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DIFFERENCES IN THE CONTENT OF SOME ELEMENTS IN SOIL, WATER AND THE ALIMENTARY CHAIN IN REGIONS CHARACTERIZED BY HIGH AND LOW INCIDENCES OF LEUKEMIA

The contents of oligo- and microelements in soil, drinking water, hay, bran, silage, cow milk, cattle and human blood in a region characterized by low incidence of leukemia and other prolific diseases have been determined and compared with analogical data from a region of an increased incidence of those diseases. The results obtained are discussed, considering the influence of the environmental factors on the incidence of the diseases mentioned.

1. INTRODUCTION

An increased incidence of neoplasmatic diseases in clayey and waterlogged regions of France was observed by Haviland [11] as early as in 1869. Numerous investigations performed later in different regions of the world allowed to state a correlation between the geochemical environment and the incidences of neoplasmatic diseases, sclerosis, hypertension, apoplexy, urolithiasis and other commonly known diseases [ef. 3, 11, 15, 16, 22, 24, 25, 33, 34, 47]. It has been shown, namely, that the deficiency or a disturbed ratio of some elements in soil and drinking water is associated with an increased incidence of these diseases among the inhabitants of these areas. Particular attention has been paid to such elements as magnesium, calcium, copper, potassium and other oligoelements. It has been found that a deficiency of magnesium and an excess of potassium in soil and soft water are associated with an increased incidence of cancer as well as some diseases of the cardiovascular system [1, 2, 3, 25, 30, 33, 34, 35].

The experiments have shown the influence of the chemical composition of the natural environment on the content of oligo- and microelements in plants [31, 35, 48]. The role of an appropriate supply of microelements in prophylaxis has been proved by epidemiological and environmental investigations combined with experiments [24, 25, 33, 34, 46,

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47, 49]. A negative influence of the deficiency of some oligo- and microelements on breedin effects has been evaluated and methods allowing the compensation of deficient elements i the forage of breeding animals developed [35, 37, 49].

Once ALEKSANDROWICZ had formulated his hypothesis on the multifactorial, environ mental etiology of leukemia [5], which was later verified by complex investigations [18-21] special attention has been paid to the contents of mineral salts in regions with differin incidences of leukemia.

In clusters of leukemia the deficiency of magnesium was stated [10, 21, 23, 24, 29] The deficit of this element due to geochemical conditions seems to be correlated with a increased incidence of leukemia in cattle ([20] and information from the lecture delivere in a symposium on "Leukemia in cattle", organized by the Institute of Veterinary, Puław 1977).

From a cycle of experiments performed it follows that in Wistar rats the deficiency o magnesium in nutrition can bring about neoplastic changes of the thymus and give a bloo picture similar to that observed in leukemia [31, 33-35].

Complex environmental investigations were conducted in regions showing exceptionall great differences in the incidence of cattle leukemia. They covered the locality Pomianow (situated in the region of the former district of Brzesko, for which the index of leukemi incidence was as high as 781.3 (100,000) in the years 1963-1966) and the locality Radłów (in the former district of Tarnów, for which the analogical index amounted to 43.5) [24] It should be noted that at Pomianowa, in the neighbourhood of a large-scale breeding far of "leukemic" cattle, as many as 8 cases of human cancer were stated within 3 years [19] whereas at Radłów (situated in the neighbouring district) neither leukemia of catlle no neoplastic diseases of inhabitants were found in this period of time.

Comparative analyses of drinking water performed in 1968 have shown a much lowe content of magnesium (6.9 mg/dm^3) and a higher content of potassium (12.9 mg/dm^3) at Pomianowa than at Radłów, when the respective values amounted to 46.5 mg/dm^3 and 3.5 mg/dm^3 [24]. ALEKSANDROWICZ and SMYK [9] have stated that in clusters of cattle leukemia and human cancers the pollution degree with *Aspergillus flavus* is significantly higher. A protective action of non-toxic concentrations of sodium selenate against the pathogenic effect of aflatoxin B_1 (the metabolite of *A. flavus*) on human lymphocytes in vitro and the teratogenic effect of this mycotoxin on *Xenopus laevis* in vivo has been demonstrated by ALEKSANDROWICZ at al. [5, 16].

The physiological role of selenium in animal and human organisms and the effect of its deficiency on the occurrence of some neoplastic diseases and lymphatic leukemia in man have been quite recently established [35, 37-39, 41]. Considering the above quoted facts, it seems interesting that FISHER at al. [19] have not detected the presence of selenium in well-water situated in farms where neoplastic diseases occurred. It appeared that the changes in the contents of other trace elements in nutrition and in organisms might also influence the frequency of some prolific as well as other diseases [21, 24, 25, 34, 37, 38, 40]. For these reasons an attempt has been made to state whether there are significant differences between regions differing in the incidence of leukemia by determining the contents of oligo- and microelements in samples of soil, drinking water and the alimentary chain as well as in the blood of cattle and the inhabitants of these two regions.

2 MATERIAL AND METHODS

The contents of physiological and toxic elements have been analysed in samples of soil, drinking water, hay, silage, bran, cow milk as well as in cattle and human blood taken from regions with a high (Pomianowa near Brzesko) and low (Radłów near Tarnów) incidences of cattle leukemia and malignant diseases of the inhabitants. Basic analyses were performed under comparable laboratory conditions and at the same season of the year 1978 (after preliminary analyses conducted in 1977). Besides routine methods and atomic absorption technique, some more modern methods have been employed. Analyses by the proton-induced X-ray emission (PIXE) method [28] was carried out in the Institute of Nuclear Physics on Cyclotrone C-48, using the detector Si/Li and CAMAC electronics with the amplitude analyser NTA-SR.

The analysis was conducted on water and blood drops and tablets (10 cm in diameter and 1 mm of thickness) formed from the solid samples. Spectra were recorded within the energy range of 2-16 kv for 10 h. Results of analyses performed on cyclotrone (obtained in the form of printouts) were calculated on the computer Odra-1305.

A new method was developed for determining the contents of elements in single blood cells [6, 7]. It consists in linear and local X-ray analyses made in microareas of the diameter smaller than 1 μ. The intensity of this radiation excited by electron flux bombardment of the sample and characteristic of the elements studied is to be determined and compared with the analogical intensity for a chemical standard with a known content of this element. Its content in a cell is calculated from the formula

$$C_p = K \frac{N_p}{N_w} C_w,$$

where:

K — coefficient for absorption corrections,

N — the number of X-ray impulses registered for a sample (blood cell),

N_w — the number of X-ray impulses registered for a chemical standard,

C_w — the weight percentage of the element examined in the standard.

The tests were performed on peripheral blood, taking 2 ml of blood for 200 units of heparine. Lymphocytes, granulocytes and erythrocytes were separated by a standard method [6, 7]. Individual fractions of the blood elements were rinsed three times in Parker's fluid, and then once again rinsed but buffered to a pH 7.3 by a 0.1 M phosphate solution. Blood cells were fixed at 4°C for 6 hours in a 2% solution of glutaric aldehyde diluted in physiological solution. Thereupon, the cells were dehydrated in a gradient of acetone concentrations, and after being three times rinsed in absolute acetone, they were left in acetone and put into a refrigerator until the preparations for determining the contents of chemical elements in cells were ready. The suspended cells were dropped on to polished high purity (99.9%) aluminium plates. The prepations were dried in a vacuum and evaporated with spectrally pure carbon. In order to improve the sensitivity of X-ray analysis, no additional evaporation with gold was applied. The tests were performed using an electron microprobe X-ray analyser combined with the scanning electron microscope ARL of the ARL and Cameca firm. The accelerating voltage and current intensity applied amounted to 15 kV and 10 μA, respectively. The results of analyses were obtained in the form of printouts, and morphological changes were photographed in a scanning microscope using "Polaroid" films. The mean contents of the elements examined were determined according to statistical requirements for representative samples and expressed in mass fractions. Significance tests of the differences between the contents of oligo- and microelements in the samples compared (soil, water, food, whole blood and its separate cells) were determined by using suitable programmes for the computer Odra-1305.

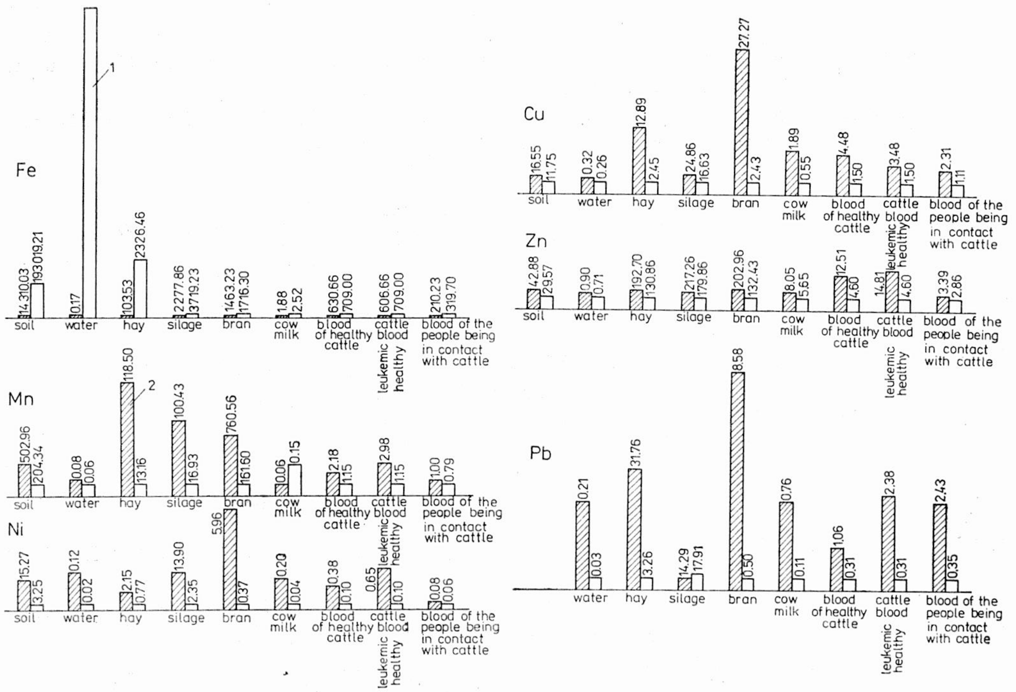
3. RESULTS

The contents of microelements obtained from analyses of fodder, cow milk, cattle blood, lymphocytes and granulocytes in cattle blood, blood of farm workers and their families as well as of single cells of peripheral blood were calculated at the levels of significance $\alpha = 0.05$ and $\alpha = 0.01$. It appeared that at $\alpha = 0.01$ the differences in the contents of some elements were significant. Results of analyses are presented in fig. and in tables 1-4.

Comparing Pomianowa, the locality with a high morbidity of cattle leukemia and human cancer, and Radłów, the locality with low morbidity of these diseases, the following was stated:

1. Considerably lower amount of iron (in soil, water, alimentary chain, cattle milk and blood) in Pomianowa. The greatest differences were found in soil and drinking-water for cattle showing that the regions examined differ with respect to their geo- and hydrochemical compositions. Pomianowa is characterized by brown and alluvial soils, while Radłów has brown-humous gley soils. At Pomianowa the water is characterized by low contents of mineral salts. At Radłów it is constantly hard with high contents of iron and, according to information obtained from assistant professor, K. OLEKSYN, it satisfies the demands required for mineral iron water.

2. Lowered content of selenium at Pomianowa and no detectable amounts of this element in bran, cow milk and blood, and in human blood. The greatest differences were found when comparing samples of silage taken from the two regions. These differences are due not only to different compositions of the soil but also of the silage, since at Radłów there is a high percentage of corn which is known to accumulate selenium from the soil.



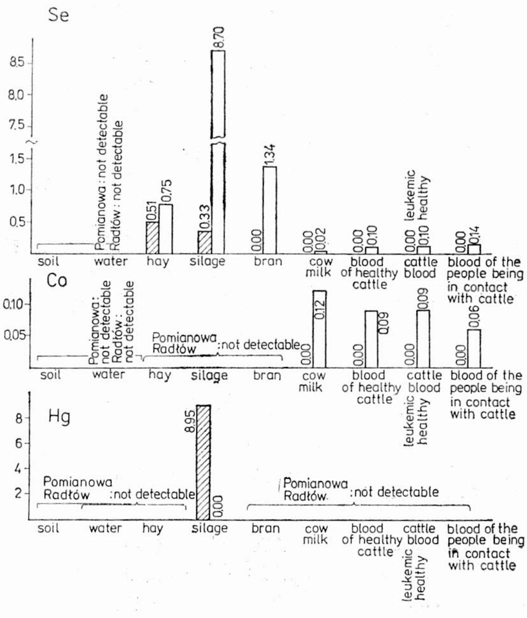


Fig. Relative contents of microelements (in ppm) determined by proton activation method

1 — leukemic area, 2 — control area

Rys. Względna zawartość mikroelementów (w ppm) oznaczonych metodą aktywacji protonowej

1 — obszar leukemiczny, 2 — obszar kontrolny

Table 1

Zinc content in peripheral blood cells in cattle from breeding farms and the people at Radłów, Pomianowa and Mydlniki (arithmetic means in weight percentage)
Zawartość cynku w komórkach krwi obwodowej bydła i ludzi w Radłowie, Pomianowej i Mydlnikach (średnie arytmetyczne wyrażone procentem wagowym)

Blood cells	Healthy cattle		Leukemic cattle	People	
	Radłów	Pomianowa	Pomianowa and Mydlniki	Radłów	Pomianowa
lymphocytes	0.1967	0.2650	0.3700	0.110	0.180
granulocytes	0.3833	0.4300	0.5633	0.170	0.257
erythrocytes	0.2833	0.3233	0.4333	0.137	0.203

Table 2

Calcium content in pheripheral blood cells in cattle from breeding farms and the people at Radłów, Pomianowa and Mydlniki (arithmetic means in weight percentage)
Zawartość wapnia w komórkach krwi obwodowej bydła i ludzi w Radłowie, Pomianowej i Mydlnikach (średnie arytmetyczne wyrażone procentem wagowym)

Blood cells	Healthy cattle		Leukemic cattle	People	
	Radłów	Pomianowa	Pomianowa and Mydlniki	Radłów	Pomianowa
lymphocytes	0.8600	0.4450	0.6550	0.827	0.393
granulocytes	0.9033	0.6500	0.5233	0.767	0.517
erythrocytes	0.8767	0.7500	0.7233	0.843	0.627

Table 3

Magnesium content in peripheral blood cells in the cattle from breeding farms and the people at Radłów Pomianowa and Mydlniki (arithmetic means in weight percentage)
Zawartość magnezu w komórkach krwi obwodowej bydła i ludzi w Radłowie, Pomianowej i Mydlnikach (średnie arytmetyczne wyrażone procentem wagowym)

Blood cells	Healthy cattle		Leukemic cattle	People	
	Radłów	Pomianowa	Pomianowa and Mydlniki	Radłów	Pomianowa
lymphocytes	0.2950	0.1683	0.3667	0.270	0.220
granulocytes	0.2967	0.3400	0.3800	0.233	0.167
erythrocytes	0.3033	0.2867	0.3300	0.280	0.207

Table 4

Sulphur content in pheripheral blood cells in the cattle from breeding farms and the people at Radłów, Pomianowa and Mydlniki (arithmetic means in weight percentage)
Zawartość siarki w komórkach krwi obwodowej bydła i ludzi w Radłowie, Pomianowej i Mydlnikach (średnie arytmetyczne wyrażone procentem wagowym)

Blood cells	Healthy cattle		Leukemic cattle	People	
	Radłów	Pomianowa	Pomianowa and Mydlniki	Radłów	Pomianowa
lymphocytes	0.3233	0.3850	0.3517	0.120	0.200
granulocytes	0.2633	0.3500	0.3833	0.147	0.240
erythrocytes	0.2933	0.4333	0.4167	0.263	0.420

3. Lowered contents of cobalt in cow milk and blood and in blood of the inhabitants at Pomianowa.

4. Elevated amounts of manganese, copper, zinc and nickel in soil, water, food, cow milk and blood and human blood at Pomianowa (tables 1-4; figure), and also of lead in drinking water, alimentary chain and blood. Striking differences are also observed in the ratios of antagonistic elements e.g. zinc, selenium, iron manganese, calcium and magnesium while comparing the samples of soil, fodder, cow milk and blood from Pomianowa and Radłów.

It may be stated that, in general, the essential differences in the contents of physiological and toxic elements in blood are associated with the differences in their contents in the natural environment and alimentary chain. It may be seen, for instance, that elevated concentrations of zinc found in soil samples from Pomianowa are accompanied with higher amounts of this element in cattle and human blood.

It seems to be worth noticing that the differences between the contents of microelements in the whole blood of healthy cattle at Pomianowa and Radłów are greater than the analogical differences between healthy and sick cattle at Pomianowa.

4. DISCUSSION

The stated influence of the composition of microelements in soil on their contents in water, fodder, milk and blood shows that disturbances in the amounts and proportions of these elements in natural environment and fodder may induce similar disturbances in whole blood as well as in particular morphological elements of that blood. This statement is consistent with available results from epidemiological, ecological, experimental and other investigations [1-13, 23-26, 30, 32-35, 37, 38, 42-48]. The above relationships refer expecially to cattle, mostly fed with forage produced within the areas studied.

It should be noted that contamination with *Aspergillus flavus*, whose carcinogenic activity is well known, was observed in some fodder both at Pomianowa and Radłów, although a higher pollution degree was found in a leukemic cattle cluster at Pomianowa (ALEKSANDROWICZ, SMYK, CZACHOR, DOBROWOLSKI — unpublished results).

In view of the protective role of selenium against the pathogenic effect of aflatoxin B_1 — the metabolite of this mould, it should be expected that the increased content of selenium in fodder and in cattle blood in the Radłów region is of substantial prophylactic importance [4, 5]. Basing on data from 26 countries, other authors [37-41] have stated the influence of the selenium deficiency in food and blood of humans and animals on the incidence of leukemia and other neoplasmic diseases.

Particularly extensive are investigations into the effect of a physiologically adequate supply of iron and cobalt on the regular course of hematopoietic processes and organism immunity [16, 17, 48].

A disturbance in the proportion of particular elements in food is a known pathogenic factor [45-48]. Such disturbances have been stated at Pomianowa which is the region of increased incidence of leukemia.

Special attention should be paid to the potential pathogenic action of the elements whose excess is characteristic of the given area.

A high concentration of manganese can worsen the metabolism of iron, whose deficiency is observed at Pomianowa, and bring about some neuro-motor disturbances [46]. It excess also results in the retention of phosphates in the organism. However, these negative results may be prevented by increasing the magnesium supply (IWATA — information in a lecture HESC, 1979) and by the higher content of iron in food [46].

An excess of copper may promote its accumulation in erythrocytes, the formation of methemoglobin and eventually lead to hemolysis. Formation of methemoglobin is also fostered by one-sided nitrogen fertilization. Because of interactions among e.g. copper, molybdenium, iron, zinc and sulphate ions [46], disturbances due to the excess of copper in the organism may be to some degree corrected by supplementing the food with antagonistic elements.

In view of the deficiency of zinc stated in patients suffering from chronic lymphocytic leukemia, the excess of this element in the natural environment, alimentary chain and cattle and human blood found in the region characterized by a higher incidence of leukemic and other proliferating diseases seems to be amazing. It is known, however, that the excess of zinc can enhance the development of neoplasmic diseases [46, 48], but it is also known that with a deficiency of iron or calcium in food, an increased supply of zinc favours the occurrence of anemia and osteoporosis [46].

With regard to the increased contents of toxic elements, such as nickel, lead and mercury stated at Pomianowa, the following conclusions may be formulated:

1. Nickel may stimulate carcinogenesis [44, 46], but carcinogenic effects may be reduced by an adequate supply of manganese [44]. This leads to the possibility for prophylactic action.

2. Long-lasting exposure to subtoxic amounts of lead may lower the morphological

indices of peripheral blood and decrease the activity of enzymes sensitive to the presence of heavy metals in erythrocytes. Enzymatic inhibition is proportional to the accumulation of lead in blood being particularly high in infants [31]. The increase of lead concentration in blood may induce the formation of lymphocytomas [18] and further the carcinogenic processes [20]. The effect of lead intoxications can be in some measure reduced by increasing the supplies of calcium and magnesium [12, 35, 45]. This is of essential importance in view of the ever increasing contamination of fodder and food with lead [14].

3. From epidemiologic investigations made by JANICKI, it follows that the incidence of leukemia is significantly higher in areas where greater amounts of mercury containing pesticides were used [26]. The influence of methylated mercury poisoning on atrophic processes and on panmyelophthisis were described [22]. In our country the mercury contents in food and fodder are highly differentiated and sometimes local contaminations with mercury fungicides occur [27]. From preliminary analyses, it follows that in "leukemic" farms the average mercury content is higher and that the mercury concentration in the hair of patients suffering from acute leukemia is higher than that in healthy persons (ALEKSANDROWICZ, JANICKI, DOBROWOLSKI and KRAŚNICKI — unpublished paper). Mercury contamination has been detected in silage in the region of the cattle leukemia cluster (Pomianowa) but not in areas without leukemia. The hazard of poisoning with methylated mercury may be reduced to some degree by a controlled supply of selenium to people and domestic animals [17, 37, 46, 48].

4. In general, it may be stated that regions differing with respect to the incidences of leukemia in cattle and proliferation diseases in people differ substantially with respect to the contents of physiological elements and contamination with metals. These differences concern the natural environment, alimentary chains and cattle and human blood. They may be reduced by administration of salts [17].

5. CONCLUSIONS

The deficiency of defined physiological microelements and the excess of some elements, especially metals, in the environment, food, as well as in cattle and human blood was found in clusters of neoplasmic diseases in people and leukemia in cattle. Therefore control examinations are indispensable to eliminate potential environmental factors conducive to the occurrence of the mentioned diseases. Prophylactic methods include a reduction in the pollution of the environment with nickel, lead and mercury as well as supplementation of deficient elements (such as iron, selenium, cobalt) in soil, in fodder for breeding animals and food for people. To this end the described analytical method should be applied to mass examinations of the contents of physiological and toxic microelements in the environment, fodder, food and blood. Results of such analyses may constitute the premises for appropriate prophylactic activity. Some attempts concerning the realization of these postulates by enriching the soil with mineral components and appropriate modification in the mineral composition of food have already been undertaken. In the complex prophylactic activity, the introduction of agrotechnical methods reducing the degree of natural environment pollution performes an essential role.

6. SUMMARY

It has been stated that in the region characterized by a high incidence of neoplasmic diseases, in the inhabitants and leukemic cattle the contents of microelements differ from those found in the regions where this incidence is low. Increased amounts of manganese, copper and zinc and a particularly high level of toxic metals (nickel and lead) have been detected in soil, water and the alimentary chain. The presence of mercury was shown in some fodders. At the same time the contents of iron, selenium and cobalt were considerably lower. Quantitative differences in the chemical composition of the environment were associated with differences in the contents of microelements and other elements in cattle and human blood coming from this region. The results obtained are discussed in the aspect of the possible influence of the environmental factors on the occurrence of some proliferating diseases.

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RÓŻNICE ZAWARTOŚCI NIEKTÓRYCH PIERWIASTKÓW W GLEBIE, WODZIE I ŁAŃCUCHU POKARMOWYM NA TERENACH O NISKIEJ ORAZ WYSOKIEJ ZACHOROWALNOŚCI NA BIAŁACZKĘ

Badano zawartość oligo- i mikroelementów w glebie, wodzie pitnej, sianie, otrębach, kiszonce, mleku krów, krwi bydła oraz ludzi na terenie o niskiej zachorowalności na białaczkę i inne choroby proliferacyjne w porównaniu z terenem o wysokiej zachorowalności. Uzyskane wyniki są dyskutowane w aspekcie wpływu środowiska na występowanie tych chorób.

DIE KONZENTRATION EINIGER ELEMENTE AUF GEBIETEN MIT HOHEN UND NIEDRIGEN LEUKEMIAVORKOMMEN

Untersucht wurden die Konzentrationen der Oligo- und Mikroelemente im Nahrungskreislauf, d.h. im Boden, Trinkwasser, Heu, Kleie, Silofutter, Kuhmilch sowie im Vieh- und Menschenblut auf Gebieten mit einem niedrigen Leukemia-Erkrankungsindex und anderer Gewebsvermehrungskrankheiten im Vergleich zu Gebieten mit einer hohen Erkrankungshäufigkeit.

Die Ergebnisse werden im Aspekt der Umwelteinflüsse auf das Vorkommen der o.e. Erkrankungen diskutiert.

РАЗЛИЧИЯ В СОДЕРЖАНИИ НЕКОТОРЫХ ХИМИЧЕСКИХ ЭЛЕМЕНТОВ В ПОЧВЕ, ВОДЕ И ПИЩЕВОЙ ЦЕПИ НА ТЕРРИТОРИИ С НИЗКОЙ И ВЫСОКОЙ ЗАБОЛЕВАЕМОСТЬЮ ЛЕЙКОМИЕЙ

Было исследовано содержание олиго- и микроэлементов в почве, питьевой воде, сене, отрубях, силосе, молоке коров, крови скота, а также обследовались люди на территории с низкой заболеваемостью лейкомией и другими пролиферативными болезнями, по сравнению с территорией с высокой заболеваемостью. Полученные результаты обсуждаются с аспекта влияния среды на появление этих болезней.