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Postnatal development and metal contamination of nestlings of Great tit (*Parus major*) from experimental study area - Ružomberok

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Abstract. We examined the appearance of trace metals from three body parts - liver, muscle and bone of 46 juveniles of Great tit (*Parus major*, Linnaeus 1758) collected at the study area Minch, Ružomberok nearby the pollution source. Our aim was to determine the metal accumulation level, distribution among body parts and age related variations. Bird sampling was conducted in May and June 2012 and 2013. Due to lack quantity of early - age samples, we were unable to do intended comparison with research from the reference area in High Tatra in 1996. In view of frequency and abundance of measured elements, the major pollutants are considered as follows: Pb, Rb, Sr, Sr, and Ni. The others have emerged in a larger extend and are perceived as essentials: P, K, Cl, S, Ca, Fe, and Zn. A remarkable trend was observed in decreasing concentration of most heavy metals in relation to increasing age. This phenomenon may be explained as the result of exogenous environmental influences - less contaminated food items in a greener spring. As well as the metabolic activity of young organism to deal with a certain level of exposure to pollutants. Beyond one abandoned brood and two dead nestlings at an early stage of development, we did not notice any visible abnormalities.

Key words: bioindicator, *Parus major*, trace metals, metal contamination, pollution, pulp mill Mondi Ružomberok

Introduction

Air pollutants have constantly been leaked into ecosystems as a consequence of urbanisation and industrial processes. Heavy metals are the most dangerous inorganic toxic substances, which have the particular concern due to the long-lasting effect, possibly irreversible direct or indirect effects.

During the last few decades, these genuine worldwide threats of these emissions have attracted the attention of many investigators. The aim of our study was to measure the metal contamination of surrounding of the pulp mill in Ružomberok town. The factory complex is the biggest integrated mill in Slovak Republic (www.mondigroup.com) and moreover, the main source of local pollution in Žilina region. The pulp and paper industry is known for the emission of non-condensed malodorous sulphurous de-gasses, which are considered to be a potential threats for local citizens and their environment. Nevertheless, the available information concerning the effects of these substances is still blurred (Costa *et al.* 2012). The evaluation of pollution effects on wildlife provides biologically meaningful information concerning environmental quality and possible impacts on population and can be used as an early warning of environmental change. As many other researchers, we used biological method with the help of bioindicator-organism, which gives adequate information on the environmental quality (Varsha and Prakash 2012).

Small insectivorous passerines are considered to be good candidates for such studies in terrestrial ecosystems. In addition, being ubiquitous, high in the food chain and due to their high metabolic rate (Morrison 1986; Root 1990). Hence, we considered Great tit (*P. major*) an appropriate species for our experimental study.

Out of a broad spectrum of pollutants emitted by pulp mill, we have focused on the investigation of Pb, Rb, Cr, Cd, Ni, Se and Hg as main pollution elements to observe possible detrimental effects on small juveniles of Great tit (*P. major*).

The aim of our research was to determine the concentration (1) and distribution pattern of some heavy metals and their effects on the metabolism of essential elements in liver, muscle and bones of Great tit *(P. major*) juveniles in the study area nearby the pollution source (2). The other objectives were to find out whether the accumulation of heavy metals depends on increasing age (3) and compare our measured Pb amounts with the samples of unencumbered high-mountain area from previous research conducted in 1996 in High Tatra (4).

Material and Methods

Source of pollution - MONDI Business Paper SCP A.S., Ružomberok

The main source of the air pollutants in our study area is the factory complex Mondi Business Paper SCP A.S., Ružomberok with main production

474.000 tons of pulp and 535.000 tons of uncoated paper per year. On that account, Mondi is the biggest pollution source in the region and integrated mill producing paper and pulp in Slovak Republic (http: //www.mondigroup.com).

The production process is based on kraft pulping. The effective substances are principally sodium hydroxide (NaOH) with the addition of sodium sulphide (Na_2S) (Drímal *et al.* 2006). During the boil of organic material accrue non-condensed de-gasses (approximately 19 main components) from which are in the main position particularly chlorine (Cl) and chlorine dioxide (ClO_2). The main source of the malodorous substances are the compounds of sulphur namely hydrogen sulphide (H_2S), dimethyl disulphide ($C_2H_6S_2$), dimethyl sulphide $(CH_3)_2S$ and methylmercaptan (CH_3SH) released during the processes of boiling, bleaching, pulp washing and chemical regeneration (Drímal *et al.* 2006).

In 1992, the factory produced 7470 tone/year of solid emission and 11 827 tone/year of vapour emission whereby took part in 80 - 85 % of global air pollution. Then years later, the modernization of the pulp mill has been occurred. The ozone beaching partly replaced the old technological method and the peroxide started to apply and in addition, new regenerative kettle and system of harvest and disposal of the malodorous substance (H_2S) were built up (Sršeň *et al.* 1992). Since then, the total dimension of pollutants has rapidly decreased. Respective atmospheric releases of metals in 2004 and 2008 were: PM_{10}, $PM_{2.5}$: 162.29 tone/year (2004), 73.69 tone/year (2008); SO_2: 1093.14 tone/year (2004), 100.65 tone/year (2008). NO_x: 892.65 tone/year (2004), 851 tone/year (2008). However the increase of CO has appeared: 67.3 tone/year (2004) to 1586 tone/year (2008) (Ministerstvo životného prostredia Slovenskej republiky *et al.* 2009). In 2008, the same values were indicated as usual averages in other parts of Slovakia. Additionally, RÚVZ (Regional Institute of Public Health) in Banská Bystrica measured the project with the results where the concentrations of the malodorous substances had no negative impact on the health of citizens (Drímal *et al.* 2006). Nevertheless, all above mentioned substances are considered to be harmful to environment, people health and wildlife during the processes of boiling, bleaching, washing of pulp and also regeneration of chemicals. Though, the available information concerning the effects of these pollutants is still uncertain. Considering the well-known hazardous effect of these elements on the environment, the regular monitoring of the study area is essential.

Study species - Great tit (P. major)

Great tit (*P. major*) is a small, (ca. 19 g, 14 cm) short-lived, eurytopic passerine bird that is resistant in our experimental area. This species is polyphagous, primarily insectivore giving preference to pests, their larvae, mostly caterpillars. Among them, noctuids and geometrids, which live in the tree foliage, are commanding as well as spiders, dipterans and lycosids. In the winter time they look for pupas and eggs of insects lying on the branches (Vilček 1984). During breeding, females are known to search for calcium-rich food items such as snail shells, which are needed for eggshell formation and skeletal growth (Graveland and Berends 1997). Sawicka-Kapusta *et al.* (1986) and Scheuhammer (1987) concluded that it is precisely food selection in the susceptibility to heavy metals. Moreover, they do not reject any oleaginous seeds or are being occurred to human facilities to feed up (Vilček 1984). The breeding season lasts from the March to the August with nestling period in the second and third decade of April. According to conditions of environment, it might breed twice a year. The size of brood is about 6-12 white eggs with rusty spots. The incubation time is approximately 13-14 days, until the nestlings hatch. Then both parents feed their offspring as long as they fledge and fly out of the nest. Even then, they keep at feeding next few days (Vilček 1984).

Study area - Minch

In May and June of 2012 (11.5 - 22.5) and 2013 (22.5 - 18.6) we conducted sampling of small Great tit (*P. major*) juveniles in a heterogeneous woodland area in the north Slovakia (49° 05' 03.14" N, 19° 19' 04.81" E). The study area (Minch, 657 m) was situated cca. 2 km north-east of base-rich expected source of acidification, the pulp mill Mondi SCP A.S. Ružomberok.

The geological base of the study area is compounded of carbonate conglomerates where the rich fauna of nummulites occurs. The numulite lime stones lie on triassic lime stones and dolomites. The western side of the area is formed by Borov formation of breccia, clay stones and sandstones. The soil types are represented by rendzina leptosoils from weathering products of solid carbonate rocks and partly calcareous fluvisoils from carbonate alluvial sediments. These indicate the existence of plentiful calcium storage (www.mineraly.sk; www.referaty.atlas.sk; www.globus.sazp.sk). Prevailing wind is especially north-western. Due to the closed valley and industrial production in town, the location is included at the top of the rankings, where is a long lasting fog (www.globus.sazp.sk). The site is located at the interface moderately cold and wet and temperate climate zone. A precipitation ranges from 720 mm to 770 mm. The average temperature in the coldest month (January) varies <-3 ° C, warmest (July) <16 ° C (www.globus.sazp.sk).

Bird sampling

The twenty-five wooden nest-boxes were hung on tree trunks 2.5 - 4 m above ground level, spaced from each about 50 meters, in area of about 2 km_2. An entrance of cca. 30 - 35 mm in diameter, was oriented to the south. Each box was colourfully and numerically differentiated to identify the brood. The data was carried out periodically in 2-3 day intervals in the morning up to afternoon, since the whole brood hatched, till the fledglings left the nest. In the selection of particular sample was taken into account the increasing size and approximate age of chicks. Each bird was euthanized after capture and individually marked, stored in polyethylene bags and transferred to the laboratory for analysis. We obtained 54 samples in total, collected on 12 successful first clutches (n=30 in 2012 from 6 clutches and n=24 in 2013 from 6 clutches as well). One brood of 10 eggs was found abandoned. Two individuals of the same clutch were found dead and eventually omitted from the study.

Sample preparation

Before dissection the chicks were stored in a freezer at -18° C. Frozen samples were somatometrical measured by ruler to determine an approximate age by comparing wing, tarsal and nib length with a growth curve of know-age nestlings. All measured data were recorded to the Excel table. Then samples were partially thawed. The respective individual was chosen from polyethylene bag, put on the dorsal side and fixed by pins on polystyrene surface. For better visibility, if it was required, the initial feathering was manually removed. Skin incision was carefully accomplished to a vertical section using the surgical scalpel and scissors. Incision was conducted from pharynx to cloaca, perpendicular to the surface of the chest. The skin was then pinned on the surface, and thus opened up the interior of the ventral side. Thereafter, the liver, part of the pectoral or femoral muscle and tibia-tarsus (in case smaller samples) or sternum (furcula) (in case of bigger samples) were dissected out. A whole heart and gizzards were collected separately and stored in diluted ethanol for DNA tests, independent of this study. Preparations had to be made very carefully under clean conditions and without contamination from instruments. Operations were carried out on all 54 samples. Three of them had decomposed liver, one entire entrails. Four nestlings (2-3 day old) were not opened because of their small size and the possibility of disruption of internal organs. Two of them were found dead. The thin layer of skin above the skull of these individuals was removed due to detection of heavy metals. Three more samples with those mentioned above were excluded because of a different method of X-ray to make data more homogenous and comparable.

The 138 body tissues of 46 nestlings were not homogenized, but directly dried in an oven 100 TCH at a constant temperature 60° C for approximately 13 hours. At the end of this time, we took them and their volumes were measured to calculate the concentrations of heavy metals. The elements in the samples were determined and measured their amount by ED-XRF Spectrometer DELTA, MA USA - Waltham based on energy-dispersion X-ray fluorescence spectrometry (EDXRF). The Spectrometer works on the principle of three components. The first is the source of excitation - X-ray tube emitting a light beam through the sample material, the detector receiving information on the identified elements and the basic unit of data collection. The calibration was made at the bottom of 5μm polycarbonate film of the cuvettes. The actual measurement of the dried tissue was carried out in a closed protective box of ED-XRF Spectrometer DELTA taking cca. 90 seconds with three repeated measurements taking 30 seconds and by averaging values obtained with a standard deviation. In the process of sample X-raying, we used the light mode matrix with multiple beam measurement to achieve very good detection limits - Soil Environmental mode with the identification of the following heavy metals: P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Zr, Mo, Ag, Cd , Sn, Sb, Ba, Pb and Hg. The metal concentrations in the different samples were determined in PPM (μg/g for dry weight).

Statistical analyse

To create the basic matrix we used PCs program Microsoft Excel. The spread sheet was created to gather the following data: the number and GPS coordinates of nest-boxes, date of sampling; wing, leg and nib length, age, note, individual organs - liver, muscle and bone with detection of heavy metals. Then we divided nestlings according to age on age classes: 8 (n=15), 12 (n=19), 16 (n=10).

For statistical analyse and processing of input quantitative data, we used a PC program called STATISTICA 8.0. The basic matrix of elements measured in the samples was standardized with the possible eccentricity. The correlation matrix followed by Tukey multiple comparisons were performed describing the different trends among the original variables. Method of principal components was used to identify the detection of effects of possible factors for the occurrence of elements in the samples. We took into account the first 11 of all 36 factors (till total variance 2.14%). Consequently, we calculated a factor scores for each bird associated with an age group. Score of three age groups was then tested by one-way ANOVA. The differences were accepted as significance $p < 0.05$.

Results

No detectable elements

Elements which demonstrated low incidence in the examined tissues, those were found only in specific individuals or tissues, or those were not detected at all, we did not include in statistical evaluation. These are the metals As, Zr, Sn, Co, Ag, Cd, and Hg, which showed negative values in all individuals and all tissues. Ba, Sb and Ti are alike. They emerged independently in five different individuals and their tissues. No mutual connections among them were discovered.

Low occurrence of elements

Elements Cu, Ni, Se and Sr appeared at low frequency of occurrence, but over-limit and under-limit ratio was relevant for the accumulation of specific organs depending on age. For demonstrating, we present the following contingency tables and graphs for each element. The number of nestlings with high level of contamination by Cu was increased in their middle-age category and gradually declined in fledglings. The soft tissues contained generally more Cu than bones. The opposite situation occurred in Ni pointed, this element was more increased in bones than in muscle or liver. The amount of Ni was also increased in the 2/3 of postnatal development. Much less comparable scheme of metal selection to Ni was observed in Selenium. More young with higher content of this element were detected with contamination in muscle and liver. The number of nestlings which were contaminated by Se in bones was low. Liver contamination by Sr was not observed at all. Otherwise, the occurrence of Sr in bone was increased in the middle-age nestlings and fledglings (Table 1).

High occurrence of elements

Twelve elements with the highest number of occurrence in the three tissues were measured for statistical evaluation (36 factors). It was found that just 3 from 11 factors (PC1-PC11) related significantly to age classes. The others were not significant (Table 2).

The positive correlations are as follows.
The first phenomenon (PC1)

The first phenomenon referred to the entire metal contamination especially soft tissues of hatched young as P (liver), K, S, Cl, Mn (all tissues), Fe (muscle), Zn (liver, muscle), Cr (liver, muscle); Pb (muscle) Mo (liver, muscle) in significant synergy. The proportional amount of named elements decreased significantly with increased levels of P and Zn, mainly Ca in bone tissue (Table 3, Fig. 1). Contamination of S, Cl, Mn, K, Cr, Mo, Zn, Fe and P equally decreasing.

The fifth phenomenon (PC5)

Phenomenon is probably related to what has been shown in other elements in contingency tables. The levels of certain elements increased in the middle age during nesting care. Paradoxically, when Ca and S in liver were low (probably as a biogenic elements), at that time Rb in bones was high in the age class of 12 (Table 4, Fig. 2).

The eleven phenomenon (PC11)

It is confirmed that the more young were developed, the less long-term Pb in bone occurred. Although the independence of this phenomenon suggested different cause as in the case of above mentioned elements (Table 5, Fig. 3).

		Liver			**Muscle**			**Bone**		
Copper (Cu)	PD	17	17	9	12	13	6	0	0	0
	ND	0	2	1	5	6	4	17	19	10
Nickel (Ni)	PD	1	0	0	1	2	0	5	8	2
	ND	16	19	10	16	17	10	12	11	8
Selenium (Se)	PD	8	15	5	3	6	0	0	1	0
	ND	9	4	5	14	13	10	17	18	10
Strontium (Sr)	PD	0	0	0	1	0	2	5	10	9
	ND	17	19	10	16	19	8	12	9	1
Age classes		**8**	**12**	**16**	**8**	**12**	**16**	**8**	**12**	**16**

Table 1. Positive (PD) and negative detection (ND) of Cu, Ni, Se and Sr in all 46 passerines for liver, muscle and bone against age classes.

Age classes	**N**	**PC2**		**PC3**		**PC4**		**PC6**		**PC7**		**PC8**		**PC9**		**PC10**	
		M	S.E.	M	S.E.	M	S.E.	M	S.E.	M	S.E.	M	S.E.	M	S.E.	M	S.E.
8	17	-0.03	0.25	0.18	0.24	-0.22	0.24	0.03	0.24	0.35	0.24	0.03	0.24	-0.12	0.24	-0.25	0.24
12	19	-0.01	0.23	-0.30	0.22	0.03	0.23	-0.16	0.23	-0.14	0.22	0.16	0.23	0.31	0.22	0.08	0.23
16	10	0.08	0.32	0.26	0.31	0.31	0.32	0.27	0.32	-0.33	0.31	-0.35	0.31	-0.39	0.31	0.26	0.31
F (2, 43)		0.04		1.53		0.89		0.61		1.85		0.88		1.88		0.96	
p		0.9593		0.2281		0.4168		0.5448		0.1689		0.4202		0.1639		0.3907	
Total %		11.49		9.69		6.38		5.19		4.45		3.80		3.53		3.11	

Table 2. No significant content for age classes. N - Number of birds, S.E. – standard error, F – Fischer test, p - significant validity, M - Mean.

Age classes	**Mean**	**S.E.**	**N**
8	-0.74	0.17	17
12	0.03	0.16	19
16	1.19	0.22	10

Table 3. F (2, 43) =23.578, p=0.00000. Total variance (%) 32.95.

Concentrations of elements against age

We created XY graphs (x=Age; y=PPM value) in STATISTICA software to demonstrate the concentration of each heavy metal in different tissue of maturing juveniles. We came to interesting observations. The elements Cl, Cr, K, S, Mn and Mo exposed the total loss of concentrations in all measured organs (Fig. 4).

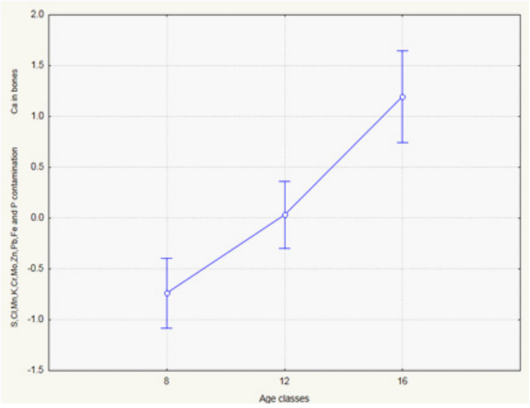


Fig. 1 Increasing Ca in bone according to age.

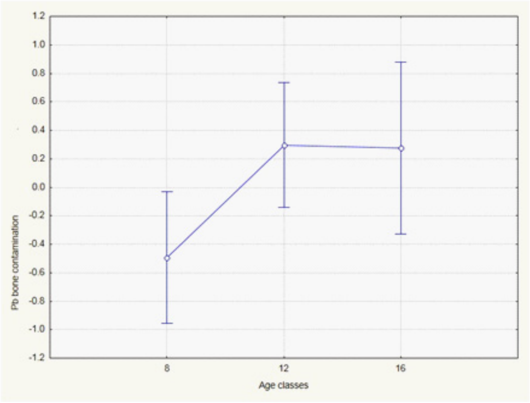


Fig. 2 Accumulation of Rb in the bone tissue in the middle age against lower level of S and Cl in liver.

Age classes	Mean	S.E.	N
8	-0.30	0.21	17
12	0.58	0.20	19
16	-0.57	0.28	10

Table 4. F (2, 43) =7.2485, p=0.00194. Total variance (%) 5.79.

Age classes	Mean	S.E.	N
8	-0.49	0.23	17
12	0.29	0.21	19
16	0.27	0.29	10

Table 5. F (2, 43) =3.6949, p=0.03305. Total variance (%) 2.13.

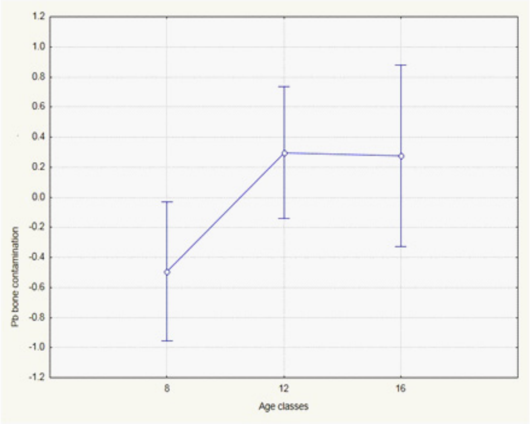
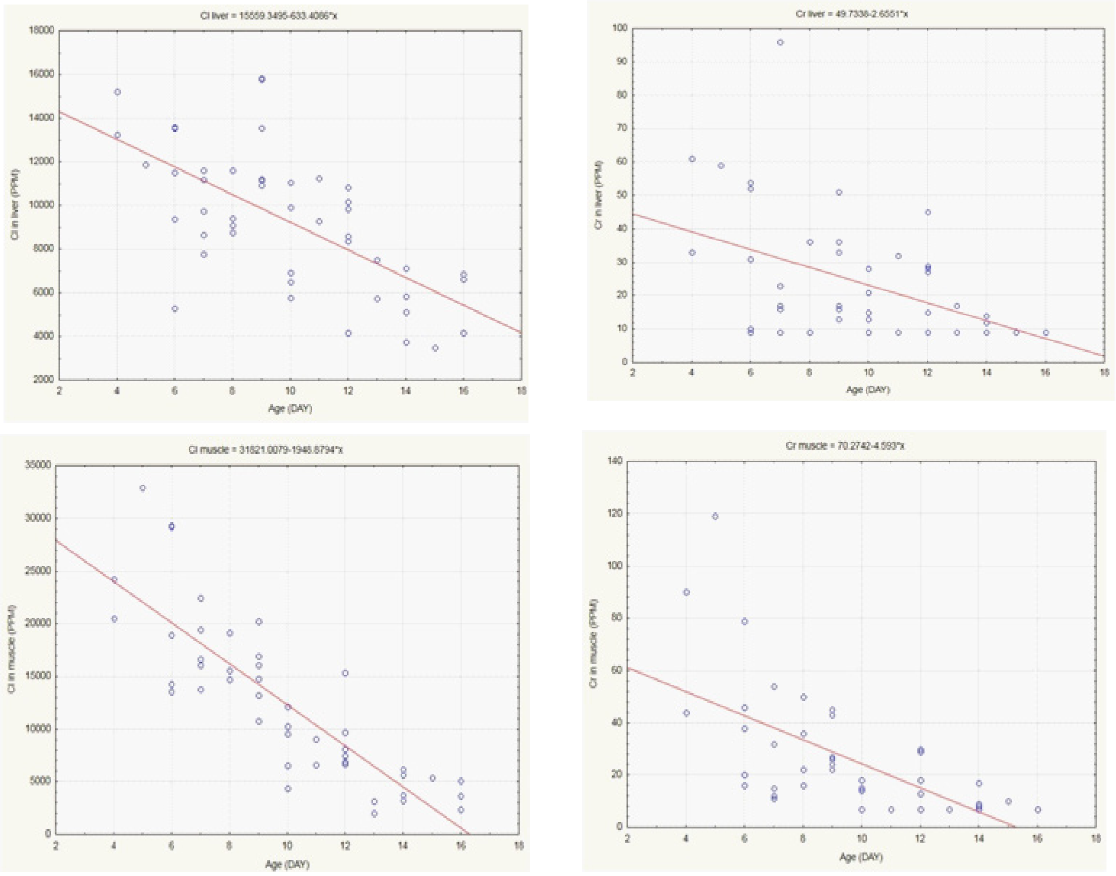


Fig. 3 Pb accumulation in bone against age.



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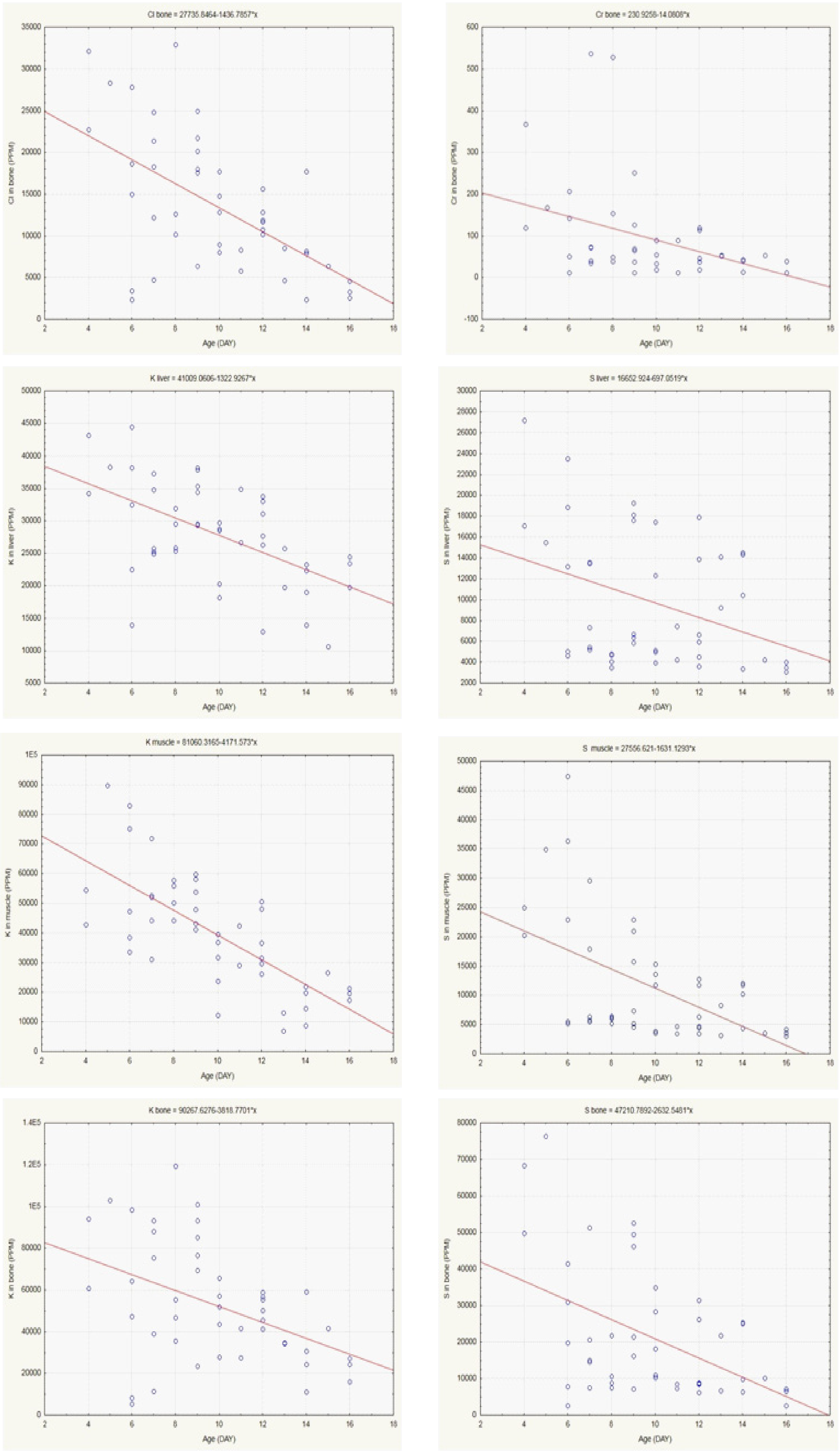
Cl bone = 27735.8464-1436.7857*x
Cl in bone (PPM)
0
5000
10000
15000
20000
25000
30000
35000
Age (DAY)
2
4
6
8
10
12
14
16
18
Cr bone = 230.9258-14.0808*x
Cr in bone (PPM)
-100
0
100
200
300
400
500
600
Age (DAY)
K liver = 41009.0606-1322.9267*x
K in liver (PPM)
5000
10000
15000
20000
25000
30000
35000
40000
45000
50000
Age (DAY)
S liver = 16652.924-697.0519*x
S in liver (PPM)
2000
4000
6000
8000
10000
12000
14000
16000
18000
20000
22000
24000
26000
28000
30000
Age (DAY)
K muscle = 81060.3165-4171.573*x
K in muscle (PPM)
0
10000
20000
30000
40000
50000
60000
70000
80000
90000
1E5
Age (DAY)
S muscle = 27556.621-1631.1293*x
S in muscle (PPM)
0
5000
10000
15000
20000
25000
30000
35000
40000
45000
50000
Age (DAY)
K bone = 90267.6276-3818.7701*x
K in bone (PPM)
0
20000
40000
60000
80000
1E5
1.2E5
1.4E5
Age (DAY)
S bone = 47210.7892-2632.5481*x
S in bone (PPM)
0
10000
20000
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Age (DAY)

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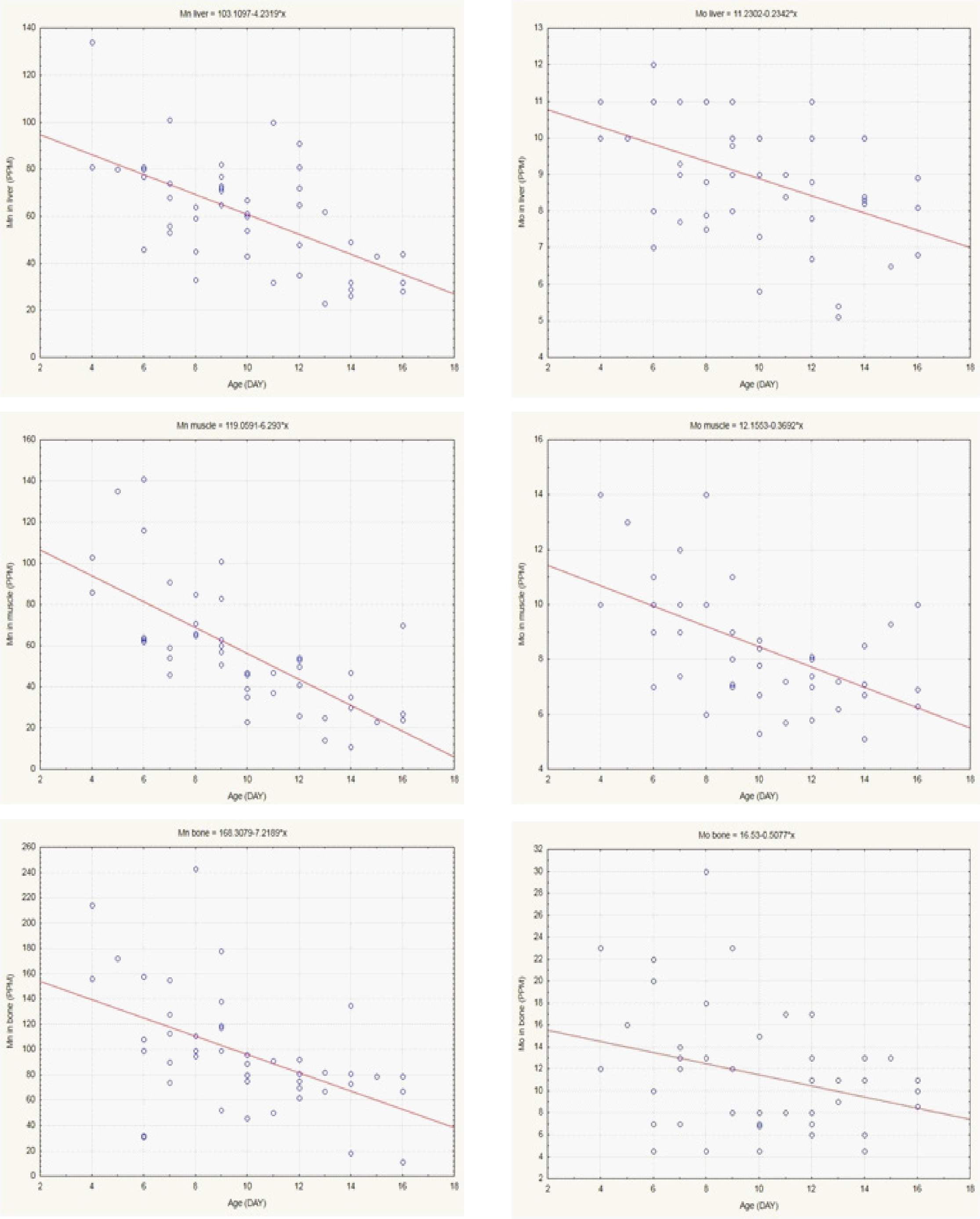
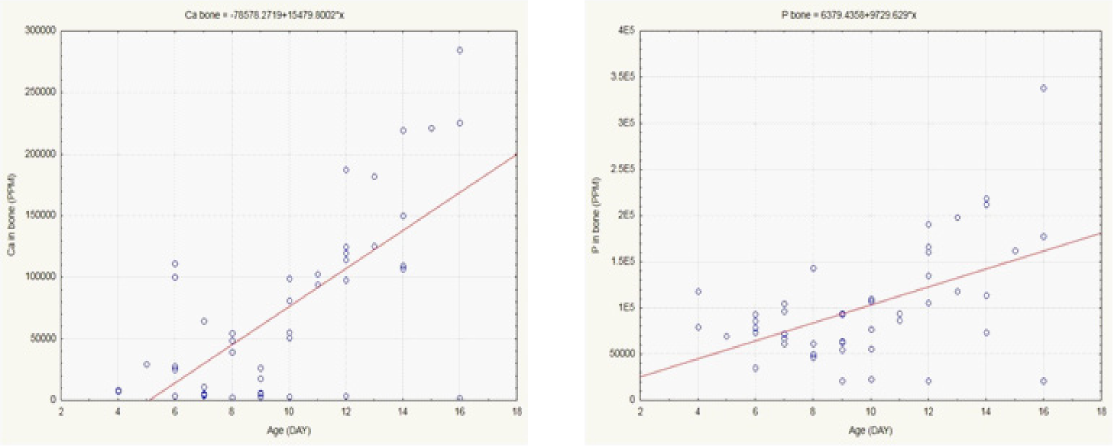


Fig. 4 Cl, Cr, K, S, Mn and Mo decreasing in all body tissues considering the age.



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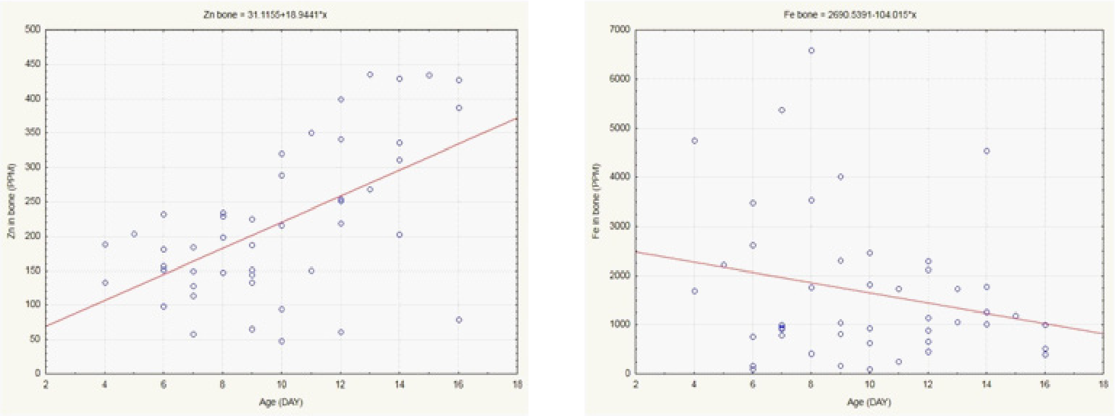


Fig. 5 Increasing concentrations of Ca, Zn and P, decreasing of Fe in bone tissue.

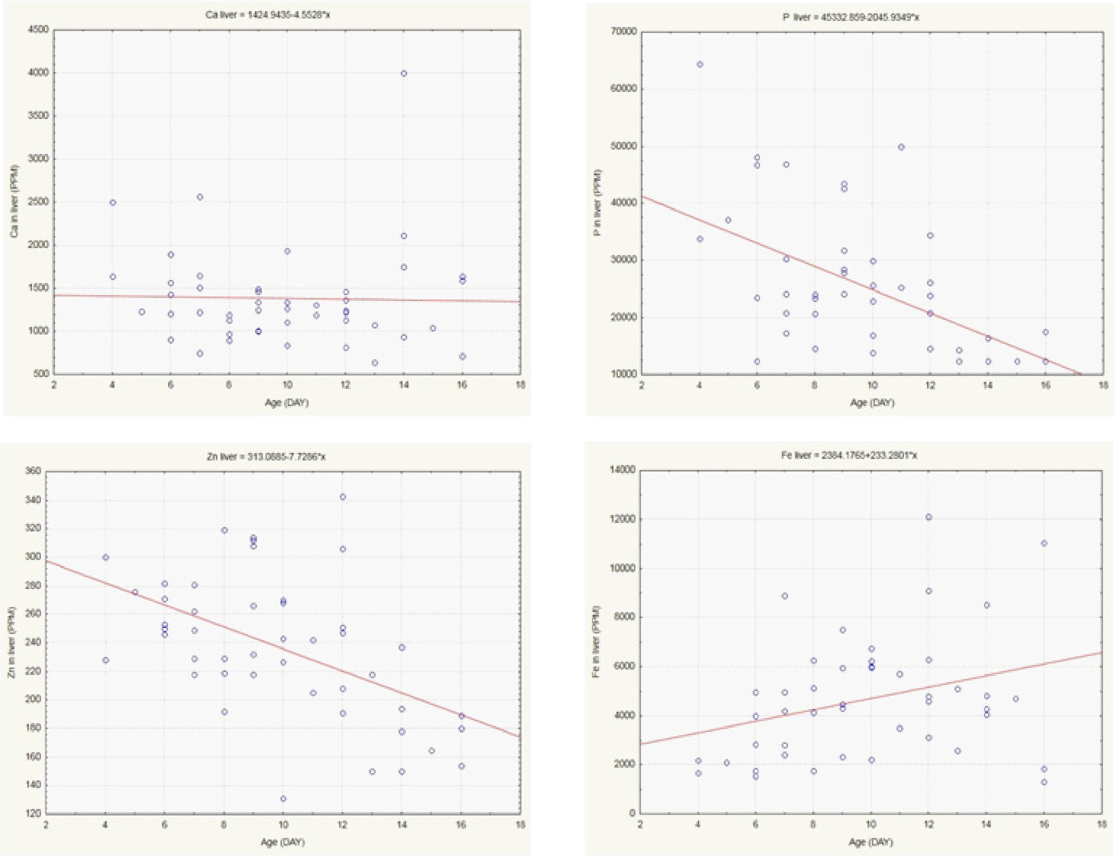
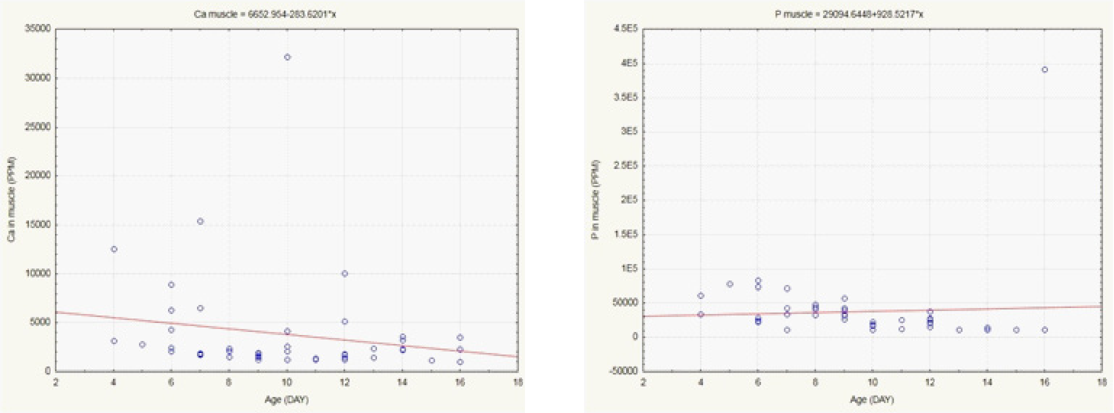


Fig. 6 Stable concentration of Ca, declining of P, Zn and increasing concentration of Fe in liver.



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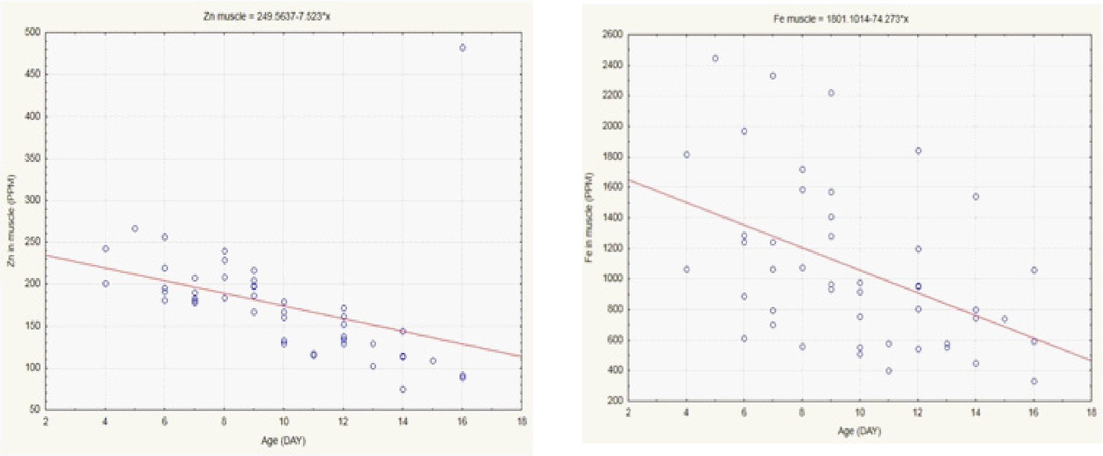

Fig. 7 Relatively stable concentration of Ca, P and decreasing of Zn and Fe in muscle.

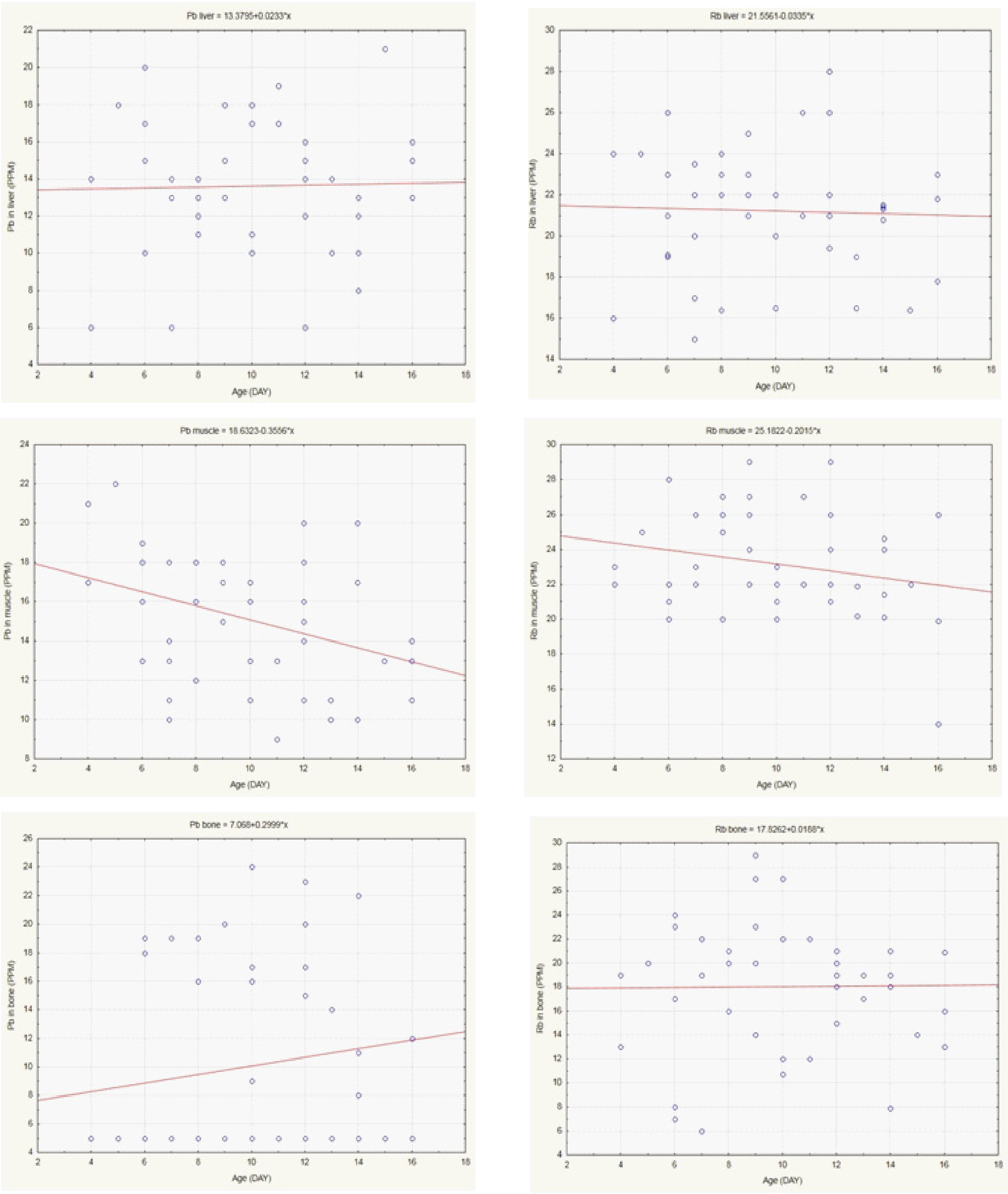

Fig. 8 Even concentrations of Pb and Rb in body tissues.

The increased concentration of Ca, P and Zn in bones was proved. On the other hand, the levels in soft tissues were decreased. Fe conducted itself antagonistically - significant increase in liver and decrease in bone (Fig. 5, 6, 7).

Lead and rubidium were specific because of relatively stable status of concentration in all tissues. The slight decrease occurred in muscle. In case of Pb, there were 26 samples of various ages, where no detection was measured. Therefore, the smallest value of measured sample (5 PPM) was automatically added. For this reason, the result cannot be considered sufficient (Fig. 8).

Discussion

Studies in Swedish heavy metal polluted forests, in the pollution gradient from a sulphide ore smelter plant, showed that adults and nestlings of insectivore passerine birds accumulated toxic elements and exhibited biological effects, reflecting the amounts of metals in aerial deposition (Nyholm 1987, 1989, 1994). Study conducted by Sawicka-Kapusta *et al.* (1986) showed increased heavy metal contamination of wintering Tits (*Parus* sp.). Another research performed in 1995 by the same authors, partially carried out in the same area, emphasized on biological (health) effects related to reproduction of Great tit. Its aim was to characterize the health state of naturally breeding birds in two differently polluted sites of forest in southern Poland and Swedish reference area. Concentrations of the non-essential elements Cd, Cu, Fe, Pb, Zn, Hg and the essentials Cu, Fe, Zn in tissues of nestlings were monitored. Results showed elevated level of non-essentials at the Polish locality reflected the degree of environmental contamination and no difference of essential elements between compared localities. Breeding results of the birds were severely affected in polluted site due to abnormal incidence of nestling mortality and high frequencies of the fledged young with an inferior health state (Nyholm *et al.* 1995).

Janssens *et al.* (2003) examined the possible effects of heavy metal exposure on the condition and health of Great tit (*P. major*) nestlings at four study sites along a pollution gradient near a large non-ferrous smelter in Belgium. Results showed that excrements of nestlings contained significantly higher concentrations of several heavy metals Ag, As, Cd, Hg, and Pb near the pollution source than at study sites farther away. In addition, number of young, nestling body mass and condition were significantly reduced. Later fledge of young bird and growth abnormalities of the legs also appeared as morphological defects caused by the destructive impact of heavy metals.

Authors Dauwe *et al.* (2002, 2005) and Deng *et al.* (2007) examined the concentrations of trace metal elements in tissues of Great tit (*P. major*) and Pied flycatcher (*Ficedula hypoleuca*) to assess the metal accumulation level, distribution among body parts and species and gender related variations. In general, the concentrations of the non-essential elements (Hg, As, Pb and Cd) were lower than those of essential elements (Zn, Se, Cu, Cr, Mn). In Dauwe's study (2002) was found significantly higher concentrations of Cd, Cu, Pb and Zn in the feathers *Parus major* at a polluted site than at a reference site. Alike no differences were observed in the metal levels in the Great tit feathers between the two studied age classes (4-9 months old and more than 16 months old). Gender variation in the level of Cd, Cu and Pb did not measure; however, Zn concentration was significantly higher in males than females. Regarding the essential elements Cu and Zn no significant correlations between levels in the feather and in any of the internal tissues were found. On the other hand, Cd and Pb concentrations in feather were significantly positively correlated with levels in the internal organs.

Deng *et al.* (2007) found the highest concentrations of Hg, Ni, Zn and Mn in the feather; Pb, Co in the bone, Cd, Cr and Se in the kidney and Cu in liver and heart. Metal concentrations had substantial interspecific variation with Great tit (*P. major*) showing higher level of Hg, Cr, Ni and Mn than Pied flycatcher (*Ficedula hypoleuca*) in tissues of the most of body parts. Gender related variation were body part and species specific. Another Dauwe's study (2005) assessed the concentrations of distribution pattern of heavy metals in internal tissues of 10 breeding Great tits (*P. major*) females and the concentrations in their eggs. The highest level of Cr, Ni, Pb, Zn was measured in bones, Cu in liver and Ag, Mn in females intestines. In the egg contents, relatively high concentrations of the essential elements Cr, Co, Cu, Mn, Ni, and Zn were found. In eggshells, concentrations of Ag, Al, As, Co, Cr, and Ni were higher compared to internal tissue concentrations, but generally concentrations in females´ tissues and eggs were poorly correlated. Adults generally accumulate the greater amount of heavy metals considering the longer-lasting exposure. Previous studies have shown, that females are able to sequester excessive amount of metals into eggs, what is an effective process for maintaining a lower body burden in their bodies. Juveniles are more susceptible to devastating effects of contaminants, though their rapid metabolism might exclude a large portion of them (Dauwe *et al.* 2002, Deng *et al.* 2007).

Costa *et al.* (2011, 2012) measured the concentrations of heavy metals (As, Cd, Cu, Hg, Ni) and Ca levels in feathers and excrements of Great tit (*P.major*) adult females. The main aim was to assess different levels of heavy metal contamination between the two study areas, industrial and rural, as well as potential detrimental effect on breeding performance. The results showed significant differences between the concentration levels of As, Hg, Pb, Ni, Cu, Cd, Zn and Se in feathers of nestlings and adult females. Only Cu and Zn levels were higher in nestlings, while As, Pb and Cd levels were higher in adult females. The concentrations of Ni in adult female feathers were presented by higher levels in the industrial area. Then significantly higher concentration of Hg was found in the polluted site. Furthermore, an interesting phenomenon emerged at breeding behaviour. The females breed earlier, laid more eggs, and produced more fledglings in the industrial area, where was also found higher biomass of caterpillars, an important food source or tits. Results showed no direct toxic effects of emission from the paper industry on the study species.

In another study situated around the pulp mill factory complex Norte *et al.* (2010) tried to prove a potential effects of exposure on the morphology, physiology, and reproductive performance of the Great tit (*P. major*). They found out, that tits from a population inhabiting the vicinity of a pulp mill had significantly higher feather Hg levels and were physiologically distinct from the others, farther away of the pollution source, which was assumed due to higher levels of red blood cell in autumn and winter time. Although no detectable effects were observed on breeding performance or nestling morphology and physiology.

A series of studies were carried out by Eeva *et al.* (1994, 1997a, 1997b) and Eeva and Lehikoinen (1995, 1996a, 1998) in 1991 - 1994 connected to the factory complex producing copper, nickel and fertilizers in the centre of Harjavalta, Finland. Studies were carried out among 12 study sites around the factory complex. The goals were to measure individual and population level effects of air pollution, both heavy metal contamination and acidification, on breeding performance and survival of two passerine bird species *Parus major* and *Ficedula hypoleuca*. Eeva performed breeding performance analyses, analyses of egg quality and female quality, nestling mortality, effects of parasites, food abundance and survival of adult birds. Studies clarified the interactions between natural and anthropogenic effects on breeding performance and the importance of direct and indirect effects. In case of *P.major* there were a several interesting conclusions as follow: (1) Variation in shell thickness or egg volume was not significantly related to the distance from the pollution source, clutch-size and hatching success did not significantly differ among study sites. (2) Decreased nestling growth and fledging success extended farther from the factory. (3) There was no reduction of survival in polluted area. (4) *P. major* received less heavy metals in food than *Ficedula hypoleuca*. The difference between species was probably due to the different diet. *P. major* forages almost exclusively in tree foliage during the nestling period. Heavy metals were perhaps accumulated more in the ground-living (mobile, often adult) food items than in the foliage-living insect food (e.g. caterpillars, spiders, aphids) of *P. major* (Hunter and Johnson 1982; Bengtsson and Rundgren 1984; Grue *et al.* 1986). This could be the main reason for the relatively high amount of Ca in the *P. major* organism. The reduced amount of Ca in the diet probably makes birds more susceptible to the detrimental effects of heavy metals. (5) No growth abnormalities were noted. Though, the strong gradient of Pb, Cu, Ni was observed in *F. hypoleuca* nestlings followed by bone defects as a potential cause by Ca-deficiency. (6) Body size of females was the same size in all areas, but at nestling time, females were heaviest in the moderately polluted area. (7) Females lay earlier in the polluted area. (8) Species preferred pine, particularly in the moderately polluted zone, where also the proportion of larvae was high. (9) The breeding success correlated positively with prey abundance, hence birds suffered from the shortage of larvae in the late nestling period.

The results of our work present the concentration of trace metals and unevenly distribution among the body tissues of *P. major* juveniles (the highest concentration of K, S, Cr, P, Zn, Ca was found in bone, Fe in liver). Generally, the amount and occurrence of non-essential elements were lower than the concentrations of essentials in all age classes. It was determined that the concentration of heavy metals went down by gradual nestling's maturity and the essential elements (P, Zn, Ca) particularly accumulated in bone tissue. An interesting phenomenon was zero detection of heavy metals Hg, As and Cd, which had been noted in previous studies as the major elements of industrial pollution (Janssens *et al.* 2003; Dauwe *et al.* 2002, 2005; Deng *et al.* 2007; Norte *et al.* 2010). Although the incidence was particularly occurred in feathers of adults, we expected the gradual accumulation at least in bone tissue of juveniles. We assumed that birds were still too young to show the level of accumulation. Copper and Selenium were discovered in both soft tissues, especially in the liver at an early stage of development. Copper is not particularly toxic, nutritionally essential element, necessary for the synthesis of new tissues (Cousins 1985), it is combined with Zn, on the other hand, it is in a contrary to Cd. It is therefore reasonable for females to accumulate these two metals to the liver during lying to transfer them to egg. Copper and Selenium have probably been transferred from the mother body to the eggs, to ensure the development of embryos (Nybo *et al.* 1996).

The organism has a tendency to get rid of these two elements from the liver and accumulate them in long-term deposition in bones and feathers, what also shows a gradual decrease in soft tissue and increase of Zn in hard tissue. Thus, the concentrations did not reflect the degree of environmental pollution in comparison with levels presented by (Deng *et al.* 2007). This was probably due to above mentioned homeostatic control mechanisms being efficiently capable of keeping normal levels of metals physiologically adequate (Nyholm *et al.* 1995). Similar observations appeared in Ni and Sr. Both tend to accumulate in hard tissues, but in much lower occurrence. In case of Ni, there is showed a rapid increase of concentration, Sr increased slowly. In the present case, we might consider both as nonessential metals from a pollution source.

Another category consists of metals with the highest level of concentration. However, the reduction of amounts in all tissues may refer to body detoxification of these metals (Cl, Cr, K, S, Mn, Mo) in age consideration. This might be the right effect of metabolism, which is enormously fast in young chicks. This is showed by maturation of the senses, the growth of skeleton and feathers. Hence, young birds are able to secrete elements into growing feathers and bones or to eliminate them via excrements or by storing them in the uropygium and salt gland (Dauwe *et al.* 2002; Deng *et al.* 2007). On the other hand, liver is in a very advantageous position to remove toxic materials from blood after their absorption because of the rapid turnover of hepatic cells and high hepatic metabolism. It may prevent their distribution to the other parts of body. Therefore, the level of heavy metals in the liver is considered to reflect the momentary exposure situation (Nybo *et al.* 1996).

We have to take into account the exogenous factors such as food sources, its abundance and availability during the incubation period and nestling's period. The breeding season lasts from March to August with the first nestling period in the second and third decade of April (divided into the pentads) (Figura 2013). During formation the avian egg is supplied with all essential materials necessary for the complete development of embryo. Especially, Ca-rich food items are beneficial for eggshell formation and skeletal growth (Eeva 1996b). In addition to essential trace elements, organic and inorganic toxicants potentially detrimental to embryonic developments are also transferred from the laying female tissues to the egg contents (Ohlendorf and Harrison 1986).

The first egg laying was accomplished in the middle of April. Since then, females had had to expend a lot of physical effort, until young have about one week. (9-10 days of egg laying, 14 days of incubating, 7 days of growth nestlings). (Noordwijk *et al.* 1995) During this time of 30 days, growing period for the caterpillars occurred. This indicated the highest food availability for 7-day-old nestlings. (Eeva and Lehikoinen 1998) The other data presented by Bureš and Weidinger (2003) said, that the intake of woodlice had increased steadily from hatching until the nestling age of about 11-12 days, decreased thereafter, which corresponded with the period of rapid skeletal growth. In the case of our study, precisely middle-age category cumulated higher amount of certain metals right in liver. This may imply that the increase is due to insect food receipt.

Another phenomenon is the accumulation of structural element - Ca in bones in regard to young growing, while decreasing the concentration of the other elements in all measured organs. We believe that the chicks were younger and still absorbing yolk for the needs of their development, the higher concentration of elements appeared in the organism. Juveniles in younger age undoubtedly received these elements from their mothers. Older nestlings reflected feeding in a new, greener habitat with abundance of insect species, with which both parents fed them.

Pb and Rb had relatively stable status of concentration in all tissues. As it is known, Pb replaces Ca in bone and cause Fe-deficiency in whole organism, (Neal and Guilarte 2012) but relatively high level of both elements point out to Pb deficiency in hard tissues. Moreover, Pb concentration in bones was not measured in more than a half of samples. On the contrary the larger concentration was measured in the soft tissues, which does not correspond with the results of previous studies (e.g. Dauwe *et al.* 2005; Deng *et al.* 2007). However, these elements are considered as pollutants coming from the external environment by the food ingestion, airways or from mother to the yolk in prenatal stage of development.

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