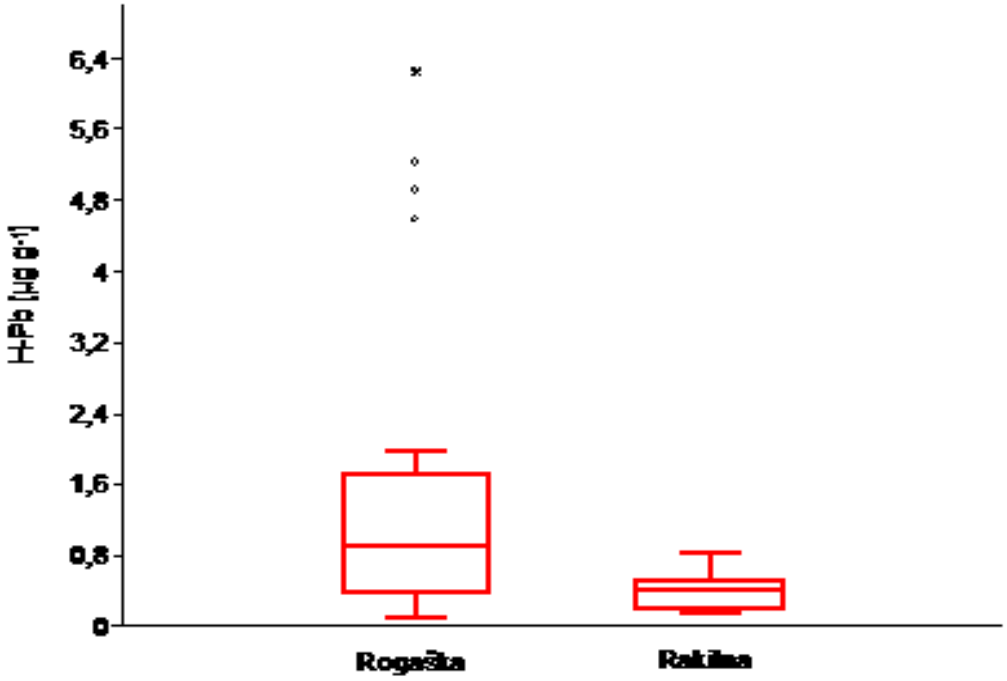


Figure 20: *Distribution of hair lead level in reference group and group in Rogaška Slatina.*

Hair generally contains less than 5 μg/g lead and concentrations greater than 25 μg/g indicate very high exposure (Furman, 2000). For children, 9 μg/g has been suggested as the allowable limit for hair lead concentration (Revich, 1994; Esteban, 1999).

Samples of hair were taken at the same time as the blood samples. In the group from Rogaška Slatina we took 36 samples and in Rakitna 16. Some of children did not want to participate in the hair sampling.

In the control group girls had higher mean values of H-Pb than boys (0.42 μg/g vs 0.39 μg/g). The average H-Pb values in both groups were higher in boys than in girls. The previous studies also reported higher values in males than female peers (Chlopicka, 1995; Chlopicka, 1998; Meng, 1998).

There is a statistically significant difference between children from Rogaška Slatina and Rakitna ($p<0.003$), using the Mann – Whitney test. None of the examined children reached or exceeded the proposed limit concentration.

4.1.3 Relationship between B-Pb and H-Pb

A statistically significant negative relationship between B-Pb and H-Pb in Rogaška Slatina was found. In the group from Rakitna, correlation is positive, but not statistically significant ($r= 0.121$; $p= 0.648$).
Relationship between B-Pb and H-Pb is presented in Figure 21.

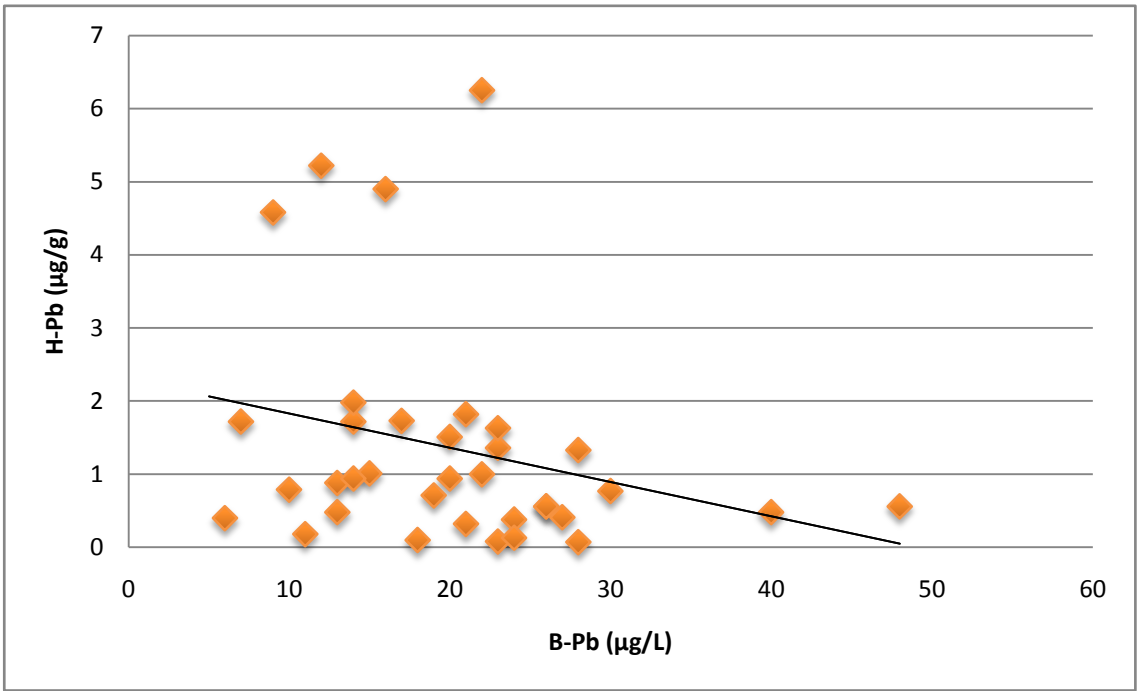


Figure 21: *The relationship between B-Pb and H-Pb in children from Rogaška Slatina (r=- 0.348; p=0.0378) (N=36).*

The results are probably caused by lag between B-Pb and H-Pb.

At the average growth rate of roughly 1 cm per month, consecutive 1-cm segments of hair recapitulate average monthly blood levels. There was approximately a 20-day lag between the concentration of trace elements in the first centimeter next to the scalp and the corresponding average monthly blood level (Clarkson, 2006). Factors, such as age, interactions between elements and genetics may, to a grater or lesser extent, modify the metabolism of the trace elements and their mobilization from the blood to the hair compartment (Chojnacka, 2006; Khalique, 2005; Pachal, 1989).

It appears that the level of environmental lead pollution considerably affects the strenght of this correlation. Niculescu et al and Clayton and Wooler found the strongest correlations for heavily exposed lead-battery workers, while the correlatons were much lower in the control population (Niculescu, 1983; Clayton, 1983). An interesting observation was reported in a study in children living in a highly polluted area in Russia (Esteban, 1999), where the mean content of B-Pb and H-Pb was 98 µg/L and 7.2 µg/g, respectively. The whole population´s correlation between log H-Pb and log B-Pb was statisticallly significant ($r=0.45$, $p<0.05$). If highly exposed children were excluded, the significance of the correlation was lost ($r=0.07$). Thus, it seems that hair may be a better biomarker in cases of high exposure. Some researchers were of the opinion that hair is not appropriate biomarker to evaluate the exposure to lead (Rodrigues, 2008).

4.2 Soil Pb

It may be inferred from data that natural (those derived from geological ground) soil-Pb levels varied considerably. Values in the range of 14 – 35 mg/g were found at different locations (Šalek valley, Rakitna). Therefore, the exact contribution of anthropogenic sources defined as ratio (R) between the measured soil Pb content (Pb_m) and natural soil Pb content (Pb_n) cannot be precisely determined. However, this contribution was shown to be substantial in the regions where the major lead emission sources are located (lead smelter, coal combustion, chemical industry, traffic).

The soil samples were taken in 2006 and 2009. In both cases, samples of soil were taken at a depth from 0 – 25 cm, depending on the sample type (filed 0 – 25 cm, cultivated garden 0 – 25, and meadow 0 - 5). First measurements of Pb in the soil were performed in the general area around Rogaška Slatina. Contrary to the expectations, measurements of lead in soils near the glass factory demonstrated that only a minor increase, insignificant to cause deleterious health effects, in lead content existed in all settlements near the factory. Only at one sampling site (No. 8, see Figure 13), relatively high (293 mg/g) soil-Pb content was found. That Pb content was above warning value, as determined by regulation (100 mg/kg), which are presented in Table 8.

Table 8: *Immision lead treshold values in soil (Decree on the limit, Warning and Critical Concentration Values of Dangerous Substances in Soil, Of. J. RS No. 68/96)*

Treshold values	Pb (mg/kg)
Limit immission value	85
Warning immission value	100
Critical immission value	530

The results of first measurement are presented in Table 9.

Table 9: *Summary of the data on soil-Pb content at different distances around the glass factory in Rogaška Slatina (general area).*

Sampling location	Type of ground	Soil profile (cm)	Distance from source (m)	Pb content (mg/g)
No. 1	Field	0 - 25	830	48
No. 2	Cultivated garden	0 - 25	420	37
No. 3	Cultivated garden	0 - 25	370	43
No. 4	Cultivated garden	0 - 25	400	37
No. 5	Meadow	0 - 5	260	37
No. 6	Meadow	0 - 5	220	32
No. 7	Meadow	0 - 5	340	55
No. 8	Field	0 - 25	300	**293**
No. 9	Field	0 - 25	740	34
No. 10	Meadow	0 - 5	870	24
No. 11	Field	0 - 25	1460	24
No. 12	Cultivated garden	0 - 25	620	40
No. 13	Meadow	0 - 5	1580	22

This sampling site was located SW of the factory at an attitude slightly higher than the height of the factory stack. Therefore, we assumed that Pb containing particles, emitted from the factory were transported across larger distances beyond the hills surrounding the village. This may be supported by the observation that sampling location No. 8 lies in the direction of prevailing winds in this region (Figure 22).

Figure 22: *Direction of prevailing winds in region.*

Thereafter, we repeated sampling in that direction. The samples were taken up from the glass factory (235 m height above sea level) to sampling site No. 8 (262 m height above sea level).

Figure 23: *Location of soil sampling near the lead glass-factory.*

Results from the second sampling are shown in Table 10.

Table 10: *Summary of the data on soil-Pb content at different MSL up from the factory chimney*

Sampling location	Type of ground	MSL (m)	Soil profile (cm)	Distance from source (m)	Pb content (mg/kg dry soil)	SD
No. 1-2	Wood	235	0 - 25	12	1193	28.9
No. 2-2	Wood	235	0 - 25	12	1140	2.8
No. 3-2	Wood	235	0 - 25	12	403	3.5
No. 4-2	Wood	247	0 - 25	24	139	20.5
No. 5-2	Wood	247	0 - 25	24	92	0
No. 6-2	Wood	247	0 - 25	24	188	1.4
No. 7-2	Field	257	0 - 10	39	214	4.2
No. 8-2	Field	257	0 - 10	39	42	0.7
No. 9-2	Field	257	0 - 10	39	32	0.7
No. 10-2	Wood	268	0 - 25	46	79	1.4
No. 11-2	Wood	268	0 - 25	46	42	9.9
No. 12-2	Wood	268	0 - 25	46	45	0.7

The highest Pb content 403 to 1192 mg/kg was at MSL which was nearest to the glass-factory chimney (10 meters from the factory), and declined with MSL (the highest point of sampling was about 60 meters from factory) (Figure 24). Soil-Pb was higher than the critical value (530 mg/kg) of sample sites in two different distances from chimney, both near the chimney. Boundary Soil-Pb levels were exceeded in three different MSL, just one sampling site was below that. The lead concentrations near chimney were a little smaller than those, measured in the Upper Meža Valley area in year 2002 (average lead concentration 1134 mg/kg dry soil) (Ivartnik, 2009).

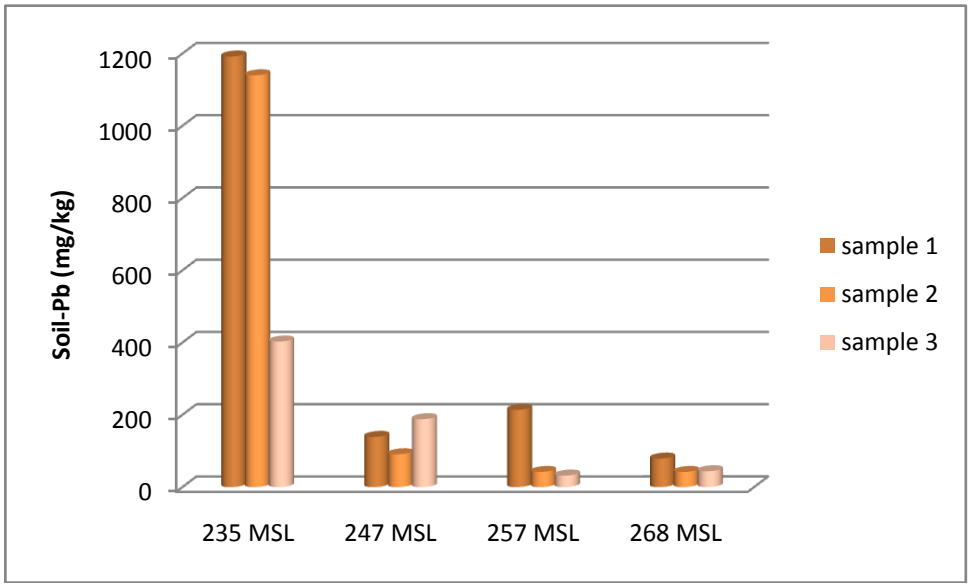
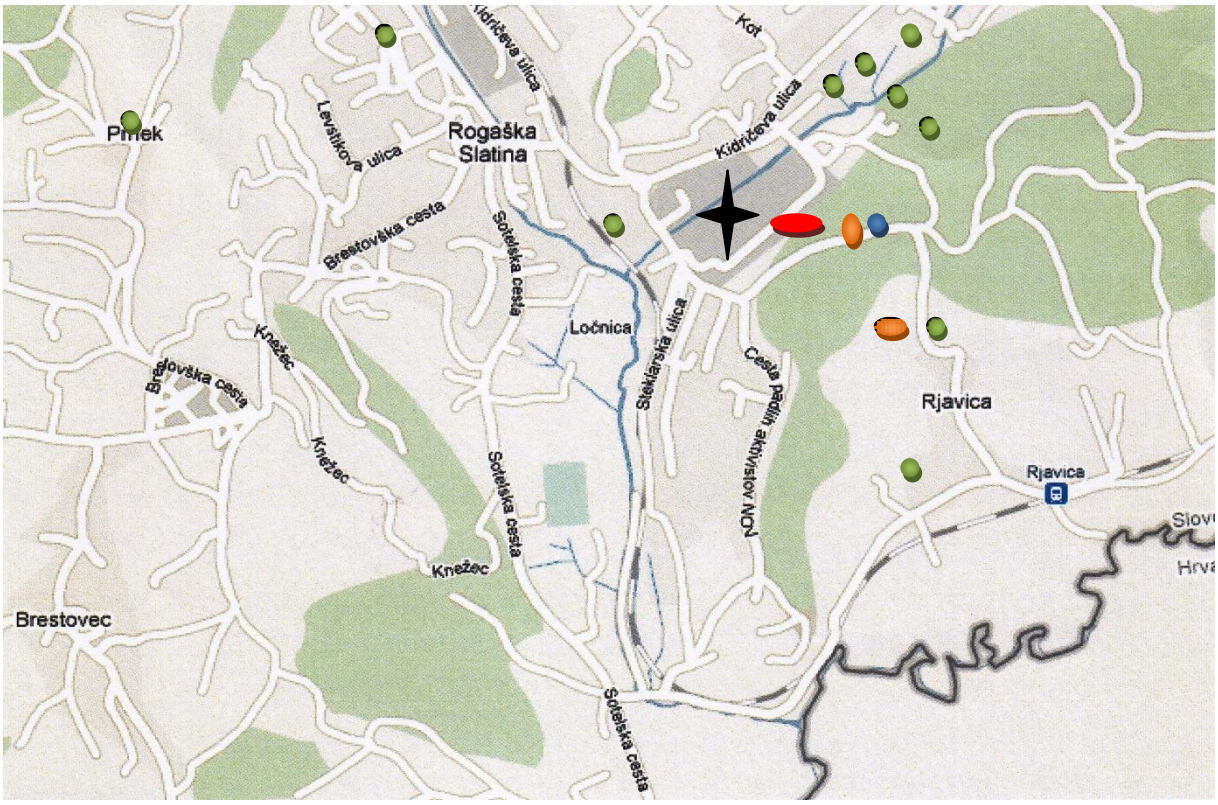


Figure 24: *Soil-Pb content at different MSL as for distance from chimney*

Distribution of lead content in the soil at Rogaška Slatina is presented in Figure 25, indicated with a red mark, which represents the highest amount of lead in the soil at that sampling point.



Legend	Pb (mg/kg)
	0 – 85
	85 – 100
	100 – 530
	> 530

Figure 25: *Areas according to different soil-Pb values around lead-glass factory.*

The average soil-Pb in Rogaška Slatina in 2006 was 58.6 mg/kg – around lead-glass factory, in 2009, 301 mg/kg – above lead-glass factory and in Rakitna 38.6 mg/kg. In the second sampling of soil in Rogaška Slatina on a hill above the lead-glass chimney, the soil-Pb content was 5-times higher than in other parts of Rogaška Slatina. If we take into consideration the average Pb content according to distance (MSL) from chimney, the highest soil-Pb was near the lead-glass factory (911 mg/kg) and lowest 46 m distance in height from the source (56 mg/kg). So, the higher we get, the lower is Pb content in soil.

The comparison between soil-Pb in both locations is presented in Figure 26.

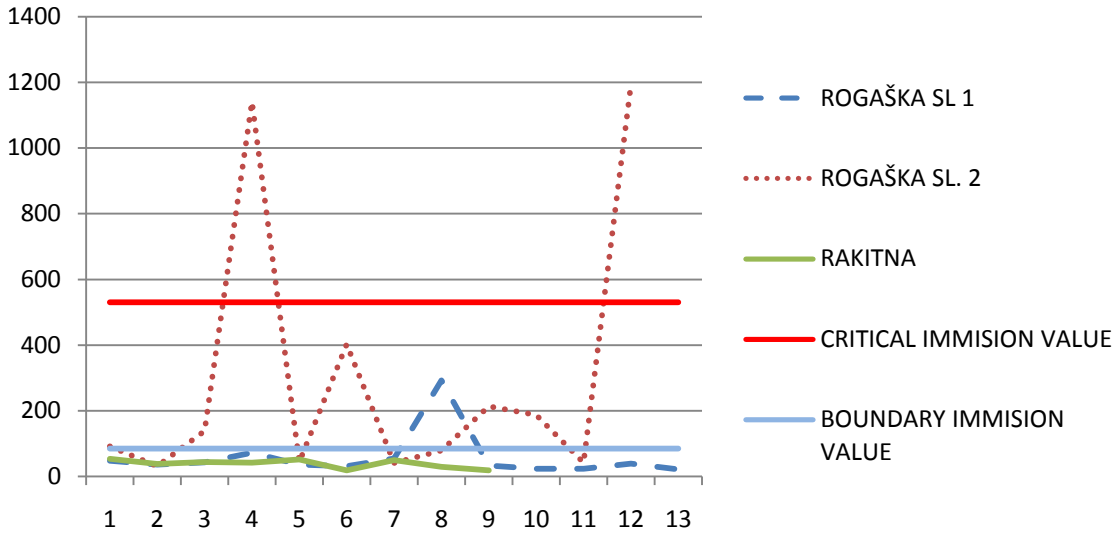


Figure 26: *Soil-Pb in Rogaška Slatina and Rakitna according to limit and critical immission values.*

The concentration of soil-Pb was lower according to the distance from source of pollution.

We can conclude that soil across the center of Rogaška Slatina is not polluted with lead, but soil right above the lead-glass factory chimney is. That is perhaps because in past, the waste material from factory was deposited uncontrolled. That is seen from looking in the cultivated field on the top of the hill, where glass is mixed with the soil (Figure 27). That soil is used for the cultivation of maize.

Figure 27: *Cultured field for maize cultivation with glass particles.*

When we compared data for the soil-Pb, there was significant differences between both locations – Rogaška Slatina and Rakitna ($p=0.007$). For conformation we used the Mann-Whitney test. Correlation between MSL and soil-Pb was highly negative, but not statistically significant.

At the top of the hill in Rjavica, where the average soil-Pb was 301 mg/kg, individual houses and farms are located. So we can conclude that people, especially children, who are living in that area are threatened by lead posioning.

The lead-contaminated house dust is a major source of lead intake for urban children (Sayre, 1974; Charney, 1980; Bornschein, 1985; Rabinowitz, 1985; Bellinger, 1986; Clark, 1991; Lanphear, 1995). Lead contamination of house dust is thought to originate from the disintegration of lead-based paint and lead-contaminated soil which is tracked indoors (Lanphear, 1995). Mouthing behaviors remain an important mechanism for lead ingestion. When reviewing the evidence, national studies usually frame the argument as follows: »Lead-based paint is the most concentrated source of lead to children, and historically, is the source most closely linked to lead poisoning in children.« (CDC, 1991b). »Lead-based paint is the largest source of high-dose lead exposure for children.« (NAS, 1993). Experiments on lead in soil and paint show that 2 to 6 times as much lead can be biologically extracted from the soil than from paint (Roberts, 1974; EPA, 1979). Literature has consistently shown that exposure to lead in soil has an effect on B-Pb levels. A reanalysis by Burgoon of 11 studies estimated a dose-response relationship between soil Pb and B-Pb of 68 μg/L per 1000 mg/kg (Burgoon, 1995). Lead in soil and house dust, but not lead-based paint, is associated with population B-Pb levels in young children. Seasonality studies strongly point to lead in soil as a significant source of population B-Pb levels. Studies of Pica children suggested that lead in soil is a greater risk factor than lead in paint (Mielke, 1998).
We also have to considere that, B-Pb has seasonal changes – children appear to receive the highest dust Pb exposure indoors and outdoors during the summer, when they have the highest blood Pb levels (Yin, 2000). The blood samples were taken in the middle of the winter. None of child who participated blood samples do not live in the Rjavica area, where the highest amount of lead in soil was discovered.
For the purpose of estimating risks to children, the EPA assumes that most children ingest relatively small quantities of soil (e.g., < 100 mg/day), while the upper 95th percentile are estimated to ingest 200 mg/day on average. The EPA has conceptually addressed the possibility that some children may display, at least on occasion, profound soil ingestion (reffered to as soil pica) in quantities far greater than the upper 95th percentile value. For such children, the EPA has proposed that risk assessors assume soil ingestion at a rate of 5 g soil/day (EPA, 1996). The results of study by Calabrese et al in 1997 showed that dose from soil (Table 11):

Table 11: *Soil intake and dose of lead from soil (Modified from Calabrese, 1997).*

Chemical	Soil screening value (mg/kg soil)	Soil intake (g soil/event)	Dose from soil[a] (mg/kg body weight)
Lead	400	5 25 50	0.2 0.8 1.5

[a] calculated as soil sreening value X soil intake / 13 kg assumed body weight.

If we calculate from these findings for soil in Rogaška Slatina the doses from soil are shown in Table 12.

Table 12: *Calculated lead doses from soil in Rogaška Slatina.*

Chemical	Soil screening value (mg/kg soil)	Soil intake (g soil/event)	Dose from soil[a] (mg/kg body weight)	Nonlethal toxic dose (mg/body weight)
Lead	900	5 25 50	0.4 1.6 3.0	0.002

That means that a child could intake 200-times the nonlethal toxic dose as for lead. Children who are 4 to 5 years old are at greatest risk due to soil lead exposure, because of hand-to-mouth activity (Ren, 2006). Exposure to lead-contaminated soil, has consistently shown a positive correlation between soil lead concentrations and population B-Pb levels (Mielke, 1998).

In comparison with data from the ROTS Report of research on ground pollution, 1989 – 2007 (Zupan, 2008), where the average soil-Pb content in Slovenia was 36 mg/kg, Pb content in soil in Rogaška Slatina was higher (58.6 mg/kg at first sampling sites and 301 mg/kg at second sampling sites). The average concentration of Pb at the second sampling above the lead-glass factory chimney was comparable to the average content of Pb in soil in the Upper Mežiška Valley (320.5 mg/kg).

4.3 Pb in dust precipitates

Pb in dust precipitates were measured at 3 sample sites in Rogaška Slatina. Results for Pb in the dust precipitates are shown in Figure 28.

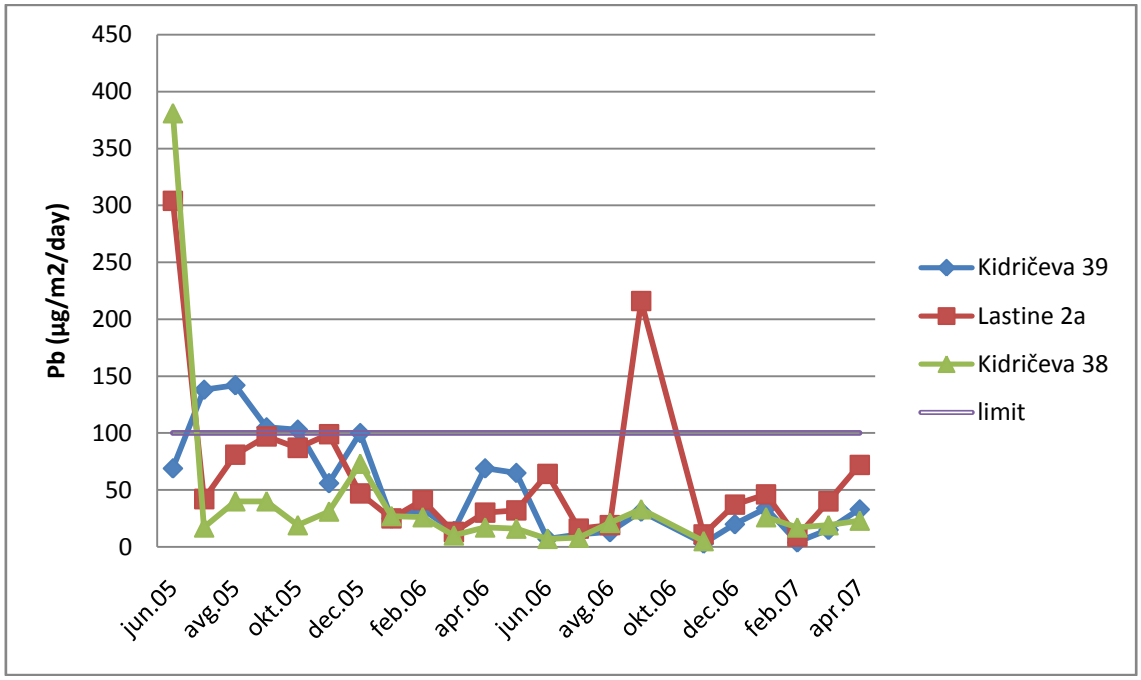


Figure 28: *Pb in dust precipitates in µg/m2/day in Rogaška Slatina from June 2005 to April 2007.*

The amount of Pb in dust precipitates was much decreased after June 2005 because the filter for cleaning the flue gases from the glass factory was installed.

Average monthly values for 2005 to 2007 are presented in Figure 29.

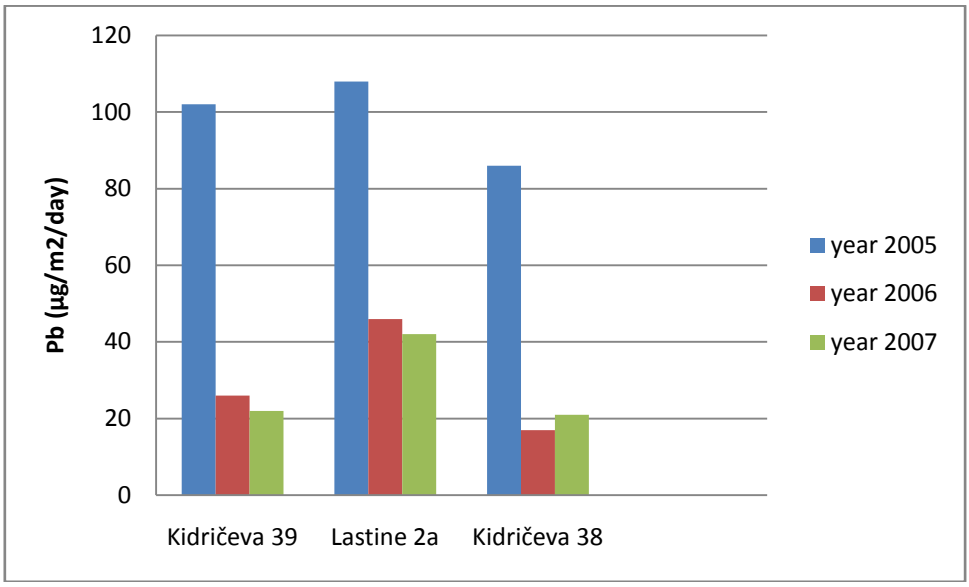


Figure 29: *Average monthly values of Pb in dust precipitates from 2005 to 2007 in µg/m2 in Rogaška Slatina.*

By the Regulation of limit, alert and critical immision values of substances in air from 1994 (expired in 2008), the limit was 100 µg/m^2/day. In year 2005 the values were above the limits, but in 2006 and 2007, the Pb in dust precipitates was below the limit.

The report »Evaluation of air pollution with SO_2, NO_2, PM_{10} particles, lead, CO, benzene, heavy metals (As, Cd, Hg, Ni) and polycyclic aromatic hydrocarbons in Slovenia« for 2009 showed that emissions of lead were reduced since 1994, when use of catalysts in cars became obligatory. In July 2001 the ban on the use of leaded gasoline entered into force. From that time the emissions of lead were significantly lower, except near some industrial installations. Too high concentratons of lead in PM10 particles were measured in the Upper Mežiška valley (ARSO, 2009).

4.4 Vegetable Pb

Measurements of Pb in vegetables was performed in the area around the glass factory (see Figure 30), where 18 samples of vegetables were taken.

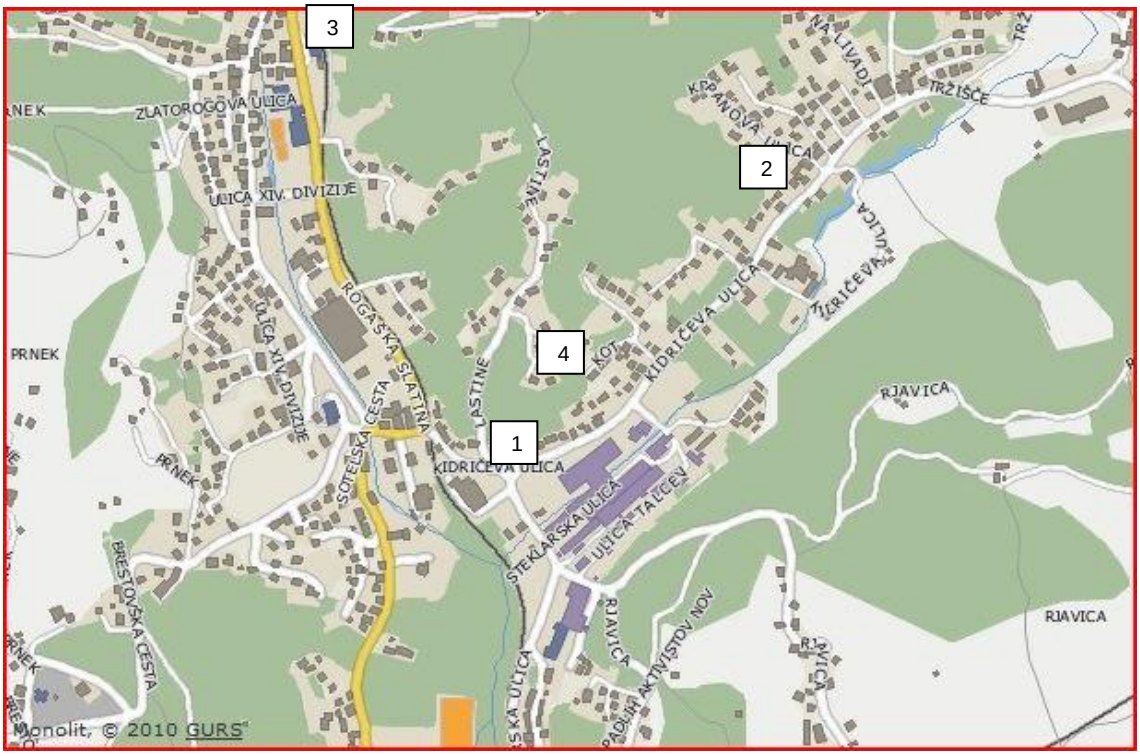


Figure 30: *Sampling sites for vegetable-Pb.*

The results of sampling the vegetable is in Table 13.

Table 13: *Summary of the data on vegetable-Pb content in different produce.*

Foodstuff	Pb content (mg/kg in DM)	Pb content (mg/kg in WW)
String bean	0.02 – 0.08	0.01 – 0.02
Chicory	0.61 – 1.33	0.04 – 0.08
Carrot	0.04 – 0.15	0.002 – 0.01
Parsley - leafs	0.59 – 2.44	0.11 – 0.44
Parsley – root	0.18 – 0.70	0.01 – 0.44
Pepper	0.05	0.005
Cabbage	0.04	0.003
Kale	0.2	0.02
Red beet	0.07	0.01

The highest amount of lead was found in leafy vegetables – parsley, followed by chicory, root of parsley, kale, carrot, red beet, string bean, pepper and cabbage. The EU Commission Regulation (EC) from 2006 determined maximum levels for lead in different foodstuffs (Table 14).

Table 14: *Maximum levels of lead in mg/kg wet weight in foodstuffs (Modified from EC No 1881/2006).*

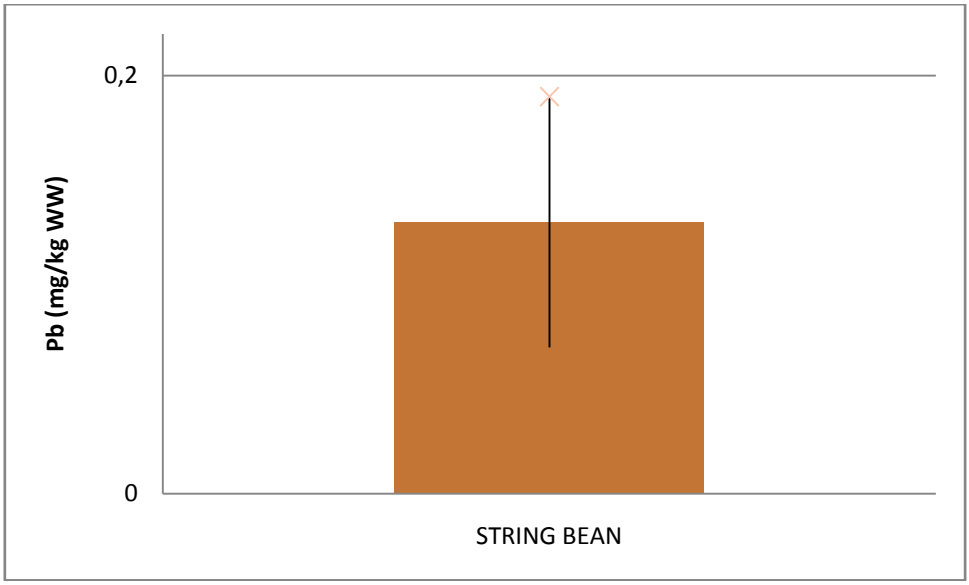
Foodstuff	Maximum levels (mg/kg in WW)
Cereals, legumes and pulses	0.20
Vegetables, excluding brassica vegetables, leaf vegetables, fresh herbs and fungi. For potatoes the maximum leve applies to peeled potatoes	0.10
Brassica vegetables, leaf vegetables and certain fungi	0.30

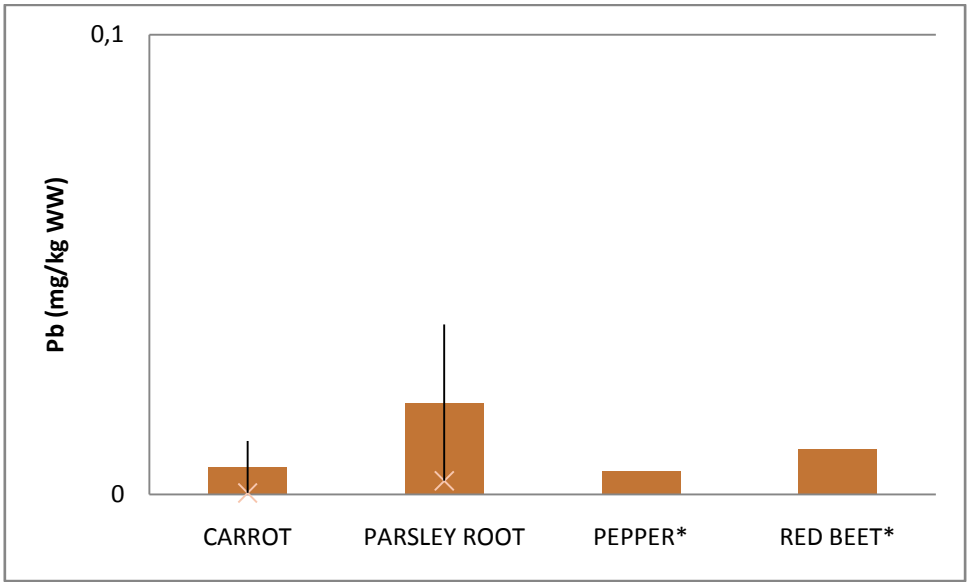
Average Pb content in wet weight was calculated by the equation

$$\text{Pb in WW} = \frac{Pb\ in\ DM \times \%\ DM\ of\ foodstuff}{100} \qquad \textit{(Equation 6)}$$

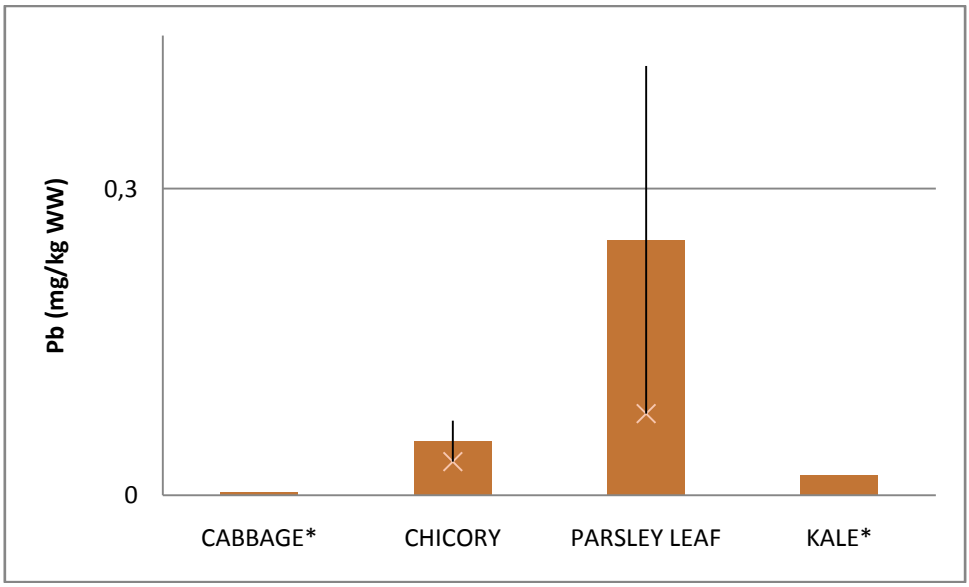
One sample of parsley – leaf – exceeded the maximum level of lead (location 4).

Average PBb levels (mg/kg WW) in certain sorts of vegetables according to maximum levels are presented in Figure 31 – 33.





* one measurement



* one measurement

Figure 31 - 33: *Amount of average lead content in different foodstuffs (mg/kg DM) in Rogaška Slatina according to maximum levels for certain groups of vegetables .*

For lead intake, the most important food are from group of cereals and cereal products, wich contribute to 1/3 of all lead intake by population in Slovenia. It was found that foodstuffs from other groups contribute smaller amount of lead, the most from the category of vegetables and products (14.2%). In comparison with the study of Eržen, the Pb amount from carrot, cabbage, pepper and red beet was lower in Rogaška Slatina. The Pb amount in string been was the same and the chicory higher (Eržen, 2004).

To determine more precise Pb amounts in different foodstuffs, more samples should be taken in the area which is most contaminated with lead. More accurate data of home

made vegetable consuption should be done, especially with children, who consume larger share of PTWI for lead than adults (Eržen, 2004).

5 SUMMARY AND CONCLUSIONS

Lead has been mined, smelted, and used in cosmetics, internal and topical medicinal preparations, and paint pigments and glazes since early recorded history (Nriagu, 1983).

Lead is potent and pervasive neurotoxicant. The toxic effects of lead are the same regardless of the route of entry into the body, which are primarily ingestion and inhalation. Once absorbed, lead bindes to erythrocytes and travels in the blood to soft tissues, such as the liver, kidneys, lungs, brain, spleen, muscles and heart. About 73% of the lead in children´s bodies is stored in their bones. Lead can stay in bones for decades (de Silva, 1981). Lead can leach out during growth and development of children constituting a significant long-term source of lead in the blood (Mahaffey, 2000).
The US Center for Disease Control and Prevention in 1991 chose 100 μg/L as an initial screening level for lead in children´s blood, although subsequent studies are still unable to find a »safe« lower level of lead, with levels bellow 100 μg/L still causing some toxicological effects (Nigg, 2008). Recent epidemiological studies have found that blood lead levels below 100 μg/L, a blood lead level, previously thought safe, can result in significant cognitive impairment in children (Canfield, 2003; Chiodo, 2007; Jusko, 2008; Schnaas, 2006; Surkan, 2007; Miranda, 2007).

Lead glass manufacture may result in lead emission. The primary pollutants from the pressed and blown glass industry are oil, fluoride, ammonia, lead and suspended solids. Fluoride and lead are added by the finishing steps (Noyes, 1993).

5.1 Main conclusion

Our hypothesis was: »The vicinity of the lead-glass factory influenced the lead burden on inhabitants of Rogaška Slatina.« Under that research topic, we examined if there was any influence on the lead burden for children, who are living near the lead-glass factory. The degree of bioburden with lead was made on basis of a median blood lead level and hair lead level. The degree of environmental pollution was made by analysis of the content of lead in soil, air and edible vegetables. We can conclude that the vicinity of the lead-glass factory has an influenced on the bioburden with lead and also on the contamination of soil, but within a currently limited area.

The aim of this study was to estimate lead intake from many different sources. For that matter two different biomarkers were chosen as an appropriate indicators, as well as lead concentrations in different environmental indicators.

5.2 Other conclusions

Children in Rogaška Slatina have not blood lead levels above 100 μg/L, or even more than 50 μg/L, so they are not »directly threatened« according to the CDC recommendation. The majority of children have B-Pb between 15 – 30 μg/L. The group

at higher risk for elevated B-Pb are boys, living in Rogaška Slatina. The median H-Pb values are greater in boys than girls. However, interpretation of blood lead levels requires recognition of which levels are anomalous compared with those found in the local population. Obviously, children in Rogaška Slatina have significantly higher blood-Pb levels compared with the control group, so it can be concluded that the assumption about differences of environmental burden with lead was justified.

Negative effects of lead are associated with lead exposure at any age. Although lead is a risk factor for developmental and behavioural problems, its presence does not indicate that these problems will necessarily occur. No characteristic developmental pattern is attributable solely to the effects of lead, and measures of the effects of lead on children are imperfected. For an individual child, neurobehavioral test performance might indicate clinically significant impairments related to lead exposure but might not fully capture the array of negative outcomes caused by lead. The effects of lead at levels approaching 100 μg/L might not be recognizable to the child´s family or clinician and might not be identified through neurobehavioral testing. The effects of exposure to lead at lower levels are best evaluated on a community-wide basis (Binns, 2007). Even small amounts of lead exposure in childhood seem to lower scores on IQ testing. A child, whose blood lead is greater than the upper limit of the 95th centile, merits investigation with relatively inexpensive blood testing.

Until 1997 the CDC and the American Academy of Pediatrics recommended universal screening of all preschool children through blood-Pb testing (CDC, 2005b). This should be performed at least twice, preferably at about ages 1 and 2 years. The concept of testing 1-year-old children is to find children at risk from lead exposure early on, hopefully before peak levels with overt toxicity would occur. Screening at age 2 years follows the observation that this critical age is characterized by increased mobility of the child combined with the continuing hand-to-mouth behaviour that provides a pathway for lead entry into the body. It is also the age of peak lead levels in the pediatric population and the best single time point predictor of the relationship between blood-Pb and later cognitive tests.

The health effects of lead are uncertain in individual children who have B-Pb measured at a single point site. So blood samples should be taken in children around Rjavica, where the highest soil-Pb amount was recognized, in order to recognize the children with higher risk for elevated blood-Pb levels. That is important because the overall weight of avaliable evidence supports an inverse (negative) association between blood-Pb levels < 100 μg/L and the cognitive function of children. A steeper slope in the dose-response curve was observed at lower rather than higher B-Pb levels.

The risk questions identify children at high risk who should be screened more frequently for B-Pb levels:

Does your child -
- Live in or regularly visit a house with peeling or chipping paint built before 1960? This could include a day-care center/ preschool, the home of a babysitter or a relative, etc. - Live in, or regularly visit, a house built before 1960 with recent, ongoing, or planned renovation or remodeling? - Have a brother or sister, housemate, or playmate being followed up or treated for lead poisoning (that is, blood level ≥150 mg/L)? - Live with an adult whose job or hobby involves exposure to lead?

- Live near an active lead smelter, battery recycling plant, or other industry likely to release lead?

The health effects of lead are uncertain in individual children who have BLL´s measured at a single point in time (CDC, 2005b). Potential compounding factors are social factors, iron status, tobacco smoke inhalation.

Vegetables adopt lead mostly from the atmosphere, so we can assume that vegetables with edible leafs could be used as an indicator of environmental pollution with lead and as indicator for the burden of local people (Eržen, 2004). Further research should be taken to identify the quantity of vegetable and which are produced domestically, are consumed by the children in Rjavica. The precise analysis should be taken in order to found out what are levels of lead in vegetables in the heavily loaded area in Rjavica.

House dust examined in houses in Rjavica would also give an answer as to how much lead is taken from outside to the indoor. The house dust and and backyard soil contribute in large proportions to the overall lead exposure and remedation strategies should be focused on them.

5.3 Some additional comments

The work has some limitations.

We did not have enough samples of soil and vegetable and data about how often the children consume home grown vegetables. With those data we could, more exactly, calculate the Pb consuption from food.

We do not know which level of lead in blood of children is typical for this area. With that information we could examine with greater detail children in the upper 95th percentila. In that group more frequent control of B-Pb is sensible in order to monitor also as an indicator of intellectual impairement.

Monitoring the larger area in the direction of Rjavica should be performed so that a more exact picture of state about Pb in the soil is done. With the determination of the polluted area we could determine if any rehabilitation of that area is necessary.

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ANNEX A:

Blood lead levels in children from Rogaška Slatina – rank list

Rang	*ID no*	*Sex*	*Location*	*B-Pb (µg/L)*
1	*187*	*M*	*Rogaška Slatina*	*4*
2	*6*	*F*	*Rogaška Slatina*	*5*
3	*180*	*M*	*Rogaška Slatina*	*6*
4	*190*	*F*	*Rogaška Slatina*	*7*
5	*186*	*M*	*Rogaška Slatina*	*9*
6	*4*	*M*	*Rogaška Slatina*	*10*
7	*177*	*M*	*Rogaška Slatina*	*10*
8	*181*	*M*	*Rogaška Slatina*	*11*
9	*179*	*M*	*Rogaška Slatina*	*12*
10	*207*	*F*	*Rogaška Slatina*	*13*
11	*13*	*M*	*Rogaška Slatina*	*13*
12	*198*	*M*	*Rogaška Slatina*	*13*
13	*7*	*M*	*Rogaška Slatina*	*13*
14	*189*	*F*	*Rogaška Slatina*	*14*
15	*197*	*M*	*Rogaška Slatina*	*14*
16	*205*	*M*	*Rogaška Slatina*	*14*
17	*191*	*F*	*Rogaška Slatina*	*15*
18	*199*	*M*	*Rogaška Slatina*	*16*
19	*11*	*F*	*Rogaška Slatina*	*16*
20	*183*	*F*	*Rogaška Slatina*	*17*
21	*195*	*M*	*Rogaška Slatina*	*19*
22	*176*	*M*	*Rogaška Slatina*	*18*
23	*8*	*M*	*Rogaška Slatina*	*19*
24	*208*	*F*	*Rogaška Slatina*	*20*
25	*201*	*M*	*Rogaška Slatina*	*20*
26	*196*	*M*	*Rogaška Slatina*	*21*
27	*200*	*M*	*Rogaška Slatina*	*21*
28	*3*	*M*	*Rogaška Slatina*	*21*
29	*192*	*M*	*Rogaška Slatina*	*22*
30	*193*	*M*	*Rogaška Slatina*	*22*
31	*202*	*M*	*Rogaška Slatina*	*23*
32	*185*	*M*	*Rogaška Slatina*	*23*
33	*182*	*M*	*Rogaška Slatina*	*23*
34	*203*	*M*	*Rogaška Slatina*	*24*
35	*1*	*F*	*Rogaška Slatina*	*24*
36	*14*	*M*	*Rogaška Slatina*	*25*
37	*206*	*F*	*Rogaška Slatina*	*26*
38	*2*	*M*	*Rogaška Slatina*	*26*
39	*209*	*M*	*Rogaška Slatina*	*26*
40	*194*	*M*	*Rogaška Slatina*	*27*
41	*9*	*F*	*Rogaška Slatina*	*27*
42	*184*	*M*	*Rogaška Slatina*	*28*
43	*210*	*M*	*Rogaška Slatina*	*28*
44	*5*	*F*	*Rogaška Slatina*	*29*
45	*188*	*F*	*Rogaška Slatina*	*30*

46	*12*	*M*	*Rogaška Slatina*	*39*
47	*204*	*M*	*Rogaška Slatina*	*40*
48	*178*	*M*	*Rogaška Slatina*	*48*

ANNEX B:

Blood lead levels in children from Rakitna – rank list

Rang	*ID no*	*Sex*	*Location*	*B-Pb (µg/L)*
1	*5*	*F*	*Rakitna*	*10*
2	*6*	*M*	*Rakitna*	*10*
3	*7*	*F*	*Rakitna*	*10*
4	*9*	*M*	*Rakitna*	*10*
5	*10*	*M*	*Rakitna*	*10*
6	*11*	*M*	*Rakitna*	*10*
7	*12*	*M*	*Rakitna*	*10*
8	*14*	*F*	*Rakitna*	*10*
9	*17*	*M*	*Rakitna*	*10*
10	*19*	*M*	*Rakitna*	*10*
11	*15*	*M*	*Rakitna*	*11*
12	*3*	*M*	*Rakitna*	*12*
13	*4*	*F*	*Rakitna*	*12*
14	*16*	*F*	*Rakitna*	*12*
15	*18*	*F*	*Rakitna*	*12*
16	*8*	*F*	*Rakitna*	*13*
17	*13*	*F*	*Rakitna*	*14*
18	*2*	*M*	*Rakitna*	*18*
19	*20*	*M*	*Rakitna*	*18*

ANNEX C:

Hair lead levels in children from Rogaška Slatina – rank list

Rang	*ID no*	*Sex*	*Location*	*H-Pb (µg/g)*
1	*189*	*F*	*Rogaška Slatina*	*0.07*
2	*190*	*F*	*Rogaška Slatina*	*0.08*
3	*178*	*M*	*Rogaška Slatina*	*0.1*
4	*13*	*M*	*Rogaška Slatina*	*0.13*
5	*205*	*M*	*Rogaška Slatina*	*0.32*
6	*6*	*F*	*Rogaška Slatina*	*0.33*
7	*4*	*M*	*Rogaška Slatina*	*0.36*
8	*208*	*F*	*Rogaška Slatina*	*0.38*
9	*199*	*M*	*Rogaška Slatina*	*0.41*
10	*210*	*M*	*Rogaška Slatina*	*0.48*
11	*2*	*M*	*Rogaška Slatina*	*0.56*
12	*5*	*F*	*Rogaška Slatina*	*0.65*
13	*200*	*M*	*Rogaška Slatina*	*0.71*
14	*193*	*M*	*Rogaška Slatina*	*0.77*
15	*179*	*M*	*Rogaška Slatina*	*0.79*
16	*203*	*M*	*Rogaška Slatina*	*0.88*

17	*183*	*F*	*Rogaška Slatina*	*0.91*
18	*1*	*F*	*Rogaška Slatina*	*0.94*
19	*197*	*M*	*Rogaška Slatina*	*1*
20	*187*	*F*	*Rogaška Slatina*	*1.36*
21	*192*	*M*	*Rogaška Slatina*	*1.37*
22	*206*	*F*	*Rogaška Slatina*	*1.51*
23	*207*	*F*	*Rogaška Slatina*	*1.63*
24	*194*	*M*	*Rogaška Slatina*	*1.72*
25	*195*	*M*	*Rogaška Slatina*	*1.72*
26	*188*	*F*	*Rogaška Slatina*	*1.73*
27	*202*	*M*	*Rogaška Slatina*	*1.98*
28	*191*	*F*	*Rogaška Slatina*	*4.58*
29	*204*	*M*	*Rogaška Slatina*	*4.9*
30	*182*	*M*	*Rogaška Slatina*	*5.22*
31	*198*	*M*	*Rogaška Slatina*	*6.25*

ANNEX D:

Hair lead levels in children from Rakitna – rank list

Rang	*ID no*	*Sex*	*Location*	*H-Pb (µg/g)*
1	*9*	*M*	*Rakitna*	*0.13*
2	*19*	*M*	*Rakitna*	*0.16*
3	*10*	*M*	*Rakitna*	*0.17*
4	*18*	*F*	*Rakitna*	*0.18*
5	*7*	*F*	*Rakitna*	*0.2*
6	*14*	*F*	*Rakitna*	*0.3*
7	*8*	*F*	*Rakitna*	*0.33*
8	*17*	*M*	*Rakitna*	*0.33*
9	*11*	*M*	*Rakitna*	*0.45*
10	*5*	*F*	*Rakitna*	*0.47*
11	*4*	*F*	*Rakitna*	*0.49*
12	*12*	*M*	*Rakitna*	*0.5*
13	*13*	*F*	*Rakitna*	*0.5*
14	*16*	*F*	*Rakitna*	*0.66*
15	*3*	*M*	*Rakitna*	*0.78*
16	*20*	*M*	*Rakitna*	*0.82*

ANNEX E:

Lead in vegetable – rank list

Rang	*Sample*	*Location*	*Pb (mg/g) in WW*
1	*Carrot*	*Rogaška Slatina*	*0.002*
2	*Cabbage*	*Rogaška Slatina*	*0.003*
3	*Pepper*	*Rogaška Slatina*	*0.005*
4	*Carrot*	*Rogaška Slatina*	*0.01*
5	*Parsley root*	*Rogaška Slatina*	*0.01*
6	*String bean*	*Rogaška Slatina*	*0.01*
7	*Parsley root*	*Rogaška Slatina*	*0.01*
8	*Red beet*	*Rogaška Slatina*	*0.01*
9	*String bean*	*Rogaška Slatina*	*0.01*
10	*String bean*	*Rogaška Slatina*	*0.02*
11	*Kale*	*Rogaška Slatina*	*0.02*
12	*Chicory*	*Rogaška Slatina*	*0.04*
13	*Parsley root*	*Rogaška Slatina*	*0.04*
14	*Chicory*	*Rogaška Slatina*	*0.04*
15	*Chicory*	*Rogaška Slatina*	*0.08*
16	*Parsley leaf*	*Rogaška Slatina*	*0.11*
17	*Parsley leaf*	*Rogaška Slatina*	*0.2*
18	*Parsley leaf*	*Rogaška Slatina*	*0.44*

ANNEX F:

ZZV CELJE šolarji

VPRAŠALNIK ZA OCENO IZPOSTAVLJENOSTI OTROK SVINCU

Priimek in ime _____ **Datum rojstva:** _____ **Spol:**
M Ž

Natančen naslov (ulica, hišna štev.,kraj)_____

Poklic matere: _____ **Kje je mati zaposlena?**
_____

Poklic očeta: _____ **Kje je oče zaposlen?**
_____

Prosimo navedite, koliko let sta se je šolala mati in oče (na primer, če je kdo zaključil šolanje po 7.razredu osnovne šole, naj napiše **7**. Če ste po osnovni šoli obiskovali še triletno poklicno šolo, napišite **11**).

Število let šolanja matere: _____ **Število let šolanja očeta:** _____

1) Kako bi označili bivališče, v katerem pretežno živite?(obkrožite ustrezen odgovor)

a) Več stanovanjska hiša (5 in več stanovanj)
b) Individualna hiša
c) Več stanovanjska hiša (2-4 stanovanj)

2) Ocenite, koliko je star stanovanjski objekt, v katerem živite, star?(vpišite ustrezen odgovor)

Starost objekta v letih_____

3) Koliko je stanovanjski objekt, v katerem živite, oddaljen od prometne ceste?(vpišite ustrezen odgovor)

Oddaljenost objekta v metrih
_____

4) Iz kakšnega materiala so okenski okviri v stanovanjskem objektu, kjer otrok živi?(obkrožite ustrezen odgovor)

a) leseni
b) kovinski
c) plastični

5) Na kakšen način ogrevate stanovanje?(obkrožite ustrezen odgovor)

S centralno kurjavo:
a) Da, na plin
c) Da, lahko kurilno olje

b) Da, na trdo gorivo (premog, drva)

Klasično ogrevanje:

d) Da, na plin
e) Da, lahko kurilno olje
f) Da, na trdo gorivo (premog, drva)

6) Ali v stanovanju, kjer živite, kdo kadi?(obkrožite ustrezen odgovor)

a) Da
c) Da-občasno
b) Da- vendar potem prezračimo
d) Ne

7) Koliko cigaret se običajno pokadi v stanovanju v enem dnevu?(vpišite število v stanovanju pokajenih cigaret na dan)

_____

8) Ocenite, koliko ur se običajno zadržujete v enem tednu na prostem! (vpišite število ur na teden)

_____

9) Kako pogosto uživate zelenjavo? (obkrožite ustrezen odgovor)

a) Vsak dan
b) 5x tedensko
c) 2-3x tedensko
d) redkeje

_____

_____

_____

_____

Datum izpolnjevanja vprašalnika skrbnika

Podpis staršev ali

_____

_____

Zahvaljujemo se vam za vaše sodelovanje!