Correlations between the Degree of Heavy Metal Contamination of Fodder and their Accumulation in Organs and Tissues

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Abstract
Research aimed at establishing an association between the level of heavy metals (Pb, Cd, Cu and Zn) in fodder, chronic diseases at cows and residual heavy metals in different organs and tissues. The toxicological investigation revealed the following main pathological states in cows: 18% marasmatic syndrome with toxic and deficiency etiology, 20% lung affections, 18% hepatopathia and under the 10% gastric diseases. The hematological and biochemical examinations revealed hyporegenerative aplastic anemia, hypophosphatemia, liver and kidney dysfunctions, bone and joint dysfunctions. Lead retention in the liver was 0.62 ppm (HAL 0.5 ppm) and cadmium was 0.82 ppm (HAL 0.05 ppm). Lead retention in the kidney was 1.05 ppm (HAL 0.5 ppm) and cadmium was 2.13 ppm (HAL 0.05 ppm).

Keywords: cadmium, cows, fodder, lead

1. Introduction

The toxic heavy metals like Cd, Pb, Hg affect biological functions, hormone system and growth. They accumulate in body organs and food animals and are transmitted through food determinate serious public health hazard. The toxicants accumulated in the vital organs like liver and kidney and exert adverse effects on domestic animals [1].
Many anthropogenic activities such as mining and metallurgy, industries or transport sectors redistribute toxic substance as heavy metals (Pb, Hg, Cd, etc.) into the environment. They persist for longer period and their metabolites affecting environment [2]. Cadmium is a non-essential element that has high rates of soil to plant transference compared with other non-essential elements, and certain plant species accumulate large amounts of cadmium from low cadmium content soils [3]. Cadmium (Cd) works by disrupting the normal state of a cell. Its similar structure to zinc and calcium allows it to interfere with the absorption of these elements, and it similarly inhibits copper and iron. Like most heavy metals, when absorbed into the body cadmium is stored in the liver and kidney of the animal. Cadmium is an accumulative poison, meaning that it builds up slowly over time in the bodies of exposed animals. Cadmium poisoning affects nearly all major organ systems and has noted reproductive effects, including decreased testes size and infertility. Additionally, because of this element's interference with the absorption of the mineral calcium, cadmium poisoning can also severely impact the healthy function of the bones [4]. Increases in cadmium levels in soil result in an increase in the uptake of cadmium by plants, although the extent to which this happens will depend on the soil pH, plant species and the part of the plant, as well as other soil characteristics [5].

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Lead is one of the commonest poisonings in farm animals [6]. Lead affects virtually every system in the animal body, including the blood, the cardiovascular, renal, endocrine, gastrointestinal, musculoskeletal, immune and reproductive systems [7]. The maximum content of lead in complete feed has been set by the European Union at 5 mg/kg feed (with 12% moisture) for all animal species, while a single dose of 200 mg Pb/kg BW is lethal to cattle [6, 8]. Lead and cadmium have been labeled as major environmental pollutants since they are easily transferred into the food chain and they are not known with any significant biological functions.

Zinc is essential trace minerals required for many biological processes, particularly enzyme positive influence on livestock growth and reproduction. It takes parts in the synthesis of DNA, proteins and insulin. It is essential for the normal functioning of the cell including protein synthesis, carbohydrate metabolism, cell growth and cell division. When the soil is polluted with zinc, many plants often have zinc uptake that their system cannot handle. Plants take up on different parts of the plants exposed to the air from polluted environments contaminated forage may absorb concentrations that are damaging to their health. Consumption of animals with elevated lead levels may lead to serious health risk. Soil zinc concentration ranging from 4.49 mg/kg dry weight to 33.39 mg/kg dry EPA maximum permissible limit (MPL) and the EU Regulation Standards for zinc in soil are 300 mg/kg respectively [9].

2. Materials and methods

This study was conducted in a polluted industrial area, following the heavy metal concentration in pastures and meadows of contaminated soils and the main manifestations of chronic toxicosis in dairy cows bred in the area. There were selected four heavy metals Pb, Cd, Cu and Zn, and in their choice was taken into account the interrelationship, such as those related to cadmium which was found to be competitive with zinc and copper, and to a lesser extents to iron.

Knowing that the heavy metals studied have cumulative properties, in order to estimate the association between heavy metals in soil and plants and their residue in animal body were conducted sampling of organs and tissues.

To determine the main syndromes associated with toxicosis caused as a result of chronic contamination in dairy cows, toxicological investigation was carried out over a period of one year, establishing the percentage of various diseases. Toxicological investigation was conducted in parallel with performing laboratory tests (hematology and biochemistry tests), knowing the action that heavy metals have on the entire body; thus Cd can cross the various biological membranes by different mechanisms (e.g metal transporters) and once inside the cells binds to ligands with exceptional affinity (e.g metallothioneins) [10], thereby reducing the absorption of copper and, to a lesser extent, of zinc [6]; for Pb, the US NRC [11], reported that retarded growth, disturbed iron metabolism, anemia.

In order to determine the load of the four heavy metals in fodder, were harvested 25 feed samples used in dairy cows, represented by: hill hay, wheat straw, corn silage, apple draff, wheat bran. Feed sampling was done according to the Regulation no. 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feeding stuffs. Analyses were made taking into account the maximum level according to Regulation no. 1275/2013 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels for arsenic, cadmium, lead, nitrites, volatile mustard oil and botanical harmful impurities.

Forage grasses were collected from different points per sampling, stored in polyethylene bags. Samples were cut into small pieces, air dried for 5 days in the laboratory and thoroughly mixed together. The samples were pulverized and passed through 1 mm sieve. Digestion of these samples (1 g each) was carried out using 5 ml of concentrated nitric acid.

The working method used was atomic absorption spectrophotometry. Atomic Absorption Spectrometry (AAS) is a technique for measuring quantities of chemical elements present in environmental samples by measuring the absorbed radiation by the chemical element of interest. Metal analysis was carried out using flame atomic absorption spectrophotometer. The calibration curves were prepared separately for all the metals by running different concentrations of standard solutions. The instrument was set to zero by running the respective reagent blanks. Average values of three replicates were taken for each determination and were subjected to statistical analysis. The metals determined includes, lead, cadmium, copper and zinc.

To determine the degree of accumulation (residue) of these heavy metals in the organism, tissues and organs samples were collected as it follows: liver, long bone and kidney. Toxicological determinations were carried out by spectrophotometry with atomic absorption method.

Blood determinations were carried out on a number of 15 blood samples collected on anticoagulant, following the next parameters: number of erythrocytes, hemoglobin, Hct, WBC count, etc., with a Coulter Counter analyzer.

For biochemical determinations were harvested 15 serum samples aiming to those parameters that can be modified in case of chronic toxicosis: serum total protein (Pt); gamma globulins; urea by urease colorimetric method; alkaline reserve by the volumetric method; glutamic oxalic-acetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) by colorimetric method with dinitrophenylhydrazine.

3. Results and discussion

Following the toxicological analyzes on samples of feed, high levels were found in all 25 samples investigated for Pb, Cu, Cd and Zn. There have been registered high concentrations, over maximum allowed (LMA) over 3 times for lead in hill hay and corn silage; more than 3 times the copper in corn silage; more than 20.5 for zinc. Cadmium concentration in all samples of fodder remained close to the bottom, knowing that "maximum content of cadmium in complete feed has been set by the European Union at 0.5 mg/kg feed (with 12% moisture) for all animal species, 1 mg/kg for ruminants and fish and 2 mg/kg for dogs and cats" [8].

The mean levels and standard deviation of lead, cadmium, copper and zinc in forage across the 25 sampling are (Table 1, Figure 1).

Table 1. Concentration (ppm) of heavy metals in feed samples toxicologically analyzed (M±SD)

Forage n=25	Pb	Cd	Cu	Zn
Hay Hill	32.0±3.1	1.14±0.10	10.5±1.1	49.54±4.12
Wheat straw	8.6±0.68	1.49±0.12	12.9±1.05	99.12±9.0
Corn silage	31.9±3.23	1.44±0.12	24.2±2.02	204.9±19.13
Apple draff	9.3±0.71	0.43±0.033	12.6±1.18	19.13±2.01
Wheat bran	2.13±0.12	0.47±0.045	10.4±1.03	27.2±2.43
M.L.A		10	1	8

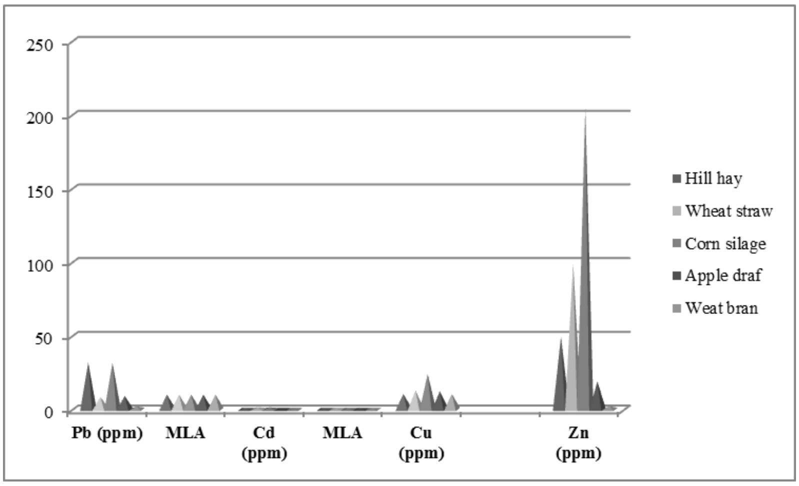

Figure 1. Pb, Cd, Cu and Zn level in fodder samples (ppm)

Toxicological investigation concluded after monitoring the cattle for one year, cases of disease in dairy cows, dependent events associated with the cumulative effect of metals such as Pb, Cu, and Cd. The main diseases recorded were: gastroenteritis, maternal toxicosis, lung and liver diseases, toxic abortion, marasmatic syndrome, nephropathy (Table 2, Figure 2).

Table 2. Incidence of syndromes associated with heavy metals toxicity

Diseases						
Gastroenteritis	Maternal and fetal toxicity	Lung affections	Hepatophaty	Toxic abortion	Marasmatic syndrome	Nephropathy
Incidence %						
10	7	19	18	2.25	18	2.75

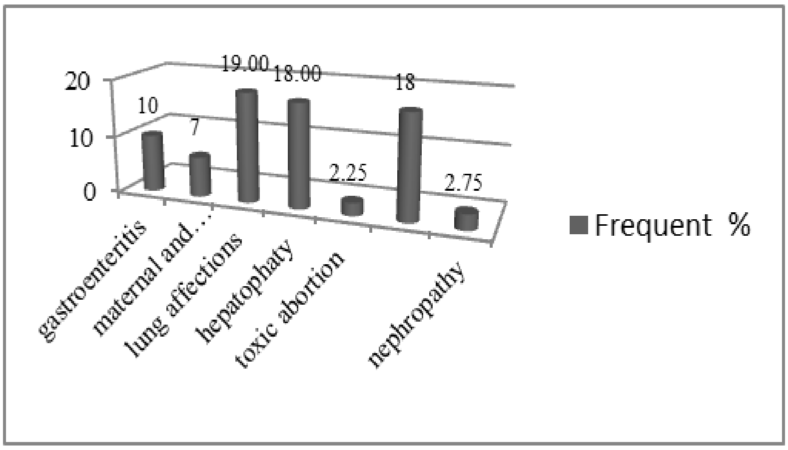

Figure 2. The most frequent diseases met in dairy cows

After biochemical and hematological examinations, the values of the considered parameters showed cumulative effect of lead, cadmium and copper, the changes being in concordance with data from the literature according to which lead containing metallo-proteins and peptides are then transferred to soft tissues (liver and kidneys mainly) and bones, where lead accumulates with age [7] and perturbation of calcium, zinc or iron homeostasis plays a key role in the toxicological action of cadmium that involves a general threat to basic cellular functions [5]. Thus were recorded: hyporegenerative aplastic anemia with low blood cells and hematocrit changes; liver and kidney disease correlated with cumulative properties of Cu and Cd; osteoarticular changes commonly found in lead poisoning. Following the clinical observations and corroborating them with the biochemical and hematological tests, it was found that the evaluated dairy cows showed a chronic pathology seen in poisoning by Pb, Cu and Cd, which is reported in other studies; thus clinical toxicity of cadmium in animals includes kidney and liver damage, anemia, retarded development or testicular degeneration, enlarged joints, scaly skin, and reduced growth and increased mortality [5]. Lead can be transferred from the mother to the fetus in utero and to the newborn animals via milk feeding [11] (Table 3).

Table 3. Values obtained by hematological and biochemical tests

Specification	UM	Lactating cows	
		Values obtained M±DS	Normal values
No erythrocytes	mil/mm^3	4.65±0.47	5–10
Hemoglobin	g/dl	8.27±0.71	8–15
Hematocrit	%	25.40±3.69	24–46
VEM	μ^3	54.13±5.45	40–60
HEM	Pg	19.10±1.08	11–21
CHEM	g/dl	34.58±3.02	30–36
No leukocytes	thousands /mm^3	11.37±1.36	7–10
Total protein	g/dl	8.79±0.80	7–8.5
Gamma globulin	g/dl	6.01±0.36	3.86–4.9
Total globulin	g/dl	1.57±0.20	1.3–1.9
Urea	mg/dl	14.80±2.41	20–40
Reserve alkaline	mEq/dl	21.7±1.30	22–27
Cholesterol	mg/dl	137.12±43.34	50-120
TGO	U/l	40.34±7.20	20–40
TGP	U/l	20.52±2.01	>10
Alkaline phosphatase	U/l	13.59±3.27	10–36
CPK	US/ml	18.59±1.21	0-14
Vitamina E	γ/ml	6.31±1.40	5-15
Ceruloplasmin	mg/dl	10.71±2.28	10–16
Calcium	mg/dl	11.21±1.51	8–11
Phosphorus	mg/dl	6.21±0.65	5–7
Magnesium	mg/dl	2.51±0.24	2.1–2.8

To determine the cumulative effect of metals, samples of bone tissue, kidney and liver tissue were analyzed. After processing the samples the following were concluded: the largest increase was that of cadmium in kidneys; lead had a maximum concentration in bones; copper and zinc were within normal limits (Table 4, Figures 3, 4, 5).

Metal intoxication is one of the most frequent diseases in farm animals, particularly in those grazed on pasture in the vicinity of metallurgic/other industrial complexes, mining communities and busy roads.

Even at soil levels above permissible limit, most of the risk is from metal contaminated soil or dust deposits on the plants rather than from uptake of metal by the plant [Heavy metal transfer from soil to plant is dependent on many factors, such as soil properties, plant species and metals bioavailability for uptake in the soil-plant system [12]. Grazing animals are directly affected by the consumption of forage and feed contaminated by airborne lead and somewhat indirectly by the up-take of lead through plant rots.

Same species of plant are capacity to accumulate high concentrations of lead [8]. Lead levels which range from 30-300 mg/ kg have been considered phytotoxic to plants. Transfer of Pb to cattle through consumption of contaminated plants was also corroborated in literature [12].

Table 4. Values of heavy metals (ppm) contained in samples of organs

Metal (ppm)	Liver M±SD	M.L.A.	Kidney M±SD	M.L.A	Bone M±SD	M.L.A
Pb	0.75±0.056	0.5	1.04±0.09	0.5	**19.16**±1.87	-
Cd	1.23±0.10	0.05	**2.13**±0.13	0.05	1.35±0.12	-
Cu	20.84±1.80	5.0	5.21±0.47	5.0	3.32±0.27	2.5
Zn	38.05±3.21	50	23.04±2.05	50	71.13±6.90	50

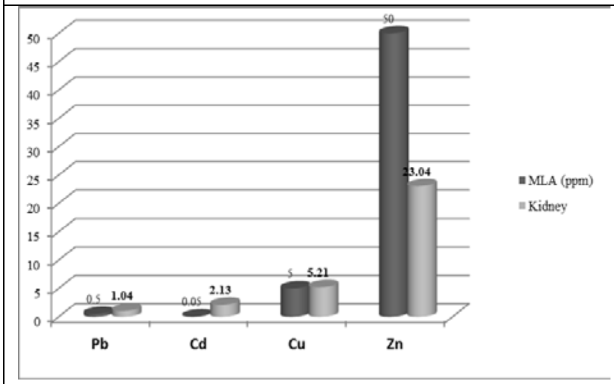

Figure 3. Heavy metals level in kidney

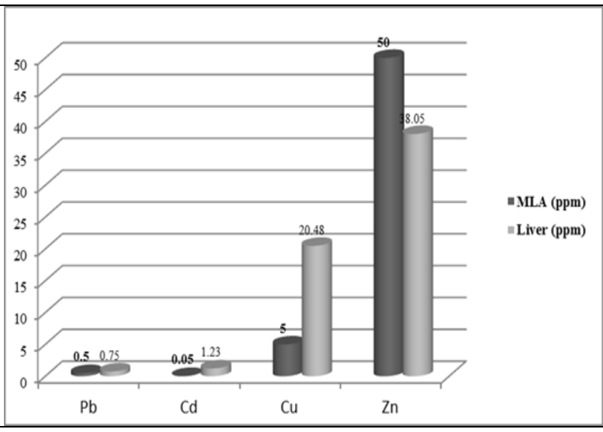

Figure 4. Heavy metals level in liver

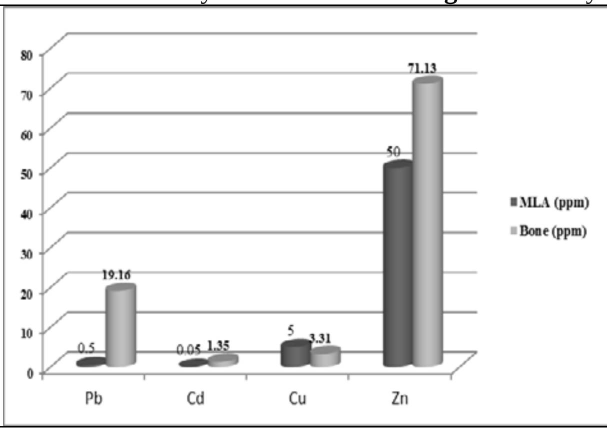

Figure 5. Heavy metals level in long bone

Conlusions

The research in an area polluted with heavy metals concluded the following issues:

1. The four heavy metals have been found over the maximum limits in all feed samples;
2. The most common chronic diseases correlated with the evolution of heavy metal contamination were marasmatic syndrome, toxic abortions, lung and gastrointestinal diseases, hepatopathy, nephropathy, hyporegenerative aplastic anemia.
3. Lead and cadmium exceeded the maximum allowed in the liver, kidneys and bones.
4. Bone tissues exhibited the highest load of lead and kidney the highest level in cadmium.
5. Following biochemical and hematological tests were set parameter changes associated with long lasting heavy metal contamination.
6. The presence of heavy metals in feed over maximum limits, the residual heavy metal accumulation in tissues for Cu, Pb or Cd, and clinical manifestations in dairy cows indicate a chronic pollution of the environment in the geographical area over which the toxicological assessment was performed.

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