Removal of Toxic Metals from the Body of Cows by Using Antidote Substances, with its Impact on Milk Productivity and Environmental Safety of Agroecosystems around the Industrial City in Ukraine

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Abstract
Toxic metals like Cd and Pb pose the greatest ecological threat to ecosystems, especially in and around the industrial cities. Four farms located around Kharkiv industrial city were chosen for scientific experiments carried out on cows feeding specially developed antidote (mineral-vitamin premix "MP-A") and subcutaneous injection of biologically active preparation "BP-9". These novelty products enhanced the urinary excretion of heavy metals Cd, Pb, Cu and Zn while ensuring the production of high quality environmentally safe milk. The toxicants are absorbed from the gastrointestinal tract into the blood, travel across the body, accumulate in organs and tissues, and pass through urine and milk. The accumulation of Cd in the blood of tested cows in control group was, on average, from 77.94 to 101.20 nmol/L, and of Pb from 4.63 to 8.32 μmol/L. The transfer of Cd from blood to urine was, on average, 1.7%-2.0%, and of Pb 5.4-7.3%. The antidote substances contributed to the exacerbation of heavy metal excretion from the body of animals and the restoration of its homeostasis. The transfer of Cd from blood to urine averaged 3.9% to 9.5% and of Pb 37.7% to 103.5% in second experimental group of cows. The same for Cd was 7.1% to 12.7% and for Pb was 70.7% to 144.1% in third experimental group. The mineral-vitamin premix and biopreparation BP-9 blocked absorption of the pollutants into the gastrointestinal tract, strengthened the protective effects on organs, and facilitated the elimination of heavy metals through urine. Dairy productivity of animals also increased in cows of the second and third experimental groups by 17-22 kg per day compared to the control group having 14 kg per day (P<0.01).

Keywords
Remix; Biopreparation; Medicinal plants; Heavy metals; Contaminated feeds; Antidote substances; Animal productivity
Introduction

Agroecosystem is a natural-anthropogenic system. It is an artificially maneuvered system of plant and animal groups having a weakly expressed self-regulation mechanism. The productivity of an agroecosystem is regulated by direct and indirect energy investments, after the termination or critical reduction of which the system degrades (Blancard and Martin, 2014; Gnatiuk, 2018). The degradation of agroecosystems also enhances the anthropogenic impact of excessive use of agrochemicals: mineral fertilizers and pesticides, and around industrial cities, along with emissions from industries, which generate pollutants and xenobiotics, among which heavy metals are the most dangerous. As a result, negative effects occur on the health of productive animals, especially dairy cows. The products like milk may contain excess quantities of cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn) and other heavy metals to be environmentally hazardous (Song et al., 2004; Al Sidawi et al., 2021; Calogero et al., 2020).

Heavy metals are among the priority environmental pollutants. The term "heavy metals" characterizes a wide group of substances, which have recently become widespread. In various scientific and applied scientific works, the scientists interpret the meaning of ‘heavy metals’ differently (Fersman, 1934-1937; Tsvetkova and Gunko, 2015). Numerous characteristics of heavy metals are used as criteria for its nomenclature: atomic mass, density, toxicity, prevalence in the environment, degree of involvement in natural and man-made cycles. In works highlighting the environmental pollution and environmental monitoring, heavy metals include more than 40 microelements with an atomic mass of over 40 atomic units. At the same time, indicators that play an important role in the classification of heavy metals are their high toxicity to living organisms at relatively low concentrations, the ability to bioaccumulate, and biomagnification (Adriano, 2001; Brygadyrenko and Ivanyshyn, 2015; Tsvetkova and Gunko, 2015). According to the Fersman (1934-1937) classification, metals with a density of more than 8 g/cm³ should be considered heavy. Formally, a large number of elements correspond to the definition; however, according to scientists’ observations, the combination of these elements is far from being equivalent to pollutants.

The excretion of heavy metals from the body of animals through faeces and urine leads to an increased content of them in organic fertilizers (manure and compost), which already contain a significant amount of heavy metals (Gutyj et al., 2016). As a result of the application of such organic fertilizers into the soil, the concentration of heavy metals such as cadmium, lead, copper, zinc, iron, and manganese increases (Brygadyrenko and Ivanyshyn, 2015).

Studies have reported bulk and mobile forms of heavy metals in polluted soils (Bigalke et al., 2017; Borah et al., 2018; Gunko, Tsvetkova and Neposshiyalenko, 2018). Heavy metals, especially cadmium and lead, accumulate in the soil in significant concentrations, which often exceed the established maximum permissible levels and, under appropriate environmental conditions, migrate to plants that are fed as a feed by farm animals. Then they get into milk and other products (for example, dry milk, where heavy metals are much more concentrated than in liquid milk) (Jolly et al., 2017), thereby, worsening its quality and environmental safety (Mamenko, Portjannyk and Yvanov, 2010; Ren-ju et al., 2015; Tahir et al., 2017). Due to intoxication by toxic elements, the milk productivity of cows decreases. Milk production in agricultural ecosystems around developed industrial cities can be significantly impacted by such environmental pollution. Agricultural milk producers strive not only to produce ecologically safe, biologically complete and high-quality milk, but also to achieve the maximum productivity of cows (Mamenko and Portiannyk, 2020).

Pollution of heavy metals Cd, Pb, Cu and Zn has a negative effect on the body of dairy cows, leading to the production of milk of poor quality and safety. In the event of increased migration of heavy metals Cd, Pb, Ni and Cu from the soil, a decrease in the mobile forms of essential mineral elements, such as Ca, P, Co, etc., is observed leading to its low content in the ration feed. As a consequent deficiency in the blood,
cows have shown symptoms of osteodystrophy, deformation of the spine and hoof, partial lysis of the last pair of ribs, deformation of the last 2-3 caudal vertebrae (Slivinska et al., 2018). On the other hand, mixed complexes of zinc, manganese and cobalt eliminate the deficiency of essential mineral elements in cows, and improve mineral metabolism, thereby, contributing to the growth of milk productivity (Bomko et al., 2018). The use of natural detoxicants of heavy metals, dry apple and dry beet pulp, in the diets of pigs helped reduce the accumulation of Cd in muscle tissues and animal products (Dyachenko et al., 2017). Therefore, a study on intoxication of dairy cows by heavy metals, and its excretion from the body by using special antidote substances (vitamin premix and biological product) was conducted by Mamenko, Portjannyk and Yvanov (2010) to produce safer and more milk.

Intakes of Cd and Pb are associated with environmental risks to the animal body because of cumulative toxicity and negative effects on internal organs and systems (Canty et al., 2014; Hashemi, 2018; Roggeman et al., 2013). As a consequence, the growth and productivity of animals decrease. The accumulation of such heavy metals in agroecosystem increases the risk of its intake into the body of cows and, thereby, poses a threat to human and animal health (Fischer et al., 2011; Rezza et al., 2018; Rahimi, 2013). As a result of chronic cadmium intoxication, (nephrotoxicity, hepatotoxicity, immunotoxicity, osteotoxicity), oxidative stress of liver and kidney cells occurs damaging them and its DNA leading to carcinogenesis and oncological diseases (Liu, Qu and Kadiiska, 2009).

The toxic environmental milieu negatively affects the efficiency of the livestock industry and, in particular, dairy farming. That is why an in-depth study is performed to demonstrate how the elimination of heavy metals from the body of animals using special anti-toxic vitamin premixes and biological products. The use of premises and bioproduct not only enhances the renal excretion of heavy metals, but also ensures an improvement in the health of cows, increases their productivity, and increases the safety of milk. The aim of this research is the quantitative assessment of the process of eliminating toxic and essential heavy metals through urine of dairy cows. Such intoxication of the animal body by Cd, Pb, Cu, Zn is reduced by feeding a specially developed mineral-flax-vitamin premix (MP-A) and subcutaneous injection of a biological product ‘BP-9’.

**Materials and Methods**

On the farms located near the industrial city of Kharkiv in Ukraine, scientific experiments were carried out on the dairy cows of Ukrainian black and red-and-white dairy breeds. For the experiments, 126 cows were selected feeding the silage-hay-concentrate type of feed. Of them, 63 fed on the silo-silage type feed, 36 fed on the silage-root crops, and 195 fed on the silage-haylage type of feed. The experimental population of cows was divided into three groups: the first as a control, and the second and third as treated groups. Cows of all groups were fed the feed having the heavy metals Cd, Pb, Cu and Zn under maximum permissible concentrations. Animals of the second experimental group were given an additional special antitoxic mineral-vitamin premix ‘MP-A’, and the third group was treated with a mineral-vitamin premix and subcutaneous injection of the biological product BP-9, which includes an extract of nine medicinal plants. The average live weight of cows was 500-545 kg; the average daily milk yield was 14.0-14.8 kg having per lactation average of 4,270-4,514 kg of milk. The comparison period was 42 days. The cows selected by the method of analogs in terms of live weight and productivity were kept in the same conditions of feeding and upkeeping. The experiment period lasted 120 days.

Mineral-vitamin premix and biologically active preparation "BP-9" were developed according to the methods invented by Portjannyk (2002a, 2002b). Biological product "BP-9" is an extract from 9 medicinal plants. 100 ml of the preparation contains *Schisandra chinensis* (15 ml), *Chamomile* (pharmacy, peeled) (3 ml), *Eleutherococcus senticosus* (15 ml), medicinal sage (pharmacy) (*Salvia officinalis*) (17 ml), common barberry (*Berberis*) (12 ml), alfalfa (*Alfalfa*) (5 ml), kidney tea (*Orthosiphon stamineus*) (15 ml), sea
buckthorn (Hippophae rhamnoides) (15 ml), and Verbena officinalis (3 ml). Subcutaneous injection of "BP-9" was applied with the dose of 20 ml/day divided into two sub-doses: 10 ml each in the morning and in the evening (at intervals of 12 hours). The frequency of administration of the BP-9 preparation was 5 times a month, with administration on an interval of 6 days. The BP-9 preparation was injected in 42 cows of the third research group feeding on silage-silage-concentrate.

The biological product BP-9 was manufactured under sterile conditions in a laboratory in accordance with the method suggested by Hmel'nic'kij, Homenko and Kanjuka (1994) and Sokolov, Andreeva and Nozdrin (2002). In the second and third research groups, the rations developed to feed dairy cows were additionally balanced and adapted to the actual daily ration with a special antitoxic mineral-vitamin premix "MP-A". The premixes were developed taking into account the synergistic and antagonistic action of macro- and micro-elements, particularly cadmium, lead, copper and zinc, in the concentration of per 1 kg of dry matter. It allowed to develop a methodological approach to rationing the content of heavy metals in the diet and the corresponding essential macro- and micro-elements. The partial deficiency of these elements was observed in the diets of cows in the control groups. The composition of the mineral-vitamin premix "MP-A" included the following mineral elements: P, Ca, Mg, S, Fe, Mn, Co, J, Se, etc.; vitamins: A, D, E, B2, B3 (niacin, PP), B4, B6, C, H (Biotin); and one sulfur-containing amino acid. 1% of the premix was introduced into the diet (Podobed et al., 1996) of animals. The diet consisted of silage-root crops and silage-hay with the rate of 250 g per head per day, silage-hay-concentrate at the rate of 290 g per day, silage-hay at the rate of 255 g per day. The premix "MP-A" was fed to 24 dairy cows of the second and third experimental groups feeding silage-root type of feed at the rate of 720 kg (250 g per head per day × 24 heads × 120 days of experience), silage-hay 3900 kg by 130 heads, silage-haylage 1285.2 kg by 42 heads, and –silage-haylage-concentrate 2923.2 kg by 84 heads. There is a large number of regional firms in the market that, according to the appropriate diet and recipe, can manufacture and sell such a premix to an agricultural enterprise. It is made quite convenient to use, which was a factor considered at the time of conducting experiments.

The recipes (formula) of preparing the "MP-A" premix and the "BP-9" were adapted from the methodologies developed by the authors themselves. Biochemical analysis of samples of plant and animal origin (feed, blood, internal organs and tissues, urine and milk for the content of macro- and micro-elements, including heavy metals, etc.) was carried out by using atomic absorption spectrophotometry (spectrophotometer AAS-30) (Praice, 1972). The results obtained were compared with physiological standards for blood (Skukovsky, 1987), for urine (Levchenko et al., 2002). Control of the quality and environmental safety of the milk was carried out in accordance with GOST 3662-97 standards (Mamenko et al., 1997), as well as taking into account the requirements of international quality standards (especially EU Regulation No. 853/2004 and No. 1881/2006).

Together with the determination of the absolute concentrations of metals in bio-substrates, their relative indicators were calculated according to the method given by Stus, Biletska and Golovkova, (2010). This method is considered as the index of renal migration (IRM). In this research, this indicator was calculated not in arbitrary units, but simply in percentage in relation to the concentration of metal in the blood as an integral internal environment of the animal organism and a leading source from which migration of heavy metals starts. The indicator allows revealing more deeply the corresponding links of the mechanism of removal of heavy metals from the body and to carry out a comparative assessment of the eliminated quantities of toxicants and micro-elements when using the antidote substances (i.e., a premix MP-A and a biological product BP-9).
All experiments with animals were carried out in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes. The ARRIVE (Animal Research Reporting of In Vivo Experiments) norms were complied with for conducting these experiments on the animals. The ARRIVE checklist is appended in Annex.

The analysis of the data was carried out considering the peculiarities of the results obtained in the study: the size of the sample and the type of distribution of the data, the nature of the variances. For each sample, the mean value of the characteristic in the sample (M) and the standard deviation (SD) were calculated: M ± SD. Disagreements between mean values were considered statistically significant at P <0.05. The calculation was carried out using the STATISTICA software package version 10.0 for the Windows 7 operating system.

**Results**

Air pollution from industrial enterprises of the city and a gas condensate station (CNG filling station) with the simultaneous use of mineral fertilizers and pesticides led to soil acidification in agroecosystems, which caused an increased migration of heavy metals from soil into the plants, and then, into feed of the cows of all experimental groups. The feed was found having cadmium, lead, copper and zinc in exceeded concentration from the maximum permissible limits. The content of heavy metals in the feed of cows with silage-root crops type of feeding exceeded the maximum permissible concentrations: Cd 2.1-3.2 times, Pb 2.4-5.7 times, Cu 1.4-2.3 times and Zn 1.2-2.4 times. The excess concentration over the maximum permissible limits for Cd and Pb was found in cereal-legume hay (3.2 and 5.7 times, respectively), for Cu in maize residue (2.3 times), and Zn in wheat straw (2.4 times). The concentration of heavy metals in the feed of cows fluctuated due to the varying concentrations of mobile forms of toxicants in the soil and the location of plants being grown, and the distance to industrial center, highways, CNG filling station, etc. Therefore, in the feed of cows with a silage-hay type of feeding, the highest content of Cd, Pb, Cu, Zn was found in fodder beets, respectively, exceeding 2.5, 3.4, 3.8, and 4.1 times over the maximum permissible limits. It is generally known that plants accumulate heavy metals in the root system to a greater extent. In fact, below-ground parts of the plants absorb fewer heavy metals; therefore, it was the fodder beets having highest level of contamination. In the feed grown on agricultural land, where cows are fed a silage-haylage-type diet, a high content of zinc (average 6.3-6.8 times) was also found in oats and peas. Among other feeds, pea bran had the highest content of cadmium and lead. Similarly, cereal-legume hay had the highest content of copper (3.9 times).

Thus, in accordance with the level of contamination of feed rations of cows, the feeds are arranged in descending order: Cd contamination - silage-root crops → silage-haylage → silage-hay → silage-haylage-concentrate; Pb contamination - silage-haylage-concentrate → silage-haylage → silage-root crops → silage-haylage → silage-hay; Cu contamination - silage-haylage-concentrate → silage-haylage → silage-hay → silage-root crops; Zn contamination - silage-haylage-concentrate → silage-haylage → silage-hay → silage-root crops. The average concentrations of heavy metals in blood, urine and their transition (from blood to urine) are given in table 1 and table 2.

Following the law of "normal" distribution (Gaussian), the Shapiro-Wilk's W test was applied. This test is considered the most powerful, especially in case of small samples (n <50) of independent groups. The concentration of cadmium in the blood and urine of cows eating silage-root crops type of feed is not

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2 https://www.nc3rs.org.uk/arrive-guidelines
subject to the law of normal distribution. The Shapiro-Wilk's W test made it possible to determine the appropriate method for further analysis of the different samples. Considering that the sample size is insignificant, the most effective method was the non-parametric analysis of variance (or H-test) (Kruskal-Wallis ANOVA). The value of the error P for the null hypothesis indicates that the extent of Cd in the blood and urine of cows in different experimental groups does not differ. In the case of P <0.05, the studied samples were statistically and significantly different from each other.

Table 1: The concentration of metals in biological media and the amount of their transition from blood to urine (M ± SD)

<table>
<thead>
<tr>
<th>Animal Feeding Type</th>
<th>Heavy Metal</th>
<th>Actual Concentration</th>
<th>Transition, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Blood</td>
<td>Urine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Experiment</td>
</tr>
<tr>
<td>Silage-root crops</td>
<td>Cd, (blood – nmol/l); (urine – μmol/l)</td>
<td>98.34 ± 2.70</td>
<td>79.11 ± 2.41</td>
</tr>
<tr>
<td></td>
<td>Pb, μmol/l</td>
<td>8.32 ± 0.99</td>
<td>3.02 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>Cu, μmol/l</td>
<td>28.93 ± 1.82</td>
<td>18.04 ± 0.83</td>
</tr>
<tr>
<td></td>
<td>Zn, μmol/l</td>
<td>29.85 ± 1.11</td>
<td>22.81 ± 1.45</td>
</tr>
<tr>
<td>Silage-hay</td>
<td>Cd, (blood – nmol/l); (urine – μmol/l)</td>
<td>101.20 ± 3.17</td>
<td>54.29 ± 2.64</td>
</tr>
<tr>
<td></td>
<td>Pb, μmol/l</td>
<td>6.54 ± 0.74</td>
<td>4.01 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>Cu, μmol/l</td>
<td>30.62 ± 1.24</td>
<td>17.07 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>Zn, μmol/l</td>
<td>17.06 ± 0.40</td>
<td>10.04 ± 0.23</td>
</tr>
<tr>
<td>Silage-haylage</td>
<td>Cd, (blood – nmol/l); (urine – μmol/l)</td>
<td>81.17 ± 0.60</td>
<td>48.19 ± 0.73</td>
</tr>
<tr>
<td></td>
<td>Pb, μmol/l</td>
<td>5.74 ± 0.32</td>
<td>2.07 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>Cu, μmol/l</td>
<td>27.62 ± 0.44</td>
<td>16.17 ± 0.37</td>
</tr>
<tr>
<td></td>
<td>Zn, μmol/l</td>
<td>26.62 ± 0.53</td>
<td>18.53 ± 0.44</td>
</tr>
<tr>
<td>Silage-haylage-concentrate</td>
<td>Cd, (blood – nmol/l); (urine – μmol/l)</td>
<td>77.94 ± 0.99</td>
<td>40.64 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>Pb, μmol/l</td>
<td>4.63 ± 0.37</td>
<td>1.72 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>Cu, μmol/l</td>
<td>25.16 ± 0.59</td>
<td>16.03 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>Zn, μmol/l</td>
<td>28.94 ± 1.60</td>
<td>18.12 ± 0.96</td>
</tr>
</tbody>
</table>

Note: The degree of reliability in comparison with the data of the control group *P<0.05; **P<0.01; n=5; Permissible limit (Levchenko et al., 2002) for urine: cadmium 0.89 μmol/l, lead 0.22 μmol/l, copper 0.79 μmol/l, zinc 0.86 μmol/l, and for blood: cadmium 20-50 μmol/l, lead 2.00 μmol/l, copper 12.6-18.9 μmol/l, zinc 15.4-23.0 μmol/l.
Table 2: Standard concentrations of metals in biological media and the amount of their transition from blood to urine

<table>
<thead>
<tr>
<th>Metal</th>
<th>Standard concentrations</th>
<th>Blood</th>
<th>Urine</th>
<th>Transition, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd, (blood – nmol/l); (urine – μmol/l)</td>
<td>20-50</td>
<td>0.89</td>
<td>1.8-4.5</td>
<td></td>
</tr>
<tr>
<td>Pb, μmol/l</td>
<td>up to 2</td>
<td>0.22</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Cu, μmol/l</td>
<td>12.6-18.9</td>
<td>0.79</td>
<td>4.2-6.3</td>
<td></td>
</tr>
<tr>
<td>Zn, μmol/l</td>
<td>15.4-23.0</td>
<td>0.86</td>
<td>3.7-5.6</td>
<td></td>
</tr>
</tbody>
</table>

The statistical analysis of the indicators studied in three experimental groups of cows feeding the silage-root type of feed is described as follows. The quantity of Cd in blood (nmol/l) (Valid N = 5; Valid obs. = 33.3%) of first (control) group cows had arithmetic mean M (Mean), median (Median), standard deviation (SD), Lower Quartile, Upper Quartile, and the minimum value, and maximum value of 98.34, 98.76, 1.03, 97.74, 98.82, 96.89, and 99.51, respectively; the same in second (treatment) group were 79.11, 78.71, 2.90, 77.52, 79.80, 75.91, and 83.6, respectively; and in third (treatment) group were 49.19, 49.26, 2.41, 48.21, 50.73, 45.74, and 52.01, respectively. Similarly, in the urine (μmol/l) of first (control) group, the Cd quantities had arithmetic mean M (Mean), median (Median), standard deviation (SD), Lower Quartile, Upper Quartile, and the minimum value, and maximum value of 1.90, 1.82, 0.17, 1.81, 2.01, 1.73, and 2.15, respectively; while second (treatment) group had 3.05, 3.15, 0.22, 2.94, 3.20, 2.72, and 3.25, respectively; and third (treatment) group had 3.48, 3.51, 0.40, 3.26, 3.63, 2.98, and 4.04, respectively. The data obtained during the experiments do not obey the law of normal distribution. Both the arithmetic mean values of the studied indicators by groups and the median with the lower and upper quartiles are depicted in figure 1 and figure 2. A small difference between the mean values of the indicators and their median is insignificant and there is no significant impact on the results of research in this experiment.

The cadmium in the blood of cows of control group feeding the silage-haylage-concentrate type of feed was determined to be 77.94 ± 0.99 nmol/l. The cadmium concentration of 101.20 ± 3.17 nmol/l was found in cows feeding the silage-hay type of feed. Similarly, the lead concentration was recorded 4.63 ± 0.37 μmol/l in cows feeding a silage-haylage-concentrate type of feed, and 8.32 ± 1.65 μmol/l in cows feeding a silage-root crops ration. At the same time, the process of accumulation of heavy metals in the body of dairy cows was observed. Cows feeding on the feeds having high concentration of pollutants confirmed in their blood a high concentration of Cd, Pb Cu and Zn. In particular, the concentration of Cd in the blood of dairy cows of the control group feeding on silage-root type of feed exceeded the permissible limit of Cd by 1.97 times. The same Cd concentration was 2.02 times to the permissible limit in cows feeding on silage-hay, 1.62 times in cows feeding on silage-haylage, and 1.56 times in cows feeding on silage-haylage-concentrate type feed. Likewise, Pb concentration in the blood was recorded 4.16 times, 3.27 times, 2.87 times and 2.32 times, respectively, to the permissible limit in the cows feeding on silage-root type, silage-hay, silage-haylage, and silage-haylage-concentrate feeds. These laboratory blood tests clearly indicate that the bodies of cows were experiencing a toxic shock as result of high concentrations of toxic heavy metals.

The concentration of cadmium in the urine of cows of the control group ranged from 1.4 μmol/l among cows feeding silage-haylage-concentrate type of feed to 1.9 μmol/l among the cows feeding on silage-root crop. These concentrations of Cd exceed permissible limit by 2.13 times among cows feeding on silage-root crop type of feed, by 1.94 times among cows feeding on silage-hay feed, by 1.83 times among cows feeding on silage-haylage, and by 1.57 times among cows feeding on silage-haylage-concentrate. In the same fashion, the concentration of Pb in the urine of cows exceeded the permissible limit by 2.09 times among the cows feeding on silage-root crop type of feed, by 1.64 times among the cows feeding on silage-hay feed, by 1.41 times among the cows feeding on silage-haylage feed, and by 1.55 times among the cows feeding on silage-haylage-
concentrate. The Cd transferred from blood to urine averaged from 1.7% to 2.0%, and Pb from 5.4% to 7.3%. It means the removal of heavy metals from the cows’ body through the urine is negligible. The likelihood of the removal of heavy metals through the urine without the use of specific antidote substance was unlikely. Therefore, distributed throughout the body of cows, the pollutants accumulate in various organs and tissues, in particular, the liver, kidneys, spleen, muscles and bone tissues. These pollutants excrete through the milk. Similarly, other elements like Cu and Zn are recorded. Thus, chronic intoxication of animals was observed as confirmed by the results of the experiments. The analysis of Cd, Pb, Cu and Zn showed that not only the milk is contaminated by these heavy metals, but also the muscle tissues and internal organs have deposits. The process of neutralization and elimination of heavy metals goes on in these tissues and organs. The highest accumulation of heavy metals was observed (in descending order) in kidneys, muscles, liver, lungs, spleen, heart and bones.

Table 3: Mineral elements and heavy metals in milk of cows at the end of the research period (M ± SD)

<table>
<thead>
<tr>
<th>Animal feeding type</th>
<th>Mineral elements (mg) per kg of milk</th>
<th>Cow groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st control</td>
</tr>
<tr>
<td>Silage-root</td>
<td>Copper, mg/kg</td>
<td>2.47 ± 0.38</td>
</tr>
<tr>
<td></td>
<td>Zinc, mg/kg</td>
<td>7.06 ± 0.32</td>
</tr>
<tr>
<td></td>
<td>Cadmium, mg/kg</td>
<td>0.087 ± 0.008</td>
</tr>
<tr>
<td></td>
<td>Lead, mg/kg</td>
<td>1.835 ± 0.093</td>
</tr>
<tr>
<td>Silage-hay</td>
<td>Copper, mg/kg</td>
<td>2.54 ± 0.42</td>
</tr>
<tr>
<td></td>
<td>Zinc, mg/kg</td>
<td>9.93 ± 0.72</td>
</tr>
<tr>
<td></td>
<td>Cadmium, mg/kg</td>
<td>0.09 ± 0.085</td>
</tr>
<tr>
<td></td>
<td>Lead, mg/kg</td>
<td>1.641 ± 0.253</td>
</tr>
<tr>
<td>Silage-haylage</td>
<td>Copper, mg/kg</td>
<td>2.36 ± 0.39</td>
</tr>
<tr>
<td></td>
<td>Zinc, mg/kg</td>
<td>7.93 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>Cadmium, mg/kg</td>
<td>0.068 ± 0.017</td>
</tr>
<tr>
<td></td>
<td>Lead, mg/kg</td>
<td>1.734 ± 0.148</td>
</tr>
<tr>
<td>Silage-haylage-concentrate</td>
<td>Copper, mg/kg</td>
<td>2.63 ± 0.42</td>
</tr>
<tr>
<td></td>
<td>Zinc, mg/kg</td>
<td>8.74 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>Cadmium, mg/kg</td>
<td>0.053 ± 0.019</td>
</tr>
<tr>
<td></td>
<td>Lead, mg/kg</td>
<td>1.794 ± 0.165</td>
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</table>

Note: The degree of reliability in comparison with the data of the control group is P <0.01; n = 5. Physiological permissible limit, according to Mamenko et al. (1997) and Levchenko et al. (2002), for cadmium is 0.03 mg/kg, for lead is 0.1 mg/kg, for copper is 0.26-0.25 to 1 mg/kg, and for zinc is 3.0-5.0 mg/kg.

The premixes developed by the authors are adapted to the actual diets of the cows. During the balancing of rations, deficit in essential macro- and micro-elements in the forages of the main diet of cows was investigated in all four experiments. At the same time, the content of heavy metals Cd, Pb, Cu, Zn in some feeds included in the diet exceeded the maximum permissible limits. Based on this, all diets are maximally balanced in terms of the composition of macro- and micro-elements, considering the mechanism of absorption and the movement of heavy metals, especially Cd and Pb, in the body of animals. In the premixes, antagonists of the ecotoxicants were included in an amount sufficient to block its absorption by the gastrointestinal tract. The introduction of vitamins into the premix helped restore the homeostasis of the cows and improve the functioning of organs and systems affected by the oligodynamic action of heavy metals. As a result, things were managed to achieve a positive effect and to reduce the content of heavy metals in milk of cows in all the farms for optimal milk quality and animal productivity. The decrease in the Cd content in milk of dairy cows feeding silage-haylage-concentrate was 0.053 ± 0.019 mg/kg in the control group, 0.024 ± 0.009 mg/kg in the second experimental group, and 0.014 ± 0.004 mg/kg in the third experimental group; and, likewise, Pb was 1.794 ± 0.165 mg/kg, 0.331 ± 0.064, and 0.032 ± 0.008 mg/kg, respectively (p <0.01), in control group, second group and third group.
of cows (Table 3). At the same time, the maximum permissible concentration of cadmium content, according to the DSTU 3662-97 standard, is set at 0.03 mg/kg, and that of lead at 0.1 mg/kg. EU Regulation No. 1881/2006 fixed maximum permissible limit at 0.02 mg/kg for Cd, at 0.26-0.35 for copper, and at 3-5 mg/kg for zinc. Table 3 also shows the total content of mineral elements, including heavy metals, in the milk of experimental cows feeding different types of animal feeds.

Figure 1: Diagram of mean values (a) and quartile diagram (b) of Cd quantities in blood (nmol/l) of cows of three experimental groups feeding on silage-root crops type of feed.
Figure 2: Diagram of mean values (a) and quartile diagram (b) of Cd content in urine (μmol/l) of cows of three experimental groups feeding silage-root crops type of feed.

Table 4 shows the average daily milk yields of experimental cows kept in agroecosystems located around the industrial city of Kharkiv.
The antidote mineral-vitamin premix MP-A in the feeds of cows in the second experimental group contributed to a slight increase in milk productivity - on average by 1.3–1.7 times. It was most effective among the cows feeding on silage-hay type of feed, where milk productivity increased 1.6 times compared to the first control group (P <0.001). Increase in dairy productivity of cows after feeding the foods having mineral-vitamin premix can be, in ascending order, as follows: silage-root (1.3 times), silage-hay-concentrate (1.3 times), silage-hay (1.4 times), silage-hay (1.6 times). The main feeding rations of animals were expected to produce at least 4,500 kg per lactation, the average daily output of 14 kg. Productivity of cows less than 4,500 kg per lactation is simply unprofitable. Supplementation of the main diet with a specially developed antidote mineral-vitamin premix MP-A contributed to a remarkable increase in milk production from 3,477 kg to 4,426 kg per lactation in the first (control) group, from 5,444 kg to 5,999 kg in the second experimental group of cows. Subcutaneous injection of the BP-9 (a biological product) significantly enhanced the antitoxic effect of the mineral-vitamin premix on toxic metals in the animal body in the third experimental group, wherein milk productivity increased by an average of 1.3–1.7 times. The most effective complex application of the premix and biological product (BP-9) was noticed among the cows of the third experimental group feeding on silage-hay type of feed. Milk productivity increased by 1.7 times in third experimental group compared to the first (control) group (P <0.001). After the complex application of the mineral-vitamin premix MP-A and biological product (BP-9), the milk productivity of animals by type of feeding can be presented as follows (in ascending order): silage-root (1.3 times) → silage-haylage (1.5 times) → silage-haylage-concentrate (1.6 times) → silage-hay (1.7 times). The applied technological method contributed to the increase of milk production to an average of 5,697–6,899 kg per lactation in the third experimental group.

Table 4: Productivity of cows during research (M ± SD)

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Feeding type</th>
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<tr>
<td></td>
<td>1st control</td>
</tr>
<tr>
<td>Average daily milk yield, kg per head</td>
<td>14.31 ± 0.74</td>
</tr>
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</table>

Thus, by using mineral-vitamin premix BP-A and biological product (BP-9) together, it is possible to significantly increase the excretion of heavy metals from the body of dairy cows in the second and third experimental groups in all farms having different types of animal feeding. The rate of transition of Cd from blood to urine averaged in the second experimental group from 3.9% to 9.5% and Pb from 37.7% to 103.5%. Similarly, in the third experiment group, this conversion of Cd and Pb were from 7.1% to 12.7% and from 70.7% to 144.1%, respectively. Thus, the combined effect of the mineral-vitamin premix and biological product gave a better result enhancing the excretion of heavy metals from the animal body than the effect of the mineral-vitamin premix alone. The transition of toxic metals from blood to urine (Table 2) is 1.8–4.5% for Cd and 11% for Pb. Increased renal excretion of cadmium and lead may be an evidence of increased metal content in animals due to its unprecedented intake with dietary food, and rapid absorption into the bloodstream through the gastrointestinal tract. Increased milk productivity of cows, i.e., 6,000-7,000 kg per lactation, due to supplementation of feeding rations with a special mineral-vitamin premix MP-A partially succeeded. However, additional subcutaneous injection of biological product (BP-9) in the third experimental group enhanced the antitoxic effect of the mineral-vitamin premix, which ultimately affected animal productivity. However, it was not possible to increase the productivity of cows...
7,000-9,000 kg per lactation, which obviously requires the use of additional zootechnical measures. The use of mineral-vitamin premix and BP-9 injection are the factors that not only determined the productivity of animals, but during the experiment, it also became possible to significantly increase the excretion of toxic metals from cows, and, at the same time, produce environmentally friendly high-quality milk, meeting domestic and international quality standards (Table 3).

Discussion

Anthropogenic impact on agroecosystems surrounding industrial cities, including from the intensification of conventional farming (using excessively the pesticides, mineral fertilizers), unfortunately, is increasing in different countries of the world (Bigalke et al., 2017). The ingress of heavy metals into the soil can lead to the accumulation of its undesirable concentrations in agricultural land. It endangers soil fertility. These pollutants, such as cadmium and lead, are transferred from the soil to the plants used for animal feed. In this way, the heavy metals are included in the diet comprising any type of feed and complicating the production of high acid milk. An increased concentration of cadmium and uranium was observed in the upper fertile soil layer of agricultural land after applying the phosphorus fertilizers and sludge from the wastewater (Bigalke et al., 2017). Bigalke et al. (2017) investigated more than 216 agricultural land plots in different regions of Switzerland, where mineral fertilizers became the dominant source of heavy metal pollution influenced by atmospheric precipitation (Bigalke et al., 2017).

Heavy metals are divided into three categories: toxic (e.g., lead and cadmium), conventionally non-essential (e.g., nickel) and essential (e.g., copper, zinc and chromium). Today, out of 92 elements found in the nature, 81 are found in the human body. At the same time, 15 (Fe, I, Cu, Zn, Co, Cr, Mo, Ni, V, Se, Mn, As, F, Si and Li) are recognized as vital, that is, essential or non-essential. However, they can have a negative effect on plants, animals and humans if the concentration of their available forms exceeds the permissible limits. Cd, Pb, Sn, Rb are considered conditionally necessary, because, most likely, these elements are not very important for plants and animals and are hazardous to human health even at relatively low concentrations (Lu et al., 2013). There are number of biogeochemical studies on trace elements exhibiting the toxicity in animals and plants. For example, Gribovsky (2000) analyzed the nickel’s toxicity in animals in the regions polluted by industrial emissions and discharges. It was found that excessive intake of nickel into the body of animals leads to the damage to the gastrointestinal tract, liver, heart, blood vessels, brain, retina, etc. Nickel has been insufficiently studied from an ecological and physiological point of view. Similarly, cadmium’s range of tolerance by animal body is not known. Much of the studies are devoted to the pathogenic effect of this heavy metal, like many others, primarily as a feed contaminant. However, due to the rapid development of industry and global technogenic pollution of the environment, diseases caused by heavy metals of industrial origin began to attract the attention. Already, in many regions of the world, the environment is becoming more and more chemically "aggressive". In recent decades, the main orientation of ecological research has been on the territories of industrial cities and the adjacent lands (Patra et al., 2008; Lu et al., 2013), which is in tune with the present research. Many scientists have found that the influence of heavy metals is quite diverse and depends on their concentration in the environment and the degree of its necessity for microorganisms, plants, animals and humans.

Cadmium exhibits toxic effects on a number of organs and systems, including the cardiovascular, reproductive, excretory (especially kidneys, liver), respiratory, musculoskeletal system (causing osteodystrophy), hematopoiesis, etc. (Gutyj et al., 2016). The most toxic effects include the carcinogenic and mutagenic effects of Cd (Gutyj et al., 2016). There is a large amount of literature on the effect of acute and chronic forms of cadmium toxicosis in humans and experimental animals (Gutyj et al., 2016; Salvatori et al., 2004). The results of many studies indicate significant differences in the metabolic effects of one-time high doses and long-term exposure to low doses of cadmium. The intoxication of the animal body with cadmium compounds causes anemia, disruption of the immune system and other disorders including
hematopoiesis (Gutyj et al., 2016). According to Neathery and Miller (1975), up to 50% of the cadmium, lead and mercury accumulate in the kidneys and liver of animals. This research has found 38% of the cadmium precisely accumulated in the kidneys and liver.

It is worth noting that the acute form of cadmium toxicosis, sometimes with a fatal outcome, rarely occurs today, but the syndrome of the chronic form of toxicosis is observed more often. Gutyj et al. (2016) have reported this in their works. Clinical signs of chronic animal poisoning are accompanied by a sharp decrease in feed intake, a decrease in body weight, a slowdown in animal growth, impaired renal function, proteinuria, liver dysfunction, anemia, and an increase in neonatal mortality.

By developing and introducing antidote vitamin premix ‘MP-A’ substances, the objective was not only to achieve an increase in the excretion of toxic heavy metals, such as cadmium and lead, from the body of dairy cows but also to test the effectiveness of the premix preparation in homeostasis of animals through the scientific experiments. Biopreparation "BP-9" is an extract from a bunch of medicinal plants with biologically active substances that are safe for the body, in contrast to the long-known synthetic preparations such as unithiol. To produce the biological product "BP-9", nine medicinal plants were used, viz. Chinese magnolia vine, medicinal chamomile (pharmacy, peeled), Eleutherococcus prickly, medicinal sage (pharmacy), common barberry, alfalfa, kidney tea, sea buckthorn, and buckthorn.

Most of the medicinal substances can penetrate cell membranes through passive transport or through active special transport systems. Fat-soluble substances penetrate the membranes through passive diffusion. The transport intensity is directly proportional to the concentration of the substance in the membrane and the fat-water distribution coefficient. Non-polar and polar substances, poorly soluble in lipids, penetrate through water pores (filtration) due to the existence of a hydrostatic and osmotic potential difference on both sides of the membrane.

Each medicinal plant e.g., Chinese magnolia vine or common barberry, which was included in the composition of the biological product, has its own biological role. Chinese magnolia vine extract protects the liver, kidneys, spleen, heart and lungs from the toxic effects of heavy metals, in addition, it reduces the negative carcinogenic processes in the mammary gland associated with the migration of cadmium and lead into the blood and milk. It also prevents the oxidation of lipids in liver. Similarly, the Eleutherococcus extract helps protect the animal body from hazardous influence of heavy metals, including Cd, Cu, Pb and Zn, which is also confirmed from the results of this research. It activates the intensity of the removal of pollutants (Cd, Pb, Cu, Zn) from blood, milk, internal organs and body systems. It normalizes blood pressure, immuno-stimulating action, resistance to stress and a stimulating effect on the adrenal glands. It contributes to the restoration of the metabolism of mineral substances in the body, simultaneously, increasing the body's resistance to various diseases, including of viral origin. The extract of kidney tea helped increase the excretion of cadmium, lead, copper and zinc, as confirmed by these experiments. It effectively counteracted the development of proteinuria, the restoration of the functioning of the renal tubules and glomeruli of nephron in the kidneys, prevented degenerative changes in this important organ during intoxication. The common barberry was 12% of the total composition of biological product, which contained high ratio of vitamins C and E, carotene, malic and citric acids, which support the normal functioning of the Krebs cycle. According to Embaby and Afifi (2016), ascorbic acid is one of the water-soluble antioxidants and has a protective function against Cd-induced histological changes (cytotoxicity) in the liver, kidneys, lungs, and bone marrow. Fat-soluble vitamin E is one of the most important ingredients having antioxidant factors. Vitamin E protects the tissues from oxidative stress, performs various physiological functions, including a free radical scavenger. It prevents lipid peroxidation, hepatoprotector of the liver from biochemical disorders, histological changes because of exposure to heavy metals (Al-Attar, 2011). It acts as anticarcinogenic and anti-thyroid remedy. Choleretic and diuretic properties strengthen the antidote effect of the preparation "BP-9".
The renal pathway for the elimination of heavy metals from the body is rather complex and dynamic process. An increase in the content of toxic metals in urine in most of the cases causes biochemical and clinical changes. At the same time, the excretion of elements is a regulating mechanism to maintain constant concentration in the body. This study has shown that, with prolonged chronic intake of heavy metals into the body, their content in biological substrates increases. That is, over time, the pathological effect of heavy metals on cellular metabolism increases, which causes damage to the morpho-functional structure of the kidneys and, if appropriate measures are not taken, then over time, this will lead to pathological conditions and kidney diseases.

If, in accordance with the results of this research, these metals are placed in a row of elimination activity with urine output conforming the value of the transition index. Considering the action of the mineral-vitamin premix and the biopreparation, the form obtained in the second and third research groups is Pb → Cu → Zn → Cd. It means that the magnitude of the transition is different for metals having different biological activity, and it characterizes the body as a self-regulating system, upon entering which the toxic metals are intensively excreted. It is observed that enhancing the excretion of the most dangerous element of cadmium through the urine was not successful. However, other metals, especially lead, were successfully removed. Lead is no less dangerous toxicant. Most intensively, Cd passed 9.5% and 12.7%, respectively, from blood to urine in the cows of the second and third experimental groups feeding silage-haylage-concentrate type. The same Cd conversion was 7.3% and 9.5%, respectively, among the cows of second and third experiments feeding on silage-haylage, and 6.6% and 9.4%, respectively, feeding on silage-hay. Those feeding on silage-root crops had this Cd conversion to 3.9% and 7.1%, respectively. On average, this Cd conversion ranged from 3.9% to 12.7%, which is a significant result for such an adverse ecological state of agroecosystems. It ultimately leads to clean production of environmentally friendly milk. A significant transition of Pb occurs from blood to urine in cows of the second and third experimental groups. It is 103.5% and 144.1%, respectively, among the cows feeding the silage-hay-concentrate type feed. The same was greater among the animals feeding silage-hay, and was 37.7% and 113.8 %, respectively, for second and third experimental groups. The conversion of lead among cows feeding silage-haylage was 81.6% and 94.1%, respectively, in second and third experimental groups. Again, the Pb conversion for animals feeding the silage-root type of feed was 42.1% and 70.7%, respectively, in second and third experimental groups. On average, the Pb transition ranged from 42.1% to 144.1%, which is a typical result for agroecosystems where the level of Pb contamination is much higher than that of Cd. Among all farms, the cows feeding the silage-haylage-concentrate type of feed ate fodder with 7.3 times higher lead content. In the absolute terms, the transfer of Pb from blood to urine was actually 10 times higher than that of Cd (3.9% Cd versus 42.1% Pb in second experimental group, and 12.7% Cd versus 144.1% Pb in third experimental group). At the same time, remarkably high-quality (ecologically safe) and biologically complete milk is produced. It corresponds to GOST 3662-97, and in terms of lead content, bacterial contamination, and the somatic cell count, it conforms to the EU Regulation No. 853/2004 and No. 1881/2006.

In addition, the elements such as copper and zinc reduce renal excretion. In cows of the control group, the transition of Zn from blood to urine averaged 3.5%-5.3%, and in animals of the second and third experimental groups, on average, it was from 10.5% to 35.7%, having average of 7-30.4%. So, the mineral-vitamin premix and the biopreparation perform their protective function, including to increase the excretion of this essential element from the body through the urine (P < 0.01) when it is consumed excessively with the feed. The biological product used in the third experimental group of cows feeding

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3GOST refers to a set of technical standards maintained by the Euro-Asian Council for Standardization, Metrology and Certification (EASC), a regional standards organization operating under the auspices of the Commonwealth of Independent States (CIS). For further information, see https://en.wikipedia.org/wiki/GOST
The test cows feeding on the silage-root ration ate the feed having highest level of Cd contamination. In the experiment, a stable tendency was achieved to increase the excretion of this mineral element in the urine (P <0.01), thereby reducing its accumulation in the body of animals. Feeding the mineral-vitamin premix and subcutaneous injection of the biological product ensured the transition of Cd from blood to urine at a low level among all four experiments. It is amounted to 3.9% in the second experimental group and 7.1% in the third experimental group, which requires further analysis or the development of new and more effective antidotes. In general, the result obtained is an important scientific achievement.

The high concentration of heavy metals in the feeds migrate rapidly into the milk output. As a result, milk produced in the breast has an increased content of Cd, Pb, Cu and Zn, which are accumulated in the alveoli, ducts and cisterns (Mamenko and Portiannyk, 2020). At the same time, the capacity of the udder, which depends on these cavities, is important. From the upper alveolar parts, the milk passes into the tank due to the contraction or relaxation of the muscle fibers. This process is controlled by the nervous system. The process of milk production is significantly influenced by excessive loading of cows with heavy metals, especially Cd and Pb (Mamenko et al., 2010; Portjannyk, 2002a; Portjannyk et al., 2003). These elements are able to block the normal functioning of the body's nervous system. The accumulation of pollutants in the internal organs and muscle tissues can subsequently adversely affect the formation of milk, its secretion, and, consequently, the milk productivity of animals. Important for the formation of milk in the breast is the diet and set of feeds. A balanced diet with a high content of succulent foods contributes to the intensive accumulation of milk in the breast, which reflexively affects the cavities of the alveoli, ducts and cisterns. By relaxing the walls of the udder cavities, its capacity increases, and thus the ability to hold more milk. Double milking, which was used in all experimental farms before the animals were tested, did not promote the development of the capacitive function of the udder of cows, which is disrupted by toxic metal intoxication reducing subsequently the animal productivity. From the beginning of the experiment, all animals were transferred to triple milking, which contributed to the intensification of the milk production process. This process is uneven. In the first hours after milking, the milk synthesis was more intense; and with increasing time from the last milking, the milk production declined. Ivanov and Obuhov (1985) show that cows of black-spotted dairy breed are characterized by more intensive milk production. In the first 6 hours after pre-milking from the beginning of lactation, 1-1.2 liter of milk was formed per hour, and after 10-12 hours only 0.61-0.65 liter per hour was formed. On the attenuation of lactation, visible milk production almost stops at 10 hours after pre-milking. In some cows having milk yield of more than 5,000 kg of milk per lactation, during their transfer from triple milking to double milking, the yield decreased by 40%; while returning to the previous triple milking regime, it was restored. Several scientists (Bucjak, 2002; Bucjak, Kravev and Bucjak, 2005; Bucjak, Cherevko and Suhors'ka, 2010; Gribovsky, 2000; Gutyj et al., 2016; Neathery and Miller, 1975; Savchenko, Savchuk and Savchenko, 2013; Savchenko, 2011; Zasekin, 2000) proved the negative impact of heavy metals on...
various organs: liver, kidneys, spleen, lymphatic system, nervous system, enzymatic production, hormonal system and circulatory systems. Heavy metals affect the secretion of the oxytocin hormone, as the nervous irritation of the peritoneal area causes the transmission of nerve impulses to the spinal cord. So, one part of the signals is sent to the brain and the other to the breast. In response to these signals, the back of the pituitary gland secretes this hormone, which appears in the blood after 20-30 seconds and reaches the breast with the flow of blood helping to contract the muscle cells that surround the alveoli and small tubules. Such reflex actions can be partially blocked by exposure to excessive concentrations of Cd and Pb in the body, which is confirmed by the presence of these elements in milk (Table 3). Transfer of oxytocin from the blood to the udder is inhibited. Morpho-biochemical analysis of blood showed an excess of heavy metals, as well as several other negative physiological changes in the body of dairy animals. In case, this system fails, the alveoli are compressed weakly, the tubules contract slowly, and their cavity does not increase. Thus, there are no favorable conditions for milk to enter the ducts of the gland.

Since the trace elements like iron, selenium, molybdenum, boron, calcium, chromium, fluorine, sodium, lithium, iodine, silicon, manganese, copper, zinc, nickel, vanadium, bromine, cobalt, cadmium and lead, and vitamins (A, C, B, PP, E, K, F) are essential, with the help of the injection of the biological product "BP-9" it was managed to enhance the anti-toxic effect of the mineral-vitamin premix "MP-A" in the tissues, organs and systems of intoxicated cows. The composition of mineral-vitamin premix and biological product is described already. Included in the "MP-A" premix, selenium performed an important function. Being a part of amino acids (selenomethionine and selenocysteine), proteins and enzymes (glutathione peroxidase), it prevents the toxic effects of peroxide radicals on cells. Glutathione peroxidase destroys not only hydrogen peroxide, but also peroxide compounds formed as a result of the oxidation of unsaturated fatty acids. This process is disrupted by Cd and Pb leading to an increase in the amount of under-oxidized products. The key role of selenium and zinc in the prevention of cadmium neurotoxicity has been proven (Branca et al., 2018).

Subcutaneous injection of the biological product "BP-9" developed by authors, made from an aqueous extract of 9 medicinal plants, with a daily dose of 20 ml per head per day applied in all four experiments, positively influenced the urinary excretion of ecotoxics Cd and Pb. The bioproduct maximized preservation in the body of dairy cows of essential macro- and micro-elements, including Cu and Zn, with varied animal feeds. Anti-inflammatory and antitumor properties of the biopreparation, decreased neurotoxicity of cadmium and lead, and hepatoprotective effect actually increased the excretion of heavy metals through the urine, thereby, reducing the contents of toxicants in the liver, kidneys, spleen, and other internal organs and tissues, including muscle tissues (P <0.01). The usage of extracts of medicinal plants has been proven by the studies of scientists. For example, the leaf extract of the perennial evergreen betel nut (Piper betle) was studied by Prabu, Muthumani and Shagirtha (2012). The extract showed a good protective effect against cadmium-induced oxidative liver damage in experimental rats. There are also known methods for removing cadmium from the body of experimental animals using betulinic acid, a natural pentacyclic triterpenoids found in the bark of some plant species, in particular, birch (Betula pubescens). Betulinic acid has anti-inflammatory and antitumor properties. It increases the excretion of cadmium from the liver and kidneys (Fan et al., 2018). A positive result on Cd and Pb intoxication was shown by an aqueous solution of tannic acid, which contains an extract of many plants (Winiaska-Mieczan, 2013), and an extract from mangosteen (an evergreen tree Garcinia Mangostana) relieves lead-induced neurotoxicity (Phyu and Tangpong, 2014). The hepatoprotective properties of royal jelly neutralizing cadmium intoxication in rats were studied preventing oxidative stress, inflammation and liver damage (Almeer et al., 2018). Bilberry (Vaccinium corymbosum L.) extract and anthocyanins isolates also have a protective, therapeutic effect against Cd intoxication at the level of metal-chelating complexes (Gong et al., 2014).

Physalis peruviana or cape gooseberry of the Solanaceae family was used in experiments that completely changed the histopathological state of liver and kidney tissues. Its good hepatorenal protective role was
proved with lipid peroxidation decreased (Dkhil et al., 2014). In the organs and tissues of cows of the second experiment group, where the biological product was not used, and of the first control group, the highest content of the heavy metals was recorded. It proves the accumulating effect of pollutants. On the other hand, the extent of elements in the organs and tissues of animals of the second experiment group tended to gradually decrease due to the mineral-vitamin premix "MP-A" (P <0.01) having blocking effect on toxicants in the gastrointestinal tract. The organs of increased contamination of heavy metals were kidneys and liver (P <0.01). Ukrainian scientists carried out research on feeding various feed additives into the diet, which played the role of a kind of adsorbent of heavy metals (Kurlyak, 2011; Savchenko, Savchuk and Savchenko, 2013; Papaioannou et al., 2005). They blocked the absorption of elements at the level of the gastrointestinal tract. In particular, they fed dairy cows of the black-and-white breed with the natural zeolite mineral from the Sokyrnytsia deposit, which has absorption, ion-exchange, catalytic and other unique properties when existing as pollutant in agroecosystems. It positively affects the physiological state, productivity of animals with 14.6% increase of yield, and the production of environmentally friendly milk free from lead content (Kurljak, 2011). Feeding animals with diet having a pectin preparation made from waste of the wine industry ensures the removal of mercury from the sheep's body. The same pectin preparation together with fodder beets given to fattening pigs helps reduce the negative effect of cadmium on the productivity of animals and its transformation into muscle tissue. In order to increase the elimination of lead, copper, zinc and iron, it is recommended to feed the cows daily for 45 days 500 ml of decoction of licorice root (Kurlyak, 2011; Savchenko, Savchuk and Savchenko, 2013; Papaioannou et al., 2005). Moreover, the intensity of the transition of heavy metals into livestock products is reduced by the introduction of additives into the diet of animals that adsorb and inhibit their absorption in the gastrointestinal tract reaching the blood. For example, saponite, glauconites, trapels, complex additives and heterosorbents (such as Belosorb – a polymer of natural origin made of cellulose) bind heavy metals, thereby, weakening their toxic effect on the body of animals, while positively affecting metabolic processes in the microbial ecosystem of the rumen. It has been experimentally proven that the use of zeolite in the diet of dairy cows helps reduce lead in milk by 56.8%, cadmium by 22.6%, and mercury by 50%, and, simultaneously, having a positive effect on productivity (Kurlyak, 2011; Savchenko, Savchuk and Savchenko, 2013; Papaioannou et al., 2005). The most promising means of preventing the migration of heavy metals in the trophic chain are complex compounds and enterosorbents, which do not exhibit toxic effects on the body and, significantly, limit the absorption of heavy metals in the gastrointestinal tract (Papaioannou et al., 2005; Savchenko, Savchuk and Savchenko, 2013).

Side by side, methodology for the development of special anti-toxic mineral and vitamin premixes "MP-A" was tested, adapted to the actual feeding ration, introduced into the daily ration of dairy cows. Such mineral-vitamin premixes are sold by a large number of enterprises in the market. Sometimes agricultural enterprises themselves have their own workshop for producing feed additives used by them. The anti-toxic effect of the premix is determined by the composition of mineral elements in an appropriate ratio. The vitamin group is aimed at maximizing the normalization of the physiological functions of animals intoxicated with heavy metals (especially Cd and Pb), and the added sulfur-containing amino acid acts as a chelating complex neutralizing the heavy metals. Along with the quality improvement, an increase in the milk productivity of animals (P <0.01) has been recorded, which averaged 17-22 kg per day in cows of the second and third experimental groups compared to the control group having 14 kg per day (P <0.01). The antidote mineral-vitamin premix MP-A and injection of biological product BP-9 with triple milking contributed to improved milk production. The secretory activity of mammary glands has also increased on average by 1.6 times, which allowed to achieve milk production of 5,697-6,899 kg per lactation (P <0.001).

**Conclusions**

The excretion of heavy metals from the urine of dairy cows indicates its load on the animal body. Heavy metals are consumed with feed exceeding the maximum permissible concentrations of the toxic elements.
The health of dairy cows has been recovered by using a special antitoxic mineral and vitamin premix used along with an injection of a biologically active preparation. These two antidote products were tested on dairy cows exposed to heavy metals having contaminated the agroecosystems near industrial city of Kharkiv. The antidotes enhanced the elimination of heavy metals (Pb, Cu, Zn and Cd) through the urine from intoxicated animals. The antidote effect of the mineral-vitamin premix and the biological product cumulatively had a positive effect on the renal excretion of Cu and Zn from the body without sacrificing the health of cows, while restoring its homeostasis, improving the quality and environmental safety of milk produced, and enhancing milk productivity of animals to 6,899 kg per lactation.

Further research is aimed at developing antidote substances that can contribute to the increased excretion of dangerous heavy metals, such as Cd and Pb, from the body of animals through the urine. It needs also to maximize the retention of essential elements in accordance with the physiological requirements, to contribute to the improvement of milk yield to 7,000-9,000 kg per lactation.

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Authors’ Declarations and Essential Ethical Compliances

Authors’ Contributions (in accordance with ICMJE criteria for authorship)

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<tr>
<th>Contribution</th>
<th>Author 1</th>
<th>Author 2</th>
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<tr>
<td>Conceived and designed the research or analysis</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Collected the data</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Contributed to data analysis &amp; interpretation</td>
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<td>Wrote the article/paper</td>
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<td>Critical revision of the article/paper</td>
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<td>Editing of the article/paper</td>
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<td>Supervision</td>
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<td>Project Administration</td>
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<td>Funding Acquisition</td>
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Research involving human bodies (Helsinki Declaration)

Has this research used human subjects for experimentation? No

Research involving animals (ARRIVE Checklist)

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