Long-term effect of diet amended by risk elements contaminated soils on risk element penetration and physiological parameters of rats

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ABSTRACT: The long-term accumulation of risk elements (As, Cd, Pb) originated from differently contaminated soils in rat organism was investigated during a model two-generation experiment. The effect of soil contamination level, gender, and length of exposure as well as the interactions between risk elements and selected essential macro- and microelements were studied. Rat diet contained 10% of individual soils (based on dry weight): (*i*) Fluvisol heavily polluted by As, Cd, Zn, and Pb, (*ii*) Luvisol contaminated by As, Cd, and Zn, and (*iii*) uncontaminated Chernozem. Male and female Wistar rats used for the experiment were housed in cages in a room with controlled temperature for 60 days and were fed *ad libitum* the mentioned diets. Subsequently, the pregnant females were continuously fed the experimental diet until weaning when the young animals were separated to male and female and fed the experimental diet till day 110 of age. The element contents in rat tissues reflected the risk element contents in contaminated soils. The bioaccessibility and bioavailability of the risk elements decreased in the order Cd>As>Pb and was affected by the soil physicochemical parameters. No significant differences were observed between male and female rats as well as between the first and the second generation. However, interactions were reported among the risk elements where the high cadmium content in Fluvisol resulted in increasing arsenic accumulation in the rat liver. Moreover, arsenic–copper interactions were observed where significant increase of the copper level was determined in kidney of the animals fed Luvisol exceeding 50-fold the maximum permissible limits for As content in agricultural soils. Among the hematological and biochemical characteristics of rats, total erythrocyte count (Er), hematocrit (Hct) increased confirming adverse effect of soil-derived risk elements especially in male rats.

Keywords: risk elements; soil; soil ingestion; liver; kidney; blood; *Rattus norvegicus*

INTRODUCTION

The potential environmental impact of risk elements originated from mining and smelting activities is widely investigated from various aspects where the potential health risk for population living in the vicinity of these locations is predominantly highlighted. There are two main factors affecting the population exposure by risk elements: the ingestion of plants produced in the contaminated area, and the ingestion or the inhalation of contaminated soil dust. Pruvot et al. (2006) demonstrated that risk elements in soils or plants of kitchen gardens, lawns, and playgrounds could be potentially transferable particularly to young children. Karadas and Kara (2011) also mentioned

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potential risk of soil ingestion in the vicinity of zinc, lead, and manganese mines. However, the potential risk of the ingestion of soil-derived risk elements depends on individual mining areas and element levels in the soils in the vicinity of the mine and also on bioavailability of individual elements in the soils (Noble et al. 2010; Szakova et al. 2012). As already mentioned in our previous work, the inhabitants living in element contaminated area are long-term exposed to the contaminated soil and the impact of risk elements should be evaluated also from biochemical, physiological, and hematological point of view (Amaral et al. 2007). Fischer et al. (2003) observed significant changes of selected hematological parameters in blood of children long-term exposed to enhanced concentrations of lead and cadmium close to a smelter. Phagocytic activity and some hematological parameters are known to be highly susceptible to alterations of nutritional and environmental factors including trace elements (Kostic et al. 1993). Ognjanovic et al. (2003) studied the impact of acute cadmium exposition of rats on hematological and immunological parameters as well as on the activities of lipid peroxidases and the potential role of vitamin E. They proved decreasing number of erythrocytes, hematocrit values, and hemoglobin concentrations in the exposed animals. The increased activity of antioxidative enzymes was observed, as well. Woods and Fowler (1977) and Bhadauria and Flora (2007) investigated the impact of mammal chronic exposition to arsenic where the adverse effect on the heme biosynthesis in liver was observed. The effect of risk elements on rat biochemical characteristics was reported in our previous investigation especially in the case of white blood cells (Szakova et al. 2012). Comparably, Castro et al. (2009) described the effect of arsenic on lymphocyte count and functions where low arsenic concentrations increased the number of T-lymphocytes whereas increasing As uptake decreased.

Instead of the direct adverse effect of the risk elements, potential effect of inter-element interactions affecting the balance of nutrients in the animal organism could be taken into account. For example, calcium and/or zinc deficiency can result in increasing resorption of lead and cadmium (Bencko et al. 1995). Increased Cd, Cu, and Pb levels were observed in the blood of anaemic children (Turgut et al. 2007). Similarly, daily rat exposition to 15 mg/kg $CdCl_2$ for 30 days resulted in anaemia followed by the changes in the metabolism and antioxidative activity of erythrocytes (Kostic et al. 1993). This effect has already been described by Prigge et al. (1977) presenting rat anaemia due to increased Cd uptake in feed (up to 31 mg/kg). Paul et al. (2002) reported higher arsenic accumulation in liver of iron-deficient rats compared to the animals with optimal iron uptake.

On the contrary, the increased Cd uptake can lead to suppressed resorption of iron, copper, zinc, cobalt etc., especially in the pregnant organism where the faetus development could be adversely affected due to the nutrient imbalance and lower bioavailability of the nutrients for the faetus (Bencko et al. 1995; Chmielnicka and Sowa 1996). The increased cadmium contents in the animal organism can affect the metabolism of many other essential elements such as selenium, manganese, chromium, magnesium, sodium, potassium, etc. (Matovic et al. 2011). Therefore, the supplementation of the organism with essential elements can lead to improvement of animal health status as described by Kusakabe et al. (2008) in the case of Fe, Cu, and Zn. Matovic et al. (2011) reported the effects and interactions of magnesium and zinc supplementation in this context.

The animals can be exposed to either single dose of contaminated soil suspension (Ellickson et al. 2001) or to long-term consumption of diet amended by the contaminated soil (Hetiarachchi et al. 2003). According to the results of the mentioned studies and our expertize, the following hypotheses were set up: (*i*) the risk elements contained in the contaminated soils can affect the whole rat organism in dependence on gender and exposition length, (*ii*) the exposure of the rat organisms to the soil amended diet can result in the imbalance of essential elements in animal organisms in accordance with gender and exposition time. This experiment follows up our previous experiment (Szakova et al. 2012) where the potential impact of soil ingestion for 60 days on element accumulation and hematological parameters in male Wistar rats (*Rattus norvegicus*) was studied. In this experiment, the response of male and female organism as well as two following generations of animals on the element uptake from the contaminated soil was investigated. The main objective of the present investigation was to assess long-term effect of risk elements (As, Cd, Pb) on selected hematological and biochemical parameters of rats. Interactions between risk elements and selected essential elements were studied as well.

MATERIAL AND METHODS

Experimental design. Twenty-four male and the same number of female Wistar rats (average body weight 251 ± 2 g for male, 185 ± 2 g for female rats, respectively) were obtained from the breeder (Velaz Ltd., Prague, Czech Republic) at 30 days of age and housed in cages (1 animal per cage) in a room with controlled temperature (varying from 23 to 25°C) under natural light conditions. The animals were randomly divided into 4 groups per 6 animals and fed a semi-synthetic diet according to the experimental design for 60 days. Subsequently, the females were fertilized by the male individuals and the males were euthanized. The whole blood samples were taken from all the animals. Pregnant females were continuously fed the experimental diet until weaning when the young animals were separated to male and female and divided again into 4 groups per 6 animals and fed the experimental diets till day 110 of age when the experiment was terminated. Feed and water were supplied to the animals *ad libitum*, feed consumption and body weight of animals were monitored weekly. The control group was fed the untreated semi-synthetic diet consisting from 50% of wheat coarse meal, 13% of fish meal, 14% of soybean meal, 0.28% of $CaHPO_4$, 1.12% of limestone, 4% of alfalfa hay, 1% of mineral additives (Aminovitan STER PLUS, Biofaktory Ltd., Prague, Czech Republic), 7.5% of feeding yeast, 4.5% of wheat germs, and 10% of oat meal. In the case of treated groups defined portions of individual soils were mixed with the mentioned semi-synthetic diet to obtain final percentage of 10% soil from final weight of the diet. The soils used for diet amendment were: (*i*) Fluvisol from the alluvium of the Litavka River, Czech Republic, heavily polluted by wastes from smelter setting pits (soil L), (*ii*) Kutná Hora soil (Luvisol) contaminated by arsenic, cadmium, and zinc mainly due to tailings of silver mining in the Middle Ages (soil K), and (*iii*) uncontaminated Chernozem (soil S). Before mixing, the soils were air-dried, homogenized, grinded, and passed through a 2-mm plastic sieve. The total element contents in soils, diet, and soil-amended diet are summarized in Table 1 and main physicochemical parameters of the soils in Table 2. After the termination of the individual parts of the experiment the animals were euthanized by exsanguination after anaesthetizing with Xylapan (xylasin) and Narketan (ketamin) and whole blood (only in the 1st generation), liver, and kidney were sampled. The sampled tissues were kept at –18°C, freeze-dried, and homogenized; aliquots of blood samples were treated by K_2EDTA, and immediately used for determination of hematological parameters whereas remaining blood was sedimented to obtain serum samples for determination of both risk and essential elements as well as the selected biochemical parameters.

Analytical methods. Pressurized wet ashing: An aliquot (~500 mg of dry matter) of the freeze-dried liver and whole kidney samples from the individual animals, or experimental diet were weighed into a

Table 1. Total content of elements in individual soils, diet, and diet amended by 10% of the soil (mg/kg of dry matter)

Element	Diet + S	Diet + K	Diet + L	Diet	Soil S	Soil K	Soil L
As	4.5 ± 2.1	130 ± 14	32.0 ± 1.0	1.51 ± 0.3	15.1 ± 0.8	1 602 ± 363	321 ± 2.2
Ca	19 244 ± 478	17 995 ± 864	18 421 ± 412	21 623 ± 216	10 031 ± 2 112	8 772 ± 212	3 013 ± 244
Cd	0.80 ± 0.05	3.05 ± 0.34	5.01 ± 0.29	0.34 ± 0.06	1.02 ± 0.03	16.7 ± 0.59	37.5 ± 1.29
Cu	39.5 ± 2.3	45.3 ± 9.9	40.5 ± 5.9	29.7 ± 2.2	156 ± 2	173 ± 1	134 ± 4
Fe	4 005 ± 123	6 220 ± 539	5 054 ± 147	445 ± 66	27 247 ± 406	43 249 ± 342	34 139 ± 129
K	18 012 ± 361	17 537 ± 175	17 113 ± 460	15 010 ± 377	24 600 ± 270	23 545 ± 18	15 233 ± 115
Mg	1 402 ± 64	1 676 ± 26	1 353 ± 80	2 190 ± 25	230 ± 18	222 ± 14	150 ± 25
Mn	218 ± 13	199 ± 17	674 ± 32	128 ± 5	912 ± 8	969 ± 11	4 941 ± 625
Mo	1.92 ± 0.07	2.34 ± 0.19	3.02 ± 0.23	2.32 ± 0.23	1.31 ± 0.37	0.88 ± 0.09	6.22 ± 0.07
Ni	5.88 ± 0.69	5.55 ± 0.61	4.73 ± 0.37	1.75 ± 0.24	37.8 ± 2.26	45.1 ± 0.24	28.8 ± 0.37
P	6 463 ± 286	5 664 ± 522	6 267 ± 288	6 697 ± 184	888 ± 50	1 700 ± 81	592 ± 13
Pb	5.14 ± 0.63	13.1 ± 1.51	522 ± 5.28	3.82 ± 1.42	105 ± 2	195 ± 2	5 074 ± 103
Zn	130 ± 27	276 ± 25	735 ± 26	117 ± 17	137 ± 32	1 970 ± 4	5 899 ± 158

soil L = Fluvisol, soil K = Luvisol, soil S = uncontaminated Chernozem

Table 2. Main physicochemical parameters of experimental soils and available contents of main nutrients according to Mehlich III soil extraction procedure (Mehlich 1984)

Soil	TOC (%)	pH	CEC (mmol/kg)	Ca (mg/kg)	Mg (mg/kg)	K (mg/kg)	P (mg/kg)
Soil L	2.31	5.8	54.5	2561	148	155	147
Soil K	6.05	7.2	346	2296	76.7	62.2	69.5
Soil S	2.21	7.2	138	7195	282	585	166

TOC = total organic carbon content, CEC = cation exchange capacity, soil L = Fluvisol, soil K = Luvisol, soil S = uncontaminated Chernozem

digestion vessel. Concentrated nitric acid (8.0 ml) and 30% H_2O_2 (2.0 ml) (both Analytika Ltd., Prague, Czech Republic) were added. The mixture was heated in an Ethos 1 (MLS GmbH, Leutkirch, Germany) microwave assisted wet digestion system at 220°C for 30 min. After cooling, the digest was quantitatively transferred into a 20 ml glass tube and filled up to the volume by deionized water (Jankovska et al. 2010). The total concentrations of trace elements in the soils were determined in the digests obtained by the following decomposition procedure: aliquots (0.5 g) of air-dried soil samples were decomposed in a digestion vessel with a mixture of 8 ml of concentrated nitric acid, 5 ml of hydrochloric acid, and 2 ml of concentrated hydrofluoric acid. The mixture was heated in an Ethos 1 (MLS GmbH) microwave assisted wet digestion system at 210°C for 33 min. After cooling, the digest was quantitatively transferred into a 50 ml Teflon® vessel and evaporated to dryness at 160°C. The digest was then dissolved in 3 ml of nitric and hydrochloric acid mixture (1 : 3), transferred into a 25 ml glass tube, filled up by deionized water, and kept at laboratory temperature until measurement. In the case of serum, the samples were diluted before measurement without any other sample preparation.

The total contents of elements in the digests were determined by optical emission spectroscopy with inductively coupled plasma (ICP-OES) with axial plasma configuration, Varian VistaPro, equipped with SPS-5 autosampler (Varian Inc., Mulgrave, Australia). Measurement conditions were for all lines: power 1.2 kW, plasma flow 15.0 l/min, auxiliary flow 0.75 l/min, nebulizer flow 0.9 l/min. Low concentrations of As, Cd, Cr, Ni, and Pb in the digests and serum were determined by electrothermal atomic absorption spectrometry (ETAAS) using the instrument VARIAN AA280Z (Varian Inc.) equipped with GTA120 graphite tube atomizer, and by inductively coupled plasma mass spectrometry using the apparatus Agilent 7700x ICP-MS (Agilent Technologies Inc., Santa Clara, USA) equipped with an auto-sampler ASX-500, a three channel peristaltic pump, and a MicroMist nebulizer. The contents of Ca, Mg, and K in the digests were determined by flame atomic absorption spectrometry (Varian 280FS; Varian Inc.).

Among the hematological parameters total number of erythrocytes (Er; T/l), hemoglobin (Hb; g/100 ml), hematocrit value (Hct; %), mean cell volume (MCV; fl), and total number of leukocytes (Le; g/l) were determined in the whole blood stabilized by K_2EDTA. All parameters were determined on a computerized analyzer MEK 5208 Celltac (NIHON KOHDEN Corp., Tokyo, Japan). For the determination of the number of leukocytes and hemoglobin values the blood hemolysis solution Isotonac 3 MEK 640 (NIHON KOHDEN Corp.) was used. In the case of biochemical parameters (aspartate transaminase (AST), alkaline phosphatase (ALP), total protein, urea, and glucose contents) and Ca, Mg, and P contents in blood serum were determined by using a computerized analyzer Cobas 6000 (Roche, Basel, Switzerland).

Statistical analysis. The data obtained were subjected to Dixon's test for identification of outliers (significance level $\alpha = 0.05$) using MS Excel 2007. Subsequently, two-way analysis of variance and linear correlation analysis were used at the significance level $\alpha = 0.05$ (STATISTICA, Version 9.1, 2011).

RESULTS

The effect of the contaminated soil addition on total element contents in the experimental diets as well as the bioavailability of individual elements determined as the element portion extractable with simulated gastric and pancreatic solution (Ruby et al. 1996) was discussed in detail in our previous paper (Szakova et al. 2012). We can summarize

Table 3. Body weight and total weight gain (g) of the animals monitored during the experiment (n = 6; mean ± standard deviation)

	1st generation								2nd generation at 110 days of age	
	male			female			litter			
	30 days	90 days	weight gain	30 days	90 days	weight gain	1 day	30 days	weight gain	male	female
Diet	250 ± 24	514 ± 24	265 ± 46	184 ± 8	336 ± 22	152 ± 19	87 ± 8	1152 ± 74	1066 ± 67	482 ± 18	285 ± 23
Diet + S	253 ± 12	502 ± 18	249 ± 20	188 ± 12	338 ± 18	150 ± 12	94 ± 8	1110 ± 210	1016 ± 206	453 ± 20	289 ± 27
Diet + K	253 ± 15	511 ± 27	258 ± 27	186 ± 14	353 ± 23	167 ± 34	101 ± 13	1120 ± 108	1099 ± 95	505 ± 99	278 ± 15
Diet + L	248 ± 7	499 ± 47	252 ± 44	183 ± 13	326 ± 22	143 ± 28	87 ± 14	1076 ± 178	989 ± 167	481 ± 41	275 ± 18

L = Fluvisol, K = Luvisol, S = uncontaminated Chernozem
values do not significantly differ at $P < 0.05$ within individual columns

that the relatively high contents of As, Cd, and Pb in the soils K and L resulted in significantly ($P < 0.05$) increased contents of these elements in the amended diets (Table 1). Moreover, the element contents extractable with simulated gastric solution confirmed higher bioaccessible contents of risk elements in the contaminated soils. The extractable portions of elements increased in the order soil S < soil K < soil L for Cd, Cu, Fe, Pb, and Zn, and opposite pattern for As, Mn, and P. The effect of soil physicochemical parameters (Table 2) was not significant.

Body weight of the animals was monitored (Table 3) as well as the total body weight gain and feed consumption. No significant changes were observed among the experimental groups although both body weight and weight gain of the litter from females fed the diet amended by the soil L tended to decrease. The contents of both risk and essential elements in liver, kidney, and serum of the animals are summarized in Tables 4–6. The arsenic contents in liver varied between 0.55 ± 0.23 mg/kg (diet) and 48.8 ± 5.8 mg/kg (diet + K), in kidney between 1.09 ± 0.60 mg/kg (diet) and 47.2 ± 9.6 mg/kg (diet + K); the cadmium content in liver varied between 0.03 ± 0.01 mg/kg (soil S) and 0.90 ± 0.15 mg/kg (diet + L), in kidney between 0.11 ± 0.05 mg/kg (diet) and 3.54 ± 1.76 mg/kg (diet + L); the lead content in liver between 0.08 ± 0.08 mg/kg (diet) and 0.74 ± 0.22 mg/kg (diet + L), in kidney between 0.09 ± 0.02 mg/kg (diet) and 20.8 ± 2.8 mg/kg (diet + L). The pattern of risk elements was comparable regardless of the generation and higher risk element contents (Cd and Pb) were found in kidney. Arsenic contents in kidney of the 2nd generation rats tended to increase especially in the case of the animals fed the As contaminated soil K.

Among the other investigated elements, significant ($P < 0.05$) increase of copper contents in kidney of the 1st generation and both liver and kidney in the 2nd generation of the animals fed the diet containing As-contaminated soil K was reported. Because the Cu contents and bioavailability of copper in this soil did not differ from the remaining experimental soils we can anticipate As-Cu interaction observed also by other authors (Birri et al. 2010). The levels of other macro- and micronutrients were not significantly ($P < 0.05$) changed due to increased risk element uptake. However, a slight decrease of Fe, Na, and Mn contents in kidney of the 2nd generation female

Table 4. Element contents (mg/kg of dry matter) in liver of the animals (n = 6; mean ± standard deviation)

	As	Ca	Cd	Cr	Cu	Fe	K
1st generation male							
Diet	1.6 ± 0.97[a]	75.9 ± 9.2[a]	0.063 ± 0.03[a]	0.17 ± 0.12[a]	75 ± 55.5[a]	386 ± 93.6[a]	12 007 ± 1 319[a]
Diet + S	4.6 ± 1.20[a]	67.7 ± 7.6[a]	0.058 ± 0.02[a]	0.14 ± 0.05[a]	40 ± 10.3[a]	397 ± 70.6[a]	11 407 ± 1 232[a]
Diet + K	49.5 ± 27[b]	73.4 ± 11[a]	0.336 ± 0.17[b]	0.15 ± 0.06[a]	99 ± 14[a]	454 ± 63.7[a]	12 175 ± 1 321[a]
Diet + L	67.9 ± 18.6	76.1 ± 14[a]	1.08 ± 0.23[c]	0.10 ± 0.05[a]	44 ± 24.6[a]	434 ± 26[a]	12 752 ± 1 823[a]
2nd generation male at 110 days of age							
Diet	4.8 ± 3.3[a]	73 ± 8.1[a]	0.07 ± 0.01[a]	0.13 ± 0.08[a]	77 ± 15[a]	533 ± 67[a]	12 850 ± 1 129[a]
Diet + S	4.5 ± 1.6[a]	76 ± 6.4[a]	0.08 ± 0.2[a]	0.14 ± 0.09[a]	25 ± 7[a]	561 ± 104[a]	12 332 ± 720[a]
Diet + K	32.6 ± 43[b]	71 ± 7.3[a]	0.42 ± 0.4[b]	0.11 ± 0.08[a]	114 ± 14[a]	629 ± 124[a]	12 438 ± 968[a]
Diet + L	45.6 ± 8.2[b]	70 ± 12.5[a]	1.45 ± 0.3[c]	0.06 ± 0.03[a]	32 ± 22[a]	532 ± 92[a]	11 734 ± 2 446[a]
2nd generation female at 110 days of age							
Diet	1.6 ± 1[a]	49 ± 2.8[a]	0.47 ± 0.2[a]	0.05 ± 0.01[a]	39 ± 18[a]	1 489 ± 197[b]	10 871 ± 422[a]
Diet + S	5.8 ± 1.5[a]	48 ± 3[a]	0.03 ± 0.006[b]	0.07 ± 0.04[a]	50 ± 19.3[ab]	1 223 ± 214[a]	11 807 ± 810[b]
Diet + K	71.1 ± 9[c]	45 ± 3.9[a]	0.71 ± 0.3[a]	0.09 ± 0.06[a]	91 ± 61[b]	1 207 ± 148[a]	11 215 ± 325[ab]
Diet + L	56.2 ± 12.8[b]	61 ± 11.5[b]	1.56 ± 0.4[c]	0.11 ± 0.09[a]	23 ± 15[a]	1 046 ± 157[a]	10 983 ± 552[a]
	Mg	Mn	Mo	Na	Ni	Pb	Zn
1st generation male							
Diet	701 ± 77[a]	7.96 ± 0.8[a]	2.07 ± 0.5[a]	3 547 ± 571[a]	0.103 ± 0.09[a]	0.04 ± 0.06[a]	84 ± 12[a]
Diet + S	684 ± 84[a]	7.98 ± 1.3[a]	1.96 ± 0.4[a]	3 681 ± 532[a]	0.083 ± 0.06[a]	0.34 ± 0.1[b]	87 ± 12[a]
Diet + K	695 ± 78[a]	7.49 ± 1.6[a]	1.95 ± 0.2[a]	4 158 ± 234[a]	0.103 ± 0.06[a]	0.11 ± 0.1[a]	89 ± 13[a]
Diet + L	700 ± 49[a]	6.95 ± 0.7[a]	1.81 ± 0.4[a]	3 830 ± 725[a]	0.100 ± 0.05[a]	1.16 ± 0.09[c]	81 ± 6[a]
2nd generation male at 110 days of age							
Diet	681 ± 31[ab]	10.6 ± 0.7[a]	2.1 ± 0.8[a]	4 057 ± 232[b]	0.15 ± 0.08[a]	0.08 ± 0.09[a]	110 ± 16[a]
Diet + S	700 ± 26[a]	11.1 ± 1.3[a]	2.2 ± 0.3[a]	4 654 ± 411[ab]	0.07 ± 0.09[a]	0.16 ± 0.06[a]	116 ± 7[a]
Diet + K	709 ± 31[a]	13.7 ± 2.8[b]	2.5 ± 0.6[a]	5 298 ± 835[a]	0.06 ± 0.05[a]	0.09 ± 0.05[a]	122 ± 12[a]
Diet + L	556 ± 212[b]	11.5 ± 1.2[a]	2.0 ± 0.3[a]	4 895 ± 702[a]	0.49 ± 0.34[b]	1.02 ± 0.15[b]	117 ± 11[a]
2nd generation female at 110 days of age							
Diet	614 ± 25[a]	14.5 ± 0.8[c]	3.4 ± 0.4[b]	5 939 ± 612[b]	0.89 ± 0.4[a]	0.04 ± 0.09[a]	126 ± 7[c]
Diet + S	632 ± 37[a]	11.7 ± 0.9[ab]	2.6 ± 0.2[a]	4 342 ± 294[a]	0.86 ± 0.5[a]	0.01 ± 0.01[a]	100 ± 6[a]
Diet + K	624 ± 23[a]	12.2 ± 1.0[b]	2.9 ± 0.3[a]	4 965 ± 295[a]	0.73 ± 0.3[a]	0.41 ± 0.47[a]	108 ± 4[b]
Diet + L	633 ± 28[a]	10.5 ± 0.9[a]	2.5 ± 0.3[a]	4 768 ± 629[a]	1.35 ± 0.9[a]	1.42 ± 0.57[b]	97 ± 5[a]

L = Fluvisol, K = Luvisol, S = uncontaminated Chernozem

[a–c]averages marked by the same letter do not significantly differ at $P < 0.05$ within individual columns

rats fed the soil-containing diets was observed most probably due to lower bioavailability of these elements in soil compared to the unamended diet. Although the Zn content in the soil L was extremely high compared to other soils, in liver and kidney it even tended to decrease. Due to high cadmium content in the soil L the potential adverse effect of Cd on Zn uptake and accumulation can be taken into account (Chmielnicka and Sowa 1996; Matovic et al. 2011).

In the case of blood serum (Table 6), where only the 1st generation of the animals was studied, similar pattern to liver and kidney occurred in the case of As and Cd whereas high lead content in the soil L did not result in increasing serum Pb concentrations. In the case of zinc, similar effect of soil Cd content as in the case of liver and kidney occurred. Moreover, similar pattern was observed also in the case of soil K with enhanced Cd and extremely high As contents. Cui and Okayasu (2008) and Saito et al. (2008) did not find significant effects of arsenic on zinc contents in animal and human organism, as well. Therefore, the suppressed Zn serum concentration in rats fed

Table 5. Element contents (mg/kg of dry matter) in kidney of the animals (n = 6; mean ± standard deviation)

	As	Ca	Cd	Cr	Cu	Fe	K
1st generation male							
Diet	1.4 ± 1.24[a]	161 ± 10[ab]	0.56 ± 0.07[a]	0.16 ± 0.12[a]	78 ± 19[a]	332 ± 76[a]	10 799 ± 770[a]
Diet + S	6.3 ± 5.3[a]	148 ± 11[a]	0.55 ± 0.17[a]	0.05 ± 0.02[a]	77 ± 50[a]	357 ± 34[a]	10 702 ± 521[a]
Diet + K	361 ± 143[b]	164 ± 10[ab]	–1.6 ± 0.5[b]	0.23 ± 0.2[a]	593 ± 77[b]	432 ± 90[a]	10 959 ± 882[a]
Diet + L	87 ± 53[a]	180 ± 16[b]	5.1 ± 0.55[c]	0.11 ± 0.1[a]	71 ± 12[a]	477 ± 159[a]	10 409 ± 408[a]
2nd generation male at 110 days of age							
Diet	3.5 ± 3.3[a]	134 ± 13[a]	0.5 ± 0.15[a]	0.15 ± 0.09[b]	79 ± 28[a]	309 ± 45[a]	9 301 ± 508[b]
Diet + S	15 ± 13.7[a]	133 ± 14[a]	0.4 ± 0.06[a]	0.05 ± 0.01[a]	81 ± 24[a]	348 ± 47[ab]	10 360 ± 1 218[ab]
Diet + K	590 ± 299[b]	156 ± 20[b]	1.9 ± 0.4[b]	0.16 ± 0.08[b]	1 085 ± 603[b]	371 ± 57[b]	11 458 ± 740[a]
Diet + L	65 ± 19.5[a]	151 ± 11[ab]	3.9 ± 0.8[c]	0.04 ± 0.01[a]	58 ± 11[a]	311 ± 39[ab]	10 763 ± 897[a]
2nd generation female at 110 days of age							
Diet	1.6 ± 0.9[a]	170.7 ± 20[a]	0.6 ± 0.3[ab]	0.15 ± 0.08[a]	89 ± 41[a]	451 ± 109[b]	10 695 ± 1 052[a]
Diet + S	12.5 ± 6[a]	163.7 ± 12[a]	0.25 ± 0.06[a]	0.07 ± 0.02[a]	48 ± 13[a]	169 ± 12[a]	11 928 ± 537.5[a]
Diet + K	461 ± 199[b]	177.7 ± 25[a]	1.05 ± 0.2[b]	0.17 ± 0.11[a]	749 ± 334	204 ± 38.5[a]	11 188 ± 898[a]
Diet + L	40.2 ± 17[a]	163.2 ± 20[a]	2.5 ± 0.8[c]	0.08 ± 0.05[a]	37 ± 10[a]	168 ± 60[a]	11 376 ± 797[a]
	Mg	Mn	Mo	Na	Ni	Pb	Zn
1st generation male							
Diet	739 ± 31[a]	4.7 ± 0.2[a]	1.1 ± 0.2[a]	12 335 ± 1 716[a]	1.02 ± 0.38[b]	0.06 ± 0.08[a]	114 ± 7[b]
Diet + S	747 ± 13[a]	4.9 ± 0.6[a]	1.0 ± 0.2[a]	12 062 ± 1 143[a]	0.03 ± 0.01[a]	0.13 ± 0.08[a]	112 ± 4[ab]
Diet + K	739 ± 47[a]	4.6 ± 0.3[a]	1.2 ± 0.1[a]	10 801 ± 908[a]	0.04 ± 0.02[a]	0.32 ± 0.03[a]	101 ± 4[a]
Diet + L	786 ± 51[a]	5.1 ± 0.4[a]	0.98 ± 0.2[a]	12 404 ± 1 661[a]	0.06 ± 0.07[a]	22 ± 3[b]	111 ± 9[ab]
2nd generation male at 110 days of age							
Diet	659 ± 25[a]	4.2 ± 0.4[b]	1.06 ± 0.2[a]	12 907 ± 2 050[a]	0.19 ± 0.09[a]	0.65 ± 0.78[a]	112 ± 7[a]
Diet + S	668 ± 43[a]	5.0 ± 0.5[a]	1.22 ± 0.19[ab]	13 754 ± 1 746[a]	0.20 ± 0.09[a]	0.88 ± 0.87[a]	126 ± 7[b]
Diet + K	727 ± 52[b]	4.9 ± 0.6[a]	1.35 ± 0.2[b]	13 519 ± 1 150[a]	0.25 ± 0.1[ab]	1.1 ± 0.8[a]	117 ± 11[ab]
Diet + L	659.2 ± 38[a]	4.6 ± 0.4[ab]	1.22 ± 0.23[ab]	11 982 ± 1 995[a]	0.37 ± 0.1[b]	20.7 ± 4[b]	109 ± 7.5[a]
2nd generation female at 110 days of age							
Diet	682 ± 58[b]	3.56 ± 0.65[c]	1.28 ± 0.2[c]	10 339 ± 1 075[c]	0.64 ± 0.26[a]	0.05 ± 0.05[a]	100 ± 15[c]
Diet + S	781 ± 48[a]	1.45 ± 0.08[ab]	0.36 ± 0.1[a]	5 301 ± 463[a]	0.42 ± 0.1[a]	0.35 ± 0.12[a]	55 ± 4[ab]
Diet + K	742 ± 65[ab]	1.89 ± 0.3[b]	0.86 ± 0.35[b]	7 414 ± 1 522[b]	0.6 ± 0.24[a]	0.45 ± 0.07[a]	63 ± 8[b]
Diet + L	784 ± 40[a]	1.27 ± 0.5[a]	0.6 ± 0.27[ab]	5 101 ± 1 435[a]	0.47 ± 0.2[a]	5.9 ± 1.8[b]	49 ± 7[a]

L = Fluvisol, K = Luvisol, S = uncontaminated Chernozem

[a–c]averages marked by the same letter did not significantly differ at $P < 0.05$ within individual columns

the diet containing soil K could be also credited to cadmium.

The contents of selected biochemical parameters determined in blood serum of the animals are summarized in Table 7. Among the enzymes indicating potential adverse effect of enhanced risk element content in feed on liver function, AST and ALP activity was assessed where no significant changes ($P < 0.05$) among the experimental groups were observed. However, the results showed a significantly ($P < 0.05$) increased concentration of urea in male rat serum indicating potential cadmium-induced renal damage. El-Demerdash et al. (2004) and Borgese et al. (2008) suggested the enhanced serum urea concentrations as the first indicator of cadmium induced reversible kidney injury. Moreover, the addition of soil itself resulted in decreasing female serum glucose whereas male samples remained unchanged. Differences between male and female glucose levels in serum of rats exposed to arsenic and lead via drinking water due to significant increase in intestinal glucose absorption by male rats was observed by Palacios et al. (2012). In our case, however, the results sug-

Table 6. Element concentrations in blood serum of the animals (n = 6; mean ± standard deviation)

	As (nmol/l)	Cd (nmol/l)	Pb (nmol/l)	Zn (µmol/l)	Ca (mmol/l)	Mg (mmol/l)	P (mmol/l)
1st generation male							
Diet	27.8 ± 15.9^{a}	0.408 ± 0.294^{b}	2.93 ± 1.75^{ab}	11.1 ± 1.2^{a}	2.28 ± 0.12^{a}	1.65 ± 0.16^{b}	1.88 ± 0.25^{a}
Diet + S	17.6 ± 5.5^{a}	0.025 ± 0.025^{a}	0.94 ± 0.61^{a}	12.0 ± 2.2^{a}	2.22 ± 0.28^{a}	0.97 ± 0.31^{a}	2.05 ± 0.32^{a}
Diet + K	2456 ± 334^{b}	0.016 ± 0.016^{a}	2.24 ± 1.19^{ab}	7.84 ± 2.20^{b}	2.32 ± 0.26^{a}	0.88 ± 0.36^{a}	1.87 ± 0.31^{a}
Diet + L	404 ± 218^{b}	0.237 ± 0.082^{ab}	3.09 ± 1.05^{b}	9.17 ± 1.74^{ab}	2.19 ± 0.16^{a}	0.92 ± 0.24^{a}	2.13 ± 0.33^{a}
1st generation female							
Diet	87.8 ± 43.9^{a}	0.155 ± 0.090^{ab}	2.74 ± 1.01^{b}	13.5 ± 4.3^{b}	2.40 ± 0.34^{a}	0.95 ± 0.35^{a}	1.79 ± 0.43^{a}
Diet + S	72.3 ± 31.9^{a}	0.074 ± 0.041^{a}	1.39 ± 0.52^{a}	16.3 ± 4.4^{bc}	2.47 ± 0.12^{a}	0.73 ± 0.06^{a}	1.71 ± 0.12^{a}
Diet + K	5112 ± 774^{b}	0.123 ± 0.033^{ab}	2.44 ± 0.37^{b}	7.69 ± 1.07^{a}	2.40 ± 0.17^{a}	0.75 ± 0.22^{a}	1.87 ± 0.33^{a}
Diet + L	912 ± 2648^{b}	0.204 ± 0.025^{b}	2.51 ± 0.55^{b}	9.62 ± 1.59^{ab}	2.38 ± 0.16^{a}	0.79 ± 0.18^{a}	1.83 ± 0.37^{a}

L = Fluvisol, K = Luvisol, S = uncontaminated Chernozem

$^{a–c}$averages marked by the same letter do not significantly differ at $P < 0.05$ within individual columns

gested suppressed bioavailability of feed-derived glucose due to presence of soil in the diet.

The investigated hematological parameters are summarized in Table 8 where a significant increase ($P < 0.05$) of hematocrit value and total erythrocyte count is evident in the case of male rats fed the diet amended by the soils K and L. Although not significant, this pattern indicated also the results of female hematological parameters. Significant increase of leukocyte count as observed in our previous experiment (Szakova et al. 2012) was not reported in this experiment.

DISCUSSION

The effect of risk element uptake on body weight and weight gain of rats was investigated by Paul et al. (2002) in the case of arsenic, Asagba (2010) in the case of cadmium, Smith et al. (2008) and Shan et al. (2009) and in the case of lead. Whereas animal exposure to arsenic and lead did not result in the animal growth suppression regardless of element rate and experiment duration, suppressed animal growth was observed after dietary cadmium exposure of rats. In our case no differences among the experimental groups were observed (Table 3) even at by one order of magnitude higher total cadmium content in the diet. However, the differences in experimental design, duration of the both experiments, and different Cd source and/or bioavailability of Cd in the diet represent serious limitations for comparison of the results from both experiments.

The arsenic content in animal tissues (Tables 4–6) was influenced by its level in experimental

Table 7. Average contents of selected biochemical parameters determined in blood serum of the animals (n = 6; mean ± standard deviation)

	AST (µkat/l)	ALP (µkat/l)	Total protein (g/l)	Glucose (mmol/l)	Urea (mmol/l)
1st generation male					
Diet	1.59 ± 0.15^{a}	3.36 ± 0.61^{a}	58.4 ± 2.0^{a}	12.6 ± 1.6^{a}	7.2 ± 0.8^{a}
Diet + S	1.70 ± 0.41^{a}	2.78 ± 1.01^{a}	60.3 ± 3.8^{a}	10.2 ± 1.6^{a}	7.6 ± 0.5^{a}
Diet + K	1.55 ± 0.12^{a}	2.47 ± 0.61^{a}	58.4 ± 4.2^{a}	9.7 ± 0.7^{a}	7.9 ± 0.4^{a}
Diet + L	1.20 ± 0.04^{a}	3.24 ± 0.76^{a}	54.1 ± 5.5^{a}	10.9 ± 0.6^{a}	9.2 ± 0.4^{b}
1st generation female					
Diet	1.89 ± 1.05^{a}	2.24 ± 0.57^{a}	63.1 ± 5.2^{a}	11.3 ± 3.0^{b}	8.5 ± 1.3^{a}
Diet + S	1.38 ± 0.07^{a}	1.83 ± 0.26^{a}	64.9 ± 6.9^{a}	9.1 ± 0.7^{a}	7.9 ± 0.3^{a}
Diet + K	1.64 ± 1.02^{a}	1.73 ± 0.55^{a}	58.3 ± 5.8^{a}	8.3 ± 0.7^{a}	7.8 ± 0.9^{a}
Diet + L	1.25 ± 0.40^{a}	2.14 ± 0.38^{a}	55.6 ± 8.1^{a}	8.3 ± 0.7^{a}	7.0 ± 0.8^{a}

AST = aspartate transaminase, ALP = alkaline phosphatase, L = Fluvisol, K = Luvisol, S = uncontaminated Chernozem

^{a,b}averages marked by the same letter do not significantly differ at $P < 0.05$ within individual columns

Table 8. Average contents of selected hematological parameters of the animals (n = 6; mean ± standard deviation)

	Er (T/l)	Hct (%)	MCV (fl)	Hb (g/100 ml)	Le (g/l)
1st generation male					
Diet	6.99 ± 1.42[a]	43.2 ± 8.5[a]	62.0 ± 1.4[a]	14.1 ± 0.8[a]	13.0 ± 2.3[a]
Diet + S	7.27 ± 0.92[a]	45.1 ± 4.9[a]	62.0 ± 3.7[a]	14.4 ± 1.1[a]	11.6 ± 3.8[a]
Diet + K	7.97 ± 0.50[ab]	50.2 ± 4.9[ab]	63.0 ± 3.4[a]	15.1 ± 1.5[a]	8.6 ± 2.6[a]
Diet + L	8.46 ± 0.70[b]	54.5 ± 4.3[b]	64.5 ± 2.3[a]	16.0 ± 1.5[a]	10.7 ± 2.1[a]
1st generation female					
Diet	7.04 ± 0.48[a]	47.5 ± 5.5[a]	69.7 ± 4.6[a]	6.92 ± 2.90[a]	13.4 ± 1.3[a]
Diet + S	8.36 ± 1.02[a]	54.9 ± 6.3[a]	65.8 ± 3.9[a]	7.02 ± 2.98[a]	16.8 ± 2.2[a]
Diet + K	8.02 ± 1.20[a]	53.5 ± 9.3[a]	66.5 ± 4.3[a]	8.85 ± 1.95[a]	15.0 ± 2.7[a]
Diet + L	8.52 ± 0.88[a]	55.6 ± 5.3[a]	65.3 ± 3.9[a]	7.92 ± 1.16[a]	16.9 ± 1.3[a]

Er = total erythrocyte count, Hct = hematocrit, MCV = mean cell volume, Hb = hemoglobin, Le = total leukocyte count
L = Fluvisol, K = Luvisol, S = uncontaminated Chernozem
[a,b]averages marked by the same letter do not significantly differ at $P < 0.05$ within individual columns

diet but the As tissue concentrations were almost comparable for both soil K and soil L amendments although the arsenic content in the soil L was significantly ($P < 0.05$) lower. As discussed in our previous experiment, the differences in As bioavailability in both soils also do not correspond to arsenic concentrations in individual tissues (Szakova et al. 2012). Therefore, potential As-Cd interactions seemed to play a substantial role. As already published, the interaction between arsenic and cadmium applied simultaneously is evident. The addition of Cd to the diet can significantly increase arsenic contents in liver and kidney of rats fed the diet containing Cd-enriched yeasts (Szakova et al. 2009). Similarly, rats treated with As + Cd had more As in heart tissue than rats treated only with arsenic (Yanez et al., 1991). Hochadel and Waalkes (1997) stated that arsenic pretreatment can reduce mortality in rats given the high dose of cadmium compared to rats given cadmium alone. The opposite effect of both elements was not observed. Moreover, arsenic pretreatment produced an 8-fold increase in hepatic levels of metallothionein. Thus, increasing contents of both elements in liver and kidney can be expected and enhanced metallothionein content in these tissues could be verified in further research. In the case of lead, low bioavailability of this element (Ellickson et al. 2001; Szakova et al. 2012) resulted in its relatively low contents in analyzed tissues where significantly ($P < 0.05$) enhanced levels were observed only in the case of extremely contaminated soil L. As explained by Smith et al. (2008), the lead uptake and transformation do not differ significantly in dependence on lead compound and/or lead-enriched matter including lead-contaminated soil. Although the lead transport through placenta and mother's milk was described by Todorovic et al. (2005), no differences between the 1st and 2nd generation were observed due to long-term uptake of the soil-amended diet after weaning.

Among the essential elements, the most apparent effect of the risk element dietary uptake was observed in the case of Cu in the tissues of animals fed As-contaminated soil K as already observed in our previous experiment (Szakova et al. 2012) and confirmed by other authors (Schmolke et al. 1992; Uthus 2001; Yu and Beynen 2001; Birri et al. 2010). Comparing the arsenic compounds, the Cu-As interaction was observed only if inorganic As compounds were the predominant species present. In our case, the mobile As pool in soils is represented by inorganic arsenate (Marin et al. 1993) suggesting possible intensification of As-Cu interaction. The other previously described interactions such as As-Fe (Paul et al. 2002), Cd-Fe (Turgut et al. 2007), or Cd-Cu (Chmielnicka and Sowa 1996) were not confirmed in our experiment. As already mentioned, the individual experiments differed in element dose and application, element source, and duration of the experiment.

The significant effect of risk element intoxication of the rats on the activity of hepatospecific enzymes such as ALT and ALP was observed after both single-dose (Dudley et al. 1982; Tzirogiannis et al. 2003), and long-term (Renugadevi and Prabu 2010) dietary exposure documenting clearly hepatotoxic effect of risk elements, especially

cadmium. In our case no changes in the enzymatic activity were observed (Table 7) but other biochemical indicator, urea concentration in the blood serum, documents adverse effect of long-term risk element exposure via the contaminated soil. The renal injury as a result of long-term cadmium exposure was frequently reported (Hiratsuka et al. 1996; El-Demerdash et al. 2004; Borgese et al. 2008). Hypoglykaemia resulting from the single-dose cadmium application was reported due to disability of gluconeogenesis caused by the liver injury by Adachi et al. (2007). However, long-term cadmium exposure resulted in the restoration of original glucose concentrations because of antioxidative mechanisms of the rats. Our results, however, do not allow us to conclude that the serum glucose suppression in female rats was caused by risk element uptake.

Decreasing hemoglobin content connected with decreasing blood iron levels and anaemia was described (El-Demerdash et al. 2004; Adachi et al. 2007). However, opposite effect was observed by Fucikova et al. (1995) where rats were fed semisynthetic diets supplemented with feeding yeast *Candida utilis* with low (3 mg/kg) or high (90 mg/kg) content of organically bound cadmium and/or cadmium in the form of $CdCl_2$ (9 mg/kg of diet). Rats on diet with yeast high in Cd or with $CdCl_2$ supplement had higher ($P < 0.05$) levels of cadmium in liver and kidney than controls. The animals fed high Cd content yeast had significantly higher hemoglobin values compared to the group fed low Cd content yeast. The effect of experimental design and duration of the experiment is documented by Fucikova et al. (1996) presenting no changes in hemoglobin values in rats fed yeast supplemented Cd-enriched diet. Also our previous experiment (Szakova et al. 2012) showed no changes in hemoglobin values but decreased iron tissue concentrations. In this experiment the iron suppression was not confirmed but hemoglobin values and total erythrocyte count increased in female rats exposed to soil L documenting unambiguous response of animal organisms to enhancement of the dietary cadmium level. Similarly, increasing white blood cells count observed after both single-dose (Kataranovski et al. 1998) and long-term (Fucikova et al. 1995; Szakova et al. 2012) cadmium exposition was not confirmed in our experiment.

The results confirmed our previous results (Szakova et al. 2012) showing increased tissue risk element levels (especially of As and Cd) as a result of regular uptake of the risk element contaminated soils. Although the shifts in the biochemical and hematological parameters of the animals did not give unambiguous results, their existence confirms adverse effect of the soil-derived risk element exposure. In the case of lead, relatively low increase of tissue levels was reported due to its low bioavaolability. However, Oktem et al. (2004) observed harmful effects (injury of red blood cells, adverse renal effects) of long-term uptake of low levels of lead in the organisms of people living in contaminated areas. Moreover, the inter-element interactions in the case of multielement contaminated area (Wang et al. 2009; Whittaker et al. 2011) can substantially modify the potential effects of the individual elements as well as the nutrient imbalance.

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