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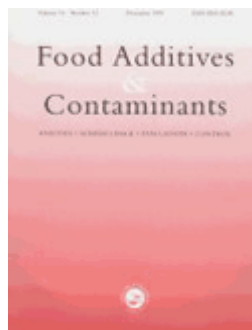
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Correlation of lead, cadmium and mercury levels in tissue and liver samples with age of cattle

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Abstract

The aim of the research was to determine the level of accumulation of selected heavy metals (Pb, Cd, Hg, As) in meat and liver of cattle. The animals were divided into four age groups which allowed the analysis of statistical-mathematical correlations between the age of the animals and contamination of meat. The research material for determining the content of heavy metals was taken from the longissimus back muscle (*m. longissimus dorsi*), and samples of liver from the tail lobe. The analysis carried out shows that contamination of meat of cattle by Cd and Pb clearly depends on the age of these animals.

Keywords: beef; heavy metals; coefficients of correlation

Introduction

Among farm animals the ruminants are most exposed to contamination of environment by heavy metals owing to the system of pasture feeding of these animals in the summer period and hay feeding in the winter period. Metals penetrate into grasses most often from the soil and are also present in hay fodder. The differences in the level of contamination of internal organs and muscles by heavy metals depend, among other things, on the species and age of animals (Hecht 1983; Krelowska-Kułas 1994; Studziński et al. 1992) and also on the degree

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3 of contamination in the region where the animals were bred and their feeds (Barghigiani and
4 Ristori 1994; Sikora 1991; Żebrowska-Rasz 1992). The degree of contamination by heavy
5 metals of the tissue of rabbits (Sikora 1991) and sheep (Studziński et al. 1992), has been
6 studied among three age groups. Other researchers (Hecht 1983; Krełowska-Kułas 1994;
7 Barghigiani and Ristori 1994; Żebrowska-Rasz 1992) have been concerned with
8 contamination by heavy metals of tissues of other species of animals, but did not include the
9 influence of age on the level of accumulation in tissues. In practice cattle are slaughtered
10 more often at the age from 1,5 to 2 years but nevertheless, meat is also obtained from a few-
11 year-old or even several-year-old animals (e.g. cows).
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21 The aim of our research was to determine the level of accumulation of selected heavy metals
22 (Pb, Cd, Hg, As) in meat and liver of cattle. The animals were divided into four age groups
23 which allowed the analysis of statistical-mathematical correlations between the age of the
24 animals and contamination of meat and liver by these elements. Additionally, the content of
25 the above-mentioned toxicants in tissues of these animals slaughtered in the years 2002 –
26 2006, presented in the paper, allows assessment of the degree of contamination by these
27 elements during this period.
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35 **Materials and methods**

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38 The research was carried out in the years 2002-2006 on cattle in the region of
39 south-eastern Poland (the areas of Podkarpackie and Lubelskie provinces), coming from the
40 farming areas, with majority of small scale commodity production, practically with no
41 industry. The region of south-east Poland has very good conditions for production of food of
42 high health and nutritional value, since as much as 95% of soils are of the first class purity in
43 respect of the content of heavy metals (Sucha and Zawada 1994). The concentration of the
44 heavy metals, both in soil as well as in drinking water of this region was within the standard.
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53 For the research there were selected clinically healthy animals, of similar slaughter weight
54 within an age group. The cattle came from individual farms and were fed traditionally (with
55 farm's own fodder).
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The slaughter of cattle was carried out in one of meat plants of south-eastern Poland in
accordance with the methodology applied by the meat industry. The samples for tests were

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3 meat and liver. Each year of research used 20 head of cattle (5 years x 20 samples of meat and
4 20 samples of liver = 100 samples of meat and 100 samples of liver). The age of animals was
5 determined on the basis of teeth and purchase documentation. Cattle of black-and-white
6 lowland breed (pre-slaughter weight 480 – 600 kg), in each age group (4 groups x 20 samples
7 of meat and 20 samples of liver = 80 samples of meat and 80 samples of liver) were divided
8 into 4 age groups:
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16 Group I – young slaughter cattle from 1.5 to 2 years,

17 Group II – middle cattle from 2 to 6 years,

18 Group III – older cattle from 6 to 12 years,

19 Group IV – old cattle over 12 years
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24 Samples for determining the content of heavy metals, i.e. arsenic, cadmium, lead and mercury
25 was taken (at least at 100 g from the same part of each tissue) from a part of the longissimus
26 muscle of back (*m. longissimus dorsi*) at the level of the last thoracic vertebrae, and samples
27 of liver from the tail lobe. The taken material was lyophilized (FreeZone6L, Labconco) and
28 homogenized .
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35 Samples were mineralized with pressure-microwave method (mineraliser MARS XPRESS,
36 CEM Corporation) in the presence of the mixture of nitric acid and hydrogen peroxide (6/1)
37 according to PN-EN 13805:2003. Simultaneously the whole procedure was carried out for a
38 reagent blanks. The digested solutions were diluted (in such a way so that the measured value
39 of concentration of a given element should be possibly in the centre of the operating range)
40 and there were determined in them: As by atomic absorption spectrometry with generation of
41 hydrides HGAAS according to PN-EN 14627:2005, Pb and Cd by atomic absorption
42 spectrometry with atomization in graphite furnace FGAAS (SpectrAA 880Z, Varian)
43 according to PN-EN 14084:2004, Hg by cold-vapours with the method of atomic absorption
44 spectrometry according to PN-EN 13806:2003. The content of heavy metals in tissues was
45 expressed in mg/kg of fresh mass, taking into account all quantitative processes during
46 preparation of the material and considering reagent blanks .
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58 The blanks were in contact with homogenizer, equipment and containers for similar time as
59 samples. A strict analytical quality control programme was employed during the study, the
60 results of which are presented in table 1. The limits of detection, expressed as a concentration

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3 in the tissue, was based on the mean sample weight for that tissue type. Quality control of
4 analytical measurements was performed using blank samples and the following certified
5 reference materials: BCR – 185R bovine liver from the Institute for Reference Materials and
6 Measurements for the determination of lead, cadmium, arsenic and NIST - RM 8414 bovine
7 muscle powder from the National Institute of Standards and Technology for the determination
8 of mercury.
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16 Results were subjected to statistical-mathematical analysis which included:

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20 1. Calculation of basic statistical measures of position (arithmetic mean) and the minimum
21 and maximum values were given.
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23 2. One-factor variance analysis which was checked by Tukey's confidence intervals, at to
24 levels of significance $P \leq 0.01$ and $P \leq 0.05$. The null hypothesis in variance analysis was
25 verified by the test formulated by Fisher Snedecor (test F).
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27 3. Calculation of the values of coefficients of simple correlation for the purpose of
28 determining the correlation between the content of examined metals in meat and liver of
29 cattle.
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35 Calculations were made basing on the program STATISTICA ver. 6.0.
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38 **Results and discussion**

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42 Variability of the chemical composition of beef depends on the sex, degree of musculature
43 and de-fatting of carcasses (Salvini et al. 1998; Williams et al. 1983) and on age, breed or
44 category of cattle (Florek and Litwińczuk 2002; Litwińczuk et al. 1999).
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49 From among farm animals, cattle are the species most susceptible to the effect of heavy
50 metals, especially lead – the element which often is the cause of poisoning with these animals
51 (Monkiewicz and Jaczewski 1990; Żmudzki et al. 1991). Susceptibility of cattle to the toxic
52 effect of Pb results, among other things, from a long period of pasture feeding during the year.
53 Particularly dangerous is pasturing cows in roadside ditches due to considerable
54 contamination of their flora with lead.
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3 The quantities of metals (Cd, Pb, Hg and As) in muscles and internal organs of farm animals
4 provide an accurate reflection of the level of these elements in the environment of the
5 animals' breeding and confirm usability of cattle as test organisms of contamination of the
6 production environment (Krupa 1997).
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12 In table 2 the content of heavy metals in tissues of cattle in the years 2002-2006 is given. The
13 data show that the content of cadmium in a muscle fluctuated from 0.012 mg/kg in 2002 to
14 0.018 mg/kg in 2005, a 50% increase (differences statistically significant $P \leq 0.05$). The
15 content of mercury both in the muscle as well as in the liver in this period did not exceed
16 0.001 mg/kg. In the tissues of cattle the presence of arsenic was not detected. In turn, the
17 content of Pb in beef liver in the years 2002-2004 was about 0.147 mg/kg. The concentration
18 of this element in the liver slightly increased in 2005 by about 6% and by 25% in 2006
19 (however, differences statistically significant $P \leq 0.05$ occurred only between the years 2002
20 and 2006). The situation was a bit different in the case of concentration of Cd in the beef
21 liver. The content of this metal slightly increased in successive years. It should be also noted
22 that the quantity of this element exceeded the permissible level 0.5 mg/kg (Commission
23 Regulation 466/2001/EC) in as many as four samples of liver.
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35 The average concentrations of lead in muscle of cattle in the years 2002 – 2006
36 (table 2) were twice the average concentration of domestic level – 0.027 mg Pb/kg,
37 determined in 1990 for muscles of this species of animals by Żmudzki et al. (1991). Also the
38 average content of Pb in the liver of cattle in the tested population in all analyzed years turned
39 out to be higher than the level of 0.135 mg/kg determined by these authors
40 (table 2). In the case of the content of mercury, both in muscles as well as in the liver, higher
41 values (about 0.003 mg/kg) than in this paper were obtained by other authors (Krupa 1997;
42 Żmudzki et al. 1991).
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51 The content of heavy metals in tissues of cattle of different age are presented in
52 table 3. The data show that the content of lead in meat of cattle was similar in groups I and II
53 and fluctuated around 0.051 – 0.054 mg/kg. The quantity of this element increased in
54 comparison with group I by 23% in group III and by 47% in group IV. Statistically significant
55 differences were confirmed between groups I and IV, II and IV, and also I and III. However,
56 the content of cadmium in meat (0.009 mg/kg) was the lowest in group I and increased in
57 successive age groups respectively by: 44%; 55% and 133% (high correlation $r=0.67$).
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3 Statistically significant differences were confirmed only for groups I and IV. In turn, the
4 concentration of mercury in meat and in the liver in particular age groups was lower than
5 0.001 mg/kg. In the analyzed age groups of cattle there was not stated the presence of arsenic
6 in meat or in the liver. In the case of the content of lead and cadmium in the liver the lowest
7 level was stated in group I respectively: 0.125 mg/kg and 0.123 mg/kg. The concentration of
8 these metals in the liver increased together with age of animals respectively by: 3%; 15% - in
9 group II, 13%; 64% - in group III, and 43%; 126% - in group IV (very high correlation
10 $r \geq 0.80$). It should be also noted that for cadmium in the liver there was exceeded the
11 permissible level (0.5 mg/kg) in two samples in group IV and in one case in group III.
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21 In the research of Krupa et al. (1995) concerning the concentration of cadmium in tissues of
22 cattle of the region of south-eastern Poland the differences in quantities of this metal in
23 muscles and organs of cows and young cattle turned out to be statistically irrelevant. The level
24 of accumulation of Cd in tissues of young cattle and cows at that time was respectively: in
25 muscles 0.016 and 0.021 mg/kg, in the liver 0.262 and 0.331 mg/kg. And in kidneys it was
26 significantly higher than in muscles and in the liver, constituting respectively: 0.836 and
27 1.212 mg/kg. In other research of this author (Krupa 1997) on bulls of the age of 18 – 22
28 months there were obtained higher values of the content of cadmium in meat (0.023 mg/kg),
29 and also of the content of mercury in meat (0.003 mg/kg) and in the liver (0.0037 mg/kg) than
30 in this paper (table 3) for the same age group of animals. In turn, this author obtained in cattle
31 smaller quantities of lead in the liver (0.077 mg/kg) and the concentration of lead in meat
32 (0.049 mg/kg) and cadmium in the liver (0.115 mg/kg) at similar level as in this paper. The
33 same author in the research (Krupa et al. 1994; 1995) concerning the assessment of
34 accumulation of lead in tissues of young cattle and cows of the region of south-eastern Poland
35 did not observe significant differences in respect of the quantity of Pb in muscles and kidneys,
36 however, significantly more of this metal accumulated in livers of older specimens. During
37 the research presented in quoted papers there also was not stated a dependence on this factor
38 despite noting some differentiation of the level of Hg with younger and older specimens.
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54 Wałkuska and Saddour (1992), however, indicate definitely higher concentration of lead in
55 the liver of young beef cattle (0.64 mg/kg) and cows (0.77 mg/kg), and lower with calves
56 (0.018 mg/kg). Salisbury and Chan (1991), assessing in the years 1982-1989 in this respect
57 the livers and kidneys of young fattening beef cattle in Canada, present as average values for
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3 this population the following ones respectively: 0.07 and 0.11 mg/kg Pb, and for calves a bit
4 higher since amounting to 0.10 and 0.15 mg/kg.
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8 Similar as in this paper (table 3) content of lead (0.050 mg/kg) and cadmium (0.004 mg/kg) in
9 meat of young slaughter cattle was obtained by Doganoc (1996). However, a lower quantity
10 of these elements respectively: (0.005 – 0.015 mg/kg) and (0.001 – 0.002 mg/kg) in beef was
11 obtained by other authors (Gonzales-Weller 2006; Jorhem et al. 1991; Larsen et al. 2002;
12 López et al. 2000; Niemi et al. 1991; Tahvonen and Kumpulainen 1994). In turn, López et al.
13 (2002) found a bit higher quantities of Pb, Cd and As in beef than in veal respectively: 0.017;
14 0.009 mg Pb/kg, 0.0014; 0.0013 mg Cd/kg and 0.005; 0.004 mg As/kg.
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23 In the research of Krupa et al. (1994) on concentration of arsenic in tissues of cattle there was
24 stated its presence at the level of thousandth parts of mg As/kg. In this research there were not
25 shown significant differences between the quantity of this element in muscles and in kidneys
26 and age. However, this factor decided on significantly higher level of arsenic in livers of
27 older animals (0.0097 mg/kg) than in young cattle (0.0065 mg/kg). In turn, with the cattle of
28 Krośnieńskie and Rzeszowskie provinces examined by the same team of authors (Krupa et al.
29 1995), and slaughtered at the age of 13-18 months and at the age of 7-11 years, a slightly
30 higher level of this metal was stated in muscles (0.0056 mg/kg) and in the liver (0.0112
31 mg/kg) of young specimens. With cows there was a bit less of arsenic, respectively: 0.0045
32 and 0.0086 mg/kg. The reverse dependence occurred in contamination of kidneys by this
33 metal, in which the quantities of arsenic were higher than in meat and in the liver, with young
34 cattle amounting on average to 0.0102 mg/kg, and in cows 0.0119 mg/kg (Krupa et al. 1995).
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46 Correlative dependences between the content of heavy metals in tissues of cattle were
47 presented in table 4. The data show that the biggest dependence occurred between the content
48 of lead in the liver and in meat ($r = 0.95$), between the concentration of cadmium in the liver
49 and in meat ($r = 0.91$), and also between the quantity of Cd and Pb in meat
50 ($r = 0.93$). However, a bit lower correlative dependencies occurred in the following pairs Cd
51 in liver – Pb in liver ($r = 0.89$), Pb in liver – Cd in meat ($r = 0.87$), and also Hg in liver – Hg
52 in meat ($r = 0.62$). Żarski et al. (1994) assessing the content of mercury in tissues of young
53 cattle obtained the strongest relation between the content of Hg in liver and muscles ($r =$
54 0.62). Krupa (1997), however, stated that in tissues of cattle the significant correlations
55 occurred most often between the content of cadmium and lead and the content of mercury and
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3 arsenic. From among the essential coefficients of correlation the highest correlation (r from
4 0.80 to 0.87) was obtained by this author between the content of lead and cadmium in
5 muscles. In turn, he noted a significant but negative correlation between the content of
6 cadmium and the content of mercury in tissues of cattle ($r =$ from -0.40 to -0.48).
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10 11 12 **Conclusions**

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16 The contamination of meat of cattle by Cd and Pb clearly depends on the age of these
17 animals. In muscles and in the liver of the animals arsenic was not detected above 0.001
18 mg/kg. However, the average concentration of mercury in these tissues was similar (≤ 0.001
19 mg/kg) in all age groups of cattle. Three samples exceeded the maximum permissible
20 concentration of cadmium in the liver of cattle of age groups III and IV. The livers from
21 these age groups should be used for consumption only to a limited extent. Between the
22 content of lead and cadmium in the liver and the concentration of these elements in muscles
23 there occurred very high positive correlative dependence.
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31 32 **References**

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Table 1. Results of the analytical quality control programme used in the determination of cadmium (Cd), lead (Pb), mercury (Hg) and arsenic (As) in bovine samples

Quality characteristics	Pb	Cd	As	Hg
Detection limit in samples (mg/kg)				
Muscle	0.006	0.001	0.001	0.0001
Liver	0.012	0.002	0.001	0.0001
Certified Reference Material BCR – 185R (Bovine Liver)				NIST - Reference Material 8414 (Bovine Muscle Powder)
Measured mean ± SD (mg/kg)	0.163 ± 0.008	0.526 ± 0.019	0.029 ± 0.003	0.0045 ± 0.0005
Certified mean ± SD (mg/kg)	0.172 ± 0.009	0.544 ± 0.017	0.033 ± 0.002	0.005 ± 0.003
Recovery (%)	94.8	96.7	87.9	90.0
Relative standard deviation (%)	5	4	10	11

Table 2. The content of heavy metals in meat and liver of cattle in the years 2002-2006 (mg/kg)

Specification	2002			2003			2004			2005			2006			The significance of differences	
	\bar{x}	x_{\min}	x_{\max}	\bar{x}	x_{\min}	x_{\max}	\bar{x}	x_{\min}	x_{\max}	\bar{x}	x_{\min}	x_{\max}	\bar{x}	x_{\min}	x_{\max}		
Meat:																	
Pb	0.060	0.047	0.069	0.062	0.052	0.072	0.062	0.051	0.073	0.066	0.057	0.089	0.060	0.041	0.086	2002<2005*	
Cd	0.012	0.006	0.015	0.015	0.007	0.021	0.016	0.007	0.019	0.018	0.009	0.031	0.015	0.005	0.034		
Hg	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.001		0.001
As	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd		
Liver:																	
Pb	0.147	0.079	0.209	0.148	0.083	0.271	0.148	0.071	0.302	0.156	0.087	0.389	0.183	0.097	0.445	2002<2006*	
Cd	0.159	0.024	0.434	0.172	0.098	0.478	0.189	0.121	0.594	0.200	0.098	0.558	0.191	0.082	0.378		
Hg	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.001		0.002
As	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd		

Explanation:

nd – presence not stated;

- As over 0.001 mg/kg;

* - differences significant at $P \leq 0.05$.

Table 3. The content of heavy metals in meat and liver of cattle of different age (mg/kg)

Specification	Groups												r	The significance of differences
	I			II			III			IV				
	\bar{X}	x_{\min}	x_{\max}	\bar{X}	x_{\min}	x_{\max}	\bar{X}	x_{\min}	x_{\max}	\bar{X}	x_{\min}	x_{\max}		
Meat:														
Pb	0.051	0.047	0.067	0.054	0.050	0.076	0.063	0.052	0.075	0.075	0.053	0.089	0.72*	I<IV**; II<IV**; I<III*
Cd	0.009	0.006	0.019	0.013	0.007	0.021	0.014	0.010	0.023	0.021	0.010	0.031	0.67*	I<IV**
Hg	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	-	-
As	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-
Liver:														
Pb	0.125	0.079	0.141	0.129	0.047	0.240	0.141	0.097	0.302	0.179	0.121	0.389	0.83*	I<IV**; I<III*
Cd	0.123	0.025	0.481	0.141	0.110	0.491	0.202	0.013	0.528	0.278	0.016	0.558	0.80*	I<IV**; II<IV**; I<III*
Hg	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	-	-
As	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-

Explanation:

nd – presence not stated;

- As over 0.001 mg/kg;

* - differences significant at $P \leq 0.05$.

** - differences significant at $P \leq 0.01$.

Table 4. Coefficients of simple correlation between the content of heavy metals in meat and liver of cattle

Pb meat	-							
Cd meat	0.93*	-						
Hg meat	0.45	0.35	-					
As meat	-	-	-	-				
Pb liver	0.95*	0.87*	0.41	-	-			
Cd liver	0.48	0.91*	0.35	-	0.89*	-		
Hg liver	0.53	0.37	0.62*	-	0.39	0.41	-	
As liver	-	-	-	-	-	-	-	-
	Pb meat	Cd meat	Hg meat	As meat	Pb liver	Cd liver	Hg liver	As liver

* - coefficients significant at $P \leq 0.05$