RESEARCH ARTICLE



Zinc, copper, cadmium, and lead levels in cattle tissues in relation to different metal levels in ground water and soil

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Abstract

The objective of the present study was to investigate the interaction between environmental (water and soil) levels of zinc, copper, cadmium, and lead levels, as well as their content in Hereford beef cattle tissues in five districts (D_1 —western area, D_2 and D_3 central area, D₄ and D₅—eastern area) of the Orenburg region. Soil metal levels were assessed using atomic emission spectrometry, whereas water and tissue (liver, kidney, muscle, heart) metal content was studied using inductively coupled plasma-mass spectrometry. The obtained data demonstrate that the highest levels Zn in soil and water (p < 0.001), as well as cattle muscle, liver, and kidney (p < 0.05) were observed in D₄ and D₅ (eastern area), exceeding the maximum permissible concentration levels (MPCL) for drinking water and muscle for all regions. Similar associations were found for Cu levels. The highest soil and water Cd and Pb content were observed in D_2 (central area) and D_5 (eastern area), respectively. At the same time, cattle tissue Cd and Pb content did not correspond to the respective environmental levels. Correlation analysis demonstrated that water and soil Zn and Cu content directly correlated with muscle, liver, and kidney, but not heart metal content. At the same time, water Cd levels were negatively interrelated with muscle cadmium content but correlated directly with hepatic metal content. Both water and soil Pb levels positively correlated with renal metal levels in cattle. In turn, soil lead content was inversely associated with muscle metal levels. Regression analysis also demonstrated a significant association between environmental and tissue levels of Zn and Cu. The models adjusted for all studied elements demonstrated a significant effect of metal interaction on tissue metal levels. Hypothetically, excessive environmental Zn, and possibly Cu, levels may affect the uptake of heavy metals including Cd and Pb from the environment.

Keywords Soil · Water · Copper · Cattle · Zinc · Cadmium

Introduction

Essential metals and trace elements are required for normal organism functioning. In particular, due to their structural, catalytic, and signaling function, trace elements play an important role in growth and development (Fraga 2005;

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Skalnaya and Skalny 2018). In turn, toxic metals and metalloids are known to induce adverse health effects due to their toxic properties both in human and animals (de Vries et al. 2007). The one of the mechanisms of metal toxicity is interference with essential elements and minerals, being most significant for zinc and cadmium (Moulis 2010).

Recent development of industry resulted in an increase in essential and toxic metal emissions into the environment. Consumption of contaminated foods is also the significant source of human metal overload. Metal accumulation in livestock was shown to be a link between environmental pollution and human exposure (Reilly 2008; Bortey-Sam et al. 2015). In particular, a significant association between tissue cattle metal levels and human exposure risk was revealed (Roggeman et al. 2014).

Environmental metal levels were associated with livestock body burden in the areas with high (Farmer and Farmer 2000; Sedki et al. 2003; Cai et al. 2009) and low pollution (Miranda

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et al. 2009). Moreover, cattle were proposed as the potential biomonitor of soil metal(loid) pollution (Alonso et al. 2002a). At the same time, data on the association between environmental levels of metals and their tissue content in livestock are unclear (Rajaganapa et al. 2011). The role of trace element interaction was also shown to have a serious impact on the kinetics of trace elements in cattle (Alonso et al. 2002b).

Orenburg region is an industrial area characterized by increased metal emissions into the environment. It has been demonstrated that different industrial activities may underlie the regional difference in environmental metal pollution in the region (Rusanov et al. 2011). Particularly, mining and smelting may result in increased heavy metal emissions (Cheng and Hu 2010; Li et al. 2014), whereas gas and petrochemical industry do not contribute significantly to environmental heavy metal levels in the region (Salnikova et al. 2018a, b). Certain studies have demonstrated that different environmental levels of metals in Orenburg region may have a significant impact on cattle tissue metal content (Miroshnikov et al. 2010).

Therefore, the objective of the present study was to investigate the interaction between environmental (water and soil) levels of zinc, copper, cadmium, and lead levels, as well as their content in beef cattle tissues in different locations of the Orenburg region with intensive industrial development.

Materials and methods

Location

The protocol of investigation was approved by the Institutional Ethics Committee (Orenburg State University, Orenburg, Russia). All information about economic and industrial development of the Orenburg region used in the present study were obtained from governmental open access sources.

The study was performed in five districts of the Orenburg region: (1) District 1—Sorochinsk district, (2) District 2-Sakmara district, (3) District 3-Sol-Iletsk district, (4) District 4-Kvarkeno district, and (5) District 5-Svetly district. According to soil characteristics and chemical content, the Orenburg region is divided into three areas: western (including D₁), central (including D_2 and D_3), and eastern (including D_4 and D_5) (Prikhozhai et al. 2004). The areas of the region are also characterized by different industrial activities (http://www. orenburg-gov.ru/Info/Economics/Industry/) with more than 80 developed mineral deposits (Antoninova et al. 2012). Particularly, the western area is characterized by oil processing industry. Gas-chemical complex, coal mining, and halite salt mining are primarily located in the central area. At the same time, the majority of industrial companies primarily heavy metal industry (copper, zinc, iron, nickel, ferronickel, steel, chromium processing) are located in the eastern area of the Orenburg region. Correspondingly, the eastern area is responsible for 73% of total atmospheric emissions of the region in 1990s (Chibilev 1999).

Soil and water sampling and preparation

A total of 15 soil and ground water samples were collected from each of the districts. Briefly, 75 soil samples from the top soils (10–15 cm) were collected in agreement with the state recommendations (Derzhavin and Bulgakov 2003). The obtained samples were cleaned from the mechanical contaminants, dried on air in dark conditions at room temperature, and sieved to 1 mm and subsequently. The obtained soil samples (5 g) were added with ammonium acetate buffer (50 ml, pH = 4.8) (Gleyzes et al. 2002) with subsequent incubation for 24 h. The resulting solution was filtered into 100-ml flask and the volume was adjusted to 100 ml with buffer solution for further analysis.

Of the water samples, 75 were collected from wells with 10-m depth of water sampling in agreement with the state recommendations (GOST R 51593–2000).

Animals and tissue sampling

A total of 75 healthy males of Hereford breed (*Bos taurus*) aged 2.5–3 years were examined (n = 15 for each district). No significant difference in the age of cattle from different regions was observed. In the stall period (September/October–May), the animals were fed primarily with wheat grass and sainfoin hay, haylage of Sudan grass, and barley grains, whereas in the pasture period (May–September/October), cattle were grazed on cereal grasses.

Similar parts of the organs were sampled from all animals including liver (lobus caudatus), left kidney (cranial part), muscle (m. biceps femoris), and heart (myocardium of the left ventricle). The organs and tissues were separated from connective tissue and rinsed with ice-cold physiological saline. Three samples of each organ from one animal were used for analysis. Subsequently, the samples were subjected to microwave acid digestion. Briefly, 50-100 mg of the tissue sample was introduced into Teflon tubes with subsequent addition of concentrated HNO₃ (Sigma-Aldrich Co., St. Louis, MO, USA) and digested in a Berghof SW-4 DAP-40 (microwave frequency, 2.46 GHz; power, 1450 W) microwave system (Berghof Products + Instruments GmbH, 72,800 Eningen, Germany) at 170-180 °C for 20 min. The obtained solutions were added with distilled deionized water to a final volume of 15 ml for further analysis.

Soil metal analysis

Assessment of mobile Zn, Cu, Cd, and Pb levels in soil samples was performed using atomic absorption spectrophotometer Formula FM 400 (LABIST, Russia) with hollow-cathode lamps. The system was calibrated with standard metal solutions with concentration of 0.5–15 mg/l. State Standard Samples were used as stock solutions for preparation of standard calibration solutions (Nos. 2293–82, 2294–82, 2295–82, 2296–82, 2297–82). Laboratory quality control of soil sample analysis was performed using spiked (with ZnSO₄, CuSO₄, Pb(CH₃COO)₂, and CdCl₂) samples. The mean recovery rates for all analyzed metals were within the range of 89–110%.

Water and tissue metal analysis

Water and tissue Zn, Cu, Cd, and Pb content was assessed using inductively coupled plasma-mass spectrometry at NexION 300D (PerkinElmer Inc., Shelton, CT, USA) equipped with seven-port FAST valve and ESI SC-2 DX4 autosampler (Elemental Scientific Inc., Omaha, NE, USA). System's calibration was performed using the metal solutions with a final concentration of 0.5, 5, 10, and 50 µg/l prepared from the Universal Data Acquisition Standards Kit (PerkinElmer Inc.). Ten-microgram-per-liter yttrium-89 and rhodium-103 solutions prepared from Yttrium (Y) and Rhodium (Rh) Pure Single-Element Standard (PerkinElmer Inc. Shelton, CT, USA) were used for internal online calibration. Laboratory quality control using ClinCheck Plasma Control, lot 129, levels 1 and 2 (RECIPE Chemicals + Instruments GmbH, Germany) and GBW09101 (Shanghai Institute of Nuclear Research, Shanghai, China) was performed regularly. The recovery rates for all studied metals were within the range of 95%-108%.

Statistical analysis

Statistica 10.0 (Statsoft, Tulsa, OK, USA) was used for data processing. Shapiro-Wilk test was used for data normality assessment. Medians and 25–75 percentile boundaries were used as descriptive statistics as the data were not characterized by Gaussian distribution. Paired group comparisons were performed using non-parametric Mann-Whitney U test. False discovery rate (FRD) adjustment for p value was applied due to multiple comparisons. Correlation between the values was evaluated using Spearman's rank correlation coefficient.

Multiple regression analysis was also performed in order to assess the contribution of the environmental factors (water and soil metal content, and geographical location) into the level of particular metals in animal tissues. Particularly, Zn, Cu, Cd, and Pb levels in cattle liver and kidneys were used as dependent variables. Model 1 includes ground water and soil levels of a particular metal (Zn, Cu, Pb, or Cd), as well as the studied district as independent variables (predictors). The model 2 was adjusted for water and soil levels of all metals studied (Zn, Cu, Pb, and Cd).

The results of all tests were considered significant at p < 0.05.

Results

The obtained data (Table 1) demonstrate that the highest levels of water zinc were observed in D_4 and D_5 , exceeding the levels in D_1 , D_2 , and D_3 by a factor of nearly 4. Similarly, the levels of Zn in soil of D_4 and D_5 significantly exceeded the respective values of D_1 and D_2 by a factor of more than 2 and those in D_3 by a factor of more than 4. In turn, soil Zn content in D_1 and D_2 was 85% and 104% higher as compared to that in D_3 .

Similar regional patterns were observed in beef cattle tissue zinc content (Table 1). Particularly, the highest muscle Zn levels were detected in D₅, being higher than the respective values in D_2 , D_3 , and D_4 by 27%, 14%, and 14%. Hepatic Zn levels in D_1 , D_2 , D_4 , and D_5 were higher as compared to D_3 (Sol-Iletsk district) values by 7%, 5%, 11%, and 13%, respectively. The highest heart Zn levels were observed in D_1 and D₄, being elevated by 18%, 17%, and 37%, and 16%, 15%, and 35%, when compared to D_2 , D_3 , and D_5 values, respectively. Finally, kidney Zn content in beef cattle from D₄ and D₅ was elevated by 12%, 18%, and 37%, as well as 9%, 16%, and 34%, as compared to the respective values in D_1 , D_2 , and D₃ districts. In turn, the lowest renal Zn content was revealed in beef cattle from D3, being lower in comparison to the values found for D1 and D2 districts by 19% and 14%, respectively.

Region-specific differences in water concentration were more significant in the case of copper (Table 2). In particular, ground water Cu levels in D_4 and D_5 exceeded the respective values in D_1 , D_2 , and D_3 by a factor of more than 150. In turn, soil Cu content was also the highest in D_4 and D_5 , being more than 5-fold and 3-fold higher than those in D_1 , D_2 , and D_3 , respectively.

However, metal levels in beef cattle tissues were differentially affected by geographic factor (Table 2). In particular, the lowest levels of muscle Cu were observed in D₂, being lower than the respective levels in D1, D3, D4, and D5 districts by 57%, 46%, 49%, and 57%. The lowest Cu levels in liver were detected for beef cattle from D₃, being decreased by 19%, 15%, 25%, and 30% when compared to the respective values from D₁, D₂, D₄, and D₅. As for heart Cu content, the lowest absolute values were detected in D₁ and D₅, although no significant group difference was observed. At the same time, maximal levels of kidney Cu were revealed in D₅, exceeding those in D₁ and D₂ by 25% and 11%.

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Sample	District 1	District 2	District 3	District 4	District 5
Water	1.178 (1.088–1.446)	1.232 (1.176–1.582)	1.144(1.092 - 1.469)	$4.690(3.915-4.995)^{1,2,3}$	$4.618(4.181 - 5.034)^{1.2.3}$
Soil	8.455 (7.565–10.235)	9.310 (8.330–11.270)	$4.560(4.176-5.520)^{1.2}$	$22.080(20.400-27.600)^{1,2,3}$	$19.950(17.850-24.780)^{1.2.3.4}$
Muscle	46.248 (42.812–47.645)	$38.818(35.866-44.336)^{1}$	43.345 (42.164–46.074) ²	43.276 (42.157–46.145)	49.444 ($46.659-55.853$) ^{2,3,4}
Liver	99.612 (96.445–102.822)	97.894 (93.758–101.323)	$92.880(89.093 - 94.894)^{1,2}$	$102.937 (96.851 - 106.896)^3$	$105.363 (97.932 - 109.797)^3$
Heart	75.340 (72.543–78.588)	$64.104 (62.041 - 69.106)^{1}$	64.647 (59.742–71.347) ¹	74.152 (66.046–78.082) ^{2,3}	55.150 (50.177–62.128) ^{1,3,4}
Kidney	88.393 (86.725–92.434)	83.351 (81.449 – 86.053) ¹	71.945 (67.232–81.051) ^{1,2}	$98.686(87.948 - 101.533)^{2,3}$	$96.708 (84.746 - 100.652)^{2,3}$

and heef cattle tissues (mo/kg) in different districts of the Orenhurg region soil (mo/l) in water Zine levels Tahle 1 ^{1, 2, 3, and 4} indicate significant group difference in comparison to districts 1, 2, 3, and 4 according to Mann-Whitney U test, respectively Data expressed as median (25–75 percentiles); numbers

Environmental cadmium levels were also region-specific (Table 3). In particular, the lowest levels of ground water Cd were detected in the eastern D_4 and D_5 , being lower than those in D₁, D₂, and D₃ by a factor of 9, 62, and 9, respectively. Similarly to water, the highest levels of Cd in soil were registered in D₂, exceeding the respective levels in D₁, D₃, D₄, and D₅ by 88%, 652%, 210%, and 151%.

Notably, tissue Cd levels in beef cattle were less variable than the environmental metal content (Table 3). Particularly, the highest levels of muscle Cd content were revealed in D₅ (Svetly district), being higher than those in D₁, D₂, D₃, and D₄ by a factor of 6–10. Oppositely, beef cattle from D₅ were characterized by the significantly decreased levels of liver Cd content, being lower than those in D_2 , D_3 , and D_4 by 37%, 35%, and 37%, respectively. No group differences in cardiac Cd levels were observed in relation to different areas. The highest renal Cd content was observed in D₂ and D₄.

Ground water Pb levels were increased in D₄ and D₅ as compared to D_2 and D_3 by a factor of more than 2. Soil Pb levels in D₅ also exceeded the respective values from other locations (D_{2,3,4}) by 168%, 62%, and 110%.

Different patterns of Pb content were observed in tissues of beef cattle (Table 4). In particular, cattle from D₅ were characterized by significantly reduced muscle Pb levels, being lower than those in D_1 , D_2 , D_3 , and D_4 by a factor of 3, 5, 4, and 7, respectively. No geographic difference in hepatic lead content in cows was observed. Cardiac Pb levels in D1 and D2 were elevated in comparison to D_3 and D_4 , by 65% and 35%, respectively. Finally, renal Pb levels in D2 were nearly 10-fold lower than the respective values of beef cattle living in other districts.

Correlation analysis revealed a significant association between environmental tissue metal levels, being more significant for essential Zn and Cu (Table 5). In particular, water and soil Zn content directly correlated with muscle, liver, and kidney metal content. At the same time, cardiac Zn levels were negatively associated with both water and soil metal levels. Similarly, soil and water Cu concentrations were positively associated with muscle, liver, and kidney metal levels, but not heart Cu content. Notably, less significant associations between environmental and beef cattle tissue levels were observed for Cd and Pb. In particular, water Cd levels were negatively interrelated with muscle metals, but correlated directly with hepatic metal content. Soil Cd levels did not significantly interact with tissue metal levels in beef cattle. Both water and soil Pb levels positively correlated with renal metal levels in cows. In turn, soil lead content was inversely associated with muscle metal levels.

The obtained data demonstrate that cattle liver and kidney metal levels significantly correlate with environmental levels. Therefore, we have performed multiple regression analysis of the potential determinants of hepatic (Table 6) and renal (Table 7) cattle metal levels. In a crude model including only

Sample	District 1	District 2	District 3	District 4	District 5
Water	0.005 (0.004-0.006)	0.006 (0.006–0.008) 1	0.004 (0.004–0.006) ²	0.965 (0.910-1.350) 1,2,3	0.991 (0.944–1.307) 1,2,3
Soil	0.466 (0.417-0.564)	0.532 (0.441-0.618)	0.532 (0.487–0.644) 1	2.870 (2.596–2.936) 1,2,3	1.788 (1.580–2.492) 1,2,3
Muscle	2.179 (1.872-3.018)	0.933 (0.816–1.189) 1	1.727 (1.188–2.144) ²	1.823 (1.267–2.135) ²	2.182 (1.636-3.008) ²
Liver	16.17 (14.64–18.32)	15.46 (13.75–17.67)	13.15 (11.42–15.34) 1,2	17.60 (13.95–18.71) ³	18.78 (16.34–19.44) ^{2,3}
Heart	12.28 (9.55–14.32)	14.82 (12.48–16.45)	16.42 (12.34–18.75)	16.42 (12.34–18.75)	12.39 (10.36–14.29)
Kidney	16.19 (14.29–18.08)	18.26 (15.65–19.58)	18.85 (16.45–19.64)	19.15 (17.29–21.29) ¹	20.29 (18.74–23.92) 1,2

Data expressed as median (25–75 percentiles); numbers ^{1, 2, 3, and 4} indicate significant group difference in comparison to districts 1, 2, 3, and 4 according to Mann-Whitney U test, respectively

water and soil zinc levels, as well as location, liver zinc was directly associated with water Zn concentrations and area of habitation. The model accounted for 28% of liver zinc variability. In an adjusted model, no particular factors were associated with hepatic Zn levels, although the model was still significant. In a crude model accounting for 16% of liver Cu variability, water Cu concentration was also associated with hepatic metal content. In an adjusted model, liver Cu levels were also significantly predicted by water (negatively) and soil (positively) Cd contents. Neither crude nor adjusted models were associated with hepatic lead levels. In turn, liver Cd levels were positively and negatively predicted by water and soil Cd levels, respectively. Although the model accounted only for 8% of the parameter variability, the overall effect was significant. Adjusted model was stronger associated with hepatic Cd levels, although no particular factors were associated with liver metal levels.

Environmental levels were also associated with kidney metal content (Table 7). A crude model accounted for 41% of kidney Zn content variability, whereas water Zn concentration and the area of habitation were significant predictors. Adjusted model was stronger associated with the parameter accounting for 54% of variability, and water Cu content was also a positive predictor of renal Zn levels. In turn, kidney Cu content was predicted by the area of investigation and both crude and adjusted model accounted for 24% and 28% of variability, respectively. Soil Pb levels were significantly associated with renal Pb content in a crude model. Adjustment for other covariates increased the predictive effect of the model up to 47%. Neither particular factors nor whole crude models were associated with renal Cd levels in beef cattle. In an adjusted model accounting for 38% of the parameter variability, soil lead levels and the area of habitation were significantly associated with renal Cd levels.

Discussion

The obtained data demonstrate that environmental and tissue levels of metals in beef cattle are region and metal specific (Fig. 1). In particular, both environmental and tissue zinc levels were characterized by an increase in the eastern areas of the region. The whole region studied could be considered as Zn-excessive, as the level of the metal in water and cattle muscle Zn content exceeded the MPCLs of 0.3 mg/l and 20 mg/kg, respectively. Nearly similar trends were observed for copper. In turn, the levels of cadmium and lead in tissues did not correspond to the environmental levels. The results of regression analysis demonstrated a significant effect of metal interaction in their uptake from the environment. At the same time, Cu, Cd, and Pb content in water and cattle muscles did not exceed the maximum permissible concentration levels (MPCL) for drinking water (MPCL: Cd-0.01 mg/l, Pb-0.1 mg/l, Cu—1.0 mg/l) and meat (MPCL: Cd—0.05 mg/kg, Pb—0.5 mg/kg, Cu—5.0 mg/kg).

The level of cadmium in ground water (mg/l), soil, and cattle tissues (mg/kg) in different districts of the Orenburg area Table 3

Sample	District 1	District 2	District 3	District 4	District 5
Water	0.0009 (0.0009–0.0011)	0.0062 (0.0059–0.0078) 1	0.0009 (0.0008-0.0011) ²	0.0001 (0.0000–0.0001) 1,2,3	0.0001 (0.0000–0.0001) 1,2,3,4
Soil	0.076 (0.068-0.092)	0.143 (0.128–0.173) ¹	0.019 (0.017–0.023) 1,2	0.046 (0.043–0.058) 1,2,3	0.057 (0.051–0.071) 1,2,3,4
Muscle	0.006 (0.004-0.010)	0.005 (0.003-0.006)	0.007 (0.004-0.010)	0.008 (0.006-0.010) ²	0.050 (0.040-0.067) 1,2,3,4
Liver	0.030 (0.026-0.034)	0.035 (0.028-0.046)	0.034 (0.028-0.039)	0.035 (0.028-0.046)	0.022 (0.018-0.031) 2,3,4
Heart	0.049 (0.023-0.078)	0.052 (0.044-0.073)	0.056 (0.028-0.087)	0.061 (0.030-0.087)	0.047 (0.033-0.071)
Kidney	0.029 (0.026–0.037)	0.045 (0.037–0.056) ¹	0.029 (0.027–0.042) ²	0.049 (0.039–0.077) 1,3	0.028 (0.019–0.037) ^{2,4}

Data expressed as median (25–75 percentiles); numbers ^{1, 2, 3, 4} indicate significant group difference in comparison to districts 1, 2, 3, and 4 according to Mann-Whitney U test, respectively

Sample	District 1	District 2	District 3	District 4	District 5
Water	0.019 (0.010-0.023)	0.010 (0.009–0.012) 1	0.009 (0.008–0.011) 1	0.022 (0.018–0.036) ^{2,3}	0.024 (0.020-0.030) ^{2,3}
Soil	3.800 (3.400-4.600)	1.587 (1.420–1.921) ¹	2.622 (2.401-3.174) 1,2	2.024 (1.870-2.530) 1,2,4	4.256 (3.808–5.286) 2,3,4
Muscle	0.031 (0.019-0.104)	0.048 (0.030-0.070)	0.044 (0.039-0.056)	0.068 (0.049–0.088) ³	0.010 (0.003–0.010) 1,2,3,4
Liver	0.487 (0.335-0.627)	0.464 (0.339-0.653)	0.482 (0.336-0.649)	0.464 (0.337-0.584)	0.503 (0.446-0.594)
Heart	0.282 (0.187-0.373)	0.233 (0.178-0.246)	0.165 (0.122–0.204) 1,2	0.174 (0.122–0.198) 1,2	0.177 (0.142-0.220)
Kidney	0.533 (0.306-0.695)	0.050 (0.040-0.065) 1	0.499 (0.404–0.517) ²	0.494 (0.277–0.693) ²	0.504 (0.373–0.676) ²

 Table 4
 Ground water (mg/l), soil, and cattle tissue (mg/kg) lead content in the studied districts

Data expressed as median (25–75 percentiles); numbers $^{1, 2, 3, \text{ and } 4}$ indicate significant group difference in comparison to districts 1, 2, 3, and 4 according to Mann-Whitney U test, respectively

The obtained data are generally in agreement with the earlier studies on soil Zn, Cu, Cd, and Pb content in Orenburg region in general (Khalitov 2008) as well as indications of higher soil and water levels of zinc and copper in the eastern area of the Orenburg region (Lestsova et al. 2009; Salnikova et al. 2018a, b). At the same time, the regional patterns of soil metal levels are shown to be dependent on industrial development of a particular area of the Orenburg region (Rusanov et al. 2011). In particular, increased levels of Zn, Cu, Pb, and Cd result from mining, smelting, and other industrial activities (Cheng and Hu 2010; Li et al. 2014), all being more developed in the eastern area of the Orenburg region (http://www. orenburg-gov.ru/Info/Economics/Industry/). Notably, multivariate analysis of metal content in pasturelands of north-west Spain demonstrated that Zn as well as Cd and Pb in soils have primarily anthropogenic origin, whereas Cu contents are dependent on geochemical factors (Franco-Uría et al. 2009).

The observed difference may be also associated with soil parameters. As stated earlier, the studied districts are located in three areas: western (including D_1), central (including D_2 and D_3), and eastern (including D_4 and D_5) (Prikhozhai et al. 2004). The predominating soil types in D1 included typical and southern chernozems; D2—alluvial soddy soils and

southern chernozem; D3—dark-chestnut soils, solonetzes, and southern chernozem; D4—typical, southern, and undeveloped chernozems; and D5—dark chestnut soils and solonetzes (Russkin et al. 1993). It has been demonstrated that different soil types contain different levels of heavy metals (Sun et al. 2013). Particularly, a number of parameters including soil acidity (Zeng et al. 2011), organic matter (Minkina et al. 2006), and salinity (Du Laing et al. 2008) may have a significant impact on metal bioavailability and its further involvement into food chain.

Zinc levels in cattle tissues were shown to be directly associated with environmental zinc levels, being in agreement with the earlier data. In particular, a study in Galicia (northwestern Spain) demonstrated that Zn levels in calves tissues are associated with soil Zn levels in a region under low environmental pollution. Particularly, liver zinc levels were characterized by a significant increase in association with elevation of soil metal levels, although no such association was revealed for muscle and kidneys. In turn, blood Zn concentrations tended to be inversely associated with soil Zn content (Alonso et al. 2002a). Similar associations were revealed in regions with higher rate of environmental pollution. A significant time dependence between cattle liver Zn content and metal exposure was revealed in a vicinity of a Kidston Gold

Sample		Zn		Cu		Cd		Pb	
		Water	Soil	Water	Soil	Water	Soil	Water	Soil
Muscle	r	0.414	0.328	0.258	0.201	-0.337	-0.160	-0.057	- 0.378
	р	< 0.001*	0.004*	0.026*	0.083	0.003*	0.171	0.626	0.001*
Liver	r	0.492	0.520	0.385	0.280	0.255	0.055	-0.015	0.078
	р	< 0.001*	< 0.001*	0.001*	0.015*	0.042*	0.639	0.902	0.505
Heart	r	-0.254	-0.172	-0.046	-0.001	-0.051	-0.119	-0.027	0.125
	р	0.028*	0.141	0.696	0.998	0.665	0.308	0.820	0.287
Kidney	r	0.538	0.5706	0.438	0.387	0.152	0.104	0.330	0.412
-	р	< 0.001*	< 0.001*	< 0.001*	0.001*	0.192	0.376	0.004*	< 0.001*

 Table 5
 Correlation between soil, water, and tissue metal content in beef cattle

Data expressed as correlation coefficients (r) and the respective p values

*Correlation is significant at p < 0.05

Independent	Zn				Cu				Pb				Cd			
Variables	Model 1		Model 2		Model 1		Model 2		Model	Model 2		Model 1			Model 2	
	β	d	β	d	β	d	β	d	β	d	β	d	β	d	β	d
Zn-water	0.769	0.041^{*}	0.807	0.150			0.719	0.226			0.961	0.162			0.216	0.720
Cu-water			-0.114	0.804	0.900	0.002^{*}	0.362	0.456			-0.778	0.168			-0.027	0.956
Pb-water			0.040	0.798			-0.066	0.688	-0.054	0.696	0.045	0.812			-0.239	0.155
Cd-water			-0.466	0.356			-1.094	0.043*			-0.164	0.791	0.570	0.008*	0.412	0.448
Zn-soil	0.209	0.438	-0.270	0.485			-0.767	0.064			-0.435	0.360			0.148	0.723
Cu-soil			0.083	0.770	-0.261	0.255	-0.203	0.499			0.056	0.871			0.251	0.411
Pb-soil			0.028	0.894			-0.266	0.236	0.088	0.477	0.037	0.887			-0.259	0.256
Cd-soil			0.638	0.148			1.165	0.014^{*}			0.243	0.651	-0.465	0.027*	-0.456	0.335
District	-0.434	0.036^{*}	-0.149	0.597	-0.340	0.086	0.059	0.845	0.029	0.826	0.118	0.733	-0.097	0.450	-0.464	0.130
Multiple R	0.562		0.606		0.445		0.537		0.091		0.221		0.344		0.515	
Multiple R^2	0.315		0.367		0.198		0.288		0.008		0.049		0.119		0.265	
Adjusted R^2	0.287		0.280		0.164		0.189		-0.034		-0.083		0.081		0.163	
d	< 0.001*		< 0.001*		0.001*		0.006*		0.897		0.945		0.029		0.013*	
Data expressed as regression coefficients (β) and individual <i>p</i> values (p^i) for a particular interaction. Model 1 includes hepatic Zn, Cu, Pb, and Cd content as dependent variable and ground water and soil levels of a particular metal, as well as the studied district as independent variables (predictors). Model 2 is adjusted for water and soil levels of other metals studied	l as regression icular metal,	n coefficient as well as t	ts (β) and ind he studied di	lividual p v strict as ind	alues (p^i) fo lependent vi	r a particula ariables (pre	r interaction. dictors). Mo	Model 1 in del 2 is adju	cludes hepat isted for wat	ic Zn, Cu, l er and soil	Pb, and Cd (levels of otl	content as d her metals :	lependent va studied	rriable and g	ground water	and soil

 Table 6
 Multiple regression analysis of the impact of water and soil metal content on hepatic metal levels in cattle

*The association is significant at p < 0.05

Independent variables	Zn				Cu				Pb				Cd			
	Model 1		Model 2		Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
	β	d	β	d	β	d	β	d	β	d	β	d	β	d	β	d
Zn-water	0.971	0.005*	- 0.376	0.399			- 0.529	0.344			0.202	0.672			0.717	0.168
Cu-water			1.534	< 0.001*	0.008	0.977	0.495	0.281			0.261	0.505			0.270	0.525
Pb-water			-0.090	0.467			-0.190	0.224	0.181	0.137	0.098	0.462			-0.191	0.187
Cd-water			-0.252	0.530			-0.834	0.101			-0.645	0.137	0.283	0.200	0.354	0.449
Zn-soil	0.145	0.556	-0.075	0.809			-0.482	0.215			-0.460	0.167			0.439	0.223
Cu-soil			0.031	0.892	-0.012	0.955	0.132	0.641			0.016	0.949			-0.255	0.332
Pb-soil			0.046	0.786			-0.214	0.311	0.339	0.003*	0.059	0.742			-0.511	0.011^{*}
Cd-soil			0.413	0.239			0.822	0.064			-0.003	0.994	-0.061	0.778	-0.448	0.272
Area	-0.665	0.001^{*}	-0.582	0.012^{*}	0.526	0.006*	0.884	0.003*	0.110	0.349	-0.191	0.430	0.167	0.210	-0.742	0.006^{*}
Multiple R	0.656		0.773		0.523		0.605		0.477		0.732		0.216		0.675	
Multiple R^2	0.430		0.597		0.274		0.366		0.228		0.536		0.047		0.456	
Adjusted R^2	0.406		0.541		0.243		0.278		0.195		0.472		0.006		0.380	
d	< 0.001*		< 0.001*		< 0.001*		< 0.001*		< 0.001*	*	< 0.001*		0.331		< 0.001*	

levels of a particular metal, as well as the studied district as independent variables (predictors). Model 2 is adjusted for water and soil levels of other metals studied *The association is significant at p < 0.05

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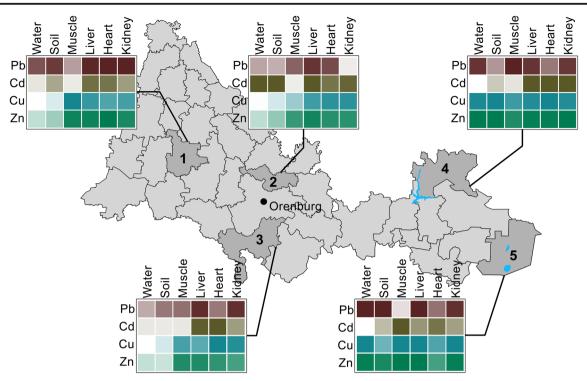


Fig. 1 Metal levels in environmental and cattle tissue samples in the studied districts of the Orenburg region. (1) District 1—Sorochinsk district, (2) District 2—Sakmara district, (3) District 3—Sol-Iletsk district,

(4) District 4—Kvarkeno district, and (5) District 5—Svetly district. The intensity of color is proportional to the maximal level of zinc obtained for each sample

Mine, North Queensland, Australia (Bruce et al. 2003). An increase in tissue Zn levels was also observed in the livestock living near a metal-production center of eastern Kazakhstan (Farmer and Farmer 2000). Particularly, the highest liver Zn content was observed in the districts with the highest hay and pasture grass Zn content, being a function of the distance from the pollution sources (Farmer and Farmer 2000). In addition, geographic difference in metal levels may be also associated with their different bioavailability due to regional variability of water parameters including pH and hardness (Golubkina et al. 2011; Salnikova et al. 2017).

Similarly, in a study by Miranda et al. (2009) in Galicia (NW Spain), it has been observed that cattle copper status also significantly correlates with environmental metal levels. Particularly, in the regression models, liver and kidney Cu contents were significantly associated with total and extractable soil copper ($R^2 = 0.873$, p = 0.001) and total and soil forage copper content ($R^2 = 0.711$, p = 0.013), respectively (Miranda et al. 2009). However, the obtained data revealed a weaker association between tissue Cu content and ground water and soil levels. Oppositely, a Spanish study by Alonso et al. (2002a) demonstrated a more significant interrelation between soil and tissue Cu content than for Zn (Alonso et al. 2002a). In addition, Cu and Zn levels were also characterized by a significant positive association both in soils (p < 0.001)and cattle liver (p < 0.05). At the same time, the results of the present study demonstrate more significant association between liver and soil Zn content as compared to Cu. This contradiction may be associated with the higher content of mobile forms of Zn in soils of the Orenburg region than of copper (Khalitov 2008). In addition, it has been demonstrated that Cu and Zn may compete for metallothionein binding in cattle, although the outcome of this competition depends on Cu/Zn ratio (López-Alonso et al. 2005).

The present findings do not correspond to the existing studies demonstrating a significant association between environmental cadmium and lead exposure and increased heavy metal body burden in cattle. Particularly, the earlier data demonstrate that elevated Cd and Pb levels from the soil are transferred to cattle liver and kidneys through a food chain. The authors also demonstrated that excessive Cd and Pb levels prevented Zn uptake from the environment (Cai et al. 2009). Proximity to the Pb-Zn mine in Kabwe, Zambia, was also directly associated with a significant increase in both Cd and Pb in liver and kidneys of cattle (Yabe et al. 2011). Similar observations were obtained in an Indian study, where increased blood and milk Cd and Pb were detected in animals living in the vicinity of a primary Pb-Zn smelter (Dwivedi et al. 2001). Moreover, significantly higher liver (29% and 84%) and kidney (67% and 141%) Pb and Cd accumulation was revealed in cattle from industrialized area as compared to the rural areas of Asturia (Spain), respectively (Miranda et al. 2005).

The absence of significant association between environmental and tissue levels of cadmium and lead may occur due to increased environmental exposure to zinc. In particular, Zn treatment was shown to reduce Cd accumulation in kidney and liver preventing its toxicity (Imed et al. 2008). The observed significant increase in Cd and Pb kidney levels in Znrich environments is generally in agreement with the observation of Zn-induced Cd retention in kidneys as the one of the mechanisms of the protective effect of Zn (Jihen et al. 2010). The molecular basis of Cd-Zn interaction includes a variety of mechanisms (Moulis 2010). It is proposed that Zn-induced metallothionein synthesis results in Cd redistribution (Brzóska and Moniuszko-Jakoniuk 2001), increasing kidney levels of Cd-metallothionein complex (Sabolić et al. 2010). Taking into account the role of metallothionein in Pb handling, similar mechanism may be proposed for Zn-Pb interaction (Kim et al. 2014). In particular, we have demonstrated that Zn supplementation results in redistribution of toxic metals including Pb, As, Sn, and Ni in rats (Skalny et al. 2015). These mechanisms may also underlie the observed inverse correlation between Cd in water and Pb in soil with Cd, Pb content in muscles. One can propose that increased intake of heavy metals may result in their tissue redistribution and higher excretion rate under zinc exposure and subsequent upregulation of metallothionein synthesis. In addition, copper status of cattle living in the Deza region (NW Spain) may also significantly affect tissue lead accumulation in cattle (Blanco-Penedo et al. 2006). In turn, Cd exposure was also shown to affect not only Zn metabolism but also Cu-Zn balance in humans (Satarug et al. 2018).

The present study has a number of limitations. First, more soil characteristics including organic matter content, acidity, and salinity should be studied in order to assess the factors affecting metal bioavailability. Second, the content of metals in animal feed (forage, hay) should be also determined. These limitations are to be addressed in further studies in order to estimate the interplay between metals and their transition from environment to the living organisms. Speciation analysis may provide additional information about the potential environmental sources and kinetics of metals in the food chain (Ajsuvakova 2018).

Conclusions

Therefore, the obtained data demonstrate that beef cattle metal contents are significantly associated with environmental Cu and especially Zn levels. Hypothetically, excessive environmental Zn, and possibly Cu, levels may affect the uptake of heavy metals including Cd and Pb from the environment. However, further studies are required to highlight the impact on environmental metal exposure on animal metal status and health. Acknowledgements The present study was supported by the Orenburg region government (Agreement No. 26, 31.07.2018).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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