Praca oryginalna ***Original paper***

Monitoring of selected heavy metals, hematological indicators and biochemical indicators in the blood of lactating dairy cows from an area with an active copper industry

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Received 21.05.2024 *Accepted 10.10.2024*

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Summary

There is a well-developed copper industry in Poland. Copper mining and processing involves processes that emit numerous chemical pollutants, including heavy metals, into the environment. Blood was collected once in the summer of 2021 and 2022 from dairy cows (aged 3–5 years) kept in small herds in the Legnica-Głogów copper district (Lower Silesia, Poland). Selected toxic metals (Cd, Cu, Hg, Pb, and Zn) were determined in the whole blood of the cows, whereas hematological parameters (WBC, RBC, PLT, MCV, MCH, MCHC), biochemical parameters (AST, GGT, TAS) and minerals (Ca, Fe, Mg, P inorganic) were measured in their blood serum. Generally, the indicators assessed were within reference values (with a few exceptions, e.g. Zn) and generally similar to those in cows kept in typically agricultural areas. This was also true of the toxic metals. The results provide no indication that the copper industry (LGOM) has a negative impact on the physiological condition of cattle.

Keywords: cooper industry, cows, blood, hematological and biochemical parameters, toxic metals

There is a developed copper industry in Poland, and the Legnica-Głogów Copper District (LGOM, Lower Silesia, Poland) is one of the largest in the world. In addition to producing copper and silver, it also produces gold, lead, palladium and platinum concentrates, as well as rare earth metals. The organizational structure includes three mining plants, three copper smelters, an ore enrichment plant and a hydrotechnical plant. Copper mining and processing involves processes that emit numerous chemical pollutants into the environment. For example, copper ore post-flotation waste is stored in the huge engineering facility Żelazny Most (Mining Waste Disposal Facility – MWDF). It covers an area of approximately 1,400 ha, and the volume of waste stored there is approximately 700 million tons, while its maximum storage capacity amounts to approximately 1.1 billion m^3 (26, 32).

Mining waste deposited in the MWDF contains many toxic metals, including lead, cadmium, nickel, arsenic and mercury, as well as compounds of copper, zinc, iron, sulfur, potassium and sodium in various concentrations, which may penetrate into the agricultural environment, contaminating soil and plants, including animal feed, through continuous aeolian and hydrological processes (4, 15, 23, 24). Gases and dust emitted by the mines and steelworks also contain heavy metals, although environmentalist measures implemented for many years have greatly limited these emissions (30).

Toxic metals (including heavy ones) easily enter the body and sometimes accumulate excessively in tissues and organs, e.g. in muscles, blood, liver (6, 25), as well as in cow's milk (8, 33) and poultry eggs (37, 52), posing a certain risk to human health when included in the diet (13, 55).

The impact of heavy metals on animal organisms is the subject of ongoing research and many publications. For example, the impact of trace element heavy metals, such as copper (Cu), cobalt (Co), manganese (Mn), iron (Fe), and (Zn) and toxic heavy metals (Cd, Pb, Hg) on metabolic processes and health of farm animals, including cattle, was presented in detail by Gupta et al. (17). Other authors (12) examined the concentration of 14 heavy metals and minerals (Al, Cr, Mn, Fe, Ni, Co, Cu, Zn, As, Se, Cd, Pb, V, Sn) in the blood serum of cows kept near and away from highways (car exhaust fumes). The levels of Cd, Ni and Pb in cows bred near highways were significantly different from those in cows bred away from highways, with the latter having significantly lower levels of Cu, Fe, and Zn. In the first group of cows, the lipid peroxidation marker-malondialdehyde (MDA) was significantly higher, and an important antioxidant-superoxide dismutase (SOD) was lower compared to the second group of cows. The relationship between these facts is difficult to explain due to the complex biochemical processes occurring in the tissues and organs (e.g. liver) of animals.

The literature provides plentiful information on the impact of various industries, metallurgy and mining on the accumulation of toxic metals in the blood and milk of animals (11, 25, 33, 44, 47), but there is less data on the impact of environmental pollutants on health indicators, e.g. cow liver metabolism (e.g. activity of aspartate transaminase, AST; lactate dehydrogenase, LDH; gamma-glutamyltransferase, GTP), enzymatic indicators of the antioxidant barrier, e.g. total antioxidant status (TAS) and glutathione reductas (GR), or at least basic hematological and endocrine parameters. One exception may be a study by Swarup et al. (47), who examined the plasma concentrations of Cd and Pb, as well as thyroid hormones (T4, T3), stress hormone (cortisol), reproductive hormones (estradiol and progesterone), and liver enzymes (AST and ALT). Their research shows that natural exposure of cows to lead in a polluted environment disrupts the hormonal profile, and higher levels of lead in the blood change serum biochemical parameters, indicating liver dysfunction. Recent studies (18) show that changes in immunological factors and antioxidant capacity in the perinatal period in cows are correlated with Pb and As levels, while Cd has no such effect.

In the LGOM region, apart from the copper industry, there is also agriculture, as the soil is fertile, crop yields are high, and farms, mostly small-scale ones, keep various species of animals, including dairy cattle. Except for a few works (7, 20-22, 29, 31), there are no recent studies on the possible impact of the copper industry on animal health, including physiological parameters, e.g. hematological or biochemical indices of cows' blood.

The aim of the study was to assess the concentration of toxic heavy metals and basic hematological and biochemical indicators (enzymes) in the blood of dairy cattle kept in an area affected by the copper industry in south-western Poland.

Material and methods

We monitored the blood of dairy cows (n = 30) kept in an area affected by the copper industry, including the MWDF Żelazny Most (communes: Rudna, Polkowice, Grębocice), in the summer of 2021 (series I) and again in 2022 (series II) (other cows, but the same herds). These studies did not require the consent of the Local Ethical Committee for Animal Experiments, because blood collection from cows took place as part of periodic herd monitoring tests carried out by the local veterinary service.

Selected cows (crossbreeds with HF blood, aged 3-5 years, with a daily productivity of approx. 15-20 liters) were kept in small herds with the use of pastures. They were in the full lactation phase, clinically healthy, and had been examined by a local veterinarian. It should be noted that production values (milkiness) were not monitored in this study. The full lactation phase was a period in which blood samples were taken and then compared in individual years. The diet was dominated by traditional roughage with the addition of cereal meal. It should therefore be noted that the animals' fodder was affected by technological processes related to the active copper industry in the area. Salt licks were used, as well as mineral premixes in winter. Blood for testing was collected once from the jugular vein (*vena jugularis externa*) to obtain fresh whole blood as well as blood serum.

Morphological indicators in the whole blood of the cows included the following hematological indicators: white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), haematocrit (HCT), platelets (PLT), average erythrocyte volume (MCV), average hemoglobin content in the blood cell (MCH), and average hemoglobin concentration in a blood cell (MCHC).

The analysis was performed by standard laboratory methods commonly used in veterinary medicine. For this purpose, an ABX Vet hematology analyzer was used (Horiba ABX, France).

The following biochemical parameters (enzymes) were determined in blood serum: aspartate aminotransferase (AST), γ-glutamyltranspeptism (GGTP), and total oxidative capacity (TAS). The following biochemical tests were used: AST was measured with an enzymatic test (UV detection) without pyridoxal phosphate as recommended by the International Federation of Clinical Chemistry (IFCC), GGTP was determined using a modified kinetic photometric test, and TAS was measured using a colorimetric test based on the ABTS reaction (2,2'-Azino-di[3-ethylbenzothiazoline sulfate]) with peroxidase. A Pentra 400 (USA) clinical chemistry analyzer from Horiba ABX was used for these determinations.

The determination of selected minerals in blood serum (total Ca, inorganic P, Mg, Fe) was performed as follows:

• calcium – photometric test using orthocresolphthalein complexone (OPC),

• inorganic phosphorus – UV test using phosphomolybdate,
• magnesium – photometric test using xylidyl blue,
• iron – photometric test using ferrozine.

Calcium was determined by a photometric test using ortho-cresolphtalein complexone (OPC). Cresolphthalein complexone reacts with calcium ions in an alkaline medium forming a red-violet color. Interference by magnesium is eliminated by addition of 8-hydroxyquinoline. Inorganic phosphorus was determined by the UV method using phosphomolybdate according to the following reaction:

Ammonium molybdate + Sulphuric acid →
→ Phosphomolybdate complex

Magnesium was determined by a photometric test using xylidyl blue. Magnesium ions form a purple colored complex with xylidyl blue in alkaline solution. In the presence of GEDTA, which complexes calcium ions, the reaction is specific. The intensity of the purple color is proportional to the magnesium concentration.

Iron was determined by a photometric test using Ferene. Iron bound to transferrin is released in an acidic medium as ferric iron and is then reduced to ferrous iron in the presence of ascorbic acid. Ferrous iron forms a blue complex with Ferene.

Ascorbic acid, Buffer

$$\text{Transferrin } (Fe^{3+})_2 \rightarrow 2\ Fe^{2+} + \text{Transferrin}$$

$$Fe^{2+} + 3\ \text{Ferene} \rightarrow \text{Ferrous Ferene (blue complex)}$$

Mineral parameters in blood serum were determined using an automatic biochemical analyzer Pentra 400 from Horiba ABX (France) and reagents from the same company.

Toxic metals (Cd, Cu, Pb, and Zn) were determined in whole blood, which had previously been mineralized in a microwave oven (Milestone, Italy). The analyses were performed by a spectrophotometric method (ASA) in an accredited chemical laboratory that meets the requirements of the PN-EN ISO/IEC 17025:2005 standard. Total Hg was determined by the spectrometric method with an AMA-254 automatic analyzer (Tesla). The smallest measurable amount of Hg was 0.2 ng.

The results of our research were analyzed statistically using the Statistica 13.1. The mean values and standard deviations were calculated, and the significance of differences between two years of data was assessed using Student's t-test.

Results and discussion

Heavy metals in whole blood. The results of our study are presented in Table. 1. The mean concentration of cadmium in the blood of cows in both series (I and II) remained at a low level, not exceeding 0.01 mg Cd/L. Nwude et al. (35) found the Cd content in the whole blood of slaughter cattle in Nigeria in the range of 0.004-0.02 mg/kg depending on the season and the type of environmental pollution sources, while Castro-Gonzales et al. (6) report an average value of 0.001 mg Cd/kg in the blood of dairy cows fed with forages from areas with industrial and volcanic activity in Mexico. Similar values are reported by other authors (45), suggesting that the best indicator of cattle exposure to cadmium is its concentration in the hair, which is confirmed by the research of Rogowska et al. (42). So far, no limit values for Cd in the blood of these ruminants have been established. Based on data from the literature and our own research, we suggest that a limit value of 0.01 mg Cd/L in the whole blood of adult cattle be adopted.

The mean copper content in the blood of cows in zone I was 0.61 mg/L in 2021 and 0.63 mg/L in 2022 respectively, with a minimum value of 0.34 mg Cu/L and a maximum of 0.90 mg Cu/L. Monkiewicz et al. (31) report values of 0.55-1.52 mg Cu/L (mean 0.98 mg/L) in the whole blood of cows from the LGOM region, which is much higher compared to our own research. However, Nwude et al. (35) report Cu concentration in blood collected during slaughter of cows from a polluted area of Nigeria, which ranged widely from 0.04 to 2.00 mg/kg, depending on the season and the degree of environmental pollution. Lopez-Alonso

Tab. 1. Heavy metals in the whole blood of cows in 2021 and 2022 (mean ± standard deviation, SD)

Statistical measures	Cd mg/L	Cu mg/L	Hg µg/L	Pb mg/L	Zn mg/L
year 2021 (series I)					
Mean	0.007[a]	0.61[a]	0.192[a]	0.065[a]	2.25[a]
SD	0.003	0.18	0.058	0.029	0.38
Min-max	0.003-0.014	0.34-0.89	0.115-0.302	0.033-0.112	1.66-2.90
year 2022 (series II)					
Mean	0.007[a]	0.63[a]	0.154[a]	0.056[a]	1.67[b]
SD	0.002	0.13	0.030	0.023	0.34
Min-max	0.003-0.010	0.48-0.90	0.115-0.211	0.024-0.108	1.24-2.48
Normal values	< 0.01[PS]	0.69-1.31*	< 0.3[PS]	< 0.10**	0.8-1.2***

Explanations: Mean values marked as a, b in the columns are statistically significant at $P < 0.05$. *Acc. to *Lopez-Alonso M., Crespo A., Miranda M.*: Assessment of some blood parameters as potential markers of hepatic copper accumulation in cattle. J. Vet. Diagn. Invest. 2006, 18, 71-75. **Acc. to *Waldner C., Checkley S., Blakley B. et al.*: Managing lead exposure and toxicity in cow–calf herds to minimize the potential for food residues. J. Vet. Diagn. Invest. 2002, 14, 481-486. ***Acc. to *Kendall N. R., Bone P.*: Fertility and trace elements – an underestimated problem. Cattle Pract. 2006, 14, 17-22. [PS]Authors' own proposals.

et al. (26) found values of 0.69-1.31 mg/L in whole blood in Spain (a Galicia region where small farm grazing pastures are fertilized with pig slurry, causing high Cu exposure) and considered them to be normal. The results of our own research are usually below 0.70 mg Cu/L, indicating a certain copper deficit in the body, which is somewhat paradoxical considering the presence of the copper industry in the region. Also Tomza-Marciniak et al. (49) found a low Cu content, averaging 0.152 mg/L, in the blood serum of dairy cows in non-industrial areas in western Poland. On the other hand, Pavlata et al. (39) provide data from 20 cattle farms in the Czech Republic, with average values of 0.65-0.86 mg/L in cattle blood serum, depending on the age and sex of cows. Copper in cattle interacts with molybdenum, sulfur, and iron. The limits between tolerance and toxicity are narrow, and the interpretation of the results should take into account its content in the liver (38).

The mercury content in the blood of cows was 0.192--0.154 µg/L in the first sampling and significantly lower (0.153 µg/L) in the second sampling, with a highest value of 0.302 µg/L. In a mining area of Brazil, the average value observed in blood was 11.7 µg Hg/L for cattle and 15.7 µg Hg/L for pigs (38), whereas in the blood of older calves in Spain, it ranged from 2.76 to 13.4 µg Hg/L, depending on the sources of contamination (3). Therefore, the values obtained in the present research should be considered as very low, indicating a trace presence of this toxic element in the environment. This is confirmed by other authors (7, 9) who conducted similar research in the LGOM region. So far, no mercury limit values have been established for the whole blood of these animals. We suggest a limit value of 0.3 µg Hg/L for adult cattle.

The mean concentration of lead in the blood of cows was 0.065 mg/L in 2021 (1st sampling) and significantly lower (0.056 mg/L) in the 2nd sampling, with a maximum value of 0.112 mg/L. Monkiewicz et al. (31) report values of 0.07-0.170 mg Pb/l (mean 0.115 mg/L) in the whole blood of dairy cows, which is much higher compared to our research, but these values were obtained many years before. Swarup et al. (46) provide values from 0.198 to 0.756 mg/L for industrially polluted areas, which are many times higher than those obtained in our research. On the other hand, Castro-Gonzales et al. (6) recorded an average content of 0.015 mg/L in dairy cows in Mexico reared in areas irrigated with wastewater. Very high concentrations of up to 10.6 mg/L were found in cows from polluted areas of Nigeria (35). According to Radostis et al. (40), a concentration of 0.025 mg/L in blood should be regarded as normal, whereas Waldner et al. (53) consider lead concentrations of up to 0.1 mg/L in cows' (whole) blood as normal, those from 0.1 to 0.3 mg/L as elevated, and those higher than 0.35 mg Pb/L as toxic.

The mean zinc content in the blood of dairy cows was 2.25 mg/L in the first sampling and 1.67 mg/L (significantly different) in the second one. The highest concentration was 2.90 mg/L. Monkiewicz et al. (1999) report values of 1.4-4.0 mg Zn/L (average 2.74 mg/L) in the whole blood of cows from the LGOM region, which are slightly higher than those in our study. According to Kendall and Bone (19), the zinc content in whole blood should be 0.8-1.2 mg/L, which is half of that obtained in own research. Relatively high concentrations of Zn in blood may be explained by an increased content of this element in fodder. Excess zinc in the fodder and blood of cows impairs the bioavailability of copper (5, 51), which is also confirmed by our research. Other studies (45) show that the level of zinc in blood may be 35% higher for highly productive cows than it is for those with low milk production.

The results are difficult to evaluate because there are no official data on the permissible limit of trace heavy metals (Cu, Zn, and others) as well as toxic heavy metals (Cd, Hg, Pb, and others) in the whole blood of cows, and there are various interactions between the elements (antagonism, synergism), which further complicates the interpretation of the results (14, 36, 41, 49). Nwude et al. (35) found significant correlations between the levels of Cd and Cu, Cd and Zn, and Co and Zn in the blood of slaughtered cattle. Similarly, various diseases may also significantly change the elemental status of blood, e.g. clinical mastitis causes a decrease in zinc concentration and an increase in copper levels in the blood of cows (1).

Hematological parameters. The hematological results, related to the lactation period, are presented in Table 2. Hematological tests are extremely important because the assessment of individual blood elements and indicators reveals the health status of the entire organism. Seven hematological parameters were determined in the cows' blood serum: WBC, RBC, PLT, MCV, MCH, and MCHC. The average WBC content (leukocytes) in the two following years (two samplings) was 7.81 and 11.98 G/L. A typical reference range for this parameter is 4-12 G/L (54). Some samples exceeded the upper limit, mainly in the second sampling, which may suggest the presence of subclinical inflammation or other factors stimulating the proliferation of the white blood cell system in some cows.

The number of RBCs (erythrocytes) was similar in the two samplings, with a mean value 6.84 and 7.95 T/L respectively, but 43.3% of samples exceeded the upper limit of the reference standard (54), i.e. 5-7 T/L, which indicates that some cows evaluated as clinically healthy (i.e. not showing clinical signs of disease) had factors (including disease factors) stimulating the proliferation of the red blood cell system.

The HGB (hemoglobin) level was similar in the two samplings (averaging 5.91 and 6.99 mmol/L) and did not exceed the reference values determined by Winnicka (54). Similarly, HCT (hematocrit) was identical in series I and II (average 0.29 1/L), with reference values of 0.24-0.46 1/L (54).

Tab. 2. Morphological indicators in the blood serum of cows in 2021 and 2022 (mean ± standard deviation, SD)

Statistical measures	WBC G/L	RBC T/L	HGB mmol/L	HCT l/L	PLT G/L	MCV fL	MCH fmol	MCHC mmol/L
year 2021 (series I)								
Mean	7.81[a]	6.84[a]	5.91[a]	0.29[a]	368.2[a]	43.1[a]	0.87[a]	20.29[a]
SD	1.60	1.03	0.79	0.04	101.2	5.64	0.11	0.42
Min-max	6.6-13.0	5.74-9.28	5.0-8.1	0.26-0.41	230-517	39-58	0.61-1.14	19.7-21.2
year 2022 (series II)								
Mean	11.98[b]	7.95[a]	6.99[a]	0.34[a]	422.3[a]	41.0[a]	1.04[b]	26.58[a]
SD	4.21	3.22	1.71	0.10	152.2	8.87	0.49	16.92
Min-max	5.0-20.1	5.09-13.60	5.30-11.1	0.20-0.59	290-750	27-52	0.65-2.60	19.3-73.8
RVs*	4.0-12.0	5.0-7.0	4.96-8.69	0.24-0.46	150-650	40-60	0.68-1.05	30-36

Explanations: RVs – reference values. Mean values marked as a, b in the columns are statistically significant at P < 0.05. * *Winnicka A.*: Wartości referencyjne podstawowych badań laboratoryjnych w weterynarii. Wyd. VII, SGGW, Warszawa 2021.

Tab. 3. Biochemical indicators in the blood serum of cows in 2021 and 2022 (mean ± standard deviation, SD)

Statistical measures	AST (U/l)	GGTP (U/l)	TAS (mmol/l)	Fe (µmol/l)	Mg (mmol/l)	P nieorg. (mmol/l)	Ca (mmol/l)
year 2021 (series I)							
Mean	70.28[a]	27.28[a]	1.37[a]	23.89[a]	1.00[a]	1.95[a]	2.37[a]
SD	12.67	10.46	0.17	6.83	0.06	0.61	0.18
Min-max	46.2-95.9	13.92-48.51	0.89-1.64	10.74-44.77	0.93-1.12	1.09-3.08	2.0-2.67
year 2022 (series II)							
Mean	73.41[a]	33.72[a]	1.39[a]	31.90[a]	0.96[a]	2.10[a]	2.43[a]
SD	18.32	16.70	0.16	8.39	0.15	0.63	0.17
Min-max	44.17-108.8	16.79-63.0	1.10-1.66	4.95-43.58	0.53-1.21	1.42-4.35	0.11-2.90
RVs*	48-100	20-48	1.30-1.77	21.5-35.8	0.78-1.23	1.48-2.90	2.25-3.03

Explanations: RVs – reference values. Mean values marked as a, b in the columns are statistically significant at P < 0.05. * *Winnicka A.*: Wartości referencyjne podstawowych badań laboratoryjnych w weterynarii. Wyd. VII, SGGW, Warszawa 2021.

The number of PLTs (platelets) in the second sampling series (2022) increased significantly (mean 422.3 G/L) compared to the first series (mean 368.2 T/L). A large part of the samples (27.67%) exceeded the upper limit of the reference standard (650 T/L), which indicates that these cows, which did not show clinical symptoms of the disease, had a blood pattern often observed in iron deficiency. MCV (Mean Corpuscular Volume) was similar in the two samplings (mean values 43.1 and 41.0 fL), with the reference standard being 40-60 fL (54). Exceeding the upper limit of reference values may indicate microcytic anemia, which usually accompanies iron or selenium deficiencies.

MCH (Mean Corpuscular Hemoglobin) values in the second sampling series were significantly higher than in the first series (2021). The average values were 0.87 and 1.04 fmol, respectively, whereas the acceptable level is 0.68-1.05 fml (54). In a significant portion of the samples (55%), the results exceeded the upper limit of the reference standard, which indicates that those cows, evaluated as clinically healthy (i.e. not showing clinical signs of disease) had factors that could cause intravascular hemolysis. MCHC (Mean Corpuscular Hemoglobin Concentration) is related to MCH. In the second sampling, MCHC was much higher (mean 26.58 mml/L) than a year earlier (20.29 mml/L), with reference values for cattle in the range of 30-36 mml/L (54). Exceeding the upper limit of this standard may also indicate the occurrence of intravascular hemolysis in cows (not showing clinical signs of disease), which was not observed in blood samples (2, 56).

Biochemical parameters. The results of measurements of biochemical indicators are presented in Table 3. The activity of enzymes in blood serum (AST, GGTP, TAS) depends largely on the physiological state of animals, including disease states, diet (nutrition), and environmental factors, including toxic substances (34, 48, 50). The mean AST activity was similar in both samplings (70.28 and 73.41 U/L). There were no statistically significant differences in mean GGTP values (27.28 and 33.72 U/L) between samples from 2021 and 2022. These values were within reference values (54). The enzymatic antioxidant barrier is created by a number of enzymes. In our research, only the total antioxidant capacity (TAS) was determined. The mean values were almost identical (1.37 and 1.39 mml/L) and slightly lower than the lower limit of the reference range (1.30 mml/L). Reduction in the activity of this enzyme (TAS) may have various causes, e.g. it may be caused by diseases (viruses), various stages of lactation, or toxic elements (10, 12, 16).

The levels of basic macronutrients in blood serum (Ca, Fe, Mg, and inorganic P) were similar in the two samplings, and the mean concentrations fell within the fairly wide reference range given by Winnicka (54). Generally, the results for the levels of minerals in the blood serum of cows are similar to other data reported in the veterinary literature, but the housing system, season, lactation level, and even health status have certain influence on their concentration (14, 39). Sayiner et al. (43) found that Ca and P levels in young cattle grazing in Northern Cyprus were significantly higher in summer than in winter, and Mg levels were significantly higher in winter than in summer, which suggests appropriate supplementation with these minerals throughout the year. Therefore, Olech et al. (36) rightly believe that the concentration of the main macronutrients in the blood serum of cattle may undergo physiologically unfavorable changes when mineral nutrition is unbalanced, especially when there is an excess or deficit of Ca or P in the diet.

In summary, it should be noted that the concentrations of heavy metals in the whole blood of dairy cows as well as the levels of selected enzymes and minerals in their blood serum were (with some exceptions) within reference values or values reported in the literature for clinically healthy cattle kept in unpolluted areas. Excessive WBC, RBC, MCH and MCHC values, as well as Zn levels, in the blood of some cows require repeated tests, especially since there were significant differences between the two samplings (in 2021 and 2022). Generally, the results do not indicate a negative impact of copper industry facilities (LGOM region) on physiological indicators in these farm animals.

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