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New Criteria for Chemical Heterogeneity of the Biosphere 2. Distribution of Cadmium and Lead in the Egg of Hens

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A B S T R A C T

There have been experiments with laying hens divided into 4 groups which receive rations with different amounts of lead and cadmium. The amount and distribution of the two toxic elements in the egg components have been studied and the environmental risk for human food use has been assessed. Two new criteria defining the chemical heterogeneity of the biosphere at sub-organismal level have been devised: Clark of distribution and Clark of safety. These criteria are proposed to increase the effectiveness of management. Disparities in the distribution of the tested elements in the egg white, egg yolk and shell and the degree of safety for human health have been found.

Introduction

The chemical heterogeneity of the biosphere is a characteristic of both the abiotic components and the living organisms. With regard to the chemical heterogeneity of agriculture and in nature, up-to-date knowledge has allowed for the study of the accumulation of toxic elements and compounds along the food chain, defined as bioaccumulation.

The increase in toxic levels continues in increasing concentrations in organisms from all units of the trophic chain. Previous studies with autotrophic and wildlife animals as well as with birds under model conditions allow for the conclusion to be made that there are differences between the bioaccumulation of toxic elements and xenobiotics in the pastoral-type trophic chain (4,5,7,10,11,12,13).

Tests carried out with laying hens (1,2,3) show that enrichment of the diet with different amounts of heavy metals leads to a significant increase in their content in the internal organs, especially in the kidneys and liver.

Additionally, it is found that the body of the hen has some mechanisms to limit the degree of toxic elements in the body, one of these mechanisms is the separation of these elements into the feathers (6, 9) and the shell of the egg (1, 2, 3, 8). More studies have provided information on the accumulation of toxic elements in bird tissues but, there is insufficient information about the accumulation of cadmium and lead in the individual parts of the egg (egg whites, yolk and egg shell).

In our present research, we have done the following:

1. Determine the content of lead and cadmium in the three main components of egg production (egg white, egg yolk and shell) and estimate the distribution by the proposed by us, Clark distribution criterion (Cd).
2. To measure the safety of human consumption for the eggs obtained from poultry who were fed diets containing different doses of lead and cadmium, measured by our proposed "Clark of Safety" (Cs).

Materials and Methods

Studies have been conducted under pre-constructed and modelled conditions. The models are mesocosmos type of the Odum classification (1986). The ecotope is a 20/10 m air-conditioned production facility for the breeding of birds, in which all groups of birds are kept simultaneously with the same parameters of the abiotic factors as prescribed in Ordinance 44 of the Ministry of Agriculture and Forests, which prescribes the technological norms of the main abiotic elements /temperature, humidity and air-speed movement, intensity of light and toxic gases content. Biotic factors are regulated by applying prophylactic programs and by optimizing the population density of the agriculturally beneficial species.

The trials were conducted with 80 laying hens divided into 4 equilateral ISA – Brown strains at 36 weeks of age for 90 days. The cohort has the content: 18% crude protein, 0,44% phosphorus, 3,83% calcium, 0,91% lysine, 0,76%, methionine + cysteine and energy of conversion off 2750 kcal/kg of feed.

The following groups were formed

Group I /control/ fed with compound feed mixture prepared with all ingredients according to the recipe containing Pb 0,45 mg/kg fodder and Cd 0,38 mg/kg feed corresponding to Maximum Residues Limit //MRL/.

Group II - fed with feed containing lead and cadmium 2,5 times more than group I.

Group III fed with feed containing lead and cadmium 20 times more than group I.

Group IV - fed with feed containing lead and cadmium 200 times more than group I.

The resulting eggs are harvested daily and stored after weighing and marked at 4 C.

The toxic chemical elements cadmium and lead were determined in drinking water and feed at the beginning of the experiment. The contents of both the toxic elements in the tissues of the laying hens is determined after measurement by the Spectra AA 220 Z-Varian (AAS) atomic absorption spectrophotometer assay using the Jorchem’s method (1993), modification of Kirov test with wet mineralization.

The static processing of the obtained results was made on the static program - Origin® 7.0 SR0, V 7.0220 (B220), and the statistical reliability criteria of all comparisons was $P < 0.05$.

Results and Discussion

The content of lead and cadmium in the control group's eggs was found to be below the detection level (0.0005 mg) of the apparatus (AAS) in all egg constituents and in all periods of time. On the tables they are marked with an "-"

Content of lead and cadmium in egg components

Lead

The data on the content of lead in the three main components of the eggs, depending on the level of feed in the fodder mixtures and feed time, are given in Table 1. Accumulation of lead in the egg white was determined to be 45 days in the both the second and third group, while in the fourth group, accumulation began after 30 days. Prior to these periods, the lead content of the egg white was below the detection level (0.0005 mg).

Figure 1 shows that the content of lead in the egg white increases in proportion to the dose in the feed and the time of action, in 2^{nd} group and 4^{th}, the highest values are at the 75^{th} day mark, after which a reduction occurs. For group 2^{nd} birds, the dynamics are different - after a characteristic increase until 75 days, followed by a peak in the content towards the 90^{th} day.

In Group 4^{th} the content of lead in yolk starts with 0.105 mg/kg of fresh weight on day 15, whereas its contents in the 2^{nd} and 3^{rd} groups were below the detection level. In the egg yolk, similar to the egg white, it appears that there is a tendency of lead to decrease at the end of the trial (0.014 and 0.08 mg (kg respectively in 2^{nd} group and 4^{th}), whereas in 3 groups its content gradually increases from the 60^{th} day until end of trial.

The content of lead in yolk was found earlier than in egg white studies: on day 15 in group 4^{th} and day 30 in group 2^{nd} and 3^{rd}, the amount of lead increased: in group 3^{rd} it was higher than in 2^{nd} but, the difference is not reliable and the difference in its quantity reliably increased in the 4^{th} group compared to the 2^{nd} group and the 3^{rd} group ($P < 0.05$). Figure 2 shows a change in dynamics that can be judged to be dependent on the level of lead in the feed and the progress of time in the experiment.

Under the experiment, it was found that the shell contains more lead than other parts of the egg (egg white and yolk).

The 4^{th} group of increased content was established on the 15^{th} day and in the 2^{nd} and 3^{th} group on the 30^{th} day from the beginning of the trial. Changes in the content of lead in the shell depending on its level in the feed mixture, the dynamics being comparable in the three experiments. As can be seen in Table 1 and Figure 3, its contents during the trial shows a difference, with an increase in the three experimental groups at day 45. The highest content is found on the 60^{th} and 75^{th} day from the beginning of the experiment, which is found to be at varying degrees in the different test groups.

Cadmium

Data on cadmium content in egg components are shown in Table 2.

The cadmium content of the egg white on the 15^{th} day in the three test groups is below the sensitivity of the apparatus (below 0.0001 mg). Cadmium content starts to increase at day 30 to 0.0073, 0.0032 and 0.0067 mg/kg of fresh mass in the 2^{nd}, 3^{rd} and 4^{th} groups respectively. There is a deviation in the curve illustrating the cadmium content depending on the dose and time. Phase effect is seen:

cadmium reaches peak values: on day 45th in both groups 2nd and 3th, and on day 75th in group 4th after which the tendency to decrease (the 3rd and 4th group) is observed (Figure 4). The difference in cadmium content between the three test groups is not reliable ($P > 0.05$).

In egg yolk, the cadmium content on the 15th day is below the detection level (0.0001 mg).

The curve reflecting the dynamics of changes in the cadmium content in yolk shows differences when compared to that of the egg white. There is evidence of cadmium in the egg yolk of the 4th group on the 15th day. In group 4th, the peak in cadmium is observed on the 30th day, after which there is a reduction to the end of the experiment. In the second and third groups, cadmium in the yolk is established on the 30th day and the maximum is on the 75th day in 2 groups and on the 30th day for all three, and in both groups there is the tendency to decrease the content of cadmium as well (Figure 5).

Cadmium was found in the bird's shell on day 15 for group 4 (0.0092 mg/kg). In the 2nd and 3rd group, the contents on the 15th day were below the detection level (0.0001 mg), but, it was determined that at day 30, in the 2nd group of cadmium at the 30th and 45th day, it is 0.005 mg/kg fresh mass and decreased to 0.003 and 0.0019 on days 60 and 75 and then increased to 0.014 mg/kg on day 90. In group 3, the cadmium content was undeniably higher than the 2nd group, with the same trend in dynamics: at day 30 with 0.0066 mg/kg which decreased to 0.0039 mg/kg on day 60 and then it is increased to 0.011 mg / kg at the end of the trial (day 90) (Figure 6).

In the 4th test group the cadmium content was higher in all studies but, the difference was not reliable ($P > 0.05$).

On day 15, its content is 0.0092 mg/kg of fresh mass, gradually increased to 0.012 and 0.0262 mg/kg on days 30 and 45, and then decreased to 0.0061 on days 60 and 75, the 90th day begins to increase (0.026).

Figure 6 shows the dynamics of cadmium in the shell in dose and time, with the same tendency to decrease its contents on the 60th and 75th day and to increase on the 90th day.

Assessment of the distribution of lead and cadmium in the egg components using criteria "Clark of distribution (Cd)"

To assess the distribution of lead and cadmium in the secondary organic production (organs and tissues of hens, eggs and fertilizer), we developed a Clark distribution criterion (Cd), which is defined as the ratio of the content of the test element to the test organ or tissue (in mg/kg of fresh mass) and the average content of the examined chemical element in the organism (Average Clark) in the same dimension. The use of the criterion Cd in the management of agro-ecological systems allows for practical problems to be solved related to the control of secondary biological production - the recommendation to test organs and tissues with the highest Cd. Cd allows for the differentiation of the safety and risk related to the production obtained from regions with a high cluster of toxic elements in the lithosphere, allowing for consumption organs and tissues for which the models were found to accumulate minimum quantities of the corresponding risk chemical factors.

The cluster data for the distribution of lead and cadmium in the egg components of the test groups are reported in Tables 3 and 4.

Lead

From Table 3, it is seen in the test groups that the distribution of lead in the egg whites is unidirectional in groups 3 and 4. Cd increased to 75 days, then gradually decreasing to the end of the trial. In the fourth group changes were less pronounced, regardless of the fact that there is a tendency for Cd to decrease towards the end of the trial.

The period of time of lead-enriched feed influences the dynamics of the distribution of lead in the egg white. For high lead groups, the Cd values in the egg white increases as time progresses to the 75^{th} day and it decreases at the end of the trial. The highest Cd score in group 3^{rd} is 0.74 (on day 74), and in group 4^{th} is 0.39, respectively. At the end of the trial, the Cd values in egg whites of the 3^{rd} and 4^{th} group, were 0.69 and 0.27, respectively.

Table.1 The content of lead in egg yolk, egg white, and shell (determined to be 15 days mg/kg mg/kg fresh mass)

Time	**Group 1**			**Group 2**			**Group 3**			**Group 4**		
	w	**y**	**sh**	**w**	**y**	**sh**	**w**	**y**	**sh**	**w**	**y*,*3**	**sh**
1	-	-	-	-	-	-	-	-	-	-	0,105± 0.013	0,107± 0.013
2	-	-	-	-	0,009± 0.001	0,0077± 0.001	-	0,012± 0.001	0,036± 0.004	0,0073± 0.001	0,095± 0.011	0,104± 0.013
3	-	-	-	0.0079	0,011± 0.001	0,015± 0.002	0,0092± 0.001	0,02± 0.002	0,035± 0.004	0,014± 0.002	0,133± 0.016	0,128± 0.015
4	-	-	-	0,0092± 0.001	0,018± 0.002	0,057± 0.007	0,02± 0.002	0,017± 0.002	0,126± 0.015	0,023± 0.028	0,209± 0.025	0,241± 0.029
5	-	-	-	0,0072± 0.001	0,053± 0.006	0,053± 0.006	0,024± 0.003	0,018± 0.002	0,128± 0.015	0,024± 0.003	0,081± 0.01	0,257± 0.031
6	-	-	-	0,031± 0.004	0,014± 0.002	0,031± 0.004	0,019± 0.002	0,042± 0.005	0,045± 0.005	0,01± 0.001	0,08± 0.01	0,097± 0.012

* the difference in comparison to the contro2^{nd} group is reliable ($P < 0.05$)
*3 the difference in comparison to the contro3^{th} group is reliable ($P < 0.05$)

Table.2 The content of cadmium in egg yolk, egg white, and shell (determined to be 15 days mg/kg mg/kg fresh mass)

Time	Group 1			Group 2			Group 3			Group 4		
	w	**y**	**sh**	**w**	**y**	**sh**	**w**	**y**	**sh**	**w**	**y*,*3**	sh
1	-	-	-	-	-	-	-	-	-	-	0,051± 0.007	**0,0092± 0.0012**
2	-	-		0,0073± 0.001	0,0092± 0.001	0,005± 0.001	0,0032± 0.0004	0,0043± 0.0006	0,0066± 0.001	0,0067± 0.001	0,07± 0.009	**0,012± 0.002**
3	-	-	-	0,012± 0.002	0,017± 0.002	0,005± 0.001	0,007± 0.001	0,0064± 0.001	0,0093± 0.001	0,0047± 0.001	0,048± 0.006	**0,026± 0.035**
4	-	-	-	0,0033± 0.0004	0,0023± 0.0003	0,003± 0.0004	0,0047± 0.001	0,006± 0.001	0,0039± 0.001	0,0075± 0.001	0,057± 0.008	**0,0061± 0.001**
5	-	-	-	0,0032± 0.0004	0,031± 0.0004	0,0019± 0.0003	0,0051± 0.001	0,0032± 0.0004	0,0061± 0.001	0,012± 0.002	0,054± 0.007	**0,0061± 0.001**
6	**-**	**-**	**-**	**0,0047± 0.001**	**0,002± 0.0003**	**0,014± 0.002**	**0.0049± 0.001**	**0,005± 0.001**	**0,011± 0.001**	**0,01± 0.001**	**0,022± 0.003**	**0,026± 0.003**

* the difference in comparison to the contro2^{nd} group is reliable ($P < 0.05$)
*3 the difference in comparison to the contro3^{th} group is reliable ($P < 0.05$)

Table.3 Assessment of the distribution of lead in the egg components using criteria “Clark of distribution (Cd)”

	I			**II**			**III**			**IV**		
	б	ж	ч	б	ж	ч	б	ж	ч	б	ж	**ч**
15	0	0	0	0	0	0	0	0	0	0,12	2,52	**2,57**
30	0	0	0	0,70	1,58	1,35	0,43	1,29	3,87	0,18	2,39	**2,61**
45	0	0	0	0,84	1,17	1,6	0,63	1,37	2,40	0,24	2,31	**2,22**
60	0	0	0	0,55	1,08	3,43	0,66	0,56	4,19	0.24	2.21	**2.54**
75	0	0	0	0,30	2,20	2,20	0,74	0,55	3,93	0,39	1,31	**4,16**
90	**0**	**0**	**0**	**1,17**	**0,53**	**1,70**	**0,69**	**1,52**	**1,63**	**0,27**	**2,17**	**2,63**

Table.4 Assessment of the distribution of cadmium in the egg components using criteria “Clark of distribution (Cd)”

Time	Group 1			Group 2			Group 3			Group 4		
	w	**y**	**sh**	**w**		**w**	**y**	**sh**	**w**		**w**	y
15	0	0	0	0	0	0	0	0	0	0,01	3,49	**0,63**
30	0	0	0	0,96	1,21	0,66	0,84	1,13	1,74	0,28	2,89	**0,50**
45	0	0	0	0,95	1,34	0,40	0,99	0,90	1,31	0,26	2.61	**1.42**
60	0	0	0	1,10	0,77	1,00	0,94	1,20	0,78	0,37	2,78	**0,30**
75	0	0	0	0,31	2,98	0,18	1,09	0,68	1,30	0,53	2,38	**0,27**
90	**0**	**0**	**0**	**0,96**	**0,41**	**2,86**	**0,89**	**0,91**	**2,00**	**0,68**	**1,50**	**1,77**

Figure.1 Content of lead in protein in egg white

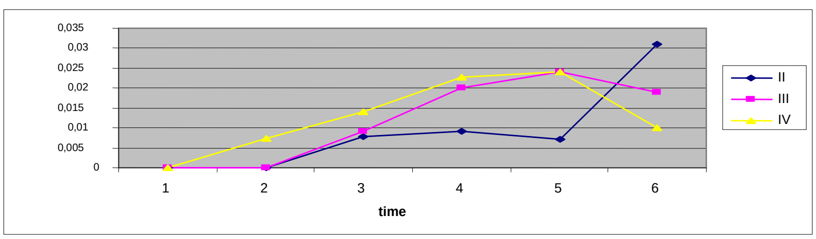


Figure.2 Content of lead in yolk

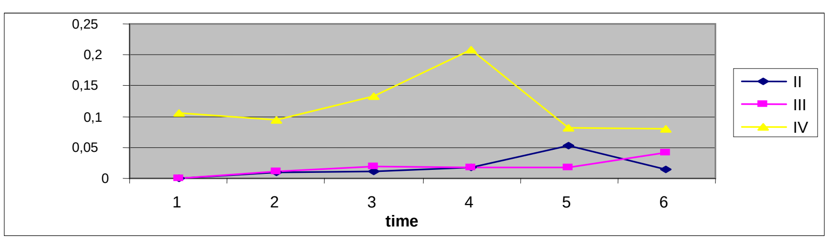


Figure.3 Content of lead in shell

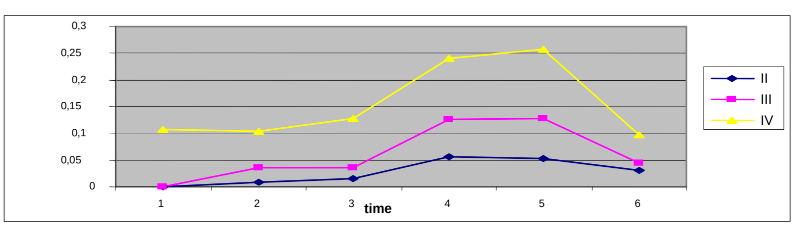


Figure.4 Content of cadmium in egg white

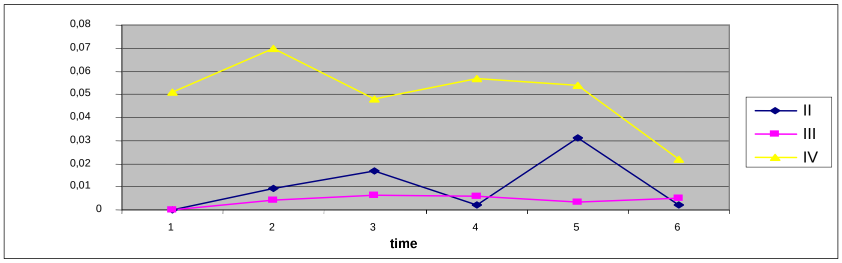


Figure.5 Content of cadmium in yolk

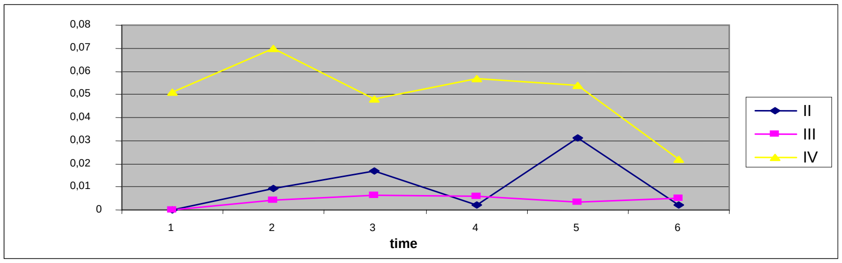
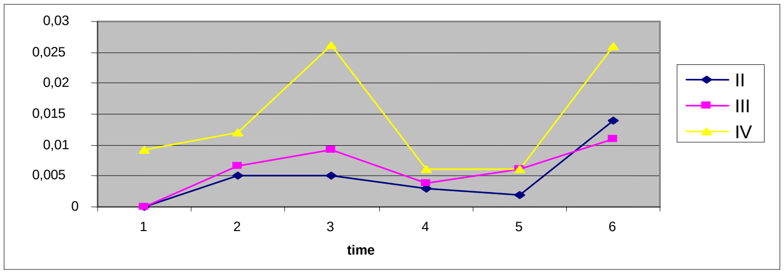


Figure.6 Content of cadmium in the shell



In the 2nd group the influence on the dynamics is different compared to the high doses of lead. Cd values increase to day 45 (0.84) and decrease on day 60 and 75 (0.55 and 0.30 respectively), with the highest values on the 90th day (1.17).

In yolk, the degree of lead distribution in group 4 increases compared to other groups but, between 3rd and 2nd group, there are differences depending on the period. On and at the highest Cd values at day 15, a gradual reduction is established until the 75th day, after which the level of the first experimental periods is restored. At the lowest dose (group 2), Cd values decrease until the end of the trial, with the exception of the 75th day when the Cd is increased. The lowest Cd value in the yolk of the 2nd group is 0.53 (on the 90th day) and the highest is 2.2 (on the 75th day). In Group 3, with exception of the 60th and 75th periods, Cd values increase as time progresses. The highest Cd value is 1.52 on the 90th day, and the lowest is a 0.55 on the 75th day.

As can be seen in Table 3, the highest values of Cd are in the shells of the eggs but, differences in the dynamics can be observed of the individual groups according to the dose and time here of depositing of the chemical element.

The distribution of lead in the egg shell is dependent on of the dose and time. At all doses, Cd of lead in yolk increases to the middle of the trial and then decreases to the end of the trial. In Group 4, the highest value of Cd is 4.16 (on the 75th day) and the lowest is 2.22 (on the 45th day). In Group 3, the highest value of Cd is 4.19 (on Day 6), and the lowest is 1.62 (on Day 90). For Group 2, the highest value is 3.43 (on the 60th day), and the lowest is 1.35 (on the 30th day).

Cadmium

The distribution of cadmium is not uniform in the egg components (egg white, yolk and egg shell) (Table 4). The highest is the value of Cd in the yolk of the experimental groups.

Cadmium in the egg white was measured on the 15th day in the 4th group and on the 30th day in the 2nd and 3th group and from that point, it was then possible to analyze Cd. Cd of cadmium in the protein is characterized by varying dynamics in the different amounts of the toxic element in the diet, decreasing as the dose increases.

In the dynamics, an increase in the degree of cadmium distribution in the 4th group was found to be proportional to the end of the trial, where Cd started at 0.01 on the 15th day and reached 0.68 on the 90th day. In Group 3, the degree of distribution of cadmium changes the same way as in group 4 but tends to decrease at the end of the trial where Cd starts at 0.84 on day 30 and increases to 1.09 on the 75th day and at the end of the trial reached a value of 0.89.

In the 2nd group there are differences in the distribution of cadmium depending on the period of the experiment. Cd of cadmium starts at 0.96 on day 30, increases to 1.1 on day 60, decreases to 0.31 and 0.96 on the 75th and 90th day respectively

Cd values in yolk of cadmium are higher than in the shell and egg whites. Increasing the dose of cadmium leads to accumulation in the group with the highest dose (4th) compared to the lowest dose group (2nd). In Group 2, Cd values increased to day 75 (2.98) and decreased to day 90 (0.41). The third group has differences in the degree of distribution of cadmium in yolks depending on time. Cd in group 3 begins at 1.21 on day 30 and reaches 1.2 on day 60, then decreases to 0.68 and 0.91 on 75th and 90th day respectively.

The 4th group, Cd of cadmium in yolk gradually decreases as the time begins, starting at 3.49 on the 15th day and reaching 2.78 on the 60th day and on 1.5 on the 90th day.

In the shell, differences in the degree of cadmium distribution depend on dose and time.

The highest cadmium distribution score values in the shell of the 2nd, 3rd and 4th groups were 2.86, 2, and 1.77 respectively (on the 90th day at all doses) and the lowest were 0.18 (day 75 for group 2), 0.78 (day 60 for group 3) and 0.27 (day 75 for group 4).

Assessment of chemical heterogeneity at sub-organismal level using the Clark of Safety Criteria (Cs)

We propose this new criterion for assessing the environmental risk due to the accumulation of toxic elements in the secondary biological production as a food resource for humans. The criterion is determined by dividing the content of the toxic factor in mg / 1 kg of fresh secondary organic production, of the maximum content in the same quantity according to the local current normative documents. When analyzing these results under criterion Cs it should be taken into account that it has been developed from a human health point of view, taking into account the movement of the toxic chemical elements and, in particular, and more specifically, their accumulation in individual tissues and organs.

Interpretation of the Cs is simple: at values above 1, the product is dangerous to human health and at values below 1 they are deemed safe. This elementary interpretation is the basis for the feasibility of the criterion, aiming at eliminating the difficulty of relying on different dimensions of toxic factors in food products: mg/kg; mcg/kg; d/t, etc. In addition, in the control laboratories extra care was taken to properly update the information on the Maximum Allowable Levels (MALs) of certain toxic factors in food.

In the latest regulations Ordinance No. 5/09.12.2015 of the Ministry of Health, which introduces Regulation (EC) 1881/2006, has differentiated requirements for cadmium and lead for individual food resources:

- Lead: in poultry meat MAL of 0.10 mg / kg; by-products - 0.50 mg / kg

- Cadmium: in poultry meat 0.050 mg/kg; in liver 0.50 mg / kg; in kidneys - 1.0 mg /kg

These important documents lack information about cadmium and lead MALs in eggs, and therefore in the current research we use the norm for poultry meat.

The legal basis is supplemented by Regulation 1275/2013 amending the European Parliament's Directive 2002/32/EC, and the MALs of toxic factors in feed are regulated as follows: lead - 5 mg/kg and cadmium - 0.5 mg/kg of feed components. In this category, we include egg shells that are used as a source of calcium in compound feed.

The studies carried out show the main possibilities for the application of criterion Cs in food safety control. The studies carried out show insufficiencies in the regulatory framework - it lists the MALs for many types of hydro biomes, but, there are no requirements for the main food product eggs. Also, missing is the differentiation necessary for effective management of anthropocenoses: MAL for the secondary biological production used for human food: egg whites and egg yolks/MAL for cadmium and lead in the shell, which we see as raw material for the production of compound feed, i.e., a product that is not directly used for human food.

Regardless of these imperfections and incompleteness, we have used the MAL regulatory documents for the assessment of cadmium and lead content in the meat / as it is missing for the eggs, we have adopted the highest value of MAL/ and of the shells, in which there is a MAL for feed components:

Pb in egg white and yolk - 0.1 mg/kg and for the shell - 5 mg/kg

Cd in egg white and yolk - 0.05 mg/kg and for the shell 0.5 mg/kg

On the basis of these norms, we found that under experimental conditions, the Cs of lead in egg white and egg yolk was less than 1 in all groups during the whole experiment, with the exception of the 4th group in which at day 15 the Cs found in yolk was 1.1 on the 15th day; 1.0 at day 30; 1.3 on day 45 and 2.1 on day 60, after which, on day 75 and day 90 it is under 1. The Cs for the shell is far below the reference value of 5 mg/kg. Similar results were also found in determining the Cs of cadmium in eggs. In all groups except group 4, values of Cs below 1 in the egg white and the yolk were determined. In the 4th group, the yolk Cs is over 1: the 15th day -1.02; on the 30th day - 1.4; at day 45 - 0.96; on the 60th day - 1.14 and on the 75th day -1.8, then it dropped to 0.44. A low Cs of cadmium in the shell was determined in all groups throughout the entirety of the experiment, with the highest, but far below the risk values (= 1) were recorded in the 4th group: at day 45 and 60, where the Cs value was 0.12.

At the end of the 19th century, Clark found that the lithosphere was a chemical non-homogeneous environment. In the twentieth century, Vernadsky /in 2/ proved, that the chemical heterogeneity is a characteristic for the entire biosphere. He stresses that non-homogeneity is a characteristic of both, the abiotic components (lithosphere, hydrosphere and atmosphere) and of the living substance. Over the half century, the focus has been on a problem that is risky for populations and biosensors: the increased content of chemical elements in the living environment that reach dangerous levels for human health and biocenoses due to accumulation in the food chain. In the present work, in order to increase the effectiveness of anthropocenosen management, new criteria are proposed, which were described in our first paper on the problem (Kirov *et al.,* 2018). They define the chemical heterogeneity of the biosphere at sub-organismal level to limit the environmental risk with increased cadmium and lead content in the living environment.

In these studies, it was found that in 1st group /control/ -feed with a combined feed mixture prepared inclusive of all components according to the recipe, the content of Pb 0.45 mg/kg feed and Cd 0.38 mg/kg of feed, which corresponds to the Maximum Residues Limit (MRLs) during the entire experimental period, minimal amounts of lead and cadmium / below the instrument's susceptibility threshold are established, which means that there is no bioaccumulation in the three tested components of the egg. In group 2 fed with feed containing lead and cadmium 2.5 times more than 1st group and 3rdgroup fed with feed containing lead and cadmium 20 times more than 1 cadmium and lead were measured on the 30th day of experimentation and in Group 4th - fed with feed containing lead and cadmium 200 times more than group 1 on the 15th day, significant amounts of the two toxic elements in yolk and shell are found.

Different dynamics of the movement of the two toxic elements is established, depending on the dose and the study period. Most probably, a systems induction of the lead and cadmium accumulation systems is likely to cause a gradual reduction in the amount of lead and cadmium at the end of the experiment after a characteristic dynamic for lead in the egg yolk and the egg white in the 2nd and 3rd group - presence of peak concentration after reduction of the amounts in the 2nd,3rd and 4tb group in cadmium. These results, under model conditions, indicate that there are mechanisms of the organism level that lead to the reduction of the two toxic elements at the end of the experiment and in the three tested components (egg white, yolk and shell).

Applying the "Clark of distribution (Cd)" criterion shows changes in the distribution of lead and cadmium, both depending on the time of the study and the amount of the two elements in the ration. The highest is Cd of lead in the 2nd,3rd and 4th group of the shell, followed by the yolk, and in cadmium there is an established dynamic related to the quantity of the element in the ration.

Cs demonstrates the need to fine-tune the regulatory framework that lacks cadmium and lead MALs for eggs, showing that there are no differentiated requirements for secondary organic production to be used for human food / egg white and egg yolk / and fodder components / shells /. Cs is found to be below 1, i.e. there is no danger to the health of the consumer from the toxic effects of cadmium and lead in the egg yolk and protein of the 1st,2nd and 3rd group, the real risk is in the 4th group. The shells have not been found to have a Cs higher than 1, i.e. there is no danger of using the egg shells in animal feed.

The conclusions of the study are as follows:

1. In the case of the raising of laying hens with lead and cadmium content under MRL, the concentrating of the two toxic elements in egg white, egg yolk and egg shell is not found.

2. When increasing the amount of lead and cadmium in the ration, the concentrating effect is shown according to the contents in the ration: in groups 2 and 3 on the 30th day of the beginning of the experiment, and in the 4th group in yolk and the shell from the 15th day.

3. Criterion Cd indicates high distribution of lead and cadmium in the shell and yolk.

4. Through applying Cs, it was indicated that in the experimental conditions, that the lead and cadmium content in the egg white and egg yolk of the 1st,2nd and 3rd group is harmless for consumption and the amount of the two elements in the egg shell is below the values that pose a risk in the feed.

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