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ORIGINAL ARTICLE

Relationship Between Lead (Pb) Concentration in Soil, Grass , Blood, Milk and δ-aminolevulinic Acid Dehydratase (ALAD) Activity , Hemoglobin (Hb) and Hematocrit (Hct) in Grazing Cows from Vicinity of Smelter "Trepça" in Kosovo

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KEYWORDS

Cows;
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ABSTRACT: This study aimed to evaluate current lead concentration (Pb) in topsoil, grass, blood and milk, ALAD activity, hemoglobin (Hb) and hematocrit (Hct) i cows (Simmental breed), rearing in three villages situated in different distances from the smelter "Trepça": Kalemend 2km, Boletin 3km and Zazhë 5km and Koliq 40km. The Pb concentration in samples is measured by atomic absorption spectrometry (AAS), blood ALAD activity is measured according the CEC standardized method. Pb concentration in topsoil and grass in the three villages from smelter is higher ($P<0.001$) than Pb concentration in reference. Blood lead level (BLL) in cows from smelter area is higher ($P<0.001$) than in control. Milk lead level (MLL) in cows from smelter area is higher compared with control. Blood ALAD activity in cows from smelter area is significantly ($P<0.001$) inhibited compared with control. There is adverse correlation ($r=-0.812$, $P<0.001$) between BLL and ALAD activity in cows from Kelmend, A positive correlation (($r=0.987$, $P<0.001$) is established between BLL and MLL in cows from Boletin. There is no significant difference of Hb and Hct values among each group of cows. There was progressive decrease of Pb concentration in topsoil, grass, blood and milk with increasing distance from the smelter. ALAD activity is proved as sensitive and useful biomarker at very low BLLs in cows. The vicinity of smelter still poses threat for livestock welfare and human health.

INTRODUCTION

Smelting of metal ores cause ambient contamination with different metals [1].Metals emitted by smelters as ultrafine particle fractions may travel in greater distance before sedimentation in the soil [2]. The residual metals in the soil may enter in the food chain. Mitrovicë is the second largest town in Kosovo. Lead and Zinc smelter "Trepça" is

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situated close to the Mitrovicë. The smelter had been in operation intermittently more than six decades. During this period of time a huge amounts of metals were released in town Mitrovicë and surrounding rural area [3]. An environmental audit ordered by the Kosovo authorities, warned that smelter should be closed as an "un-acceptable" source of air contamination with metals especially by lead. The smelter "Trepça" was closed in 2000 year [4].

Several studies reported high levels of heavy metals concentration in agricultural soils which are located in mining and smelting area in the Mitrovicë region [4, 5, 6 and 7]. The results of studies showed high concentration of heavy metals in agricultural crops and vegetables from Mitrovicë region [8, 9 and 10].

Toxic metals and trace elements are studied in cow, goat, sheep and buffalo milks from different regions such as Spain [11], Iran [12 and 13], India [14 and 15], Turkey [16], Croatia [17 and 18], Poland [19], China and Japan [20], Argentine [21]. Earlier study - 1968 [22] showed that milk of sheep from vicinity of smelter "Trepça" contained, on average, about 132 γ per 100 g lead (Pb), a much larger quantity than in other districts. Lambs showed paralysis of extremities and tongue, temporary diarrhea, anemia and general weakness. In cattle no marked lead poisoning were observed, although their milk in this area showed about the same concentrations as that in sheep [22] .

Blood lead level (BLL) and blood ALAD activity is considered valuable biomarkers for evaluation of Pb toxicity [23].

Lead is a multi-targeted toxicant, with hematotoxicity a primary effect, specifically inhibition of heme synthesis. ALAD is an enzyme a primary of heme synthesis pathway, whose inhibition in humans and animals has served as an important, and sensitive, biological marker for Pb exposure and injury [23]. BLL and ALAD activity are studied in cows environmentally exposed to lead [24, 25, 26, 27, 28 and 29].

Grazing dairy cows would ingest daily less than 250 g of dry soil under grazing conditions. The majority of Pb consumption through most of the year at moderately and highly contaminated environments with lead relatively higher amounts of soluble soil-Pb can be ingested at rates exceeding safety threshold limits [30].

In Kosovo, cattle are the most numerous of the different kinds of livestock and are fed particularly on grass and locally grown fodder. The aim of this study was to analyze lead concentrations in soil, grass, blood and milk , ALAD activity , Hb and Hct values in grazing cows from vicinity of smelter "Trepça" in Kosovo.

MATERIAL AND METHODS

Study area

Mitrovicë is the second largest town in Kosovo. Lead and Zinc smelter "Trepça" is situated close to the Mitrovicë. The smelter had been in operation intermittently more than six decades. During this period of time a huge amounts of metals were released in town Mitrovicë and surrounding rural area [3]. An environmental audit ordered by the Kosovo authorities, warned that smelter should be closed as an "un-acceptable" source of air contamination with metals especially by lead. The smelter "Trepça" was closed in 2000 year [4].

Sample collection and analysis

This study was conducted during May-June 2018. In total, 59 topsoil, 62 grass samples were collected in land and pastures, 103 blood and 68 milk samples were collected in cows (Simmental breed) from smelter area within a radius of 2-3-5 km from smelter "Trepça": Kelemnd village – 2 km (10 cows), Boletin village - 3 km (19 cows), Zazhë village – 5 km (46 cows) and Koliq village control - 40 km from smelter -29 cows (Figure 1). All cows reared in the local pastures, they were also given locally produced hay.

Topsoil samples from the pastures of villages (Kelmend, Boletin, Zhahzë and Koliq) were collected according to European guidelines. After surface litter (0 horizons) was removed, top soil samples were collected from each pasture. The weight of soil e sample was 1 kg. Soil samples were air-dried in laboratory for two weeks. Air-dried soil samples then n gently disaggregated, cleaned of extraneous material and sieved through a nylon sieve of 2 mm. Sieved

samples of soil were was homogenized and a quarter was milled in an agate mill to an analytical grain size of <0. 125 mm. Grass were collected in pastures, dried at 80°C for 48 h and ground to a fine powder.

Soil samples were mineralized with a microwave acid digestion apparatus -Milestone Ethos with internal temperature sensor, 640-260 terminal with easy CONTROL software installed and HPR1000/105 high pressure segmented rotor. Soil samples were solubylized by acid digestion adding in Teflon bombs 3 ml HNO_3 65%, 9 ml of HCl 37 % to 500 mg of powered samples. Solutions were collected in flasks and adjusted to 25 ml with distilled water.

Grass samples were mineralized with a microwave acid digestion apparatus - Milestone Ethos. Grass samples were solubylized by acid digestion adding in Teflon bombs 6 ml HNO_3 65%, 2 ml of H_2O_2 30 % to 500 mg powder samples. Solutions were collected in flasks and adjusted to 25 ml with distilled water.

Milk samples were collected grazing cows between 7.00 to 9.00 a.m. in clean polyethylene bottles. Each milk sample was stored at – 20 ^{0} C until analysis. Milk samples were mineralized with a microwave acid digestion apparatus - Milestone Ethos. Samples were solubylized by acid digestion adding in Teflon bombs: 6 ml HNO_3 65%, 2 ml of H_2O_2 30 % to 500 mg powder samples. Solutions were collected in flasks and adjusted to 25 ml with distilled water. Solutions were collected in flasks and adjusted to 25 ml with distilled water. A 5.0 ml of blood samples were obtained by jugular vein puncture from all cows by a veterinary trained phlebotomist. The blood was stored in BD vacationer (LH 170 I.U; UK.). Blood samples during transportation were stored at 4^0C. The total content of Pb in soil, grass, milk and blood samples was analyzed by graphic furnace atomic absorption spectrometry GF-AAS (Varian spectra AA 640Z Zeeman AAS, equipped with a GTA 100 graphite furnace – Varian USA and PSD 100 auto-sampler –Varian, USA).

Blood ALAD activity was measured according the European standardized method [31], Hematocrit values (Hct) were determined in the heparinized capillary tubes, centrifuged for 8 minutes at 10.000 rpm (Heamofuge Heraues). Blood hemoglobin (Hgb) concentrations were measured by standard cyan-methemoglobin method.

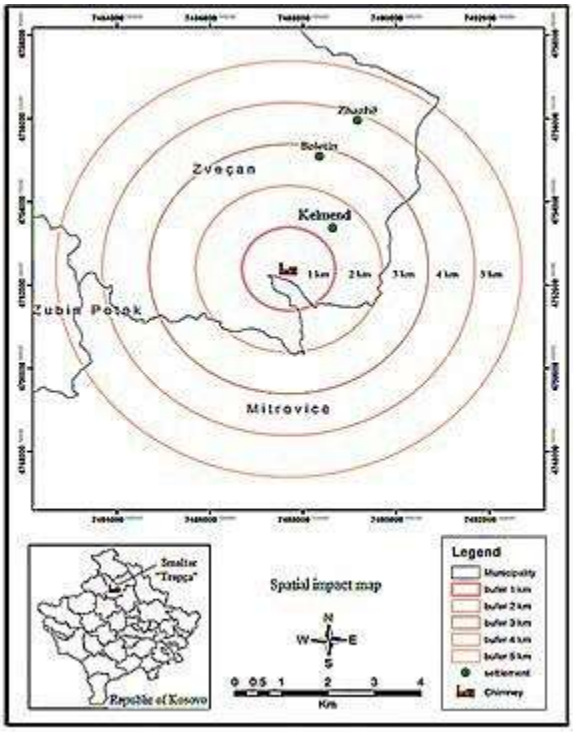

Figure 1. Map of the spatial impact in the study area: Kelmend, Boletin and Zhazhë (smelter area).

RESULTS AND DISCCUSION

The results of lead (P) concentration in topsoil, grass from three different contaminated localities (Kelmend, Boletin and Zhazhë), and control locality, Koliq village, blood lead level (BLL), milk lead levels (MLL), ALAD activity, Hb and Hct contents in grazing cows from these localities are presented in the Table 1 and Figure 2) . The results of correlations between Pb concentration in the soil, grass, blood and milk and ALAD activity are presented in Table 2.

The results (Table 1) show that Pb concentrations in the topsoil (PbS) in three localities situated at different proximity (Kelmend 2 km, Bolerin 3 km and Zhazhë 5 km) from former smelter "Trepça" are significantly higher (P<0.001) compared with (184.9±114; 75±15; 83±45; 40±16 mg kg^{-1}, respectively) their concentrations in a topsoil from control locality-Koliq. There was also found significant concentration of lead in the topsoil of Kelmend (2 km) compared with lead in the topsoil's from Boletin (P<0.05;184.9±114; 75±15 mg kg^{-1}, respectively) and Zhazhë (P<0.001; 184.9±114; 83±45.0 mg kg^{-1}, respectively). Lead concentration in grass (PbG) form Kelmed, Boletin and Zhazhë is higher (P< 0.001; P<0.006: P< 0.03; 37±10.7; 34±8.3; 27.3±14.07; 24.8±5.2 mg kg^{-1}, respectively), compared with concentration in grass form control locality-Koliq. Blood lead level (BLL), in the grazing cows from Kelmend, Boletin and Zhazhë is significantly higher (P<0.001; 0.0403±0.0277; 0.0268±0.0113; 0.0248±0.0168; 0.0128±0.0041 mg kg^{-1}, respectively). There is higher BLL in grazing cows form Kelmed compared with BLL in the grazing cows from Zhazhë (P<0.02; 0.0403±0.0277; 0.0268±0.0113; 0.0248±0.0168 mg kg^{-1}, respectively).

Table 1. Mean value of lead concentration (Pb mg kg^{-1}) in soil, grass, blood, milk and δ-aminolevulinic acid dehydratase (ALAD) activity, hemoglobin (Hb) and hematocrit (Hct) in cows (Simmental breed) from vicinity of former smelter "Trepça" in Kosovo.

Parameters	Distance from smelter			Control
	2 km (Kelmend)	3 km (Boletin)	5 km (Zhazhë)	40 km (Koliq)
Soil: Pb mg kg^{-1}	184±114 ***	75±15 ***	83±45 ***	40±16
Grass: Pb mg kg^{-1}	37.0±10 (15)	34±8.3 (5)	27±14 (22)	24±5.0 (20)
Blood: Pb mg kg^{-1}	0.040±0.027 (10)	0.0268±0.011 (19)	0.0248±0.016 (46)	0.0128±0.0041 (28)
Milk: Pb mg kg^{-1}	0.045±0.033 (5)	0.052±0.032 (36)	0.054±0.058 (20)	0.033±0.016 (7)
ALAD: U/LE	3.7±1.3 (11)	4.6±1.6 (19)	7.3±2.9 (46)	6.0±2.9 (29)
Hb: g/l	104±5.0 (11)	104±9.0 (19)	104±22 (46)	99±25 (29)
Hct %	30.7±5.0 (11)	30.4±8.0 (19)	31.6±4.0 (46)	30.7±6.0 (29)

Note: The results are presented as Mean - X: Standard deviation - Sd±: * P<0.05; ** P<0.01; *** P<0.001. Number of samples in parenthesis.

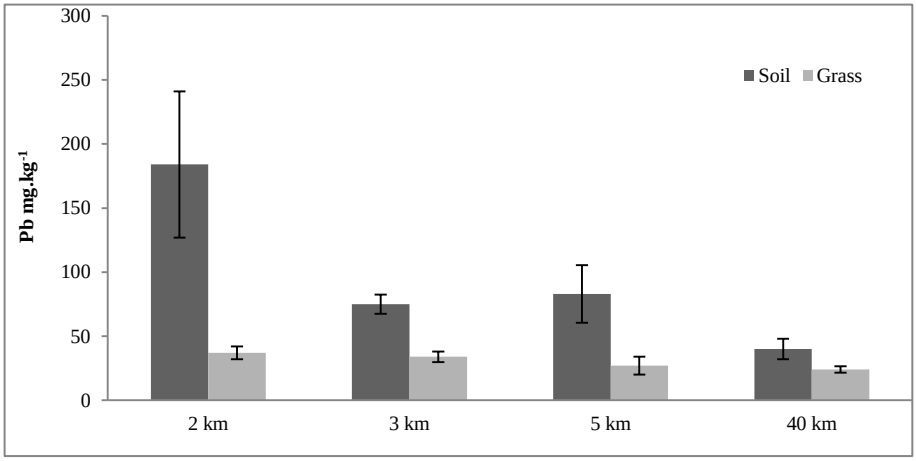

Figure 2. Lead (Pb) concentration (mg/kg) in the soil and grass from smelter Trepca – Mitrovicë

Table 2. Speman correlation (r) between Pb soil-Pb grass, Pb soil-Pb blood, Pb grass-Pb blood, Pb grass-Pb milk and Pb-blood –milk concentrations (mg kg^{-1}) in three localities from vicinity of former smelter "Trepça" in Kosovo.

Area	Pb: Soil/Grass	Pb: Soil/Blood	Pb: Grass/Blood	Pb: Grass/Milk	Pb: Blood/Milk
Kelmend (2 km)	-0.315	0.675	-0.265	-0.177	0.099
Boletin (3 km)	-0.353	0.716	-0.303	-0.025	0.987***
Zhazhë (5 km)	0.159	0.171	0.319	0.319	-0.031
Koliq (40 km)	-0.153	-0.132	---	0.008	-0.439

*P<0.05, ***P<0.001

MLL in grazing cows from three localities from smelter contaminated area: Kelemnd, Boletin and Zhazhë although is higher compared with MLL in the grazing cows from control locality-Koliq (0.045±0.33; 0.052±0.32; 0.054±0.58; 0.033±0.16 mg kg^{-1}, respectively), a statistically significant higher MLL was found (P<0.05) only between MLL in a grazing cows from Boletin and grazing cows from control locality-Koliq.

The results of ALAD activity in the blood of grazing cows from Kelmend and Boletin is inhibited (P<0.001) in comparison of blood ALAD activity of grazing cows from Zhazhë (3.7±1.3; 4.6±1.6; 7.3±2.9, respectively). Blood ALAD activity in the cows from Kelemd and Boletin is also significantly inhibited (P<0.01; 3.7±1.3; 4.6±1.6; 6.0±2.9 U/LE, respectively) compared with ALAD activity in the blood of grazing cows from control area-Koliq. There is adverse correlation between BLL and ALAD activity (r=-0.812; P<0.004) in grazing cows from locality of Kelemed situated 2 km from former smelter "Trepça". Significant positive correlation (P<0.001) was found between BLL and MLL in grazing cows with BLL above >0.026 mg L^{-1} – Boletin village. The Hb content and hematocrit values did not show significant difference between grazing cows from different localities.

Our results of mean values of Pb concentrations in the topsoil from contaminated area: Kelmend, Boletin and Zhazhë (184.9±114; 75±15; 83±45 mg/ kg, respectively), are several times higher compared with worldwide average of soil Pb concentration [32] and the upper limit of Pb concentration for unpolluted soils [33].

A study results undertaken in 2009 in soils of Mitrovicë region- northern Kosovo [3], showed the Pb concentration mainly between 100 and 300 mg/kg , 5% below 100 mg/kg, and 10% above 2000 mg/kg.Studies in the soil from the narrower vicinity of Mitrovicë [4] showed 20-fold higher Pb concentration compared with compared with the European median soil Pb concentration. The above authors note that concentrations of some metals (Pb, Cd, As, Hg,

Zn and Cu) in the soil from towns of Mitrovicë and Zveçan, are higher than the intervention values, according to the New Dutch list [4].

Our results of decrease of Pb concentration in topsoil with proximity from smelter "Trepça" are in accordance with results of Nanoni [5], who also found decrease of Pb concentration in soil with proximity e from smelter "Trepça" , Trepça battery factory and urban center of Mitrovicë.

Study results of heavy metal concentrations in agricultural soils in Kosovo [6] established that soil Pb concentrations vary from 15.5 to 2206.3 mg kg^{-1}, with mean 163.3 mg kg^{-1}.

Decrease of Pb concentration in topsoil with distance from the smelter "Trepça" area (Kelmend, Boletin and Zhazhë villages) are consistent with results of other authors [7], who reported a progressive decrease of mean Pb soil concentration in villages of Kelemend, Boletin and Zhazhë in order: 1309.20 > 496.22 > 366.39 mg kg^{-1}. Higher Pb concentrations in the soils from the same villages [7] compared with Pb soil concentrations in our study can be as e result that we collected the topsoil samples from 0-5 cm while they collected up to 20 cm.

Our results of Pb concentration in topsoil, grass and BLL in grazing cows within 1-5 km around smelter "Trepça" are consistent with results of Zadnik [25], who during long period of studua (1975-2002) of Pb concentration in topsoil, forage and Pb concentration in the blood, liver and kidneys in cows from farms situated in the distance from 1-10 km from the lead mine and smelter (Meža River Valley) after lead filtration in 1978, found progressive decrease of Pb lead concentration decrease. Results of this study showed that filters influenced on prominent decrease of lead concentration from 1975-2002 in the soil (192-1,558 to 347.5±300 mg kg^{-1}), forage (584.0±324.9; 5.6±3.3 mg kg^{-1}) and mean blood lead level (1.251±0.580 to 0.069±0.041 mg kg^{-1}) 36.

Our results of decrease of Pb concentration in grass with from smelter area (Kelmend 37 mg kg^{-1}; Boletin 34 mg kg^{-1} and Zahazhë 27 mg kg^{-1}) and Koliq (12.8) control area, are lower than Pb concentrations founded in crops from of different locations (Rudare: Lucerne 52.50 mg kg^{-1}, Hay 62.50 mg kg^{-1} and Spinach 100 mg kg^{-1} and in Shupkovc: Lucene 46.25, Hay 46.25 and Spinach 31.73) in the vicinity of "Trepça" plant in Mitrovicë [8].

On the other hand results of Pb concentration in leaves of onion plants grown (in laboratory) in soil samples with Pb concentrations 2516.54 mg/kg from Shupkovc and 2122.09 Mitrovicë town showed mean values of lead concentration 8.35 mg kg^{-1} and 5.69 mg kg^{-1} respectively [10].

Studies in cattle from lead-zinc smelter in Guizhou, China, showed Cd and Pb concentration in bovine kidney and liver slightly over Chinese standard [34].

The results of study [35] found that grazing cows can ingest 1% to nearly 18 % of their dry matter as soil [35]. When grazing conditions are severe, dry soil intake in cows grazing in intensive rearing systems can increase up to 1kg/day and individual intakes can even exceptionally reach 1.3 kg/day [30].

Our results of progressive decrease of blood lead level (BLL) in grazing cows within 2-5 km around smelter "Trepca" are consistent with the results established in the 25-year long systemic investigation (1975-2002) in the blood of cows from the vicinity smelter in Meza Valley – Slovenia [36].The results of their study showed progressive decline of BLL in cows from mining and smelter area from 1975 – to 2002. The BLL in 2002 was 18.13 times lower compared with blood level in 1975 (BLL in 1975: 1.251±0.580 mg kg^{-1}; BLL in 2002: 0.069±0.41 mg kg^{-1}, respectively) [36].

A 16 year retrospective study (1998-2013) of acute Pb poisoning in Canadian cattle [37] showed that mean Pb concentrations in the blood, liver and kidney were several times higher (1.30±1.70 mg kg^{-1} w.t., 33.5± 80.5 mg kg^{-1} w.t., 55.5 ± 39.7 mg kg^{-1} w.t. respectively), compared with mean " normal" Pb concentrations a liver and kidney (0.03±0.003 mg kg^{-1}, w.t. 0.16±0.63 mg kg^{-1}, w.t., and 0.41±0.62 mg kg^{-1} w.t.) [37].

Results of study [38], found significantly higher Pb values in the blood and fair in cattle from lead-zinc smelter area in India compared with control (blood: 0.097 mg kg^{-1}, 0.07 mg kg^{-1}, respectively. hair: 156 mg kg^{-1} and 3 mg kg^{-1}, respectively). In cattle from lead-zinc area the accumulation of Pb in the hair compared with Pb level in

blood was 160.8 times higher, while in the control group of cattle it was 42.8 times higher. Results of herds clinically poisoned with PB (39) proved that 7-40 of A previous study [39] of herds with clinical cases of Pb poisoning found that 7-40% of asymptomatic cattle had BLL ≤10 µg dl^{-1}.

Our results of BLL and ALAD activity in the grazing cows from smleter "Trepça" areconsistent with results of Prpic-Majic [24], who in a 5 year study (1976, 1978, 1982, 1984, 1988) in cows smelter in Mezica Valley-Slovenia recorded progressive decrease of BLL from 1976 to 1980 (1976: 3.09 µmol/L, 1978: 1.44 µmol/L, 1982: 1.44 0.27 µmol L^{-1}, 1984: 0.75 µmol L^{-1}, and in 1988: 0.66 µmol L^{-1}, respectively), while blood ALAD activity was increased from 0.6 U/LE in 1976 to 1.5 U/LE 1982 , 2.7 U/LE µmol/L in 1984 and 3.4 U/LE in 1988, meanwhile the EP was progressively decrease: 1976: 14.55 µm L^{-1}, 1982: 3.47 µm L^{-1}, 1984: 3.48 µm L^{-1} and in 1988: 2.66 µm L^{-1} [24] .

Study results of in the cows from the vicinity of lead and zinc plant showed [23], higher BLL (155 µg L^{-1}, 30 µg L^{-1}) , inhibition of ALAD activity (2.4 U/LE, 6.4 U/LE respectively; P<0.01), and lower Hb amount (99.8 g l^{-1}, 104.0 respectively; P< 0.001). The authors of this study showed that the relationship between ALAD activity and BLL, in cows is not linear but is exponential (log ALAD/Pb). After them could be related to the fact that ALAD is more sensitive than BLL in reflecting the chronic Pb exposure and accumulation in bone as a "critical organ" of lead exposure. On the other hand results of study in cow-calf herds found an exponential, rather than linear concentration of Pb in milk with respect to the BLLs in cows [39].

Results of study on the cattle and sheep from mining polluted area Spain, shoved BLL 160 times higher in cattle (15.45 µ dl^{-1}; 0.1 µ dl^{-1} respectively), in sheep's d 35 times higher compared with their respective controls (2.41 µ dl^{-1}; 0.06 µ dl^{-1} respectively). The blood ALAD activity of cattle and sheep from mining area were significantly inhibited compared with ALAD activity in respective control groups (cattle ALAD: 7.95 nmol ml^{-1} RBC; 3.280 nmol ml^{-1} RBC, respectively) [26].

Our results of milk lead levels (MLL) in the cows from smelter contaminated area are consistent with results [12] recorded in the milk of livestock animals from different regions of Iran [12]. Analyses of lead level in the buffalo, cows from Township: Urmia. Khoy, Salmas, Naghadeh and Miandoab showed the highest MLL in buffalo 0.018±0.002, moderate in cow 0.008±0.01 mg kd^{-1} and the lowers in ewe 0.002±0.001 mg kg^{-1} [13] , after them the concentration of lead in the milk do not exceed the safety limits [13].

Results of study of Pb contents in Chinese commercial and raw milks from China was several times higher than in Japanese commercial milks [1] [20].

Results of study [15] of cows environmentally exposed to higher level of lead and cadmium around different industrial units in India, found the higher lead and cadmium in their mil. The authors of this study argue that increased blood and milk Pb or Cd level as a result of natural exposure of lactating cows significantly influences trace minerals compositions of milk [15].

The results of study of metals (Pb,As, Zn, Co and Fe) in row milk from industrial, rural and a heavy traffic intensity region from Bursa, in Turkey [41], found highest metal content in the milk samples collected from industrial region, followed by traffic intensive region and rural region [41].

Results of cow's milk from fifteen farms near Kniž in Zagreb region – Croatia showed [42], Pb and Cd mean values (0.27 ± 0.06 mg kg^{-1} and 0.037±0.007 mg kg^{-1} DM).

The results of study in six individual cows accidentally exposed to lead showed that BLL ranged from 0.8822 to 1.216 mg l^{-1} BLLs of these samples ranged (from six individual cows) from 0.8822 to 1.216 mg l^{-1}. The BLL of 22 samples collected within the first 90 days showed decrease of BLL > 0.025 mg l^{-1} . On the first day of testing MLL was 0.0362 mg l^{-1} while 12 days later MLL was 0.0406 mg l^{-1} [43]. The MLL of cows from smelter area is more than two times higher compared with the permissible concentration (0.02 µg mL^{-1}) of the raw milk given by the standards of the European Commission Regulation [44].

CONCLUSIONS

Soil, grass, blood and milk lead content in the grazing cows from smelting area progressively decreased with the proximity of smelter "Trepça". ALAD activity is proved as a sensitive and useful biomarker at a relatively low blood lead levels in cows. Milk lead levels in cows from smelter area are more than two times higher than given standards by the European Commission Regulation.The vicinity of smelter still poses threat for livestock welfare and human health

CONFLICT OF INTERESTS

The authors declare that there are no competing interests associated with the manuscript. Manuscript was self-funded by authors.

REFERENCES

1. Nriagu J.O., 1996. A history of global meta l pollution. Science. 272, 223-224.

2. Csavina J., Taylor M.P., Félix O., Rine K.P., Sáez A.E., Betterton E. A. 2014. Size –resolved dust and aerosols contaminants associated with copper and lead smelting emissions implications for emission management and human health. Sci Total Environ. 493, 750-7 56.

3. Borgna L., Di Lella L.A., Nanon F., Pisani A., Pizzetti E., Protano G., Riccobono F., RRossi S., 2009. The high contents of lead in soils of northern Kosovo. J Geochem Explor. 101, 137-146.

4. Šajn R., Aliu M., Stafilov T., Alijagic J., 2013. Heavy metal contamination of topsoil around lead and zinc smelter in Kosova Mitrovica/Mitrovicw, Kosovo/Kosovw. Journal of Geochemical Exploration. 134, 1-16.

5. Nanoni F., Protano G., Riccobono., 2011. Fractionation and geochemical mobility of heavy elemnts in soils of mining area in northern Kosovo. Geoderma .161. 63-73.

6. Zogaj M., Pacarizi M., During R.A., 2014. Spatial distribution of heavy metals and assessment of their bioavaibility in agricultrural soils in Kosovo. Carpatian Journal of Earth Sciences. 9(1). 221-230.

7. Kelmendi M., Sadiku M., Kadriu S., Dobroshi F., Igrishta L., Baruti B., 2018. Research of heavy metals on the agricultural land in Bajgora region, Kosovo. Acta Chemica IASI. 21(1), 105-122.

8. Deda Sh., Kastrati R., Elezi Xh., Bakalli R., Demaj A., Konjufca V., 1996. Lead concentrations in crops from vicinity of lead industry of "Trepca" plant. Environmental Letters (Special issue). 29-32.

9. Nanoni F., Rosi S., Protano G., 2016. Potentially toxic element contamination in soil and accumulation in mize plants in a smelter area in Kosovo. Environ Sci Pollut Res. 23(12), 11937-11946. doi 10.1007/s11356-016-6411-0

10. Gashi B., Osmai M., Aliu S., Zogaj M., Kastrati F., 2020. Risk assessment of heavy metal toxicity by sensitive biomarker δ-aminolevulinic acid dehydratase (ALA-D) for onion plants cultivated in polluted areas in Kosovo. Journal of Environmental Science and Health, Part B. 1-8. Doi.org/10.1080/03601234.2020.1721229

11. Gonzalez-Montana J.R., Senis E., Gutierrez A.. Prieto F., 2012. Cadmium and lead in bovine milk in the mining areof the Caudal River (Spain). Environ Monit Assess.184, 4029-4034.

12. Rahimi E., 2013. Lead and cadmium concentrations in goat, cow, sheep, and buffalo milks from different regions from Iran. Food Chemistry. 136, 389-391.

13. Najarnezhad V., Jalilzahed –Amin G., Anassori E., Zeinali V., 2015. Lead and cadmium in raw buffalo, cow and eve milk from the west Azerbaijan, Iran. Food Additives & Contaminants: Part B. 8(2), 123-127. doi: 1o.1080/1939210.2015.10077396

14. Swarup D., Patra R.C., Naresh R., Kumar P., Shekhar., 2005. Blood lead levels in lactating cows reared around polluted localities; transfer of lead into milk. Science of the Total Environment. 347, 106-110.

15. Patra R.C., Swarup D., Kumar P., Nandi D., Naresh R., Ali S.L., 2008. Milk trace elements in lactating cows environmentally exposed to higher level of lead and cadmium around different industrial units. Science of the Total Environment. 404, 36-43.

16. Temiz H., Soylu A., 2012. Heavy metal concentrations in raw milk collected from different regions of Samsun, Turkey. International Journal of Diary Technology. 65, 1-7.

17. Bilandzic N., Dokic M., Sedak M., Solomun B., Varenina I., Knezevic Z., Benic M., 2011. Trace element levels in raw milk from northern and southern Croatia. Food Chemistry.127, 63-66.

18. Bilandzic N., Dokic M., Sedak M., Calopek B., Bozic-Liburic D., Solomun-Kolanovic B., Varenina I., Dokic M., Kmetic I., 2016. Lead concentrations in raw cow and Goat milk collected from rural areas in Croatia from 2010 to 2014. Bull Environ Contam Toxicol. 96(5), 645-649. doi: 10.107/soo128-016-1749-z.

19. Pilarczyk R.,Vojcik J., Czerniak P., Sadlik P., Pilarezyk B., Tomza-Marciniak A., 2013. Concentrations of toxic heavy metals and trace elements in raw milk of Simmental and Holstein- Friesian cows from organic farms. Environ Mont Assess. 185, 8383-8329.

20. Qin L.Q., Wang X.P., Li W., Tong X., Tong W.J., 2009 . The Minerals and Heavy Metals in Cows's Milk from China and Japan. 55(2), 300 - 305.

21. Rubio M.R., Sigrist M.E., Encinas E.E., Baroni J.E., Caronel J.C., Boggio H.R., Beldomenico.,1998. Cadmium and lead levels in cow's milk from milking region in Santa Fe, Argentina. Bull Environ Contam Toxicol. 60, 164-167.

22. Stamatovic S., Milic D., 1968. Problems of air pollution in Yugoslavia. Proccedings of the First European Congress on the Influence of Air Pollution on Plants and Animals. Wageningen, Aprill 22 to 27, 255-257.

23. Telišman S., Prpic-Majic D., Keršanc A., 1990. Relationships between blood lead and indicators of effect in cows environmentally exposed to lead. Toxicology Letters. 52, 347-356.

24. Prpic-Majic D., Karacic V., Skender Lj., 1990. A follow-up study of lead absorbtion in cows as an indicator of environmental lead pollution. Bull Environ Contam Toxicol. 45, 19-24.

25. Zadnik T., 2007. A 25-year long monitoring of lead content in the blood cows from a stationaty polluted area. Int J Environment and Pollut. 31(1/2), 34-40.

26. Rodriguez-Estival J., Jose E., Barasona A., Mateo R., 2012. Blood Pb and δ-ALAD inhibition in cattle and sheep from polluted mining area. Environmenatl Pollution. 100, 118-124.

27. Elezaj I.R., Selimi Q.I., Letaj K.Rr., Millaku L.B., Sefaja L., 2013. Metal Accumulation, Blood δ-Aminolevulinic Acid Dehydratase Activity and Micronucleated Erithrocytes of Feral pigeons (*Columba livia*) Living Near Former Lead-Zinc Smelter "Trepça"-Kosovo.2013. E3S web Conf. 1, 34001. DOI:10.1051/ e3sconf/2013014001, 2013.

28. Bischoff K., Hillebrandt J., Erb H.N., Thompson B., Johns S., 2016. Comparison blood and tissue lead concentrations from cattle with known lead exposure. Food Additives & Contaminants: Part A. 33(10), 1563-1569.

29. Aluc Y., Ekici H., 2019. Investigation of Hevay Metal Levals in Blood of Three Cattle Breeds in Turkey. Bull Environ Contam Toxicol. 103(5), 739-744.

30. Jurjanz S., Feidt C., Perez-Prieto L.A., Ribeiro-Hilho H.M.N., Rychen G., Delagarde R., 2012. Soil intake in lactating dairy cows in intensive strip grazing systems. Animal. 6(8), 1350-1359.

31. Berlin A., Schaller K.H., 1974. European standardized method for the determination of delta- aminolevulinic acid dehydratase activity in blood. Z Klin Chem Klin Biochem. 12, 389-390.

32. Koljonen T., 1992. Geochemical Atlas of Finland, Part 2: Till.Geological Survey of Finland, Espoo, Finland. pp. 218.

33. Fergusson J.E., 1990. The Heavy Elements: Chemistry, Environmental Impact and Health Effects. Pergamon Press, Oxford. pp. 614.

34. Cai Q., Long L., Zhu M., Zhou Q.z., Zhang L., Liu. 2009. Food chain transfer of cadmium and lead to catle in lead-zinc smelter in Guizhau, China. Environmental Pollution. 157, 3078-3082.

35. Thornton T., Abrahams P., 1986. Soil ingestion – a major pathway of heavy metals into livestock grazing contaminated land. The Science of the Total Environment. 28, 287-294.

36. Zadnik T. Monitoring of lead in topsoil, forage, blood, liver, and kidneys in cows in a lead polluted area in Slovenia (1975-2002) and a case of lead poisoning in 1993.

International Journal of Chemical Engineering. 10, 1-9. doi:10.1155/2010/940206.

37. Cowan V., Blakley B., 2016. Acute lead poisoning in western Canadian cattle- A 16-year retrospective study of diagnostic case records. CVJ. 57, 421-426.

38. Patra R.C., Swarup D., Sharma M.C., Naresh R., 2006. Trace mineral profile in blood and hair from cattle environmentally exposed to lead and cadmium around different industrial units. J Vet Med. A53, 311-317.

39. Waldner C., Checkley S., Blakley B., 2002. Managing lead exposure and toxicity in cow-calf herds to minimize the potential for food residues. J Vet Diag Invest. 14, 481-486.

40. Telišman S., Pongracic J., Cretnik R., Prpic-Majic D., 1985. Lead in milk and indicators of lead absorption in cows from lead contaminated and control areas. In: T.D. Lekkas (Ed.), Heavy Metals in the Environment. Vol. 1. CEP Consultants Ltd., Edinburgh. pp. 417419. 2

41. Simsek O., Gultekin R., Oksuz O., Kurultay S., 2000. The effect of en vironmental pollution on the heavy metal content of raw milk. Nahrung. 44(5), 360-363.

42. Pavlovic I., Sikirc M., Havranek J.L., Plavljanic N., Brajenovic N., 2004. Lead and cadmium levels in raw cow's milk from and industrial Croatian region determined be electrothermal atomic absorption spectrometry. Czech J Anim Sci. 49(4), 164-168.

43. European Commission. 2006. Commission regulation (EC) no 1181/2006: setting maximum levels for certain contaminants in foodstuffs [Internet]. [place unknown]; [cited 2015 Jul 7]. Available from: http://eur-lex.europa. eu/legal-content EN/ALL/?uri=CELEX:02006R1881-20100701

44. Bischoff K., Higgins W., Thompson B., Ebel J.G., 2014. Lead excretion in milk of accidentally exposed dairy cattle. Food Additives & Contaminants: Part A. 31(5), 839-884.