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Contamination of *Apodemus flavicolis* in the experimental study area - Ružomberok

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Abstract. The aim of this study was to determine the impact of the paper mill factory MONDI SCP Inc. on the yellow - necked mouse *Apodemus flavicollis*. The level of impact was defined by the levels and temporal changes of the pollutants and their distribution in the bodies of mice. The relation to the sex and locality was also investigated. Samples were collected on the hill Mních, which is significantly polluted due to its close proximity to the factory. Samples were also collected from control area Hrboltová. Subsequently they were examined by the X – ray Spectrometer DELTA. The highest extent of S, Cl, K, Ca, Mn, Cu, Zn, Mo and Pb pollution in liver was detected in spring season, as well as pollution of Fe and Cu with an opposite trend of Rb and Ca. Female liver contained more Mo in relation to the amount of Pb and Zn, whereas the liver of males exhibited proportionally higher amounts of Pb, Zn and lower amounts of Mo. In the control area Hrboltová, our mice tended to contain more Cu and less S, Cl and K, while in Mních it was *vice versa*. Pb prevailed in the kidneys of mice found in Mních while Rb prevailed in the control area. An inverse relationship between Rb, Mo versus Cl was detected in kidneys. Increase of Cl is dominant mainly in spring and autumn season, while its decrease is dominant in summer. The same pattern was detected of an inverse relationship between Rb, Ca versus Fe, Zn. Rb and Ca are absorbed mainly by kidneys of males, whilst Mo is absorbed more by females. Individuals from Mních contained more Fe and K, while animals from the control site Hrboltová contained more Ca and Rb when related to Fe and K.

Key words: *Apodemus flavicolis*, metal contamination, heavy metals, paper mill factory, bioaccumulation, liver, kidney

Introduction

Increase of the human population in the past resulted in formation of the cities, which have brought with them a new way of living. Industry, development of agriculture, increase the traffic, consumption and pollution was resulted. Pollution was visible, but also invisible. Even though people noticed that rivers were no longer pristine and the soil was less rich, some effects remained unnoticed. Air was considered to still be clean and nature still healthy. But recent studies have proved that pollution strongly affects the climate and the world's fauna and flora. The main pollutants include carbon monoxide, ozone, nitrogen and sulfur oxides (www.epa.gov 2015) and heavy metals.

Paper mills are no exceptions. Indeed, they belong to the industries with the highest pollution in the world. Polluting especially fresh water to a great extent. Sulfids, phenols and chlorinated compounds are just a few of the total polluting chemicals (www.novapublishers.com 2015). Paper mill production is a source of heavy metal contamination too (Beauchamp *et al.* 2002).

Paper production includes several other environmental problems such as an extensive deforestation, air and soil pollution or waste production, which represents, for example, in the USA about 40 % of total waste of the country (www.en.wikipedia.org 2015).

Last but not least are affects on the health of the fauna, flora and people. Pollution can enter the body in different ways: through respiration, skin contact, ingestion of food or water in terrestrial animals (www.phac-aspc.gc 2015) and by gills or digestion of sediments in aquatic animals (Muir and Servos 1996). Consequently it may cause pulmonary, skin diseases or diseases of internal organs. These diseases are just a result of important elements missing, whose absorption is obstructed by high levels of heavy metals (Przyslawski *et al.* 1998).

To determine the level of impact bioindicator species are used. However, not every species can serve as a bioindicator and they have to satisfy certain requirements (Holt and Miller 2011).

Mammals are considered to be very suitable for heavy metal analysis because of their higher trophic level, which means that they are able to store more of the pollutants than the organisms of the lower trophic level (Metcheva *et al.* 1996). The issue of heavy metals in the food chain was studied by many authors (Baby *et al.* 2010, Metcheva *et al.* 1996).

Since higher numbers of samples are needed to attain a study, it would be environmentally unsustainable to use bigger mammals which are literally reservoirs of energy which would be lost from the forest. In terms of ecology, economy and efficiency we decided to study small mammals as they are easy to catch and manipulate. Therefore, use of the laboratory mice is so common. But of course, it is necessary to take into account, that laboratory mice are not suitable for many researches, when the free living animals are needed. Their advantage for our research is that they are more stressed than laboratory ones, which means they are more sensitive to toxic elements (Salinska *et al.* 2012, Tete *et al.* 2014).

In addition, a large amount of similar research has already been done. On the hill Mních it is represented mainly by rodents, which are able to accumulate higher amounts of contamination in comparison with other mammals (Martiniakova *et al.* 2010; www.ubm.ro 2015). Due to their small size few pollutants are needed for the mice to be poisoned and for us to be able to record it. The big advantage for them and also for our study is that they have good reproductive potential (www.arkive.org 2015). The most used species of small mammals are bank vole, common vole, common shrew, yellow-necked mouse or wood mouse.

Even though some heavy metals, such as Pb, accumulate the most with rising altitude, which is caused mainly by precipitation and wind (Zechmeister 1995), other studies found the highest concentrations on the lowest sites (Metcheva *et al.* 1995). Therefore, our study site Mních should be suitable.

We chose *Apodemus flavicollis* for its abundance in the study area. They have adequate length of life (about one year), omnivorous diet, intensive metabolism which could increase their sensitivity to pollution (Martiniakova *et al.* 2010) and also small range size in comparison to bigger mammals, thus enabling us to study specific site (www.ubm.ro 2015; Metcheva *et al.* 1995). Another advantage is similarity of their metabolism to the metabolism of a man (Damek-Poprawa and Sawicka-Kapusta 2004; Martiniakova *et al.* 2010).

However, amongst the rodents, their home range is relatively large (Brink *et al.* 2011). They don´ t hibernate (www.arkive.org 2015), which is good since the levels of pollutants tend to be highest during the hibernating period (Klonecki *et al.* 2003). Except when eating seeds they are carnivorous. It was found that carnivorous species can absorb more pollutants than herbivorous ones (Wijnhoven *et al.* 2007).

The yellow-necked mouse was used as a bioindicator in several other studies, often compared with other rodent species. Whilst in some studies it proved to be a more suitable bioindicator than other species in certain parameters, other times it didn´ t. Differences between the species were caused by different ecology, which includes different diet or habitat requirements.

To investigate the contamination levels in the bodies of the yellow-necked mouse we observed different body tissues. We were interested especially in liver, kidneys and bone. Nevertheless we decided to abandon the bone analysis on account of difficulties with achieving a homogenity of the skull samples.

After the findings, which demonstrate that these parts of the bodies accumulate the most pollution, studies have focused on this topic (Atanassov 1998; Arromba de Sousa *et al.* 2013; Lanocha *et al.* 2013). The liver and kidneys are the key organs in detoxification (Lanocha *et al.* 2013) and metabolism (Atanassov 1998). Thus, these parts are very sensitive to environmental changes and therefore they are good indicators (Atanassov 1998).

The fact, that man can not survive without healthy environment and knowledge of connections of natural processes, which are returning us all the pollution we produced since we are on highest trophic level together with other animals, forces us to face this problem.

The objective of this study was to measure an impact of paper factory Mondi SCP Inc. Ružomberok on surrounding environment, which was presented by the accumulation of toxic metals in the liver and kidneys of the yellow-necked mouse (*A. flavicollis*). Considering the data acquired, another aim was to determine the correlation of accumulation rate in the organs and other factors such as locality, sex and season.

Material and Methods

Our research was carried out from January 2015 to April 2015 on the hill Mních, which is situated just few hundred metres from the paper mill MONDI SCP Inc. and thus is considered to be more polluted. Boundary between the factory and Mních forms a river Váh. Less contaminated, while more remote was the study site Hrboltová. Distance between the sites and also from site Hrboltová to source of the pollution is about 8 kilometeres. North and west slopes of Mních are dropping slightly, formed by meadows and mixed forest, south and east are rocky and steep, formed by boulders, rocks and pine forests (www.mineraly.sk 2015).

Mice were captured on south and south - west slopes. The highly developed ability to climb allows *A. flavicolis* to inhabit rocky localities (www.mammal.org.uk 2015). Basically, we could divide the character of the studied slopes into two types. One, south - west, consists predominantly of pine forest and rocks; second, south, is formed by residue of landslide, which consists of rubble, rocks and islands of pine.

Since Mních belongs to Choč's nappe, it consists of limestone as well. This feature is important for the occurrence and maintenance of the pines on this locality. Limestone is an important nutritional resource for the diverse flora which is resulting in high faunal biodiversity. Besides that alkaline substrate improves the health of environmental components and also helps to balance the low pH caused by acidic rains, in association with heavy metals the high level of Ca in the body correlates with decreasing resorption of Pb and Cd (Cibulka *et al.* 1986). Most of the Pb accumulates in the upper 5 cm of soil, where most of the life of mice takes place (Cibulka *et al.* 1986).

In addition to the yellow - necked mouse we can find here also bank vole, which was often found in our traps. That indicates their similar diet habits. According to several studies investigating the correlation of the eating habits with concentrations of pollutants in their bodies, yellow - necked mouse has the most diverse omnivorous diet including seeds, herbs and plants, mosses and insects (Metcheva *et al.* 1996).

Plants, especially above ground reproductive parts, together with seeds, absorb the least of the pollutants and most of them concentrate in the roots (Cibulka *et al.* 1986), which are mostly not available to the mice. In contrast, Pb in skin of fruit contains most of the pollutants as well as mosses which enter their tissue directly. This finding was reported by Beltcheva *et al.* (2011) studying the bioaccumulation in herbivorous snow voles from food. The reason of the higher accumulation is lack of cuticle (Cibulka *et al.* 1986), which serve as a protective layer. That means that mosses are very sensitive to contamination and thus serve as good bioindicators. However, despite the fact that plants do not absorb as much of the pollutants, they remain on the surface. Insects are responsible for increase of the proteins in bodies of mice, which is another factor correlating with Pb to resorbtion by organisms (Cibulka *et al.* 1986). Therefore, insectivores proved to be able to accumulate the highest concentrations of heavy metals, omnivorous species lower and herbivours the least concentrations (Baby *et al.* 2010; Metcheva *et al.* 1998).

Three different types of traps were used for capture: Sherman aluminium traps, wooden box traps and snap traps. They were used approximately in ratio of 3:2:1 = 80:40:18. Originally only the common mouse traps were used, but our study was combined with another study investigating hematological parameters in yellow - necked mouse using two other types of the traps. For their study the living mice were needed, whereas for our study the dead ones were needed. Therefore, in the event of death of mice captured by their traps we were provided the corpses.

The most frequently used traps were Sherman's traps both for the reliability when set and for their ability to be folded. The second most reliable were the wooden box traps, of which we had only 18. The least reliable and least successful were the snap traps, because it was very difficult to set them correctly. It was caused mainly by the abrasion of the traps after several uses as twisted metal parts. It happened often, when we checked the traps in the morning, that either the bait was gone, without the mouse or trap was snapped, but without any trace of mouse. We baited them with a piece of apple, walnut and oatmeal which was the most delicious for them. The traps were set near the rocks, roots, mouse holes or other places with the evident mouse traces. Distance between them was approximately 10 to 15 meters. To cover quite wide home range of the mice, which is according to available sources about 0,5 ha (www.mammal.org.uk), 40 common mouse traps were needed. We set the traps each time right before sunset and collected them right after sunrise, since the mice are nocturnal (www.mammal.org.uk 2015). The lowest numbers of mice caught were during the snowing or freezing below -5 °C. Highest numbers were observed during the warmer nights, especially from March. Some of the trapped mice were eaten by some predator present in the study area. It could be a cat, common buzzard or fox from the predators we had a chance to see, or any other carnivore hunting mice. The presence of the carnivores was obvious from the many burrows we found. Dead mice were collected, put in plastic bags separately, properly marked and put in the freezer. When all the mice were caught and we were finally prepared for their detailed examination we defrosted and subsequently measured them. We measured the length of the hindpaw, ear, body (from nose to anus) and tail (if not cut). Mice were of different ages, which allowed us to examine the patterns of accumulation related to age. The high accumulation in younger mice unlike older ones was shown. This finding refers to increase of metabolic rate in young mice (Blagojević *et al.* 2012). The age was determined on the basis of the length of the hindpaw. Gender was determined either on basis of external characteristics or internal characteristics after dissection. Dissection was carried out in sterile laboratory conditions. For cutting we used the scalpel and scissors with the help of tweezers. Before the cutting we fixed a dead mouse on a dorsal side with four pins, two for front paws and two for hind ones on a polystyrene square.

We cut the abdomen from sternum to genitals and from sternum to front paws. After opening the abdomen we removed the liver and kidneys and additionally checked the reproductive organs to determine the gender. We placed the liver and kidneys to petri dishes and put them in the drier TCH 100 at a temperature of 53 °C for 13 hours. Afterward we homogenized the dried samples with mortar to powder. Powders of liver and kidneys were placed separately. Homogenizing of the samples is a common technique used in many studies.

For the analysis of elements present in samples we used ED - XRF Spectrometer DELTA MA USA - Waltham device. The device was connected to a fully shielded portable workstation. Before the measurements the calibration was needed. We used the CONOSTAN calibration standard. The calibration correlation coefficient was 0.999833. Calibration error was 0.9 mg/kg. We put each sample in the cuvette which had 5 μm polycarbonate film on the bottom. We used multiple - beam measurement, in which every measurement consisted of 3 beams for 30 seconds, repeated three times, and then averaged. The following elements were detected: P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Zr, Mo, Ag, Cd, Sn, Sb, Ba, Hg and Pb. The results were given in PPM (part per milion) units. Data of all individuals such as number of individual, day, month, locality, sex, values of elements and length of the ear, tail, body and hind paw were collected in a matrix with information necessary for statistics. Measurement results were then processed in STATISTICA 8 including only the average data. Some elements which were under the limit of detection we didn't take into account. Others were standardized and analyzed by using principal component analysis based on determination of factor scores which identify the correlation between the individual factors and occurrence of elements. Basically, this method converts possibly correlated variables to uncorrelated variables (Holcová and Maslowská, 1994). Variance was determined using eigenvalues and eigenvectors. Functionality of factor scores was determined by ANOVA analysis of variance. Values of $p < 0.05$ were the most interesting for us. In the statistics we included also data from (Hadidová 2014).

Results

Röntgen analysis exhibited different representation of each element. We sorted them by rate of representation to four groups. First group contains no detected elements in any of studied individuals. Ele-

	Eigen vectors of correlation matrix					
Variable	**Factor 1**	**Factor 2**	**Factor 3**	**Factor 4**	**Factor 5**	**Factor 6**
S	-0.382	0.045	-0.168	-0.315	0.179	0.029
Cl	-0.380	0.216	-0.140	0.106	-0.012	0.138
K	-0.409	-0.007	-0.240	-0.064	0.005	0.072
Ca	-0.286	-0.381	-0.118	-0.348	-0.373	0.059
Mn	-0.399	-0.096	-0.255	0.008	0.050	0.056
Fe	-0.109	0.477	0.377	-0.408	0.488	0.116
Cu	-0.254	0.478	-0.079	0.289	-0.091	-0.088
Zn	-0.238	0.253	0.470	-0.081	-0.601	-0.429
Rb	-0.185	-0.428	0.529	-0.245	0.141	0.064
Mo	-0.260	-0.125	0.404	0.569	-0.029	0.547
Pb	-0.257	-0.278	0.061	0.352	0.444	-0.668

Table 1. Eigenvectors for the first six principal components (factors) from the standardized correlation matrix of measured elements in liver.

Variable	N	Mean score*	SD
January	11	-0.14 a, b	0.27
February	5	-0.39 a, b	0.4
March	16	0.62 b	0.26
May-July	9	-0.96 a	0.4
October	12	0.14 a, b	0.37

Table 2. Synergic variation of S, Cl, K, Ca, Mn, Cu, Zn, Mo and Pb in the liver of the yellow-necked mice in dependence on seasons. The variation is described by the mean prinicipal component scores and their standard deviations (SD). *Different letter indices (a, b) denote statistically significant differences at Tukey multiple-range test ($p<0.05$).

Variable	N	Mean score*	SD
January	11	-0.14 a, b	0.27
February	5	-0.39 a, b	0.4
March	16	0.62 b	0.26
May-July	9	-0.96 a	0.4
October	12	0.14 a, b	0.37

Table 3. Means Factor 2 scores (and SD) desribing the different pattern of Rb, Ca and Fe, Cu accumulation in the liver of *A. flavicollis*. *Different letter indices (a, b) denote statistically significant differences at Tukey multiple-range test ($p<0.05$).

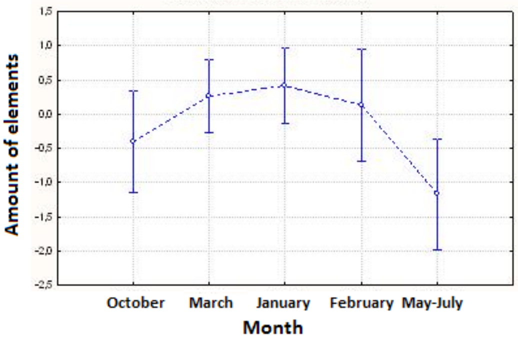

Fig. 1. The effects of mutual increase or decrease of S, Cl, K, Ca, Mn, Cu, Zn, Mo and Pb in the liver of *A. flavicollis* in different seasons (F (4,34)=3.31, p=0.02).

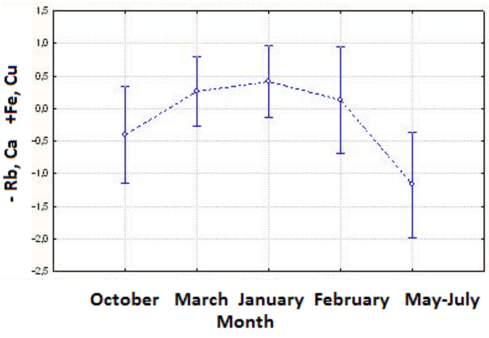

Fig. 2. The effects of mutual increase or decrease of Rb, Ca and Fe, Cu in the liver of *A. flavicollis* in different seasons (F (4,34)=3.31, p=0.02).

ments found in liver were: Ti, Co, Ni, As, Sr, Zr, Ag, Cd, Sn, Ba, Hg. Elements found in kidneys were: Ti, Co, As, Se, Sr, Zr, Ag, Cd, Sn, Hg. Second group contains elements detected only in few individuals. In liver, they were: P, Cr, Se, Sb. In kidneys, they were: Ni, Se, Sb, Ba. Third group contains elements detected in most of the individuals. In liver, it was Cu whereas in kidneys they were: P, Cr, Cu, Pb. Forth group contains elements detected in all individuals. In liver they were: S, Cl, K, Ca, Mn, Fe, Zn, Rb, Mo, Pb. In kidneys they were: S, Cl, K, Ca, Mn, Fe, Zn, Rb, Mo. These elements were statistically analyzed.

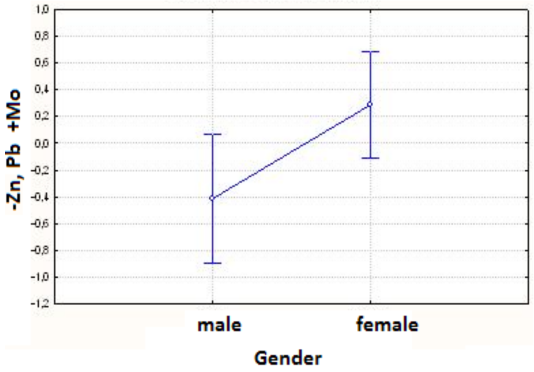

Fig. 3. The effects of mutual increase or decrease of Zn, Pb and Mo in the liver of *A. flavicollis* related to sex (F (1,37)=5.23, p=0.02).

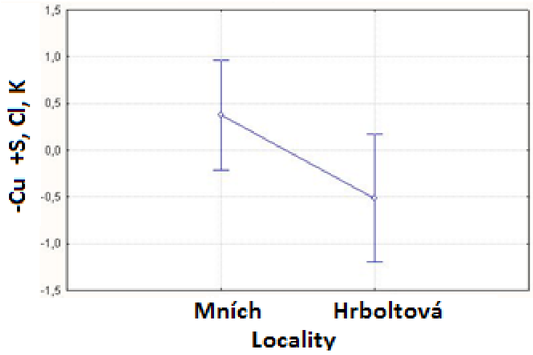

Fig. 4. At the locality Mnich liver of *A. flavicollis* contained more Cl, S, K related to Cu than at the control area (F (1,17)=4.35, p=0.05).

Eigen vectors of correlation matrix

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
S	-0.323	0.403	0.210	-0.009	0.137	-0.145
Cl	-0.028	0.630	-0.101	0.029	-0.052	0.193
K	0.056	0.621	-0.141	0.026	-0.069	0.167
Ca	-0.390	-0.026	0.238	-0.158	-0.317	0.104
Mn	-0.434	-0.010	0.217	-0.032	0.105	-0.128
Fe	-0.340	0.020	0.431	0.186	0.259	-0.222
Cu	-0.338	-0.216	-0.277	0.160	0.187	0.689
Zn	-0.421	-0.053	-0.213	0.200	0.029	0.168
Rb	-0.213	-0.021	-0.419	0.541	-0.481	-0.440
Mo	-0.128	0.042	-0.536	-0.226	0.620	-0.368
Pb	-0.288	-0.038	-0.227	-0.727	-0.378	-0.074

Table 4. Eigenvectors for the first six principal components (factors) from the standardized correlation matrix of measured elements in liver.

Multivariate analysis - liver

In this statistics we combined data published by Hadidová (2014) together with our own measurements. Thus, they create more complex and accurate results of correlation of pollutants and month/sex. We focused on six most significant factors listed in Table 1.

Seasonal effects

Factor 1 summarized approximately 47% of total variance and describes the mutual variation of following elements: S, Cl, K, Ca, Mn, Cu, Zn, Mo and Pb. The amount of these elements in the livers mainly increased in the spring season (Table 2, Fig. 1). Factor 2 describes the bipolar trend between elements: Rb, Ca and Fe, Cu. In winter, Fe, Cu increased in relation to Rb, Ca, in summer decreased (Table 3, Fig. 2).

Difference between sexes

Under the Factor 6, the significant relation between the sex of animals and bipolar Pb, Zn versus Mo was found. While female liver contained more Mo in relation to the amount of Pb and Zn, liver of males exhibited proportionally higher amounts of Pb, Zn and lower amounts of Mo (Fig. 3).

Local effects – experimental versus control area

In this statistics we used only data from our own research. Data from locality Mních and control locality Hrboltová were compared. The principal components matrix was calculated (Table 4). We focused on six most significant factors. Only factor 2 and factor 4 exhibited $p < 0.05$.

Sulfur and chlorus at studied locality (Factor 2)

In Hrboltová, mice tend to contain more Cu and less S, Cl and K in their livers than in Mních (Fig. 4).

Lead and Rubidium (Factor 4)

An inverse relationship between elements Pb and Rb is described by factor 4. Pb prevailed in experimental control area while Rb in experimental area (Fig. 5).

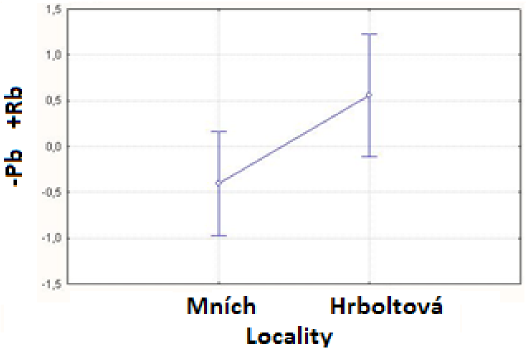

Fig. 5. At the locality Mnich liver of *A. flavicollis* contained more Rb related to Pb than at the control area Hrboltová (F (1,17)=5.4, p=0.03).

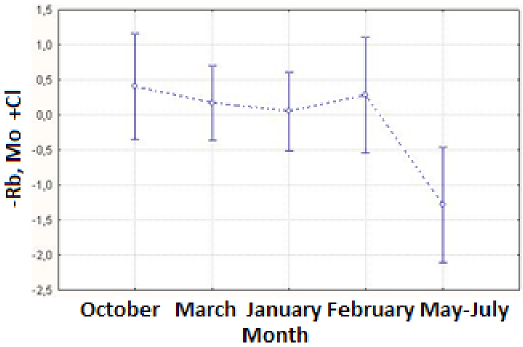

Fig. 6. The effects of mutual increase or decrease of Rb, Mo and inverse variation of Cl in the kidneys of *A. flavicollis* in different seasons (F (4, 34)=3.05, p=0.02).

Eigen vectors of correlation matrix						
Variable	**Factor 1**	**Factor 2**	**Factor 3**	**Factor 4**	**Factor 5**	**Factor 6**
P	-0.345	0.231	-0.261	0.024	0.189	0.083
S	-0.365	0.243	-0.063	-0.139	0.113	-0.115
Cl	-0.329	0.377	0.035	-0.185	0.161	0.021
K	-0.363	0.228	-0.116	-0.128	0.018	0.016
Ca	-0.235	-0.259	-0.507	0.407	-0.496	0.096
Cr	-0.341	-0.132	-0.019	0.098	-0.262	-0.379
Mn	-0.370	-0.125	-0.053	-0.026	-0.002	0.075
Fe	-0.254	-0.182	0.576	-0.214	-0.353	-0.405
Zn	-0.290	-0.290	0.366	-0.089	-0.067	0.781
Rb	0.011	-0.535	-0.411	-0.702	0.167	-0.087
Mo	-0.226	-0.436	0.138	0.455	0.673	-0.199

Table 5. Eigenvectors for the first six pricipal components from the standardized correlation matrix of measured elements in kidneys.

Variable	N	Mean score*	SD
January	11	0.04 a, b	0.27
February	5	0.29 a, b	0.41
March	16	0.16 a	0.26
May-July	9	-1.28 b	0.41
October	12	0.41 a	0.37

Table 6. Means Factor 2 scores (and SD) desribing the different pattern of Rb, Ca and Fe, Cu accumulation in the liver of *A. flavicollis*. Different letter indices (a, b) denote statistically significant differences at Tukey multiple-range test ($p < 0.05$).

Variable	N	Mean score*	SD
January	11	0.34 a, b	0.26
February	5	-0.36 a	0.38
March	16	0.48 a, b	0.25
May-July	9	-1.29 b	0.38
October	12	-0.21 a	0.35

Table 7. Means Factor 3 scores (and SD) describing the different pattern of Rb, Ca and Fe, Zn accumulation in the kidneys of *A. flavicollis* in different seasons. Different letter indices (a, b) denote statistically significant differences at Tukey multiple-range test ($p < 0.05$).

Multivariate analysis - kidneys

The eigenvectors of first six factors are in Table 5. We used combination of our data and from Hadidová (2014).

Cl versus Rb, Mo accumulation (Factor 2)

This component describes the inverse relationship between Cl and Rb, Mo accumulation. It was evident, the relative proportion of Cl versus Rb, Mo decreased in summer (Table 6, Fig. 6).

Rb, Ca versus Fe, Zn in different seasons

Factor 3 describes an inverse relation between Rb, Ca and Fe, Zn. This bipolar vector divided seasonal pattern of accumulation to spring and autumn versus summer season (Fig. 7).

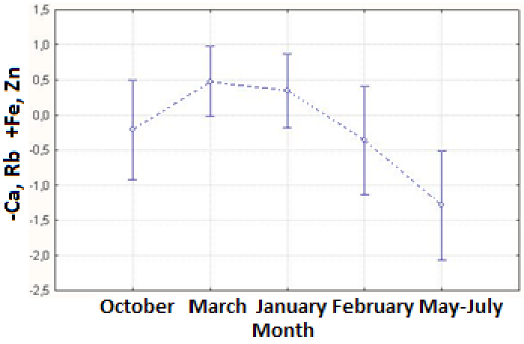

Fig. 7. The effects of inverse variation of Ca, Rb verus Fe, Zn in the kidneys of *A. flavicollis* in different seasons (F (4,34)=4.54, p=0.004).

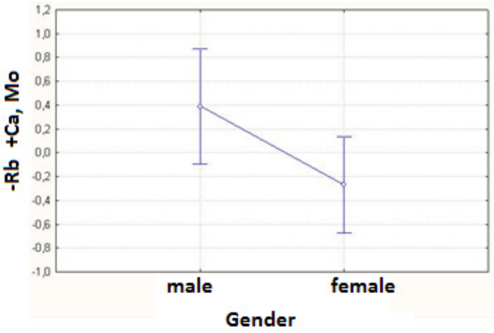

Fig. 8. The effects of mutual increase or decrease of Rb and Ca, Mo in the kidneys of *A. flavicollis* related to sex (F (4,34)=4.54 p=0.004).

Eigen vectors of correlation matrix

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
S	-0.426	0.178	0.228	0.108	0.073	-0.010
Cl	-0.439	0.027	-0.234	0.175	-0.300	-0.148
K	-0.405	0.298	-0.036	0.157	-0.364	0.041
Ca	-0.249	-0.533	0.196	-0.342	0.077	-0.313
Mn	-0.280	0.129	0.435	0.128	0.678	-0.093
Fe	-0.127	0.348	0.264	-0.659	-0.289	-0.322
Cu	-0.416	0.081	-0.273	0.139	0.183	0.116
Zn	-0.287	-0.187	-0.172	-0.508	0.105	0.719
Rb	-0.219	-0.640	0.028	0.199	-0.204	-0.179
Pb	0.039	-0.085	0.700	0.218	-0.371	0.451

Table 8. Eigenvectors for the first six pricipal components (factors) from the standardized correlation matrix of measured elements in kidneys.

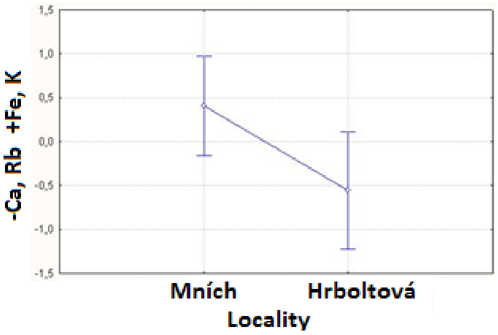

Fig. 9. At the locality Mnich kidneys of *A. flavicollis* contained more Fe, K related to Ca, Rb than at the control area (F (1,37) = 4.48, p=0.04).

The amount of Rb proportionally increased in summer accompanied with Ca (Table 7).

Difference between sexes

Factor 4 exhibited an inverse relationship of accumulation of Rb and Ca, Mo in the mouse kidneys. Male kindney contained relatively higher amount of Ca, Mo when related to Rb than female kidneys (Fig.8).

Experimental and control study area

In this statistic we used only data from our own research. Data from Mních study site and control site Hrboltová were compared (Table 8). We focused on the six most important factors but only factor 2 has p < 0,05. Factor 2 describes an inverse relation between Ca, Rb and Fe, K. Individuals from Mních contained more Fe, K versus Ca, Rb in the kidneys while the animals from control area contained more Ca, Rb when related to Fe, K (Fig. 9).

Discussion

Essential elements as S, K, Ca, Mn or Rb were represented both in liver and kidneys in highest extent, trace elements as Ni or Pb in lower extent or not detected at all, for example Cd, Hg or Se. It´ s because of determination of detection limits of these elements, which wasn´ t exceed. The most significant differences between liver and kidneys was found in lower level of P and Cr in liver than in kidneys and lower level of Pb in kidneys than in liver. Compared to other similar studies, our results of Pb in kidneys varied from 11 to 23 mg/kg, whilst in another study (Metcheva *et al.* 1996) they found only 5 mg/kg both in kidneys of yellow-necked mouse and common shrew, which might contain more because of his higher trophic level (Metcheva *et al.* 1996). Similarly, we found Cr levels to be from 16-79 mg/kg, but other study (Metcheva *et al.* 1998) found only about 16 mg/kg.

In our study we observed several significant factors indicating pollution. Different pattern of accumulation of elements in liver correlated with season, locality and sex. Increase of elements in liver as S, Cl, K, Ca, Mn, Cu, Zn, Mo and Pb in spring months against decrease of these elements in summer and increase a little in autumn probably because of long exposure of food confirm the fact, that pollutants cumulate in winter and spring (Klonecki *et al.* 2003).

Regression of Zn and Mo is related to their antagonistic interaction, when Mo helps to utilise Zn. Thus, when Mo is low and Zn high, organism fails in utilization of Zn (www.blog.garymoller.com 2015). This pattern was exhibited by males. On the other hand, females exhibited the opposite pattern. The same factor showed a proportional increase/decrease of Pb and Zn. According to (Salinska *et al.* 2012) Zn is present to protect the liver from Pb. Mo deficiency tends to occur in acidic environment (Weir 1984). In our research the trend occurred in males.

Dependence on study area was shown in two factors. First factor exhibited increase of S, Cl, K and decrease of Cu on a site Mních against the opposite trend on a site Hrboltová. Presence of S can cause decrease of Cu and that can lead to several diseases (Zhou *et al.* 2009). Acidic S grew proportionally with alkaline K, which is an opposite character (www.naei.defra.gov.uk 2015). Despite, K induced decrease of Cu. Most of the K originate from using fertilizers, which could be likely in an agricultural surrounding (www.euwfd.com 2015).

Second factor exhibited increase of heavy metal Pb synchronously with decrease of important trace element Rb on a Mních site. Deficiency of Rb cause diseases (Lombeck *et al.* 1980). Hrboltová proved to be less contaminated with opposite trend.

In kidney analysis dependence of season, locality and sex was investigated as well. Level of contamination related to season was found in two factors. In first, increase of Cl at the expense of Rb and Mo on a Mních site. High extent of Cl may end up in kidney damage (Cuthbertson *et al.*, 1945) and in our study was detected in spring and autumn season. Conversely, deficiency of Cl may lead to reduced growth (Cuthbertson and Greenberg 1945). Getting enough of Ca is essential for normal growth (Thacker, 1943) detected increase of Ca when shortage of Cl occured. This finding is opposite to ours. In second, increase of Zn together with Pb and decrease of Ca and Rb on Mních site during spring and autumn was detected. Very strong correlations were detected between Zn and Ca (Alonso *et al.* 2004). Shortage of Ca can results in renal diseases (Lewis 2013). Correlation between Fe and Ca is due to Ca inhibiting Fe absorbtion (Lynch 2000). Our results exhibited increase of Fe with decrease of Rb. Findings of Yokoi *et al.* (1996) refer to different trend, when Fe increased together with Rb. They also found higher Rb when lower Ca, while we found antagonistic reaction between them. However they confirmed in their study our antagonistic Zn and Rb. Dependence on sex was detected in one factor. Males exhibited higher levels of Ca and Mo, lower levels of Rb, while females followed opposite pattern (Noggle *et al.* 1964) refer to higher uptake of Rb by roots than uptake of Ca. Similar pattern could be thus characteristic also for rodents.

Last significant factor detected in kidneys depended on study site. Mních exhibited increase of Fe and K against decrease of Ca and Rb. It´s interesting, that in this factor Ca and Rb act totally different than in previous factor and increase/decrease proportionally. Ca and Fe pattern again confirm the finding of Lynch (2000). Low Ca could be affected by high utility of fertilizers. (www.pthorticulture.com 2015). Low Rb related to high K can again relate to higher utility of fertilizers, which have impact on deficiency of Rb and consequent diseases (Lombeck *et al.* 1980). Even though, that we provided quite high amount of biological material, we still have to think objectively, because there is a lot of factors around influencing us all the time. I would suggest to collect data at least from four sites orientate on east, west, north and south to be able to detect impact of wind distributing pollutants in several directions and also control site to each one of these four remoted from the factory. Slopes of Mních itself can cumulate pollutants differently in association with orientation (Fritsch *et al.* 2011) and soil type or hydrological changes (Metcheva *et al.* 2003). We decided to investigate soft tissues of yellow-necked mouse. But it has one big disadvantage, which is actually killing the individuals and thus disruption of ecosystem´s balance of Mních study site.

Some studies already ran less harming methods of investigating pollutants from feces or bone tissue (Beltcheva *et al.* 2011). Bone tissue shown to be even more reliable in study of (Ward *et al.* 1978), when sheeps grazing near highway shown significant increase of Pb in liver, kidneys and bones. Although, after the return to natural pasture levels in liver and kidneys returned to normal, bones remained unchanged. Bones are able to store informations about pollutants levels from past (Martiniaková *et al.* 2010) and we can collect them without doing any harm. However, bones cumulate the pollutants slowly and can tell us more about long exposure than about the acute poisoning of the animals (Cibuľka *et al.* 1986).

Killing the animals, if any, should be at least followed by use in greater extent than only for liver and kidneys. Our proposition is to use more organs and bones. There is also some researches studying for example transfer of pollutants from fetus to mother through placenta which could be also used in case of capture of pregnant mouse (Cibuľka *et al.* 1986) found a Cd and Pb to inhibit trasition of Zn from milk to mother and (Bukovjan *et al.* 1993) that these heavy metals negatively affect a fetus continuously during the pregnancy, mainly in first phase of pregnancy and later to placenta and cause different diseases.

Another important informations to consider are age and sex (Cibuľka *et al.* 1986) claims that females cumulate more heavy metals than males and young mice 4 to 100 times more of Cd than adults. These levels then increase with age what could be due to reduced ability to deal with pollutants. Above all (Beamer *et al.* 2001) stated genetic to be more important than environmental factors (40-93%). Adaptation of liver to polluted environment, possible due to stress hormones (Baby *et al.* 2010) is another possible factor which can cause unthrustworthiness of the results. All of these suggestions concern investigation of impact of pollution which already exist. If our goal is to maintain the healthy environment and not only to observe and investigate, we could also consider an option of using zeolites (Beltcheva *et al.* 2011) or plants (Baby *et al.* 2010) to eliminate pollutants.

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