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Rank Non-Parametric Correlation Analysis of Indicators of Heavy Metal Transition from Blood to Cow's Milk to Assess its Environmental Safety

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Abstract. Correlation analysis constitutes an essential method of statistical processing of the obtained scientific research results. Its proper application using special computer software and reliable results allow practically facilitating the work of the veterinary and livestock service in the production of environmentally safe high-quality cow's milk. The purpose of this study is to analyse the correlation between the content of toxic metals Cd and Pb in the blood and milk of cows using the STATISTICA computer software version 10.0. Scientific and economic experiments were conducted on dairy cows with different types of feeding. All animals selected according to the analogue method in terms of live weight and productivity were divided into three groups: the first control group and the second and third experimental groups. The diet included feeds with an excess of heavy metals, especially cadmium and lead. The high biological activity of pollutants affected their migration from the feed of the diet through the gastrointestinal tract to milk. To reduce intoxication of the animal body, premix "MP-A" was introduced into the main diet of cows of the second and third experimental groups, and in the third experimental group – premix "MP-" and injection of the biological product "BP-9". First, using the Shapiro-Wilk's W test, the study verified the obtained data from laboratory tests of blood and milk for the concentration of toxic metals, the law of "normal" (Gaussian) distribution, and then the necessary Spearman's non-parametric rank correlation coefficient was selected for calculation. The analysis revealed a high correlation between the variables, which was within $r=0.82-0.91$ (Cd) and $r=0.78-0.96$ (Pb) with probability ($p<0.05$) in animals with different types of feeding. The discovered high correlation allows veterinary medicine specialists to quickly apply measures to reduce the toxic load of the body with elements only by analysing blood for cadmium and lead, and timely prevent the production of low-quality dairy raw materials, including using premix and phytobiopreparation tested in the experiment. Further research is aimed at analysing the correlation between other indicators of the quality and environmental safety of milk and feed, constructing regression equations that will practically contribute to the activities of specialists whose task is to ensure the production of high-quality environmentally safe cow's milk

Keywords: correlation, correlation coefficient, non-parametric correlation coefficient, Spearman correlation coefficient, dairy products, dairy cows, toxic metals



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INTRODUCTION

The danger of harmful effects of toxic heavy metals through food products, including water, milk, etc. On the human body is world-known due to the studies of J. Song et al. [1], W. Fischer et al. [2], C. Rezza et al. [3], E. Rahimi [4], Lili Liu et al. [5]. There are situations when pollutants enter the body of animals with feed containing an increased concentration of elements. Cd and Pb intake is associated with environmental risks to the body due to cumulative toxicity and negative effects on internal organs and systems, as proven by the studies of M. Canty et al. [6], S. Hashemi [7], S. Roggeman et al. [8]. Toxic metals, especially cadmium and lead, are freely absorbed in the gastrointestinal tract into the blood, and enter milk, which has been investigated in the studies of Y. Jolly et al. [9], D. Adriano [10], S. Ren-ju Tahir et al. [12]. It is challenging for veterinary and livestock specialists to assess the risks of dangerous effects of pollutants on animal health, quality, and environmental safety of milk produced, dairy raw materials that are used for processing and production of dairy products for human nutrition.

Conducting scientific and economic experiments to study the migration of toxic metals in the trophic chain and obtaining the corresponding array of digital data on numerous indicators of laboratory tests performed may not always indicate a high regularity of certain changes. Scientists have long and quite widely used various methods of correlation analysis of the obtained research results. At the same time, various computer software and analysis algorithms are used. It is essential not only to analyse the correlation strength between the indicators, but to choose the correct indicators as well. For example, scientists Lili Liu et al. [5] used a correlation study to identify the correlation between heavy metals in Hong Kong marine sediments over a long period of time from 1991 to 2011. Therewith, Spearman's, and then Pearson's rank correlations were used at the first stage. The bay and harbour (Victoria) were contaminated with heavy metals Pb and Cu, which correlated with Cr, Ni, and Zn contamination. Lili Liu et al. [5] believe that this is the first time they have used such an analysis to investigate environmental pollution, in addition to the fact that such methods have already been widely used in biology, sociology, and computer science. It is important that the investigated correlation analysis can be applied not only to marine sediments, but in other ecological systems as well, which is precisely what the authors of this study did. Other scientists Mingtao Xiang et al. [13] performed a correlation analysis of the heavy metals content in soil and plants grown to assess the risk of crop contamination and predict the danger of harmful effects of toxic metals, especially mercury and cadmium, on the human body. Scientists mostly paid attention to the contamination of soil and plants with heavy metals and did not pay attention to the correlation between them. The results indicated that the toxic metals studied in the soil had the greatest effect on zinc

levels in plants, while lead and chromium had a synergistic effect on zinc uptake by plants. Experiments prove the importance of correlation assessment for controlling the risk of contamination, which was not previously considered. The scientist Mariusz Rudy [14] from Poland investigated the correlation analysis of heavy metals' bioaccumulation in the tissues and organs (liver) of sheep, including muscle tissue, with age. Sheep were kept in eastern Poland. The experiment results demonstrated that with the age of sheep, the water content in muscle tissue decreases, the content of protein, fat, and ash increases. Contamination of meat and liver with cadmium and lead depends on the age of the animals. Between the youngest animals and the oldest, a difference of several times in the level of pollution was recorded. Examples of the use of correlation analysis can be given both in agriculture and in other areas related to environmental pollution in different countries, but the authors of this paper have investigated the correlation between the content of toxic metals in the blood of productive cows and milk for the first time.

Blood tests of cows on farms are regularly conducted by veterinary doctors, and analysis of the quality and environmental safety of milk is mainly conducted by livestock workers. The study of the correlation between the content of toxic metals in the blood and milk of animals is a convenient and fairly reliable indicator of predicting the safety of manufactured products (risk assessment) in the household. Therefore, *the purpose of this study* is to establish a correlation between the content of toxic metals Cd and Pb in the blood and milk of cows that consume feed with an increased concentration of environmentally hazardous toxicants for various types of feeding using the computer software for statistical data processing.

MATERIALS AND METHODS

In the farms located around the industrial city, during 2000-2007, scientific and economic experiments were conducted on dairy cows of Ukrainian black- and red- piebald dairy breeds. 126 heads of cows with silage-haylage-concentrate type of feeding, 63 – with silage-haylage, 36 – with silage and root crop, and 195 – with silage-hay type of feeding were selected for experiments, respectively. The experimental livestock was divided into three groups: the first control and the second and third experimental groups. Cows of all groups were fed feed containing heavy metals Cd, Pb, Cu, and Zn above the established maximum permissible concentrations. Animals of the second experimental group received an additional special antitoxic mineral and vitamin premix "MP-A", and the third – premix and subcutaneous injection of the biological product "BP-9". The average live weight of cows is 500-545 kg, the average daily milk yield is 14.0-14.8 kg. The duration of the comparison period was 42 days. Cows selected according to the method

of analogues in terms of live weight, productivity, were in the same conditions of feeding and keeping. The trial period lasted 120 days.

Biochemical analysis of samples of plant and animal origin, including blood and milk, for the content of macro-, microelements, toxic metals, etc. was performed according to Atomic Absorption Spectrophotometry method (AAS-30 spectrophotometer) [15]. Quality and environmental safety of milk was controlled pursuant to DSTU 3662-97 [16], as well as considering the requirements of international quality standards (Regulations (EC) No 853/2004 [17] and No 1881/2006 [18]). All manipulations with animals were conducted in accordance with the European Convention for the Protection of Vertebrates used for Experimental and Scientific Purposes (Strasbourg, 1986).

The data was analysed considering the features of the results obtained in the study: the sample size and type of data distribution, the nature of variances. For each sample, the average value of the feature in the sample (M) and the standard deviation (SD) are calculated, and the estimate is given as $M \pm SD$. Discrepancies between the average values were considered statistically

significant for $P < 0.05$. Correlation analysis – using the Spearman's non-parametric rank correlation coefficient, factoring in the Shapiro-Wilk's W test. The calculation was performed using the STATISTICA software package version 10.0 for the Windows 7 operating system.

RESULTS

Feeding dairy cows feed containing an increased content of toxic metals Cd and Pb affected their transition from the gastrointestinal tract to the blood, then to milk. To establish the strength of the correlation between the content of toxic metals in blood and milk, under the appropriate conditions of a scientific experiment, one of the non-parametric correlation coefficients was used – Spearman's rank correlation coefficient, since the Shapiro-Wilk's W test, which is considered the most powerful to establish the correspondence of the obtained data to the law of "normal" (Gaussian) distribution, especially in small samples ($n < 50$) of independent groups, indicated that the content of cadmium and lead in blood and milk cows with different types of feeding do not obey the law of normal distribution (Figs. 1-4).

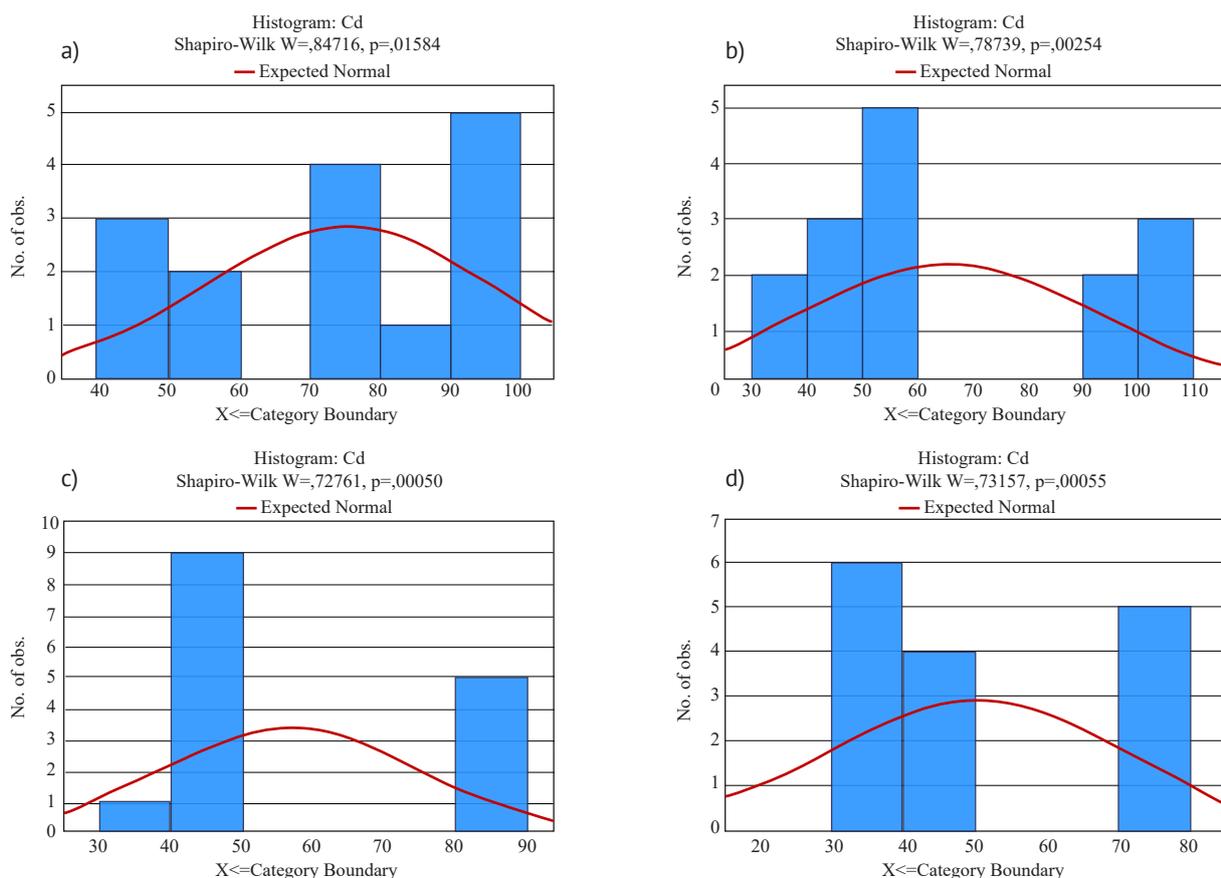


Figure 1. The result of analysing the distribution of the studied indicators of Cd content in the blood (nmol/l) of cows according to the Shapiro-Wilk's W test: a – silage-root crop type of feeding; b – silage-hay; c – silage-haylage; d – silage-haylage-concentrate

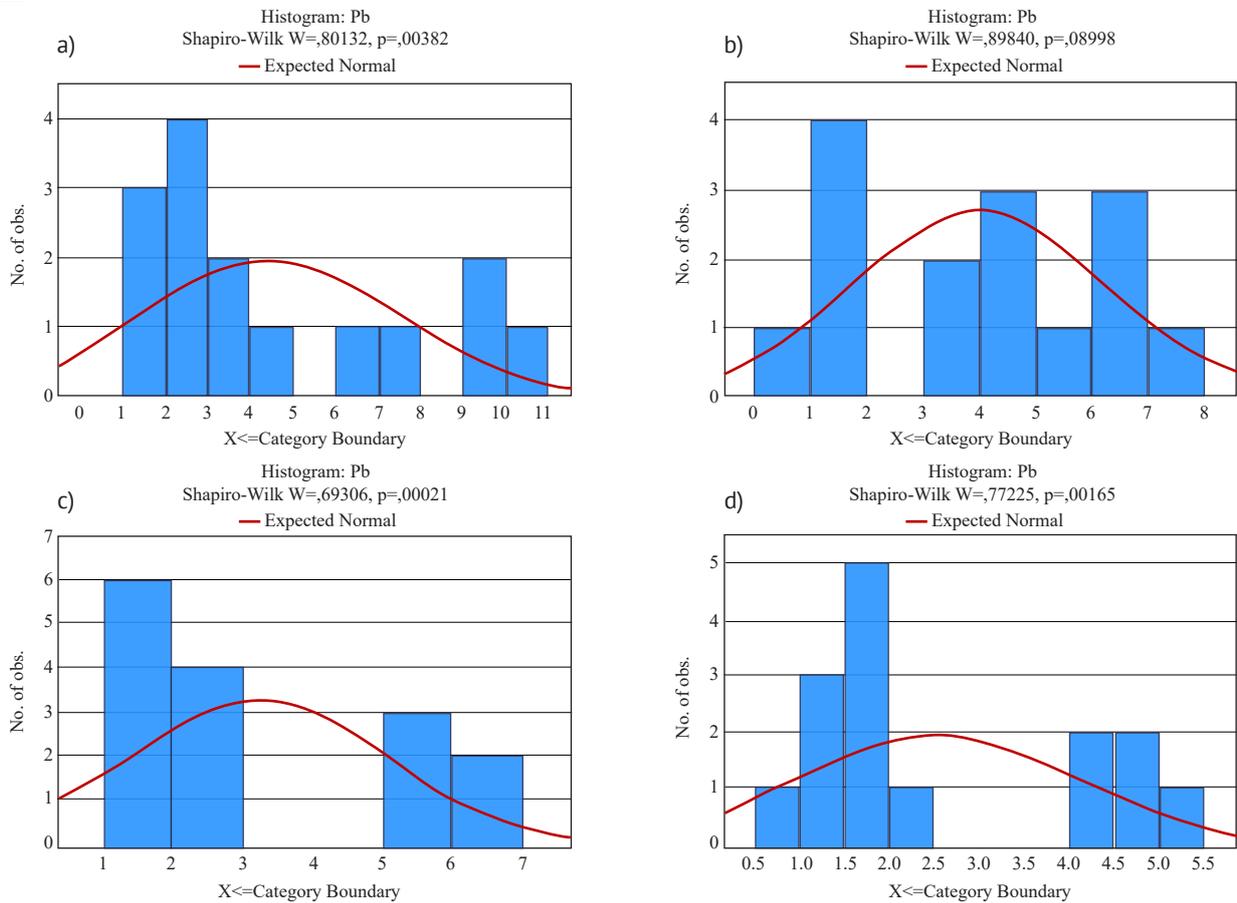


Figure 2. The result of analysing the distribution of the studied indicators of Pb content in the blood (nmol/l) of cows according to the Shapiro-Wilk's W test: a – silage-root crop type of feeding; b – silage-hay; c – silage-haylage; d – silage-haylage-concentrat

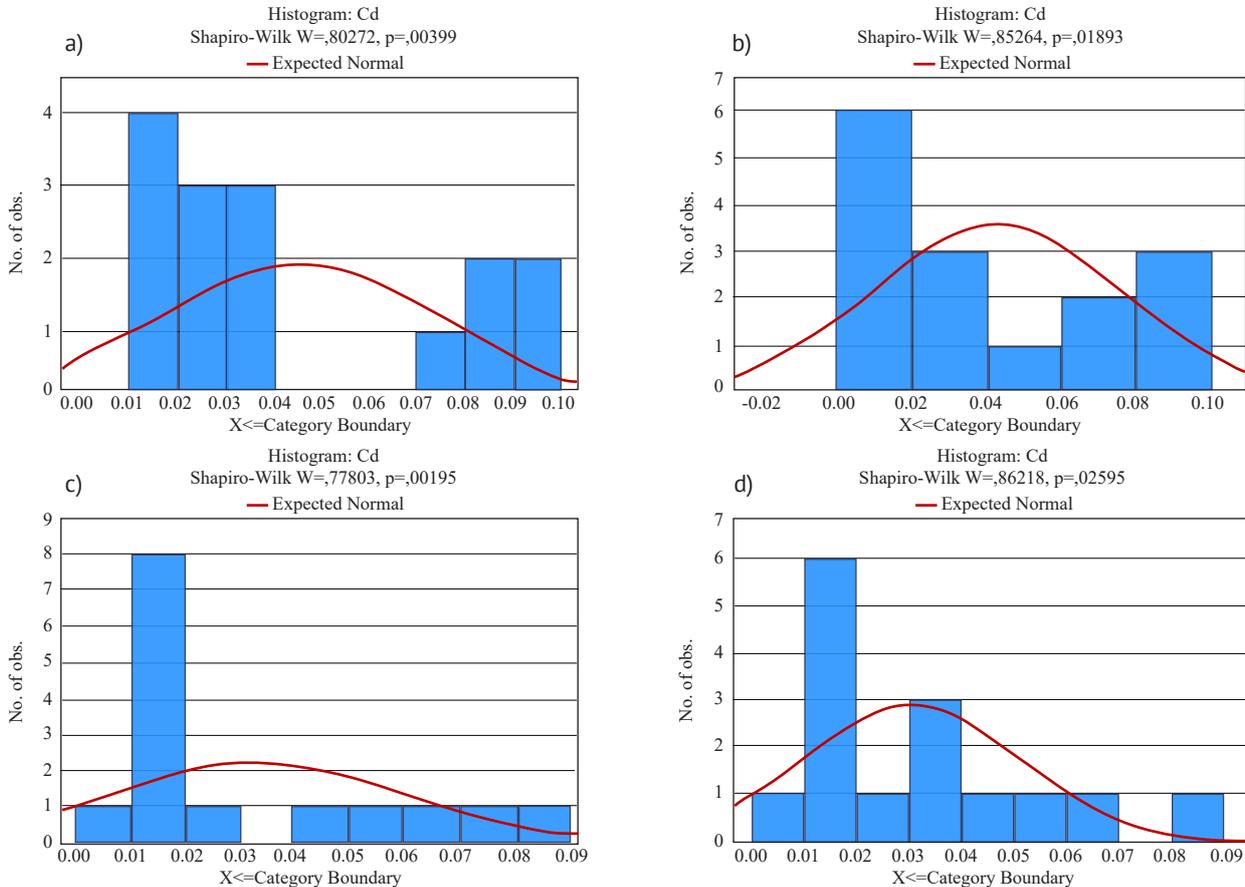


Figure 3. The result of analysing the distribution of the studied indicators of Cd content in the milk (mg/kg) of cows according to the Shapiro-Wilk's W test: a – silage-root crop type of feeding; b – silage-hay; c – silage-haylage; d – silage-haylage-concentrate

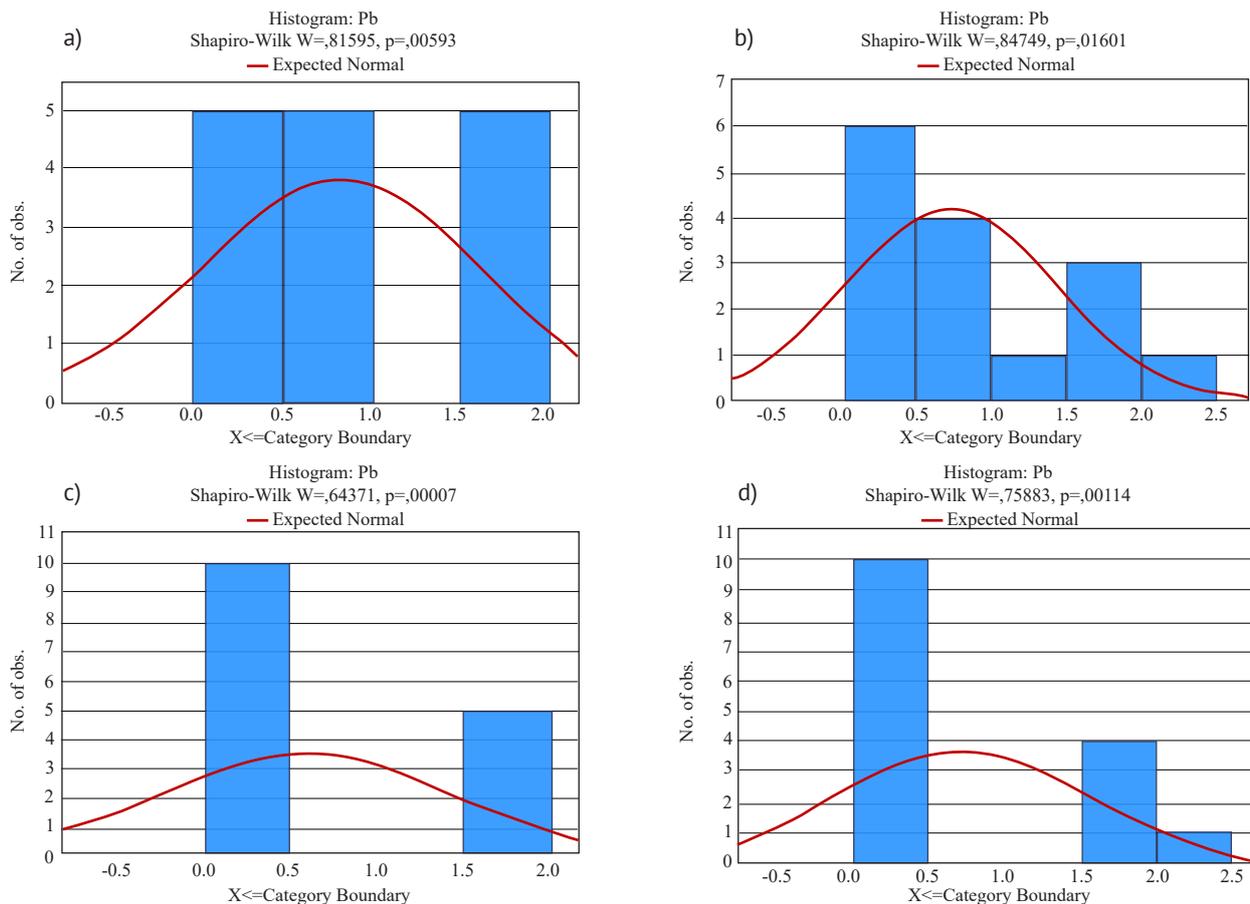


Figure 4. The result of analysing the distribution of the studied indicators of Pb content in the milk (mg/kg) of cows according to the Shapiro-Wilk's W test: a – silage-root crop type of feeding; b – silage-hay; c – silage-haylage; d – silage-haylage-concentrate

Application of the Pearson's coefficient in such situation entails conclusions that do not correspond to reality. It is more appropriate to apply the Pearson's correlation coefficient when the studied indicators correspond to the law of "normal" (Gaussian) distribution.

In the Statistics / Nonparametrics menu of the

STATISTICA software version 10.0, the Correlations module is launched (Spearman, Kendall tau, gamma). Next, in Variables, the columns containing the necessary data are selected and the Spearman R icon is clicked. A table with the analysis results will appear (Table 1 and Figs. 5-6).

Table 1. Toxic metals in cow's blood and milk at the end of the study period (M±SD)

| Type of animal feeding | Mineral elements | Group of cows | | |
|------------------------|------------------|---------------|-----------------|-----------------|
| | | 1. Control | 2. Experimental | 3. Experimental |
| Silage and root crops | Blood | | | |
| | Cadmium, nmol/l | 98.34±1.03 | 79.11±2.90 | 49.19±2.41 |
| | Lead, nmol/l | 8.32±1.65 | 3.02±0.99 | 1.98±0.16 |
| | Milk | | | |
| | Cadmium, mg/kg | 0.087±0.008 | 0.031±0.005 | 0.018±0.002 |
| | Lead, mg/kg | 1.835±0.093 | 0.614±0.085 | 0.014±0.003 |
| Silage-hay | Blood | | | |
| | Cadmium, nmol/l | 101.20±3.17 | 54.29±2.64 | 40.72±1.98 |
| | Lead, nmol/l | 6.54±0.45 | 4.01±0.64 | 1.38±0.28 |
| | Milk | | | |
| | Cadmium, mg/kg | 0.09±0.085 | 0.031±0.008 | 0.011±0.003 |
| | Lead, mg/kg | 1.641±0.253 | 0.515±0.064 | 0.027±0.012 |

Table 1, Continued

| Type of animal feeding | Mineral elements | Group of cows | | |
|----------------------------|------------------|---------------|-----------------|-----------------|
| | | 1. Control | 2. Experimental | 3. Experimental |
| Silage-haylage | | Blood | | |
| | Cadmium, nmol/l | 81.17±0.60 | 48.19±0.73 | 41.61±1.05 |
| | Lead, nmol/l | 5.74±0.32 | 2.07±0.16 | 1.87±0.09 |
| | | Milk | | |
| | Cadmium, mg/kg | 0.068±0.017 | 0.017±0.004 | 0.012±0.002 |
| | Lead, mg/kg | 1.734±0.148 | 0.016±0.004 | 0.014±0.004 |
| Silage-haylage-concentrate | | Blood | | |
| | Cadmium, nmol/l | 77.94±0.99 | 40.64±0.54 | 32.14±0.55 |
| | Lead, nmol/l | 4.63±0.37 | 1.72±0.17 | 1.27±0.22 |
| | | Milk | | |
| | Cadmium, mg/kg | 0.053±0.019 | 0.024±0.009 | 0.014±0.004 |
| | Lead, mg/kg | 1.794±0.165 | 0.331±0.064 | 0.032±0.008 |

Note: the degree of probability compared to the data of the control group $P < 0.01$; $n = 5$

The correlation coefficient between the cadmium content in the blood and milk of cows for all types of feeding is positive and very high ($r = 0.82-0.91$), which indicates a direct and very high degree of correlation. The experiment established a lower correlation between the indicators in animals with silage-haylage-concentrate type of feeding $r = 0.82$ and silage-haylage – $r = 0.86$. The highest correlation coefficient was discovered in animals with silage-root feeding type ($r = 0.91$). The software highlights statistically significant correlation coefficients in red. Apart from calculating the correlation coefficient, the software allows simultaneously evaluating the statistical significance, which in all cases was $p < 0.05$. The correlation coefficient between the lead content in blood and milk ranged from 0.78 to 0.96. In cows with the silage-haylage type of feeding, the correlation was the weakest, but high ($r = 0.78$). The highest level is found in animals with silage-haylage-concentrate type ($r = 0.96$).

Thus, the use of Spearman's rank correlation coefficient, using the STATISTICA software version 10.0, made it possible to calculate and thereby reliably establish a high strength of correlation between the indicators essential for the experiment – the content of toxic metals Cd and Pb in the blood and milk of dairy cows with different types of feeding, which were received with a diet of feed with a high content of these dangerous elements.

DISCUSSION

Due to the complexity of ecological systems, the method of modelling using appropriate computer software is frequently employed to investigate them. Abstract models are more widely used in environmental research. In this case, the model refers to some abstract descriptions of

a particular object or phenomenon in the real world, which allows analysing its properties and making forecasts. The advantages of such models are that they enable the analysis of the state of ecological systems in relatively simple and inexpensive ways and predict the nature of their changes when certain changes are introduced to the ecosystem, for example, heavy metal pollution. The main requirement for abstract environmental models is accuracy and sufficient generality. In many cases, the accuracy of abstract models depends on the number of elements and system parameters selected for its construction. It should be borne in mind that inclusion of a multitude of components in the model complicates its analysis and creates "noise". Conversely, too small a number of elements greatly distances the model from reality. Depending on the research apparatus, abstract models are as follows: verbal, graphic, mathematical, etc. For the purposes of this paper, it was decided to employ the construction of mathematical models based on the calculation of the correlation dependence between the indicators under study and the construction of the corresponding regression equations. In scientific research, it is often necessary to search for relationships between different indicators of the groups under study (precipitation and crop yield; body temperature and pulse rate, etc.). In this case, the content of mineral elements in blood and milk was analysed. Various types of correlation analysis are used to solve this type of problem. Correlation analysis allows assessing the direction of the correlation between two indicators (direct or inverse), as well as quantifying it using the correlation coefficient. The closer the correlation coefficient is to 1 (in modulus), the stronger the correlation between the indicators.

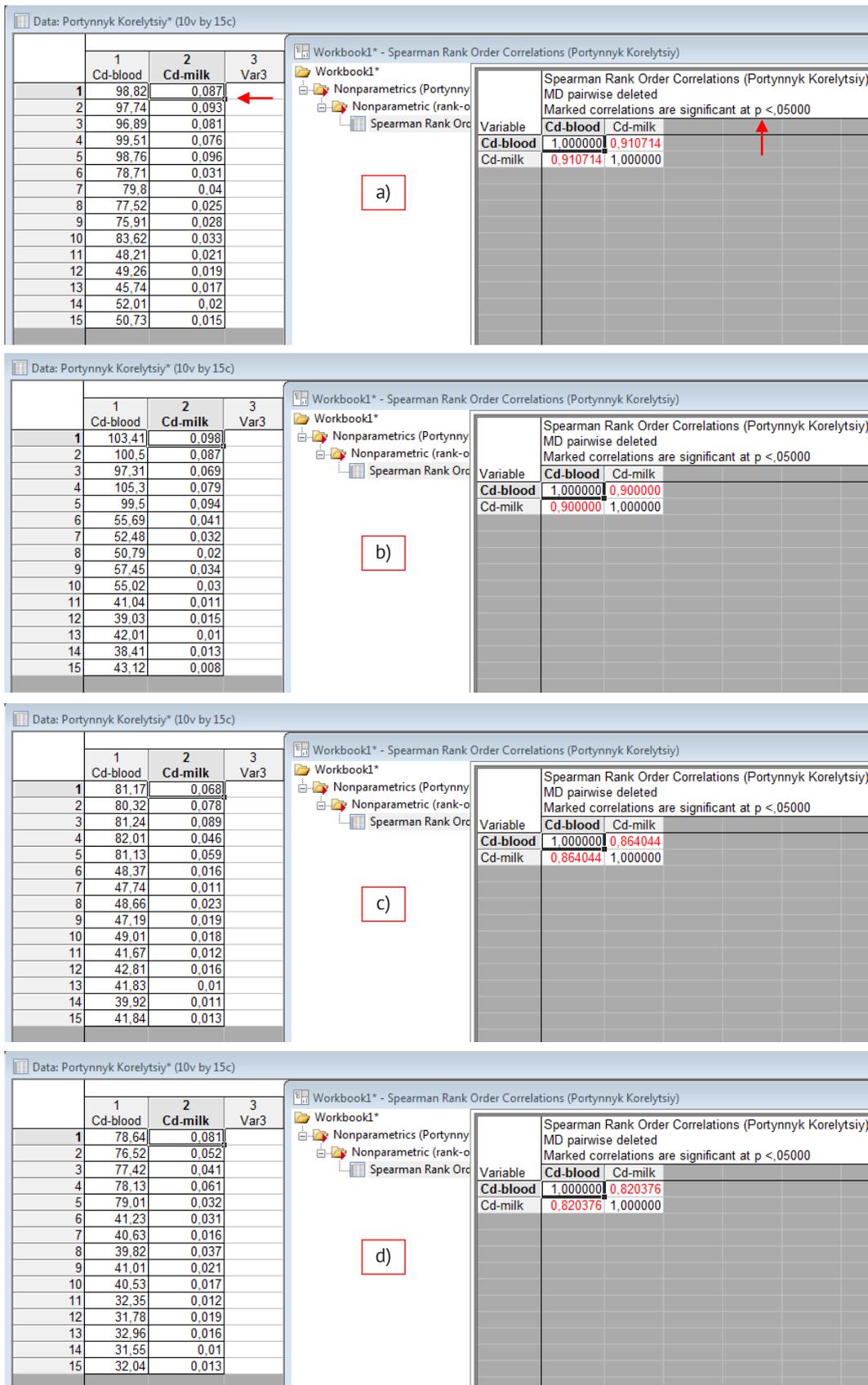


Figure 5. The indicators of cadmium content in the blood and milk of cows of groups 1, 2, 3 entered in the software window and the result – Spearman’s correlation coefficient between the content of the element in the blood and milk of animals: a – silage-root type of feeding; b – silage-hay; c – silage-haylage; d – silage-haylage-concentrate

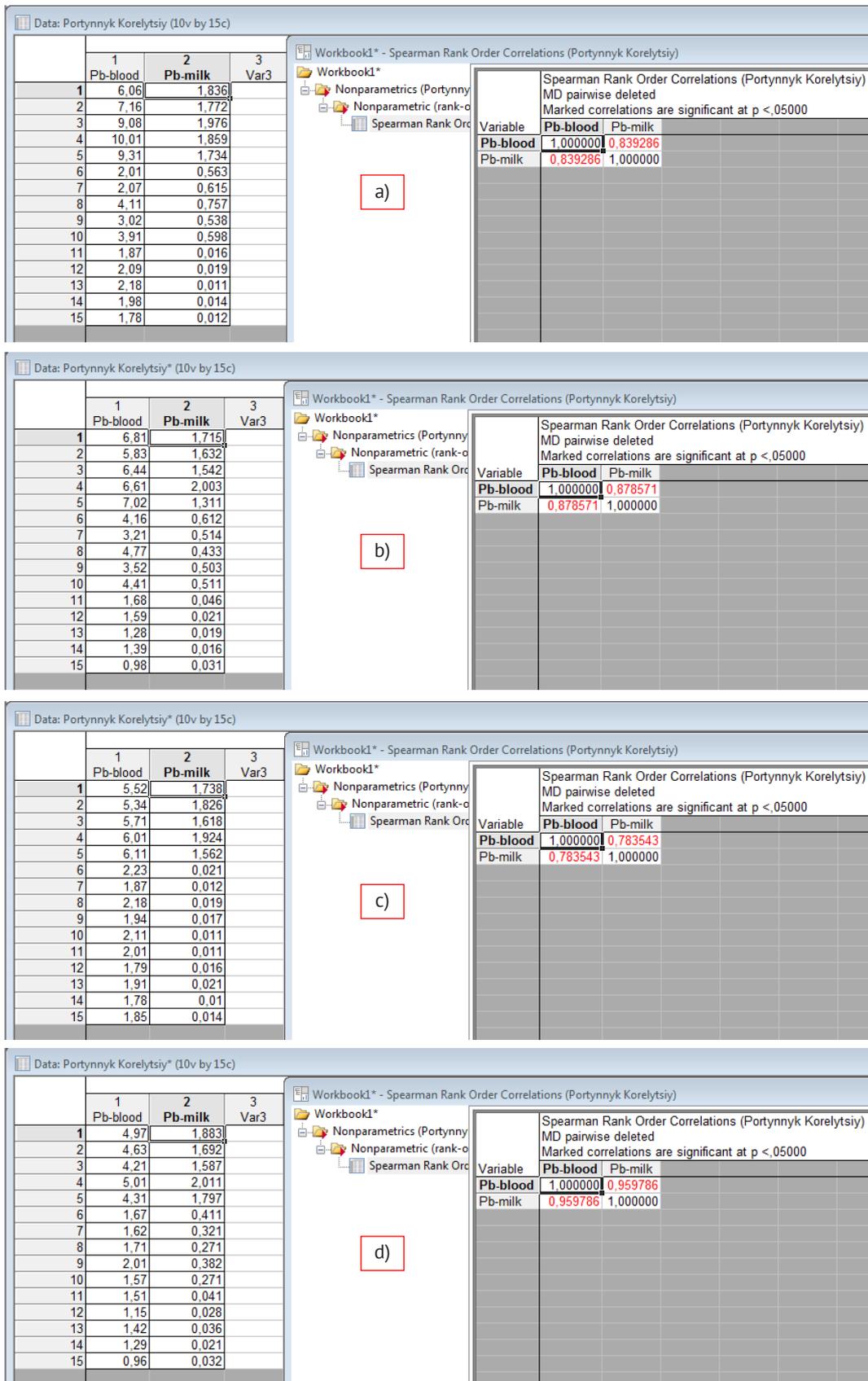


Figure 6. The indicators of lead content in the blood and milk of cows of groups 1, 2, 3 entered in the software window and the result – Spearman's correlation coefficient between the content of the element in the blood and milk of animals: a – silage-root type of feeding; b – silage-hay; c – silage-haylage; d – silage-haylage-concentrate

The correlation between the content of Cd and Pb in the blood and milk of cows is essential, which was investigated by the authors of this study in the experiment. The authors believe that regular medical examination of dairy cows by a veterinary doctor in agricultural enterprises located near industrial centres, ecosystems contaminated with toxic metals with simultaneous blood sampling for biochemical analysis and analysis of the Cd and Pb content will allow assessing the risks of the transition of pollutants to the dairy products – milk sold on the market as a product for human consumption, or dairy raw materials for the production. At the same time, it is difficult, sometimes even impractical, and generally unusual in the practice of a veterinary specialist to conduct a chemical analysis of blood and milk for heavy metals. For example, scientists S. Offor et al. [19] prove that the practice of analysing donated blood for people for the content of toxic metals is also unusual. Donor blood is tested for various pathogens, but not for the content of toxic metals. However, it is clearly established that a donor can accumulate these elements in the blood from various sources (professional activity, environment). Once in the human body, such blood can cause intoxication. The researchers emphasise that access to safe and adequate blood transfusions with minimal risk of exposure to toxic metals for recipients is also a public health issue. Scientists recommend reducing the risks of dangerous exposure to toxic metals – blood intended for transfusion, especially in vulnerable individuals, such as children, should be regularly examined for the concentration of toxicants. The main thing in the work of a veterinarian is prevention, to avert undesirable changes in the state of animal health, including to prevent deterioration of the quality and environmental safety of milk. The results of the correlation analysis made by the authors can substantially facilitate the production of high-quality milk. Timely detection of toxic metals in the blood will help specialists to take timely measures to exclude contaminated feed from the diet, if this is not possible – to balance the diet by timely introducing feed additives, or mineral-vitamin premixes of the type “MP-A”, as was done by the authors of this study in the experiment, mineral elements antagonists of cadmium and lead, to reduce their transition to milk, the introduction of proven drugs (protectors) of the type “BP-9” to reduce intoxication of the animals' bodies, improve their health, prevent poisoning. The high correlation between the content of toxicants in blood and milk established by the researchers at $r=0.82-0.91$ (Cd) and $r=0.78-0.96$ (Pb) ($P<0.05$), allows specialists to act effectively in production conditions.

Correlation analysis is widely and, most importantly, effectively used in the practice of scientists from different countries of the world, in various industries – not only in agriculture. Ying Han et al. [20] conducted a study of the effect of goat's milk enriched with oligosaccharides on the structures of the microbiota in the large

intestine of mice. Spearman's correlation analysis investigated the strength of the correlation between the microbiota and short-chain fatty acids in experimental animals. Spearman's correlation analysis demonstrated that propionic, isobutyric, and Valerian acids positively correlate with a particular microbiota. Accordingly, it is proved that goat's milk is useful for improving the microflora of the large intestine. Furthermore, Spearman's rank correlation coefficient was successfully applied in the analysis of indicators by scientists Han Young Joo et al. [21], who investigated the correlation between the dose of natural radiation and the incidence of human cancer in the Republic of Korea. Radiation dose rate data were taken from 171 observation points in 113 administrative districts of the country for 10 years from 2007 to 2016. The relative number of cancer cases for this analysis was the difference in the number of cancer patients per hundred thousand people per year in administrative districts with the five highest and five lowest natural gamma dose rates per year over the same period. An analysis of the correlation between two variables of Spearman's rank correlation coefficient was obtained using R. The result of the analysis indicated that Spearman's rank correlation coefficient exceeded 0.05, and the correlation between the two variables was not statistically significant.

Evidently, international scientific practice demonstrates that the Spearman's rank non-parametric correlation coefficient can establish both a high-strength correlation between the indicators under study, which is proved by Ying Han et al. [20], and vice versa, the low-strength correlation, as well as the low statistical probability, as proved by Han Young Joo et al. [21].

Computer software currently developed and available on the market, including STATISTICA version 10.0 for the Windows 7 operating system used by the authors of this study for correlation analysis proves its effectiveness. Given the preliminary verification of the obtained indicators of laboratory blood and milk analysis according to the Shapiro-Wilk's W test, it is advisable to use the Spearman's non-parametric rank correlation coefficient.

CONCLUSIONS

Using computer software of statistical data processing and the function of analysing the Spearman's non-parametric rank correlation, a high correlation was established between the transition of toxic metals cadmium and lead from blood to milk, which allows specialists to more confidently predict the environmental safety of produced milk (dairy raw materials) in case of dairy cattle breeding in conditions of increased anthropogenic load on agroecosystems and timely apply appropriate countermeasures to improve product quality.

The STATISTICA software version 10.0 used for statistical processing of biometric data, establishing the strength of correlation between the studied indicators,

and the reliability of research results is quite reliable, convenient for scientists and practitioners, and is as informative as possible. Correctly entered laboratory test data is quickly processed and simultaneously displayed both in the form of digital data and in the form of the necessary visual graphs.

Further research is aimed at conducting correlation and regression analysis using the same software, but with other indicators of environmental safety and quality of milk produced that are crucial in veterinary and livestock practice.

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Використання рангового непараметричного кореляційного аналізу показників переходу важких металів з крові в молоко корів для оцінки його екологічної безпеки

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Анотація. Кореляційний аналіз є важливим методом статистичної обробки отриманих наукових результатів досліджень. Правильне його застосування з використанням спеціальних комп'ютерних програм і вірогідні результати дають змогу практично полегшити роботу ветеринарної і зоотехнічної служби під час виробництва екологічно безпечного високоякісного коров'ячого молока. Метою досліджень був кореляційний аналіз між вмістом токсичних металів Cd і Pb у крові та молоці корів за допомогою комп'ютерної програми STATISTICA версії 10.0. Науково-господарські дослідження проведено на дійних коровах з різними типами годівлі. Всіх тварин, відібраних методом аналогів за живою масою, продуктивністю, було поділено на три групи: першу контрольну та другу і третю дослідні групи. До складу раціону входили корми з надлишком важких металів, особливо, таких як кадмій і свинець. Висока біологічна активність полютантів вплинула на міграцію їх з кормів раціону через шлунково-кишковий тракт у молоко. Для зменшення інтоксикації організму тварин до основного раціону корів другої і третьої дослідних груп вводився премікс «МП-А», а в третій дослідній групі – премікс «МП-» та ін'єкція біопрепарату «БП-9». Спочатку за допомогою тесту Шапіро-Уїлка (Shapiro-Wilk's W test) було перевірено відповідність отриманих даних лабораторних аналізів крові та молока на концентрацію токсичних металів, закону «нормального» розподілу (Гауссова), а потім вибрано для розрахунку необхідний, у такому випадку, непараметричний ранговий коефіцієнт кореляції Спірмена. Аналіз встановив високу кореляційну залежність між змінними показниками, котра знаходилася в межах $r=0,82-0,91$ (Cd) та $r=0,78-0,96$ (Pb) з вірогідністю ($p<0,05$) у тварин з різними типами годівлі. Виявлений високий кореляційний зв'язок дасть змогу спеціалістам ветеринарної медицини лише за аналізом крові на вміст кадмію та свинцю швидко застосовувати заходи зі зниження токсичного навантаження організму елементами, вчасно попереджувати виробництво неякісної молочної сировини в т.ч. із застосуванням перевірених в експерименті преміксу та фітобіопрепарату. Подальші дослідження спрямовані на аналіз кореляційного зв'язку між іншими показниками якості та екологічної безпеки молока, кормів, побудову рівнянь регресії, котрі практично сприятимуть діяльності спеціалістів, завданням яких є забезпечення виробництва високоякісного екологічно безпечного коров'ячого молока

Ключові слова: кореляція, коефіцієнт кореляції, непараметричний коефіцієнт кореляції, коефіцієнт кореляції Спірмена, молочна сировина, дійні корови, токсичні метали
